

Supplementary Information S1: desorption of protein by extractants

Background

An experiment was undertaken to see if 0.5 M K₂SO₄ could be used to effectively recover ¹⁴C-labelled protein from litter and soil.

Method

Litter and soil (0.8 cm³) collected from Site 2 were placed in 20-mL polypropylene vessels and heated at 80 °C for 1 h to sterilise them. ¹⁴C-labelled protein isolated from tobacco leaves (0.1 mL; 11 kBq mL⁻¹) was then added to the litter or soil samples. As a control, ¹⁴C labelled protein was also added directly to 5 mL of distilled water or 0.5 M K₂SO₄ (i.e. no soil or litter present). The samples were incubated for 30 min at 20 °C. At the end of the incubation period, litter and soil were shaken with 5 mL of either 0.5 M K₂SO₄ or distilled water for 15 min (250 rev min⁻¹). The samples were then centrifuged (18,000 × *g*) and the amount of ¹⁴C-labelled protein in the supernatant determined by liquid scintillation counting. All treatments were carried out in triplicate. A two-way ANOVA (fixed effects models) with Fisher's LSD post-hoc test ($P \leq 0.05$) was used to separate groups. The statistical analysis was performed in SAS.

Results

Table S1 clearly shows that extraction with 0.5 M K₂SO₄ leads to a poor recovery of ¹⁴C-labelled protein from both litter and soil. Even in the absence of litter or soil (i.e. protein added directly to the water or 0.5 M K₂SO₄ solution), the presence of concentrated K₂SO₄ induced the precipitation of the protein through the salting-out phenomenon. The lower recovery from the soil treatment also suggests that some of the protein also became bound to the soil matrix and was not recovered. Although no difference was verified in protein recovery between K₂SO₄ and water in the soil, in the case of litter, greater recovery was observed when water was used as an extractant.

Table S1

Recovery of ¹⁴C-labelled protein from litter and soil (both heat-sterilised) when extracted with either 0.5 M K₂SO₄ or distilled water. Values represent means ± SEM ($n = 3$). Different lowercase letters indicate differences within columns, according to Fisher's LSD test ($P \leq 0.05$).

Extractant	Recovery of ¹⁴ C-labelled protein (%)		
	Control	Litter	Soil
Water	86.2 ± 0.3 a	61.9 ± 3.3 a	6.0 ± 0.2
K ₂ SO ₄	22.1 ± 0.7 b	21.2 ± 0.5 b	5.6 ± 0.3