

ISOTOPIC CHARACTERISATION OF ORGANIC MATERIALS LEACHED FROM LEAVES IN WATER

(Karakterisasi Isotop Senyawa-Senyawa Organik Dari Daun yang Terlarut dalam Air)

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ABSTRAK

Komposisi isotop dari senyawa organik yang berasal dari peristiwa penguraian beberapa jenis daun tumbuhan dalam air bervariasi yang disebabkan perubahan aktifitas mikrobiologi. Nilai rata-ran $\delta^{13}\text{C}$ sampel daun sebelum perlakuan diperoleh berada pada kisaran nilai $\delta^{13}\text{C}$ tumbuhan daratan secara umum yang menggunakan jalur fotosintesis C3. Komposisi isotop dari senyawa organik yang mengalami penguraian dari daun segar wandoo eucalyptus ditemukan mengalami pengurangan yang kontinyu dari nilai $\delta^{13}\text{C}$ selama tiga bulan periode penguraian. Perubahan nilai $\delta^{13}\text{C}$ juga berhubungan dengan perubahan jenis dan komposisi senyawa makromolekul. Sebagai contoh, lignin yang memiliki nilai $\delta^{13}\text{C}$ rendah berperan dalam menurunkan nilai $\delta^{13}\text{C}$ dari residu sampel setelah periode pengamatan.

Kata kunci: isotop, bahan organik, fotosintesis.

INTRODUCTION

Isotopes can be divided into two types; stable and unstable (radioactive). For example, carbon exists as two stable isotopes, ^{12}C and ^{13}C , distinct from radioactive ^{14}C . ^{12}C and ^{13}C are called the “light” and “heavy” isotopes of carbon account for 98.899 and 1.111 weight % of the total global carbon pool, respectively. The difference in mass between isotopes of the same element results in measurable isotopic fractionation during chemical, biochemical and physical processes. Bonds formed by the ^{12}C isotope are weaker than bonds involving the ^{13}C isotope. During a chemical reaction, molecules containing the ^{12}C isotope will, in general, react slightly more readily than those containing the ^{13}C isotope. The main goal in stable isotope analysis is to quantitatively convert a sample to a suitable purified gas analyte, which can be analysed by an isotope ratio mass spectrometer. For stable carbon isotope analysis the sample is combusted to gaseous carbon dioxide and water. The water is

removed and only the carbon dioxide enters the mass spectrometer. The isotope ratio mass spectrometer measures the relative abundance of ^{13}C to ^{12}C from the abundances of ions m/z 44 ($^{12}\text{CO}_2$), 45 ($^{13}\text{CO}_2$) and 46 [$^{12}\text{CO}_2$ (^{18}O)]. The ratio of abundances of ions 45/44 gives the stable carbon isotope composition. Correction is made for m/z 46 ($^{12}\text{CO}_2$ containing ^{18}O). Methods of determining stable isotope composition of a sample include, bulk stable isotope analysis and compound specific isotope analysis (CSIA).

As organic matter (OM) depends upon the relative proportions and isotopic compositions of marine and terrigenous OM, isotopic effects associated with organisms producing the OM, and the nature and degree of genetic alteration and reworking of OM can be predicted with some confidence from $^{13}\text{C}/^{12}\text{C}$ analysis. Carbon isotope composition of bulk lacustrine OM has been widely used to reconstruct paleoenvironmental conditions (e.g. Grice *et al.*, 2001; Hayes *et al.*, 1990; Summons *et al.*, 1994). In addition, the

nitrogen isotope composition of organic matter also has been widely used to trace biogeochemical cycling in marine and lacustrine environments (Altabet and Francois, 1994; Lund *et al.*, 2000). Nitrogen isotopic ratios have been used as a recorder of changes in the degree of nitrate utilization, denitrification and N₂-fixation (Aranibar *et al.*, ; Lehmann *et al.*, 2002; Zhou *et al.*, 2006). Although N isotopes are increasingly used as tracers for food-chain and bulk OM present in the system, N isotope studies of lacustrine OM are scarce and rarely perhaps due to the more complex nature of the N cycle compared to that of C (Bernasconi *et al.*, 1997).

The aim of this project is to investigate the utility of stable carbon and nitrogen isotopic compositions for establishing the precursors of DOC from different plant precursors by characterising the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of residues from plants leached in water samples.

Experimental

Plant Samples

Three different plant species were sampled from the vegetation surrounding Mundaring Weir Dam. These included *wandoo eucalyptus*, *pinus radiata* (conifer) and *corymbia calophylla*. The bark, wood, fresh and dead leaves, fresh and dead flowers, and nut elements of each plant were sampled and stored in plastic bags. In preparation for the leaching experiments, wood samples were cut into thin slices with a saw. The *corymbia calophylla* leaves were cut in half and the central woody stem was removed.

Leached Plant Material Used in Isotope Analysis

Leached plant materials were collected during each leachate experiment. The samples were allowed to dry at room temperature before being placed in an oven and heated to 50°C for 3 days. Dried-plant materials were ground into a very fine powder with a mortar and pestle.

Elemental Analysis-Isotope Ratio Mass Spectrometer (EA-irMS)

Stable carbon and nitrogen isotope analyses of the raw, residue and leached plant materials were performed using a EuroVector EuroEA3000

elemental analyser interfaced to a micromass IsoPrime isotope ratio mass spectrometer.

$\delta^{13}\text{C}$ Analysis

For bulk carbon isotope analysis, 0.02-0.05 mg of the powdered samples was weighed into a small tin capsule, which was then folded and compressed to remove atmospheric gases. Care was taken to ensure no finger grease came into contact with the sample or tin capsule. An autosampler was used to drop the sample capsule into a combustion reactor held at 1025°C. Both the sample and capsule melt in an atmosphere temporarily enriched with oxygen, where the tin promotes flash combustion. The combustion products are carried through an oxidation catalyst (chromium oxide) by a constant flow of helium. The oxidation products are then passed through a reduction reactor, containing copper granules at 650°C, where any oxides of nitrogen (NO, N₂O and NO₂) are reduced to N₂ and excess oxygen is removed. The resulting gas species then pass through a magnesium perchlorate filter to remove water. The remaining CO₂, together with N₂ and SO₂ (if present) are separated on a 3 m chromatographic column (Poropak Q), before the remaining gas is passed through a thermal conductivity detector (TCD) and then into an irMS. The isotopic compositions are calculated by integration of the ion currents obtained from measuring masses 44, 45 and 46 representing the CO₂ peak. The $\delta^{13}\text{C}$ composition is reported relative to that of a reference gas pulse produced by measuring the isotope ratio of carbon dioxide of known $\delta^{13}\text{C}$ content. Isotopic compositions are recorded in the delta notation relative to the VDPB standard. Average and standard deviation values obtained from at least three analyses of each sample were calculated.

$\delta^{15}\text{N}$ Analysis

The analysis of bulk nitrogen isotopes is undertaken in a similar way to the carbon analysis as described above. Slightly more sample is used (4.2-6.6 mg) due to the typically lower concentration of N. After chromatographic separation from CO₂ and SO₂ (if present), the isotopic composition of the N is calculated by integration of the ion currents obtained from the masses 28, 29 and 30 of N₂ peak. As with carbon, the $\delta^{15}\text{N}$ composition is reported relative to that

of a reference gas pulse of N₂ of known δ¹⁵N content and the isotopic compositions are given in delta notation relative to an air standard.

Results and Discussion

δ¹³C and δ¹⁵N of Raw and Residue Plant Materials

The averaged data of δ¹³C and δ¹⁵N for raw and residue plant materials collected at selected sampling times are summarised in Table 1 and Table 2 respectively.

Table 1. δ¹³C (‰) of plant materials.

Sample Name	δ ¹³ C (‰) ^{*)}					
	Sampling time (days)					
	0	14	29	56	84	138
Wandoo fresh leaf	-28.3 (0.24) ³	-28.4 (0.31) ⁴	-28.5 (0.21) ²	-28.7 (0.30) ²	-29.3 (0.18) ²	-28.4 (0.23) ²
Wandoo dead leaf	-27.9 (0.18) ²	-28.3 (0.13) ³	-28.7 (0.11) ²	-28.5 (0.18) ³	-28.8 (0.15) ²	-28.4 (0.10) ²
Conifer fresh leaf	-27.5 (0.23) ²	-27.7 (0.23) ²	-29.2 (0.12) ²	-28.8 (0.23) ²	-28.7 (0.09) ²	-27.9 (0.22) ³
Conifer dead leaf	-26.7 (0.27) ²	-27.4 (0.19) ²	-28.6 (0.09) ²	-27.7 (0.18) ²	-27.7 (0.34) ³	-27.6 (0.10) ²

Table 2. δ¹⁵N (‰) of plant materials.

Sample Name	δ ¹⁵ N (‰) ^{*)}					
	Sampling time (days)					
	0	14	29	56	84	138
Wandoo fresh leaf	-0.1 (0.24) ²	0.4 (0.22) ²	0.6 (0.04) ²	-0.6 (0.04) ²	-0.8 (0.29) ²	-0.0 (0.00) ²
Wandoo dead leaf	0.1 (0.09) ²	0.1 (0.01) ²	1.6 (0.01) ²	0.3 (0.03) ²	-0.5 (0.13) ²	-0.4 (0.04) ²
Conifer fresh leaf	2.4 (0.03) ²	1.6 (0.23) ²	1.3 (0.21) ²	1.0 (0.06) ²	1.4 (0.06) ²	2.2 (0.01) ²
Conifer dead leaf	0.7 (0.04) ²	0.7 (0.13) ²	1.1 (0.14) ²	1.7 (0.13) ²	1.4 (0.23) ²	0.3 (0.08) ²

^{*)} Numbers in parentheses are standard deviations; superscript numbers of replicate analyses.

^{**)} δ¹⁵N of conifer wood could not be carried out due to technical difficulties.

The similar δ¹³C (Table 1) for most of the raw plant samples collected supports the notion that terrestrial plant carbon is relatively homogeneous (Eadie *et al.*, 1978). The average δ¹³C for the raw plant materials is -27‰, covering a range from -26.7 to -28.3‰, in accordance with the C3 type of photosynthetic pathway.

Throughout the experiments, small changes in the δ¹³C of decomposing plant materials were evident. Figure 1 shows variations in δ¹³C of

plant materials from raw and residual plant materials. It is apparent that the plant materials with progressive leaching became more depleted in ¹³C between 0 and 84 days, with the exception of the wandoo flowers and bark. The δ¹³C values of *wandoo eucalyptus* dead leaf after 138 days of leaching vary between -27.9 and -28.8‰ and δ¹⁵N values range from 1.6 to -0.5‰. The conifer dead leaf, on the other hand, shows δ¹³C and δ¹⁵N with values ranging from -26.7 to -28.6‰ and from 1.7 to 0.7‰ after 138 days, respectively. Potential reasons for the differences observed in δ¹³C of various plant components is attributed to species-specific differences in plant tissue and microbial utilisation of leachates (Machas *et al.*, 2006). The changes in the stable isotopic compositions relate to a combination of differences in (i) leaching rate of the relatively more soluble compounds (ii) microbial degradation of organic matter, and (iii) subsequent leaching of hydrolysed substances.

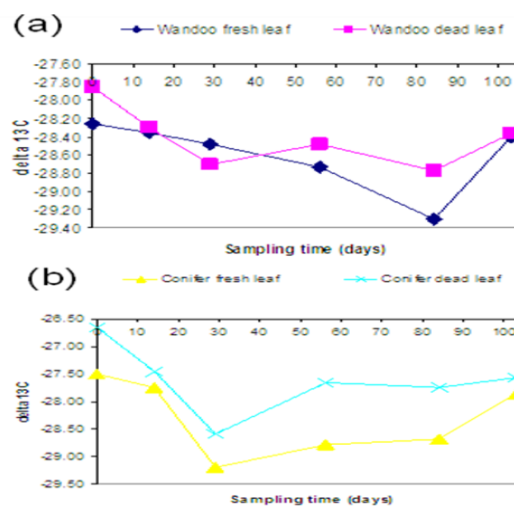


Figure 1. Variation of δ¹³C values of Wandoo and Conifer leaves from selected sampling times during the leaching experiments. Vertical bars – standard deviation.

Furthermore, small changes in δ¹³C may result from the comparative resistance of more refractory lignocelluloses that are isotopically depleted compared to the bulk δ¹³C of plant biomass and more readily metabolized compounds to decay (Benner and Hodson, 1985). From day 84 onwards the δ¹³C of the decomposing plant materials is relatively

enriched in ^{13}C . This could be attributed to the regrowth of bacteria contributing to the overall $\delta^{13}\text{C}$ of plant biomass (Macko and Estep, 1984). The role of microbial growth is attributed to removal or structural alteration of organic carbon resulted in changes in $\delta^{13}\text{C}$ also reported by Macko and Estep (1984). In their study it was shown that bacterial cells are generally enriched in ^{13}C to substrate compounds due to loss of light carbon in CO_2 . Furthermore, it has been well known that bacterial biomass has a $\delta^{13}\text{C}$ signature similar to that of the assimilated substrate (Machas *et al.*, 2006). It is often reported that decomposed soil organic matter is ^{13}C , and ^{15}N enriched relative to fresh litter and recent organic matter (Muzuka and Shunula, 2006).

Mean $\delta^{15}\text{N}$ values for the raw plant materials ranged from -3.6‰ to 5.9‰ (Table 2), supporting atmospheric nitrogen fixation by the plant having values close to 0‰ (Hoefs, 1987; Létolle, 1980). Values above 0‰ are consistent with the decomposition of leaves and the vascular plant detritus being metabolized by micro-organisms. During this process, some carbon is lost from the biomass *via* respiration of the micro-organisms but the dynamics of nitrogen are more complex and may be controlled by the initial composition of the plant material. Changes in $\delta^{15}\text{N}$ are thought to arise from assimilation of dissolved inorganic nitrogen (DIN) by bacterial communities during decomposition (Caraco *et al.*, 1998), and the amount and trend of the $\delta^{15}\text{N}$ changes during decomposition are dependent on the nature of the microbial community (Lehmann *et al.*, 2002) and the isotopic composition of the DIN (Caraco *et al.*, 1998).

Figure 2 compares $\delta^{15}\text{N}$ of the Wando leaves and Conifer leaves over time. The shifts in $\delta^{15}\text{N}$ values of leached plant elements from wandoo eucalyptus (leaves) were found to be similar. The $\delta^{15}\text{N}$ of these leached plant materials show a marked enrichment in ^{15}N content in the first month of leaching, followed by a significant depletion of ^{15}N during the following two-months of leaching and a final slight enrichment in ^{15}N at the end of the experiment. Changes in $\delta^{15}\text{N}$ are thought to arise from assimilation of dissolved inorganic nitrogen (DIN) by bacterial communities during decomposition (Caraco *et al.*,

1998), and the nature of the microbial communities.

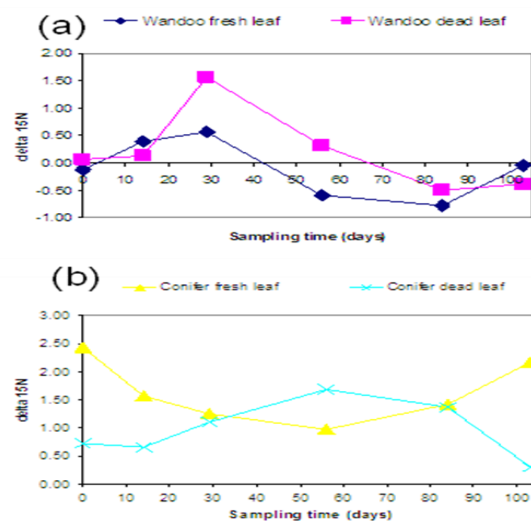


Figure 2. Variation of $\delta^{15}\text{N}$ values of plant elements from selected sampling time during the leaching experiments. Vertical bars – standard deviation.

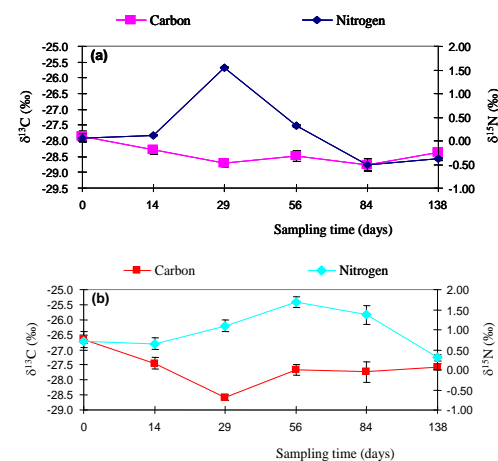


Figure 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of: (a) wandoo eucalyptus dead leaf, (b) conifer dead leaf. Vertical bars – standard deviation.

Different isotopic trends are shown for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ represented by wandoo eucalyptus and conifer dead leaves (Fig. 3.13). A different behaviour in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compositions during decomposition has been reported before for other vascular plants. Machas *et al.*, (2006), for example, reported contrasting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results from *Zostera noltii* leaves following decomposition over 60 days. Zieman *et al.* (1984)

also reported the differences between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of mangroves.

Conclusion

Measurements of the variation in $\delta^{13}\text{C}$ values of leached plant elements over the five month leaching experiments revealed a significant depletion in ^{13}C in the first month. Moreover, *wandoo eucalyptus* fresh leaf showed continually depleted $\delta^{13}\text{C}$ values over the first 3 months. The range in $\delta^{13}\text{C}$ data from the plant elements studies are due to the varied isotope composition of DOC leached from different plant elements reflecting the variations in $\delta^{13}\text{C}$ of source carbon. The $\delta^{13}\text{C}$ of the DOC could also be influenced by utilisation of different molecular classes of the plants (e.g. lipids, carbohydrates and protein) by bacteria.

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