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1 **Research Paper**

2 **Carbon and nitrogen dynamics in decaying wood: paleoenvironmental implications**

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

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

32 EN16049

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34 Impact of early diagenesis on organic matter

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

## 36 **Introduction**

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

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

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

## 70 **Experimental**

### 71 *Samples*

72 A treetop branch of *Sciadopitys verticillata* (Cupressaceae, conifer) was collected in December  
73 2012 from a garden in Tokyo, Japan, after having lain for 6 months on the ground to ensure the  
74 natural presence of decay organisms. We chose this endemic specimen because it has been chemically  
75 related to the morphogenus conifer *Xenoxylon* Gothan, which was widespread during Jurassic and  
76 Cretaceous times and is of paleoclimatic interest nowadays.<sup>[34,35]</sup> Both extremities of the branch and  
77 the bark were removed to clean the branch and avoid heterogeneities that were too large between its  
78 different parts (Fig. 1). The branch (~60 cm long; diameter ~3 cm) was then sliced in ~2-mm-thick  
79 pieces, which were air-dried and weighed.

### 80 *Experimental design*

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

93 The incubation experiment was carried out for 73 weeks and wood pieces were collected after 2, 4,  
94 8, 16, 32, 52 and 73 weeks, corresponding to  $t_1$  to  $t_7$  respectively. Initial RW was stored in a 10-L  
95 canister in the dark and water was bubbled in order to keep it oxygenated. Filtered RW or DW was  
96 added to Erlenmeyers at  $t_3$ ,  $t_5$  and  $t_6$  to counterbalance evaporation in Erlenmeyers.

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

### 100 *Analyses*

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

104 analyser coupled with a Thermo Scientific Delta V Plus mass spectrometer connected to a ConFlo IV  
105 dilution system. Systems precision and accuracy were monitored using tyrosine (N = 7.73 %,  $\delta^{15}\text{N}$  =  
106 10.01 ‰ and C = 59.66 %,  $\delta^{13}\text{C}$  = -23.20 ‰,) as internal laboratory standard that was calibrated with  
107 international standards IAEA-N-1 ( $\delta^{15}\text{N}$  = 0.4 ‰), IAEA-N-2 ( $\delta^{15}\text{N}$  = 20.3 ‰) and IAEA-NO-3 ( $\delta^{15}\text{N}$   
108 = 4.7 ‰) and expressed relative to  $\text{N}_2$ -AIR for nitrogen and relative to Vienna Pee Dee Belemnite  
109 (VPDB) for carbon. Nitrogen analyses were systematically performed in duplicate ( $n = 51$ ), whereas  
110 for carbon analyses, samples were analysed in duplicate ( $n = 12$ ) every 10 samples, precision being  
111 usually better for carbon isotopes. The overall precision ( $2\sigma$ ) was <0.002 and <0.6 % for N and C  
112 content respectively, and <0.2 ‰ for N and C isotopes.

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

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

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

### 124 *Water chemistry control*

125 Water chemistry ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ) was controlled in each type of water at  $t_0$ ,  $t_2$ ,  $t_3$ ,  $t_5$ ,  $t_6$   
126 and  $t_7$  by spectrophotometry using a Gallery system. As expected, DW contained no or trace amounts  
127 of these chemical compounds, with no forms of nitrogen (always <0.2 ppm). In contrast, RW did  
128 contain significant amount of nitrates at  $t_0$  (~20 ppm), which disappeared after  $t_2$  (<0.1 ppm  
129 remained), whereas other nutrients remained in trace amounts.

### 130 *Statistical treatment and data sets*

131 Significance of the linear regressions and of the mean comparisons (e.g. differences in N content  
132 between samples in RW and DW) were tested using Pearson correlations and Student's tests  
133 respectively, with the programming language R (version 3.2.2, R Foundation for Statistical Computing,  
134 Vienna, Austria). Hypotheses were tested on the basis of a 5 % significance level ( $P$  value), meaning  
135 that a relation or comparison is significant when  $P < 0.05$  for  $n$  observations. Original data sets are  
136 given in the Supplementary material, whereas condensed results used for figures are reported in Table  
137 2.

## 138 Results

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

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

152 The degradation features mentioned became increasingly marked at the end of the experiment (Fig.  
153 2c and d). Growth-rings of wood pieces in DW became increasingly dark, while they were  
154 progressively erased in RW, with increasing numbers of floating woody particles, suggesting two  
155 different microbial communities.

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

### 162 *Carbon patterns*

163 The carbon content of wood pieces in both DW and RW increased between  $t_0$  and  $t_1$  (2 weeks),  
164 from 47.4 to 48.5 and 48.9 % respectively (Fig. 4a). After 32 weeks ( $t_5$ ), they progressively tended  
165 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  
166  $t_4$ ), it was in the range of the analytical precision ( $\pm 0.2\text{‰}$ ; Fig. 4b). In addition, the floating woody  
167 particles were sampled at  $t_5$  in an Erlenmeyer containing RW. They significantly differed from the  
168 original piece of wood with (i) carbon content of 35 v. 50 % in the original piece of wood (Fig. 4a),  
169 and (ii)  $\delta^{13}\text{C}$  value of  $-22.6$  v.  $-24.0\text{‰}$  (Fig. 4b).

## 170 *Nitrogen patterns*

171 In contrast to carbon content, the nitrogen content of wood pieces depicted two different patterns  
172 according to the water type (Fig. 4c). The average %N of wood pieces in DW was 0.07 % for the  
173 whole duration of the degradation, significantly lower than the initial value (0.08 %;  $n = 21$ ;  $P <$   
174 0.001), whereas the average %N of wood pieces in RW was significantly higher (0.09 %;  $n = 21$ ;  $P <$   
175 0.02). In addition, N content of wood pieces in DW remained close to 0.07 % after a rapid decrease ( $t_0$   
176 to  $t_3$ ) from the initial value (0.08 %). In contrast, the N content of samples in RW almost linearly  
177 increased from the initial value to 0.12 % at  $t_7$  (Fig. 4c). This reflects an accumulation of nitrogen in  
178 the remaining wood, called the ‘immobilisation phase’.<sup>[15,21]</sup>

179 As a consequence, the atomic C/N ratio decreased linearly from ~650 to ~400 in RW wood as  
180 degradation advanced (Fig. 5). In the same time, the C/N ratio of wood pieces in DW increased to  
181 >800 until  $t_3$ , and then remained stable until the end of incubation. Those very high C/N ratios of  
182 wood pieces are characteristic of fresh wood.<sup>[11]</sup> In contrast, floating woody particles sampled at  $t_5$  in  
183 RW showed a C/N ratio of 30 (Fig. 5), typical of substantially decayed wood.<sup>[11]</sup>

184 The immobilisation phase in RW is associated with significantly higher  $\delta^{15}\text{N}$  values (+1.7 ‰ on  
185 average) of the remaining wood, when compared with the initial value (-0.2 ‰;  $n = 21$ ;  $P < 0.001$ ),  
186 whereas  $\delta^{15}\text{N}$  values of wood pieces in DW remained almost constant at approximately -0.5 ‰ (Fig.  
187 4d). In addition, the floating woody particles (in RW) sampled at  $t_5$  showed a higher nitrogen content  
188 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  
189 nitrogen content suggests that wood particles were invaded by N-rich microorganisms.

## 190 **Discussion**

### 191 *Decomposers and degradation rate*

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

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

#### 220 *Carbon dynamics*

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

236 If carbohydrates were effectively degraded, their C would have been reused by microbes, which  
237 would be integrated in the floating woody particles or in the remaining wood. In RW, the woody  
238 particles were analysed at  $t_5$ . Their high %N (1.37 v. 0.09 %) and low atomic C/N ratio (30 v. 769)  
239 relative to the remaining wood suggest a high content of microbial biomass because it is concentrated  
240 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



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

#### 249 *Nitrogen dynamics*

250 Nitrogen dynamics are much more complex either in DW or RW. The C/N ratio tracks changes in  
251 chemical composition of samples and thus reflects degradation processes.<sup>[12]</sup> Considering similar  
252 behaviours in C content of samples in both types of waters (Fig. 4a), C/N variations likely reflect  
253 changes in N content (Fig. 5). Consequently, the rapid decrease in N content of samples in DW (Fig.  
254 4c), and the resulting increase of C/N ratio (Fig. 5), are most likely due to N release. In contrast, the  
255 opposite pattern was observed in samples incubated in RW, with an increase in N content (Fig. 4C)  
256 leading to a decrease of C/N (Fig. 5). This points to N accumulation in the remaining wood. In both  
257 cases, the microenvironment of samples should be considered and its implications are discussed  
258 below. It should also be noted that substantial isotopic fractionation processes like denitrification can  
259 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  
260 fractionation factor.<sup>[42]</sup>

#### 261 *Distilled water*

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

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

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

#### 284 *River water*

285 Nitrogen dynamics in RW samples showed an opposite pattern compared with samples in DW,  
286 with N accumulation in the remaining wood (Fig. 4c) associated with significantly higher  $\delta^{15}\text{N}$  values  
287 (1.5 ‰ on average v. -0.2 ‰ at  $t_0$ ; Fig. 4d). N immobilisation has been commonly reported during  
288 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  
289 *Fagus sylvatica* inoculated with basidiomycete fungi.<sup>[44]</sup> According to those authors, it reflects  
290 accumulation of nitrogen during an immobilisation phase of nutrients. Two main processes are able to  
291 supply nitrogen in those decaying materials: (i)  $\text{N}_2$  fixation by specialised microorganisms,<sup>[21,47]</sup> and  
292 (ii) uptake by cord-forming fungi from the surrounding environment.<sup>[21,44,47]</sup> However, rates of  $\text{N}_2$   
293 fixation are too low to explain N accumulation in decaying wood,<sup>[21,47]</sup> because fungi are not able to  
294 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  
295 major process involved in N accumulation. Alternatively, cord-forming fungi have the ability to  
296 scavenge N from the surrounding environment, and further import it into the decaying material via a  
297 translocating network of mycelium that interconnects discontinuous nutrient sources.<sup>[21,44,47]</sup> Thus,  
298 wood-decaying fungi are likely the mediators of N accumulation into decaying wood in RW. In the  
299 present study, the source of exogenous N is RW and the cord-forming fungi are most probably white-  
300 rot fungi (Basidiomycetes), their activity being the cause of the continuous decrease in C/N ratio (Fig.  
301 5).

302 The  $\delta^{15}\text{N}$  variations in wood samples are likely due to the isotopic composition of the N imported  
303 by fungi (N source) and/or influence of metabolism effects leading to internal fractionation of  $^{15}\text{N}$   
304 during N incorporation.<sup>[12,28,49–51]</sup> However, metabolism effects in fungi are thought to be low because  
305 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  
306 be seriously considered here. The only form of inorganic N detected in RW was  $\text{NO}_3^-$ . The rapid  
307 decrease in  $\text{NO}_3^-$  concentration in RW from ~20 ppm to 0 (within 8 weeks; Fig. 4c) supports its  
308 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  $t_7$ ,  
309 although original RW was added at  $t_3$ ,  $t_5$  and  $t_6$ . It probably reflects the complete consumption of  $\text{NO}_3^-$   
310 by microorganisms because (i) water sampling was performed before water addition in the  
311 Erlenmeyers, and (ii) time largely exceeded 8 weeks between each sampling. Two lines of evidence

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

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

#### 341 *Paleoenvironmental implications*

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

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

350 Fogel and Tuross<sup>[45]</sup> concluded that changes in  $\delta^{15}\text{N}$  values during early diagenesis ‘may preclude  
351 the use of  $\delta^{15}\text{N}$  in plant fossil material or sedimentary organic matter for reconstructing  
352 paleoenvironments’. By extrapolating the linear regression between  $\delta^{15}\text{N}$  values and N content of  
353 samples in RW (Fig. 6a) to typical values of lignites rich in organic matter (~1 % in Paleocene–  
354 Eocene lignites)<sup>[6]</sup> and recently peatified organic matter (~2 %),<sup>[26]</sup> the expected  $\delta^{15}\text{N}$  values (~45 and  
355 ~94 ‰) would be unrealistic, when compared with those recorded elsewhere (–6 to +7 ‰).<sup>[6,26]</sup> This  
356 discrepancy suggests that early diagenesis, leading to the ‘geological’ value, is not linear whether due  
357 to incorporating exogenous N<sup>[23]</sup> or microbial processing.<sup>[24,26,45]</sup> Thus, the effect of early diagenesis  
358 on  $\delta^{15}\text{N}$  values is very fast and can be considered as instantaneous at the geological time scale. A  
359 similar non-linear relationship was reported in peatified *sphagnum*, where  $\delta^{15}\text{N}$  values increased from  
360 –6 to 0 ‰ during the first 40 years, whereas it increased by +1 ‰ during the next 180 years of  
361 burial.<sup>[26]</sup> Based on the associated decrease in C/N ratio along the peat core, those authors attributed  
362 the increase in  $\delta^{15}\text{N}$  values partly to microbially mediated isotopic fractionation and partly to retention  
363 of  $^{15}\text{N}$ -enriched substrates. Similarly, the incorporation of N in the present wood samples and  
364 exportation of N from them are likely driven by microbial community activity (e. g. fungi): N is  
365 incorporated when available in the surrounding environment (i.e. in RW) and exported when non-  
366 available (i.e. in DW). The incorporation (exportation) of N in (from) wood illustrates the rapid  
367 response of microbial processes to environmental conditions.<sup>[50]</sup> In that case, early diagenesis  
368 homogenises initial  $\delta^{15}\text{N}$  values of individuals (i.e. wood pieces) and  $\delta^{15}\text{N}$  values of the surrounding  
369 environment (i.e.  $\text{NO}_3^-$  in water). Therefore, when utilising organic nitrogen isotopes ( $\delta^{15}\text{N}_{\text{org}}$ ) for  
370 paleoenvironmental reconstructions, the so called ‘primary signal’ of paleoenvironmental conditions  
371 is unlikely to be recorded. However, the smoothed environmental signal (in space and time), such  
372 millennial or multimillennial-scale climatic change may be preserved thanks to early diagenesis  
373 homogenisation as suggested by previous paleoenvironmental studies.<sup>[6]</sup> It could explain why the  
374 range of terrestrial  $\delta^{15}\text{N}_{\text{org}}$  values in prequaternary sediments are so limited, with most values between  
375 –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  
376 plants (–8 to +22 ‰).<sup>[60]</sup>

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

## 382 **Conclusion**

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

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

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

## 405 **Supplementary material**

406 Content and isotopic data of carbon and nitrogen used for statistical tests xxx are available from the  
407 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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575 **Fig. 1.** Outlines of the method used to evaluate nitrogen and carbon dynamics during wood decay of the initial  
 576 coniferous branch. DW, distilled water. RW, river water; t, time.

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

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

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

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

590 **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  
 591 relative to the inverse of the N content of the same eight groups of samples: 10 samples for the initial values and  
 592 3 samples for each grey point. Both linear regression are significant with  $P < 0.05$ .

593

**Table 1. Initial variability**

594

%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

595

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

596

Remaining organic matter (OM), %C, %N, atomic C/N ratio,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values during the whole

597

experiment.  $\sigma$  represents the standard deviation of the values based on triplicate measurements. DW,

598

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