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Q1 **Response of C and N cycles to N fertilization in**  
 2 **Sphagnum and Molinia-dominated peat**  
 3 **mesocosms**

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## ABSTRACT

Plant communities play an important role in the C-sink function of peatlands. However, global change and local perturbations are expected to modify peatland plant communities, leading to a shift from *Sphagnum* mosses to vascular plants. Most studies have focused on the direct effects of modification in plant communities or of global change (such as climate warming, N fertilization) in peatlands without considering interactions between these disturbances that may alter peatlands' C function. We set up a mesocosm experiment to investigate how Greenhouse Gas (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) fluxes, and dissolved organic carbon (DOC) and total dissolved N (TN) contents are affected by a shift from *Sphagnum* mosses to *Molinia caerulea* dominated peatlands combined with N fertilization. Increasing N deposition did not alter the C fluxes (CO<sub>2</sub> exchanges, CH<sub>4</sub> emissions) or DOC content. The lack of N effect on the C cycle seems due to the capacity of *Sphagnum* to efficiently immobilize N. Nevertheless, N supply increased the N<sub>2</sub>O emissions, which were also controlled by the plant communities with the presence of *Molinia caerulea* reducing N<sub>2</sub>O emissions in the *Sphagnum* mesocosms. Our study highlights the role of the vegetation composition on the C and N fluxes in peatlands and their responses to the N deposition. Future research should now consider the climate change in interaction to plants community modifications due to their controls of peatland sensitivity to environmental conditions.

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56 **1. Introduction**

57 Peatlands currently act as a major long-term carbon (C) sink  
58 ecosystem. Although these wetlands cover only 3% of the land  
59 area, they have stored a third of the global soil C since the  
60 early Holocene (Turunen et al. 2002). Most *Sphagnum*  
61 peatlands (up to 80%) are located at high latitudes of the  
62 northern hemisphere in the cool temperate zone in associa-  
63 tion with waterlogged, nutrient poor conditions and the  
64 presence of *Sphagnum* mosses (e.g. Gorham 1991). To cope  
65 with low nutrient concentrations, *Sphagnum* mosses have  
66 developed mechanisms to efficiently use nutrients thanks to  
67 their high cation exchange capacity, nutrient translocation  
68 and atmospheric interception, reducing the nutrient avail-  
69 ability to vascular plants (e.g. Turetsky et al. 2012). However,  
70 northern temperate ecosystems receive four times more  
71 airborne nitrogen (N) today than 150 years ago (Holland et al.  
72 1999; Lamarque et al. 2005). Increased N deposition leads to a  
73 progressive N saturation of *Sphagnum* mosses, thus favoring  
74 the invasion of vascular plants and reducing *Sphagnum* moss  
75 growth (Limpens et al. 2011). Such changes seem to reduce the  
76 C sequestration rates in peatlands (Bragazza et al. 2006;  
77 Gunnarsson et al. 2008), even if they increase the vascular  
78 plants' productivity (Wu et al. 2015). However, the effect of the  
79 increase in N loads on stocks and exchanges of N and C are  
80 still understudied in peatlands, although they are known to  
81 generally increase N<sub>2</sub>O emissions to the atmosphere (e.g.  
82 Nykänen et al. 2002; Francez et al. 2011). Peatland C-storage  
83 capacity is often considered alone to assess the effects of  
84 climate change on peatlands without considering the N stored  
85 in the ecosystems that could account for a significant N<sub>2</sub>O  
86 source and therefore act as a positive feedback to climate  
87 change (Repo et al. 2009).

88 The increase in vascular plant cover due to human  
89 activities such as nutrient supply, e.g., atmospheric N  
90 deposition, or drainage, increases organic matter decomposi-  
91 tion (Gogo et al. 2016) and modulates CO<sub>2</sub> and CH<sub>4</sub> emissions  
92 in peatlands (Ward et al. 2013; Leroy et al. 2017). The  
93 combined effects of vascular plant invasion with N deposition  
94 on both C and N cycles and stocks still remain to be  
95 elucidated. N fertilization generally stimulates the vascular  
96 plant biomass, thereby contributing to higher primary pro-  
97 duction. However, it also leads to a higher decomposition rate  
98 due to a reduction in the C/N ratio and more root exudates  
99 that generate additional respiration (Wu et al. 2015). Our aim  
100 was therefore to assess the effect of N supply on both C and N  
101 dynamics in peat mesocosms collected in a *Sphagnum*-  
102 dominated peatland invaded by a vascular plant, *Molinia*  
103 *caerulea*. All the peat mesocosms contained *Sphagnum*  
104 *rubellum*, and half of them also contained *M. caerulea*. Half of  
105 each plant community mesocosm was subjected to an  
106 increase in N deposition by a weekly amendment to reach  
107 an addition of 3.2 g N/(m<sup>2</sup>·year). Thus, the hypotheses  
108 investigated are that N deposition will lead to the following  
109 processes under the two plant communities:

110 (i) Processes involving the C cycle: (a) an increase in C  
111 fluxes by promoting ecosystem respiration (ER) due to a  
112 faster decomposition of plant tissues containing more

N (Bragazza et al. 2006); (b) stimulation of the gross 113  
primary production (GPP) by an enhancement of both 114  
*Sphagnum* mosses and graminoid biomass (e.g. 115  
Tomassen et al. 2003; Granath et al. 2009); (c) a rise in 116  
CH<sub>4</sub> emissions through a higher OM decomposition and 117  
increase in root exudates. 118

(ii) Processes involving the N cycle: (a) higher concentra- 119  
tions of the dissolved NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> and of the N stored 120  
by *Sphagnum* mosses; (b) an increase in N<sub>2</sub>O emissions 121  
under both plant communities (Roobroeck et al. 2010). 122

(iii) Processes involving *M. caerulea* occurrence: an increase 123  
in the C fluxes in peatlands (CO<sub>2</sub>, CH<sub>4</sub>) and DOC content 124  
and a decrease in the ecosystem C sink function 125  
compared to *Sphagnum*-dominated peatland due to the 126  
promotion of peat decomposition (Leroy et al. 2017). 127

**2. Materials and methods** 128**2.1. Experimental design** 130

131 Twelve peat mesocosms (depth and diameter: 30 cm) were  
132 collected in March 2015 at La Guette peatland, an acidic fen  
133 invaded by *M. caerulea* (pH about 4, 47°19'44"N, 2°17'04"E,  
134 France). The mean annual precipitation and temperature of La  
135 Guette peatland are 883 mm and 11 °C, respectively (Gogo et al.  
136 2011). The mesocosms were buried outdoors (N 47°50'01", E  
137 1°56'34", ISTO, Orléans) and surrounded with a tarpaulin  
138 containing water from the peatland. Air and soil temperature  
139 at 5 and 20 cm depth were monitored in each mesocosm at 15  
140 minute intervals. The water table level (WTL) was measured  
141 by using piezometers in all the mesocosms. For each gas  
142 measurement, peat water was collected from the piezometer  
143 and filtered at 0.45 μm to analyze DOC and TN concentrations  
144 ([DOC] and [TN]) with a Shimadzu TOC-5000 analyzer and NH<sub>4</sub><sup>+</sup>  
145 and NO<sub>3</sub><sup>-</sup> concentrations by Dionex ICS 900 and 1100 ion  
146 chromatography. The mesocosms were first separated into  
147 two different plant communities: six containing only *S.*  
148 *rubellum* (called '*Sphagnum*' mesocosms) and six containing  
149 both *S. rubellum* and *M. caerulea* (called '*Sphagnum* + *Molinia*'  
150 mesocosms). *Molinia caerulea* growth started in May. The plant  
151 covered up to 60% of mesocosms until its senescence in  
152 November. Mesocosms of both plant communities were  
153 separated into two treatments with (called 'Fertilized'  
154 mesocosms) and without (called 'Control' mesocosms) addi-  
155 tions of NH<sub>4</sub>NO<sub>3</sub>, commonly used as an agricultural fertilizer.  
156 A powder of NH<sub>4</sub>NO<sub>3</sub> was dissolved in peat water and added  
157 every week to reach 3.2 gN/(m<sup>2</sup>·year), which represents 3.7 gN/  
158 (m<sup>2</sup>·year) during the 14 months of the experiment. This  
159 enrichment is higher than that currently observed in  
160 peatlands (Bragazza et al. 2004), but could reflect the N  
161 deposition expected for 2100 (Lamarque et al. 2005).

162 At the end of the mesocosm experiment (June 2016), C and  
163 N content (%) in *Molinia* leaves and litter, *Sphagnum* capitula  
164 (0–0.5 cm), living *Sphagnum* (0.5–2.5 cm) and in peat cubes (5 × 5  
165 × 5 cm) at 2.5–7.5, 7.5–12.5, 12.5–17.5 and 17.5–22.5 cm depth  
166 were measured for each mesocosm with an elementary  
167 analyzer (Thermo-126 FLASH 2000 CHNS/O Analyzer). For  
168 each mesocosm at the previously mentioned depths, cubes

169 measuring 5 cm per side were prepared and oven dried at 50°C  
170 to calculate peat bulk density ( $\text{g}/\text{cm}^3$ , Table S1) in order to  
171 evaluate the C and N stock in each layer (Eq. (1)):

$$\text{C or N stocks (g/m}^3\text{)} = \text{C or N content (\%)} \times \text{Peat bulk density (1)}$$

172

## 174 2.2. Greenhouse gas (GHG) measurements

175 GHG measurements were performed with the closed cham-  
176 ber method between once to twice per week during the  
177 growing season (April–October 2015 and April–June 2016) and  
178 every 2 weeks during winter (November 2015–March 2016).  
179  $\text{CO}_2$  fluxes were measured during 5 minute using a GMP343  
180 Vaisala probe inserted in a transparent PVC chamber  
181 (D'Angelo et al. 2016). A clear chamber was used to measure  
182 the net ecosystem exchange (NEE), the balance between GPP  
183 (absorption of  $\text{CO}_2$  by photosynthesis) and ER (release of  $\text{CO}_2$   
184 into the atmosphere). ER was measured by placing an opaque  
185 cover on the chamber to block photosynthesis (D'Angelo et al.  
186 2016).  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions were measured during 15 minute  
187 by using SPIRIT, a portable infrared laser spectrometer  
188 (Guimbaud et al. 2011).

## 189 2.3. Data analysis and modeling

190 C fluxes (in  $\text{g C}/(\text{m}^2\text{-year})$ ), including the GPP, ER and  $\text{CH}_4$   
191 emissions, were derived for the entire year at a 15 minute time  
192 step following Leroy et al. (in prep) by using  $\text{CO}_2$  and  $\text{CH}_4$   
193 measurements to calibrate and validate equations based on  
194 Bortoluzzi et al. (2006) and Kandel et al. (2013) with:

$$\text{ER} = \left[ \left( a^* \frac{\text{WTL}}{\text{WTL}_{\text{ref}}} \right) + (b^* \text{M}_{\text{claves}}) \right] * \left( \frac{(T_a - T_{\text{min}})}{(T_{\text{ref}} - T_{\text{min}})} \right)^c \quad (2)$$

196 ER is the ecosystem respiration flux ( $\mu\text{mol CO}_2/(\text{m}^2\text{-s})$ ).  $T_{\text{ref}}$  is  
197 the reference air temperature and  $T_{\text{min}}$  the minimum air  
198 temperature. These two parameters were set as in Bortoluzzi et  
199 al. (2006) at 15 and  $-5^\circ\text{C}$ , respectively.  $T_a$  refers to the measured  
200 air temperature ( $^\circ\text{C}$ ). The reference for the WTL ( $\text{WTL}_{\text{ref}}$ ) was set  
201 at  $-15\text{ cm}$  corresponding to the deepest WTL recorded in the  
202 mesocosms. The coefficients  $a$ ,  $b$  and  $c$  (temperature sensitivity  
203 parameters) are empirical parameters.

204 An equation similar to Eq. (1) was used to model the  
205 emissions (Eq. (2)):

$$\text{CH}_4 = \left[ d^* \frac{\text{WTL}}{\text{WTL}_{\text{ref}}} + e \right] * \left( \frac{(T_s - T_{\text{min}})}{(T_{\text{ref}} - T_{\text{min}})} \right)^f \quad (3)$$

206 where,  $\text{WTL}_{\text{ref}}$ ,  $T_{\text{min}}$ ,  $T_{\text{ref}}$  and  $T_{\text{min}}$  were set as for the ER  
207 equation.  $T_s$  refers to the measured soil temperature ( $^\circ\text{C}$ ).

208 The GPP was modeled by using a rectangular hyperbola  
209 saturation curve with the photosynthetic photon flux density  
210 (PPFD) and by taking into account the effect of temperature  
211 and vegetation with the Eq. (3):

$$\text{GPP} = \frac{\text{GPP}_{\text{max}} * \text{PPFD}}{k + \text{PPFD}} * \text{RVI} * \frac{(T - T_{\text{min}})(T - T_{\text{max}})}{(T - T_{\text{min}})(T - T_{\text{max}}) - (T - T_{\text{opt}})^2} \quad (4)$$

212 where  $\text{GPP}_{\text{max}}$  ( $\mu\text{mol}/(\text{m}^2\text{-s})$ ) represents the GPP at light  
213 saturation, the parameter  $k$  ( $\mu\text{mol}/(\text{m}^2\text{-s})$ ) is the half saturation  
214 value and RVI are a vegetation index to include the effect  
215 of *Molinia* leaves number on photosynthesis.  $T_{\text{min}}$ ,  $T_{\text{opt}}$  and

216  $T_{\text{max}}$  represent the minimum, optimum and maximum air  
217 temperature for photosynthesis and were set at 0, 20 and 40  
218  $^\circ\text{C}$ , respectively (Kandel et al. 2013). 219

220 N fluxes (concerning only  $\text{N}_2\text{O}$  emissions,  $\text{g N}/(\text{m}^2\text{-year})$ )  
221 were extrapolated for the entire year from the mean values of  
222 12 months of measurements (in  $\mu\text{mol N}_2\text{O}/(\text{m}^2\text{-s})$ ). Three-way  
223 repeated-measure ANOVAs were used to compare C and N  
224 fluxes and differences in environmental parameters due to  
225 the main effect of 'Vegetation' between *Sphagnum* and  
226 *Sphagnum + Molinia* mesocosms, and 'Nitrogen' between the  
227 Fertilized and Control mesocosms over the measurement  
228 period (Table 1). Two-way ANOVAs were used to compared C  
229 and N content, peat bulk density and C and N stocks per depth  
230 for the main effect of 'Vegetation' between *Sphagnum* and  
231 *Sphagnum + Molinia* mesocosms, and 'Nitrogen' between the  
232 Fertilized and Control ones (Tables 2, S1, S2). 233

## 234 3. Results

### 235 3.1. C and N fluxes

236 No significant differences in ER, GPP,  $\text{CH}_4$  emissions or [DOC]  
237 were observed between the Control and Fertilized mesocosms  
238 for the two plant communities (Table 1). Hypothesis (i), which  
239 assumed a promotion of ER, GPP and  $\text{CH}_4$  emissions, must  
240 therefore be rejected. Differences were driven only by the  
241 plant communities: the presence of *M. caerulea* increased the  
242 gaseous C fluxes (ER, GPP,  $\text{CH}_4$  emissions) compared to  
243 *Sphagnum* mesocosms (Table 1). Furthermore, the number  
244 and height of *M. caerulea* leaves were similar between the  
245 Control and Fertilized mesocosms and no stimulation of  
246 *Molinia* growth with addition of N was observed (Table 1).  
247 Neither  $\text{NH}_4^+$  nor  $\text{NO}_3^-$  concentrations were significantly  
248 influenced by the increase in N deposition, refuting hypoth-  
249 esis (ii, a), i.e. higher  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations in peat  
250 water due to  $\text{NH}_4\text{NO}_3$  additions (Table 1). Water in both plant  
251 communities contained low  $\text{NO}_3^-$  concentrations, and  $\text{NH}_4^+$   
252 concentrations varied with the vegetation cover (Table 1, Fig.  
253 2), the presence of *M. caerulea* significantly reducing the  
254  $\text{NH}_4^+$  concentrations (and also the TN content) compared to  
255 *Sphagnum* mesocosms (Table 1). 256

257 The only significant differences due to the  $\text{NH}_4\text{NO}_3$  addi-  
258 tions concerned  $\text{N}_2\text{O}$  emissions that increased in Fertilized  
259 mesocosms compared to Control ones for both plant commu-  
260 nities. This confirms our hypothesis (ii, b), i.e. that a higher N  
261 would increase  $\text{N}_2\text{O}$  emissions under both plant communities  
262 (Table 1, Figs. 1, 2 and S1). Furthermore,  $\text{N}_2\text{O}$  emissions were  
263 also affected by the vegetation composition with a decrease in  
264  $\text{N}_2\text{O}$  emissions in the *Sphagnum + Molinia* mesocosms compared  
265 to the *Sphagnum* mesocosms (Table 1, Fig. 1). 266

### 267 3.2. C and N stocks

268 Increasing N deposition triggered a short-term response with  
269 an increase in N concentrations in *Sphagnum* capitula (0–0.5 cm  
270 depth) and in its living tissues (0.5–2.5 cm depth) and in  $\text{N}_2\text{O}$   
271 emissions (Tables 1, 2 and Fig. 1). These effects modified the N  
272 cycles and stocks in the peat mesocosms (Table S2, Fig. 2).  
273 Extrapolation of  $\text{N}_2\text{O}$  fluxes (in  $\text{g N}/(\text{m}^2\text{-year})$ ) showed that  
274 275

**Table 1 – Mean values of 12 months measurements of Net Ecosystem Exchange (NEE), Gross Primary Production (GPP), Ecosystem Respiration (ER), CH<sub>4</sub> emissions (CH<sub>4</sub>), DOC, N<sub>2</sub>O emissions, TN, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> contents, air temperature (Ta), Water Table Level (WTL), number and height of *Molinia* leaves in *Sphagnum* and *Sphagnum* + *Molinia* mesocosms with (Fertilized) or without (Control) NH<sub>4</sub>NO<sub>3</sub> addition**

|  | <i>Sphagnum</i> |                  | <i>Sphagnum</i> + <i>Molinia</i> |                 | Significance |             |
|--|-----------------|------------------|----------------------------------|-----------------|--------------|-------------|
|  | Control         | Fertilized       | Control                          | Fertilized      | Nitrogen     | Vegetation  |
|  | Interaction     |                  | C cycle                          |                 |              |             |
| NEE (μmol/(m <sup>2</sup> ·s))   | 1.55 ± 0.26     | 1.29 ± 0.22      | 6.50 ± 1.26                      | 7.11 ± 1.32     |              | ***         |
| GPP (μmol/(m <sup>2</sup> ·s))   | 2.61 ± 0.37     | 2.28 ± 0.33      | 10.24 ± 1.92                     | 10.56 ± 1.93    |              | ***         |
| ER (μmol/(m <sup>2</sup> ·s))  | 1.06 ± 0.26     | 0.99 ± 0.22      | 3.75 ± 0.81                      | 3.50 ± 0.75     |              | ***         |
| CH <sub>4</sub> (μmol/(m <sup>2</sup> ·s))   | 0.018 ± 0.007   | 0.019 ± 0.008    | 0.130 ± 0.032                    | 0.133 ± 0.03    |              | ***         |
| DOC (mg/L)   | 58.38 ± 8.29    | 41.71 ± 7.24     | 35.95 ± 7.34                     | 24.14 ± 5.37    |              | *           |
| N cycle  |                 |                  |                                  |                 |              |             |
| N <sub>2</sub> O (μmol/(m <sup>2</sup> ·s))  | 5.26–5 ± 1.48–5 | 19.41–5 ± 5.38–5 | –0.89–5 ± 1.21–5                 | 2.86–5 ± 5.94–6 | *            | *           |
| TN (mg/L)  | 5.27 ± 0.56     | 4.01 ± 0.36      | 1.70 ± 0.31                      | 1.14 ± 0.22     |              | **          |
| NH <sub>4</sub> <sup>+</sup> (mg/L)  | 5.15 ± 0.67     | 4.11 ± 0.35      | 0.27 ± 0.13                      | 0.16 ± 0.059    |              | **          |
| NO <sub>3</sub> <sup>-</sup> (mg/L)  | 0.51 ± 0.13     | 0.46 ± 0.11      | 0.26 ± 0.09                      | 0.55 ± 0.175    |              |             |
| Environmental parameters   |                 |                  |                                  |                 |              |             |
| Ta (°C)  | 12.06 ± 1.50    | 12.14 ± 1.525    | 14.54 ± 1.57                     | 14.66 ± 1.52    |              |             |
| WTL (cm)   | –6.32 ± 0.51    | –3.28 ± 0.40     | –7.06 ± 0.49                     | –7.25 ± 0.54    |              |             |
| <i>Molinia</i> leaves number   | n. a.           | n. a.            | 233.54 ± 46.0                    | 269.94 ± 48.7   |              | n. a. n. a. |
| <i>Molinia</i> leaves height   | n. a.           | n. a.            | 13.04 ± 2.29                     | 12.02 ± 2.14    |              | n. a. n. a. |
| n. a.: not applicable. Data are presented as mean ± SE, n = 12. Significant differences of repeated-measure ANOVAs are expressed as *p < 0.05, **p < 0.01, ***p < 0.001. |                 |                  |                                  |                 |              |             |

increasing N deposition produced an increase in N<sub>2</sub>O emissions of 0.125 gN/(m<sup>2</sup>·year) in *Sphagnum* mesocosms and of 0.033 gN/(m<sup>2</sup>·year) in *Sphagnum* + *Molinia* mesocosms (Fig. 2). However, the increase in N<sub>2</sub>O emissions represents only 4 and 1% of the total N additions, respectively, in *Sphagnum* mesocosms and in *Sphagnum* + *Molinia* mesocosms. Most of the NH<sub>4</sub>NO<sub>3</sub> load was stored in *Sphagnum* mosses with an estimated increase of 2.9 g N/m<sup>2</sup> in the stocks of *Sphagnum*+*Molinia* mesocosms and of 4.0 g N/m<sup>2</sup> in *Sphagnum* ones in the two first layers of the Fertilized mesocosms compared to the Control ones.

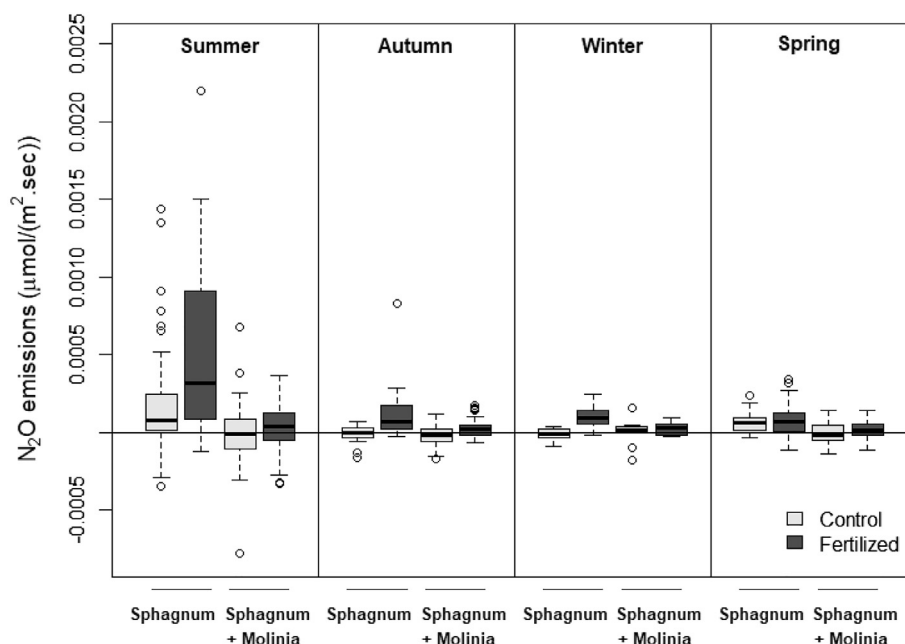
The NH<sub>4</sub>NO<sub>3</sub> load did not significantly impact C cycles and stocks (Table 1, Fig. 3). C compartments were only affected by

the vegetation cover which modified C absorption and mineralization (Fig. 3). The shift from *Sphagnum* to *Molinia* dominated peatland increased C emissions and absorption and led to an increase in the C balance (Table 1, Fig. 3, Leroy et al., in prep). This refutes our hypothesis (iii) which assumed a decrease in the ecosystem C balance with *M. caerulea* occurrence. Only the *M. caerulea* effect was modeled on the CO<sub>2</sub> and CH<sub>4</sub> fluxes (Fig. 2, Leroy et al., in prep). C contents were similar between vegetation treatments (Table 2), but the density was higher in *Sphagnum* plots than in *Sphagnum* + *Molinia* mesocosms (Table S1). Consequently, the C stock was

**Table 2 – C and N content (%) of *Molinia* and *Sphagnum* mesocosms with (Fertilized) or without (Control) NH<sub>4</sub>NO<sub>3</sub> addition per depth (in cm),**

|           | <i>Sphagnum</i> |              | <i>Sphagnum</i> + <i>Molinia</i> |              | Significance |            |             |
|-----------|-----------------|--------------|----------------------------------|--------------|--------------|------------|-------------|
|           | Control         | Fertilized   | Control                          | Fertilized   | Nitrogen     | Vegetation | Interaction |
| C cycle   |                 |              |                                  |              |              |            |             |
| 0–0.5     | 40.80 ± 0.39    | 40.41 ± 0.28 | 40.13 ± 0.20                     | 39.37 ± 0.72 |              | –          |             |
| 0.5–2.5   | 37.60 ± 1.02    | 34.69 ± 3.83 | 39.63 ± 2.59                     | 38.40 ± 2.32 |              | –          |             |
| 2.5–7.5   | 32.54 ± 3.07    | 31.67 ± 2.43 | 34.77 ± 1.70                     | 31.42 ± 2.41 |              |            |             |
| 7.5–12.5  | 37.91 ± 1.05    | 32.21 ± 2.61 | 38.05 ± 0.37                     | 37.22 ± 1.95 | –            |            |             |
| 12.5–17.5 | 34.87 ± 1.37    | 34.14 ± 2.76 | 37.80 ± 0.26                     | 36.38 ± 1.05 |              |            |             |
| 17.5–22.5 | 37.29 ± 0.38    | 33.45 ± 4.37 | 34.58 ± 2.43                     | 36.65 ± 0.98 |              |            |             |
| N cycle   |                 |              |                                  |              |              |            |             |
| 0–0.5     | 1.10 ± 0.11     | 2.01 ± 0.13  | 1.34 ± 0.05                      | 1.73 ± 0.10  | ***          |            | *           |
| 0.5–2.5   | 0.68 ± 0.09     | 0.90 ± 0.11  | 0.74 ± 0.06                      | 1.14 ± 0.12  | *            |            |             |
| 2.5–7.5   | 0.86 ± 0.19     | 0.98 ± 0.07  | 0.67 ± 0.12                      | 0.77 ± 0.10  |              |            |             |
| 7.5–12.5  | 0.99 ± 0.05     | 1.18 ± 0.28  | 1.12 ± 0.07                      | 1.51 ± 0.36  |              |            |             |
| 12.5–17.5 | 1.53 ± 0.08     | 1.31 ± 0.19  | 1.49 ± 0.10                      | 1.72 ± 0.18  |              |            |             |
| 17.5–22.5 | 1.60 ± 0.14     | 1.37 ± 0.06  | 1.49 ± 0.05                      | 1.64 ± 0.07  |              |            | –           |

Data are presented as mean ± SE, n = 3. Significant differences are expressed as – for 0.05 < p < 0.1, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Fig. 1 – N<sub>2</sub>O emissions measured in summer (n=19), autumn (n=8), winter (n=5) and spring (n=8) in *Sphagnum* and *Sphagnum* + *Molinia* mesocosms with (Fertilized) or without (Control) NH<sub>4</sub>NO<sub>3</sub> addition (n=3).**

300 higher in *Sphagnum* mesocosms than in *Sphagnum* + *Molinia*  
 301 mesocosms (Table S2 and Fig. 3). The variability of peat  
 302 density between vegetation did not affect the stoichiometry of  
 303 the peat. The C/N ratio was only affected by the N treatment  
 304 in the top two layers (0–0.5 and 0.5–2.5 cm) with a lower ratio  
 305 in the Fertilized plots than in the Control ones (Fig.4).

## 306 4. Discussion

### 308 4.1. N retention by *Sphagnum*

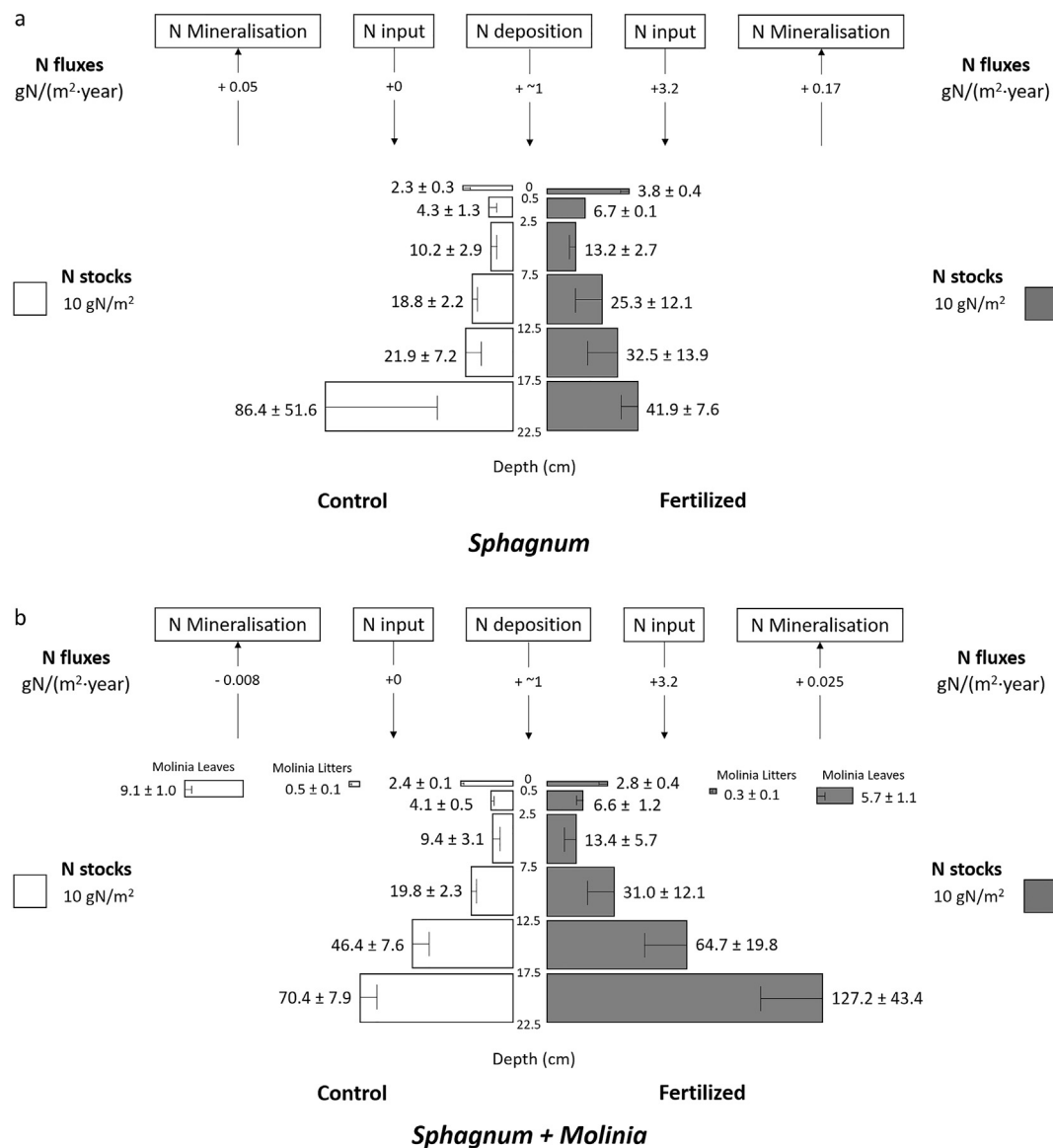
309 The number and height of *M. caerulea* leaves were similar  
 310 between the Control and the Fertilized mesocosms and no  
 311 stimulation of *Molinia* growth was observed. This is in agreement  
 312 with the results of Tomassen et al. (2003) who found an effect of  
 313 N addition on *M. caerulea* biomass only after 3years of N input. In  
 314 addition, the different forms of N dissolved in peat water were  
 315 not affected by the N addition treatment (Table 1). However, N  
 316 addition induced an increase in the concentration and stock of N  
 317 in the living parts of *Sphagnum* (the first two layers of the peat  
 318 mesocosms: 0–0.5 cm and 0.5–2.5 cm; Table 2, Fig. 2). Thus, the  
 319 lack of a short-term effect of N fertilization on *M. caerulea* growth  
 320 can be explained by the high capacity of *Sphagnum* mosses to  
 321 retain N deposition (van Breemen, 1995). Indeed, *Sphagnum*  
 322 species can capture the atmospheric N supply, limiting its  
 323 availability for the surrounding vascular plants (van Breemen,  
 324 1995; Tomassen et al. 2003). Such a mechanism can have a long-  
 325 term effect on OM decomposition. The N enrichment of living  
 326 *Sphagnum* by increased N atmospheric deposition leads to a  
 327 lower C:N ratio (Fig. 4). Such a change in peat stoichiometry could  
 328 increase the decomposition rate of *Sphagnum* litters and in the  
 329 long term, could negatively affect the C balance of *Sphagnum*-  
 330 dominated peatlands (Aerts et al. 1992).

Despite the N retention by *Sphagnum* mosses, increasing  
 331 NH<sub>4</sub>NO<sub>3</sub> inputs enhances N<sub>2</sub>O emissions under both plant  
 332 communities (Fig. 1). When error terms are considered, the  
 333 amount of N added in *Sphagnum* mesocosms (3.7 g N/m<sup>2</sup>) is  
 334 recovered in the amount of N found in the combined N<sub>2</sub>O  
 335 emissions and N stocks in *Sphagnum* layers (4.0 g N/m<sup>2</sup> on  
 336 average; Fig. 2). No other N output or stock is required to close the  
 337 N balance. These results suggest that increased N<sub>2</sub>O emissions  
 338 may be generated by a stimulation of denitrification triggered by  
 339 an increase in N availability (Hayden and Ross 2005; Francez et  
 340 al. 2011). This stimulation is modulated by the vegetation. 341

### 4.2. Effect of *Molinia caerulea* on the N cycle 342

Vegetation composition was also found to impact N<sub>2</sub>O  
 343 emissions (Table 1) with lower N<sub>2</sub>O emissions in the presence  
 344 of *M. caerulea* (Fig. 1). The dissolved NO<sub>3</sub> concentration (Table  
 345 1) and the N content of living *Sphagnum* (Table S1) did not  
 346 differ between *Sphagnum* and *Sphagnum* + *Molinia* mesocosms.  
 347 Furthermore, the above-ground biomass production of *M.*  
 348 *caerulea* was not stimulated by N addition (Table 1; Fig. 2). The  
 349 combined increase in N<sub>2</sub>O emissions and N stocks (2.9 gN/m<sup>2</sup>)  
 350 in Fertilized mesocosms compared to the Control ones  
 351 represents 80% of the N additions. The fraction of N lacking  
 352 may have been either denitrified to N<sub>2</sub> (not possible to observe  
 353 with our technique) or incorporated into the root biomass. 354

The competition between *M. caerulea*, a nitrophilous grass  
 355 (Tomassen et al. 2004), and denitrifiers for mineral N could  
 356 limit the substrate's availability for denitrification, leading to  
 357 a reduction in N<sub>2</sub>O emissions (Repo et al. 2009; Roobroeck et al.  
 358 2010). The utilization of N for biomass building by *M. caerulea*  
 359 is supported by the reduction in dissolved NH<sub>4</sub><sup>+</sup> concentration  
 360 in presence of *M. caerulea*, as this plant can use NH<sub>4</sub><sup>+</sup> as an N  
 361 source (Troelstra et al. 1995). In any case, these results clearly 362



**Fig. 2 – N stocks (g N/m<sup>2</sup>) and fluxes (black arrow, g N/(m<sup>2</sup>-year) in *Sphagnum* (a) and *Sphagnum* + *Molinia* mesocosms (b) with (Fertilized) or without (Control) NH<sub>4</sub>NO<sub>3</sub> addition (±SE, n = 3) from July 2015 to June 2016. N deposition represents the rain N deposition, N input are the amount of NH<sub>4</sub>NO<sub>3</sub> added and N Mineralization represents the quantity of N-N<sub>2</sub>O emissions.**

363 showed that the occurrence of *M. caerulea* modifies the C cycle  
 364 either by increasing root biomass (with further modification  
 365 of the C cycle) or by modulating the denitrifier microbial  
 366 communities (from N<sub>2</sub>O to N<sub>2</sub> emission), or both. Further  
 367 studies should thus focus on the OM dynamics associated  
 368 with the *M. caerulea* rhizosphere.

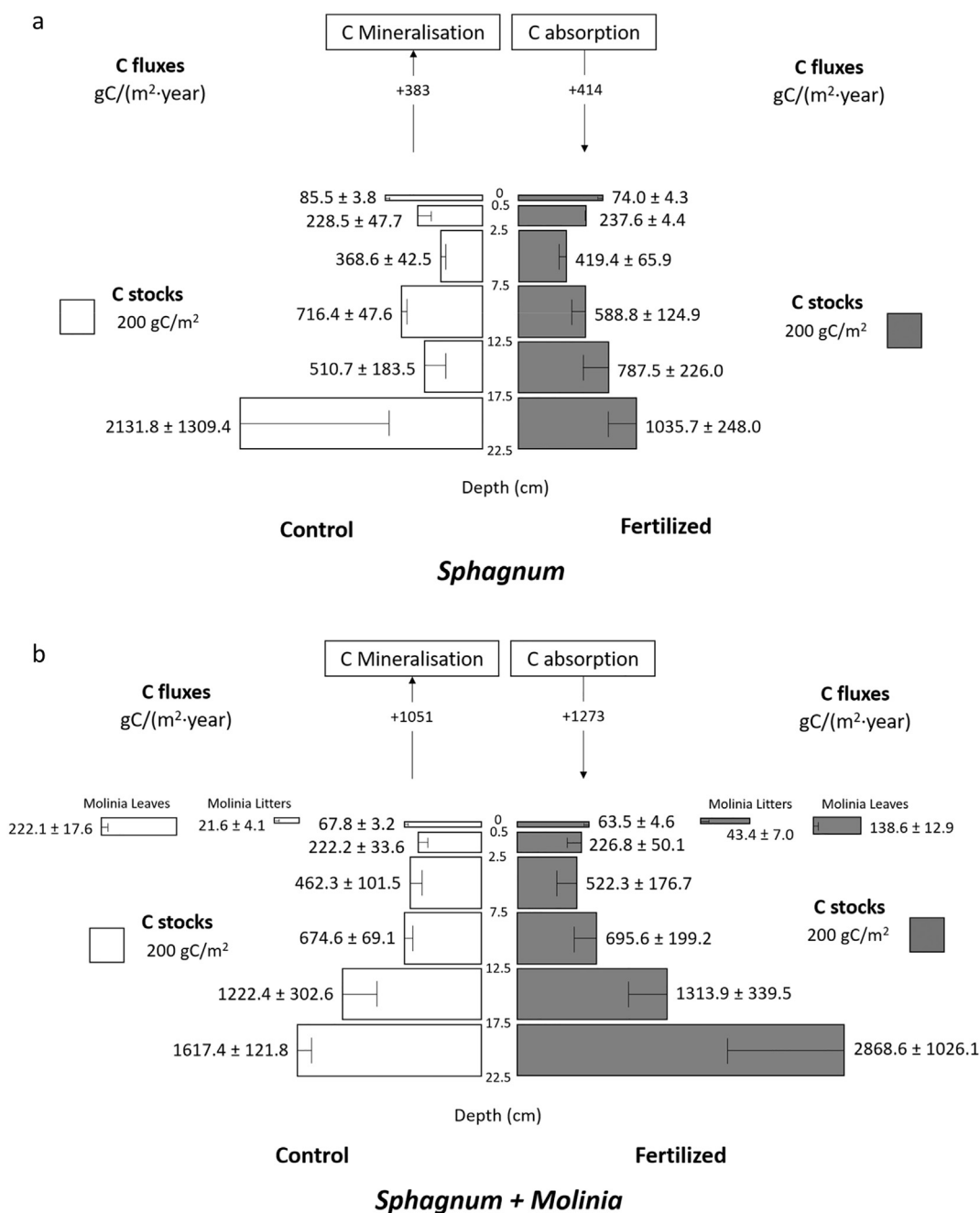
## 369 5. Conclusions

371 Increasing N deposition did not impact the C fluxes (CO<sub>2</sub>, CH<sub>4</sub>),  
 372 [DOC], stocks or above-ground biomass of *M. caerulea* in this  
 373 short-term experiment. This was due to the high capacity of  
 374 *Sphagnum* mosses to intercept atmospheric N, limiting the N  
 375 input effect. Despite the low N availability, NH<sub>4</sub>NO<sub>3</sub> addition  
 376 promoted N<sub>2</sub>O emissions, which were also influenced by the

vegetation composition with the lowest emissions with *M. caerulea*  
 377 occurrence. This modification in N<sub>2</sub>O emissions probably  
 378 results from an alteration of the denitrification activity linked to  
 379 the availability of mineral N. Our results indicate that N  
 380 deposition alters the N cycling in peatlands with also an  
 381 important regulatory role of plant communities on C and also  
 382 on N dynamics. Nonetheless, peatland ecosystem reactions to N  
 383 deposition should be considered in the longer term, especially in  
 384 peatlands with a *Sphagnum* layer N-saturated. 385

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**Fig. 3 – C stocks (per depth in g C/m<sup>2</sup>) and fluxes (black arrow, g C/(m<sup>2</sup>·year)) in *Sphagnum* (a) and *Sphagnum + Molinia* mesocosms (b) with (Fertilized) or without (Control) NH<sub>4</sub>NO<sub>3</sub> addition (±SE, n = 3) from July 2015 to June 2016.**

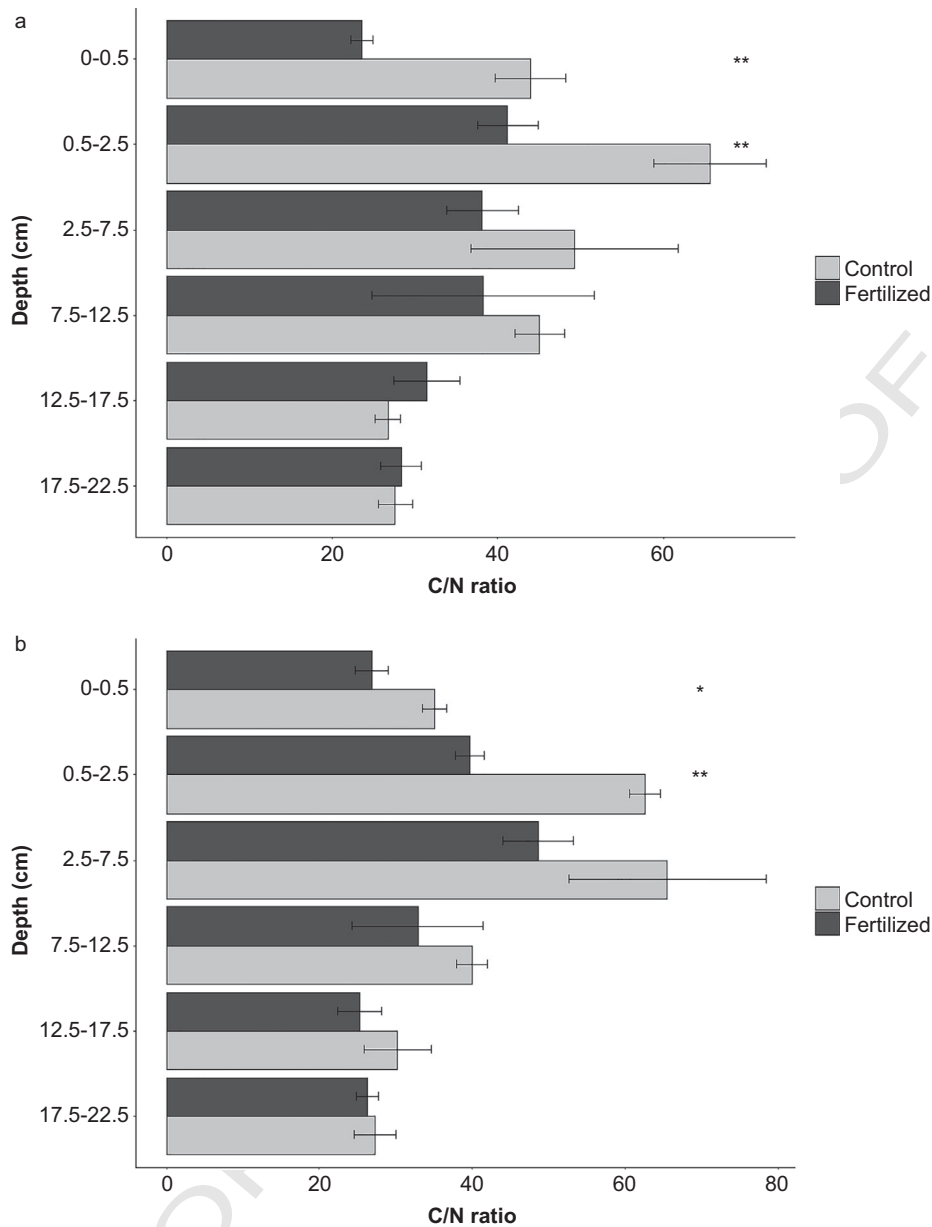
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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at  
 407 <https://doi.org/10.1016/j.jes.2018.08.003>. 408





**Fig. 4 – C/N ratio per layer in *Sphagnum* (a) and *Sphagnum* + *Molinia* mesocosms (b) with (Fertilized) or without (Control)  $\text{NH}_4\text{NO}_3$  addition ( $\pm$ SE,  $n = 3$ ). Significant differences per layer are expressed as – for  $0.05 < p < 0.1$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .**

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