Microbial turnover of above and belowground litter components in shrublands

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Supplementary materials and methods

SM_1: Soil characteristics

Topsoil was collected from the Ah horizon (0-10 cm depth) and placed in gas-permeable plastic bags and transferred to the laboratory. Further details of the site, soil and management regime can be found in Glanville et al. (2016). The field-moist soil was then sieved to pass 2 mm to remove stones, plant roots and earthworms prior to analysis. Soil moisture content was measured by oven drying soil (10 g, 105°C, 16 h). Soil pH and electrical conductivity (EC) were determined in a 1:2 (w/v) soil:distilled water extracts (1 h, 250 rev min^{-1}). Water soluble N was determined by shaking field-moist soil (5 g) with 25 ml of distilled water (1 h, 250 rev min^{-1}, 20°C), centrifuging the extracts (16,000 *g*, 5 min) and recovery of the supernatant. Nitrate in the extract was determined using the vanadate method of Miranda et al. (2001) while ammonium was determined using the salicylate-nitroprusside and hypochlorite procedure of Mulvaney (1996). Free amino acids were determined by the fluorometric OPAME procedure (Jones et al., 2002), and dissolved organic C (DOC) and total dissolved N (TDN) were determined with a Multi N/C 2100S analyser (Analytik Jena, Jena, Germany). Total C and N were determined with a TruSpec® analyser (Leco Corp., St Joseph, MI, USA). Soil general properties are presented in Table S_1.

Table S_1

General properties of the soil used in the mineralisation studies. Values represent means ± standard error (n = 3).

Soil parameter	
Water content (% of field moist soil weight)	26.2 ± 0.1
pH (1:2 H_2O)	5.4 ± 0.1
EC (1:2 H_2O µS cm^{-1})	169.4 ± 4.1
Available NO_3^- (mg N kg soil)	10.2 ± 0.2
Available NH_4^+ (mg N kg^{-1} soil)	0.2 ± 0.1
Free amino acids (mg C kg^{-1} soil)	2.6 ± 0.9
Dissolved organic C (mg kg^{-1} soil)	26.8 ± 4.3
Dissolved organic N (mg kg^{-1} soil)	11.9 ± 2.5
Total C (g kg^{-1} soil)	37.7 ± 2.45
Total N (g kg^{-1} soil)	3.77 ± 0.11

SM_2: Growing and ^{14}C-labelling of plants

Cistus monspeliensis L. plants were propagated through cuttings, and grown in a hydroponic system consisting of 50% strength Long Ashton nutrient solution (KNO_3, 2 mM; Ca $(NO_3)_2.4H_2O$, 2 mM; NaH_2PO_4. $2H_2O$, 0.67 mM; $MgSO_4$. $7H_2O$, 0.75 mM; EDTA Fe III (CH_2.N (CH_2.COO)$_2$ I_2 Fe Na), 0.05 mM; micronutrients $MnSO_4.4H_2O$, 5 µM; $CuSO_4$ $5H_2O$, 0.5 µM; $ZnSO_4.7H_2O$, 0.5 µM; H_3BO_3, 25 µM; $Na_2MO_4.2H_2O$, 2.5 µM; NaCl, 100 µM) under laboratory conditions. Lighting was provided using a light bank that offers photosynthetically active radiation (400-700 nm) of approximately 201 µmol m^{-2} s^{-1} at plant height (SKP-200, Skye Instruments Ltd, UK).

An electronic timer was set to give a photoperiod of 16 h light and 8 h dark. Once the plant cuttings were established, they were placed in hydroponic troughs in a sealed acrylic chamber (410 × 410 × 500 mm) and were labelled with $^{14}CO_2$. $^{14}CO_2$ was generated by adding 1 ml of 1 M acetic acid to a 1.5 ml microcentrifuge tube containing 200 µl of 2 MBq $NaH^{14}CO_3$ and left to fix the $^{14}CO_2$ for 5 h. The process was repeated on 2 separate days, 3 days apart to get sufficient translocation of ^{14}C to all plant components. To ensure uniform distribution of generated $^{14}CO_2$ throughout the chamber, a small battery operated fan was provided inside the labelling chamber (Farrar et al., 2012).

SM3: Distribution and identification of ^{14}C in labelled plant material

The distribution of ^{14}C-label within plant material was determined by performing a sequential chemical extraction (Jones and Darrah, 1994). Briefly, 50 mg of ground, labelled plant material was sequentially extracted by adding 8 ml of deionised water for 30 min at 85°C, 8 ml of 20% ethanol for 30 min at 80°C, 5 ml of 0.3% HCl for 3 h at 95°C and 5 ml of 1 M NaOH for 1 h at 95°C. Samples were centrifuged at each step (5000 *g*, 15 min) and the supernatant collected after each extraction. The ^{14}C content of the extracts was determined using a Wallac 1409 liquid scintillation counter (EG&G, Milton Keynes, UK) after mixing with Scint Safe 3 scintillation fluid (Fisher Scientific, UK). Water and ethanol soluble compounds together approximately represent neutral detergent fibre (e.g. proteins, sugars and lipids), whereas HCl soluble represents hemicellulose and cellulose, NaOH soluble and insoluble fractions together represent the lignin fraction (Simfukwe et al., 2011). These results were tested with unlabelled plants simultaneously, by determining the neutral detergent fibre, hemicellulose, cellulose, and lignin content in leaf and roots, according

to Van Soest et al. (1991), using an Ankom 2000 (Ankom Technology, USA) automated fibre analyser (Jančík et al., 2008).

SM_4: Extraction of soluble and insoluble fractions in plant material

Hot water extraction was done by sub sampling 2 g each of ^{14}C-labelled leaves, stem and roots. These were subsequently placed into 50 cm^3 polypropylene tubes. 15 ml of deionised water was added and the samples placed in a hot water bath at 85°C for 1 h. Samples were then centrifuged (5000 *g*, 10 min) and soluble and insoluble fractions separated using a Whatman 40 filter paper (modified from Ahn et al., 2009). Soluble fractions of root, stem and leaf were sub-sampled and measured for ^{14}C content as described above. Similarly, dried insoluble root, stem, leaf fractions were oxidized to measure their total ^{14}C content using an OX-400 biological oxidizer (RJ Harvey Instrument Corp., Hillsdale, NJ) by collecting the $^{14}CO_2$ evolved in Oxosol scintillation fluid (National Diagnostics, Hessle, UK) and measured using a Wallac 1409 liquid scintillation counter (EG&G, Milton Keynes, UK). A similar extraction process was conducted for unlabelled plant components and soluble fraction from each component was analysed for distribution of low molecular weight (≤ 300 Da) compounds using MALDI-TOF mass spectrometry (Bruker Reflex IV; Bruker Corp., Billerica, MA) with TiO_2 as a matrix (Ke et al., 2010). Briefly, 25 mg of TiO_2 was mixed with 1 ml of distilled water and 10 µl of the solution dispersed on to the MALDI target plate. Subsequently, 10 µl of sample was added and dried for 1 h at room temperature and then used for MALDI-TOF MS analysis.

SM_5: ^{14}C mineralisation

Mineralisation of ^{14}C from soluble and insoluble fractions of plant litter components was studied over a 150 d period. Briefly, either 1 ml of the soluble fraction or 1 g of the insoluble fractions derived from either root, stems, or leaves were added to 50 cm^3 polypropylene tubes containing 10 g of field-moist soil. Deionised water (1 ml) was added to the insoluble fractions to equalise the moisture content with the soluble fractions. A vial containing 1 ml of 1 M NaOH was then added to each polypropylene tube to collect the $^{14}CO_2$ evolved (Jan et al., 2009; Glanville et al., 2016). Traps were changed at regular time intervals (1 h, 3 h, 6 h, 24 h, 48 h, 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months and 5 months) and the amount of $^{14}CO_2$ captured was determined as described above. Mineralization vessels were maintained at 10°C throughout the experimental period.

SM_6: Extraction of residual ^{14}C

At the end of the incubation period we estimated the amount of soluble ^{14}C remaining in the soil by extracting the soil with 0.5 M K_2SO_4 (Rousk and Jones, 2010). This ^{14}C can include soluble substrate not utilised by the soil microbial community and also soluble transformation products excreted by the microbial biomass. Briefly, 3 g of soil was subsampled, to which 15 ml of 1°C, 0.5 M K_2SO_4 was added. This was shaken for 15 min (250 rev min^{-1}) and 1.5 ml supernatant transferred into a microcentrifuge tube and centrifuged for 10 min (15,000 *g*). 1 ml of the supernatant was removed and its ^{14}C content determined as described above.

SM7: Data and statistical analysis

Many earlier studies have indicated that the mineralisation of ^{14}C-labelled organic substrates follows a biphasic kinetic pattern (Farrar et al., 2012; Glanville et al., 2012; Hill et al., 2008, 2012). Following this, a double exponential first order kinetic decay model was fitted to the experimental data:

$$Y = [a_1 \times \exp^{(-k_1 t)}] + [a_2 \times \exp^{(-k_2 t)}] \quad \text{(equation 1)}$$

where Y represents the amount of ^{14}C remaining in the soil, a_1 describes the first rapid mineralisation pool, k_1 is the exponential decay coefficient for a_1, while a_2 describes the second slower mineralisation pool and k_2 is the exponential decay coefficient for a_2 and t is time after ^{14}C-substrate addition to soil.

The soluble ^{14}C-labelled plant fractions are likely to be primarily composed of low molecular weight solutes such as sugars, organic acids, amino acids and peptides with some higher molecular weight proteins also present. In the case of the soluble ^{14}C-labelled plant fractions, pool a_1 was attributed to the rapid use of ^{14}C-substrate in catabolic processes leading to the loss of $^{14}CO_2$ in respiration while pool a_2 was attributed to the slower turnover of ^{14}C, assumed to be initially immobilised in the microbial biomass via anabolic processes (Simfukwe et al., 2011). The assumptions and validation of this modelling approach is provided in Glanville et al. (2016).

In the case of the insoluble ^{14}C-labelled plant material, pool a_1 was attributed to the rapid use of labile, non-water soluble C compounds (e.g. proteins, lipids) while a_2 was largely attributable to the slower turnover of the ^{14}C of more recalcitrant high molecular weight insoluble polymers (e.g. cellulose, hemicellulose, lignin; Simfukwe et al., 2011).

The half-life period for the first mineralisation pool a_1 can be calculated by using the following equation:

$$t_{1/2} = \ln(2)/k_1 \quad \text{(equation 2)}$$

However, the added C substrate to soil may be transformed by several microbial processes, and calculating the half-life period for the second phase (k_2) is subject to uncertainty due to the complexity over the connectivity between pool a_1 and a_2 (Boddy et al., 2008; Glanville et al., 2016). Therefore the combined half-life period for both pools ($a_1 + a_2$) together for each C substrate was calculated numerically as presented in Oburger and Jones (2009). Equation 1 cannot be solved explicitly for t and so the Newton-Raphson algorithm was applied to the double exponential decay equations to calculate the time when the overall ^{14}C remaining in the soil is half the initial amount (i.e. 50% remaining in the $a_1 + a_2$ pools combined); from here on this is referred to as the substrate halving-time ($Y_{½}$).

Following Glanville et al. (2016) the microbial carbon use efficiency (CUE) for the soluble plant material can be calculated as follows:

$$CUE = a_2/(a_1 + a_2)$$

CUE cannot be calculated for the insoluble component as it is not possible to distinguish between C remaining in the plant material and that transformed into soil microbial biomass.

The temporal mineralisation of the soluble, insoluble plant fractions and whole leaves were plotted using SigmaPlot v12.3 (Systas Software Inc., Chicago, IL). A least sum of squares curve fitting algorithm in SigmaPlot v12.3 was then used to fit the kinetic equation to the experimental data (Glanville et al., 2016). A one way ANOVA with PostHoc least significant difference test using SPSSv20.0 (SPSS Inc., Chicago, IL) was used to compare the size of the mineralisation pools a_1 and a_2, their corresponding decay rate constants k_1 and k_2 and half-life period values of pool a_1 for the different substrates. We accepted $P < 0.05$ as an indication of statistical significance.

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Table S2. Distribution of ^{14}C in plant material (% of total ^{14}C in the whole plant material) based on the sequential extraction compared with unlabelled plant material. Value represents means ± standard error mean. Differing lower case letters identify signs of significant difference ($P < 0.05$; $n = 3$) within each column for ^{14}C-labelled plants only. NA indicates data not available.

Plant component	Neutral detergent soluble		Cellulose + Hemicellulose		Lignin	
	^{14}C-labelled	Unlabelled	^{14}C-labelled	Unlabelled	^{14}C-labelled	Unlabelled
Root	45 ± 2[a]	40.3 ± 2.6	36.3 ± 3.8[a]	40.6 ± 2.5	18.7 ± 3.9[a]	19.1 ± 0.3
Stem	76.7 ± 1.8[b]	NA	8.6 ± 0.2[b]	NA	14.6 ± 1.6[a]	NA
Leaf	62.4 ± 2.6[c]	61.2 ± 1.5	19.1 ± 0.7[c]	26 ± 0.5	18.4 ± 2.6[a]	12.7 ± 1.5

Fig. S1. Relative distribution of low molecular weight compounds in soluble fractions of shrub roots, stems and leaves. Values represent means ± SEM (n = 3). Columns with different letters indicate significant differences within each group ($P < 0.05$).

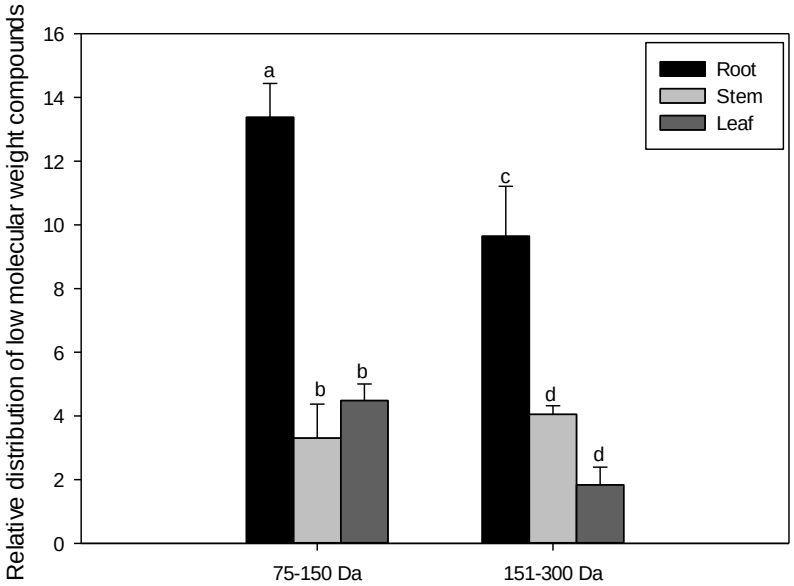

Fig. S2. ^{14}C mineralisation during the first hour following additions of ^{14}C-labelled shrub root, stem and leaf to soil. Values represent means ± SEM (n = 3). Columns with different letters indicate significant differences within each group ($P < 0.05$).