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# Acid hydrolysis to partition plant material into decomposable and resistant fractions for use in the Rothamsted carbon model

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### Abstract

Using various plant materials, we identified two conceptual pools of plant litter, decomposable plant material (DPM) and resistant plant material (RPM), in the Rothamsted Carbon Model (RothC) by comparing the default proportions of DPM and RPM in the RothC and proportions in plant material fractions as determined by two-step acid hydrolysis with  $H_2SO_4$ . We collected 37 plant samples from 15 species at six sites on arable land, grassland, or forest in Japan. Carbon in the plant materials was divided into three pools by acid hydrolysis: (a) Labile Pool I (LP I), obtained by hydrolysis with 5 N  $H_2SO_4$  at 105 °C for 30 min; (b) Labile Pool II (LP II), obtained by hydrolysis with 26 N  $H_2SO_4$  at room temperature overnight, and then with 2 N  $H_2SO_4$  at 105 °C for 3 h; and (c) Recalcitrant Pool (RP), the unhydrolyzed residue. The average proportion of LP I in crops and grasses was 59%, which was the same as the proportion of DPM defined in the RothC as the default value for crops and grasses. The remaining 41% (23% LP II + 18% RP) was consequently the same as the RPM proportion defined in the RothC. Similarly, the average proportion of LP I in all tree leaves (19%) was very close to the proportion of DPM in the RothC (20%) for trees. These results indicate that DPM in the RothC can be identified as LP I from the acid hydrolysis analysis and RPM as LP II + RP. We conclude that, at least theoretically, the use of an independent DPM:RPM ratio, as determined by acid hydrolysis analysis for each plant material, should enable more reliable modeling of SOM dynamics than the use of default DPM:RPM values provided by the model, even though the practical advantages of this method require further evaluation.

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# 1. Introduction

Soil organic matter (SOM) turnover models are very effective at simulating changes in SOM associated with different agricultural management systems or with climatic changes. Many SOM turnover models have been developed (e.g. review by McGill, 1996). However, a limitation of such models—namely, the fact that most of the conceptual pools they contain do not correspond to experimentally measurable fractions—has been stressed (Christensen, 1996; Elliot et al., 1996). If these pools could be related to measurable fractions, it would be possible to initialize the model without the need to input historical data or run the model repeatedly assuming equilibrium conditions; it would also be possible to validate the model using the size of each pool as well as the total soil organic carbon (SOC).

The Rothamsted Carbon Model (RothC: Coleman and Jenkinson, 1996) is one of the leading SOM turnover models and is widely used worldwide. It contains five compartments of SOC, including two plant litter compartments (decomposable plant material, DPM; and resistant plant material, RPM) and three other SOC pools (microbial biomass, BIO; humified organic matter, HUM; and inert organic matter, IOM). BIO is measurable by a fumigation– extraction method (Vance et al., 1987), but the other four pools are basically conceptual.

Several studies (Balesdent, 1996; Ludwig et al., 2003; Skjemstad et al., 2004) have tried to match measurable fractions with some of the conceptual pools of the RothC. However, the two plant litter compartments (DPM and RPM) have not been matched with experimentally measurable fractions.

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The proportions of various compounds (e.g. carbohydrate, protein, hemicellulose, cellulose, and lignin) in plant materials can be determined by a series of chemical extractions (e.g. the classical method of Waksman and Stevens, 1930). Among such compounds, lignin is known as one of the more recalcitrant. As it is usually quantified as the residue of hydrolysis with concentrated H<sub>2</sub>SO<sub>4</sub>, an attack with strong acid could be used as a criterion to separate labile and recalcitrant fractions. Rovira and Vallejo (2000) reported that two-step acid hydrolysis with H<sub>2</sub>SO<sub>4</sub> was the best predictor of organic matter decomposition, rather than hydrolysis with 6 N HCl, and was much better than other indices such as the C/N ratio or lignin/N ratio. It is therefore possible that the proportions of fractions determined by the acid hydrolysis analysis of Rovira and Vallejo (2000) could be matched with the proportions of DPM and RPM in the RothC.

Our objective was to identify the DPM and RPM in the RothC by comparing the default proportions of DPM and RPM in the RothC and the proportions of fractions obtained by acid hydrolysis analysis of various plant materials.

## 2. Materials and methods

#### 2.1. Plant samples

Thirty-seven plant samples from 15 species were collected from six sites in Japan (Table 1). Three crops (Triticum aestivum, Zea mays, and Brassica campestris) were sampled from upland crop fields at NIAES (National Institute for Agro-Environmental Sciences; N36°01', E140°07') on 8 May 2002, 12 August 2002, and 19 November 2002, respectively. They were then separated into nine samples of different plant parts (ears, leaves+ stems, roots, etc.). Three wild grasses (Imperata cylindrica, Solidago altissima, and Miscanthus sinensis) were sampled from grassland at TERC (Terrestrial Environment Research Center, University of Tsukuba; N36°07', E140°06') on 20 December 2002 and then separated into eight samples of different parts. Samples of two wild grasses (Miscanthus sinensis and Sasa spp.) and two trees (Quercus mongolica and Pinus densiflora), used to make seven samples of different plant parts, were taken from grassland and adjacent forest at Sugadaira (Sugadaira Montane Research Center, University of Tsukuba; N36°31', E138°21') on 25 May 2004. Samples of Sasa spp. and four trees (Quercus mongolica, Betula ermanii, Betula platyphylla, and Acer palmatum), making up six samples of different parts, were taken from a forest at Takayama (N36°08', E137°25') on 21 October 2003. Samples of two trees (Pinus densiflora and *Ilex pedunculosa*), making four samples of different parts, were taken from a forest at Fuji-yoshida (N35°27', E138°46'), and two trees (Chamaecyparis obtusa and Tsuga sieboldii), making three samples of different parts,

were taken from a forest at Aokigahara (N35°28′, E138°40′) on 9 June 2004.

#### 2.2. Analysis

Samples of plant materials were dried and ground to pass 250-µm mesh. Acid hydrolysis analysis was conducted by the procedure of Rovira and Vallejo (2000, 2002).

Three hundred milligrams of ground sample was hydrolyzed with 20 ml of 5 N  $H_2SO_4$  for 30 min at 105 °C in a sealed Pyrex tube. The hydrolysate was recovered by centrifugation and decantation. The residue was washed twice with 25 ml of water, and the wash water added to the hydrolysate. This hydrolysate was taken as Labile Pool I (LP I).

The remaining residue was dried at 60 °C, and then hydrolyzed with 2 ml of 26 N  $H_2SO_4$  overnight at room temperature, under continuous shaking. Thereafter, water was added to dilute the acid to 2 N and the sample was hydrolyzed for 3 h at 105 °C, with occasional shaking. The hydrolysate was recovered by centrifugation and decantation. The residue was washed twice with 25 ml of water, and the wash water added to the hydrolysate. This hydrolysate was taken as Labile Pool II (LP II).

The remaining residue was transferred to a tared beaker and dried at 60 °C. This fraction was taken as the Recalcitrant Pool (RP).

The total C in LP I and LP II was analyzed with a TOC analyzer (TOC-V, Shimadzu Co., Tokyo, Japan). The total C in the RP was analyzed by a dry combustion method using an NC-Analyzer (Sumigraph NC-900, Sumika Chemical Analysis Service, Ltd, Osaka, Japan).

## 3. Results

Table 1 shows the proportions of each of the three pools (LP I, LP II, and RP) in the 37 plant samples from Japan (average of two replicated measurements), together with the results for four samples from Barcelona, as derived from data in Rovira and Vallejo (2002).

Proportions of LP I were highest in crops and grasses, lowest in trees, and intermediate in wild grasses. Proportions of RP were highest in trees, intermediate in wild grasses and lowest in crops and grasses. These results indicate that the plant materials in crops and grasses are the most labile and those in trees are the most recalcitrant. In addition, in the trees, woody materials (barks and branches) were more recalcitrant than leaves. Among tree leaves, conifer tree leaves were more recalcitrant than broadleaf tree leaves. These tendencies are similar to those found in previous studies of the decomposability of plant materials (e.g. Swift et al., 1979).

The average proportion of LP I in crops and grasses was 59%. This proportion was the same as the proportion of DPM (59%) defined in the RothC (Table 2) as a default value for crops and grasses. The remaining 41% (23% LP

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