Supplementary material

Proteins are a major component of dissolved organic nitrogen (DON) leached from terrestrially aged *Eucalyptus camaldulensis* leaves

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Fig. S1. PCO analysis of amino acid distribution in whole leaf and leachate samples (azide and no azide) after 24 hours.





Fig. S2. Time course leaching experiments with (\circ) and without (\bullet) azide addition showing average concentration of Individual amino acids over a 24 hour leaching period.



Fig. S3. Average proportion of total amino acids leached after 24 hours (a) and relative DON contribution of leached amino acids after 24 hours (b) in the azide addition experiment.



Fig. S4. Time course leaching experiments with (\circ) and without (\bullet) azide addition showing average concentration of dissolved organic nitrogen (DON) (a) and inorganic nutrients (NO_x) (b) and ammonium (NH₄⁺) (c) over a 24 hour period for *Eucalyptus camaldulensis* leaves. Data points are the means of five replicate samples ± 2 SE.



Fig. S5. (a) Simulation of DCAA-nitrogen leaching kinetics in the presence of azide according to first-order process, and (b) Simulation of DCAA-nitrogen leaching kinetics in the absence of azide, according to a surface site-induction and re-adsorption process. Mechanisms and fit parameters shown below.

Azide

AA-N_bound $\xrightarrow{k=0.149 hr^{-1}}$ AA-N_free

AA-N _{total}	AA-N_bound _{Initial}	χ^2
(mg-N/g)	(mg-N/g)	
0.880	0.742	0.827

Non-azide

AA-N_bound
$$\xrightarrow{k=0.149 hr^{-1}}$$
 AA-N_free
Surf $\xrightarrow{k=0.757 hr^{-1}}$ Surf* (×7)

AA-N_free + Surf* $\xrightarrow{k=3.91 hr^{-1}}$ AA-N_adsorbed

AA-N Total	AA-N_bound Initial (mg-	Surf Initial	χ^2
(mg-N/g)	N/g)	(mg-N /g)	
0.880	0.830	0.250	4.18



Fig. S6. Bright field micrographs and FPA-FTIR transmission images of transverse sections of Eucalyptus camaldulensis leaves, leached for 0 hours (**T0**) and 24 hours (**T24**) from mid-vein regions of leaf. IR maps are shown for wavenumber regions corresponding to: (i) stretching modes of Amide I region (1705-1570 cm⁻¹), (ii) C-O-C stretching modes of carbohydrates (1180 – 950 cm⁻¹), and (iii) C-O and C-C stretching modes (characteristic of lignin) (1260 – 1210 cm⁻¹).

Rep	Leaf Image	(i) v(C=O)	(ii) v(C-O-C)	(iii) v(C-O) v(C-C)
T0-1				12 8
T24-1				
T0-2	236	190 - 2.4 1.2.1 - 2.4	de al	04 3 5
T24-2		44.15	2	2
T0-3			\$ -	& .
T24-3	010			
T0-4	Cor.	ne m	6 L.	
T24-4				
T0-5				
T24-5			٩	\$

Fig. S7. Bright field micrographs and FPA-FTIR transmission images of transverse sections of Eucalyptus camaldulensis leaves, leached for 0 hours (**T0**) and 24 hours (**T24**) from mesophyll regions of leaf. IR maps are shown for wavenumber regions corresponding to: (i) stretching modes of Amide I region (1705-1570 cm⁻¹), (ii) C-O-C stretching modes of carbohydrates (1180 – 950 cm⁻¹), and (iii) C-O and C-C stretching modes (characteristic of lignin) (1260 – 1210 cm⁻¹).

Fig. S8. (a) PCA scores plot for FTIR spectra $(1800 - 1400 \text{ cm}^{-1})$ extracted from transverse sections of unleached and leached *E. camaldulensis* leaves. (b) Loading plots for PC-1 and PC-2 which combined explain 97% of the observed variance.

Rep	%N	N (mg/g leaf)	%C	C (mg/g leaf)
1	1.65	16.5	50	500
2	1.76	17.6	49.5	495
3	1.71	17.1	50	500

Table S1. Total nitrogen analysis of three terrestrially ages Eucalyptus camaldulensis leaves

Table S2. Amino acid three letter abbreviation

Amino acid	Three letter abbreviation	Amino acid	Three letter abbreviation
Aspartic acid	ASP	Cysteine	CYS
Serine	SER	Tyrosine	TYR
Glutamic acid	GLU	Valine	VAL
Glycine	GLY	Methionine	MET
Histidine	HIS	Lysine	LYS
Arginine	ARG	Isoleucine	ILE
Threonine	THR	Leucine	LEU
Alanine	ALA	Phenylalanine	PHE
Proline	PRO		