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# Research Paper

## Carbon and nitrogen dynamics in decaying wood: paleoenvironmental implications

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**Environmental context.** Carbon and nitrogen isotopes of terrestrial organic matter are widely used for reconstructing past environments, but organic matter is exposed to degradation as soon as it is deposited during what is called early diagenesis. This study explores the effects of this process on organic carbon and nitrogen isotopes, and concludes that it homogenises an environmental signal by integrating all their components. Thus, early diagenesis may not preclude paleoenvironmental reconstructions.

**Abstract.** The effect of early diagenesis on carbon and, especially, nitrogen isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of organic matter is not well understood and is of interest for accurate paleoenvironmental reconstructions. Wood samples were incubated in distilled water and river water in order to assess the effects of early diagenesis on carbon and nitrogen dynamics. Elemental content and isotopic composition of carbon and nitrogen as well as mass loss of wood pieces were determined. Mass loss in river water was three times greater than in distilled water. This difference was attributed to the development of two different types of fungi characterised by various degradation rates. Carbon dynamics of wood samples showed similar patterns in both type of water: (i) a sharp increase in carbon content, possibly related to carbohydrate degradation, before it slowly returned towards initial values, and (ii) no significant changes in  $\delta^{13}\text{C}$  values. In contrast, nitrogen dynamics of samples showed complex patterns: (i) N release associated with  $^{15}\text{N}$  depletion in distilled water, attributed to uptake of  $^{15}\text{N}$ -enriched pool (i.e. proteins) by fungi, and (ii) N accumulation associated with  $^{15}\text{N}$  enrichment in river water. . The latter pattern was attributed predominantly to microbially mediated importation of  $^{15}\text{N}$ -enriched nitrate from river water. Although challenging, the present results suggest that early diagenesis may average an environmental signal by integrating individual signals (woods, fungi, water) and microbial processes. Considering the non-linear behaviour of early diagenesis, this integration is probably almost instantaneous on the geological time scale, which may not preclude paleoenvironmental reconstructions.

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Impact of early diagenesis on organic matter

**Additional keywords:**  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , early diagenesis, fungi, degradation

## Introduction

The carbon and nitrogen isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of terrestrial organic matter is widely used for reconstructing past environments for time periods from hundreds to million years.<sup>[1–8]</sup> However, organic matter is exposed to degradation as soon as it is transported, integrated into soils or deposited in sediments. Degradation processes of organic matter constitute the earliest stage of diagenesis, which leads in turn to the geological archive as diagenesis stages advance.<sup>[9,10]</sup> In this context, paleoenvironmental investigations require consideration of the potential alteration of the primary isotopic signal during early diagenesis because most of the organic matter is degraded during this stage.<sup>[11–13]</sup>

Changes of  $\delta^{13}\text{C}$  values during early diagenesis of terrestrial organic matter (e. g. plant or litter material) are well known and mainly attributed to a preferential loss of  $^{13}\text{C}$ -enriched carbohydrates.<sup>[14–18]</sup> In contrast, nitrogen dynamics during terrestrial organic decay are more complex and consequences on  $\delta^{15}\text{N}$  values are not well constrained.<sup>[12,15,19–21]</sup> Indeed, they involve complex interactions of biotic and abiotic factors: microbial processing *vs* N sources and environmental conditions.<sup>[12,22–28]</sup> For example, the effect of microbial processing on  $\delta^{15}\text{N}$  values depends on microbial communities (biotic), themselves dependent on the oxygenation conditions of the system.<sup>[12]</sup> However, changes in  $\delta^{15}\text{N}$  values during early diagenesis of terrestrial organic matter in a depositional context that allows preservation at the geological time scale (immersed environments) have not been assessed yet because most studies have dealt with litterbag experiments on field or soil incubation.<sup>[15,19,23,24,26,28–30]</sup> In addition, considering the importance of coarse woody debris in terrestrial organic matter<sup>[11,31]</sup> and of immersed environments for the geological archive (sediments), it is essential to assess the effect of early diagenesis on potential paleoenvironmental proxies, such as  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ , for this material and in these kinds of environments. Because fungi are the most important decomposers of woody material,<sup>[32,33]</sup> we designed a laboratory experiment that mainly focussed on biotic factors involved during early diagenesis of woody material, by limiting the action of abiotic factors (by use of constant temperature, oxygenation, pH and absence of light).

Centimetric pieces of wood were incubated in water in order to assess the effects of early diagenesis on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of woody terrestrial organic matter in immersed environments. Wood samples were immersed either in (i) distilled water (DW) or (ii) river water (RW). Incubations conducted in DW were used as a control treatment, because microbial activity was considered to be minimal. Mass-loss rates of decaying wood and carbon dynamics (C content and  $\delta^{13}\text{C}$  values) were determined to characterise the biotic processes involved during degradation of woody debris. Then, their consequences on  $\delta^{15}\text{N}$  values are discussed to evaluate the preservation potential of the initial  $\delta^{15}\text{N}$  signal in fossil and terrestrial sedimentary organic matter.

## Experimental

### Samples

A treetop branch of *Sciadopitys verticillata* (Cupressaceae, conifer) was collected in December 2012 from a garden in Tokyo, Japan, after having lain for 6 months on the ground to ensure the natural presence of decay organisms. We chose this endemic specimen because it has been chemically related to the morphogenus conifer *Xenoxylon* Gothan, which was widespread during Jurassic and Cretaceous times and is of paleoclimatic interest nowadays.<sup>[34,35]</sup> Both extremities of the branch and the bark were removed to clean the branch and avoid heterogeneities that were too large between its different parts (Fig. 1). The branch (~60 cm long; diameter ~3 cm) was then sliced in ~2-mm-thick pieces, which were air-dried and weighed.

### Experimental design

Samples were submitted to degradation in two different types of water: (i) DW, and (ii) RW from La Murette (Essonne, France), sampled in October 2013. The pH of the water samples was checked with pH paper before each sampling. It remained close to neutral and constant for both types of waters during the whole experiment. RW was filtered with a Whatman filter (22 µm; Prolabo, Paris) before filling Erlenmeyers. Samples were placed individually into Erlenmeyers, with 75 mL of DW and RW (Fig. 1). Erlenmeyers were covered with finely perforated aluminium foil to allow gas exchange between water and the atmosphere and avoid external contamination by dust. They were then continuously shaken in the dark in ambient air (room temperature conditioned at 22 °C) to keep the water oxygenated and inhibit the development of photosynthetic microorganisms that could interfere with degrading heterotroph microorganisms. An oxygenated environment has been preferred to promote relatively efficient degradation when compared with oxygen-depleted environments and to prevent denitrification, which leads to high loss of <sup>14</sup>N.

The incubation experiment was carried out for 73 weeks and wood pieces were collected after 2, 4, 8, 16, 32, 52 and 73 weeks, corresponding to  $t_1$  to  $t_7$  respectively. Initial RW was stored in a 10-L canister in the dark and water was bubbled in order to keep it oxygenated. Filtered RW or DW was added to Erlenmeyers at  $t_3$ ,  $t_5$  and  $t_6$  to counterbalance evaporation in Erlenmeyers.

After sampling, pieces of wood were rinsed with distilled water to remove superficial microorganisms. They were then frozen, freeze-dried, weighed, scanned (with EPSON scanner) and ground (<40 µm) for analysis with an ultracentrifugal ZM-200 mill.

### Analyses

Carbon content and  $\delta^{13}\text{C}$  values were determined by isotope-ratio mass spectrometry using a Vario Pyro Cube analyser, coupled with an IsoPrime Micromass mass spectrometer connected to a Micromass dilution system. Nitrogen content and  $\delta^{15}\text{N}$  values were measured with a Flash 2000

analyser coupled with a Thermo Scientific Delta V Plus mass spectrometer connected to a ConFlo IV dilution system. Systems precision and accuracy were monitored using tyrosine ( $N = 7.73\%$ ,  $\delta^{15}N = 10.01\%$  and  $C = 59.66\%$ ,  $\delta^{13}C = -23.20\%$ ), as internal laboratory standard that was calibrated with international standards IAEA-N-1 ( $\delta^{15}N = 0.4\%$ ), IAEA-N-2 ( $\delta^{15}N = 20.3\%$ ) and IAEA-NO-3 ( $\delta^{15}N = 4.7\%$ ) and expressed relative to  $N_2$ -AIR for nitrogen and relative to Vienna Pee Dee Belemnite (VPDB) for carbon. Nitrogen analyses were systematically performed in duplicate ( $n = 51$ ), whereas for carbon analyses, samples were analysed in duplicate ( $n = 12$ ) every 10 samples, precision being usually better for carbon isotopes. The overall precision ( $2\sigma$ ) was  $<0.002$  and  $<0.6\%$  for N and C content respectively, and  $<0.2\%$  for N and C isotopes.

#### *Initial variability of the branch and sampling strategy*

Before beginning the experiment, 11 pieces of wood, randomly distributed along the branch, were selected to evaluate the initial variability of %C,  $\delta^{13}C$ , %N and  $\delta^{15}N$ . Carbon content and isotopes were on average  $47.4 \pm 0.5$  and  $-24.1 \pm 0.1\%$  respectively, whereas nitrogen content and isotopes were on average  $0.08 \pm 0.01\%$  and  $-0.2 \pm 0.5\%$  respectively (Table 1). In agreement with literature, initial variability of  $\delta^{15}N$  values was much higher than for carbon.<sup>[36–38]</sup> In addition, the  $\delta^{15}N$  values were randomly distributed along the branch and showed extreme values from  $-0.8$  to  $+1.1\%$ .

Considering the initial variability of  $\delta^{15}N$  values, experiments were run in triplicate for each type of water, meaning that six wood pieces were sampled (three per type of water). Within triplicates, individual wood pieces were chosen according to their position along the branch, with a maximum distance between them to be representative of the overall initial variability along the branch.

#### *Water chemistry control*

Water chemistry ( $PO_4^{3-}$ ,  $NO_3^-$ ,  $NO_2^-$  and  $NH_4^+$ ) was controlled in each type of water at  $t_0$ ,  $t_2$ ,  $t_3$ ,  $t_5$ ,  $t_6$  and  $t_7$  by spectrophotometry using a Gallery system. As expected, DW contained no or trace amounts of these chemical compounds, with no forms of nitrogen (always  $<0.2$  ppm). In contrast, RW did contain significant amount of nitrates at  $t_0$  ( $\sim 20$  ppm), which disappeared after  $t_2$  ( $<0.1$  ppm remained), whereas other nutrients remained in trace amounts.

#### *Statistical treatment and data sets*

Significance of the linear regressions and of the mean comparisons (e.g. differences in N content between samples in RW and DW) were tested using Pearson correlations and Student's tests respectively, with the programming language R (version 3.2.2, R Foundation for Statistical Computing, Vienna, Austria). Hypotheses were tested on the basis of a 5 % significance level ( $P$  value), meaning that a relation or comparison is significant when  $P < 0.05$  for  $n$  observations. Original data sets are given in the Supplementary material, whereas condensed results used for figures are reported in Table 2.

## Results

### *Macroscopic observations and mass-loss patterns*

At the initial stage, all wood pieces showed multimillimetre-sized zones with reddish-brown colour, which may be attributed to black-rot or soft-rot fungi (Fig. 2a and b, feature i).<sup>[32]</sup> Fungi were probably already within the wood before branch sampling because decay starts as soon as a tree is cut,<sup>[39]</sup> or even before because wood of living conifers is known for hosting fungi.<sup>[32]</sup> Alternatively, the branch may have been colonised while it was on the ground. Translucent and whitish fungi developed within 2 weeks on wood pieces in DW, whereas no visible change occurred in RW. After 8 weeks ( $t_3$ ), fungi turned brown-black on wood pieces in DW, and became widespread after 16 weeks ( $t_4$ ; Fig. 2a, features i and ii). At this time, the RW in the Erlenmeyers became cloudy and yellowish, with millimetre-sized floating particles (Fig. 2b, feature iii). In addition, white-rot fungi developed on wood pieces in RW as indicated by their mottled appearance, their whitish-yellow coloration and their brittle and spongy traits as well as their loss of color contrast (Fig. 2b, features iv and v).<sup>[32]</sup> Those features were not observed on samples in DW, suggesting that no or less white-rot fungi developed.

The degradation features mentioned became increasingly marked at the end of the experiment (Fig. 2c and d). Growth-rings of wood pieces in DW became increasingly dark, while they were progressively erased in RW, with increasing numbers of floating woody particles, suggesting two different microbial communities.

Consequently, wood pieces in RW seemed to have been degraded faster than those in DW. The higher decay rate of samples in RW is confirmed by a weight loss of ~30 % after 73 weeks ( $t_7$ ), whereas it reached less than 10 % for samples in DW (Fig. 3). Considering that the amount of inorganic material is negligible in wood, the mass loss is attributed to organic matter loss. In addition, mass loss is linearly linked to time ( $t$ ) in both waters with a slope three times steeper for wood pieces in RW ( $-0.36t$ ) than in DW ( $-0.11t$ ), where  $t$  is expressed in weeks (Fig. 3).

### *Carbon patterns*

The carbon content of wood pieces in both DW and RW increased between  $t_0$  and  $t_1$  (2 weeks), from 47.4 to 48.5 and 48.9 % respectively (Fig. 4a). After 32 weeks ( $t_5$ ), they progressively tended towards initial values. Although a small increase in  $\delta^{13}\text{C}$  values occurred ( $-24.1\text{‰}$  at  $t_0$  to  $-23.9\text{‰}$  at  $t_4$ ), it was in the range of the analytical precision ( $\pm 0.2\text{‰}$ ; Fig. 4b). In addition, the floating woody particles were sampled at  $t_5$  in an Erlenmeyer containing RW. They significantly differed from the original piece of wood with (i) carbon content of 35 v. 50 % in the original piece of wood (Fig. 4a), and (ii)  $\delta^{13}\text{C}$  value of  $-22.6$  v.  $-24.0\text{‰}$  (Fig. 4b).

## Nitrogen patterns

In contrast to carbon content, the nitrogen content of wood pieces depicted two different patterns according to the water type (Fig. 4c). The average %N of wood pieces in DW was 0.07 % for the whole duration of the degradation, significantly lower than the initial value (0.08 %;  $n = 21$ ;  $P < 0.001$ ), whereas the average %N of wood pieces in RW was significantly higher (0.09 %;  $n = 21$ ;  $P < 0.02$ ). In addition, N content of wood pieces in DW remained close to 0.07 % after a rapid decrease ( $t_0$  to  $t_3$ ) from the initial value (0.08 %). In contrast, the N content of samples in RW almost linearly increased from the initial value to 0.12 % at  $t_7$  (Fig. 4c). This reflects an accumulation of nitrogen in the remaining wood, called the ‘immobilisation phase’.<sup>[15,21]</sup>

As a consequence, the atomic C/N ratio decreased linearly from ~650 to ~400 in RW wood as degradation advanced (Fig. 5). In the same time, the C/N ratio of wood pieces in DW increased to >800 until  $t_3$ , and then remained stable until the end of incubation. Those very high C/N ratios of wood pieces are characteristic of fresh wood.<sup>[11]</sup> In contrast, floating woody particles sampled at  $t_5$  in RW showed a C/N ratio of 30 (Fig. 5), typical of substantially decayed wood.<sup>[11]</sup>

The immobilisation phase in RW is associated with significantly higher  $\delta^{15}\text{N}$  values (+1.7 ‰ on average) of the remaining wood, when compared with the initial value (−0.2 ‰;  $n = 21$ ;  $P < 0.001$ ), whereas  $\delta^{15}\text{N}$  values of wood pieces in DW remained almost constant at approximately −0.5 ‰ (Fig. 4d). In addition, the floating woody particles (in RW) sampled at  $t_5$  showed a higher nitrogen content than the original piece of wood (1.37 v. 0.09 %) and  $\delta^{15}\text{N}$  value (2.7 v. 1.0 ‰; Fig. 4d). The very high nitrogen content suggests that wood particles were invaded by N-rich microorganisms.

## Discussion

### *Decomposers and degradation rate*

Basidiomycetes fungi have been the major decomposers of wood since at least the upper Devonian.<sup>[32]</sup> They are classified as white-rot or black-rot fungi, based on macroscopic aspects.<sup>[33]</sup> In contrast, soft-rot fungi comprise Ascomycetes and Deuteromycetes, which are less efficient than Basidiomycetes for degrading wood.<sup>[32,33]</sup> Black-rot and soft-rot fungi have the same morphological characteristics, but black-rot fungi do not support very wet conditions, whereas soft-rot fungi do.<sup>[32]</sup> Consequently, based on morphological characteristics of the samples after incubation, soft-rot fungi are the most probable decomposers in DW, whereas white-rot fungi are more likely in RW (Fig. 2).<sup>[32,33]</sup> White-rot fungi are able to selectively degrade lignin and hemicellulose before cellulose – especially in gymnosperms, which is the case in the present study – or to simultaneously degrade those molecules.<sup>[33]</sup> In contrast, soft-rot fungi preferentially decompose cellulose and hemicellulose, while lignin is only slightly degraded by those fungi, leading to less brittle traits when compared with white-rot fungi.<sup>[32,33]</sup> The low efficiency of soft-rot fungi for degrading wood when compared with Basidiomycetes could thus explain the lower mass loss of wood pieces in DW than in RW.



The mass-loss rate of wood pieces in RW ( $-0.36t$ ; Fig. 3) is close to that described during the first degradation phase of litterbags containing red pine litter, with a slope of  $-0.45t$  with  $t$  in weeks.<sup>[15]</sup> In the study mentioned, this first degradation phase was described as a constant mass loss (linear) dominated by the loss of carbohydrates, and lasted 44 months to reach 80 % of mass loss.<sup>[15]</sup> Then, the second phase was characterised by very slow or negligible mass loss during 33 additional months. Those two phases were also reported in different wood species after artificial aging by thermal treatment.<sup>[18]</sup> However, the second phase was not observed in wood samples of the present study, suggesting that the first phase of degradation was not entirely completed (Fig. 3). Extrapolating results to 80 % of mass loss for wood pieces in RW, the first phase of degradation would have been complete in  $\sim 220$  weeks, which is  $\sim 50$  weeks longer than reported in soils.<sup>[15]</sup> Thus, the degradation rate of wood pieces in RW is slightly lower than measured in soils,<sup>[15]</sup> and in contradiction with another study<sup>[29]</sup> that showed significantly higher degradation rates in aerobic water than in soils. This discrepancy could be due to the difference in the plant material used in both studies, which dealt with needles<sup>[15]</sup> and green leaves from angiosperms.<sup>[29]</sup> Nevertheless, the mass loss of wood pieces obtained here confirms that this parameter is a good indicator of the state of degradation.<sup>[15,29]</sup>

#### *Carbon dynamics*

Natural decomposing and artificial aging of plant material has often shown C enrichment and  $^{13}\text{C}$  depletion in the remaining organic matter, mainly attributed to the preferential loss of carbohydrates (cellulose and hemicellulose) during early diagenesis.<sup>[14,16,18]</sup> In the present study, the increase in carbon content of the samples (remaining wood) incubated in both types of water probably reflects the increasing relative predominance of C-rich compounds resulting from loss of carbohydrates. Considering the fungi type deduced above from macroscopic observations, this would imply that soft-rot fungi and white-rot fungi preferentially degraded cellulose and hemicellulose.<sup>[33]</sup> However,  $\delta^{13}\text{C}$  values did not significantly change. The absence of expected  $^{13}\text{C}$  depletion in the remaining wood after carbohydrate loss may be linked to the low amount of material affected by degradation, with a maximum mass loss of  $\sim 30$  % for samples in RW and  $\sim 10$  % in DW. Alternatively, the loss of  $^{13}\text{C}$ -enriched carbohydrates may have been counterbalanced by the loss of hydrolysable  $^{13}\text{C}$ -depleted compounds<sup>[15]</sup> or by the simultaneous loss of lignin and carbohydrates, in agreement with decay abilities of Basidiomycetes in RW.<sup>[32,33]</sup> Leaching of hydrolysable compounds may have occurred as soon as samples were immersed, but its influence is hard to discuss here because of the lack of sampling data during the first days of the incubation.

If carbohydrates were effectively degraded, their C would have been reused by microbes, which would be integrated in the floating woody particles or in the remaining wood. In RW, the woody particles were analysed at  $t_5$ . Their high %N (1.37 v. 0.09 %) and low atomic C/N ratio (30 v. 769) relative to the remaining wood suggest a high content of microbial biomass because it is concentrated in N.<sup>[40]</sup> In addition, the lower %C (35 v. 49 %) and higher  $\delta^{13}\text{C}$  values ( $-22.6$  v.  $-24.0$  ‰) in those



floating particles, when compared with the remaining wood (Fig. 4a and b), support the idea that carbonaceous molecules mainly originate from C-depleted and  $^{13}\text{C}$ -enriched carbohydrates of the remaining wood.<sup>[14]</sup> Additional loss of  $^{12}\text{C}$  might be associated with microbial respiration.<sup>[28]</sup> The isotopic effect of preferential degradation and microbial respiration during the incubation is probably not sufficient to be recorded in the slightly degraded remaining wood or it is counterbalanced by concomitant loss of  $^{13}\text{C}$ -enriched and  $^{13}\text{C}$ -depleted compounds. The latter hypothesis may be favoured, at least in RW, because Basidiomycetes (white-rot fungi) are able to simultaneously degrade  $^{13}\text{C}$ -depleted lignin and  $^{13}\text{C}$ -enriched carbohydrates.<sup>[33]</sup>

### *Nitrogen dynamics*

Nitrogen dynamics are much more complex either in DW or RW. The C/N ratio tracks changes in chemical composition of samples and thus reflects degradation processes.<sup>[12]</sup> Considering similar behaviours in C content of samples in both types of waters (Fig. 4a), C/N variations likely reflect changes in N content (Fig. 5). Consequently, the rapid decrease in N content of samples in DW (Fig. 4c), and the resulting increase of C/N ratio (Fig. 5), are most likely due to N release. In contrast, the opposite pattern was observed in samples incubated in RW, with an increase in N content (Fig. 4C) leading to a decrease of C/N (Fig. 5). This points to N accumulation in the remaining wood. In both cases, the microenvironment of samples should be considered and its implications are discussed below. It should also be noted that substantial isotopic fractionation processes like denitrification can be ruled out as it only occurs under anoxic conditions<sup>[41]</sup> and that  $\text{N}_2$  fixation, if any, has a very small fractionation factor.<sup>[42]</sup>

### *Distilled water*

N loss of the remaining wood in DW was associated with significant lower  $\delta^{15}\text{N}$  values ( $-0.5\text{‰}$  on average), compared with the initial one ( $-0.2\text{‰}$ ;  $n = 21$ ;  $P < 0.05$ ; Fig. 4d), suggesting the preservation of a  $^{15}\text{N}$ -depleted pool and/or preferential degradation of a  $^{15}\text{N}$ -enriched pool. Such N loss has already been observed during the first 120 days of incubation of *Spartina* plants.<sup>[23]</sup> However, it was attributed to leaching without  $^{15}\text{N}$  fractionation, which is not consistent with the  $^{15}\text{N}$  depletion measured in samples incubated in DW (Fig. 4d). Consequently, leaching is probably not appropriate to solely explain N dynamics in DW.

Alternatively, the extraction of N by fungi is suspected because field incubations showed they are able to extract N from the colonised wood by gaining access to proteinaceous material, which results in concentrating N in their mycelium.<sup>[43,44]</sup> Thus, rapid N release from wood pieces in DW is probably associated with the development of mycelium that grew partly within the wood and partly in the water (Fig. 2a). Fungi probably used nitrogen from the wood for their metabolism, leaving it depleted in N.<sup>[44]</sup> This is supported by the absence of an external N source because DW is nutrient-free. Because N depletion occurred rapidly (within 2 weeks) and did not substantially change during the rest of the

incubation (e.g. stable C/N ratio; Fig. 5), microbial communities may have reached an equilibrium within 2 weeks, in response to the low nutrient availability. The  $^{15}\text{N}$  depletion of the remaining wood is not currently well understood, but preferential degradation of a  $^{15}\text{N}$ -enriched pool is suspected. In particular, a large range of positive  $\delta^{15}\text{N}$  values (2–8 ‰) in individual amino acids of a spatterdock plant has been reported<sup>[45]</sup> and it has also been demonstrated that proteins were 3 ‰-enriched relative to cells.<sup>[46]</sup> Proteins and amino acids may therefore correspond to the  $^{15}\text{N}$ -enriched compounds preferentially extracted by fungi as a nutrient source,<sup>[47]</sup> all the more because their preservation potential is low.<sup>[10]</sup>

#### River water

Nitrogen dynamics in RW samples showed an opposite pattern compared with samples in DW, with N accumulation in the remaining wood (Fig. 4c) associated with significantly higher  $\delta^{15}\text{N}$  values (1.5 ‰ on average v. -0.2 ‰ at  $t_0$ ; Fig. 4d). N immobilisation has been commonly reported during decomposition of leaf-litter,<sup>[15,21,45]</sup> sphagnum litter<sup>[26]</sup> and woody litter<sup>[47]</sup> or in decaying wood of *Fagus sylvatica* inoculated with basidiomycete fungi.<sup>[44]</sup> According to those authors, it reflects accumulation of nitrogen during an immobilisation phase of nutrients. Two main processes are able to supply nitrogen in those decaying materials: (i)  $\text{N}_2$  fixation by specialised microorganisms,<sup>[21,47]</sup> and (ii) uptake by cord-forming fungi from the surrounding environment.<sup>[21,44,47]</sup> However, rates of  $\text{N}_2$  fixation are too low to explain N accumulation in decaying wood,<sup>[21,47]</sup> because fungi are not able to fix  $\text{N}_2$  given  $\text{N}_2$ -fixing organisms are limited to prokaryotes.<sup>[48]</sup> Hence,  $\text{N}_2$  fixation is probably not the major process involved in N accumulation. Alternatively, cord-forming fungi have the ability to scavenge N from the surrounding environment, and further import it into the decaying material via a translocating network of mycelium that interconnects discontinuous nutrient sources.<sup>[21,44,47]</sup> Thus, wood-decaying fungi are likely the mediators of N accumulation into decaying wood in RW. In the present study, the source of exogenous N is RW and the cord-forming fungi are most probably white-rot fungi (Basidiomycetes), their activity being the cause of the continuous decrease in C/N ratio (Fig. 5).

The  $\delta^{15}\text{N}$  variations in wood samples are likely due to the isotopic composition of the N imported by fungi (N source) and/or influence of metabolism effects leading to internal fractionation of  $^{15}\text{N}$  during N incorporation.<sup>[12,28,49–51]</sup> However, metabolism effects in fungi are thought to be low because no or little change in  $\delta^{15}\text{N}$  values was detected after N transfer to their host.<sup>[49]</sup> Thus, N sources must be seriously considered here. The only form of inorganic N detected in RW was  $\text{NO}_3^-$ . The rapid decrease in  $\text{NO}_3^-$  concentration in RW from ~20 ppm to 0 (within 8 weeks; Fig. 4c) supports its incorporation into the wood by fungi. After  $t_3$  (8 weeks), no  $\text{NO}_3^-$  in RW was detected at  $t_5$ ,  $t_6$  and  $t_7$ , although original RW was added at  $t_3$ ,  $t_5$  and  $t_6$ . It probably reflects the complete consumption of  $\text{NO}_3^-$  by microorganisms because (i) water sampling was performed before water addition in the Erlenmeyers, and (ii) time largely exceeded 8 weeks between each sampling. Two lines of evidence

support the incorporation of N-NO<sub>3</sub><sup>-</sup> by fungi within the wood. First,  $\delta^{15}\text{N}$  values of the remaining wood were positively correlated with N content (Fig. 6a), attesting that nitrate was <sup>15</sup>N-enriched relative to the wood. Second, the y intercept of a Keeling plot of the data enabled estimating the isotopic signature of the source of N,<sup>[52]</sup> thus suggesting a  $\delta^{15}\text{N-NO}_3^-$  equal to 6.6 ‰ (Fig. 6b), which is in agreement with the typical  $\delta^{15}\text{N-NO}_3^-$  values measured in rivers of the region (+4 to +13 ‰).<sup>[53]</sup> However, according to Miller and Tans,<sup>[54]</sup> this value is only valid when considering a stable geochemical background of the wood, which is the case here because ‘background’ refers to its initial  $\delta^{15}\text{N}$  and %N values. Because N recycling also occurs during microbial decomposition,<sup>[45]</sup> part of the N within wood could have been ‘replaced’ by exogenous N. In this case, the  $\delta^{15}\text{N-NO}_3^-$  value can be lower than 6.6 ‰.

Organic nitrogen in RW is also a valuable external N source.<sup>[55]</sup> However, nitrate is likely the main source of N for microorganisms because nitrates are precursors of dissolved organic nitrogen, leading to a similar  $\delta^{15}\text{N}$  signature for dissolved organic matter and nitrates.<sup>[56,57]</sup> Alternatively, other microbial processes mediated by bacteria can account for the observed increasing  $\delta^{15}\text{N}$  values of wood pieces in RW. Microbial processes in general and bacterial processes in particular are often considered as the main drivers of  $\delta^{15}\text{N}$  values under natural conditions (modulated by the oxygenation conditions), over the importation of exogenous N.<sup>[12,15,24,26,28,58]</sup> In this context, it has been proposed that increasing  $\delta^{15}\text{N}$  values of the remaining organic material under oxic conditions was the result of the liberation of <sup>15</sup>N-depleted compounds during microbial (meaning bacterial) processing, leading to an increasing C/N ratio.<sup>[12]</sup> In the same way, other authors have suggested that <sup>15</sup>N enrichment of their incubated litter was best explained by ‘microbial-mediated’ isotopic fractionation.<sup>[24,26]</sup> In all cases, N loss was invoked, which is not observed here. Even though bacterial growth within the remaining wood is in agreement with decreasing C/N values, their main nitrogen source must be the river water. However, we cannot completely exclude bacterial-mediated isotopic fractionation in the present study. Besides, bacterial-mediated isotopic fractionation could be one of the sources of the non-explained variance (43 %) in the relationship between the  $\delta^{15}\text{N}$  values and the %N of samples in RW (Fig. 6a), which is best explained by the incorporation of exogenous N.<sup>[23,30]</sup> The heterogeneous distribution of fungi on samples may also be a major source of that non-explained variance and of the large variability in wood masses (Fig. 3).

#### *Paleoenvironmental implications*

Previous studies with litterbags concluded that  $\delta^{15}\text{N}$  values are more variable than  $\delta^{13}\text{C}$  values during early diagenesis, because of nutrient availability (exogenous N) and microbial processing.<sup>[15,24,26,45]</sup> The same conclusions were drawn for lacustrine sediments.<sup>[12,58]</sup> According to those authors, selective preservation of refractory compounds (e.g. lignin), and preferential loss of labile or biodegradable compounds, should not drastically modify  $\delta^{13}\text{C}$  values, whereas microbial processing and subsequent <sup>15</sup>N discrimination result in large modifications of  $\delta^{15}\text{N}$  values. The present

incubation of wood samples does agree with those assumptions,  $\delta^{13}\text{C}$  values being indistinguishable from the initial value (Fig. 4b), whereas  $\delta^{15}\text{N}$  values were highly variable (Fig. 4d).

Fogel and Tuross<sup>[45]</sup> concluded that changes in  $\delta^{15}\text{N}$  values during early diagenesis ‘may preclude the use of  $\delta^{15}\text{N}$  in plant fossil material or sedimentary organic matter for reconstructing paleoenvironments’. By extrapolating the linear regression between  $\delta^{15}\text{N}$  values and N content of samples in RW (Fig. 6a) to typical values of lignites rich in organic matter (~1 % in Paleocene–Eocene lignites)<sup>[6]</sup> and recently peatified organic matter (~2 %),<sup>[26]</sup> the expected  $\delta^{15}\text{N}$  values (~45 and ~94 ‰) would be unrealistic, when compared with those recorded elsewhere (–6 to +7 ‰).<sup>[6,26]</sup> This discrepancy suggests that early diagenesis, leading to the ‘geological’ value, is not linear whether due to incorporating exogenous N<sup>[23]</sup> or microbial processing.<sup>[24,26,45]</sup> Thus, the effect of early diagenesis on  $\delta^{15}\text{N}$  values is very fast and can be considered as instantaneous at the geological time scale. A similar non-linear relationship was reported in peatified *sphagnum*, where  $\delta^{15}\text{N}$  values increased from –6 to 0 ‰ during the first 40 years, whereas it increased by +1 ‰ during the next 180 years of burial.<sup>[26]</sup> Based on the associated decrease in C/N ratio along the peat core, those authors attributed the increase in  $\delta^{15}\text{N}$  values partly to microbially mediated isotopic fractionation and partly to retention of  $^{15}\text{N}$ -enriched substrates. Similarly, the incorporation of N in the present wood samples and exportation of N from them are likely driven by microbial community activity (e. g. fungi): N is incorporated when available in the surrounding environment (i.e. in RW) and exported when non-available (i.e. in DW). The incorporation (exportation) of N in (from) wood illustrates the rapid response of microbial processes to environmental conditions.<sup>[50]</sup> In that case, early diagenesis homogenises initial  $\delta^{15}\text{N}$  values of individuals (i.e. wood pieces) and  $\delta^{15}\text{N}$  values of the surrounding environment (i.e.  $\text{NO}_3^-$  in water). Therefore, when utilising organic nitrogen isotopes ( $\delta^{15}\text{N}_{\text{org}}$ ) for paleoenvironmental reconstructions, the so called ‘primary signal’ of paleoenvironmental conditions is unlikely to be recorded. However, the smoothed environmental signal (in space and time), such as millennial or multimillennial-scale climatic change may be preserved thanks to early diagenesis homogenisation as suggested by previous paleoenvironmental studies.<sup>[6]</sup> It could explain why the range of terrestrial  $\delta^{15}\text{N}_{\text{org}}$  values in prequaternary sediments are so limited, with most values between –1 and +7 ‰<sup>[6,59]</sup> compared with the range of  $\delta^{15}\text{N}_{\text{org}}$  values recorded worldwide in modern soils and plants (–8 to +22 ‰).<sup>[60]</sup>

Changes in oxygenation conditions during burial may also influence  $\delta^{15}\text{N}_{\text{org}}$  values in an opposite way, with decreasing values in oxic environments and increasing values in anoxic environments, due to the presence of different microbial communities.<sup>[12,26]</sup> Thus, evaluating the effect of early diagenesis under oxic and anoxic conditions is of interest, but may be challenging because anoxic conditions strongly slow down degradation processes.<sup>[3,12]</sup>

## Conclusion

This original incubation experiment was conducted with wood pieces immersed in river water and distilled water in order to evaluate the effects of early diagenesis on carbon and nitrogen isotopes. The significant development of fungi in DW was not expected and enabled the comparison of degradation rates, and carbon and nitrogen dynamics of decayed wood between soft-rot and white-rot fungi. It also highlighted the incorporation of prediagenesis fungal material. In all samples,  $\delta^{13}\text{C}$  values did not significantly change, in agreement with low modification of  $\delta^{13}\text{C}$  values generally reported during early diagenesis of organic matter.

Nitrogen dynamics of wood samples in DW and RW showed two different patterns: N release, associated with slight  $^{15}\text{N}$  depletion ( $-0.3\text{‰}$ ), was in evidence in samples immersed in distilled water. It was attributed to the extraction of a  $^{15}\text{N}$ -enriched pool (i.e. proteins) by degrading fungi. Conversely, N accumulation, associated with  $^{15}\text{N}$  enrichment of the remaining wood, was seen in samples immersed in RW. This was mostly attributed to the importation of  $^{15}\text{N}$ -enriched nitrate of the RW into the remaining wood. The primary signal of organic nitrogen isotopes may not be recorded in the fossil archive, but early diagenesis seems to average an environmental signal by integrating individual signals (i.e. wood, fungi,  $\text{NO}_3^-$ ) and microbially mediated isotopic fractionation. This integration could be almost instantaneous at the geological time scale, hence potentially enabling paleoenvironmental reconstructions.

However, because the marine setting and oxygen-depleted environments represent large areas and conditions favourable to terrestrial organic matter accumulation, this kind of incubation experiment should also be performed in salty water and under anoxic conditions, although the latter conditions can be a limit of the methodology used, because of the long time needed when considering the low degradation rate in anoxic environments.

## Supplementary material

Content and isotopic data of carbon and nitrogen used for statistical tests xxx are available from the journal online (see [http://www.publish.csiro.au/?act=view\\_file&file\\_id=EN16049\\_AC.pdf](http://www.publish.csiro.au/?act=view_file&file_id=EN16049_AC.pdf)).

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**Fig. 1.** Outlines of the method used to evaluate nitrogen and carbon dynamics during wood decay of the initial coniferous branch. DW, distilled water. RW, river water; t, time.

**Fig. 2.** (a and b) Morphology of samples before and after 16 weeks ( $t_4$ ) of degradation. (c and d) Morphology of samples after 73 weeks ( $t_7$ ). DW, distilled water; RW, river water; i, reddish-brown colour characterising black-rot or soft-rot; ii, black fungi floating from the tree-ring limits; iii, floating woody particles; iv, white spots characterising white-rot fungi; v, increasing uniformity of the coloration.

**Fig. 3.** Mass loss of samples v. time. Error bars are the standard deviation ( $2\sigma$ ), based on triplicates. 100 % corresponds to the initial value. DW, distilled water. RW, river water; t, time.

**Fig. 4.** Carbon and nitrogen dynamics of wood samples v. incubation time. Error bars correspond to  $2\sigma$ . Because woody particles were analysed at  $t_5$  without replicates, the error bar is the average error of analytical devices:  $\pm 0.6$  and  $\pm 0.002$  % for C and N content respectively, and  $\pm 0.2$  ‰ for carbon and nitrogen isotopes.  $\text{NO}_3^-$  in river water (RW) remained at trace levels after  $t_2$ .

**Fig. 5.** Atomic C/N ratio of wood pieces. Only the correlation between C/N and time in river water (RW) is significant. The red star correspond to the floating particles sampled at  $t_5$  in river water (RW). Error bars ( $2\sigma$ ) are within the symbols. DW, distilled water.

**Fig. 6.** (a)  $\delta^{15}\text{N}$  values relative to N content of wood samples incubated in river water (RW). (b)  $\delta^{15}\text{N}$  values relative to the inverse of the N content of the same eight groups of samples: 10 samples for the initial values and 3 samples for each grey point. Both linear regression are significant with  $P < 0.05$ .

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**Table 1. Initial variability**

%C, %N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values along the initial branch.  $\sigma$  represents the standard deviation

Elements	<i>n</i>	Elemental content (%)			Isotopic composition (‰)		
		Average	$2\sigma$	Range	Average	$2\sigma$	Range
Carbon	11	47.4	0.9	46.7 to 48.3	-24.1	0.2	-24.3 to -23.9
Nitrogen	10	0.08	0.02	0.07 to 0.10	-0.2	1.0	-0.8 to 1.0

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**Table 2. Carbon and nitrogen contents and isotopic compositions**

Remaining organic matter (OM), %C, %N, atomic C/N ratio,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during the whole experiment.  $\sigma$  represents the standard deviation of the values based on triplicate measurements. DW, distilled water; RW, river water

Remaining OM	$2\sigma$	Content (%)				Atomic C/N ratio	Isotopes (‰)		
		Carbon	$2\sigma$	Nitrogen	$2\sigma$		Carbon	$2\sigma$	Nitrogen
98.7	1.5	48.5	1.0	0.07	0.01	827	-24.1	0.3	-0.6
98.6	2.3	48.8	0.5	0.07	0.00	810	-24.1	0.2	-0.8
100.7	1.3	48.4	0.5	0.07	0.00	869	-24.1	0.1	-0.6
100.7	1.7	48.7	1.1	0.07	0.02	804	-24.0	0.1	0.1
96.8	1.8	48.4	1.0	0.07	0.02	807	-24.0	0.4	-0.6
95.4	2.5	48.4	0.3	0.07	0.01	821	-24.1	0.1	-0.9
91.3	1.9	47.9	1.0	0.07	0.01	785	-24.1	0.1	-0.4
—	—	48.5	—	0.07	—	—	-24.1	—	-0.5
98.6	1.3	48.9	1.0	0.08	0.01	719	-24.0	0.1	-0.3
97.8	2.7	48.0	0.3	0.08	0.01	730	-23.9	0.2	0.8
97.4	1.3	48.5	0.7	0.09	0.01	601	-23.9	0.3	2.5
93.8	0.9	48.1	0.8	0.10	0.01	471	-23.9	0.1	2.1
86.5	8.4	48.9	1.9	0.09	0.01	545	-24.0	0.3	1.0
—	—	34.9	—	1.37	—	30	-22.6	—	2.7
80.6	6.0	48.0	1.7	0.11	0.03	444	-24.0	0.2	1.9
73.3	8.3	47.6	1.2	0.13	0.03	371	-23.9	0.2	2.3
—	—	48.3	—	0.10	—	—	-23.9	—	1.5

599 ← What is number? It does not appear in the original table