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ISOTOPIC CHARACTERIZATION OF ORGANIC MATERIALS LEACHED FROM LEAVES IN WATER OF MUNDARING WEIR DAM

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ABSTRACT

This study examined the organic constituents aquatically leached from leaf components of two tree species (wandoo eucalyptus and pinus radiate). In particular this study aimed to assess the stable isotope composition behaviour of dissolved organic carbon (DOC) from the residue leaves after leaching over five months. The changes in the stable carbon and nitrogen isotope compositions of the leached leaves materials were investigated using an elemental analyzer-isotope ratio mass spectrometry (EA-irMS). The stable isotope compositions were found to vary according to microbially-mediated alteration and decomposition. The average $\delta^{13}C$ content of the raw plant elements was consistent with the $\delta^{13}C$ values of terrestrial plants using a C3 photosynthetic pathway. The isotope compositions of leached materials of wandoo eucalyptus fresh leaf were continually depleted in $\delta^{13}C$ over the leaching period of three months. These variations correlated well with its DOC profile. Changes in $\delta^{13}C$ values may also relate to the differential leaching of the macromolecular precursors of the original material. Lignin, for example, has a typically low $\delta^{13}C$ and probably contributed to the decrease of $\delta^{13}C$ in residue of the plant materials.

Keywords: *isotope composition, leached materials, C3 plant*

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 [1-2]. The difference in mass between isotopes of the same element results in measurable isotopic fractionation during chemical, biochemical and physical processes [1,3-5]. 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 analytic, which can be analyzed 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 [6-9]. The isotope ratio mass spectrometer measures the relative abundance of ^{13}C to ^{12}C from the abundances of ions *m/z* 44 ($^{12}CO_2$), 45 ($^{13}CO_2$) and 46 [$^{12}CO_2$ (^{18}O)]. The ratio of abundances of ions 45/44 gives the stable carbon isotope composition. Correction is made for *m/z* 46 ($^{12}CO_2$ 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 $^{13}C/^{12}C$ analysis. Carbon isotope composition of bulk lacustrine OM has been widely used to reconstruct paleoenvironmental conditions [10-13]. In addition, the nitrogen isotope composition of organic matter also has been widely used to trace biogeochemical cycling in marine and lacustrine environments [14-16]. Nitrogen isotopic ratios have been used as a recorder of changes in the degree of nitrate utilization, denitrification and N_2-fixation [17-18]. 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 [19].

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

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EXPERIMENTAL SECTION

Materials

Two different plant species were sampled from the vegetation surrounding Mundaring Weir Dam, located in the Darling Ranges (approximately 40 km east of Perth, Western Australia). These included *wandoo eucalyptus* and *pinus radiata* (conifer). Fresh and dead leaves of each plant were sampled and stored in plastic bags. The *corymbia calophylla* leaves were cut in half and the central woody stem was removed.

Instrumentation

Stable carbon and nitrogen isotope analyses of the raw, residue and leached plant materials were performed using a EuroVector EuroEA3000 elemental analyzer interfaced to a micromass IsoPrime isotope ratio mass spectrometer (Elemental Analysis-Isotope Ratio Mass Spectrometer, EA-irMS).

Procedure

Leached Plant Material Used in Isotope Analysis

Leached plant materials were collected during each leaching 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 grounded into a very fine powder with a mortar and pestle.

$\delta^{13}C$ 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 was melting in an atmosphere temporarily enriched with oxygen, where the tin promotes flash combustion. The combustion products were carried through an oxidation catalyst (chromium oxide) by a constant flow of helium. The oxidation products were then passed through a reduction reactor, containing copper granules at 650 °C, where any oxides of nitrogen (NO, N_2O and NO_2) were reduced to N_2 and excess oxygen was removed. The resulting gas species then passed through a magnesium perchlorate filter to remove water. The remaining CO_2, together with N_2 and SO_2 (if present) were separated on a 3 m chromatographic column (Poropak Q), before the remaining gas was passed through a thermal conductivity detector (TCD) and then into an irMS. The isotopic compositions were calculated by integration of the ion currents obtained from measuring masses 44, 45 and 46 representing the CO_2 peak. The $\delta^{13}C$ composition was reported relative to that of a reference gas pulse produced by measuring the isotope ratio of carbon dioxide of known $\delta^{13}C$ content. Isotopic compositions were 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.

$\delta^{15}N$ Analysis

The analysis of bulk nitrogen isotopes was undertaken in a similar way to the carbon analysis as described above. Slightly more sample was used (4.2-6.6 mg) due to the typically lower concentration of N. After chromatographic separation from CO_2 and SO_2 (if present), the isotopic composition of the N was calculated by integration of the ion currents obtained from the masses 28, 29 and 30 of N_2 peak. As with carbon, the $\delta^{15}N$ composition was reported relative to that of a reference gas pulse of N_2 of known $\delta^{15}N$ content and the isotopic compositions were given in delta notation relative to an air standard.

RESULT AND DISCUSSION

$\delta^{13}C$ and $\delta^{15}N$ of Raw and Residue Plant Materials

The averaged data of $\delta^{13}C$ and $\delta^{15}N$ for raw and residue plant materials collected at selected sampling times are summarized in Table 1 and Table 2 respectively.

The similar $\delta^{13}C$ (Table 1) for most of the raw plant samples collected supports the notion that terrestrial plant carbon is relatively homogeneous [20]. The average $\delta^{13}C$ 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 $\delta^{13}C$ of decomposing plant materials were evident. Fig 1 shows variations in $\delta^{13}C$ of plant materials from raw and residual plant materials. It is apparent that the plant materials with progressive leaching became more depleted in ^{13}C between 0 and 84 days, with the exception of the wandoo flowers and bark. The $\delta^{13}C$ values of *wandoo eucalyptus* dead leaf after 138 days of leaching vary between -27.9 and -28.8‰ and $\delta^{15}N$ values range from 1.6 to -0.5‰. The conifer dead leaf, on the other hand, shows $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ of various plant components is attributed to species-specific differences

Table 1. $\delta^{13}C$ (‰) of plant materials

Sample Name	$\delta^{13}C$ (‰)*)					
	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. $\delta^{15}N$ (‰) of plant materials

Sample Name	$\delta^{15}N$ (‰)*)					
	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.

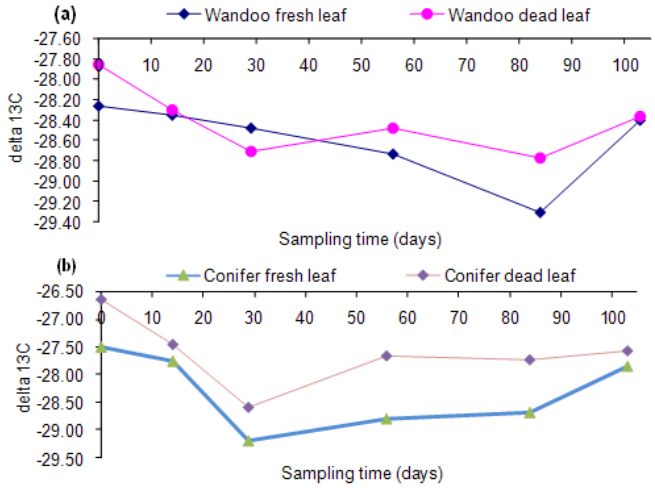

Fig 1. Variation of $\delta^{13}C$ values of Wando and Conifer leaves from selected sampling times during the leaching experiments

in plant tissue and microbial utilisation of leachates [21]. 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.

Furthermore, small changes in $\delta^{13}C$ may result from the comparative resistance of more refractory lignocelluloses that are isotopically depleted compared to the bulk $\delta^{13}C$ of plant biomass and more readily metabolized compounds to decay [22]. From day 84 onwards the $\delta^{13}C$ of the decomposing plant materials is relatively enriched in ^{13}C. This could be attributed to the regrowth of bacteria contributing to the overall $\delta^{13}C$ of plant biomass [23]. The role of microbial growth is attributed to removal or structural alteration of organic carbon resulted in changes in $\delta^{13}C$ also reported by [23]. 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 $\delta^{13}C$ signature similar to that of the assimilated substrate [24]. It is often reported that decomposed soil organic matter is ^{13}C, and ^{15}N enriched relative to fresh litter and recent organic matter [25].

Mean $\delta^{15}N$ 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‰ [26]. 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 $\delta^{15}N$ are thought to arise from assimilation of dissolved inorganic nitrogen (DIN) by bacterial communities during decomposition [6], and the amount and trend of the $\delta^{15}N$ changes during decomposition are dependent on

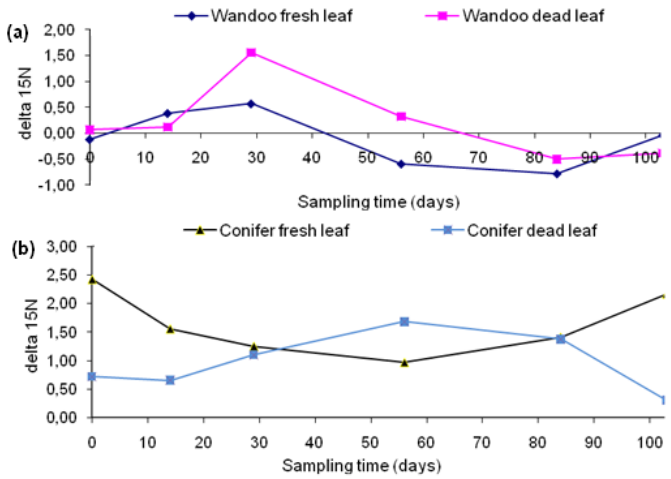

Fig 2. Variation of $\delta^{15}N$ values of plant elements from selected sampling time during the leaching experiments

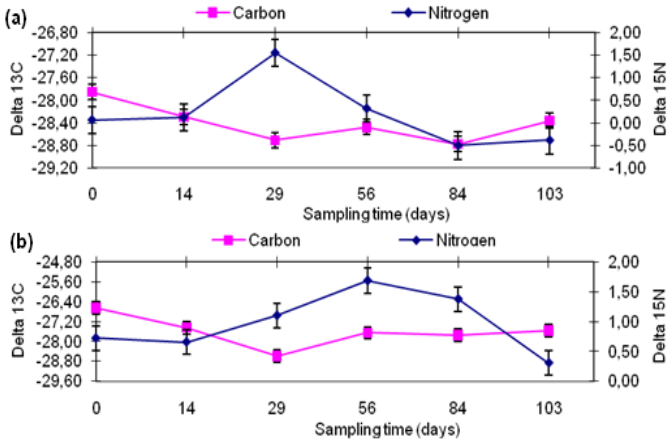

Fig 3. $\delta^{13}C$ and $\delta^{15}N$ of: (a) *wandoo eucalyptus* dead leaf, (b) conifer dead leaf. Vertical bars – standard deviation

the nature of the microbial community [17] and the isotopic composition of the DIN [6].

Fig 2 compares $\delta^{15}N$ of the Wando leaves and Conifer leaves over time. The shifts in $\delta^{15}N$ values of leached plant elements from *wandoo eucalyptus* (leaves) were found to be similar. The $\delta^{15}N$ 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 $\delta^{15}N$ are thought to arise from assimilation of dissolved inorganic nitrogen (DIN) by bacterial communities during decomposition [6], and the nature of the microbial communities.

Different isotopic trends are shown for $\delta^{13}C$ and $\delta^{15}N$ represented by *wandoo eucalyptus* and conifer dead leaves (Fig 3). A different behavior in $\delta^{13}C$ and $\delta^{15}N$ compositions during decomposition has been reported before for other vascular plants. Machas *et al*. [21], for example, reported contrasting $\delta^{13}C$ and $\delta^{15}N$ results from *Zostera noltii* leaves following decomposition over 60 days. Zieman et al. [27] also reported the differences between $\delta^{13}C$ and $\delta^{15}N$ of mangroves.

CONCLUSION

Measurements of the variation in $\delta^{13}C$ 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 $\delta^{13}C$ values over the first 3 months. The range in $\delta^{13}C$ data from the plant elements studies are due to the varied isotope composition of DOC leached from leaves elements reflecting the variations in $\delta^{13}C$ of source carbon. Comparisons of $\delta^{15}N$ values of leachates revealed that both *wandoo eucalyptus leaves* had the most depleted ^{15}N composition, while $\delta^{15}N$ of conifer dead leaf showed enrichment in ^{15}N content in the three months of leaching. Changes in $\delta^{15}N$ are due to the assimilation of dissolved inorganic nitrogen by bacterial communities during decomposition and the nature of the microbial communities.

The stable carbon and nitrogen isotope compositions of residual leaves leached in water present the potential of the use of stable isotope studies for diagnosis of particular organic precursor.

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