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# Response of C and N cycles to N fertilization in

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- mesocosms 3

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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 (CO2, CH4, N2O) 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 N2O 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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## **ARTICLE IN PRESS**

#### 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 area, they have stored a third of the global soil C since the 59 early Holocene (Turunen et al. 2002). Most Sphagnum 60 peatlands (up to 80%) are located at high latitudes of the 61 northern hemisphere in the cool temperate zone in associa-62 tion with waterlogged, nutrient poor conditions and the 63 presence of Sphagnum mosses (e.g. Gorham 1991). To cope 64 with low nutrient concentrations, Sphagnum mosses have 65 developed mechanisms to efficiently use nutrients thanks to 66 their high cation exchange capacity, nutrient translocation 67 and atmospheric interception, reducing the nutrient avail-68 ability to vascular plants (e.g. Turetsky et al. 2012). However, 69 northern temperate ecosystems receive four times more 70 71 airborne nitrogen (N) today than 150 years ago (Holland et al. 72 1999; Lamarque et al. 2005). Increased N deposition leads to a progressive N saturation of Sphagnum mosses, thus favoring 73 the invasion of vascular plants and reducing Sphagnum moss 74 growth (Limpens et al. 2011). Such changes seem to reduce the 75 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 still understudied in peatlands, although they are known to 80 generally increase N<sub>2</sub>O emissions to the atmosphere (e.g. 81 Nykänen et al. 2002; Francez et al. 2011). Peatland C-storage 82 capacity is often considered alone to assess the effects of 83 climate change on peatlands without considering the N stored 84 in the ecosystems that could account for a significant  $N_2O$ 85 source and therefore act as a positive feedback to climate 86 87 change (Repo et al. 2009).

88 The increase in vascular plant cover due to human activities such as nutrient supply, e.g., atmospheric N 89 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 in peatlands (Ward et al. 2013; Leroy et al. 2017). The 92 combined effects of vascular plant invasion with N deposition 93 94 on both C and N cycles and stocks still remain to be elucidated. N fertilization generally stimulates the vascular 95 96 plant biomass, thereby contributing to higher primary pro-97 duction. However, it also leads to a higher decomposition rate due to a reduction in the C/N ratio and more root exudates 98 that generate additional respiration (Wu et al. 2015). Our aim 99 was therefore to assess the effect of N supply on both C and N 100 dynamics in peat mesocosms collected in a Sphagnum-101 dominated peatland invaded by a vascular plant, Molinia 102 caerulea. All the peat mesocosms contained Sphagnum 103 rubellum, and half of them also contained M. caerulea. Half of 104 105 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  $CH_4$  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<sup>+</sup><sub>4</sub> and NO<sup>-</sup><sub>3</sub> 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

2.1. Experimental design

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#### 130

Twelve peat mesocosms (depth and diameter: 30 cm) were 131 collected in March 2015 at La Guette peatland, an acidic fen 132 invaded by M. caerulea (pH about 4, 47°19'44"N, 2°17'04"E, 133 France). The mean annual precipitation and temperature of La 134 Guette peatland are 883 mm and 11 °C, respectively (Gogo et al. 135 2011). The mesocosms were buried outdoors (N 47°50′01″, E 136 1°56'34", ISTO, Orléans) and surrounded with a tarpaulin 137 containing water from the peatland. Air and soil temperature 138 at 5 and 20 cm depth were monitored in each mesocosm at 15 139 minute intervals. The water table level (WTL) was measured 140 by using piezometers in all the mesocosms. For each gas 141 measurement, peat water was collected from the piezometer 142 and filtered at  $0.45\,\mu m$  to analyze DOC and TN concentrations 143 ([DOC] and [TN]) with a Shimadzu TOC-5000 analyzer and  $NH_4^+$  144 and  $NO_3^-$  concentrations by Dionex ICS 900 and 1100 ion 145 chromatography. The mesocosms were first separated into 146 two different plant communities: six containing only S. 147 rubellum (called 'Sphagnum' mesocosms) and six containing 148 both S. rubellum and M. caerulea (called 'Sphagnum + Molinia' 149 mesocosms). Molinia caerulea growth started in May. The plant 150 covered up to 60% of mesocosms until its senescence in 151 November. Mesocosms of both plant communities were 152 separated into two treatments with (called 'Fertilized' 153 mesocosms) and without (called 'Control' mesocosms) addi- 154 tions of NH<sub>4</sub>NO<sub>3</sub>, commonly used as an agricultural fertilizer. 155 A powder of NH<sub>4</sub>NO<sub>3</sub> was dissolved in peat water and added 156 every week to reach 3.2 gN/(m<sup>2</sup>·year), which represents 3.7 gN/ 157 (m<sup>2</sup>·year) during the 14 months of the experiment. This 158 enrichment is higher than that currently observed in 159 peatlands (Bragazza et al. 2004), but could reflect the N 160 deposition expected for 2100 (Lamarque et al. 2005). 161

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

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measuring 5 cm per side were prepared and oven dried at 50 °C to calculate peat bulk density ( $g/cm^3$ , Table S1) in order to

171 evaluate the C and N stock in each layer (Eq. (1)):

C or N stocks  $(g/m^3) = C$  or N content  $(\%) \times$  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 growing season (April-October 2015 and April-June 2016) and 177 every 2 weeks during winter (November 2015-March 2016). 178 179 CO<sub>2</sub> fluxes were measured during 5 minute using a GMP343 Vaisala probe inserted in a transparent PVC chamber 180 181 (D'Angelo et al. 2016). A clear chamber was used to measure the net ecosystem exchange (NEE), the balance between GPP 182 (absorption of CO<sub>2</sub> by photosynthesis) and ER (release of CO<sub>2</sub> 183 into the atmosphere). ER was measured by placing an opaque 184 cover on the chamber to block photosynthesis (D'Angelo et al. 185 2016). CH<sub>4</sub> and N<sub>2</sub>O emissions were measured during 15 minute 186 by using SPIRIT, a portable infrared laser spectrometer 187 188 (Guimbaud et al. 2011).

#### 189 2.3. Data analysis and modeling

190 C fluxes (in g C/( $m^2$ ·year)), including the GPP, ER and CH<sub>4</sub> 191 emissions, were derived for the entire year at a 15 minute time 192 step following Leroy et al. (in prep) by using CO<sub>2</sub> and CH<sub>4</sub> 193 measurements to calibrate and validate equations based on 194 Bortoluzzi et al. (2006) and Kandel et al. (2013) with:

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

196 ER is the ecosystem respiration flux ( $\mu$ mol CO<sub>2</sub>/(m<sup>2</sup>·s). T<sub>ref</sub> is 198 the reference air temperature and T<sub>min</sub> the minimum air 199 temperature. These two parameters were set as in Bortoluzzi et al. (2006) at 15 and -5 °C, respectively. T<sub>a</sub> refers to the measured 200 air temperature (°C). The reference for the WTL (WTL<sub>ref</sub>) was set 201 at -15 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 emissions (Eq. (2)):

$$CH_{4} = \left[d^{*}\frac{WTL}{WTL_{ref}} + e\right] * \left(\frac{(T_{s} - T_{min})}{(T_{ref} - T_{min})}\right)^{f}$$
(3)

where,  $WTL_{ref}$ ,  $T_{min}$ ,  $T_{ref}$  and  $T_{min}$  were set as for the ER equation.  $T_s$  refers to the measured soil temperature (°C).

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

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

**217** where GPPmax ( $\mu$ mol/(m<sup>2</sup>·s)) represents the GPP at light 218 saturation, the parameter k ( $\mu$ mol /(m<sup>2</sup>·s)) is the half satura-219 tion value and RVI are a vegetation index to include the effect 220 of Molinia leaves number on photosynthesis.  $T_{min}$ ,  $T_{opt}$  and  $T_{\rm max}$  represent the minimum, optimum and maximum air 221 temperature for photosynthesis and were set at 0, 20 and 40 222 °C, respectively (Kandel et al. 2013). 223

N fluxes (concerning only N<sub>2</sub>O emissions, g N/( $m^2$ ·year)) 224 were extrapolated for the entire year from the mean values of 225 12 months of measurements (in µmol N<sub>2</sub>O/( $m^2$ ·s)). Three-way 226 repeated-measure ANOVAs were used to compare C and N 227 fluxes and differences in environmental parameters due to 228 the main effect of 'Vegetation' between *Sphagnum* and 229 *Sphagnum* + Molinia mesocosms, and 'Nitrogen' between the 230 Fertilized and Control mesocosms over the measurement 231 period (Table 1). Two-way ANOVAs were used to compared C 232 and N content, peat bulk density and C and N stocks per depth 233 for the main effect of 'Vegetation' between *Sphagnum* and 234 *Sphagnum* + Molinia mesocosms, and 'Nitrogen' between the 235 Fertilized and Control ones (Tables 2, S1, S2). 236

#### 3. Results

3.1. C and N fluxes

No significant differences in ER, GPP, CH<sub>4</sub> emissions or [DOC] 240 were observed between the Control and Fertilized mesocosms 241 for the two plant communities (Table 1). Hypothesis (i), which 242 assumed a promotion of ER, GPP and CH<sub>4</sub> emissions, must 243 therefore be rejected. Differences were driven only by the 244 plant communities: the presence of M. caerulea increased the 245 gaseous C fluxes (ER, GPP, CH4 emissions) compared to 246 Sphagnum mesocosms (Table 1). Furthermore, the number 247 and height of M. caerulea leaves were similar between the 248 Control and Fertilized mesocosms and no stimulation of 249 Molinia growth with addition of N was observed (Table 1). 250 Neither NH<sub>4</sub><sup>+</sup> nor NO<sub>3</sub><sup>-</sup> concentrations were significantly 251 influenced by the increase in N deposition, refuting hypoth- 252 esis (ii, a), i.e. higher  $NH_4^+$  and  $NO_3^-$  concentrations in peat 253 water due to NH<sub>4</sub>NO<sub>3</sub> additions (Table 1). Water in both plant 254 communities contained low NO3 concentrations, and NH4 255 concentrations varied with the vegetation cover (Table 1, Fig. 256 2), the presence of M. caerulea significantly reducing the  $NH_4^+$  257 concentrations (and also the TN content) compared to 258 Sphagnum mesocosms (Table 1). 259

The only significant differences due to the NH<sub>4</sub>NO<sub>3</sub> addi- 260 tions concerned N<sub>2</sub>O emissions that increased in Fertilized 261 mesocosms compared to Control ones for both plant commu- 262 nities. This confirms our hypothesis (ii, b), i.e. that a higher N 263 would increase N<sub>2</sub>O emissions under both plant communities 264 (Table 1, Figs. 1, 2 and S1). Furthermore, N<sub>2</sub>O emissions were 265 also affected by the vegetation composition with a decrease in 266 N<sub>2</sub>O emissions in the Sphagnum+Molinia mesocosms compared 267 to the Sphagnum mesocosms (Table 1, Fig. 1). 268

#### 3.2. C and N stocks

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

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t2 1

t1.1Table 1 – Mean values of 12 months measurements of Net Ecosystem Exchange (NEE), Gross Primary Production (GPP),t1.2Ecosystem Respiration (ER),  $CH_4$  emissions ( $CH_4$ ), DOC)  $N_2O$  emissions, TN,  $NH_4^+$  and  $NO_3$  contents, air temperature (Ta),t1.3Water Table Level (WTL), number and height of Molinia leaves in Sphagnum and Sphagnum + Molinia mesocosms witht1.4(Fertilized) or without (Control)  $NH_4NO_3$  addition

t1.6	Sphag		num Sphagnum -		⊦ Molinia		ignificance	
t1.7		Control	Fertilized	Control	Fertilized	Nitrogen	Vegetation	
t1.8	Interaction		C cycle					
t1.9	NEE (µmol/(m²·s))	1.55±0.26	1.29±0.22	$6.50 \pm 1.26$	7.11±1.32		***	
t1.10	GPP (µmol/(m <sup>2</sup> ⋅s))	$2.61 \pm 0.37$	$2.28 \pm 0.33$	$10.24 \pm 1.92$	$10.56 \pm 1.93$		***	
t1.11	ER (µmol/(m <sup>2</sup> ·s))	$1.06 \pm 0.26$	$0.99 \pm 0.22$	$3.75 \pm 0.81$	$3.50 \pm 0.75$		***	
t1.12	CH₄ (µmol/(m²⋅s))	$0.018 \pm 0.007$	$0.019 \pm 0.008$	$0.130 \pm 0.032$	$0.133 \pm 0.03$		***	
t1.13	DOC (mg/L)	$58.38 \pm 8.29$	$41.71 \pm 7.24$	$35.95 \pm 7.34$	$24.14 \pm 5.37$		*	
t1.14 t1.15	N cycle							
t1.16	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	*	*	
t1.17	TN (mg/L)	$5.27 \pm 0.56$	$4.01 \pm 0.36$	$1.70 \pm 0.31$	$1.14 \pm 0.22$		**	
t1.18	NH4 (mg/L)	$5.15 \pm 0.67$	$4.11 \pm 0.35$	$0.27 \pm 0.13$	$0.16 \pm 0.059$		**	
t1.19	NO <sub>3</sub> (mg/L)	$0.51 \pm 0.13$	$0.46 \pm 0.11$	$0.26 \pm 0.09$	$0.55 \pm 0.175$			
t1.20 t1.21	Environmental paramete	ers						
t1.22	Ta (°C)	$12.06 \pm 1.50$	$12.14 \pm 1.525$	$14.54 \pm 1.57$	$14.66 \pm 1.52$			
t1.23	WTL (cm)	$-6.32 \pm 0.51$	$-3.28 \pm 0.40$	$-7.06 \pm 0.49$	$-7.25 \pm 0.54$			
t1.24	Molinia leaves number	n. a.	n. a.	$233.54 \pm 46.0$	$269.94 \pm 48.7$		n. a.	n. a.
t1.25	Molinia leaves height	n. a.	n. a.	$13.04 \pm 2.29$	$12.02 \pm 2.14$		n. a.	n. a.

t1.26n. a.: not applicable. Data are presented as mean  $\pm$  SE, n=12. Significant differences of repeated-measure ANOVAs are expressed as \*p < 0.05, \*\*p < 1.280.01, \*\*p < 0.001.

276 increasing N deposition produced an increase in N<sub>2</sub>O emissions 277 of 0.125gN/(m<sup>2</sup>·year) in Sphagnum mesocosms and of 0.033gN/ 278 (m<sup>2</sup>·year) in Sphagnum + Molinia mesocosms (Fig. 2). However, 279 the increase in N<sub>2</sub>O emissions represents only 4 and 1% of the 280 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 281 stored in Sphagnum mosses with an estimated increase of 2.9g 282 N/m<sup>2</sup> in the stocks of Sphagnum+Molinia mesocosms and of 4.0g 283 N/m<sup>2</sup> in Sphagnum ones in the two first layers of the Fertilized 284 mesocosms compared to the Control ones. 285

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 288 mineralization (Fig. 3). The shift from *Sphagnum* to Molinia 289 dominated peatland increased C emissions and absorption 290 and led to an increase in the C balance (Table 1, Fig. 3, Leroy et 291 al., in prep). This refutes our hypothesis (iii) which assumed a 292 decrease in the ecosystem C balance with M. *caerulea* 293 occurrence. Only the M. *caerulea* effect was modeled on the C 294 flux since it was the only significant effect observed on the 295  $CO_2$  and  $CH_4$  fluxes (Fig. 2, Leroy et al., in prep). C contents 296 were similar between vegetation treatments (Table 2), but the 297 density was higher in *Sphagnum* plots than in *Sphagnum* + 298 *Molinia* mesocosms (Table S1). Consequently, the C stock was 299

t2.2	depth (in ci	m), `	•	1 9	,	,	· · · · ·	
ŧ2.3		Sphagnum		Sphagnum + Molinia		Significance		
t2.5		Control	Fertilized	Control	Fertilized	Nitrogen	Vegetation	Interaction
t2.6	C cycle							
t2.7	0–0.5	$40.80 \pm 0.39$	$40.41 \pm 0.28$	$40.13 \pm 0.20$	$39.37 \pm 0.72$		-	
t2.8	0.5-2.5	$37.60 \pm 1.02$	$34.69 \pm 3.83$	$39.63 \pm 2.59$	$38.40 \pm 2.32$		-	
t2.9	2.5-7.5	$32.54 \pm 3.07$	$31.67 \pm 2.43$	$34.77 \pm 1.70$	$31.42 \pm 2.41$			
t2.10	7.5-12.5	$37.91 \pm 1.05$	$32.21 \pm 2.61$	$38.05 \pm 0.37$	$37.22 \pm 1.95$	-		
t2.11	12.5-17.5	$34.87 \pm 1.37$	$34.14 \pm 2.76$	$37.80 \pm 0.26$	$36.38 \pm 1.05$			
t2.12	17.5-22.5	$37.29 \pm 0.38$	$33.45 \pm 4.37$	$34.58 \pm 2.43$	$36.65 \pm 0.98$			
t2.13 t2.14	N cycle							
t2.15	0-0.5	$1.10 \pm 0.11$	$2.01 \pm 0.13$	$1.34 \pm 0.05$	$1.73 \pm 0.10$	***		*
t2.16	0.5-2.5	$0.68 \pm 0.09$	$0.90 \pm 0.11$	$0.74 \pm 0.06$	$1.14 \pm 0.12$	*		
t2.17	2.5-7.5	$0.86 \pm 0.19$	$0.98 \pm 0.07$	$0.67 \pm 0.12$	$0.77 \pm 0.10$			
t2.18	7.5-12.5	$0.99 \pm 0.05$	$1.18 \pm 0.28$	$1.12 \pm 0.07$	$1.51 \pm 0.36$			
t2.19	12.5-17.5	$1.53 \pm 0.08$	$1.31 \pm 0.19$	$1.49 \pm 0.10$	$1.72 \pm 0.18$			
t2.20	17.5–22.5	$1.60 \pm 0.14$	$1.37 \pm 0.06$	$1.49 \pm 0.05$	$1.64 \pm 0.07$			-

Table 2 - C and N content (%) of Molinia and Sphaanum mesocosms with (Fertilized) or without (Control) NH4NO2 addition per

t2.22 Data are presented as mean  $\pm$  SE, n = 3. Significant differences are expressed as – for 0.05 , <math>\*p < 0.05, \*\*p < 0.01.



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).

higher in Sphagnum mesocosms than in Sphagnum + Molinia
mesocosms (Table S2 and Fig. 3). The variability of peat
density between vegetation did not affect the stoichiometry of
the peat. The C/N ratio was only affected by the N treatment
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

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

Despite the N retention by Sphagnum mosses, increasing 331  $NH_4NO_3$  inputs enhances  $N_2O$  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  $N_2O$  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  $N_2O$  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.

#### 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><sup>-</sup> 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<sup>4</sup><sub>4</sub> concentration 360 in presence of *M. caerulea*, as this plant can use NH<sup>4</sup><sub>4</sub> as an N 361 source (Troelstra et al. 1995). In any case, these results clearly 362

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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)  $NH_4NO_3$  addition (± SE, n = 3) from July 2015 to June 2016. N deposition represents the rain N deposition, N input are the amount of  $NH_4NO_3$  added and N Mineralization represents the quantity of  $N-N_2O$  emissions.

showed that the occurrence of M. caerulea modifies the C cycle either by increasing root biomass (with further modification of the C cycle) or by modulating the denitrifier microbial communities (from  $N_2O$  to  $N_2$  emission), or both. Further studies should thus focus on the OM dynamics associated with the M. caerulea rhizosphere.

#### 369 5. Conclusions

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

vegetation composition with the lowest emissions with M. 377 caerulea occurrence. This modification in  $N_2O$  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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386

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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)  $NH_4NO_3$  addition (±SE, n = 3) from July 2015 to June 2016.

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#### Appendix A. Supplementary data

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

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