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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

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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 (CO₂ exchanges, CH₄ 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 N₂O 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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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 N₂O 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 N₂O
 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 CO₂ and CH₄ 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/(m²·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
 CH₄ 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 NH₄⁺ and NO₃⁻ and of the N stored 120
 by *Sphagnum* mosses; (b) an increase in N₂O 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 (CO₂, CH₄) 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 NH₄⁺
 145 and NO₃⁻ 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 NH₄NO₃, commonly used as an agricultural fertilizer.
 156 A powder of NH₄NO₃ was dissolved in peat water and added
 157 every week to reach 3.2 gN/(m²·year), which represents 3.7 gN/
 158 (m²·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 (g/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 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 CO_2 by photosynthesis) and ER (release of 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). CH_4 and N_2O 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 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 CO_2 and 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_{ref} is
197 the reference air temperature and T_{min} the minimum air
198 temperature. These two parameters were set as in Bortoluzzi et
199 al. (2006) at 15 and -5°C , respectively. T_a refers to the measured
200 air temperature ($^\circ\text{C}$). The reference for the WTL (WTL_{ref}) 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
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, WTL_{ref} , T_{min} , T_{ref} and T_{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 GPP_{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_{min} , T_{opt} and

216 T_{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 N_2O 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, 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 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, 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 NH_4^+ nor NO_3^- concentrations were significantly
248 influenced by the increase in N deposition, refuting hypoth-
249 esis (ii, a), i.e. higher NH_4^+ and NO_3^- concentrations in peat
250 water due to NH_4NO_3 additions (Table 1). Water in both plant
251 communities contained low NO_3^- concentrations, and NH_4^+
252 concentrations varied with the vegetation cover (Table 1, Fig.
253 2), the presence of *M. caerulea* significantly reducing the
254 NH_4^+ concentrations (and also the TN content) compared to
255 *Sphagnum* mesocosms (Table 1). 256

257 The only significant differences due to the NH_4NO_3 addi-
258 tions concerned N_2O 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 N_2O emissions under both plant communities
262 (Table 1, Figs. 1, 2 and S1). Furthermore, N_2O emissions were
263 also affected by the vegetation composition with a decrease in
264 N_2O 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 N_2O
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 N_2O 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), CH₄ emissions (CH₄), DOC, N₂O emissions, TN, NH₄⁺ and NO₃ 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) NH₄NO₃ addition

	<i>Sphagnum</i>		<i>Sphagnum</i> + <i>Molinia</i>		Significance	
	Control	Fertilized	Control	Fertilized	Nitrogen	Vegetation
Interaction	C cycle					
NEE (μmol/(m ² ·s))	1.55 ± 0.26	1.29 ± 0.22	6.50 ± 1.26	7.11 ± 1.32		***
GPP (μmol/(m ² ·s))	2.61 ± 0.37	2.28 ± 0.33	10.24 ± 1.92	10.56 ± 1.93		***
ER (μmol/(m ² ·s))	1.06 ± 0.26	0.99 ± 0.22	3.75 ± 0.81	3.50 ± 0.75		***
CH ₄ (μmol/(m ² ·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 ₂ O (μmol/(m ² ·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 ₄ ⁺ (mg/L)	5.15 ± 0.67	4.11 ± 0.35	0.27 ± 0.13	0.16 ± 0.059		**
NO ₃ ⁻ (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.

276 increasing N deposition produced an increase in N₂O emissions
 277 of 0.125 gN/(m²·year) in *Sphagnum* mesocosms and of 0.033 gN/
 278 (m²·year) in *Sphagnum* + *Molinia* mesocosms (Fig. 2). However,
 279 the increase in N₂O emissions represents only 4 and 1% of the
 280 total N additions, respectively, in *Sphagnum* mesocosms and in
 281 *Sphagnum* + *Molinia* mesocosms. Most of the NH₄NO₃ load was
 282 stored in *Sphagnum* mosses with an estimated increase of 2.9 g
 283 N/m² in the stocks of *Sphagnum*+*Molinia* mesocosms and of 4.0 g
 284 N/m² in *Sphagnum* ones in the two first layers of the Fertilized
 285 mesocosms compared to the Control ones.

286 The NH₄NO₃ load did not significantly impact C cycles and
 287 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 C
 flux since it was the only significant effect observed on the
 CO₂ and CH₄ 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) NH₄NO₃ 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.

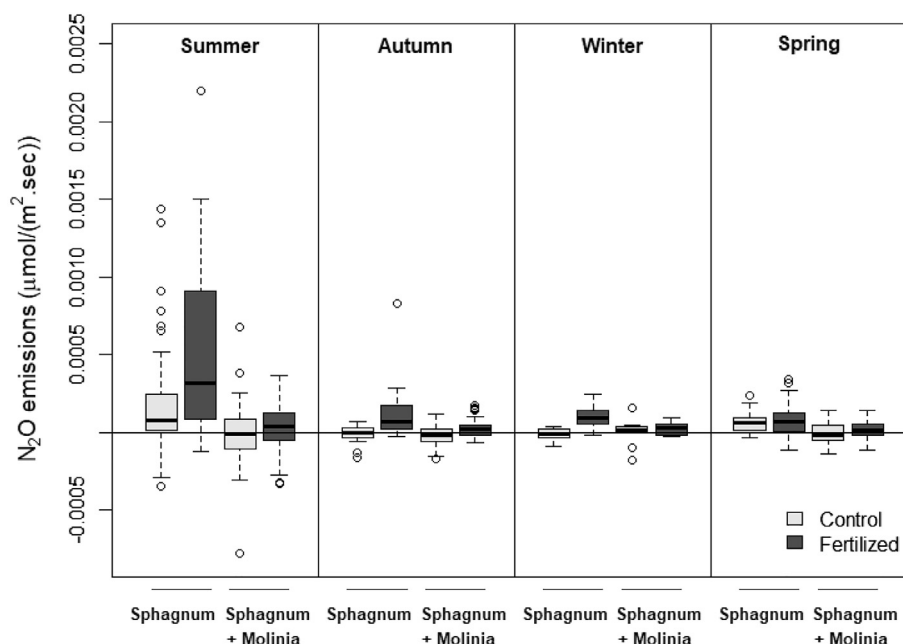


Fig. 1 – N₂O emissions measured in summer (n=19), autumn (n=8), winter (n=5) and spring (n=8) in *Sphagnum* and *Sphagnum* + *Molinia* mesocosms with (Fertilized) or without (Control) NH₄NO₃ addition (n=3).

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
 NH₄NO₃ inputs enhances N₂O 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/m²) is 334
 recovered in the amount of N found in the combined N₂O 335
 emissions and N stocks in *Sphagnum* layers (4.0 g N/m² on 336
 average; Fig. 2). No other N output or stock is required to close the 337
 N balance. These results suggest that increased N₂O 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 N₂O 343
 emissions (Table 1) with lower N₂O emissions in the presence 344
 of *M. caerulea* (Fig. 1). The dissolved NO₃ 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 N₂O emissions and N stocks (2.9 gN/m²) 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 N₂ (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 N₂O 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 NH₄⁺ concentration 360
 in presence of *M. caerulea*, as this plant can use NH₄⁺ as an N 361
 source (Troelstra et al. 1995). In any case, these results clearly 362

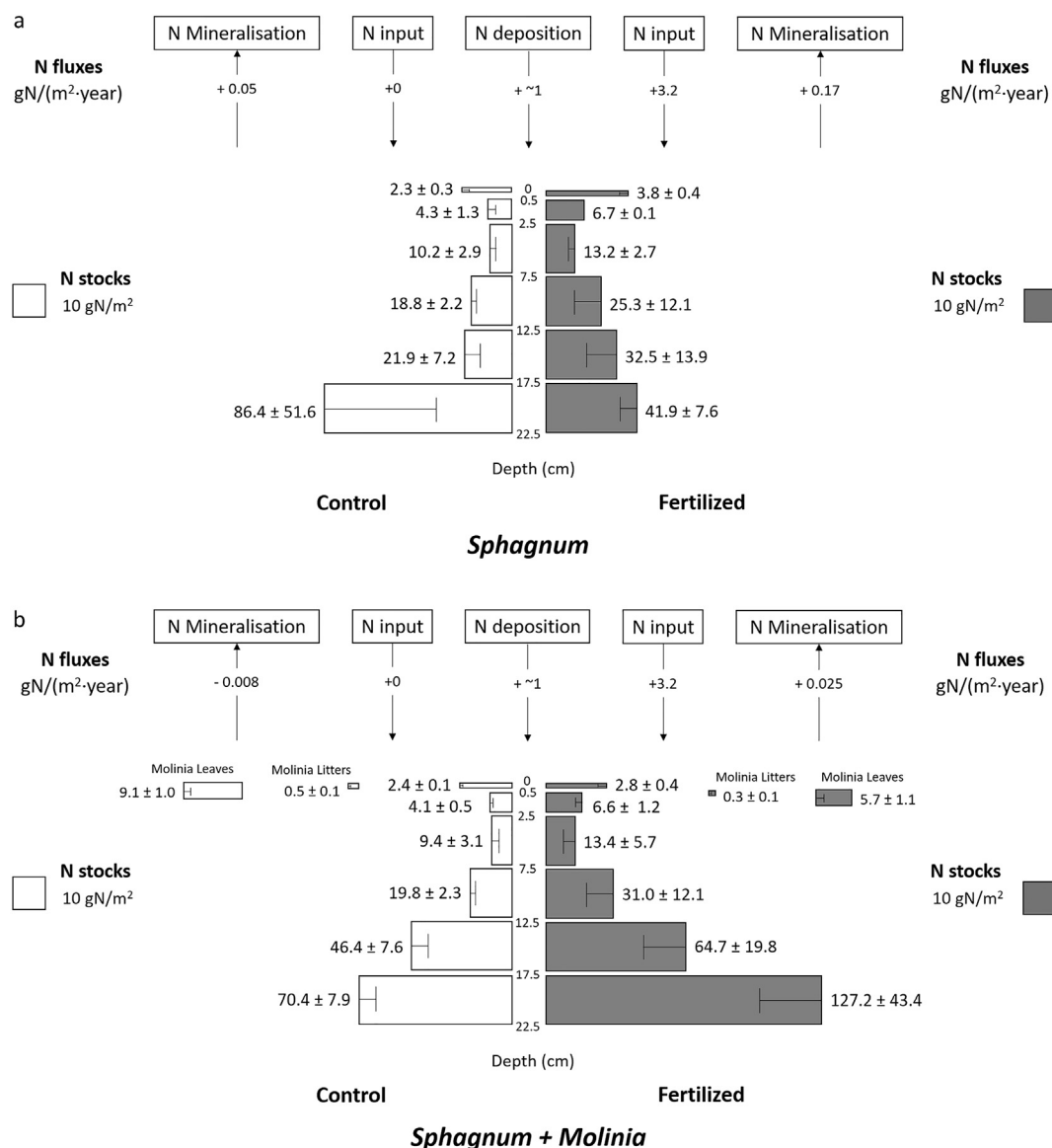


Fig. 2 – N stocks (g N/m²) and fluxes (black arrow, g N/(m²-year) in *Sphagnum* (a) and *Sphagnum + Molinia mesocosms* (b) with (Fertilized) or without (Control) NH₄NO₃ addition (±SE, n = 3) from July 2015 to June 2016. N deposition represents the rain N deposition, N input are the amount of NH₄NO₃ added and N Mineralization represents the quantity of N-N₂O 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 N₂O to N₂ 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 (CO₂, CH₄),
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, NH₄NO₃ addition
376 promoted N₂O emissions, which were also influenced by the

vegetation composition with the lowest emissions with *M. caerulea*
377 occurrence. This modification in N₂O 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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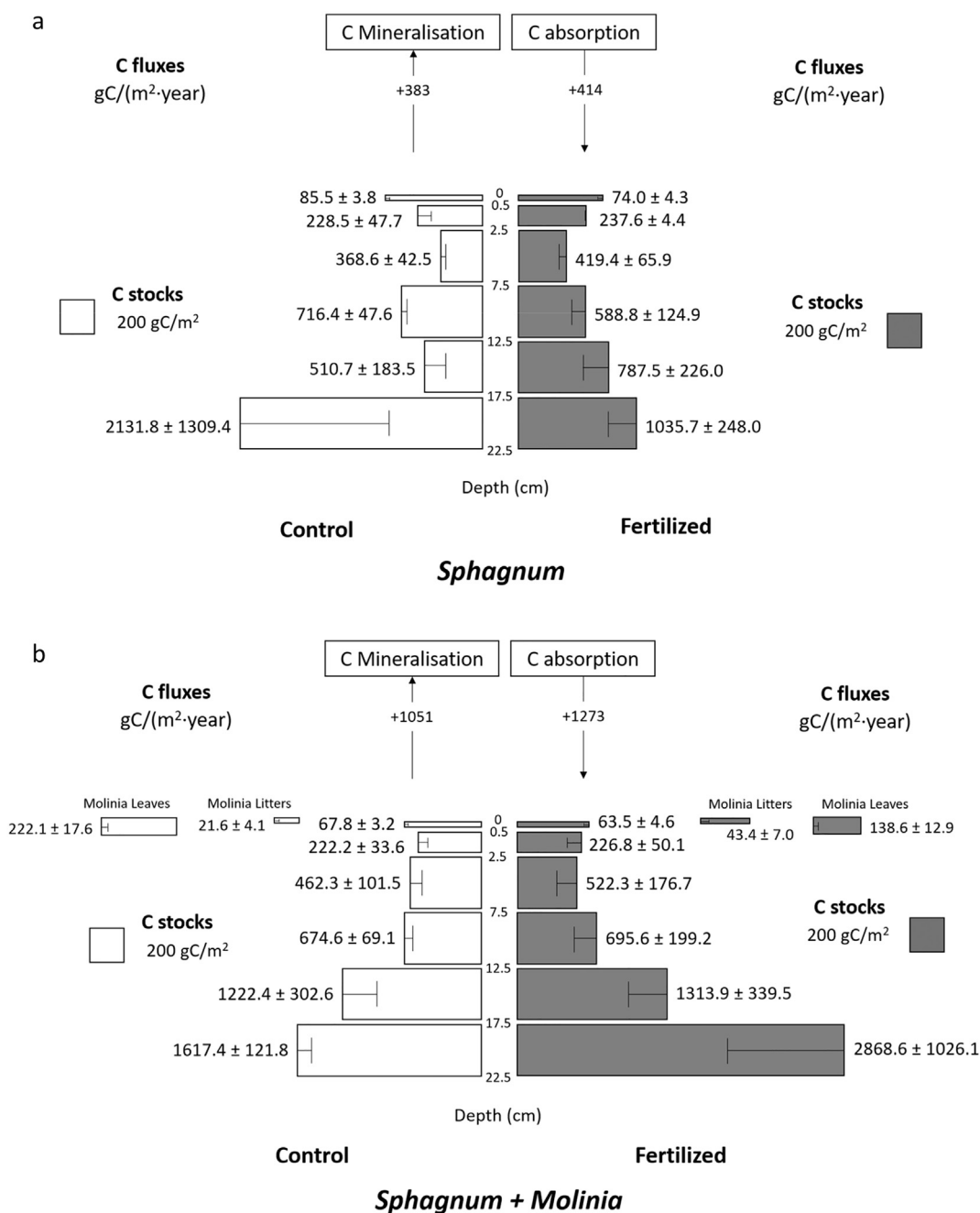


Fig. 3 – C stocks (per depth in g C/m²) and fluxes (black arrow, g C/(m²·year)) in *Sphagnum* (a) and *Sphagnum + Molinia* mesocosms (b) with (Fertilized) or without (Control) NH₄NO₃ addition (±SE, n = 3) from July 2015 to June 2016.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at
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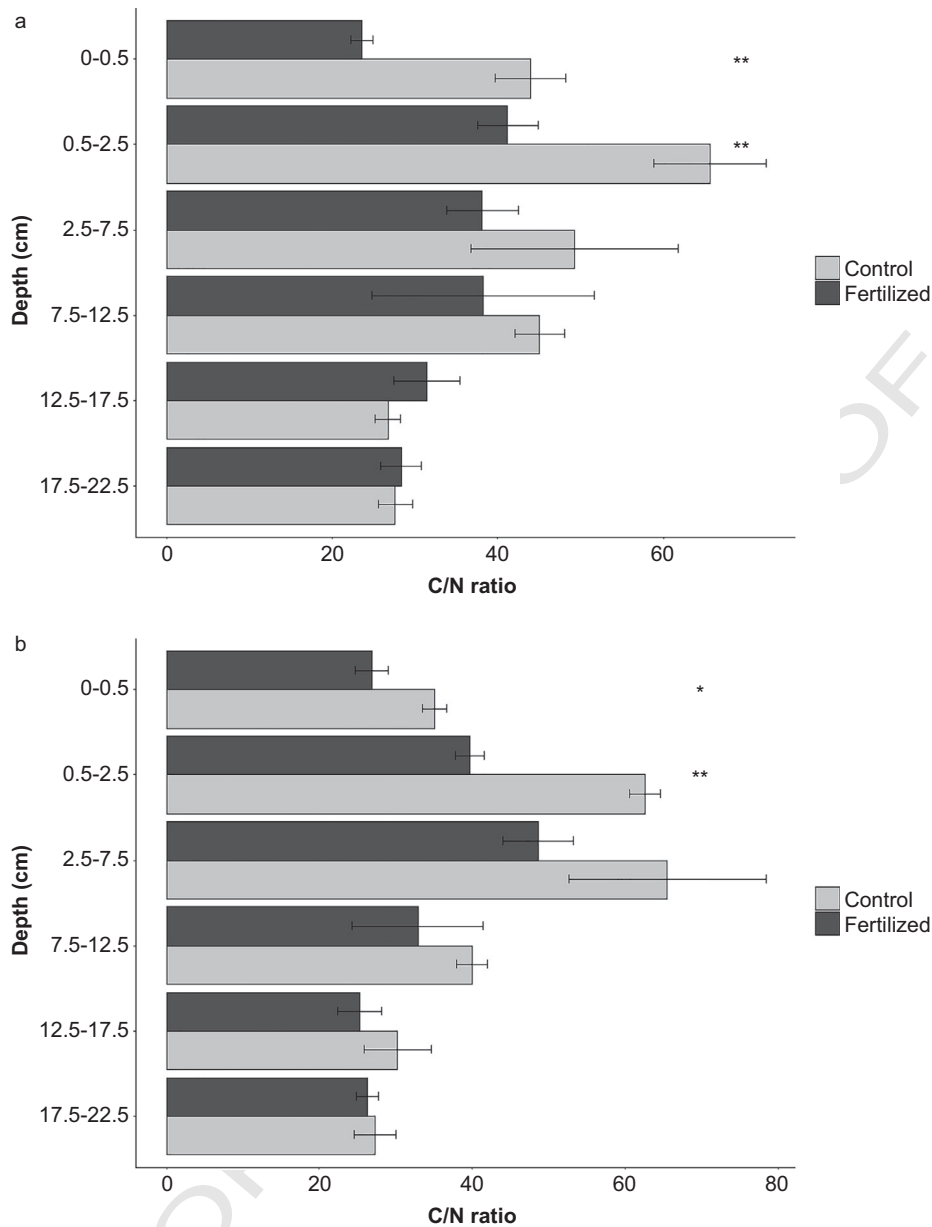


Fig. 4 – C/N ratio per layer in *Sphagnum* (a) and *Sphagnum* + *Molinia* mesocosms (b) with (Fertilized) or without (Control) NH_4NO_3 addition (\pm SE, $n = 3$). Significant differences per layer are expressed as – for $0.05 < p < 0.1$, * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$.**

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