Microbial turnover of above and belowground litter components in shrublands

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Abstract

Shrublands cover a large proportion of the world's land surface, yet they remain poorly studied in comparison to other ecosystems. Within shrublands, soil organic matter (SOM) is replenished from inputs of both above- and below-ground plant litter, however, their relative importance depends on their respective turnover rates. To critically address this, we measured the biodegradation rates of the soluble and insoluble components of ^{14}C-labelled above- and below-ground plant litter in soil. During the 150 day incubation, the amount of plant-derived soluble-C lost as $^{14}CO_2$ was similar for the different plant parts being 64.7 ± 2.3% for roots, 72.1 ± 7.4% for stems, and 72.4 ± 1.8% for leaves. In comparison, the turnover of the insoluble fraction was much slower. However, again little difference in mineralisation was seen for the different plant parts with the total losses being 21.1 ± 0.9% for roots, 19.5 ± 1.6% for stems, and 19.6 ± 1% for leaves. A double exponential first order kinetic model fitted well to the experimental data. It also allowed the partitioning of C between microbial anabolic and catabolic processes for the soluble C component. Using this model, we deduced that the soluble fraction turns over *ca.* 40 times annually, whereas it takes *ca.* 2.5 years to turnover the insoluble fraction. For the soluble plant component, the overall microbial carbon use efficiency (CUE) was estimated to be greater for root-derived C in comparison to that derived from aboveground (no difference was observed for the insoluble component). From this, we tentatively suggest that C sourced from belowground plant components may persist longer in soil than C derived from aboveground plant components.

Key words: belowground carbon storage, mineralisation, nutrient cycling, litter decomposition, root turnover

Soil organic matter (SOM) represents a major store of terrestrial carbon (C) (Schlesinger, 1997) and its turnover and replenishment represents a critical component of the global C cycle. SOM is primarily derived from the continual input of above- and below-ground plant components, however, their relative importance, particularly in shrubland ecosystems, remains poorly understood (Vogt et al., 1986). Earlier studies have suggested that plant roots contribute a larger proportion of C to soil organic carbon (SOC) than plant shoots, due to their greater chemical recalcitrance in relation to microbial enzymatic breakdown (Broadbent and Nakashima, 1974; Jane et al., 2007). In contrast, within some agroecosystems, significant contributions by crop shoots have also been observed (Barber, 1979).

The input of organic matter to the soil can be broadly classified into two pools (van Hees et al., 2005). The first pool is described as the dissolved organic C component that includes low molecular weight, highly bioavailable compounds such as organic acids, peptides, amino acids, mono- and oligo-saccharides, amino sugars, phenolics and siderophores (McKeague et al., 1986). The second pool consists of plant polymers such as cellulose, hemicellulose, lignin and some proteins, which are relatively resistant to microbial attack (Kalbitz et al., 2000). These two pools can have vastly different C:N:P ratios which may subsequently influence their rate of processing and also microbial carbon use efficiency (CUE; Schmidt et al., 2011).

Numerous studies have described the mineralisation of individual low molecular weight compounds (Glanville et al., 2012), plant material (Simfukwe et al., 2011) and have measured the subsequent rates of $^{14}CO_2$ evolution and/or microbial incorporation. These studies have enhanced our understanding of the ^{14}C mineralisation process of single or occasionally combinations of simple C compounds by the microbial

community. However, plant material consists of vast range of compounds (Buckingham, 1993) and the mineralisation capacity of microorganisms to act upon more complex suite of substrates provides a more representative estimate of the potential for C storage in soil. Therefore, the aim of this study was to assess the microbial turnover of the soluble and insoluble fractions of above- and below-ground plant components (root, stem, leaf) from a common shrubland plant to assess their persistence in the soil under laboratory conditions.

Soil was obtained from the Henfaes experimental station located in Abergwyngregyn, Gwynedd, North Wales (53°14'N, 4°01'W) UK. The sandy clay loam textured soil is classified as a Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy) (see SM_1 and Table S_1). *Cistus monspeliensis* L. plants were grown in a hydroponic system consisting of 50% strength Long Ashton nutrient solution under laboratory conditions. Plants were labelled with ^{14}C twice, 3 days apart for 5 h each time to get sufficient translocation of ^{14}C to all plant components (see SM_2). Immediately after the second labelling, the plant components were separated into leaves, stem, and roots and air-dried. The dried plant parts were finely ground using a ball mill and stored in 50 ml polypropylene tubes at 20°C for further analysis. The distribution of ^{14}C label among soluble and structural fractions of plant material was determined by performing a sequential chemical extraction. These results were tested in parallel with unlabelled plants, using an automated fibre analyser (see SM_3). The soluble and insoluble fraction from each of the three plant components were separated using a hot water extract (see SM_4) and amended to field-moist soil contained in 50 cm^3 polypropylene tubes. The mineralisation of the ^{14}C-labelled components was studied for 150 days and values were expressed as a percentage of the initial amount of ^{14}C applied to the soil (see SM_5). Similar extraction process was conducted with unlabelled

plant components and the soluble fraction from each component was analysed for distribution of low molecular weight (≤ 300 Da) compounds using MALDI-TOF mass spectrometry (Bruker Reflex IV) with TiO_2 as a matrix. At the end of the incubation period, the amount of soluble ^{14}C remaining in the soil either as unaltered plant material or fixed in the microbial biomass was determined by extracting the soil in 0.5 M K_2SO_4 (see SM_6). A double exponential first order decay model was then fitted to the experimental data (Glanville et al., 2016). Substrate-C pool distribution within the microbial community, decay constants, CUE and half-lives (Newton-Raphson iteration method) (Oburger and Jones, 2009) were calculated (see SM_7). The data was analysed by one-way ANOVA with Post-Hoc least significant difference test using SPSSv20.0 (SPSS Inc., Chicago, IL) using $P < 0.05$ as an indication of statistical significance.

Following the labelling process, the distribution of ^{14}C into soluble and structural fractions of the different plant components was broadly similar to the total amount of unlabelled ^{12}C in each chemical fraction, although the data for stems is not available (Table S_2). This indicates a fairly uniform dilution of the ^{14}C isotope within the plant. The addition of ^{14}C-labelled soluble and insoluble fractions to soil caused an initial rapid phase of $^{14}CO_2$ evolution followed by a secondary slower phase, irrespective of plant tissue type (Fig. 1). The overall amount of ^{14}C mineralisation in soils amended with soluble fractions was substantially higher compared to the values obtained for the insoluble fractions ($P < 0.001$). This was presumably due to the presence of more labile low molecular weight compounds in the soluble fractions. Conversely, insoluble fractions broadly consist of structural polymers which require enzymatic depolymerisation to promote solubilisation prior to uptake and assimilation by the microbial community (van Hees et al., 2005). Among the soluble fractions, root-derived ^{14}C showed the fastest mineralisation rate followed by stem and leaf ^{14}C during

122 the first hour, presumably because of relatively higher quantities of low molecular
123 weight compounds which exist in roots (Figs. S_1 and S_2). After 24 h, the amount of ^{14}C
124 mineralisation of the root soluble fraction (19.7 ± 0.4%) was substantially higher than
125 for the stems (8.7 ± 0.3%) and leaves (5.7 ± 0.3%). Similarly, among the insoluble
126 fractions, the root-derived ^{14}C fraction had the highest initial mineralization rate (0.62
127 ± 0.2%) within 24 h, followed by the stems (0.43 ± 0.02%) and leaves (0.26 ± 0.01%).
128 However, at the end of 150 days, the pattern had changed with 64.7% ± 2.3, 72.1 ±
129 7.4%, and 72.4 ± 1.8% of the soluble fraction lost for the root, stem and leaf-derived
130 ^{14}C, respectively. In contrast, for the three insoluble fractions the amount recovered as
131 $^{14}CO_2$ after 150 d was very similar, being 21.1 ± 0.9%, 19.5 ± 1.6%, and 19.6 ± 1% of
132 the total ^{14}C added for the root, stem and leaves respectively.

133 The amount of ^{14}C allocated to the rapid mineralisation pool (a_1) and
134 corresponding decay constant values (k_1) were much higher for soluble fractions than
135 insoluble fractions (Table 1), presumably due to their rapid assimilation by microbial
136 biomass (Boddy et al., 2007). This is supported by the lack of soluble-^{14}C recovered
137 from the soil after 150 d (Fig. 2). The half-life periods calculated from k_1 for the
138 insoluble fractions were 3-5 fold longer than that of the soluble fraction. However, the
139 k_2 values were very low (100-200 times lower than the k_1 values) for both soluble and
140 insoluble fractions and were significantly different. Using the Newton-Raphson
141 iteration method, the combined half-life period for both pools together (a_1+a_2) was *ca.*
142 9 and 930 d for the soluble and insoluble fractions respectively (Oburger and Jones,
143 2009). Thus, soluble fractions turnover *ca.* 40 times annually, whereas insoluble
144 fractions take *ca.* 2.5 years to turnover.

145 It was interesting to note that approximately 20% more soluble C derived from
146 the aboveground plant components (leaf and stem) was allocated to microbial catabolic

C pools (pool a_1) than soluble C derived from the belowground component (despite having an initial slower ^{14}C mineralisation rate). Conversely, more root-derived soluble ^{14}C was allocated to anabolic microbial processes (pool a_2) thus resulting in a higher CUE for the below-ground soluble component (Glanville et al., 2016). Hence, microbes have shown more efficient usage of root soluble ^{14}C compared to leaf and stem which could be major driver for ecosystem C storage potential (Sinsabaugh et al., 2013). Thus, we tentatively suggest that C sourced from belowground plant components persists longer than the above ground plant components in soil. However, overall contributions can only be calculated once the total flux of each component into the ecosystem is known. In addition, the amount of C associated with mycorrhizal turnover and root exudation would be needed to complete the budget. Nevertheless, the results obtained here highlight the importance of roots in soil C storage especially as plants in most shrublands heavily invest in belowground biomass in the form of a deeper root system (Meyer, 2011). Results also support suggestions that increased allocation of C to roots under elevated atmospheric CO_2 may partially mitigate atmospheric CO_2 rise by increasing soil C storage (Madhu and Hatfield, 2013).

In conclusion, this study has clearly demonstrated the faster mineralisation of soluble fractions compared to the insoluble fractions. Additionally, modelling of the C pools tentatively suggests the longer persistence of belowground components in soil relative to shoots and leaves.

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