



**HAL**  
open science

## Response of C and N cycles to N fertilization in Sphagnum and Molinia-dominated peat mesocosms

Fabien Leroy, Sébastien Gogo, Christophe Guimbaud, Andre-Jean Francez,  
Renata Zocatelli, Christian Défarge, Léonard Bernard-Jannin, Zhen Hu,  
Fatima Laggoun-Défarge

### ► To cite this version:

Fabien Leroy, Sébastien Gogo, Christophe Guimbaud, Andre-Jean Francez, Renata Zocatelli, et al..  
Response of C and N cycles to N fertilization in Sphagnum and Molinia-dominated peat mesocosms.  
Journal of Environmental Sciences, 2018, Accepted, 8 p. 10.1016/j.jes.2018.08.003 . insu-01864513v1

**HAL Id: insu-01864513**

**<https://insu.hal.science/insu-01864513v1>**

Submitted on 30 Aug 2018 (v1), last revised 14 Sep 2018 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

[www.elsevier.com/locate/jes](http://www.elsevier.com/locate/jes)

SciVerse S

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

Q3 Q2 **Fabien Leroy<sup>1,2,3,\*</sup>, Sébastien Gogo<sup>1,2,3</sup>, Christophe Guimbaud<sup>4,5</sup>, André-Jean Francez<sup>6</sup>,**  
 5 **Renata Zocatelli<sup>7</sup>, Christian Défarge<sup>1,2,3,7</sup>, Léonard Bernard-Jannin<sup>1,2,3</sup>, Zhen Hu<sup>8</sup>,**  
 6 **Fatima Laggoun-Défarge<sup>1,2,3</sup>**

7 1. Université d'Orléans, ISTO, UMR 7327, 45071 Orléans, France

8 2. CNRS, ISTO, UMR 7327, 45071 Orléans, France

9 3. BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France

10 4. Université d'Orléans, LPC2E, UMR 7328, 45071 Orléans, France

11 5. CNRS, LPC2E, UMR 7328, 45071 Orléans, France

12 6. UMR CNRS 6553 ECOBIO, Université de Rennes 1, Rennes, France

13 7. Université d'Orléans, Cellule R&D CETRAHE, 45072 Orléans, cedex 2, France

14 8. School of Environmental Science and Engineering, Shandong University, Jinan, China

## ARTICLE INFO

## ABSTRACT

## Article history:

20 Received 3 August 2017

26 Revised 31 July 2018

22 Accepted 16 August 2018

28 Available online xxxx

## Keywords:

34 CO<sub>2</sub> and CH<sub>4</sub>

35 N<sub>2</sub>O

36 Peatland

37 Plant community composition

38 Sphagnum moss

39 Graminoid

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.

© 2018 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

Published by Elsevier B.V.

Q5 \* Corresponding author at: Université d'Orléans, ISTO, UMR 7327, 45071 Orléans, France.

Q4 E-mail address: [lerfabien@gmail.com](mailto:lerfabien@gmail.com). (F. Leroy).

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:

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

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  $\text{NH}_4^+$   
254 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.

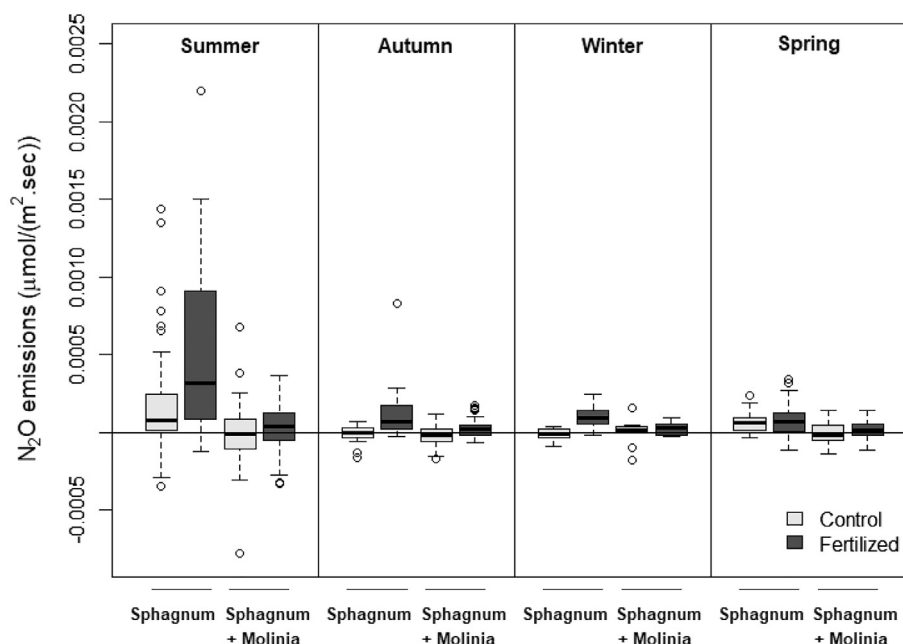
The NH<sub>4</sub>NO<sub>3</sub> load did not significantly impact C cycles and stocks (Table 1, Fig. 3). C compartments were only affected by

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*

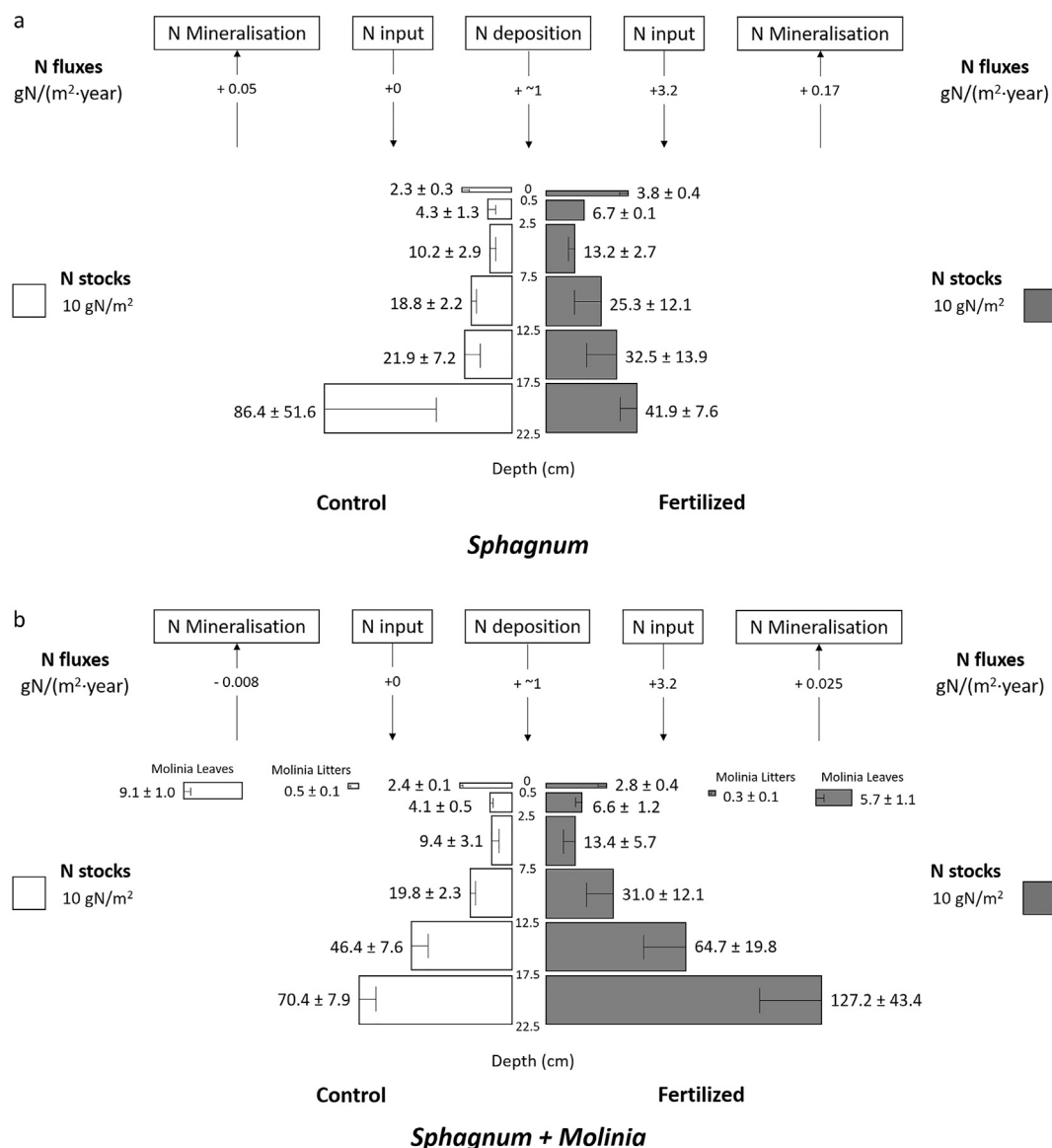
309 The number and height of *M. caerulea* leaves were similar  
 310 between the Control and the Fertilized mesocosms and no  
 311 stimulation of *Molinia* growth was observed. This is in agreement  
 312 with the results of Tomassen et al. (2003) who found an effect of  
 313 N addition on *M. caerulea* 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

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.

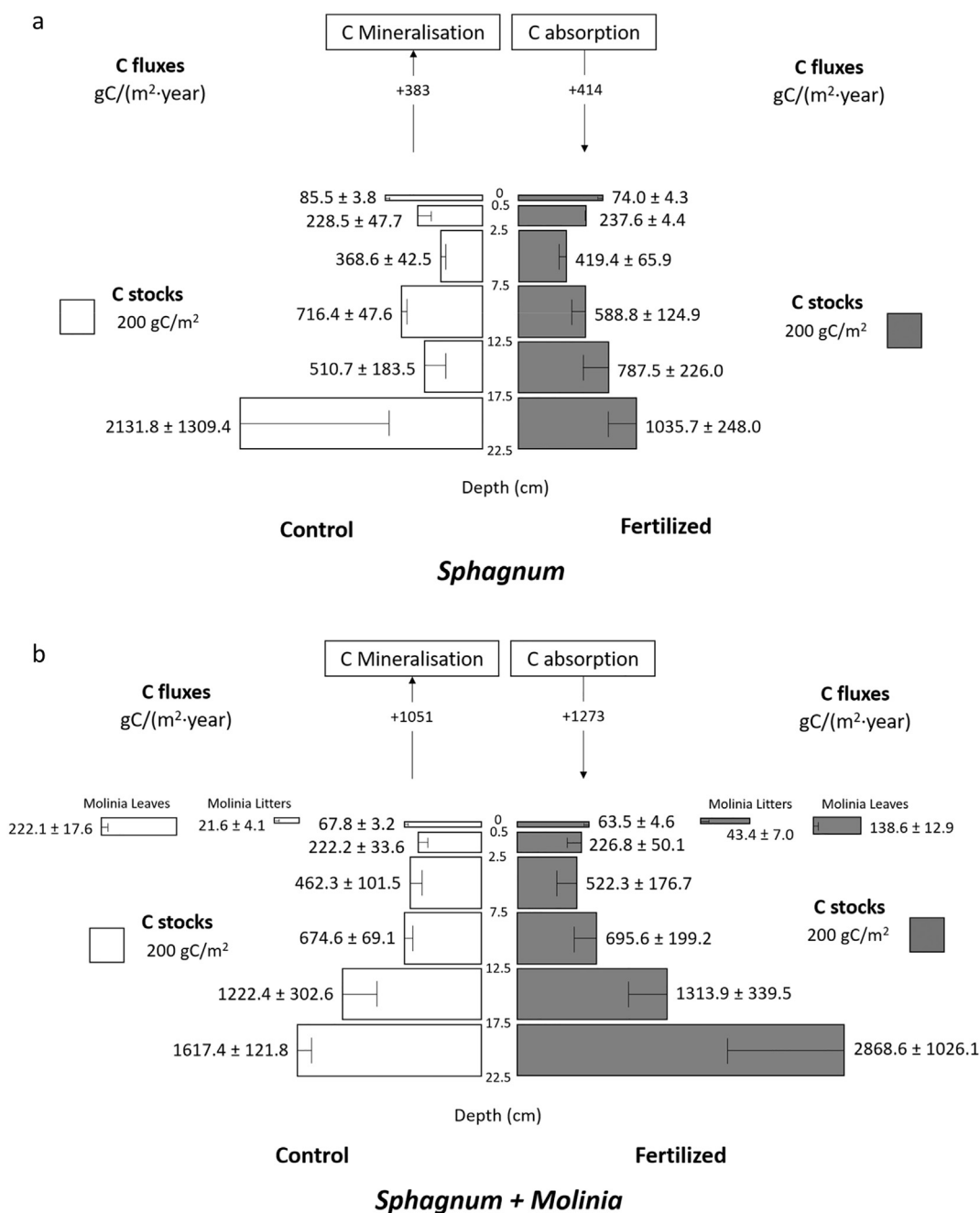
## 369 5. Conclusions

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

## Acknowledgments

This paper is a contribution to the research conducted in the  
 Labex VOLTAIRE (ANR-10-LABX-100-01). The authors gratefully  
 acknowledge the financial support provided to the



**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.

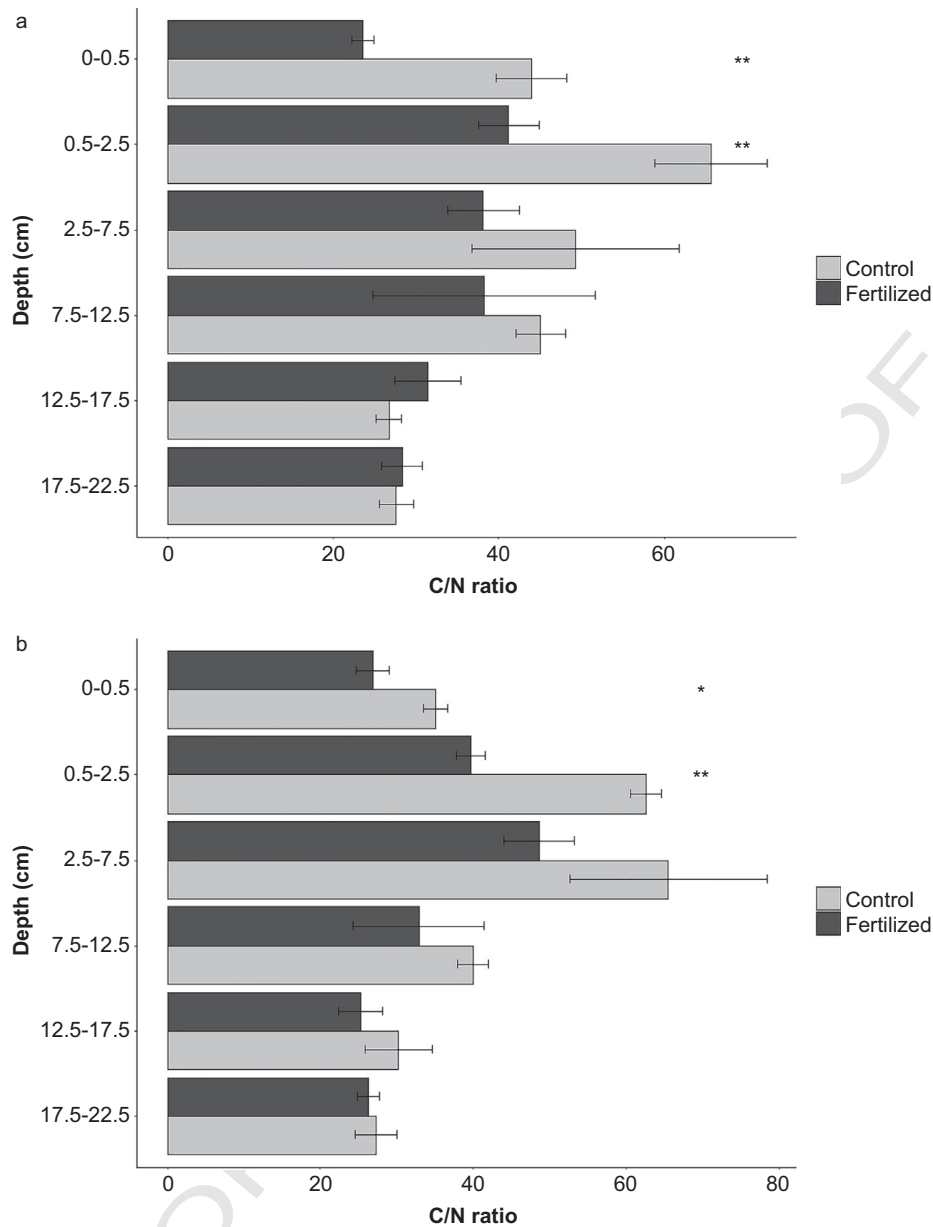
391 PIVOTS project by the Région Centre – Val de Loire: ARD 2020  
 392 program, CPER 2015 -2020 and the European Union who  
 393 invests in Centre-Val de Loire with the European Regional  
 394 Development Fund. This work is also supported by the AMIS  
 395 (Fate and Impact of Atmospheric PollutantS) project funded  
 396 by the European Union, under the Marie Curie Actions IRSES  
 397 (International Research Staff Exchange Scheme), within the  
 398 Seventh Framework Programme FP7-PEOPLE-2011-IRSES. The  
 399 authors would like to thank X. Yin, G. Belot, A. Mennequerre  
 400 for their contribution to gas measurements, P. Jacquet and C.

Robert for providing assistance in SPIRIT maintenance, N.  
 401 Lothier for DOC and TN analysis and A. Guirimand-Dufour for  
 402 contribution to NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> analyses. We also thank E.  
 403 Rowley-Jolivet for revision of the English version. **Q6**

#### 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  $0.05 < p < 0.1$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .**

#### 4 0 9 REFERENCES

- 410 Aerts, R., Wallen, B., Malmer, N., 1992. Growth-limiting nutrients  
412 in *Sphagnum*-dominated bogs subject to low and high atmo-  
413 spheric nitrogen supply. *J. Ecol.* 131–140.
- 414 Bortoluzzi, E., Epron, D., Siegenthaler, A., Gilbert, D., Buttler, A.,  
415 2006. Carbon balance of a European mountain bog at con-  
416 trasting stages of regeneration. *New Phytol.* 172 (4):708–718.  
417 <https://doi.org/10.1111/j.1469-8137.2006.01859.x>.
- 418 Bragazza, L., Tahvanainen, T., Kutnar, L., Rydin, H., Limpens, J.,  
419 Hájek, M., et al., 2004. Nutritional constraints in  
420 ombrotrophic *Sphagnum* plants under increasing  
421 atmospheric nitrogen deposition in Europe. *New Phytol.* 163  
422 (3), 609–616.
- 423 Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner,  
424 N., et al., 2006. Atmospheric nitrogen deposition promotes  
carbon loss from peat bogs. *Proc. Natl. Acad. Sci. U. S. A.* 103  
(51), 19386–19389.
- D'Angelo, B., Gogo, S., Laggoun-Défarge, F., Le Moing, F., Jégou, F.,  
Guimbaud, C., 2016. Soil temperature synchronisation  
improves representation of diel variability of ecosystem  
respiration in *Sphagnum* peatlands. *Agric. For. Meteorol.* 223,  
95–102.
- Francez, A.J., Pinay, G., Josselin, N., Williams, B.L., 2011. Denitri-  
fication triggered by nitrogen addition in *Sphagnum*  
*magellanicum* peat. *Biogeochemistry* 106 (3), 435–441.
- Gogo, S., Laggoun-Défarge, F., Delarue, F., Lottier, N., 2011.  
Invasion of a *Sphagnum*-peatland by *Betula* spp and *Molinia*  
*caerulea* impacts organic matter biochemistry. Implications for  
carbon and nutrient cycling. *Biogeochemistry* 106 (1), 53–69.
- Gogo, S., Laggoun-Défarge, F., Merzouki, F., Mounier, S.,  
Guirimand-Dufour, A., Jozja, N., et al., 2016. In situ and  
laboratory non-additive litter mixture effect on C dynamics of

- 442 *Sphagnum rubellum* and *Molinia caerulea* litters. *J. Soils Sedi-*  
 443 *ments* 16 (1), 13–27.
- 444 Gorham, E., 1991. Northern peatlands: role in the carbon cycle and  
 445 probable responses to climatic warming. *Ecol. Appl.* 1 (2),  
 446 182–195.
- 447 Granath, G., Wiedermann, M.M., Strengbom, J., 2009. Physiological  
 448 responses to nitrogen and sulphur addition and raised  
 449 temperature in *Sphagnum balticum*. *Oecol* 161 (3), 481–490.
- 450 Guimbaud, C., Catoire, V., Gogo, S., Robert, C., Chartier, M., Laggoun-  
 451 Défarge, F., et al., 2011. A portable infrared laser spectrometer for  
 452 flux measurements of trace gases at the geosphere–atmosphere  
 453 interface. *Meas. Sci. Technol.* 22 (7), 075601.
- 454 Gunnarsson, U., Boresjö Bronge, L., Rydin, H., Ohlson, M., 2008.  
 455 Near-zero recent carbon accumulation in a bog with high  
 456 nitrogen deposition in SW Sweden. *Glob. Chang. Biol.* 14 (9),  
 457 2152–2165.
- 458 Hayden, M.J., Ross, D.S., 2005. Denitrification as a nitrogen  
 459 removal mechanism in a Vermont peatland. *J. Environ. Qual.*  
 460 34 (6), 2052–2061.
- 461 Holland, E.A., Dentener, F.J., Braswell, B.H., Sulzman, J.M., 1999.  
 462 Contemporary and pre-industrial global reactive nitrogen  
 463 budgets. *Biogeochemistry* 46 (1), 7–43.
- 464 Kandel, T.P., Elsgaard, L., Lærke, P.E., 2013. Measurement and  
 465 modelling of CO<sub>2</sub> flux from a drained fen peatland cultivated  
 466 with reed canary grass and spring barley. *GCB Bioenergy* 5 (5):  
 467 548–561. <https://doi.org/10.1111/gcbb.12020>.
- 468 Lamarque, J.F., Kiehl, J.T., Brasseur, G.P., Butler, T., Cameron-  
 469 Smith, P., Collins, W.D., et al., 2005. Assessing future nitrogen  
 470 deposition and carbon cycle feedback using a multimodel  
 471 approach: analysis of nitrogen deposition. *J. Geophys. Res.*  
 472 Atmos. 110 (D19).
- 473 Leroy, F., Gogo, S., Guimbaud, C., Bernard-Jannin, L., Hu, Z.,  
 474 Laggoun-Défarge, F., 2017. Vegetation composition controls  
 475 temperature sensitivity of CO<sub>2</sub> and CH<sub>4</sub> emissions and DOC  
 476 concentration in peatlands. *Soil Biol. Biochem.* 107, 164–167.
- 477 Leroy, F., Gogo, S., Guimbaud, C., Bernard-Jannin, L., Yin, X., Belot,  
 478 G., Shuguang, W., Laggoun-Défarge, F., In prep Carbon balance  
 479 and global warming potential modifications in *Sphagnum*-  
 480 dominated peat mesocosms invaded by *Molinia caerulea*.  
 481 Submitted to Biogeosciences.
- 482 Limpens, J., Granath, G., Gunnarsson, U., Aerts, R., Bayley, S.,  
 483 Bragazza, L., et al., 2011. Climatic modifiers of the response to  
 nitrogen deposition in peat-forming *Sphagnum* mosses: a  
 meta-analysis. *New Phytol.* 191 (2), 496–507.
- Nykänen, H., Vasander, H., Huttunen, J.T., Martikainen, P.J., 2002.  
 Effect of experimental nitrogen load on methane and nitrous  
 oxide fluxes on ombrotrophic boreal peatland. *Plant Soil* 242  
 (1), 147–155.
- Repo, M.E., Susiluoto, S., Lind, S.E., Jokinen, S., Elsakov, V., Biasi, C.,  
 et al., 2009. Large N<sub>2</sub>O emissions from cryoturbated peat soil in  
 tundra. *Nat. Geosci.* 2 (3), 189–192.
- Roobroeck, D., Butterbach-Bahl, K., Brüeggemann, N., Boeckx, P.,  
 2010. Dinitrogen and nitrous oxide exchanges from an  
 undrained monolith fen: short-term responses following  
 nitrate addition. *Eur. J. Soil Sci.* 61 (5), 662–670.
- Tomassen, H., Smolders, A.J., Lamers, L.P., Roelofs, J.G., 2003.  
 Stimulated growth of *Betula pubescens* and *Molinia caerulea* on  
 ombrotrophic bogs: role of high levels of atmospheric nitrogen  
 deposition. *J. Ecol.* 91 (3), 357–370.
- Tomassen, H., Smolders, A.J., Limpens, J., Lamers, L.P., Roelofs, J.  
 G., 2004. Expansion of invasive species on ombrotrophic bogs:  
 desiccation or high N deposition? *J. Appl. Ecol.* 41 (1), 139–150.
- Troelstra, S.R., Wagenaar, R., Smant, W., 1995. Nitrogen utilization  
 by plant species from acid heathland soils: I. Comparison  
 between nitrate and ammonium nutrition at constant low pH  
 1. *J. Exp. Bot.* 1103–1112.
- Turetsky, M.R., Bond-Lamberty, B., Euskirchen, E., Talbot, J.,  
 Froelking, S., McGuire, A.D., et al., 2012. The resilience and  
 functional role of moss in boreal and arctic ecosystems. *New*  
*Phytol.* 196 (1), 49–67.
- Turunen, J., Tomppo, E., Tolonen, K., Reinikainen, A., 2002.  
 Estimating carbon accumulation rates of undrained mires in  
 Finland—application to boreal and subarctic regions. *The*  
*Holocene* 12 (1), 69–80.
- Van Breemen, N., 1995. How *Sphagnum* bogs down other plants.  
*Trends Ecol. Evol.* 10 (7), 270–275.
- Ward, S.E., Ostle, N.J., Oakley, S., Quirk, H., Henrys, P.A., Bardgett,  
 R.D., 2013. Warming effects on greenhouse gas fluxes in  
 peatlands are modulated by vegetation composition. *Ecol. Lett.*  
 16 (10), 1285–1293.
- Wu, Y., Blodau, C., Moore, T.R., Bubier, J., Juutinen, S., Larmola, T.,  
 2015. Effects of experimental nitrogen deposition on peatland  
 carbon pools and fluxes: a modelling analysis. *Biogeosciences*  
 12 (1), 79–101.