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Response of C and N cycles to N fertilization in

- Sphagnum and Molinia-dominated peat
- mesocosms
- Fabien Leroy^{1,2,3,*}, Sébastien Gogo^{1,2,3}, Christophe Guimbaud^{4,5}, André-Jean Francez⁶, Renata Zocatelli⁷, Christian Défarge^{1,2,3,7}, Léonard Bernard-Jannin^{1,2,3}, Zhen Hu⁸,
- Fatima Laggoun-Défarge^{1,2,3}
- 1. Université d'Orléans, ISTO, UMR 7327, 45071 Orléans, France
- 2. CNRS, ISTO, UMR 7327, 45071 Orléans, France
- 3. BRGM, ISTO, UMR 7327, BP 36009, 45060 Orléans, France
- 4. Université d'Orléans, LPC2E, UMR 7328, 45071 Orléans, France 10
- 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
- 8. School of Environmental Science and Engineering, Shandong University, Jinan, China

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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 (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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Corresponding author at: Université d'Orléans, ISTO, UMR 7327, 45071 Orléans, France. E-mail address: lerfabien@gmail.com. (F. Leroy).

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1. Introduction

Peatlands currently act as a major long-term carbon (C) sink ecosystem. Although these wetlands cover only 3% of the land area, they have stored a third of the global soil C since the early Holocene (Turunen et al. 2002). Most Sphagnum peatlands (up to 80%) are located at high latitudes of the northern hemisphere in the cool temperate zone in association with waterlogged, nutrient poor conditions and the presence of Sphagnum mosses (e.g. Gorham 1991). To cope with low nutrient concentrations, Sphagnum mosses have developed mechanisms to efficiently use nutrients thanks to their high cation exchange capacity, nutrient translocation and atmospheric interception, reducing the nutrient availability to vascular plants (e.g. Turetsky et al. 2012). However, northern temperate ecosystems receive four times more airborne nitrogen (N) today than 150 years ago (Holland et al. 1999; Lamarque et al. 2005). Increased N deposition leads to a progressive N saturation of Sphagnum mosses, thus favoring the invasion of vascular plants and reducing Sphagnum moss growth (Limpens et al. 2011). Such changes seem to reduce the C sequestration rates in peatlands (Bragazza et al. 2006; Gunnarsson et al. 2008), even if they increase the vascular plants' productivity (Wu et al. 2015). However, the effect of the increase in N loads on stocks and exchanges of N and C are still understudied in peatlands, although they are known to generally increase N2O emissions to the atmosphere (e.g. Nykänen et al. 2002; Francez et al. 2011). Peatland C-storage capacity is often considered alone to assess the effects of climate change on peatlands without considering the N stored in the ecosystems that could account for a significant N2O source and therefore act as a positive feedback to climate change (Repo et al. 2009).

The increase in vascular plant cover due to human activities such as nutrient supply, e.g., atmospheric N deposition, or drainage, increases organic matter decomposition (Gogo et al. 2016) and modulates CO2 and CH4 emissions in peatlands (Ward et al. 2013; Leroy et al. 2017). The combined effects of vascular plant invasion with N deposition on both C and N cycles and stocks still remain to be elucidated. N fertilization generally stimulates the vascular plant biomass, thereby contributing to higher primary production. However, it also leads to a higher decomposition rate due to a reduction in the C/N ratio and more root exudates that generate additional respiration (Wu et al. 2015). Our aim was therefore to assess the effect of N supply on both C and N dynamics in peat mesocosms collected in a Sphagnumdominated peatland invaded by a vascular plant, Molinia caerulea. All the peat mesocosms contained Sphagnum rubellum, and half of them also contained M. caerulea. Half of each plant community mesocosm was subjected to an increase in N deposition by a weekly amendment to reach an addition of 3.2 g N/(m²·year). Thus, the hypotheses investigated are that N deposition will lead to the following processes under the two plant communities:

(i) Processes involving the C cycle: (a) an increase in C fluxes by promoting ecosystem respiration (ER) due to a 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.
- (ii) Processes involving the N cycle: (a) higher concentra- 119 tions of the dissolved NH_4^+ and NO_3^- and of the N stored 120 by *Sphagnum* mosses; (b) an increase in N_2O 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

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2. Materials and methods

2.1. Experimental design

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 NO₃ 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 NH₄NO₃, commonly used as an agricultural fertilizer. 155 A powder of NH₄NO₃ was dissolved in peat water and added 156 every week to reach 3.2 gN/(m²·year), which represents 3.7 gN/ 157 (m²·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).

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/cm³, Table S1) in order to 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)

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2.2. Greenhouse gas (GHG) measurements

GHG measurements were performed with the closed chamber method between once to twice per week during the growing season (April–October 2015 and April–June 2016) and every 2 weeks during winter (November 2015–March 2016). CO₂ fluxes were measured during 5 minute using a GMP343 Vaisala probe inserted in a transparent PVC chamber (D'Angelo et al. 2016). A clear chamber was used to measure the net ecosystem exchange (NEE), the balance between GPP (absorption of CO₂ by photosynthesis) and ER (release of CO₂ into the atmosphere). ER was measured by placing an opaque cover on the chamber to block photosynthesis (D'Angelo et al. 2016). CH₄ and N₂O emissions were measured during 15 minute by using SPIRIT, a portable infrared laser spectrometer (Guimbaud et al. 2011).

189 2.3. Data analysis and modeling

C fluxes (in g C/(m^2 -year)), including the GPP, ER and CH₄ emissions, were derived for the entire year at a 15 minute time step following Leroy et al. (in prep) by using CO₂ and CH₄ measurements to calibrate and validate equations based on Bortoluzzi et al. (2006) and Kandel et al. (2013) with:

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

ER is the ecosystem respiration flux (μ mol CO₂/(m²·s). T_{ref} is the reference air temperature and T_{min} the minimum air temperature. These two parameters were set as in Bortoluzzi et al. (2006) at 15 and – 5 °C, respectively. T_a refers to the measured air temperature (°C). The reference for the WTL (WTL_{ref}) was set at –15 cm corresponding to the deepest WTL recorded in the mesocosms. The coefficients a, b and c (temperature sensitivity parameters) are empirical parameters.

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

$$CH_4 = \left[d^* \frac{WTL}{WTL_{ref}} + e \right] * \left(\frac{(T_s - T_{min})}{(T_{ref} - T_{min})} \right)^f \tag{3}$$

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):

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

where GPPmax (μ mol/(m^2 ·s)) represents the GPP at light saturation, the parameter k (μ mol/(m^2 ·s)) is the half saturation value and RVI are a vegetation index to include the effect 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).

N fluxes (concerning only N_2O emissions, g $N/(m^2\cdot year)$) 224 were extrapolated for the entire year from the mean values of 225 12 months of measurements (in μ mol $N_2O/(m^2\cdot s)$). 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).

3. Results

3.1. C and N fluxes

No significant differences in ER, GPP, CH₄ 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 CH₄ 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 NH₄⁺ nor NO₃⁻ concentrations were significantly 251 influenced by the increase in N deposition, refuting hypoth- 252 esis (ii, a), i.e. higher NH₄ and NO₃ concentrations in peat 253 water due to NH₄NO₃ 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 NH₄ 257 concentrations (and also the TN content) compared to 258 Sphagnum mesocosms (Table 1).

The only significant differences due to the NH_4NO_3 addi- 260 tions concerned N_2O 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_2O emissions under both plant communities 264 (Table 1, Figs. 1, 2 and S1). Furthermore, N_2O emissions were 265 also affected by the vegetation composition with a decrease in 266 N_2O emissions in the *Sphagnum+Molinia* mesocosms compared 267 to the *Sphagnum* mesocosms (Table 1, Fig. 1).

3.2. C and N stocks

Increasing N deposition triggered a short-term response with 270 an increase in N concentrations in Sphagnum capitula (0–0.5cm 271 depth) and in its living tissues (0.5–2.5 cm depth) and in N₂O 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 N₂O fluxes (in g N/(m²·year) showed that 275

t1.1

t1.7 t1.8

t1 9 t1.10 t1.11 t1.12 t1.13 t1.14 t1.15 t1.16 t1.17 t1 18

t1.22 t1.23 t1.24 t1.25

t1.19 t1.20 t1 21

t1.28 276

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t1.26

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t2.1 t2.2 £2:34

t2.5 t2.6

t2.7 t2.8 t2.9 t2.10 t2.11

t2.14 t2 15 t2.16 t2.17 t2.18

t2.12 t2 13

t2.20

t2.22

t2.19

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, NH⁺₄ 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

	Spha	gnum	Sphagnum + Molinia		Significance		
	Control	Fertilized	Control	Fertilized	Nitrogen	Vegetation	
Interaction		C cycle					
NEE (μmol/(m²·s))	1.55 ± 0. 26	1.29 ± 0.22	6.50 ± 1.26	7.11 ± 1.32		***	
GPP (μmol/(m²·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))	$5.26-5 \pm 1.48-5$	$19.41 - 5 \pm 5.38 - 5$	$-0.89-5 \pm 1.21-5$	$2.86-5 \pm 5.94-6$	*	*	
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 paramete	ers						
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			
Molinia leaves number	n. a.	n. a.	233.54 ± 46.0	269.94 ± 48.7		n. a.	n. a.
Molinia 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 N₂O emissions of 0.125 gN/(m²·year) in Sphagnum mesocosms and of 0.033 gN/ (m²·year) in Sphagnum + Molinia mesocosms (Fig. 2). However, the increase in N₂O emissions represents only 4 and 1% of the total N additions, respectively, in Sphagnum mesocosms and in Sphagnum + Molinia mesocosms. Most of the NH₄NO₃ load was stored in Sphagnum mosses with an estimated increase of 2.9 g N/m² in the stocks of Sphagnum+Molinia mesocosms and of 4.0g N/m² in Sphagnum ones in the two first layers of the Fertilized mesocosms compared to the Control ones.

The NH₄NO₃ 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 CO₂ and CH₄ 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

Table 2 – C and N content (%) of Molinia and Sphagnum mesocosms with (Fertilized) or without (Control) NH_4NO_3 addition per

	Sphagnum		Sphagnum + Molinia		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				
1								
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 \pm SE, n = 3. Significant differences are expressed as - for 0.05 , *<math>p < 0.05, **p < 0.01, ***p < 0.001.

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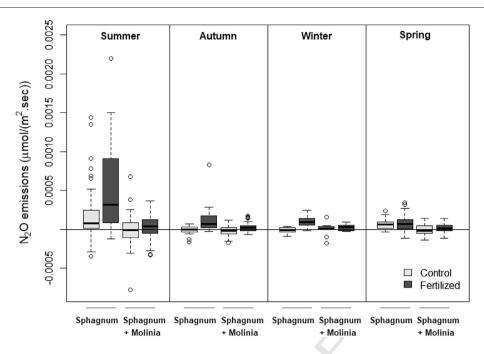


Fig. 1 – N_2O emissions measured in summer (n=19), autumn (n=8), winter (n=5) and spring (n=8) in Sphagnum and Sphagnum+Molinia mesocosms with (Fertilized) or without (Control) NH_4NO_3 addition (n=3).

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 in the Fertilized plots than in the Control ones (Fig.4).

4. Discussion

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4.1. N retention by Sphagnum

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

Despite the N retention by *Sphagnum* mosses, increasing 331 $\mathrm{NH_4NO_3}$ inputs enhances $\mathrm{N_2O}$ 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/m²) is 334 recovered in the amount of N found in the combined $\mathrm{N_2O}$ 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 $\mathrm{N_2O}$ 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_2O 343 emissions (Table 1) with lower N_2O emissions in the presence 344 of M. caerulea (Fig. 1). The dissolved NO_3^- 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_2O 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 N_2 (not possible to observe 353 with our technique) or incorporated into the root biomass.

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_2O 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_4^+ concentration 360 in presence of M. caerulea, as this plant can use NH_4^+ as an N 361 source (Troelstra et al. 1995). In any case, these results clearly 362

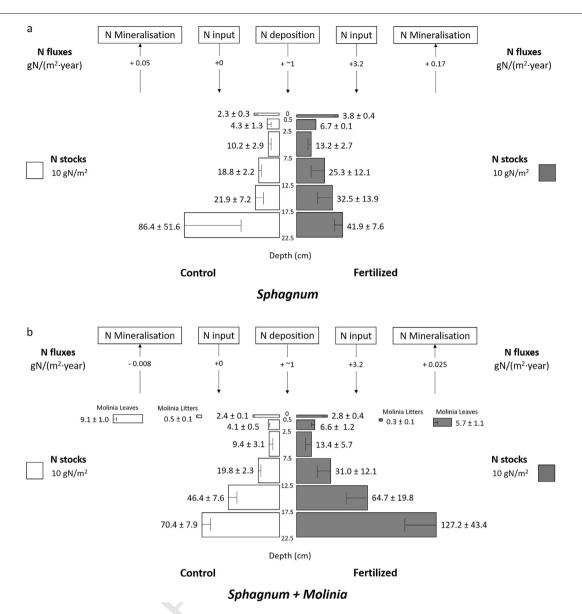


Fig. 2 - N stocks (g N/m2) and fluxes (black arrow, g N/(m2·year)) in Sphagnum (a) and Sphagnum + Molinia mesocosms (b) with (Fertilized) or without (Control) NH_4NO_3 addition (\pm SE, n=3) from July 2015 to June 2016. N deposition represents the rain N deposition, N input are the amount of NH_4NO_3 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 N2O to N2 emission), or both. Further studies should thus focus on the OM dynamics associated with the M. caerulea rhizosphere.

5. Conclusions

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Increasing N deposition did not impact the C fluxes (CO₂, CH₄), [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, NH₄NO₃ addition promoted N2O emissions, which were also influenced by the

vegetation composition with the lowest emissions with M. 377 caerulea 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

Acknowledgments

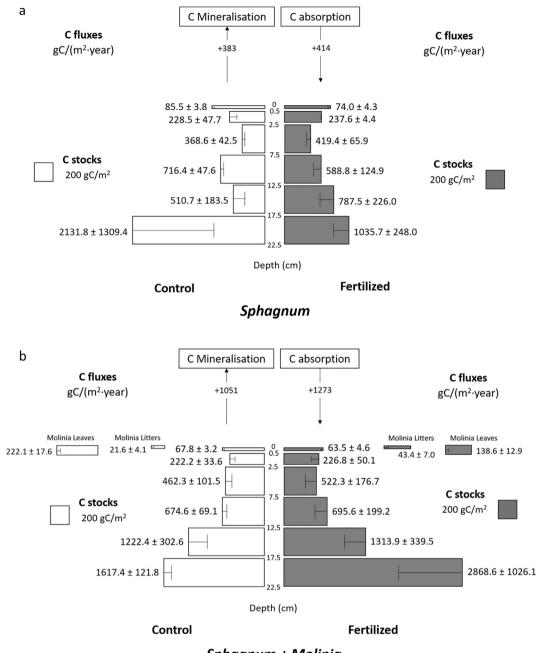
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Sphagnum + Molinia

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 (\pm SE, n = 3) from July 2015 to June 2016.

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Appendix A. Supplementary data

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

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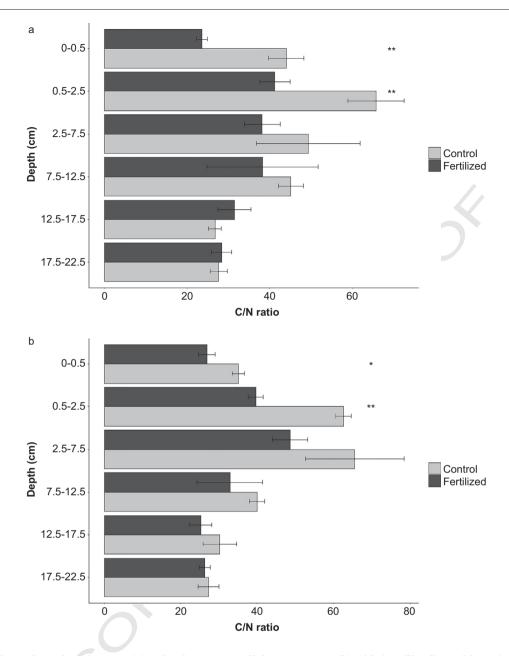


Fig. 4 – C/N ratio per layer in Sphagnum (a) and Sphagnum + Molinia mesocosms (b) with (Fertilized) or without (Control) NH₄NO₃ addition (\pm SE, n = 3). Significant differences per layer are expressed as – for 0.05 < p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001.

409 REFERENCES

424

419 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. Bortoluzzi, E., Epron, D., Siegenthaler, A., Gilbert, D., Buttler, A., 414 415 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.Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, 423

N., et al., 2006. Atmospheric nitrogen deposition promotes

carbon loss from peat bogs. Proc. Natl. Acad. Sci. U. S. A. 103 425 (51), 19386-19389. 426 D'Angelo, B., Gogo, S., Laggoun-Défarge, F., Le Moing, F., Jégou, F., 427 Guimbaud, C., 2016. Soil temperature synchronisation 428 improves representation of diel variability of ecosystem 429 respiration in Sphagnum peatlands. Agric. For. Meteorol. 223, 430 95-102 431 Francez, A.J., Pinay, G., Josselin, N., Williams, B.L., 2011. Denitri-432 fication triggered by nitrogen addition in Sphagnum 433 magellanicum peat. Biogeochemistry 106 (3), 435-441. 434 Gogo, S., Laggoun-Défarge, F., Delarue, F., Lottier, N., 2011. 435 Invasion of a Sphagnum-peatland by Betula spp and Molinia 436 caerulea impacts organic matter biochemistry. Implications for 437 carbon and nutrient cycling. Biogeochemistry 106 (1), 53-69. 438 Gogo, S., Laggoun-Défarge, F., Merzouki, F., Mounier, S., 439 Guirimand-Dufour, A., Jozja, N., et al., 2016. In situ and 440 laboratory non-additive litter mixture effect on C dynamics of 441

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- 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
- Granath, G., Wiedermann, M.M., Strengbom, J., 2009. Physiological 447 448 responses to nitrogen and sulphur addition and raised 449 temperature in Sphagnum balticum. Oecol 161 (3), 481-490.

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- Guimbaud, C., Catoire, V., Gogo, S., Robert, C., Chartier, M., Laggoun-Défarge, F., et al., 2011. A portable infrared laser spectrometer for flux measurements of trace gases at the geosphere-atmosphere interface. Meas. Sci. Technol. 22 (7), 075601.
- Gunnarsson, U., Boresjö Bronge, L., Rydin, H., Ohlson, M., 2008. Near-zero recent carbon accumulation in a bog with high nitrogen deposition in SW Sweden. Glob. Chang. Biol. 14 (9), 2152-2165.
- Hayden, M.J., Ross, D.S., 2005. Denitrification as a nitrogen removal mechanism in a Vermont peatland. J. Environ. Qual. 34 (6), 2052-2061.
- Holland, E.A., Dentener, F.J., Braswell, B.H., Sulzman, J.M., 1999. Contemporary and pre-industrial global reactive nitrogen budgets. Biogeochemistry 46 (1), 7-43.
- Kandel, T.P., Elsgaard, L., Lærke, P.E., 2013. Measurement and modelling of CO2 flux from a drained fen peatland cultivated with reed canary grass and spring barley. GCB Bioenergy 5 (5): 548-561. https://doi.org/10.1111/gcbb.12020.
- Lamarque, J.F., Kiehl, J.T., Brasseur, G.P., Butler, T., Cameron-Smith, P., Collins, W.D., et al., 2005. Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: analysis of nitrogen deposition. J Geophys. Res: Atmos. 110 (D19).
- Leroy, F., Gogo, S., Guimbaud, C., Bernard-Jannin, L., Hu, Z., Laggoun-Défarge, F., 2017. Vegetation composition controls temperature sensitivity of CO2 and CH4 emissions and DOC concentration in peatlands. Soil Biol. Biochem. 107, 164-167.
- Leroy, F., Gogo, S., Guimbaud, C., Bernard-Jannin, L., Yin, X., Belot, G., Shuguang, W., Laggoun-Défarge, F., In prep Carbon balance and global warming potential modifications in Sphagnumdominated peat mesocosms invaded by Molinia caerulea. Submitted to Biogeosciences.
- Limpens, J., Granath, G., Gunnarsson, U., Aerts, R., Bayley, S., 482 483 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., 490 et al., 2009. Large N₂O emissions from cryoturbated peat soil in 491 tundra. Nat. Geosci. 2 (3), 189-192.
- Roobroeck, D., Butterbach-Bahl, K., Brueggemann, 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 499 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: 502 desiccation or high N deposition? J. Appl. Ecol. 41 (1), 139-150.
- Troelstra, S.R., Wagenaar, R., Smant, W., 1995. Nitrogen utilization 504 by plant species from acid heathland soils: I. Comparison between nitrate and ammonium nutrition at constant low pH 506 1. J. Exp. Bot. 1103-1112.
- Turetsky, M.R., Bond-Lamberty, B., Euskirchen, E., Talbot, J., Frolking, 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, 518 R.D., 2013. Warming effects on greenhouse gas fluxes in 519 peatlands are modulated by vegetation composition. Ecol. Lett. 520 16 (10), 1285-1293. 521
- Wu, Y., Blodau, C., Moore, T.R., Bubier, J., Juutinen, S., Larmola, T., 2015. Effects of experimental nitrogen deposition on peatland 523 carbon pools and fluxes: a modelling analysis. Biogeosciences 12 (1), 79-101.

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