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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}N depletion in distilled water, attributed to uptake of ^{15}N -
26 enriched pool (i.e. proteins) by fungi, and (ii) N accumulation associated with ^{15}N enrichment in river water. .
27 The latter pattern was attributed predominantly to microbially mediated importation of ^{15}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.

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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.^[1–8]
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.^[9,10] 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.^[11–13]

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}C -enriched carbohydrates.^{[14–}
47 ^{18]} In contrast, nitrogen dynamics during terrestrial organic decay are more complex and consequences
48 on $\delta^{15}\text{N}$ values are not well constrained.^[12,15,19–21] Indeed, they involve complex interactions of biotic
49 and abiotic factors: microbial processing *vs* N sources and environmental conditions.^[12,22–28] 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.^[12] 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.^[15,19,23,24,26,28–30] In
55 addition, considering the importance of coarse woody debris in terrestrial organic matter^[11,31] 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,^[32,33] 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.^[34,35] 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 ¹⁴N.

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 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 (2σ) 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 ± 0.5 and -24.1 ± 0.1 ‰ respectively, whereas nitrogen content and isotopes
117 were on average 0.08 ± 0.01 % and -0.2 ± 0.5 ‰ respectively (Table 1). In agreement with literature,
118 initial variability of $\delta^{15}\text{N}$ values was much higher than for carbon.^[36–38] 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 (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
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).^[32] Fungi were
142 probably already within the wood before branch sampling because decay starts as soon as a tree is
143 cut,^[39] or even before because wood of living conifers is known for hosting fungi.^[32] 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).^[32] 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‰ at t_0 to -23.9‰ 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‰ (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’.^[15,21]

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.^[11] 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.^[11]

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.^[32] They are classified as white-rot or black-rot fungi, based on macroscopic aspects.^[33] In
194 contrast, soft-rot fungi comprise Ascomycetes and Deuteromycetes, which are less efficient than
195 Basidiomycetes for degrading wood.^[32,33] 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.^[32]
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).^[32,33] 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.^[33] 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.^[32,33] 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.^[15] 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.^[15] 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.^[18] 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 ~ 220 weeks, which is ~ 50 weeks longer than reported in soils.^[15] Thus, the degradation
215 rate of wood pieces in RW is slightly lower than measured in soils,^[15] and in contradiction with
216 another study^[29] 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^[15] and green leaves from angiosperms.^[29] Nevertheless, the mass loss of wood pieces obtained
219 here confirms that this parameter is a good indicator of the state of degradation.^[15,29]

220 *Carbon dynamics*

221 Natural decomposing and artificial aging of plant material has often shown C enrichment and ^{13}C
222 depletion in the remaining organic matter, mainly attributed to the preferential loss of carbohydrates
223 (cellulose and hemicellulose) during early diagenesis.^[14,16,18] 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.^[33] However, $\delta^{13}\text{C}$
228 values did not significantly change. The absence of expected ^{13}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 ~ 30 % for samples in RW and ~ 10 % in DW. Alternatively, the loss of ^{13}C -
231 enriched carbohydrates may have been counterbalanced by the loss of hydrolysable ^{13}C -depleted
232 compounds^[15] or by the simultaneous loss of lignin and carbohydrates, in agreement with decay
233 abilities of Basidiomycetes in RW.^[32,33] 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.^[40] 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}C -enriched carbohydrates of the
243 remaining wood.^[14] Additional loss of ^{12}C might be associated with microbial respiration.^[28] 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}C -enriched and ^{13}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}C -depleted lignin and ^{13}C -enriched carbohydrates.^[33]

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.^[12] 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^[41] and that N_2 fixation, if any, has a very small
260 fractionation factor.^[42]

261 *Distilled water*

262 N loss of the remaining wood in DW was associated with significant lower $\delta^{15}\text{N}$ values (-0.5‰ on
263 average), compared with the initial one (-0.2‰ ; $n = 21$; $P < 0.05$; Fig. 4d), suggesting the
264 preservation of a ^{15}N -depleted pool and/or preferential degradation of a ^{15}N -enriched pool. Such N
265 loss has already been observed during the first 120 days of incubation of *Spartina* plants.^[23] However,
266 it was attributed to leaching without ^{15}N fractionation, which is not consistent with the ^{15}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.^[43,44] 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.^[44] 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}N depletion of the remaining wood
278 is not currently well understood, but preferential degradation of a ^{15}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^[45] and it has also been demonstrated that proteins were 3 ‰-enriched relative
281 to cells.^[46] Proteins and amino acids may therefore correspond to the ^{15}N -enriched compounds
282 preferentially extracted by fungi as a nutrient source,^[47] all the more because their preservation
283 potential is low.^[10]

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,^[15,21,45] sphagnum litter^[26] and woody litter^[47] or in decaying wood of
289 *Fagus sylvatica* inoculated with basidiomycete fungi.^[44] 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) N_2 fixation by specialised microorganisms,^[21,47] and
292 (ii) uptake by cord-forming fungi from the surrounding environment.^[21,44,47] However, rates of N_2
293 fixation are too low to explain N accumulation in decaying wood,^[21,47] because fungi are not able to
294 fix N_2 given N_2 -fixing organisms are limited to prokaryotes.^[48] Hence, 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.^[21,44,47] 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}N
304 during N incorporation.^[12,28,49–51] 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.^[49] Thus, N sources must
306 be seriously considered here. The only form of inorganic N detected in RW was NO_3^- . The rapid
307 decrease in 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 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 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-NO₃⁻ by fungi within the wood. First, δ¹⁵N values of the remaining
313 wood were positively correlated with N content (Fig. 6a), attesting that nitrate was ¹⁵N-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,^[52] thus suggesting a δ¹⁵N-NO₃⁻ equal to 6.6 ‰ (Fig. 6b), which
316 is in agreement with the typical δ¹⁵N-NO₃⁻ values measured in rivers of the region (+4 to +13 ‰).^[53]
317 However, according to Miller and Tans,^[54] 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 δ¹⁵N and %N values. Because N recycling also occurs during microbial decomposition,^[45] part of the
320 N within wood could have been ‘replaced’ by exogenous N. In this case, the δ¹⁵N-NO₃⁻ value can be
321 lower than 6.6 ‰.

322 Organic nitrogen in RW is also a valuable external N source.^[55] 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 δ¹⁵N signature for dissolved organic matter and nitrates.^[56,57] Alternatively, other
325 microbial processes mediated by bacteria can account for the observed increasing δ¹⁵N 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 δ¹⁵N values under natural conditions (modulated by the oxygenation
328 conditions), over the importation of exogenous N.^[12,15,24,26,28,58] In this context, it has been proposed
329 that increasing δ¹⁵N values of the remaining organic material under oxic conditions was the result of
330 the liberation of ¹⁵N-depleted compounds during microbial (meaning bacterial) processing, leading to
331 an increasing C/N ratio.^[12] In the same way, other authors have suggested that ¹⁵N enrichment of their
332 incubated litter was best explained by ‘microbial-mediated’ isotopic fractionation.^[24,26] 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 δ¹⁵N values and the %N of samples in RW
338 (Fig. 6a), which is best explained by the incorporation of exogenous N.^[23,30] 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 δ¹⁵N values are more variable than δ¹³C values
343 during early diagenesis, because of nutrient availability (exogenous N) and microbial
344 processing.^[15,24,26,45] The same conclusions were drawn for lacustrine sediments.^[12,58] 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 δ¹³C values, whereas microbial
347 processing and subsequent ¹⁵N discrimination result in large modifications of δ¹⁵N 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^[45] 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)^[6] and recently peatified organic matter (~2 %),^[26] the expected $\delta^{15}\text{N}$ values (~45 and
355 ~94 ‰) would be unrealistic, when compared with those recorded elsewhere (–6 to +7 ‰).^[6,26] This
356 discrepancy suggests that early diagenesis, leading to the ‘geological’ value, is not linear whether due
357 to incorporating exogenous N^[23] or microbial processing.^[24,26,45] 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.^[26] 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}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.^[50] 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. 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.^[6] 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 ‰^[6,59] compared with the range of $\delta^{15}\text{N}_{\text{org}}$ values recorded worldwide in modern soils and
376 plants (–8 to +22 ‰).^[60]

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.^[12,26] 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.^[3,12]

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}N depletion (-0.3‰), was in evidence in samples immersed in distilled water.
392 It was attributed to the extraction of a ^{15}N -enriched pool (i.e. proteins) by degrading fungi.
393 Conversely, N accumulation, associated with ^{15}N enrichment of the remaining wood, was seen in
394 samples immersed in RW. This was mostly attributed to the importation of ^{15}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, 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).

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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 (2σ), 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 2σ .
 584 Because woody particles were analysed at t_5 without replicates, the error bar is the average error of analytical
 585 devices: ± 0.6 and ± 0.002 % for C and N content respectively, and ± 0.2 % for carbon and nitrogen isotopes.
 586 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 (2σ)
 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. σ represents the standard deviation

Elements	<i>n</i>	Elemental content (%)			Isotopic composition (‰)		
		Average	2σ	Range	Average	2σ	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. σ represents the standard deviation of the values based on triplicate measurements. DW,

598

distilled water; RW, river water

Remaining OM	2σ	Content (%)				Atomic C/N ratio	Isotopes (‰)			
		Carbon	2σ	Nitrogen	2σ		Carbon	2σ	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