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# 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

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## A B S T R A C T

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

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

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

## 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 N<sub>2</sub>O 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 N<sub>2</sub>O 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 CO<sub>2</sub> and CH<sub>4</sub> 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 *Sphagnum*-dominated 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/(m<sup>2</sup>·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 primary production (GPP) by an enhancement of both *Sphagnum* mosses and graminoid biomass (e.g. Tomassen et al. 2003; Granath et al. 2009); (c) a rise in CH<sub>4</sub> emissions through a higher OM decomposition and increase in root exudates.

- (ii) Processes involving the N cycle: (a) higher concentrations of the dissolved NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> and of the N stored by *Sphagnum* mosses; (b) an increase in N<sub>2</sub>O emissions under both plant communities (Roobroeck et al. 2010).
- (iii) Processes involving *M. caerulea* occurrence: an increase in the C fluxes in peatlands (CO<sub>2</sub>, CH<sub>4</sub>) and DOC content and a decrease in the ecosystem C sink function compared to *Sphagnum*-dominated peatland due to the promotion of peat decomposition (Leroy et al. 2017).

## 2. Materials and methods

### 2.1. Experimental design

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

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

measuring 5 cm per side were prepared and oven dried at 50°C to calculate peat bulk density (g/cm<sup>3</sup>, Table S1) in order to evaluate the C and N stock in each layer (Eq. (1)):

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

172

## 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). CO<sub>2</sub> 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 CO<sub>2</sub> by photosynthesis) and ER (release of CO<sub>2</sub> into the atmosphere). ER was measured by placing an opaque cover on the chamber to block photosynthesis (D'Angelo et al. 2016). CH<sub>4</sub> and N<sub>2</sub>O emissions were measured during 15 minute by using SPIRIT, a portable infrared laser spectrometer (Guimbaud et al. 2011).

## 2.3. Data analysis and modeling

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

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

ER is the ecosystem respiration flux (μmol CO<sub>2</sub>/(m<sup>2</sup>·s)). T<sub>ref</sub> is the reference air temperature and T<sub>min</sub> the minimum air 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 air temperature (°C). The reference for the WTL (WTL<sub>ref</sub>) 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)):

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

where, WTL<sub>ref</sub>, T<sub>min</sub>, T<sub>ref</sub> and T<sub>min</sub> were set as for the ER equation. T<sub>s</sub> refers to the measured soil temperature (°C).

The GPP was modeled 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):

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

where GPP<sub>max</sub> (μmol/(m<sup>2</sup>·s)) represents the GPP at light saturation, the parameter k (μmol/(m<sup>2</sup>·s)) is the half saturation value and RVI a vegetation index to include the effect of *Molinia* leaves number on photosynthesis. T<sub>min</sub>, T<sub>opt</sub> and

T<sub>max</sub> represent the minimum, optimum and maximum air temperature for photosynthesis and were set at 0, 20 and 40 °C, respectively (Kandel et al. 2013).

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

## 3. Results

### 3.1. C and N fluxes

No significant differences in ER, GPP, CH<sub>4</sub> emissions or [DOC] were observed between the Control and Fertilized mesocosms for the two plant communities (Table 1). Hypothesis (i), which assumed a promotion of ER, GPP and CH<sub>4</sub> emissions, must therefore be rejected. Differences were driven only by the plant communities: the presence of *M. caerulea* increased the gaseous C fluxes (ER, GPP, CH<sub>4</sub> emissions) compared to *Sphagnum* mesocosms (Table 1). Furthermore, the number and height of *M. caerulea* leaves were similar between the Control and Fertilized mesocosms and no stimulation of *Molinia* growth with addition of N was observed (Table 1). Neither NH<sub>4</sub><sup>+</sup> nor NO<sub>3</sub><sup>–</sup> concentrations were significantly influenced by the increase in N deposition, refuting hypothesis (ii, a), i.e. higher NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>–</sup> concentrations in peat water due to NH<sub>4</sub>NO<sub>3</sub> additions (Table 1). Water in both plant communities contained low NO<sub>3</sub><sup>–</sup> concentrations, and NH<sub>4</sub><sup>+</sup> concentrations varied with the vegetation cover (Table 1, Fig. 2), the presence of *M. caerulea* significantly reducing the NH<sub>4</sub><sup>+</sup> concentrations (and also the TN content) compared to *Sphagnum* mesocosms (Table 1).

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

### 3.2. C and N stocks

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



**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> (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.

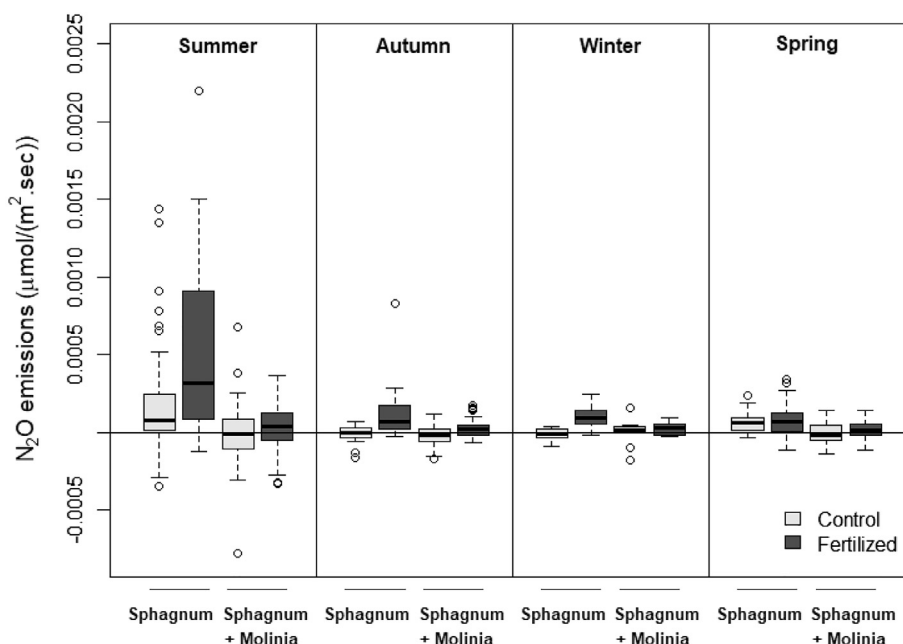
The NH<sub>4</sub>NO<sub>3</sub> load did not significantly impact C cycles and stocks (Table 1, Fig. 3). C compartments were only affected by

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 C flux since it was the only significant effect observed 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).**

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

### 4.1. N retention by *Sphagnum*

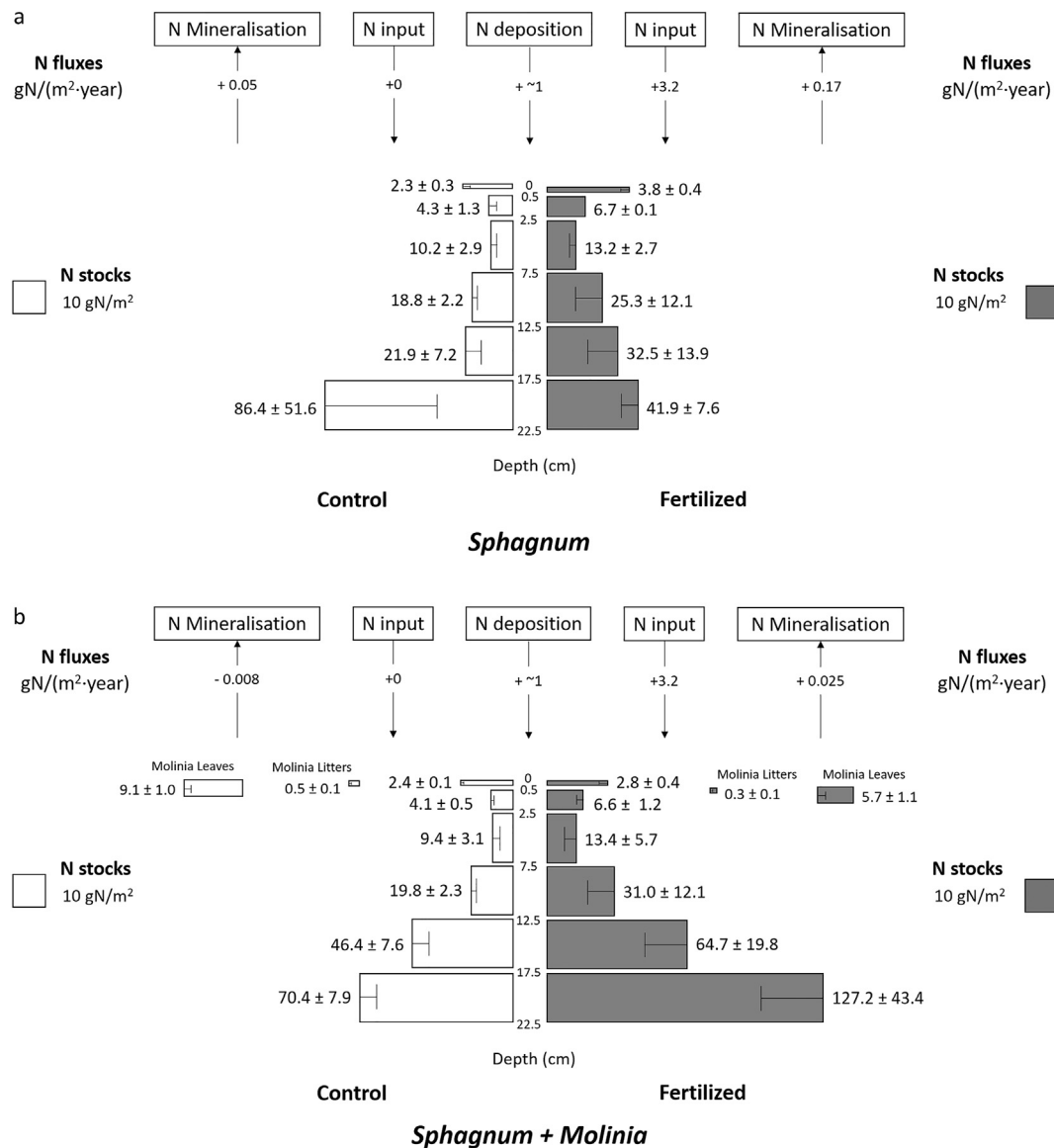
The number and height of *M. caerulea* leaves were similar between the Control and the Fertilized mesocosms and no stimulation of *Molinia* growth was observed. This is in agreement with the results of Tomassen et al. (2003) who found an effect of N addition on *M. caerulea* biomass only after 3 years 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 long-term 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 *Sphagnum*-dominated peatlands (Aerts et al. 1992).

Despite the N retention by *Sphagnum* mosses, increasing NH<sub>4</sub>NO<sub>3</sub> inputs enhances N<sub>2</sub>O emissions under both plant communities (Fig. 1). When error terms are considered, the amount of N added in *Sphagnum* mesocosms (3.7 g N/m<sup>2</sup>) is recovered in the amount of N found in the combined N<sub>2</sub>O emissions and N stocks in *Sphagnum* layers (4.0 g N/m<sup>2</sup> on average; Fig. 2). No other N output or stock is required to close the N balance. These results suggest that increased N<sub>2</sub>O emissions may be generated by a stimulation of denitrification triggered by an increase in N availability (Hayden and Ross 2005; Francez et 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 emissions (Table 1) with lower N<sub>2</sub>O emissions in the presence of *M. caerulea* (Fig. 1). The dissolved NO<sub>3</sub> concentration (Table 1) and the N content of living *Sphagnum* (Table S1) did not differ between *Sphagnum* and *Sphagnum* + *Molinia* mesocosms. Furthermore, the above-ground biomass production of *M. caerulea* was not stimulated by N addition (Table 1; Fig. 2). The combined increase in N<sub>2</sub>O emissions and N stocks (2.9 gN/m<sup>2</sup>) in Fertilized mesocosms compared to the Control ones represents 80% of the N additions. The fraction of N lacking may have been either denitrified to N<sub>2</sub> (not possible to observe with our technique) or incorporated into the root biomass.

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



**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.**

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<sub>2</sub>O to N<sub>2</sub> emission), or both. Further studies should thus focus on the OM dynamics associated with the *M. caerulea* rhizosphere.

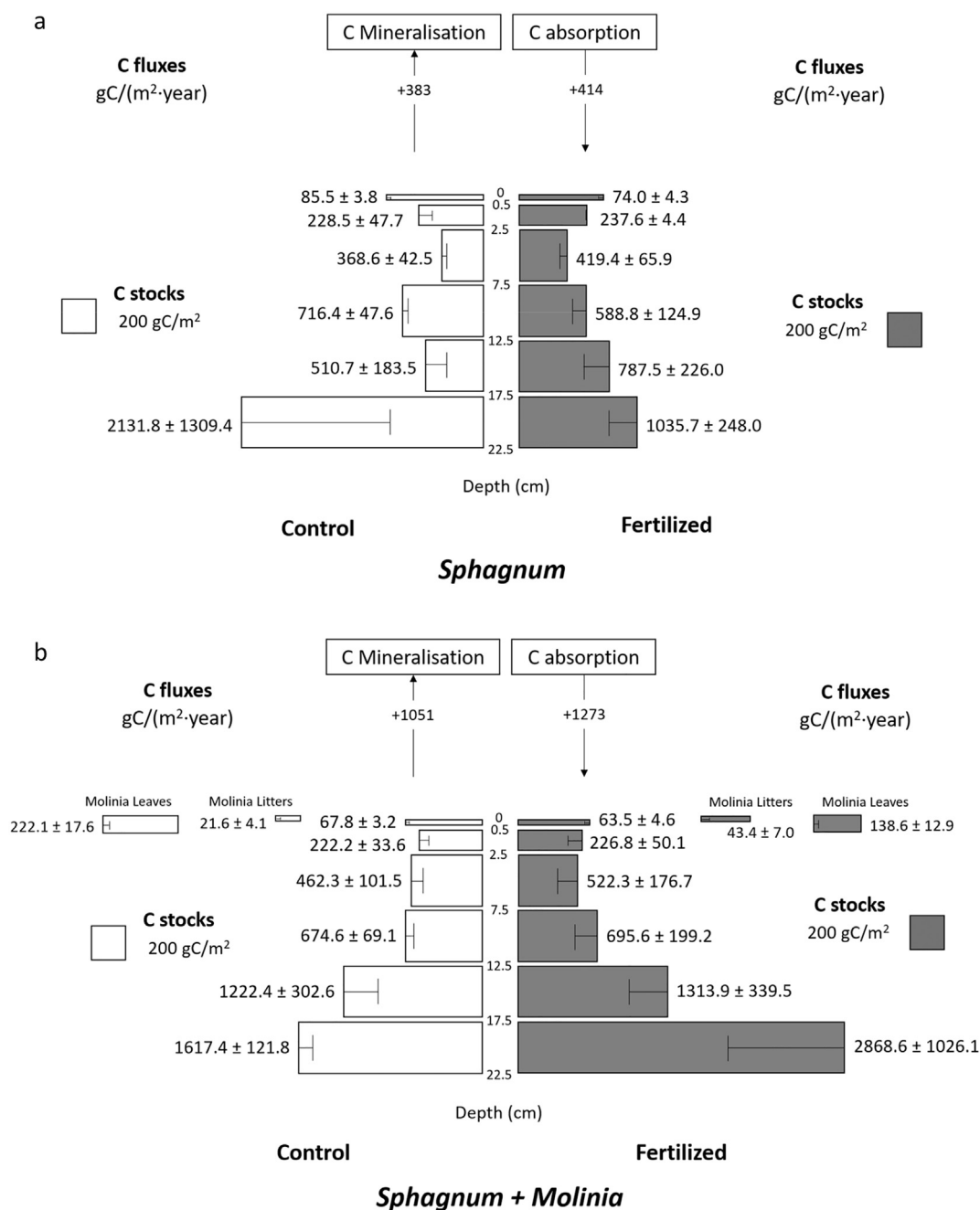
## 5. Conclusions

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

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

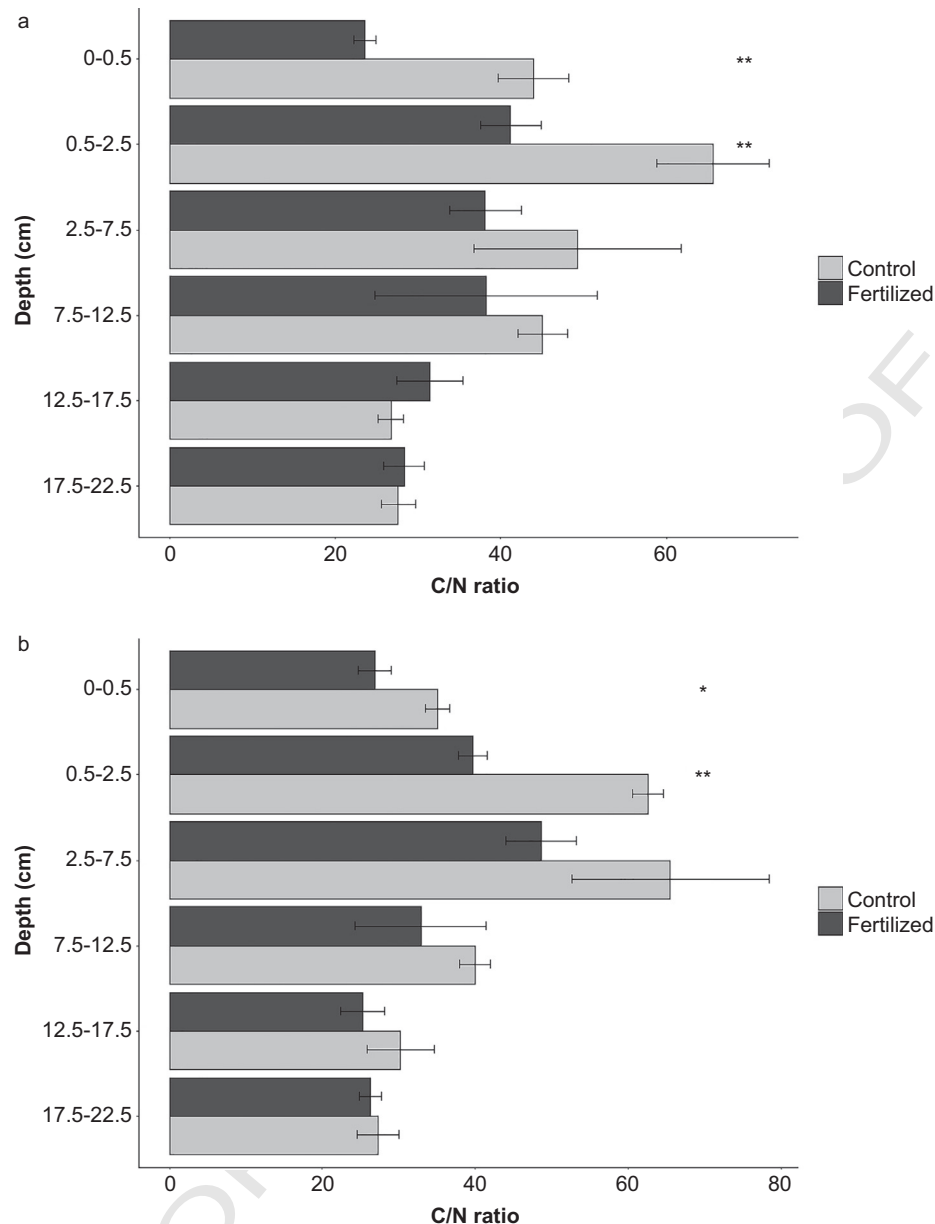
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## Appendix A. Supplementary data

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





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

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