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Stoichiometric response of shrubs and mosses to long-term nutrient (N, P and K) addition in an ombrotrophic peatland

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Abstract

Background and aim Although ombrotrophic peatlands are nutrient deficient, it is not clear to what extent plants will respond to changes in nutrient availability.

Methods We examined the changes in foliar stoichiometry and species abundance of four shrub species and moss after a decade of nitrogen (N), phosphorus (P) and potassium (K) fertilization at the Mer Bleue bog, eastern Canada.

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Results Shrub abundance increased and moss cover decreased after fertilization with 6.4, 5 and 6.3 g m^{-2} yr^{-1} of N, P and K, respectively; foliar concentrations of N, P, K and calcium (Ca) and magnesium (Mg) were affected. Stoichiometry showed mainly N limitation after P and K fertilization and P (co)limitation after high levels of N addition in shrubs; moss showed consistent K or KN-co-limitation, even with PK and NPK additions. Shrubs exhibited the strongest homeostasis (the maintenance of an organism's tissue chemical composition with changes in environmental resources) to N, with the homeostatic regulation coefficient (H)>9.7, compared to 1.4 in moss. For P and K, shrubs showed weaker homeostasis than N, while moss had a stronger homeostasis.

Conclusions The strong homeostasis of shrubs may be an adaptive strategy to limited availability of soil N and P.

Introduction

Northern peatlands store large amounts of carbon (C) and nitrogen (N) in soil organic matter (436 and 11 Gt, respectively; Loisel et al. 2014), but generally have low availability of nutrients arising from little nutrient input from the atmosphere or underlying mineral soil,

and slow rates of organic matter decomposition and nutrient release (Charman 2002). Bogs, which cover 0.7 million km^2 in Canada (Tarnocai 2006) have average foliar N:P ratios of dominant species of 14.8 (±0.9, mean±SE, $n=7$ sites) (Pakarinen and Gorham 1983; Malmer et al. 1992; Thormann and Bayley 1997; Walbridge and Navaratnnam 2006; Wang and Moore 2014) and plants are often N limited or co-limited by N and P (Koerselman and Meuleman 1996). Increased atmospheric deposition of N (Turunen et al. 2004) and P (Tipping et al. 2014) as well as accelerated organic matter decomposition through warming and lowered water tables (Charman 2002) may shift nutrient limitation regimes, stimulate plant growth in the short term (Aerts et al. 1992; Bragazza et al. 2004) and alter community composition and ecosystem nutrient cycling in the long term (Berendse et al. 2001; Bubier et al. 2007). In addition, there may be potassium (K) limitation, especially under increasing ambient CO_2 levels and N enrichment (Hoosbeek et al. 2002).

To assess the effect of increased nutrient availability on bog vegetation, we conducted a long-term nutrient fertilization experiment over a decade at Mer Bleue bog in eastern Ontario, Canada. The experimental site was located in the ombrotrophic part of the peatland, where the vegetation was mainly comprised of dwarf evergreen shrubs with an underlying ground layer dominated by *Sphagnum* mosses and *Polytrichum strictum*. We measured foliar nutrient concentrations of N, P, K, calcium (Ca) and magnesium (Mg) and species abundance of four shrub species and a combination of three moss species in response to N, P and K fertilization, at varying levels and combinations.

We applied three approaches to the analysis of the results:

i)   Comparison of differences of single element concentrations between fertilization treatments and the control, and among different species.
ii) Ecological stoichiometry to establish changes in ratios among nutrient elements in leaf tissue, providing insight into nutrient limitation of net primary productivity (Sterner and Elser 2002).
iii) Stoichiometric homeostasis, the degree to which an organism maintains its tissue chemical composition constant, despite changes in the relative availability of element resources in the external

environment (Kooijman 1995). Little is known about the stoichiometric homeostasis of terrestrial plants, which are developmentally more complex and expected to show stronger homeostasis than algae or fungi (Sterner and Elser 2002). Sterner and Elser (2002) defined a regulation coefficient (H) to quantify the degree of stoichiometric homeostasis from the power function of $y=cx^{1/H}$, where y and x are the stoichiometric element ratios or concentrations (masses or ratios of substances) of the organism and the external conditions (e.g. food or external environment), respectively, and c is a constant. Yu et al. (2010) reported average H_N (elements as subscript), H_P and $H_{N:P}$ of 5.6 (±2.6, SD) in a Mongolian grassland community, noting the association between strong homeostasis and a conservative nutrient strategy. This suggests that species with strong homeostasis are dominant, leading to large productivity and stability, which may be an important ecological strategy in other nutrient-deficient ecosystems.

Elevated N deposition over the past decades in eastern Canada (Turunen et al. 2004) may have alleviated the predominant N limitation and may account for the N and P co-limitation at Mer Bleue bog (Wang et al. 2014). The single addition of N or P will probably lead to P or N-limitation, respectively. Therefore, we anticipated that N-only additions would not facilitate further uptake of N proportionally, owing to the deterioration of P (or K-co) limitation compared to the addition with NPK together. Similarly, the PK-only addition would not lead to the uptake of P or K proportionally, owing to the deterioration of N limitation compared to the addition with NPK together. As a result, we hypothesized:

(1) Concentrations of N, P and K in foliar tissues would increase in response to the type and magnitude of addition, and concentrations of Ca and Mg would decrease, resulting in a shift of nutrient limitation, based on stoichiometric ratios.
(2) Plants would be less responsive to N-only fertilization than to NPK fertilization, that is, in terms of stoichiometric homeostasis, $H_{N(+N)} > H_{N(+NPK)}$.
(3) Plants would be less responsive to PK-only fertilization than to NPK fertilization, that is, $H_{P(+PK)} > H_{P(+NPK)}$.

(4) There would be no difference in the degree of homeostasis to K between two sets of experiments (PK-only *versus* NPK) with large *H*, as K is not a limiting nutrient.

(5) Strong stoichiometric homeostasis will enhance productivity and increase community biomass.

Methods

Study site and species description

This study was conducted at Mer Bleue bog, located 10 km east of Ottawa, eastern Ontario, Canada (45.41° N, 75.52° W). The monthly average air temperature in January and July is −10.8 and 20.9 °C, respectively, with a mean annual temperature 6.0 °C. The average precipitation from May to August is 350 mm and the mean annual precipitation is 943 mm with 76 % as rainfall (Canadian Climate Normal 1981–2010). The total atmospheric wet N deposition is ~0.8 g m^{-2} yr^{-1} (Turunen et al. 2004) and the total wet P deposition in eastern Canada ranges from 0.006 to 0.026 g P m^{-2} yr^{-1} (R. Vet personal communication).

Two sets of fertilization experiments were established with triplicate 3×3 m plots on the hummock microtopography at Mer Bleue bog (Table 1). Experiment 1 started in 2000 with an initial set of four fertilization treatments (C1, PK, 5N and 5NPK), complemented with an additional two treatments (10NPK and 20NPK) in 2001. To study the effect of larger N deposition, we initiated the second experiment in 2005 without the addition of P and K (C2, 10N and 20N). All the experiments followed a stratified random design to avoid hollow microtopography within the plots (Bubier et al. 2007). The 10NPK and 20NPK plots were aligned adjacent to each other to minimize contamination. All the plots were separated by 1 m buffer zones with a total of 27 plots (9 treatments×3 replicates/treatment) and a total spatial extent of ~600 m^2 (~30× 20 m). Nutrients were applied in soluble form: N as ammonium nitrate (NH_4NO_3), and P and K as monopotassium phosphate (KH_2PO_4), dissolved in 2 mm deionized water, every 3 weeks from May to August.

The dominant vascular plants are evergreen shrubs: *Chamaedaphne calyculata* (L.) Moench and *Rhododendron groenlandicum* (Oeder) K.A. Kron & W.S. Judd (formerly *Ledum groenlandicum* Oeder),

with deciduous *Vaccinium myrtilloides* Michx. and evergreen *Kalmia angustifolia* L. distributed sparsely in all the plots. The dominant mosses are *Sphagnum capillifolium, S. magellanicum* and *Polytrichum strictum* Brid.

Foliar sampling and chemical analysis

Two samples (~40 leaves each, excluding petioles) of fully expanded and sun-exposed current year's leaves of evergreen shrubs and mature leaves of deciduous *V. myrtilloides* from each plot were collected between 9 and 11 July 2012. These two samples were averaged prior to further statistical analyses (*n*=3). The capitulum of *S. capillifolium, S. magellanicum* and the upper 1 cm of new growth of *P. strictum* were collected and combined to one sample (i.e. 'moss') from each plot (*n*=3). Each sample consisted of over 90 % of *Sphagnum* mosses and less than 10 % of *P. strictum*. Samples were oven-dried at 60 °C to a constant weight and ground (Wiley Mini Mill 3383-L10, Thomas Scientific, USA) to fine powder (60-mesh sieve). Carbon and N concentrations were determined by dry combustion on an elemental analyzer (Leco CNS 2000, Leco Corporation, St. Joseph, Michigan, USA). Total P concentration was measured colorimetrically by ammonium molybdate-ascorbic acid method (Murphy and Riley 1962) on a LarchatQuik-Chem AE flow-injection auto-analyzer (Lachat Instruments, Milwaukee, Wisconsin, USA), after digestion in concentrated sulphuric acid and hydrogen peroxide with selenium and lithium sulphate as catalysts (Parkinson and Allen 1975). After digestion, samples were filtered through 0.45 μm glass fibre filters (MN 85/90, MACHEREY-NAGEL GmbH & Co. KG, Germany) before analyses. Potassium, Ca and Mg concentrations were determined on an atomic absorption spectrophotometer (Perkin Elmer model 2380, Perkin Elmer Inc., USA).

Soil nutrient availability

Plant root simulator (PRS$^{™}$) ion-exchange resin probes (Western Ag Innovations, Saskatoon, Canada) were used to determine the availabilities of N (the total of ammonium and nitrate), P (phosphate), K, Ca and Mg in peat soil at the peak growing season (1 to 31 July 2012).

Table 1 The fertilization experimental design at Mer Bleue bog

We have annually applied 5 (1.6 g N m^{-2}), 10 (3.2 g N m^{-2}), and 20 times (6.4 g N m^{-2}) the estimated summer time atmospheric wet N deposition with or without P (5 g m^{-2}) and K (6.3 g m^{-2}). Treatments are abbreviated accordingly. C1 and C2 are the controls (added distilled water only) for the two sets of experiment, respectively

Treatment	Start year	N (g m^{-2} yr^{-1})	P (g m^{-2} yr^{-1})	K (g m^{-2} yr^{-1})
Experiment 1				
C1	2000	0	0	0
PK	2000	0	5	6.3
5N	2000	1.6	0	0
5NPK	2000	1.6	5	6.3
10NPK	2001	3.2	5	6.3
20NPK	2001	6.4	5	6.3
Experiment 2				
C2	2005	0	0	0
10N	2005	3.2	0	0
20N	2005	6.4	0	0

Four pairs of PRS™ probes, one set adsorbing cations and the other adsorbing anions, were installed 10 cm below the peat surface in each plot for a period of 1 month. After retrieval, all soil and litter residues were brushed off the probes and the probes were rinsed with deionized water and stored at 4 °C until analysed. Each set of probes per plot were combined and extracted with 0.5 M HCl and ammonium and nitrate concentration in the extractant were analyzed colorimetrically using an automated flow injection analysis system (Technicon Autoanalyzer II, Technicon Instrument Corporation, Tarrytown, New York, USA). Phosphate, K, Ca and Mg were measured by inductively-coupled plasma spectrometry (Perkin Elmer Optima 3000-DV ICP, Perkin Elmer Inc., Shelton, Connecticut, USA). More details about the preparation and analysis of PRS™ probes can be found in Hangs et al. (2004). Nutrient availabilities are reported as mg m^{-2} of the membrane surface area over 1 month (Supplementary Table S1). As an additional approach to nutrient availability, particularly for mosses, we used the annual addition, comprising the estimated ambient atmospheric deposition (0.6 g N m^{-2} yr^{-1}, 0.02 g P m^{-2} yr^{-1} and 0.15 g K m^{-2} yr^{-1}) plus the amount added in the fertilizer treatments (Supplementary Table S2).

Point intercept measurements

Vegetation structure and species composition were measured using a point intercept method (Buttler 1992). The number of times ('hits') a plant species and organ (e.g. leaf/woody stem/flower/moss shoot) came in contact with a metal rod (radius 4 mm) over 61 grid points in preinstalled 60×60 cm collars was recorded in each plot (n=3/treatment). We analyzed the among-treatment differences in abundance (the total hits of leaf and woody stem) of *C. calyculata*, *R. groenlandicum*, *K. angustifolia* and *V. myrtilloides* and in the cover of mosses.

Statistics

All data were log(x+1) transformed for statistical analysis, but the raw (untransformed) data were reported in Figures and Tables. The overall variation among treatments and species in nutrient concentrations and stoichiometric ratios, and in species composition, were examined using one-way ANOVA for experiment 1 and 2 separately. Levene's test was conducted to examine the homoegeneity of the variance. Difference between individual treatments and corresponding control were assessed by Dunnett's post hoc test for experiment 1 and 2 separately, with α=0.01 to control the Type I error when performing multiple comparisons. Differences among species under the same treatment were examined by Fisher's Least Significant Difference test with Bonferroni adjustment (adjusted P values were 0.01).

We acknowledge the term 'homeostasis' has broader implications in other fields and it may be controlled by factors other than nutrient availabilities and interactions. Here, we confined homeostasis to the stability of nutrient concentrations and stoichiometric ratios in photosynthetically active tissues (i.e. leaves of shrubs and capitulum of mosses) in response to different levels of fertilization. The

homeostatic regulation coefficient (H) was estimated by linearizing the power function ($y = cx^{1/H}$) using logarithms (Sterner and Elser 2002):

$$log(y) = log(c) + \frac{log(x)}{H}$$

Where y is the nutrient concentration or stoichiometric ratio in leaves or capitula, x is the nutrient availability or stoichiometric ratio in the peat from PRS™ probes and c is a constant. As H was expected to be equal or greater than 1 (Sterner and Elser 2002), the slope ($1/H$) should be between 0 and 1. We used a one-tailed t-test with $\alpha = 0.1$ to examine the significance of the slope to be greater than 0. When the regression relationship was insignificant (i.e. $P > 0.1$), $1/H$ was usually set to 0 (i.e. H approaches infinity, e.g. Makino et al. 2003) and the organism was considered strictly homeostatic. However, simply considering the organism as strictly homeostatic when the ordinary least squared regression slope is insignificant ($P > 0.1$) does not discriminate the cases of strict homeostasis from highly variable stoichiometry in organisms arising from noisy data and other factors controlling an organism's stoichiometry, other than resource stoichiometry. For each case when the regression model was insignificant, we compared the root mean square of error (RMSE) (i.e. the residual error, Zar 2009) for each nutrient concentration and stoichiometric ratio with their standard deviation. If these two metrics did not differ remarkably, we considered it appropriate to classify this as strict homeostasis (Persson et al. 2010). The normality of all the data was examined by Shapiro-Wilk test.

For each aspect of H (H_N, H_P and H_K), we examined the differences in H that were derived from different sets of treatments (i.e. +N, +PK or +NPK) for each species. As each H was derived from the reciprocal of a regression slope, we tested the differences in H by comparing the difference between/among regression slopes (Zar 2009). To assess the significance of H, we only included plots that had direct fertilization effect on the relative availability of element resources. Specifically, for H_N, we included N-only treatments (5N, 10N and 20N), NPK treatments (5NPK, 10NPK and 20NPK) and controls. For H_P and H_K, we included PK-only treatment, NPK treatments and controls. If there was no significant difference in H between/among different sets of treatments, H was reported by combining all data in the regression model.

We also examined the inter-specific difference in each aspect of H by comparing the regression slopes among *C. calyculata*, *K. angustifolia*, *R. groenlandicum*, *V. myrtilloides* and moss. The results showed that there were significant differences between H_P derived from PK-only treatment (i.e. $H_{P(+PK)}$) and NPK treatments (i.e. $H_{P(+NPK)}$) for *C. calyculata* and *K. angustifolia* (Table 2). Therefore, we used $H_{P(+PK)}$ of *C. calyculata* and *K. angustifolia* for the multiple comparison among species by including treatments that only had direct fertilization effect on the resource stoichiometry.

All statistical analyses were conducted in JMP 10 software (SAS Institute, Cary, NC).

Results

Fertilization effects on nutrient concentrations among species

The N concentration in the leaves of the shrubs (8.5 to 15.7 mg g^{-1}) was not significantly different among fertilized plots and the controls ($P > 0.05$; Fig. 1a). The N concentration in moss varied from 3.6 to 26.1 mg g^{-1}: 20NPK plots showed a significantly larger concentration (26.1 ± 1.6 mg g^{-1}, mean ± SE) than in C1 plots (3.6 ± 2.1 mg g^{-1}) ($P = 0.009$).

The P concentration varied from 0.7 to 2.5 mg g^{-1} in shrubs and 0.5 to 4.6 mg g^{-1} in moss under different levels of fertilization (Fig. 1b). The P concentration in all species was significantly larger in PK and NPK plots (1.6 to 4.6 mg g^{-1}) than in C1 plots (1.0 to 1.1 mg g^{-1}) ($P < 0.001$), except for *V. myrtilloides* ($P > 0.01$). The P concentration in moss was significantly larger in 20N plots (0.9 ± 0.1 mg g^{-1}) than in C2 plots (0.7 ± 0.04 mg g^{-1}) ($P = 0.007$).

The K concentration varied from 3.2 to 8.3 mg g^{-1} in shrubs and 1.0 to 1.6 mg g^{-1} in moss with different levels of fertilization (Fig. 2a). The K concentration in *C. calyculata* and *K. angustifolia* were significantly larger in PK and NPK plots (5.7 to 8.4 mg g^{-1}) than in C1 plots (3.6 to 5.5 mg g^{-1}) ($P < 0.01$). There was no significant difference in K concentration between fertilized plots and the controls in moss ($P > 0.5$), which showed a significantly smaller K concentration than in the shrubs in all plots by up to 86 % ($P < 0.001$).

The Ca concentration did not vary substantially between fertilized plots (2.4 to 6.0 mg g^{-1}) and the controls (3.7 to 5.6 mg g^{-1}) (Fig. 2b). Although not significant,

Table 2 Results of regression analysis between nutrients and stoichiometric ratios in foliar tissues and soil based on PRS™ probe measurements

Species	Aspects	Slope	H	R^2	RMSE	SD	P value
C. calyculata	N	0.092	10.870[A]*	0.087	0.094	0.096	0.081
	$P_{(+PK)}$	0.153	6.536[Aa]	0.822	0.067	0.148	<0.001
	$P_{(+NPK)}$	0.211	4.739[b]	0.922	0.053	0.183	<0.001
	K	0.173	5.780	0.415	0.067	0.084	0.004
K. angustifolia	N	0.090	11.074[A]	0.070	0.105	0.106	0.090
	$P_{(+PK)}$	0.167	5.988[Aa]	0.951	0.036	0.151	<0.001
	$P_{(+NPK)}$	0.222	4.505[b]	0.918	0.057	0.193	<0.001
	K	0.166	6.024	0.359	0.072	0.087	0.009
R. groenlandicum	N	0.103	9.709[A]	0.248	0.079	0.089	0.013
	P	0.215	4.651[BC]	0.931	0.049	0.180	<0.001
	K	0.147	6.803	0.533	0.045	0.063	<0.001
V. myrtilloides	N	0.080	∞[A]	0.048	0.112	0.112	0.302
	P	0.199	5.025[AB]	0.770	0.091	0.184	<0.001
	K	0.276	3.623	0.425	0.104	0.133	0.003
Moss	N	0.739	1.353[B]	0.286	0.370	0.428	0.007
	P	0.425	2.353[C]	0.926	0.100	0.358	<0.001
	K	0.059	∞	0.052	0.082	0.081	0.361

RMSE: root mean square of error. SD: standard deviation of the dependent variables. The homeostatic regulation coefficient (H) is calculated as the inverse of the regression slope. Different capitalized letters indicate significant differences in the same aspect of H among species at $P<0.01$. Different low case letters indicate significant differences in the same aspect of H derived from different sets of experiment for the same species at $P<0.01$. The subscripts of the aspects indicate the fertilization treatments: +N, N-only treatment; +PK, PK-only treatment; +NPK, NPK treatments. Treatment abbreviations as described in Table 1

* An example for the calculation of H was shown in Supplementary Figure 2

the highest level of N addition alone (20N) or with PK (20NPK) reduced Ca concentrations in some species, whereas no similar fertilization effect occurred for Mg (Fig. 2c). *V. myrtilloides* showed significantly larger Mg concentration than the other shrubs ($P<0.01$). The Mg concentration in moss (4.9 to 9.6 mg g^{-1}) was significantly larger than in shrubs (0.7 to 2.3 mg g^{-1}) in all plots by up to ten-fold ($P<0.001$).

Changes in species abundance in response to fertilization

The moss cover decreased significantly in NPK plots compared to C1 ($P<0.01$; Fig. 3e). In 20NPK plots, moss cover was substantially reduced by 99.7 % compared to C1 ($P<0.001$). In general, *C. calyculata* was the dominant shrub in most of the plots, comprising 35 to 92 % of the shrub abundance (Fig. 3a), followed by *R. groenlandicum* at 5 to 50 %. *Rhododendron groenlandicum* dominated in 20N plots (50 %) (Fig. 3c). The total abundance of shrubs was significantly larger in

10NPK and 20NPK plots than in C1 by 87 and 81 % respectively ($P<0.001$; Fig. 3f).

Stoichiometry and homeostasis

Shrubs showed similar N:P:K stoichiometric characteristics to each other in response to fertilization in mid-July (Fig. 4), showing N limitation after P and K addition (i.e. PK, 5NPK, 10NPK and 20NPK), and P or P, N-co-limitation after high levels of N addition (i.e. 10N and 20N) (Fig. 5a). Moss species showed a consistent pattern of K or K, N-co-limitation, even in PK and NPK plots (Fig. 5b).

In general, when using the PRS data for nutrient availability, all shrubs showed large values for H_N, ranging from 9.7 (*R. groenlandicum*) to infinity (*V. myrtilloides*) and moss showed a significantly smaller H_N of 1.4 ($P<0.001$; Table 2). All shrubs showed weaker homeostasis to P and K than N, with values of 4.5 to 6.5 (H_P) and 3.6 to 6.8 (H_K), whereas moss showed a stronger homeostasis to P and K with H of

Fig. 1 Nitrogen (N) and phosphorus (P) concentrations in the foliar tissues of different species in response to long-term N, P and K fertilization. Bars are mean with SE (n=3/treatment). Asterisks indicate a significant difference at $P<0.01$ between individual treatments and the control in experiment 1 (C1) and experiment 2 (C2) separately for the same species. Different lower-case letters indicate a significant difference among species under the same treatment. Treatment abbreviations as described in Table 1
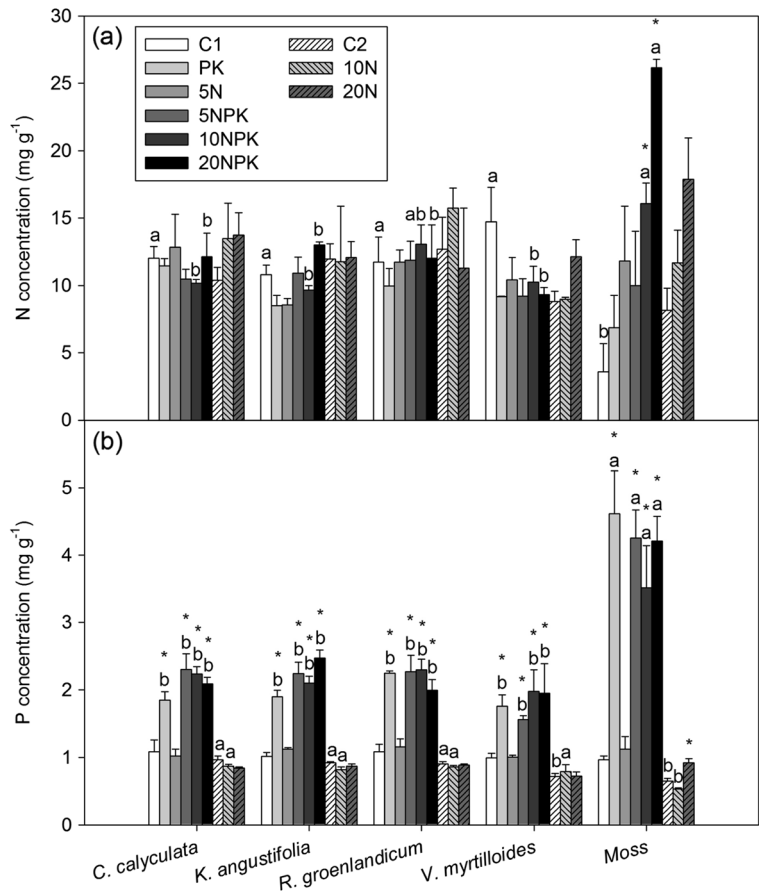
2.4 (H_P) and infinity (H_K), compared to N. Moss showed a significantly smaller H_P than shrub species ($P<0.001$). The interaction between N and PK treatments showed a significant effect on some aspects of the homeostatic coefficient in *C. calyculata* and *K. angustifolia*: H_P was significantly larger in PK plots than in NPK plots (i.e. $H_{P(+PK)}>H_{P(+NPK)}$) (Table 2).

When annual nutrient input was used to assess nutrient availability, essentially similar patterns of homeostasis were recorded (Supplementary Table S2) and there was a strong correlation between the H values based on PRS probe and annual nutrient input (Supplementary Figure S1).

Discussion

Stoichiometric homeostasis in bog vegetation

The strong homeostasis in shrub leaves in our study may be related to phenotypic plasticity, nutrient translocations and the allocation of biomass and nutrients to belowground tissues. Yu et al. (2011) noted that strong homeostasis in aboveground biomass was associated with weak homeostasis in the belowground biomass and *vice versa*. At Mer Bleue, hummock shrubs allocate a large proportion of their biomass belowground with the aboveground/belowground biomass ratio of 0.56±0.30 (mean±SD) (Murphy et al. 2009). This suggests a potential for storing nutrients belowground biomass to keep foliar elemental composition stable.

In contrast, moss showed weak homeostasis in the photosynthetically active tissues except for K. Mosses have powerful capillary networks, especially in *Sphagnum*, which can transport water and metabolites upwards to the growing capitulum (Rydin and Clymo 1989; Aldous 2002; Rydin and Jeglum 2006). The addition of large amounts of N (6.4 g N m^{-2} yr^{-1}) with P and K did not increase the N concentration in shrub leaves, but increased N in moss capitula. These loads of N addition are much larger than the 1 g m^{-2} yr^{-1} critical threshold suggested by Lamers et al. (2000) and

Fig. 2 Potassium (K), calcium (Ca) and magnesium (Mg) concentrations in the foliar tissues of different species in response to long-term N, P and K fertilization. Bars are mean with SE (*n*=3/ treatment). Asterisks indicate a significant difference at *P*<0.01 between individual treatments and the control in experiment 1 (C1) and experiment 2 (C2) separately for the same species. Different lower-case letters indicate a significant difference among species under the same treatment. Treatment abbreviations as described in Table 1
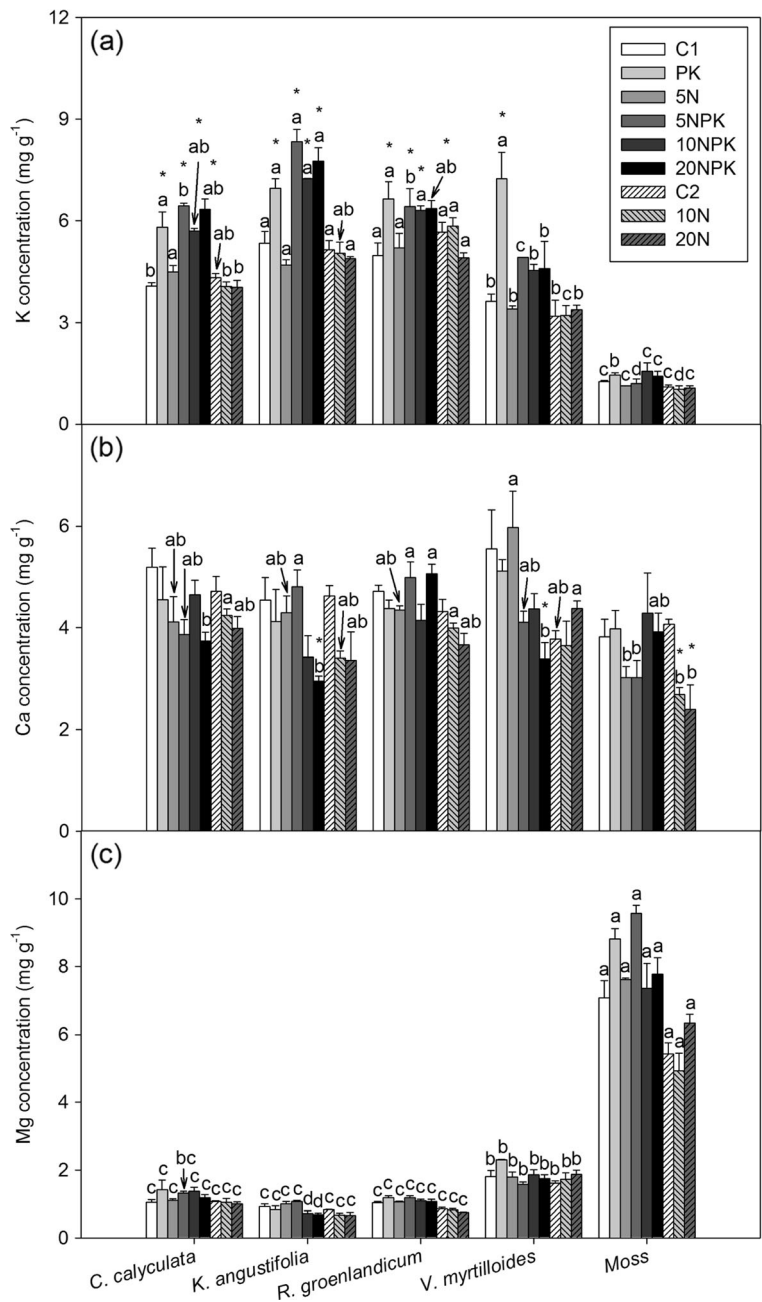
Berendse et al. (2001), above which the capacity of moss to filter excess N fails. Vitt et al. (2003) found the critical toxic load for *Sphagnum* to be 1.7 to 3.4 g N m^{-2} yr^{-1} in western Canada. The substantial decrease of moss cover in 20N and 20NPK plots at Mer Bleue may be related to toxicity arising from large N load (> 6.4 g N m^{-2} yr^{-1}, including atmospheric N deposition). With a large increase in N input,

Sphagnum moss may not be able to assimilate all the N, resulting in the storage of N as amino acids (Limpens and Berendse 2003) and accounting for the weak N homeostasis in moss. Furthermore, moss continuously creates cation exchange sites that may efficiently capture the nutrients from fertilization without assimilating all of them into living cells (Rydin and Jeglum 2006), further contributing to weak homeostasis.
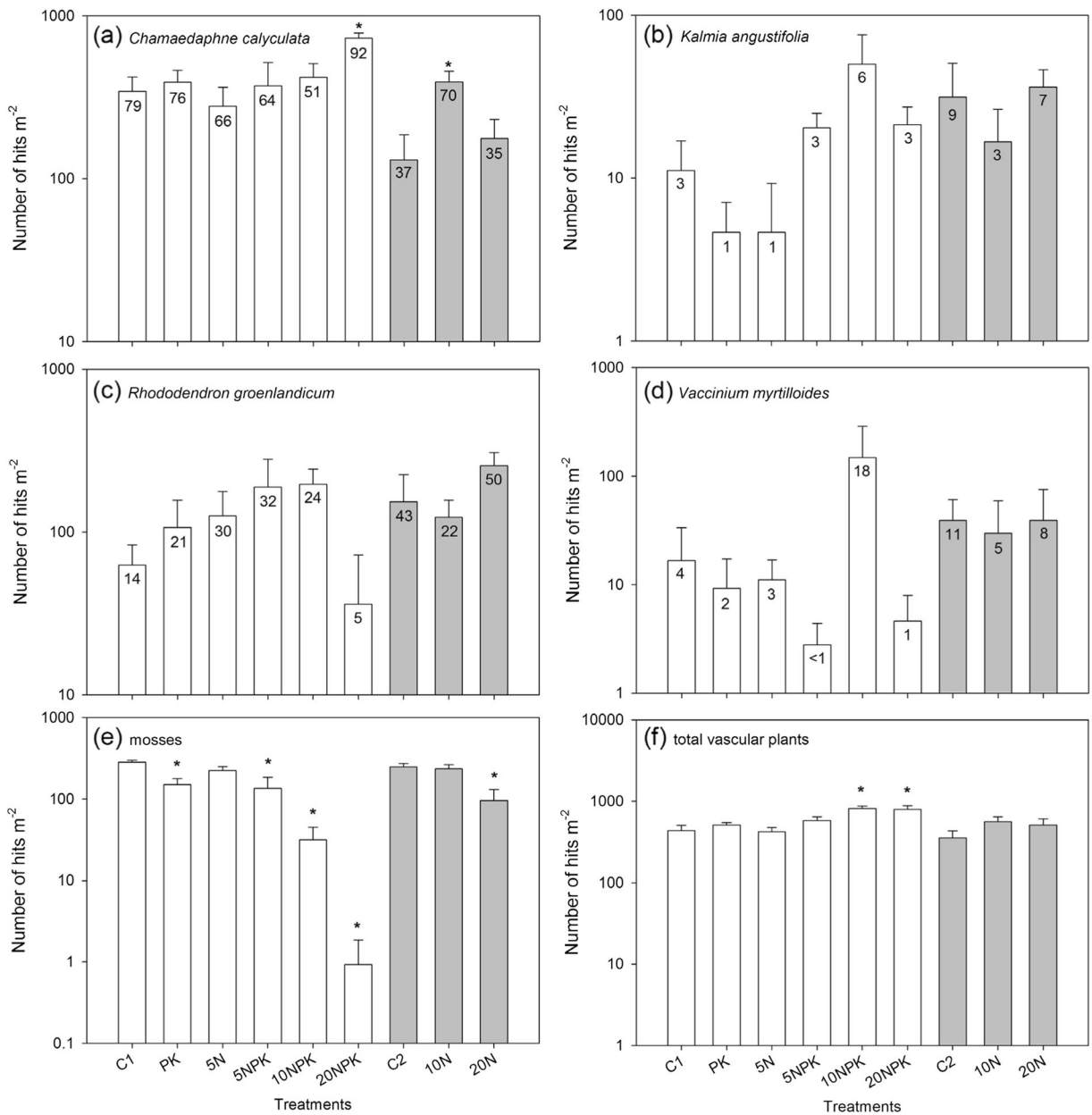
Fig. 3 Vegetation abundance of *C. calyculata* (**a**), *K. angustifolia* (**b**), *R. groenlandicum* (**c**), *V. myrtilloides* (**d**), mosses (**e**) and total vascular plants (**f**) in response to long-term N, P and K fertilization. Data are expressed on a logarithmic (base$_{10}$) basis. Bars are mean with SE (*n*=3/treatment). *Asterisks* indicate a significant difference at *P*<0.01 between individual treatments and the control in experiment 1 (C1, open bars) and experiment 2 (C2, filled bars) separately for the same species. Numbers within the bars indicate the percentage of the abundance of each vascular plant over the total abundance of all vascular plants under each treatment. Treatment abbreviation as described in Table 1

Contrary to our second hypothesis that plants would show stronger homeostasis to N in N-only plots than in NPK plots due to N and P interaction, there was no difference in H_N between the two sets of treatments in either shrubs or moss, resulting from N saturation. The third hypothesis that plants would show stronger homeostasis to P in PK-only plots than in NPK plots is observed only in *C. calyculata* and *K. angustifolia*. The large increase of P concentration and shrub abundance in response to (N)PK addition supports the notion of P
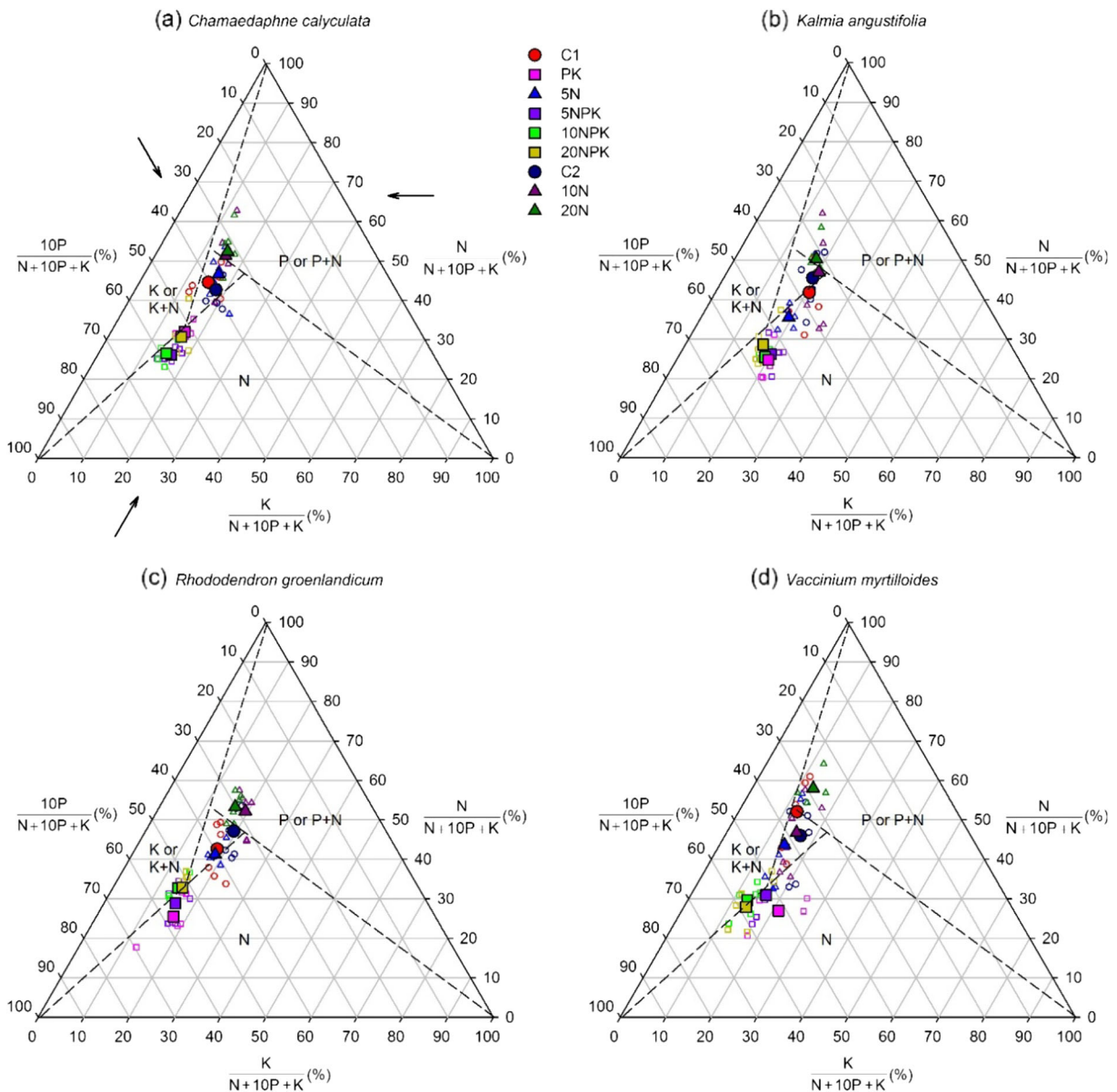
Fig. 4 Ternary diagram showing the stoichiometric relationship of N, P and K in shrub species in response to long-term N, P and K fertilization. Open and filled symbols are individual replicate and mean under each treatment, respectively. Dashed lines indicate the critical ratios of N:P (14.5), N:K (2.1) and K:P (3.4) (Olde Venterink et al. 2003). These lines divide the plot into four sections, three of which indicate N limitation (N section), P or P, N-co-limitation (P or P+N section) and K or K, N-co-limitation (K or K+N section), respectively. For the central triangle section, the N:P:K stoichiometric ratios cannot be used to determine the type of nutrient limitation or this is non-NPK limitation. For visual reasons, the P concentration is multiplied by a factor of 10. Arrows indicate the direction in which the axes should be read. Treatment abbreviations as described in Table 1

(co)limitation at Mer Bleue. Like N, the substantive amount of P and K added exceeds the capacity of moss to filter, making them available to shrubs (Lamers et al. 2000). The competitive advantage of shrubs over moss after nutrient enrichment is detrimental for the function of *Sphagnum* to engineer the acidic, nutrient-deficient

and wet environment in bogs. Bragazza et al. (2013) have shown that soil warming and the subsequent drought due to climate change facilitated the expansion of ericaceous shrubs and reduced the productivity of peat mosses by affecting the interactions between shrubs and soil microbes and consequently altered soil C and N
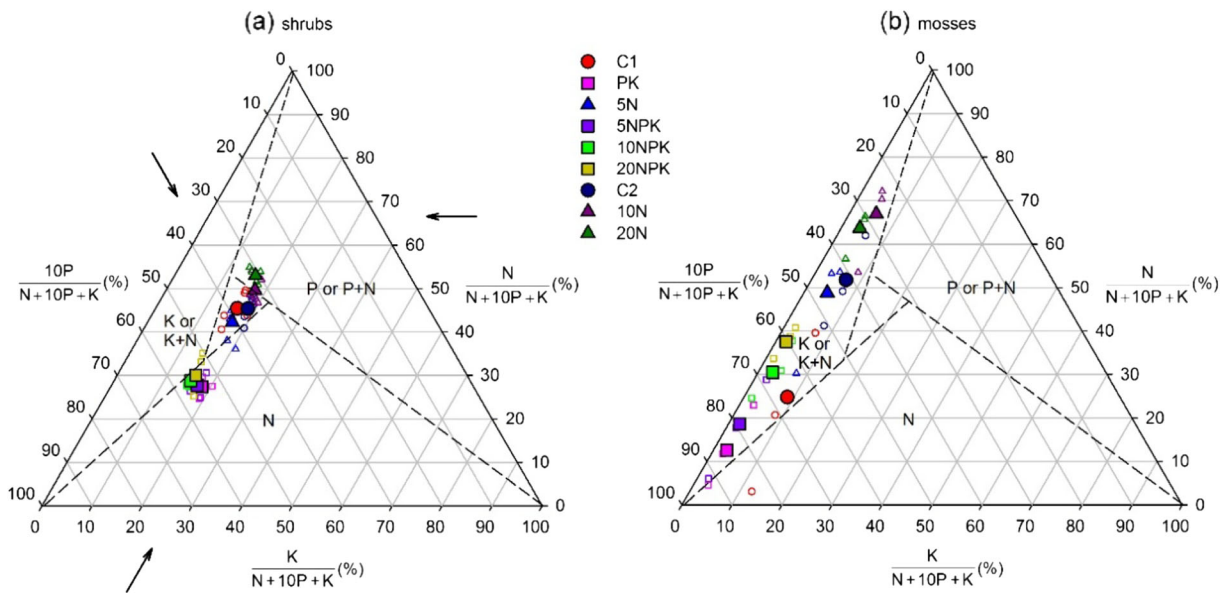
Fig. 5 Ternary diagram showing the stoichiometric relationship of N, P and K in response to long-term N, P and K fertilization in shrubs (**a**) and mosses (**b**). Open and filled symbols are individual replicate and mean under each treatment, respectively. Treatment abbreviations as described in Table 1

dynamics in peatlands. These climate-induced changes in plant community composition increased vascular plant cover at the expense of losing peat mosses and promoted the decomposition of soil organic matter by influencing soil enzymatic activities (Bragazza et al. 2013).

Bogs are poorly connected to the underlying mineral substrates, impeding the upward replenishment of mineral nutrients, and the sources of P to bogs are atmospheric P deposition. The few available atmospheric P deposition data for the area occupied by northern peatlands generally fall in the range of 7 to 34 mg m^{-2} yr^{-1} (Schindler et al. 1976; Newman 1995; Tipping et al. 2014) and the modeled total P atmospheric deposition estimate is <5 mg m^{-2} yr^{-1} (Mahowald et al. 2008). Northern peatlands accumulate P at approximately the same rate as they gain P from the atmosphere (Wang et al. 2015) and thus P may play a critical role in C and N cycling.

Vaccinium myrtilloides had larger Mg concentration than the other shrubs, suggesting that deciduous species may be better adapted to richer nutrient environments than evergreens; Mg deficiency may develop in acidic bogs (Rydin and Jeglum 2006). With a significantly larger Mg concentration, moss showed an extremely small K concentration attributed to the predominant K limitation in moss, perhaps because of leaching under

acidic conditions or replacement by Mg from the cation exchange sites, which prefer di-valent over uni-valent cations (Pakarinen and Tolonen 1977). The increase of foliar N, P and K concentrations and decrease of Ca concentration in response to the type and magnitude of fertilization partly supports our first hypothesis. Even though we accept our fourth hypothesis that the degree of homeostasis to K did not differ between PK-only and NPK treatments, it seems to be associated with the leaching of K under acidic condition or replacement by Mg in the cation exchange sites rather than the nature of K limitation at Mer Bleue.

Foliar N:P:K ratios can suggest different types of nutrient limitation for wetland plants (Olde Venterink et al. 2003). With lower atmospheric N deposition than European equivalents, Canadian bogs are often N limited (Malmer et al. 1992; Thormann and Bayley 1997) or co-limited by N and P (Pakarinen and Gorham 1983; Malmer et al. 1992), based on the foliar stoichiometry of the dominant species. In the current study, the N:P:K stoichiometry in shrubs suggests N limitation after P and K fertilization, and P (co)limitation after high levels of N addition, supporting our first hypothesis. Without extensive P inputs and with historically elevated atmospheric N deposition, P co-limitation develops in bogs and the shift from N to P (co)limitation in response to N enrichment has been observed in Europe (Aerts et al. 1992;

Bragazza et al. 2004; Jiroušek et al. 2011). Larmola et al. (2014) showed that N inputs from N_2 fixation in peatlands can far exceed those from atmospheric deposition and supported the notion that N_2 fixation is the primary mechanism linking N and P availability. The degree to which the N_2 fixation can mediate nutrient stoichiometry in peatlands deserves further attention. There was no evidence of K limitation based on leaf N:P:K stoichiometry in shrubs, but moss showed strong K limitation. Intriguingly, K concentration in moss did not increase after PK and NPK fertilization, perhaps due to the heavy leaching under acidic condition or replacement by Mg in cation exchange sites.

Homeostasis and species abundance: shrubs differ from moss

Yu et al. (2010) have suggested that species with strong homeostasis should dominate a plant community and lead to a larger productivity: hence our fifth hypothesis that strong homeostasis shrubs would replace weak homeostasis moss. At Mer Bleue, the heavy loads of N, P and K fertilization increased the abundance of shrubs at the expense of moss cover (Bubier et al. 2007; Juutinen et al. 2010; Larmola et al. 2013), noted in other bogs under elevated N input (Heijmans et al. 2001; Limpens et al. 2003; Tomassen et al. 2003). This is probably due to the toxic effect on moss growth (Limpens and Berendse 2003; Bragazza et al. 2005) and/or shading from increased shrub LAI and litter accumulation (Heijmans et al. 2002; Chong et al. 2012).

However, this change at Mer Bleue did not enhance ecosystem productivity. Larmola et al. (2013) found that the plot-level gross photosynthesis rates did not change significantly with fertilization as the greater shrub biomass compensated for the loss of the *Sphagnum* cover with the largest addition of N at Mer Bleue. In these heavily fertilized (20N and 20NPK) plots, net CO_2 assimilation per leaf area in the top shrub canopy was largely unaltered (Bubier et al. 2011). In particular under high NPK addition the net ecosystem uptake of CO_2 was substantially diminished owing to an increase in ecosystem respiration, a combination of plant respiration and microbial degradation of organic matter (Larmola et al. 2013). Granath et al. (2009) reported that the production of *Sphagnum* mosses was governed more by environmental factors, such as water content and light availability, than photosynthetic capacity, which explained the lack of relationship between the

maximum photosynthetic rate and biomass production. *Sphagnum* mosses can adjust physiologically to the high level of N deposition (Granath et al. 2009) before it exceeds the critical load (~1–1.5 g N m^{-2} yr^{-1}, e.g. Lamers et al. 2000; Bragazza et al. 2004) when the growth of *Sphagnum* starts to decrease.

There are several methodological concerns regarding homeostasis. As this long-term fertilization experiment has been running for 7 to 12 years, the dominant perennial shrubs with large nutrient stores belowground and fairly short leaf life-span (2–3 years) may have 'adapted' to elevated nutrient availability, which may influence the strong homeostasis observed here. A weaker homeostasis may appear if tested 1 or 2 years after the first fertilization. Factors other than nutrient availabilities, such as light, temperature and water availabilities, may affect stoichiometric homeostasis, which we are not able to rule out.

We have demonstrated strong and weak homeostasis in shrubs and moss, respectively, in a bog. The strong homeostasis of shrubs may be an evolutionary strategy or a long-term adaptation to impoverished soil fertility. Moss has a weak homeostasis and only survives from the limited availabilities of nutrients and the harsh micro-environment it establishes. Substantial nutrient enrichment from anthropogenic disturbance may break the balance between shrubs and moss; yet the dominance of shrubs with strong homeostasis has not increased ecosystem productivity at Mer Bleue.

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