

Microbial turnover of above and belowground litter components in shrublands

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Pedobiologia

DOI:
[10.1016/j.pedobi.2016.07.001](https://doi.org/10.1016/j.pedobi.2016.07.001)

Published: 01/07/2016

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Marella, V. S., Hill, P., Jones, D., & Roberts, P. (2016). Microbial turnover of above and belowground litter components in shrublands. *Pedobiologia*, 59(4), 229-232.
<https://doi.org/10.1016/j.pedobi.2016.07.001>

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1 **Title: Microbial turnover of above and belowground litter components in**
2 **shrublands**

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8 **Author contributions:** All authors contributed equally to the research and manuscript

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22 **Supplementary materials and methods**

23 **SM₁: Soil characteristics**

24 Topsoil was collected from the Ah horizon (0-10 cm depth) and placed in gas-
25 permeable plastic bags and transferred to the laboratory. Further details of the site, soil
26 and management regime can be found in Glanville et al. (2016). The field-moist soil
27 was then sieved to pass 2 mm to remove stones, plant roots and earthworms prior to
28 analysis. Soil moisture content was measured by oven drying soil (10 g, 105°C, 16 h).
29 Soil pH and electrical conductivity (EC) were determined in a 1:2 (w/v) soil:distilled
30 water extracts (1 h, 250 rev min⁻¹). Water soluble N was determined by shaking field-
31 moist soil (5 g) with 25 ml of distilled water (1 h, 250 rev min⁻¹, 20°C), centrifuging
32 the extracts (16,000 g, 5 min) and recovery of the supernatant. Nitrate in the extract was
33 determined using the vanadate method of Miranda et al. (2001) while ammonium was
34 determined using the salicylate-nitroprusside and hypochlorite procedure of Mulvaney
35 (1996). Free amino acids were determined by the fluorometric OPAME procedure
36 (Jones et al., 2002), and dissolved organic C (DOC) and total dissolved N (TDN) were
37 determined with a Multi N/C 2100S analyser (Analytik Jena, Jena, Germany). Total C
38 and N were determined with a TruSpec[®] analyser (Leco Corp., St Joseph, MI, USA).
39 Soil general properties are presented in Table S₁.

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47 **Table S1**

48 General properties of the soil used in the mineralisation studies. Values represent

49 means \pm standard error ($n = 3$).

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Soil parameter	
Water content (% of field moist soil weight)	26.2 \pm 0.1
pH (1:2 H ₂ O)	5.4 \pm 0.1
EC (1:2 H ₂ O μ S cm ⁻¹)	169.4 \pm 4.1
Available NO ₃ ⁻ (mg N kg soil)	10.2 \pm 0.2
Available NH ₄ ⁺ (mg N kg ⁻¹ soil)	0.2 \pm 0.1
Free amino acids (mg C kg ⁻¹ soil)	2.6 \pm 0.9
Dissolved organic C (mg kg ⁻¹ soil)	26.8 \pm 4.3
Dissolved organic N (mg kg ⁻¹ soil)	11.9 \pm 2.5
Total C (g kg ⁻¹ soil)	37.7 \pm 2.45
Total N (g kg ⁻¹ soil)	3.77 \pm 0.11

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52 **SM2: Growing and ¹⁴C-labelling of plants**

53 *Cistus monspeliensis* L. plants were propagated through cuttings, and grown in a
 54 hydroponic system consisting of 50% strength Long Ashton nutrient solution (KNO₃,
 55 2 mM; Ca (NO₃)₂.4H₂O, 2 mM; NaH₂PO₄. 2H₂O, 0.67 mM; MgSO₄. 7H₂O, 0.75 mM;
 56 EDTA Fe III (CH₂.N (CH₂.COO)₂ I₂ Fe Na), 0.05 mM; micronutrients MnSO₄.4H₂O,
 57 5 μ M; CuSO₄ 5H₂O, 0.5 μ M; ZnSO₄.7H₂O, 0.5 μ M; H₃BO₃, 25 μ M; Na₂MO₄.2H₂O,
 58 2.5 μ M; NaCl, 100 μ M) under laboratory conditions. Lighting was provided using a
 59 light bank that offers photosynthetically active radiation (400-700 nm) of
 60 approximately 201 μ mol m⁻² s⁻¹ at plant height (SKP-200, Skye Instruments Ltd, UK).

61 An electronic timer was set to give a photoperiod of 16 h light and 8 h dark. Once the
62 plant cuttings were established, they were placed in hydroponic troughs in a sealed
63 acrylic chamber (410 × 410 × 500 mm) and were labelled with $^{14}\text{CO}_2$. $^{14}\text{CO}_2$ was
64 generated by adding 1 ml of 1 M acetic acid to a 1.5 ml microcentrifuge tube containing
65 200 μl of 2 MBq $\text{NaH}^{14}\text{CO}_3$ and left to fix the $^{14}\text{CO}_2$ for 5 h. The process was repeated
66 on 2 separate days, 3 days apart to get sufficient translocation of ^{14}C to all plant
67 components. To ensure uniform distribution of generated $^{14}\text{CO}_2$ throughout the
68 chamber, a small battery operated fan was provided inside the labelling chamber (Farrar
69 et al., 2012).

70

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

72 The distribution of ^{14}C -label within plant material was determined by
73 performing a sequential chemical extraction (Jones and Darrah, 1994). Briefly, 50 mg
74 of ground, labelled plant material was sequentially extracted by adding 8 ml of
75 deionised water for 30 min at 85°C, 8 ml of 20% ethanol for 30 min at 80°C, 5 ml of
76 0.3% HCl for 3 h at 95°C and 5 ml of 1 M NaOH for 1 h at 95°C. Samples were
77 centrifuged at each step (5000 g, 15 min) and the supernatant collected after each
78 extraction. The ^{14}C content of the extracts was determined using a Wallac 1409 liquid
79 scintillation counter (EG&G, Milton Keynes, UK) after mixing with Scint Safe 3
80 scintillation fluid (Fisher Scientific, UK). Water and ethanol soluble compounds
81 together approximately represent neutral detergent fibre (e.g. proteins, sugars and
82 lipids), whereas HCl soluble represents hemicellulose and cellulose, NaOH soluble and
83 insoluble fractions together represent the lignin fraction (Simfukwe et al., 2011). These
84 results were tested with unlabelled plants simultaneously, by determining the neutral
85 detergent fibre, hemicellulose, cellulose, and lignin content in leaf and roots, according

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

88

89 **SM4: Extraction of soluble and insoluble fractions in plant material**

90 Hot water extraction was done by sub sampling 2 g each of ¹⁴C-labelled leaves,
91 stem and roots. These were subsequently placed into 50 cm³ polypropylene tubes. 15
92 ml of deionised water was added and the samples placed in a hot water bath at 85°C for
93 1 h. Samples were then centrifuged (5000 g, 10 min) and soluble and insoluble fractions
94 separated using a Whatman 40 filter paper (modified from Ahn et al., 2009). Soluble
95 fractions of root, stem and leaf were sub-sampled and measured for ¹⁴C content as
96 described above. Similarly, dried insoluble root, stem, leaf fractions were oxidized to
97 measure their total ¹⁴C content using an OX-400 biological oxidizer (RJ Harvey
98 Instrument Corp., Hillsdale, NJ) by collecting the ¹⁴CO₂ evolved in Oxosol scintillation
99 fluid (National Diagnostics, Hesse, UK) and measured using a Wallac 1409 liquid
100 scintillation counter (EG&G, Milton Keynes, UK). A similar extraction process was
101 conducted for unlabelled plant components and soluble fraction from each component
102 was analysed for distribution of low molecular weight (≤ 300 Da) compounds using
103 MALDI-TOF mass spectrometry (Bruker Reflex IV; Bruker Corp., Billerica, MA) with
104 TiO₂ as a matrix (Ke et al., 2010). Briefly, 25 mg of TiO₂ was mixed with 1 ml of
105 distilled water and 10 μ l of the solution dispersed on to the MALDI target plate.
106 Subsequently, 10 μ l of sample was added and dried for 1 h at room temperature and
107 then used for MALDI-TOF MS analysis.

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111 **SM₅: ¹⁴C mineralisation**

112 Mineralisation of ¹⁴C from soluble and insoluble fractions of plant litter
113 components was studied over a 150 d period. Briefly, either 1 ml of the soluble fraction
114 or 1 g of the insoluble fractions derived from either root, stems, or leaves were added
115 to 50 cm³ polypropylene tubes containing 10 g of field-moist soil. Deionised water (1
116 ml) was added to the insoluble fractions to equalise the moisture content with the
117 soluble fractions. A vial containing 1 ml of 1 M NaOH was then added to each
118 polypropylene tube to collect the ¹⁴CO₂ evolved (Jan et al., 2009; Glanville et al., 2016).
119 Traps were changed at regular time intervals (1 h, 3 h, 6 h, 24 h, 48 h, 1 week, 2 weeks,
120 1 month, 2 months, 3 months, 4 months and 5 months) and the amount of ¹⁴CO₂
121 captured was determined as described above. Mineralization vessels were maintained
122 at 10°C throughout the experimental period.

123

124 **SM₆: Extraction of residual ¹⁴C**

125 At the end of the incubation period we estimated the amount of soluble ¹⁴C
126 remaining in the soil by extracting the soil with 0.5 M K₂SO₄ (Rousk and Jones, 2010).
127 This ¹⁴C can include soluble substrate not utilised by the soil microbial community and
128 also soluble transformation products excreted by the microbial biomass. Briefly, 3 g of
129 soil was subsampled, to which 15 ml of 1°C, 0.5 M K₂SO₄ was added. This was shaken
130 for 15 min (250 rev min⁻¹) and 1.5 ml supernatant transferred into a microcentrifuge
131 tube and centrifuged for 10 min (15,000 g). 1 ml of the supernatant was removed and
132 its ¹⁴C content determined as described above.

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136 **SM7: Data and statistical analysis**

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

141
$$Y = [a_1 \times \exp^{-k_1 t}] + [a_2 \times \exp^{-k_2 t}] \quad (\text{equation 1})$$

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

146 The soluble ^{14}C -labelled plant fractions are likely to be primarily composed of
147 low molecular weight solutes such as sugars, organic acids, amino acids and peptides
148 with some higher molecular weight proteins also present. In the case of the soluble ^{14}C -
149 labelled plant fractions, pool a_1 was attributed to the rapid use of ^{14}C -substrate in
150 catabolic processes leading to the loss of $^{14}\text{CO}_2$ in respiration while pool a_2 was
151 attributed to the slower turnover of ^{14}C , assumed to be initially immobilised in the
152 microbial biomass via anabolic processes (Simfukwe et al., 2011). The assumptions
153 and validation of this modelling approach is provided in Glanville et al. (2016).

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

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

161 $t_{1/2} = \ln(2)/k_1$ (equation 2)

162 However, the added C substrate to soil may be transformed by several microbial
163 processes, and calculating the half-life period for the second phase (k_2) is subject to
164 uncertainty due to the complexity over the connectivity between pool a_1 and a_2 (Boddy
165 et al., 2008; Glanville et al., 2016). Therefore the combined half-life period for both
166 pools ($a_1 + a_2$) together for each C substrate was calculated numerically as presented in
167 Oburger and Jones (2009). Equation 1 cannot be solved explicitly for t and so the
168 Newton-Raphson algorithm was applied to the double exponential decay equations to
169 calculate the time when the overall ^{14}C remaining in the soil is half the initial amount
170 (i.e. 50% remaining in the $a_1 + a_2$ pools combined); from here on this is referred to as
171 the substrate halving-time ($Y_{1/2}$).

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

174 $\text{CUE} = a_2/(a_1 + a_2)$

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

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

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244

245 **Table S2.** Distribution of ^{14}C in plant material (% of total ^{14}C in the whole plant
 246 material) based on the sequential extraction compared with unlabelled plant material.
 247 Value represents means \pm standard error mean. Differing lower case letters identify
 248 signs of significant difference ($P < 0.05$; $n = 3$) within each column for ^{14}C -labelled
 249 plants only. NA indicates data not available.
 250

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

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

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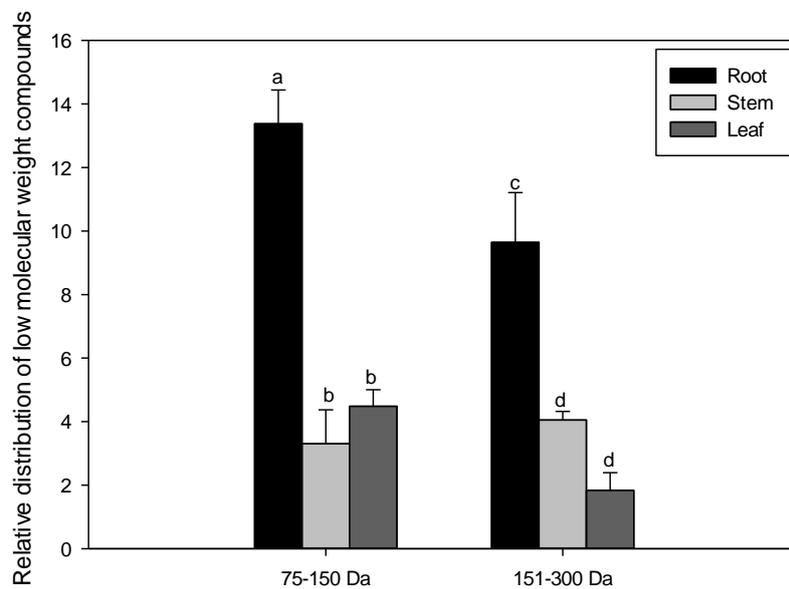
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267 **Fig. S2.** ^{14}C mineralisation during the first hour following additions of ^{14}C -labelled
268 shrub root, stem and leaf to soil. Values represent means \pm SEM ($n = 3$). Columns with
269 different letters indicate significant differences within each group ($P < 0.05$).
270

