The C:N:P:K stoichiometry in an ombrotrophic peatland

Meng Wang

Department of Geography
McGill University, Montreal

February 2014

A thesis submitted to McGill University in partial fulfillment of the requirement of the degree of Doctor of Philosophy

Abstract

Ombrotrophic peatlands are nutrient-deficient systems and important carbon (C) sinks, yet the stoichiometry of nitrogen (N), phosphorus (P) and potassium (K), essential for plant growth and decomposition, has rarely been studied in these systems. Under contemporary anthropogenic environmental changes, such as enhanced atmospheric deposition, the balance among multiple elements during ecological processes and interactions, i.e. ecological stoichiometry, is altered. The stoichiometry in plants and soils in peatlands and the response of these stoichiometric characteristics to nutrient enrichment are critical in a world of elemental imbalance between organisms and their environment. The objectives of this research were to examine the seasonal variation in C:N:P:K stoichiometry among different plant functional types (PFTs), the effects of nutrient addition on nutrient resorption and the degree of stoichiometric homeostasis, and the decomposition cascade of C:N:P stoichiometry from mature tissues to peat at Mer Bleue Bog, eastern Ontario, Canada. Along with a greater variation in nutrient concentrations and stoichiometric ratios among PFTs than across the growing season, a convergence of C:N:P:K to a mass ratio of 445:14:1:9 was found at peak growing season, indicating N and P co-limitation. A substantial resorption of N, P and K during leave senescence was observed and was significantly different between the two dominant shrub species, *Chamaedaphne calyculata* and *Rhododendron groenlandicum*. The interaction between N, P and K addition on their resorption reflected the stoichiometric coupling of nutrient cycling. In response to long-term N, P and K fertilization, shrubs and mosses showed strong and weak homeostasis, respectively; whereas the dominance of shrubs with strong homeostasis did not enhance the stability or the productivity of the plant community. The examination of the decomposition cascade of C:N:P stoichiometry confirmed the resorption-associated decrease in C:N and C:P ratios from mature to senescent tissues. A possible mechanism related to plant/mycorrhizae uptake of P may account for the increase in C:P and N:P ratios in peat. This thesis contributes new knowledge on the stoichiometry of plants and soils in an ombrotrophic peatland. Specifically, it emphasizes the important role of P in peatland biogeochemical cycles, especially the plant and mycorrhizal uptake of P in these nutrient-deficient ecosystems. The substantial variation in nutrient resorption and stoichiometric homeostasis between species and among PFTs shed new light on the potential shifts in plant community composition under environmental changes.

Résumé

Les tourbières ombrotrophes sont des systèmes déficients en nutriments et des puits importants de carbone (C). Cependant, la stoichiométrie de l'azote (N), du phosphore (P) et du potassium (K), nutriments essentiels pour la croissance des plantes et la décomposition, est peu étudiée. Dans un contexte de changements environnementaux anthropogéniques, incluant une augmentation de la déposition atmosphérique, l'équilibre entre les différents éléments dans le cadre des processus écologiques et des interactions (i.e. la stoichiométrie écologique) est altéré. La stoichiométrie des plantes et du sol dans les tourbières et la façon dont ces caractéristiques stoichiométriques répondent à l'addition de nutriments sont critiques dans un monde de débalancement des éléments entre les organismes et leur environnement. L'objectif de cette recherche était d'examiner les variations saisonnières dans la stoichiométrie C:N:P:K parmi différents types fonctionnels végétaux (TFVs), les effets de l'addition de nutriments sur la résorption des nutriments et le niveau d'homéostasie stoichiométrique, et la cascade de décomposition de la stoichiométrie du C:N:P des tissus matures à la tourbe à la tourbière Mer Bleue, Ontario, Canada. Avec une variation plus grande des concentrations en nutriment et des ratios stoichiométriques parmi les TFVs que le long de la saison de croissance, une convergence du C:N:P:K vers un ratio de masse de 445:14:1:9 lors du sommet de la saison de croissance a été observé, indiquant une co-limitation N-P. Une résorption substantielle de N, P et K pendant la sénescence des feuilles fut observée avec une variation interspécifique significative entre deux espèces dominantes d'arbustes, *Chamaedaphne calyculata* et *Rhododendron groenlandicum.* L'interaction entre l'addition de N, de P et de K sur leur résorption reflétait le couplage stoichiométrique du cyclage des nutriments. En réponse à la fertilisation à long terme du N, P et K, les bryophytes et les arbustes ont démontré une homéostasie forte et faible, respectivement; alors que la dominance des arbustes avec une forte homéostasie n'a pas amélioré la stabilité et la productivité de la communauté végétale. L'examen de la cascade de décomposition de la stoichiométrie du C:N:P a confirmé la diminution, associée à la résorption, des ratios C:N et C:P des tissus matures aux tissus sénescents. Un mécanisme relié au captage de P par une association plante/mycorhize pourrait expliquer l'augmentation des ratios C:P et N:P dans la tourbe. Cette thèse contribue à la connaissance de la stoichiométrie des plantes et des sols dans une tourbière ombrotrophe. Plus spécifiquement, elle met l'emphase sur le rôle important du P dans les cycles biogéochimiques des tourbières, en particulier le captage du P par les plantes et les mycorhizes dans ces systèmes déficients en nutriments. La variation substantielle entre les espèces et les TFVs en fait de résorption des nutriments et d'homéostasie stoichiométrique met en lumière les changements potentiels de composition des communautés végétales dans un contexte de changements environnementaux.

Table of Contents

List of Figures

List of Tables

Acknowledgements

There are a numerous people to whom I would like to thank for their help during my journey to finish this doctoral thesis. First and foremost, I would like to thank my supervisor, Professor Tim Moore (Department of Geography, McGill University), for his intellectual and financial supports that make this journey possible. I am grateful to Professor Moore for always being available to help. Professor Moore is a great mentor with his hospitality, generosity, practicality, strong skills of organization and a good sense of humour, all of which are the excellent characteristics that I would like to inherit for developing my future career and supervising my own students someday.

I am also indebted to my supervisory committee members, Professors Changhui Peng (Institut des sciences de l'environnement, Université du Québec à Montréal) and Nigel Roulet (Department of Geography, McGill University) who provided excellent intellectual support and offered constructive comments that greatly advanced the development of this thesis.

Special thanks to Professors Nathan Basiliko (Living with Lake Centre, Laurentian University), Christian Blodau (Department of Hydrology, University of Münster), Jill Bubier (Environmental Studies Department, Mount Holyoke College), Steve Frolking (Department of Earth Sciences, University of New Hampshire), Pierre Richard (Département de géographie, Université de Montréal) and Julie Talbot (Département de géographie, Université de Montréal) who provided useful advice and discussion relevant to my thesis. I would like to thank Professor Elyn Humphreys (Department of Geography and Environmental Studies, Carleton University) for providing data from the eddy covariance tower at Mer Bleue site and National Capital Commission for access to Mer Bleue.

I would like to thank Chinese Scholarship Council for providing me a 4-year Ph.D. fellowship. I appreciate the financial support from McGill University through McGill International Doctoral Student Awards (MIDAs), Provost's Graduate Fellowship, Campus Community Bursary and University International Tuition Support Bursary. Financial supports also generously came from Department of Geography, providing me Graduate Excellent Awards

and Graduate Research Enhancement and Travel (GREAT) Awards. Professor Moore offered me research assistantships and paid for research expense from his Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants. I also thank Global Environmental and Climate Change Centre (GEC^3) for graduate student stipend and student travel grants, and PEATNET for travel funding to support my presentation at international conferences.

I gratefully acknowledge the laboratory and field assistance from Agnieszka Adamowicz-Walczak (Centre de recherche en géochimie et géodynamique (GÉOTOP), Université du Québec à Montréal), Hicham Benslim (Department of Natural Resource Sciences, McGill University), Zhaojun Bu (Institute for Peat and Mire Research, Northeast Normal University), Vi Bui (Environmental Studies Department, Mount Holyoke College), Mike Dalva (Department of Geography, McGill University), Leanne Elchyshyn (Department of Biology, McGill University), Kellie Foster (Department of Chemical Engineering, McGill University), Angela Grant (Faculty of Agricultural and Environmental Sciences, McGill University), Hélène Lalande (Department of Natural Resource Sciences, McGill University), Tuula Larmola (Environmental Studies Department, Mount Holyoke College), Sheng-ting Lin (Department of Anatomy and Cell Biology, McGill University), Cori Magnusson (Environmental Studies Department, Mount Holyoke College), Cheenar Shah (McGill School of Environment, McGill University) and Zheng Wang (Department of Geography, McGill University).

Many thanks to my friends and the supporting staffs at Department of Geography and Global Environment and Climate Change Centre, including Amanda Alfonso, Christopher Amyot, Mandy Chong, June Connolly, Elisa David, Allison De Young, Ellie Goud, Silvie Harder, Lorna Harris, Benjamin Heumann, Paula Kestelman, Youngil Kim, Angela Kross, Derrick Yuk Fo Lai, Melanie Lazazzera, Mark Lalonde, Angela Mansi, Maria Marcone, Franca Mancuso, Avni Malhotra, Angie Mansi, Meaghan Murphy, Pauline Nesbitt, Drew Pinsonneault, Nancy Secondo, Ruilan Shi, Joseph Vacirca, Dongqi Wang, Weifeng Wang, Zheng Wang, Paul Wilson, Jianghua Wu and Tanja Živković.

Last, but not least, I sincerely thank my parents, Hongming Wang and Meihua Ren, and parents-in-law, Baoshan Shao and Jianli Zhang, who supported me unconditionally. My deepest gratitude goes to my beloved wife, Xiaoqian Shao, who was supportive with love, encouragement and understanding all the time. Her everlasting love and eternal commitment to our family made this dissertation possible. Finally, thanks go to my princess Emma and my prince Eason, who brought me a handful of happiness and hope. The miracle of life brought me the courage to cheer up on the way to success.

Contribution of authors

This thesis consists of four main chapters (Chapters 3 to 6) that are prepared as manuscripts following the standard requirements for publication in scientific journals. For all the four manuscripts, I developed the scientific questions, carried out the experimental designs, conducted the field work and laboratory analyses, analyzed the data, interpreted the results and wrote the manuscript as lead author. As my Ph.D. supervisor, Tim R. Moore offered suggestions on developing scientific questions, experimental designs and interpretation of results, helped with field work and laboratory analyses, and provided detailed comments on each manuscript. The roles of other co-author(s) are described as follows:

Manuscript #1 (Chapter 3): “Carbon, nitrogen, phosphorus and potassium stoichiometry in an ombrotrophic peatland reflects plant functional type” by Meng Wang and Tim R. Moore (published on *Ecosystems*, doi: 10.1007/s10021-014-9752-x). This study was conducted by me and my Ph.D. supervisor with no other co-author.

Manuscript #2 (Chapter 4): “Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog” by Meng Wang, Meaghan T. Murphy and Tim R. Moore (published on *Oecologia*, 2014, 174, 365–377). MTM was involved in experimental design, field sampling, and maintained the long-term fertilization experiment at Mer Bleue Bog where this study was conducted. All authors were involved in manuscript preparation.

Manuscript #3 (Chapter 5): “Differences in stoichiometric homeostasis between shrubs and bryophytes in an ombrotrophic peatland in response to a long-term fertilization” by Meng Wang, Tuula Larmola, Meaghan T. Murphy, Tim R. Moore and Jill L. Bubier (submitted to *Plant and Soil*, in revision). TL collected species abundance data by point intercept measurement. TL and MTM were involved in the maintenance of the long-term fertilization experiment at Mer Bleue Bog and helped with collecting leaf samples. JLB initiated and maintained the long-term fertilization experiment. All authors involved in manuscript preparation.

Manuscript #4 (Chapter 6): “The cascade of C:N:P stoichiometry in an ombrotrophic peatland: from plants to peat” By Meng Wang, Tim R. Moore, Julie Talbot and Pierre J. H. Richard (published on *Environmental Research Letters*, 2014, 9, 024003). JT helped analyze and interpret palaoecological data. RJHR collected two peat profiles and conducted palaoecological analyses. All authors were involved in manuscript preparation.

Chapter 1. Introduction

1.1 Research context

Because of the combined effects of nutrient limitation, low temperature and waterlogged soils (Clymo 1984), the slow rates of plant production and litter and organic matter decomposition make northern peatlands important carbon (C) sinks, which store 547 (473-621) Gt of C (Yu *et al*. 2010b) yet cover only ~3% of the earth's land surface (4×10^6 km^2) (Gorham 1991; Joosten & Clarke 2002). In Canada, peatlands cover ~1.1 million km^2 of the land surface and 760,000 km^2 are ombrotrophic (i.e. rain-fed) bogs (Tarnocai 2006).

Nitrogen (N) limitation is often prevalent in bogs under low levels of atmospheric N deposition (Aerts *et al*. 1992; Gunnarsson & Rydin 2000). The increase in N availability owing to atmospheric deposition and accelerated organic matter decomposition may stimulate plant growth and shift the previously N-limited ecosystems to be phosphorus (P), potassium (K) or P, K co-limited in the short term (Aerts *et al*. 1992; Verhoeven *et al*. 1996; Bragazza *et al*. 2004; von Oheimb *et al*. 2010), and alter community composition and ecosystem nutrient cycling in the long term (Aerts & Berendse 1988; Bobbink *et al*. 1998; Berendse *et al*. 2001; Bubier *et al*. 2007).

The possible shift in N, P and K interactions highlights that the relative balance among multiple elements, i.e. ecological stoichiometry (Sterner & Elser 2002), in addition to their absolute amounts, governs the metabolic functioning, plant growth and ecosystem processes. Ecological stoichiometry deals with the balance of multiple elements in the ecological processes and interactions and links the physiological constraints at the cellular or organismal levels to the biogeochemical patterns at the global scale (Sterner & Elser 2002). The application of ecological stoichiometry in terrestrial ecosystems is less developed than the aquatic equivalents. For example, Koerselman & Meuleman (1996) suggest plant growth is N- or P-limited when the plant N:P ratio is smaller than 14 or larger than 16, respectively. Graminoid litter decomposition is affected by N and P concentrations and their ratio (Güsewell & Verhoeven 2006). Although this approach is often criticized (e.g. von Oheimb *et al*. 2010), it has been successfully applied to bryophytes (Aerts *et al*. 1992; Bragazza *et al*. 2004; Jiroušek *et al*. 2011) and vascular plants (Güsewell & Koerselman 2002; Olde Venterink *et al*. 2003).

Most stoichiometric studies in mid- and high-latitude northern regions have been conducted in fen meadows, heathlands, boreal forests and tundra. No study of nutrients in the sense of stoichiometry has been conducted in ombrotrophic bogs. To determine the stoichiometry in plants and soil in these nutrient-deficient ecosystems and how these stoichiometric characteristics respond to nutrient enrichment are critical in a world of elemental imbalance between organisms and the external conditions (Sterner & Elser 2002; Frost *et al*. 2005).

1.2 Research objectives

My overall research objective is to fill some of the above scientific gaps by examining different aspects of C:N:P:K stoichiometry in plants and soils in a bog ecosystem. I first look into the seasonal variation in C:N:P:K stoichiometry in the major species belonging to different plant functional types (PFTs) at Mer Bleue Bog. Because of the essential roles of nutrient resorption in controlling seasonal variation in plant stoichiometry and nutrient cycling, I quantify the resorption efficiency and proficiency of C, N, P, K, calcium (Ca) and magnesium (Mg) at Mer Bleue Bog and examine the effects of long-term N, P and K fertilization and their interactions on nutrient resorption. This well-established long-term fertilization experiment offers a great opportunity to investigate the fundamental concept of the theory of ecological stoichiometry, i.e. stoichiometric homeostasis, which is the essence of life and defined as the degree of an organism to maintain its body stoichiometry constant despite major fluctuation in the stoichiometry of its resources (Kooijman 1995). I demonstrate the decomposition cascade of C:N:P stoichiometry from mature plant tissues through senescent litters to peat, and associate the stoichiometry of plants with that of the soil. To summarize, my specific research objectives are to:

(1) Explore the seasonal variation in C:N:P:K stoichiometry among different PFTs;
(2) Investigate the effects of long-term N, P and K fertilization on nutrient resorption of the major macronutrients between two dominant evergreen shrubs;
(3) Determine the degree of stoichiometric homeostasis and the difference in homeostasis between shrubs and bryophytes;
(4) Demonstrate the decomposition cascade of C:N:P stoichiometry from mature plant tissues, through senescent litters to peat.

1.3 Study site

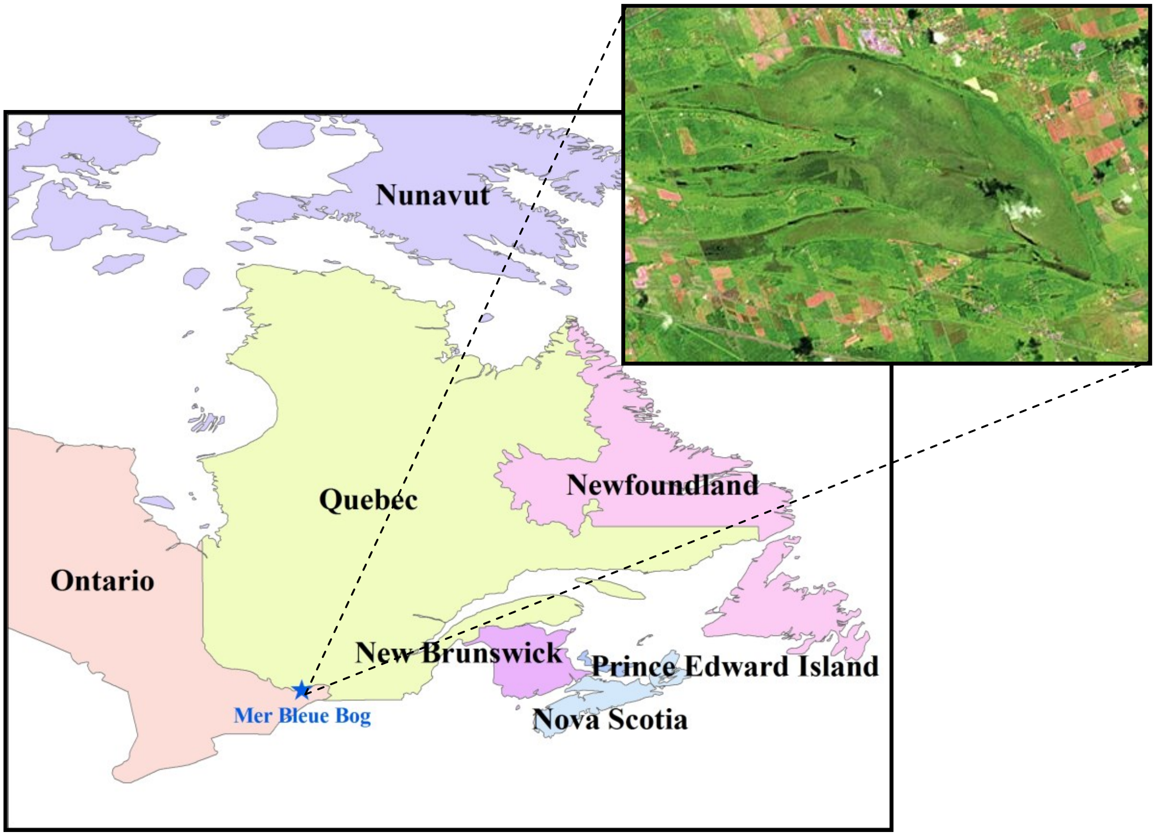
My research was conducted at Mer Bleue peatland (Figure 1.1), which is primarily an ombrotrophic bog with hummock and hollow micro-topography and a beaver pond with a total area of 28 km^2. The peat began to accumulate ~8500 years ago, leading to peat depth ranging from 2 m at the edge to greater than 5 m in the central area (Roulet *et al*. 2007). The site is located in the Ottawa River Valley, 10 km east of Ottawa, Canada (45.41°N, 75.52°W). Mean annual temperature is 6.0 °C and ranges from -10.8 °C in January to 20.9 °C in July (Bubier *et al*. 2007). Mean annual precipitation is 943 mm (76% as rainfall), 350 mm of which falls from May to August (Environment Canada; Canadian Climate Normals, 1981-2010). The estimated background inorganic wet N deposition in this region is approximate 0.8 g N m^{-2} yr^{-1} (Turunen *et al*. 2004) and total wet P deposition ranges from 6 to 26 mg m^{-2} yr^{-1} (R. Vet personal communication).

There are deciduous trees - birch (*Betula populifolia* Marshall) and tamarack (*Larix laricina* (Du Roi) K. Koch.). On the bog hummock, the dominant shrubs are evergreens *Chamaedaphne calyculata* (L.) Moench, *Rhododendron groenlandicum* (Oeder) K.A. Kron & W.S. Judd (formerly *Ledum groenlandicum* Oeder) and *Kalmia angustifolia* L., with the deciduous *Vaccinium myrtilloides* Michx. *Sphagnum capillifolium* (Ehrh.) Hedw. occurs in hummocks and hollows contain *S. magellanicum* Brid., *S. fallax* (Klinggr.) Klinggr. and *S. angustifolium* (C.E.O. Jensen ex Russow) C.E.O Jensen, with sedge (*Eriophorum vaginatum* L.) and forb (*Maianthemum trifolium* (L.) Sloboda). *Typha latifolia* L. and *S. cuspidatum* Ehrh. ex Hoffm. occur in the beaver pond, with *S. fallax* at the edge of the beaver pond.

1.4 Thesis structure

This thesis consists of 7 chapters. Chapter 1 introduces the research background about northern peatlands and the scientific gaps of studies on ecological stoichiometry in ombrotrophic bogs, and proposes 4 scientific questions. Chapter 2 provides a comprehensive literature review on some essential principles of ecological stoichiometry and the applications in wetlands, particularly focuses on northern peatlands. Chapters 3 to 6 show my original research findings presented in a collection of scientific manuscripts accepted or to be submitted for publication. Specifically, Chapter 3 explores the seasonal variation in C:N:K:P stoichiometry in photosynthetically active tissues of 14 species belonging to 5 different PFTs. Chapter 4 investigates the resorption efficiency and proficiency of C, N, P, K, Ca and Mg of two dominant evergreen shrubs, and the effects of long-term N, P and K fertilization. Chapter 5 determines the variation in the degree of stoichiometric homeostasis between shrubs and mosses and how they are related to changing species abundance after long-term N, P and K fertilization. Chapter 6 demonstrates the decomposition cascade of C:N:P from mature plant tissues, through senescent litters to peat. Chapter 7 summarizes the main conclusions of my research, emphasizes the scientific contributions to current knowledge and proposes future research directions.

Figure 1.1 The location of Mer Bleue peatland (45.41°N, 75.52°W).



Chapter 2. Literature review

2.1 What are wetlands?

Wetlands are "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters" (Ramsar 1987). The Canadian Wetland Classification has a narrower definition that confines wetlands as "land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soil, hydrophytic vegetation and various kinds of biological activity which are adapted to a wet environment" (National Wetlands Working Group 1997). In either definition, there are three specific features that distinguish wetlands from other landforms: (1) water present at or near the land surface; (2) anaerobic soil conditions at least during part of the year; (3) the particular biota adapted to the wet and sometimes waterlogged soils (Charman 2002; Mitsch & Gosselink 2007).

2.2 Northern peatlands: the dominant wetlands in northern hemisphere

Literally, peat-land is the "land" covered by "peat", which is the residuals of plant and animal constituents accumulating under more or less water-saturated conditions because of incomplete decomposition, owing to cool temperature, anaerobic soil condition, functionally limited decomposer communities and the recalcitrant nature of litters (Rydin & Jeglum 2006). Usually a minimum depth of peat is required to be classified as peatland, which is 40 cm in Canadian classification and 30 cm in the definition from the International Mire Conservation Group (Joosten & Clarke 2002). Globally, peatlands cover ~4 × 10^6 km^2 (Gorham 1991; Joosten & Clarke 2002) and ~87% of the world's peatlands are distributed in the boreal and subarctic regions. Five out of six countries where peatlands cover more than 50,000 km^2 are predominantly boreal (Russia, Canada, USA, Finland and Sweden), with 270,000 km^2 of tropical peatlands located in Indonesia (Joosten & Clarke 2002). Canada contains the second largest area (1.06 × 10^6 km^2) of peatlands in the world, accounting for approximately 30% of the total peatlands area on Earth. Peatlands cover ~12% of the land surface of Canada, with 97% occurring in the boreal (64%) and subarctic regions (33%) (Tarnocai *et al*. 2002).

From a hydrological perspective, peatlands that receive the supply of water only from rain or snow are considered to be ombrogenous, and these peatlands are influenced by low concentrations of dissolved cations and anions ("minerals") that provides an ombrotrophic condition for the development of a bog (Vitt 2006). Other peatlands are influenced by the supply from ground and/or surface water and considered as geogenous peatlands, where the geogenous water contains dissolved cations and anions that strongly affect the vegetation, flora and functioning of peatlands and provide a minerotrophic basis that is favorable to form a fen. From an ecological perspective, bogs are ombrotrophic peatlands and fens are minerotrophic peatlands. In Canada, bogs are the most important peatland type, which cover 7.62×10^5 km^2 (Tarnocai 2006).

Other than the ecological significance of hosting a wide diversity of species and life forms, northern peatlands are substantial C sinks, which attracts scientific interest. In Canada, peatlands contain ~147 Gt soil C, which is approximately 56% of the organic C stored in all Canadian soils (Tarnocai 2006). Since most of the peatlands are located in the high latitudes, where the expected global warming effect is greatest, 60% of the total area of Canadian peatands and 51% of the total organic C mass in all Canadian peatlands is predicted to be severely to extremely severely affected by climate change (Tarnocai 2006). Therefore, more and more scientific attention has been dedicated to northern peatlands in order to understand the responses of peatland ecosystems to global changes from different perspectives.

2.3 Ecological stoichiometry and its application in wetlands

2.3.1 Ecological stoichiometry: an introduction

The word 'stoichiometry' comes from the Greek root 'stoicheion' which refers to the quantitative relationship between reactants and products during chemical reaction (Sterner & Elser 2002). As two of the most important laws in chemistry, the laws of definite proportions and conservation of mass, play important roles in all branches of stoichiometry. In order to understand complicated ecosystems, we can consider the living organisms to be highly evolved chemical substances that interact with each other or with the non-living world in a way comparable to a complex chemical reaction. For example, Elser *et al.* (1996) used the reaction of $(N_xP_y)_{predator} + (N_aP_b)_{prey} \rightarrow Q(N_xP_y)_{predator} + (N_{a'}P_{b'})_{waste}$ as an abstraction of a complex ecological process of the predation with N and P recycling, where a prey item with a specific nutrient composition (a:b) was consumed by a predator with fixed elemental composition (x:y) to increase predator's biomass by a factor of Q, and at the same time produced the waste with different elemental composition (a′:b′) involved in the resource competition among primary producers.

As a subfield of stoichiometry, ecological stoichiometry deals with the balance of multiple chemical substances in ecological interactions and processes and links the biogeochemical patterns at the global scale to physiological constraints that operate at cellular or organism levels (Sterner & Elser 2002). Ecological stoichiometry focuses on the relative elemental composition of major participants during ecological interactions in ecosystems (Elser *et al*. 1996), especially N, P and K. Several important aspects and their applications in wetlands, particularly peatlands, will be discussed in the following.

2.3.2 Stoichiometric homeostasis: the essence of life

Ecological stoichiometry exists owing to one fundamental concept, homeostasis, which is the degree to which an organism maintains its body chemical composition constant, despite the changes in the chemical composition in the external environment, and is the essence of life (Kooijman 1995). The degree to which organisms remain homeostatic is critical to food webs and ecosystem functioning in the context of elemental imbalance between organisms and the external conditions (Frost *et al*. 2005). The homeostatic regulation coefficient (H) can be estimated by growing organisms in a wide range of environmental or dietary conditions (x), determining the consequent organismal elemental composition (y) and linearizing the power function ($y = cx^{1/H}$) using logarithms (Sterner & Elser 2002):

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

where c is a constant.

At least in theory, there are two extremes for homeostasis: strict homeostasis (H approaches infinity, e.g. Levi & Cowling 1969; Goldman *et al*. 1987; Zauke *et al*. 1998) and non-homeostasis ($H = 1$, e.g. Rhee 1978). The degree of homeostasis is a function of several critical factors: the organism itself, its life stage, the aspects of elements and their ratios under consideration (e.g. N, P concentration, or N:P ratio), and last and the most important one, the range of x, since the homeostasis may only happen within a specific range of resource availability (Sterner & Elser 2002). As reported in freshwater invertebrates, the macronutrients, such as N, P and K, are more homeostatic than micronutrients and non-essential metals are not homeostatic (Karimi & Folt 2006). Heterotrophs are generally more homeostatic than autotrophs, particularly in the case of multicellular metazoans (Sterner & Elser 2002).

Although there are a few studies examining the stoichiometric homeostasis on a wide range of taxa, little is known about the stoichiometric homeostasis of higher plants, especially in wetlands. Plants generally show stronger homeostasis than algae or fungi (Rhee 1978; Sterner & Elser 2002; Yu *et al*. 2011). For example, Güsewell (2004) reported a range of 1.7 to 4.6 in the *H* value of N:P ratio in herbaceous plants from marshes, fen meadows, poor and rich fens, heathlands and grassland (recalculated from Shaver & Melillo 1984; Ryser & Lambers 1995; Güsewell & Bollens 2003; Güsewell 2005b). Yu *et al*. (2011) reported the *H* values of N, P and N:P ratio ranging from 1.9 to 14.5 in grass and forb species in grasslands and the association between strong homeostasis and conservative nutrient use suggests an important ecological strategy in other nutrient-deficient ecosystems. However, plant homeostasis in bogs remains unknown, especially for peat-forming *Sphagnum* moss, which is the "ecosystem engineer" in northern peatlands and contributes to their capacity of C sequestration (van Breemen 1995).

2.3.3 Relatively conservative stoichiometric ratios

Because of the essential stoichiometric homeostasis of life, people have sought a single molecule to simplify our understanding of nature. For example, the stoichiometric formula for a living human can be:

$$\mathrm{H_{375000000}O_{132000000}C_{85700000}N_{6430000}Ca_{1500000}P_{1020000}S_{206000}Na_{183000}K_{177000}Cl_{127000}Mg_{40000}Si_{38600}}$$
$$\mathrm{Fe_{2680}Zn_{2110}Cu_{76}I_{14}Mn_{13}F_{13}Cr_{7}Se_{4}Mo_{3}Co_{1}}$$

It means there are 375 million hydrogen atoms for every cobalt atom in a human body (Sterner & Elser 2002). A similar approach in marine ecosystems gives rise to the famous Redfield ratio. Redfield's critical C:N:P mass ratio of 41:7:1 in phytoplankton and seawater, and his seminal theory of "the biota in the ocean control their environments" (Redfield 1958) have been considered a landmark of ecological stoichiometry. Recently, an intriguing study showed that the canonical Redfield ratio of N:P equalling 7:1 was not a coincidence but rather a consequence of the conservative balance between protein and ribosome RNA synthesis in prokaryotic and eukaryotic microbes with intrinsic cell N:P ratio around 7:1 under optimal growth condition (Loladze & Elser 2011). Although the plastic allocation of plant biomass to organs with distinct stoichiometry may prevent a ubiquitous optimum stoichiometric ratio in plants as observed by Redfield in ocean, the promising experimental validation of the possible existence of such critical stoichiometric ratio in microbes is still appealing and provides an opportunity to examine nutrient limitation by looking into the stoichiometric ratios of foliage or aboveground biomass, as discussed in the following section.

2.3.4 Relative nutrient limitation

In the early nineteenth century, Liebig proposed that organisms will become limited by whatever resources in the shortest supply compared to their demands (Liebig's law of minimum) and it has been accepted as a universal principle in ecological stoichiometry (Ågren 2008). The subsequent idea of estimating specific types of nutrient limitation based on the deviation of organismal stoichiometric ratio from a critical threshold is a popular focus of contemporary research. Nitrogen versus P limitation is unequivocally the hottest topic in both terrestrial and aquatic ecosystems (Schindler 1977; Vitousek & Howarth 1991; Elser *et al*. 1996; Koerselman & Meuleman 1996; Downing 1997; Tyrrell 1999; Olde Venterink *et al*. 2003; Ågren 2004; Güsewell 2004; McGroddy *et al*. 2004; Wright *et al*. 2005; Elser *et al*. 2007; Filippelli 2008; Yuan & Chen 2009).

The application of the "Redfield-like" ratio to wetland ecosystems advanced our understanding of the balance of multiple elements which regulate nutrient cycling in these ecosystems. A number of influential studies have been published since the 1990's. Koerselman & Meuleman (1996) reviewed 40 fertilization studies and revealed that N and P concentrations in plant tissue were mainly determined by external supply. They proposed that it was the N:P ratio that determined the nature of nutrient limitation. At the community level, the N:P mass ratio > 16 indicated P limitation and N:P ratio < 14 indicated N limitation, with N:P ratio between 14 and 16 indicating N and P-colimitation (Koerselman & Meuleman 1996).

With the same dataset, Verhoeven *et al*. (1996) looked more specifically into the variation in vegetation N:P ratios among different types of peatlands and showed that plant growth in herbaceous peatlands was mainly limited by N or P with a minority of sites co-limited by N and P, or by N and K. However, wet heathlands and bogs are mostly limited by P (Verhoeven *et al*. 1996).

Güsewell & Koerselman (2002) revisited this topic by compiling a larger dataset of wetland plants and suggested that although the N:P ratios in the vegetation did not exactly mirror the nutrient availability in the soil, they showed a clear response to fertilization in the field, making them reliable indicators of nutrient limitation.

Including bryophytes in their studies of nutrient limitation and plant diversity in North American wetlands, Bedford *et al*. (1999) demonstrated the variation of nutrient limitation in different types of wetlands by virtue of N:P ratios in plant tissues and soil. They highlighted the necessity of understanding nutrient limitation at both community and individual species level which was also suggested by Rozbrojová & Hájek (2010).

By taking K into consideration, Olde Venterink *et al*. (2003) introduced a ternary diagram to distinguish nutrient limitation and its relationship with species richness-productivity patterns, through N:P, N:K and K:P ratios. This ternary diagram has far-reaching implications for future stoichiometry research. The main results were that P (co)-limitation only occurred under low productivity, K (co)-limitation can happen up to intermediate productivity, and N-limitation may exist along the whole productivity gradient. Based on the ternary diagram, Gotelli *et al*. (2008) examined the stoichiometric ratios of N:P:K in three common bog plant genera from 24 ombrotrophic peatlands in the northeastern regions of the United States, and suggested plant growth in these bogs was limited by P, or co-limited by all three macronutrients.

Bragazza *et al*. (2004) investigated the response of N:P:K stoichiometry in *Sphagnum* moss to atmospheric N deposition in 15 peatlands from 11 European countries and proposed a threshold of N deposition of ~1 g m^{-2} yr^{-1} above which *Sphagnum* moss shifted from N-limited to be K+P co-limited, at N:P ratio > 30 and N:K ratio > 3. Nevertheless, Jiroušek *et al*. (2011) argued that *Sphagnum* plants still seemed to be N-limited despite their N saturation, when investigating the N:P:K stoichiometry in *Sphagnum* capitula along a N deposition gradient in Central-East Europe. Similar thresholds (~1 g N m^{-2} yr^{-1}) have been reported by Gunnarsson & Rydin (2000) and Lamers *et al*. (2000) with regard to N saturation or shift in N-limitation of *Sphagnum* moss in ombrotrophic bogs.

Carnivorous plants, with their special features of prey, gain the benefit of growing in wetlands where light and moisture are abundant and soil macronutrients, especially N, P and K, are limiting, such as ombrotrophic bogs (Givnish *et al*. 1984). Ellison (2006) reviewed nutrient limitation and stoichiometry of different carnivorous species, primarily grown in wetlands, and found that P-limitation or N and P co-limitation were common factors that restrained the growth of carnivorous plants. A manipulative experiment with the addition of frozen houseflies to carnivorous pitcher plant *Sarracenia purpurea* revealed that P was the primary nutrient from prey and their feeding shifted previous P-limitation under ambient circumstance to N-limitation (Wakefield *et al*. 2005).

Based on the recalculation of foliar N:P ratios of few dominant species in several Canadian bogs (Walbridge & Navaratnnam 2006) under lower atmospheric N deposition than their European equivalents, Canadian bogs are either N limited (Malmer *et al*. 1992; Thormann & Bayley 1997) or co-limited by N and P (Pakarinen & Gorham 1983; Malmer *et al*. 1992).

However, without taking K into consideration, this approach may overestimate the possibility of N or P limitation and overlook the possibility of K limitation, especially under the circumstance of increasing ambient CO_2 levels and N enrichment (Hoosbeek *et al*. 2002). Moreover, the seasonal variation in nutrient concentration and stoichiometric ratios in plant tissues (Chapin *et al*. 1980; Güsewell & Koerselman 2002) and among different PFTs (Sardans *et al*. 2012) calls for caution when reaching a conclusion based on samples that are restricted to specific PFTs and are not taken from the peak growing season.

2.3.5 The C:nutrient stoichiometry after senescence

The stoichiometry of mature tissues has an "after-life" effect on the subsequent biogeochemical processes after senescence. Nutrient resorption during leaf senescence has been considered as a significant nutrient conservation strategy, ensuring nutrients are available for future plant growth and reducing the dependence on external nutrient availability (Aerts 1996; Vergutz *et al*. 2012). The resorption of N and P in wetland graminoids is related to the type of nutrient limitation and contributes to the dominance of graminoids with high resorption capacity in P-limitation wetlands (Güsewell 2005a).

The depleted nutrient concentration in senescent leaves accounts for the increase in litter C:nutrient ratios, which have been used to indicate litter quality for decomposition. For example, in a 12-year litter decomposition study at three pairs of upland forest and peatland sites, Moore *et al*. (2008) found that N and P were retained by decomposing litter until critical C:N and C:P ratios were reached, after which N and P were lost and the N:P ratio converged to 12:1 to 16:1. Similarly, Limpens & Berendse (2003) found the critical C:N ratio of 66.7 above which the N started to be lost from *Sphagnum* litter of two hummock and two hollow species from a Ireland bog. Moreover, Güsewell & Verhoeven (2006) collected foliar litters from 11 graminoid species in Swiss and Dutch wetlands, the N:P ratio of which indicates whether the decomposition is more likely to be N- or P-limited. The critical N:P ratio of N- and P-limitation for graminoid foliar litter appeared to be ~25.

The mismatch between the stoichiometry of litter and that of decomposers, primarily bacteria and fungi in northern peatlands (Winsborough & Basiliko 2010), governs ecosystem nutrient cycling and thus C accumulation (Sterner & Elser 2002; Hessen *et al*. 2004). For example, the substantially larger C:N:P mass ratio of 1000:20:1 in subarctic flora (Aerts *et al*. 2012) than 180:6:1 in soil microbial biomass in tundra (Cleveland & Liptzin 2007) indicates a possible long term net N and P immobilization in soil until a critical litter C:N and C:P ratio is reached.

The slow rate of litter and organic matter decomposition in peatlands contributes to the substantial accumulation of organic matter (Moore & Basiliko 2006). These partially decomposed litters accumulate in peatlands mostly during the Holocene, contributing to the massive C storage in these ecosystems. There have been a noticeable effort to examine C accumulation rate in peatlands by the palaoecological community, with less attention given to N, and little is known about the accumulation of other essential macronutrients, such as P, K, Ca and Mg. Applying the mean C:N ratio of 46 for all peat types to the estimate of global peat C pool of 481 Gt, Loisel *et al*. (2014) assesses the global peat N storage of 10.5 Gt. Unlike the C:N ratio, little attention has been applied to other C:nutrient ratios, making it difficult to estimate the role of northern peatlands as the pool of other macronutrients that merits future research.

2.3.6 Ecological stoichiometry in the context of global change

Recently, there are more and more studies focusing on the effects of global changes on plant stoichiometry, especially the spatially varied N and P supplementation. Through the combustion of fossil fuel, wide-spread use of fertilizer (N and P), cultivation of N-fixing legumes and land use changes, human activities have doubled the input of reactive N on the Earth's land surface (Vitousek *et al*. 1997; Galloway *et al*. 2008; Gruber & Galloway 2008) and doubled the net transfer of dissolved and particulate P from land to ocean (Smil 2000). The increasing availability of N and P, which overcompensates the requirement of growth-limiting nutrients, can shift nutrient limitation types and stimulate plant growth, increasing nutrient concentration in plant tissues in the short term (Aerts *et al*. 1992; Verhoeven *et al*. 1996; Turunen *et al*. 2004; Bragazza *et al*. 2006; Breeuwer *et al*. 2009; Vicars *et al*. 2010). However, in the long term, the overgrowing of fast-growing species may out-compete these stress-tolerant species and shift plant community composition and reduce plant diversity, which will ultimately affect ecosystem nutrient cycling (Aerts & Berendse 1988; Tilman 1996; Berendse *et al*. 2001; Malmer & Wallén 2005; Nordin *et al*. 2005; Bubier *et al*. 2007).

Chapter 3. Carbon, nitrogen, phosphorus and potassium stoichiometry in an ombrotrophic peatland reflects plant functional type

(Published on *Ecosystems*, doi: 10.1007/s10021-014-9752-x)

Context within the thesis

As demonstrated in Chapter 1, no study of nutrient stoichiometry has been conducted in ombrotrophic bogs, which are important C sinks under extremely low nutrient availabilities. To explore the stoichiometric relationship among multiple elements in ombrotrophic bogs through time and across space, this chapter aims to fill this scientific gap by examining the seasonal variation in C:N:P:K stoichiometry among 14 different species belonging to 5 different PFTs (mosses, deciduous trees/shrubs, evergreen shrubs, graminoids and forb) at Mer Bleue Bog. This comprehensive evaluation of the C:N:P:K stoichiometry in foliar tissues will advance our knowledge of the well-known ecological theory (i.e. ecological stoichiometry) in the context of peatland biogeochemistry. Besides, the N:P:K stoichiometry in photosynthetically active tissues at peak growing season can suggest the primarily limited nutrient(s) for plant growth, which will be used to guide the experimental design of Chapters 4 and 5 when investigating the effect of long-term N, P and K fertilization on characteristics of nutrient resorption and homeostasis.

3.1 Summary

Ombrotrophic bog peatlands are nutrient-deficient systems and important C sinks yet the stoichiometry of N, P and K, essential for plant growth and decomposition, has rarely been studied. We investigated the seasonal variation in C, N, P and K concentrations and their stoichiometric ratios in photosynthetically active tissues of 14 species belonging to 5 PFTs (mosses, deciduous trees/shrubs, evergreen shrubs, graminoids and forb) at Mer Bleue Bog, an ombrotrophic peatland in eastern Ontario, Canada. Although we observed variations in stoichiometry among PFTs at peak growing season, there was convergence of C:N:P:K to an average mass ratio of 445:14:1:9, indicating N and P co-limitation. Nitrogen, P and K concentrations and stoichiometric ratios showed little seasonal variation in mosses, evergreens and graminoids, but in forb and deciduous species were the largest in spring and decreased throughout the growing season. Variations in nutrient concentrations and stoichiometric ratios among PFTs were greater than seasonal variation within PFTs. Plants exhibit N and P co-limitation and adapt to extremely low nutrient availability by maintaining small nutrient concentrations in photosynthetically active tissues, especially for evergreen shrubs and *Sphagnum* mosses. Despite strong seasonal variations in nutrient availabilities, few species show strong seasonal variation in nutrient concentrations, suggesting a strong stoichiometric homeostasis at Mer Bleue Bog.

3.2 Introduction

Ecological stoichiometry deals with the elemental balance in ecological interactions and processes, providing an integrative nutrient framework linking biogeochemical patterns at the global scale to physiological constraints that operate at cellular or organismal levels (Sterner & Elser 2002). Compared to aquatic ecosystems, the application of the principles of ecological stoichiometry in terrestrial ecosystems is less developed. For example, the N:P ratios in vegetation are considered reliable indicators of nutrient limitation, with the mass N:P ratio < 14 or > 16 indicating N or P limitation respectively, and between 14 and 16 indicating N and P co-limitation (Koerselman & Meuleman 1996; Güsewell & Koerselman 2002). Although this approach is often criticized (e.g. von Oheimb *et al*. 2010), it has been successfully applied to bryophytes (Aerts *et al*. 1992; Bragazza *et al*. 2004; Jiroušek *et al*. 2011) and vascular plants (Güsewell & Koerselman 2002; Olde Venterink *et al*. 2003). Potassium limitation is rarely studied but could also occur in peatlands, especially after N and carbon dioxide enrichments (Hoosbeek *et al*. 2002).

Most stoichiometric studies in mid- and high-latitude northern regions have been conducted in fen meadows, heathlands, boreal forests and tundra. Little attention has been given to ombrotrophic peatlands such as bogs, which are nutrient-deficient systems with slow rates of plant production and litter and soil organic matter decomposition (e.g. Moore *et al*. 2008) resulting in important C sinks, storing ~547 Gt C (Yu *et al*. 2010b) and covering about 1 million km^2 in Canada (Tarnocai 2006). The resorption of nutrients from senesced leaves and subsequent translocation to newly grown tissues (Killingbeck 1986) is important to reduce the dependence on external nutrient availability (Aerts 1996; Aerts & Chapin 2000). The resorption of nutrients is essential in supporting spring growth in deciduous species and forbs (Chapin *et al*. 1980), which are expected to show strong seasonal variations in nutrient concentrations and stoichiometry, whereas evergreen species can retain leaf nutrients and resorption can occur gradually over the growing season (Chapin *et al*. 1980). Although *Sphagnum* mosses are able to resorb nutrients (Aldous 2002), these evergreen-like non-vascular species may show little seasonal variation in stoichiometry. The seasonal variation in organic matter mineralization and nutrient release may contribute to the seasonal variation in plant stoichiometry.

Nitrogen limitation is often prevalent in bogs under low atmospheric N deposition (Aerts *et al*. 1992; Gunnarsson & Rydin 2000). Increased N availability from atmospheric deposition and accelerated organic matter decomposition may shift nutrient limitation types and stimulate plant growth in the short term (Aerts *et al*. 1992; Verhoeven *et al*. 1996; Bragazza *et al*. 2004) and alter community composition and ecosystem nutrient cycling in the long term (Aerts & Berendse 1988; Bobbink *et al*. 1998; Berendse *et al*. 2001; Bubier *et al*. 2007).

In this study, we examined the seasonal variation in nutrient concentrations and their stoichiometric ratios in photosynthetically active tissues (i.e. leaves of vascular plants and capitula of *Sphagnum* mosses) in 14 species belonging to 5 PFTs (moss, deciduous tree/shrub, evergreen shrub, graminoid and forb) at Mer Bleue Bog, an ombrotrophic peatland in eastern Ontario, Canada. Atmospheric N deposition at Mer Bleue is ~0.8 g N m^{-2} yr^{-1} (Turunen *et al*. 2004), close to the critical value of 1 g N m^{-2} yr^{-1}, above which *Sphagnum* mosses in Europe shifted from N limitation to co-limitation by P or K (Gunnarsson & Rydin 2000; Bragazza *et al*. 2004). We hypothesized that plant growth at Mer Bleue Bog is co-limited by N and P or K, as indicated by the N:P:K ratios in photosynthetically active tissue at the peak growing season. In addition, we hypothesized that deciduous species, forbs and graminoids would show stronger seasonal variation in their nutrient concentrations and stoichiometric ratios than evergreen species and *Sphagnum* mosses.

3.3 Materials and methods

3.3.1 Study sites and species description

This study was conducted at Mer Bleue peatland located 10 km east of Ottawa, eastern Ontario, Canada (45.40°N, 75.50°W). Mean annual temperature is 6.0 °C with monthly averages of -10.8 °C in January and 20.9 °C in July (Bubier *et al*. 2007). Mean annual precipitation is 943 mm (76% as rainfall), 350 mm of which falls from May to August (Canadian Climate Normals 1981-2010). The atmospheric wet inorganic N deposition in this site was ~0.8 g N m^{-2} yr^{-1} (Turunen *et al*. 2004) and the total wet P deposition in eastern Canada is estimated to range from 6 to 26 mg P m^{-2} yr^{-1} (R. Vet personal communication).

The central portion of the peatland is an ombrotrophic bog with hummock and hollow micro-topography and a beaver pond. There are deciduous trees - birch (*Betula populifolia* Marshall) and tamarack (*Larix laricina* (Du Roi) K. Koch.). On the bog hummock, the dominant shrubs are evergreens *Chamaedaphne calyculata* (L.) Moench, *Rhododendron groenlandicum* (Oeder) K.A. Kron & W.S. Judd (formerly *Ledum groenlandicum* Oeder) and *Kalmia angustifolia* L., with the deciduous *Vaccinium myrtilloides* Michx. *Sphagnum capillifolium* (Ehrh.) Hedw. occurs in hummocks and hollows contain *S. magellanicum* Brid., *S. fallax* (Klinggr.) Klinggr. and *S. angustifolium* (C.E.O. Jensen ex Russow) C.E.O Jensen, with sedge (*Eriophorum vaginatum* L.) and forb (*Maianthemum trifolium* (L.) Sloboda). *Typha latifolia* L. and *S. cuspidatum* Ehrh. ex Hoffm. occur in the beaver pond, with *S. fallax* at the edge of the beaver pond. The classification of PFTs and species scientific names was based on United States Department of Agriculture (USDA) plant database (http://plants.usda.gov) with the exception of *T. latifolia*, which was classified as a graminoid rather than a forb (e.g. Končalová 1990; Kercher & Zedler 2004; Vernescu *et al*. 2005). Fine-scale vegetation distribution and biomass at Mer Bleue were investigated by Bubier *et al*. (2006), while Moore *et al*. (2002) estimated net primary productivity.

3.3.2 Vegetation sampling and chemical analyses

Samples of 14 species were collected with 5 replicates (1 × 1 m plots) for each species and 70 plots in total. The plots for *T. latifolia* and *S. cuspidatum* were located in the open water of the beaver ponds with the plots of *S. fallax* at the edge of the beaver pond. All the other 55 plots were located in the central portion of the bog. For each species, the 5 plots were randomly chosen and separated as far as possible to cover a large spatial extent. All the plots were chosen to include a monoculture of each species if possible. Fully expanded and sun-exposed current year's leaves (exclusive of petioles) of the shrubs (*C. calyculata*, *R. groenlandicum*, *K. angustifolia* and *V. myrtilloides*), forb (*M. trifolium*) and tree species (*B. populifolia* and *L. laricina*), the leaf blades of graminoids (*E. vaginatum* and *T. latifolia*) and the capitula of *Sphagnum* mosses (*S. capillifolium*, *S. angustifolium*, *S. magellanicum*, *S. fallax* and *S. cuspidatum*) were sampled on July 6 and September 27 of 2010, May 18, June 17, July 18, August 18, September 16 and October 30 of 2011. A subset of samples were collected and covered by aluminum foil immediately after defoliation to determine chlorophyll concentration after being stored at -20 °C until analyzed. The rest of the tissues 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 concentration was determined by dry combustion on an elemental analyzer (Leco CNS 2000, Leco Corporation, St. Joseph, Michigan, USA). Total P was determined colorimetrically by the ammonium molybdate-ascorbic acid method (Murphy & Riley 1962) on a Lachat Quik-Chem AE flow-injection auto-analyzer (Lachat Instruments, Milwaukee, Wisconsin, USA), after digested in concentrated sulphuric acid and hydrogen peroxide with selenium and lithium sulphate as catalysts (Parkinson & Allen 1975). Digested samples were filtered through 0.45 µm glass fibre filters (MN 85/90, MACHEREY-NAGEL GmbH & Co. KG, Germany) before analysis. Total iron (Fe) and K concentration was measured on an atomic absorption spectrophotometer (PerkinElmer model 2380, PerkinElmer Inc., USA). The chlorophyll concentration were extracted by the dimethyl sulphoxide (DMSO) (Hiscox & Israelstam 1979) with the absorbance being measured by a visible spectrophotometer (GENESYSTM 10 Scanning UV/Visible, Thermo Electron Corporation, USA) under two different wave lengths: 645 and 663 nm, respectively. The total chlorophyll concentration (g L^{-1}) is calculated according to the equation from Arnon (1949):

$$\textit{Total chlorophyll concentration} = 0.0202 \times A645 + 0.00802 \times A663$$

where *A645* and *A633* are the absorbance under the wavelength of 645 and 643 nm respectively. Total chlorophyll concentration is reported as mg g^{-1} dry mass.

3.3.3 Statistics

Shapiro-Wilk and Levene's tests were used to examine the normality of data and the homogeneity of variance, respectively. All data were logarithmically transformed before statistical analyses but the raw (untransformed) data were reported in the figures. As there was no significant difference in nutrient concentrations and stoichiometric ratios in July and September between 2010 and 2011 (*t* test, $P > 0.05$), we considered 2011 a typical year (Appendix 3.1) and do not show data from 2010. The variation among PFTs and seasonal variation in nutrient concentrations and stoichiometric ratios were examined by a coefficient of variation (CV) derived from $\log_{10}$-transformed data (Güsewell & Koerselman 2002):

$$\mathrm{CV}_{\mathrm{log-normal}} = \frac{\frac{1}{2}\left(10^{(\overline{\mathrm{X}}+\mathrm{SD})} - 10^{(\overline{\mathrm{X}}-\mathrm{SD})}\right)}{10^{\overline{\mathrm{X}}}} = \frac{10^{\mathrm{SD}} - 10^{-\mathrm{SD}}}{2}$$

where $\overline{\mathrm{X}}$ and SD are the mean and standard deviation of log10-transformed data. Compared to the usual calculation of CV, this approach has the advantage that $\overline{\mathrm{X}}$ and SD are derived from normally distributed data and hence the CV follows a normal distribution (Güsewell & Koerselman 2002). Standard deviations were calculated in different ways to quantify the variation among PFTs and the seasonal variations in nutrient concentrations and stoichiometric ratios. For 'among functional type means' (Table 3.1), nutrient concentrations and stoichiometric ratios from all sampling months were first averaged for each PFT (moss, evergreen, deciduous, graminoid and forb) and SD was then calculated with the means of each PFT. For 'among sampling month means', SD was calculated for each PFT separately. For example, for 'mosses', the nutrient concentrations and stoichiometric ratios of all mosses were averaged within one sampling month, and SD was calculated with the means of individual sampling month. For 'across all functional types and months', SD was calculated with all individual PFTs from all sampling months.

The overall variation among different species in nutrient concentrations and stoichiometric ratios was examined by principal component analysis (PCA), using the means from all sampling months for each species. Linear mixed model with repeated measures was conducted to examine the seasonal variation in nutrient concentrations and stoichiometric ratios among PFTs in 2011. Compound symmetry covariance structure was adopted to minimize Schwarz's Bayesian Criterion (BIC). If the interaction between PFTs and sampling month (PFT × M) was significant ($P < 0.05$), multiple comparisons were conducted to discriminate the difference among PFTs within each sampling month and among different sampling months for each PFT with Bonferroni adjustment. As there was only one species in forb (*M. trifolia*) and only one of the graminoids (*E. vaginatum*) grew in May, forb in all months and graminoids in May were excluded from all statistical analyses and only means are shown for visual comparison.

Principal component analysis was conducted using PC-ORD for Windows (Version 5.0, MjM Software, Gleneden Beach, Oregon, USA) and all other analyses were conducted in IBM SPSS Statistics Version 20.0 for Windows (IBM Corp., Armonk, New York State, USA).

3.4 Results

3.4.1 Principal component analysis

The 14 plant species were separated by their nutrient concentrations and stoichiometric ratios and converged in PFTs (Figure 3.1). The first and second principal axes explained 51% and 36% of the total variation among species, respectively. The first axis separated the graminoids, forb and *S. cuspidatum* (pond moss) from the other species, reflecting larger N, P, K and chlorophyll concentrations and smaller C:N, C:P and N:P ratios. The second axis separated mosses and forb from the other PFTs, reflecting smaller C concentrations and N:K ratios and larger Fe concentrations and K:P ratios (i.e. poorer in structural C and richer in K and Fe).

3.4.2 Stoichiometric ratios at peak growing season and over the full season

The N:P:K stoichiometric ratios at peak growing season (July) converged to the boundary of N and P co-limitation (Figure 3.2a). Forbs and mosses were richer in K than other PFTs. No substantial variation was observed among species within the same PFT, except for *S. cuspidatum*, which was in the N-limited section (Figure 3.2a) owing to smaller N:P ratios than other *Sphagnum* mosses (Appendix 3.2a). The average mass ratio of C:N:P:K in moss, evergreen, deciduous, graminoid and forb at peak growing season were 612:13:1:11, 708:16:1:8, 640:16:1:7, 292:14:1:8 and 303:14:1:12 respectively, giving an overall average of 445:14:1:9.

The N:P:K stoichiometric ratios also converged to the boundary of N and P co-limitation throughout the growing season for mosses, evergreens and graminoids (Figure 3.2b). For forb and deciduous species, the N:P:K stoichiometric ratios in May deviated from other months, showing a richer in P than other PFTs.

3.4.3 Seasonal variations in nutrient concentrations and stoichiometric ratios

The interactions between PFTs and sampling months were significant in all nutrient concentrations and stoichiometric ratios ($P < 0.05$; Figures 3.3 and 3.4). Mosses, evergreens and graminoids showed little seasonal variation. In contrast, deciduous species showed significant seasonal variation in all nutrient concentrations and stoichiometric ratios ($P < 0.01$) except for N:K ($P = 0.710$) and K:P ratios ($P = 0.061$). Specifically, N, P and K concentrations were the largest in May and decreased throughout the growing season to the smallest in October ($P < 0.001$; Figure 3.3a,b,c). The C:N and C:P ratios were the smallest in May, increased to the largest in October ($P < 0.001$; Figure 3.4a,b) and the smallest N:P ratios occurred in May ($P < 0.001$; Figure 4c). A similar trend was observed for forb (Figures 3.3 and 3.4). There was little seasonal variation in Fe concentration (Figure 3.3d). Chlorophyll concentration was the largest in May for forb and significantly smaller in October for deciduous species ($P < 0.001$; Figure 3.3e).

As the seasonal variation was mainly related to varied nutrient concentrations and stoichiometric ratios in May for forb and in May and October for deciduous species (Figures 3.2b, 3.3 and 3.4), we excluded these sampling months from the calculation of CV resulting in a substantially reduced seasonal variation. Specifically, the seasonal variation was significantly reduced by ~20% when May was excluded for forb (CV = 16.7 ± 2.1%, mean ± 1 SE; *t* test, $P = 0.019$) and by ~80% when May and October were excluded for deciduous species from the calculation of CV (CV = 10.6 ± 1.7%; $P = 0.010$), in which there was no seasonal variation from June to September ($P > 0.05$; Figures 3.3 and 3.4).

3.4.4 Variation among PFTs

There were more substantial differences among PFTs in nutrient concentrations and stoichiometric ratios than seasonal variation (Table 3.1, Figures 3.3 and 3.4). Graminoids and forb showed a trend of larger N and P concentrations and smaller C:N and C:P ratios than mosses, evergreen and deciduous species, except for May and October, when deciduous species showed the largest N and P concentrations and smallest C:N and C:P ratios in May ($P < 0.001$), and the smallest N and P concentrations and the largest C:N and C:P ratios in October ($P < 0.05$; Figures 3.3a,b and 3.4a,b). There was no significant difference among PFTs in chlorophyll concentration ($P > 0.5$; Figure 3.3e) and N:P ratios except in May when the N:P ratio was significantly larger in mosses and evergreen species than in deciduous species ($P < 0.05$; Figure 3.4c). In general, mosses showed the largest Fe concentration across all PFTs ($P < 0.05$; Figure 3.3d), and smaller N:K ratio (Figure 3.4d) and larger K:P ratios (Figure 3.4e) than evergreen and deciduous species.

The variation in nutrient concentrations and stoichiometric ratios among PFTs (i.e. 'among functional type means') was generally larger than among species within each PFT (Appendix 3.3). The variation among PFTs was also significantly larger than the seasonal variation (i.e. 'among sampling month means, all functional types') (*t* test, $P = 0.002$; Table 3.1). The overall variation (i.e. 'across all functional types and months') in N concentration was smaller than in P and K concentrations; Fe and chlorophyll concentrations varied the most. The ratios of N:P, N:K and K:P varied less than did N, P and K concentration. The variation in C:N and C:P ratios were comparable to that in N and P concentration respectively, with C concentration varying the least. On average, mosses and evergreen species showed significantly lower seasonal variation than deciduous species ($P < 0.05$; Table 3.1).

3.5 Discussion

3.5.1 N and P co-limitation

Foliar stoichiometric ratios can suggest nutrient limitation, especially N, P and K, in wetlands (Koerselman & Meuleman 1996; Güsewell & Koerselman 2002; Hoosbeek *et al*. 2002; Olde Venterink *et al*. 2003; Güsewell 2004). Güsewell (2004) suggested re-setting the critical value of N and P limitation to be N:P < 10 and > 20 respectively, instead of N:P < 14 and > 16 suggested by Koerselman & Meuleman (1996). Our N:P ratios suggest a consistent pattern of N and P co-limitation at Mer Bleue Bog based on the critical ratio suggested by Güsewell (2004) (Figure 3.2), supporting P limitation or N and P co-limitation in temperate North American wetlands (Bedford *et al*. 1999). Nitrogen and P co-limitation is supported by the long-term fertilization experiment at Mer Bleue Bog: the addition with only N or P+K did not significantly change species abundance of *C. calyculata* and *R. groenlandicum* after a decade compared to the control, whereas their abundance was increased by ~50% when N, P and K were applied together (Larmola *et al*. 2013). Under lower atmospheric N deposition than their European equivalents, Canadian bogs are either N limited (Malmer *et al*. 1992; Thormann & Bayley 1997) or co-limited by N and P (Pakarinen & Gorham 1983; Malmer *et al*. 1992), based on the foliar stoichiometry of the dominant species.

With foliar N:K ratio < 2.1 and K:P ratio > 3.4 (Olde Venterink *et al*. 2003) at peak growing season, none of the PFTs showed K limitation at Mer Bleue Bog. However, under elevated CO_2 and/or N deposition, K could primarily limit the potential growth of bog vegetation (Hoosbeek *et al*. 2002) and the depletion of K could result under acidic conditions (Rydin & Jeglum 2006).

While most ecosystems in the northern hemisphere are N limited (Walker & Syers 1976; LeBauer & Treseder 2008), ombrotrophic peatlands are weakly connected to the underlying mineral soil, impeding the upward replenishment of mineral nutrients and P may occur as occluded P or organic P, leaving it less available to plants and microbes (Rydin & Jeglum 2006; Walbridge & Navaratnnam 2006). An *in situ* soil mineralization study at Mer Bleue Bog revealed that 4.9 ± 0.7% and 0.4 ± 0.02% of the total soil N and P was available, as potassium chloride extractable ammonium and nitrate, and Mehlich III extractable orthophosphate, respectively (M. Wang unpublished data). Nitrogen was mineralized but P was either immobilized by soil microorganisms or occluded by soil organic matter after three months' incubation and strong P immobilization by soil microorganisms has been reported in fen and pocosin peatlands (Richardson & Marshall 1986; Walbridge 1991). In the absence of significant external P inputs and historically elevated atmospheric N deposition, P co-limitation develops in ombrotrophic bogs and a shift from N to P limitation in response to N enrichment has been demonstrated in Europe (Aerts *et al*. 1992; Gunnarsson & Rydin 2000; Bragazza *et al*. 2004; Jiroušek *et al*. 2011).

3.5.2 Well constrained C:N:P:K stoichiometry

Although the C:nutrients, N:K and K:P ratios at peak growing season may vary (up to three-fold among PFTs), the N:P ratio varies within a narrow range, and on average, the mass C:N:P:K ratio (445:14:1:9) is convergent at Mer Bleue Bog. This ratio is similar to the global average of C:N:P = 469:13:1 in forest foliage (McGroddy *et al*. 2004), 427:17:1 in Canadian forest litter with 20% original C remaining (Moore *et al*. 2011), the average of global terrestrial plants of 375:13:1 (Elser *et al*. 2000) and 384:13:1 in subarctic flora (Aerts *et al*. 2012). This convergence in stoichiometry, especially N:P ratio, in our ombrotrophic bog may have a cause: Loladze & Elser (2011) suggested that the Redfield mass ratio of N:P = 7:1 was not a coincidence but rather a consequence of the conservative balance between protein and ribosome RNA (rRNA) synthesis in prokaryotic and eukaryotic microbes with intrinsic cell N:P ratio around 7:1 under optimal growth condition. Although the N:P ratio of 14:1 in photosynthetically active tissues at peak growing season at Mer Bleue Bog slightly declined to 12:1 after senescence (Wang *et al*. 2014b), the plastic allocation of plant biomass to organs with distinct stoichiometry may shift the ratios, preventing an ubiquitous optimum stoichiometric ratio.

A similar convergence in C:N:P stoichiometry has been observed in soil microbial biomass in the top 30 cm peat at Mer Bleue Bog (mass ratio 31:3:1) (Basiliko 2004), supporting the convergence of C:N:P stoichiometry in both total soil pools (72:6:1) and soil microbial biomass (23:3:1) globally (Cleveland & Liptzin 2007). The C:N, C:P and N:P ratios are substantially smaller in soil microbes than in plant tissues, the imbalance between which governs the overall nutrient cycling and C sequestration (Sterner & Elser 2002; Hessen *et al*. 2004). In ombrotrophic bogs, the imbalance between the C:N:P stoichiometry in soil microbes and C-rich plant litters may immobilize N and P by soil microorganisms, while mineralizing C until critical C:nutrients ratios are reached, for examples litter mass C:N ratio between 33 and 68 and C:P ratio between 800 and 1200 in Canadian forest litter (Moore *et al*. 2011). The larger N:P ratios in plant tissues than in soil microbes indicate the mineralization of organic matter in bogs is primarily limited by P, as supported by the immobilization of P in our *in situ* mineralization study discussed above.

Overall at Mer Bleue Bog, N concentration varied less than P and K concentrations, consistent with the results from broad reviews of wetland plants (Bedford *et al*. 1999; Güsewell & Koerselman 2002; Olde Venterink *et al*. 2003). Güsewell & Koerselman (2002) attributed these more variable P and K plant concentrations to a larger variation in P and K availability in soil. Our results suggest that the overall variation in nutrient concentration and stoichiometry is mainly driven by PFTs that coexist in similar habitats. This implies either little variation in soil nutrient availability or weak relationships between soil nutrient availability and nutrient concentration in plants, which is strong homeostasis (Sterner & Elser 2002). The variation in P concentration among PFTs was consistently larger than in N, probably because of the inherently tighter physiological regulation on N than on P (Aerts & Chapin 2000). The smaller variation of stoichiometric ratios than nutrient concentrations for N, P and K may be explained by the fact that the stoichiometric ratios are not affected by C-rich structural materials. The variation in C:N and C:P ratios was largely caused by the variation in N and P concentrations, shown by the negative correlations ($P < 0.001$; $r = -0.973$ and -0.984, respectively).

3.5.3 Seasonal variation among PFTs

A more pronounced seasonal variation of nutrient concentration and stoichiometry was found in deciduous species than in evergreen species and mosses, whereas graminoids and forb showed intermediate variation (Table 3.1). We did not observe significant differences in N:P ratio among PFTs, in contrast to graminoids having a larger N:P ratio than forbs (Güsewell 2004), and herbaceous plants having a smaller N:P ratio than woody plants (Kerkhoff & Enquist 2006). Although statistically insignificant, N, P and K concentrations in graminoids and forb tended to decrease, and therefore C:N and C:P ratios increased, over the growing season, as noted in sedges by Konings *et al*. (1992) and Thormann & Bayley (1997). Mosses, deciduous or evergreen species showed little seasonal variation in nutrient concentrations and stoichiometry over the major part of the season (June to September). Ion-exchange resin membranes (PRS^{TM}, Western Ag Innovations of Saskatchewan, Canada) captured the seasonal variation in available N (ammonium and nitrate), P (orthophosphate) and K in peat soil at Mer Bleue Bog in May, July and September: N availability was doubled and tripled, and K availability was increased by 40 and 80% in July and September compared to May, whereas no change in P availability was observed (Appendix 3.4). This supports our earlier argument of strong homeostasis in bog plants, especially evergreens and mosses, which can keep their tissue stoichiometry stable, despite the changes in the chemical composition of the environment (Sterner & Elser 2002). The uniformly small concentrations of Fe in all PFTs except for mosses are within the range of Fe deficiency (0.05 to 0.15 mg g^{-1}) (Marschner 1995). The larger Fe concentrations in mosses indicates either their greater physiological need or Fe is just left behind as Fe-rich water is drawn up from the water table and is evaporated, leaving Fe in the mosses.

The substantial seasonal variation in nutrient concentration in deciduous species at the beginning (May) and the end (October) of the growing season is attributed to nutrient translocation and resorption respectively, as is suggested in tundra (Chapin *et al*. 1980). Deciduous species translocate a substantial amount of N, P and K into new leaves in the beginning of the growing season and then resorb a large portion of them in the end of the growing season. Evergreen species translocate these nutrients into new leaves gradually throughout the growing season, especially in the second year's leaves, resulting in relatively stable nutrient concentration and stoichiometry (Chapin *et al*. 1980). We observed a 10-50% decrease in N, P and K concentration in the second year's leaves of *C. calyculata* and *R. groenlandicum* during senescence, compared to mature leaves (Wang *et al*. 2014b), supporting the relationship between seasonal variation and nutrient resorption. The lack of seasonal variation in evergreen species and resorption-induced seasonality in deciduous species has been widely reported in arctic and subarctic tundra (Chapin *et al*. 1980; Arndal *et al*. 2009).

3.5.4 Implications for bogs

Bogs, covering 760,000 km^2 in Canada (Tarnocai 2006), are important C sinks with low productivity and slow rates of litter and peat decomposition. Located in a region of relatively high atmospheric N deposition in Canada (~0.8 g N m^{-2} yr^{-1}), Mer Bleue Bog shows N and P co-limitation. The absence of significant external P inputs and historically elevated atmospheric N deposition may trigger further shift to P-limitation of Canadian bogs, as demonstrated in Europe, and a restraint on organic matter mineralization which is primarily P-limited. By adapting to extremely low nutrient availability, bog plants at Mer Bleue maintain small nutrient concentrations in photosynthetically active tissues, especially in evergreen shrubs and *Sphagnum* mosses. Unlike deciduous species, the dominant evergreen shrubs and *Sphagnum* mosses at Mer Bleue Bog do not show a strong seasonal variation in plant stoichiometry, despite seasonal variations in nutrient availability, indicating a strong ecological homeostasis.

Table 3.1 Coefficients of variation (%) for C, N, P, K, Fe and chlorophyll (Chl) concentrations and C:N, C:P, N:P, N:K and K:P ratios. Different letters indicate significant difference in mean coefficient of variation of all nutrient concentrations and stoichiometric ratios among PFTs at $P = 0.05$. See *Materials and Methods* for details of calculations.

Coefficients of variation (%)	C	N	P	K	Fe	Chl	C:N	C:P	N:P	N:K	K:P	Mean	SE
Among functional type means	7.2	39.0	47.4	65.6	58.0	33.4	40.8	50.1	9.7	39.7	32.5	38.4	5.4
Among sampling month means													
mosses	4.5	10.4	10.7	15.9	14.5	13.7	13.6	17.5	9.7	14.5	20.7	13.2a	1.3
evergreen species	4.9	9.5	11.8	36.0	39.4	27.1	10.7	14.8	13.7	29.5	31.3	20.8a	3.6
deciduous species	5.5	50.7	67.7	42.4	37.4	152.8	54.4	69.2	20.9	14.6	24.7	49.1b	12.1
graminoids	7.2	30.6	41.5	66.4	29.8	37.4	37.2	48.8	13.3	34.6	27.9	34.1ab	4.8
forb*	5.3	22.5	45.0	30.1	21.1	41.8	24.8	48.4	22.6	20.2	27.5	28.1ab	3.8
all functional types	4.5	17.5	18.8	24.4	24.9	28.6	18.7	21.3	10.4	13.5	18.7	18.3	2.1
Across all functional types and months	9.0	39.3	52.0	51.3	81.3	71.1	38.7	52.7	20.4	44.3	42.9	45.7	6.1

* The coefficient of variation for forb is calculated from *M. trifolia* only.

Figure 3.1 PCA biplots of C, N, P, K, Fe, chlorophyll (Chl) concentration and C:N, C:P, N:P, N:K and K:P ratios in 14 species. The first two axes explained 87% of the total variance. The means of multiple sampling months for each species were used in this analysis (triangle symbols). Arrows are the eigenvectors of nutrients and their stoichiometric ratios. Abbreviations are: *SC*, *Sphagnum capillifolium; SM*, *S. magellanicum; SA*, *S. angustifolium; SF*, *S. fallax; MT*, *Maianthemum trifolium; SCU*, *S. cuspidatum; LL*, *Larix laricina; RG*, *Rhododendron groenlandicum; KA*, *Kalmia angustifolia; VM*, *Vaccinium myrtilloides; CC*, *Chamaedaphne calyculata; EV*, *Eriophorum vaginatum; TL*, *Typha latifolia; BP*, *Betula populifolia*.

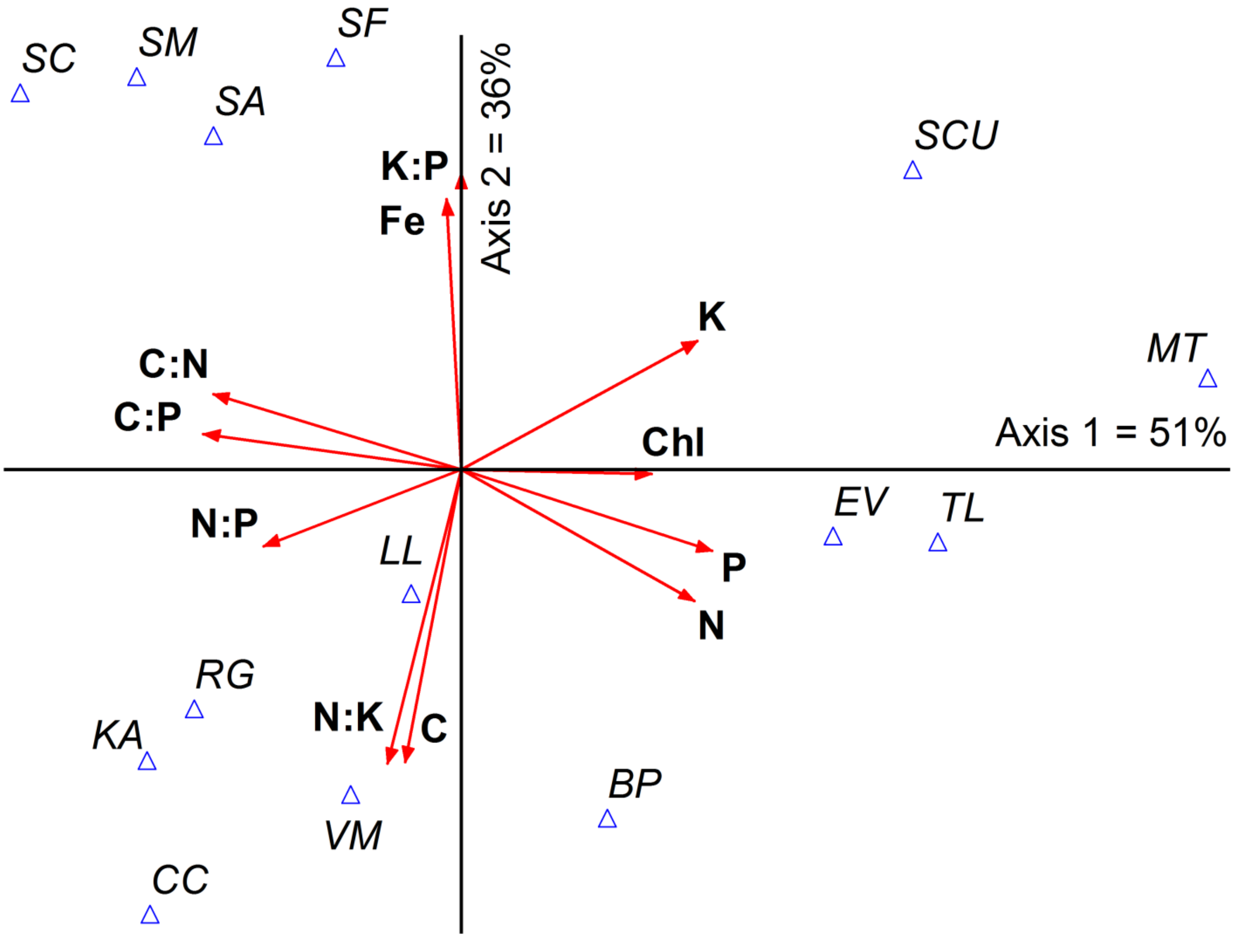


Figure 3.2 Ternary diagram showing the stoichiometric relationship of N, P and K in moss, graminoids, deciduous, forb and evergreen species at peak growing season (a) and over the full season (b). Each symbol represents one species within each PFT in (a) and the average of all species for each PFT in (b). 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 parts, and three of them indicate N-limitation (N section), P or P, N-colimitation (P or P+N section) and K or K, N-colimitation (K or K+N section), respectively. For the central triangle section, the stoichiometric ratio 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.

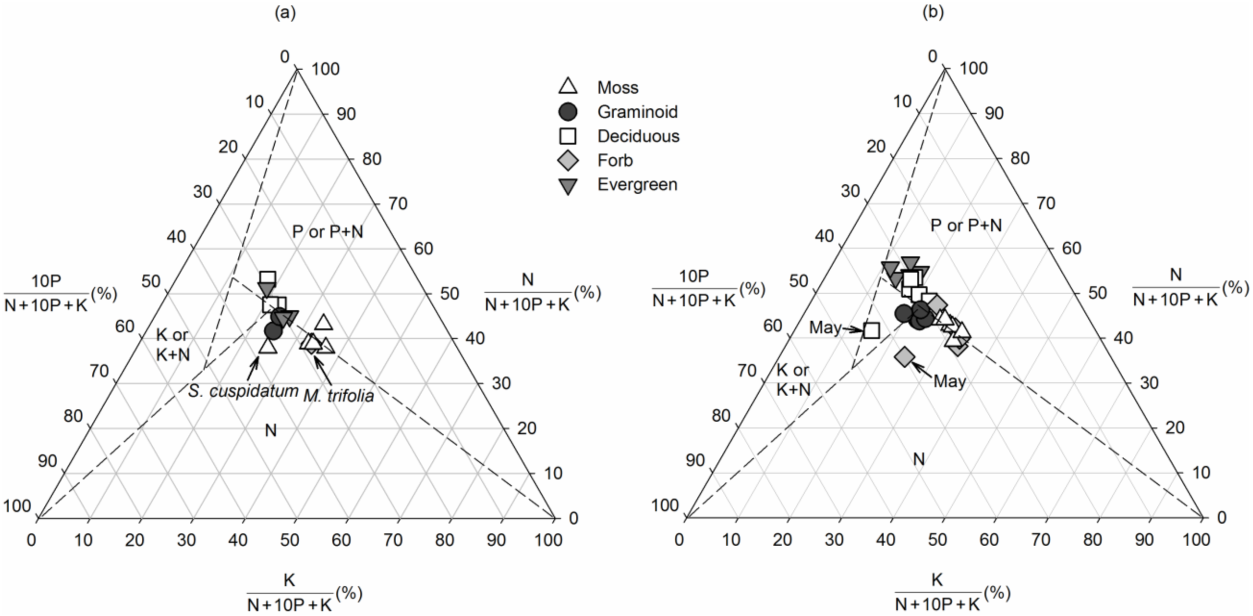
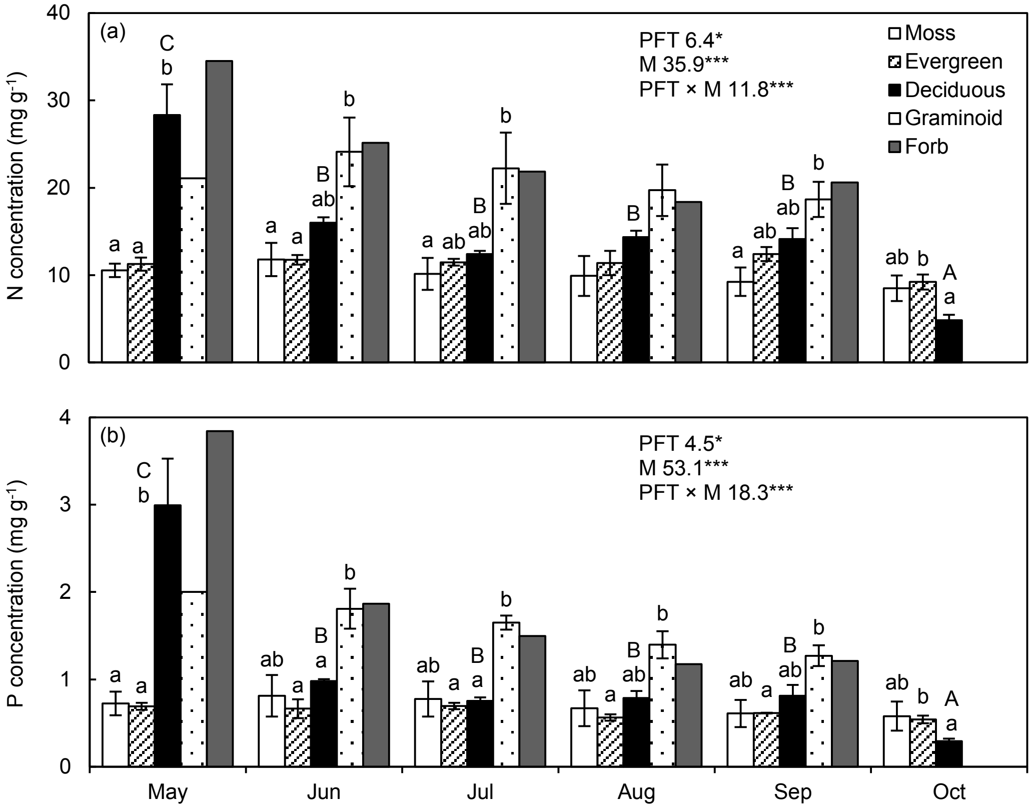


Figure 3.3 The seasonal course of C, N, P, K, Fe and chlorophyll concentration (means ± SE) in moss, evergreen, deciduous, graminoids and forb. Linear mixed model with repeated measures analyzing the difference among PFTs, sampling months (M) and the interaction (PFT × M) in nutrient concentrations are shown. NS, $P < 0.05$; *, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$. Standard error is not calculated for graminoid in May and forb, as they contain only one species respectively. Different upper case letters indicate significant difference between sampling months for each PFT. Different lower case letters indicate significant difference among PFTs for each sampling month.



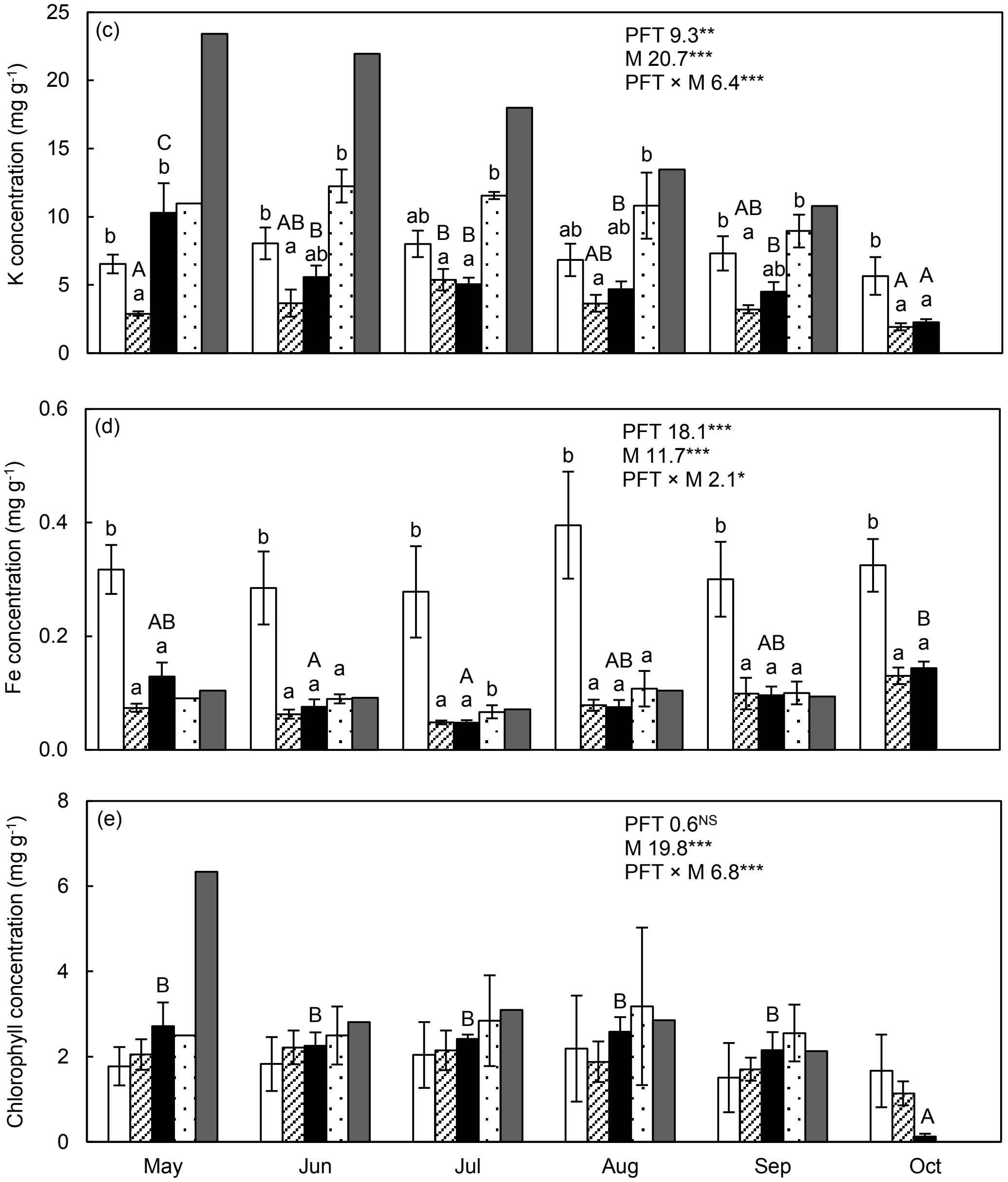
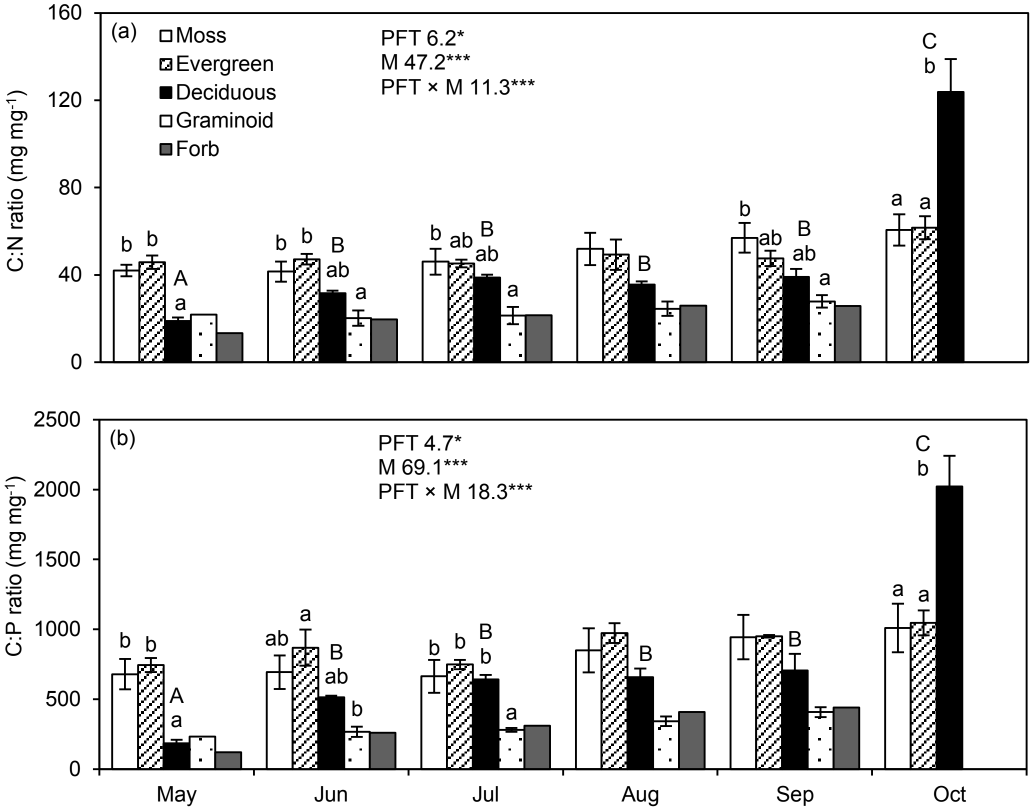
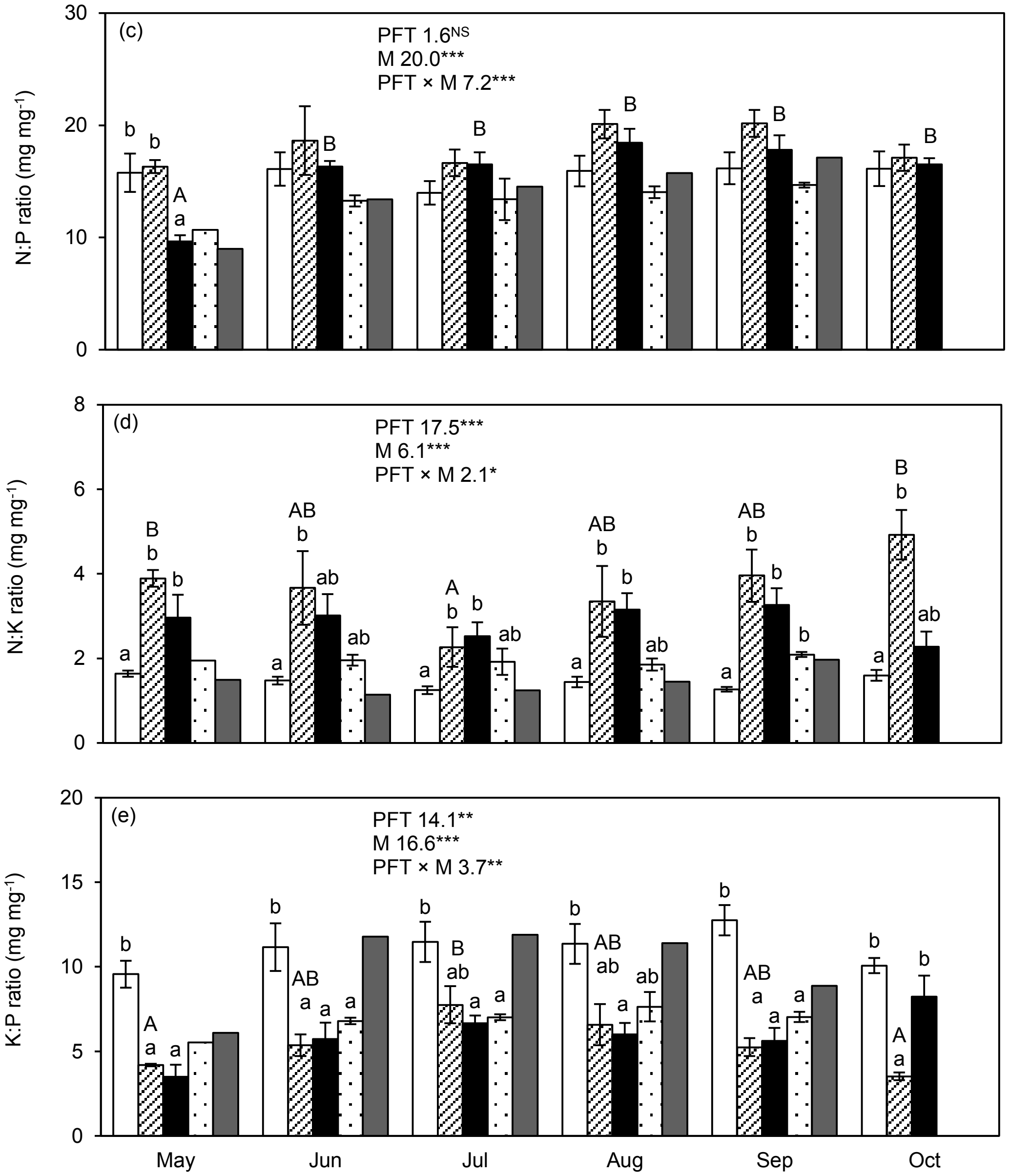

(c)
PFT 9.3**
M 20.7***
PFT × M 6.4***
K concentration (mg g-1)
0
5
10
15
20
25
b
A
a
C
b
b
AB
a
B
ab
b
ab
B
a
B
a
b
ab
AB
a
B
ab
b
b
AB
a
B
ab
b
b
A
a
A
a
(d)
PFT 18.1***
M 11.7***
PFT × M 2.1*
Fe concentration (mg g-1)
0.0
0.2
0.4
0.6
b
a
AB
a
b
a
A
a
a
b
a
A
a
b
b
a
AB
a
a
b
a
AB
a
a
b
a
B
a
(e)
PFT 0.6NS
M 19.8***
PFT × M 6.8***
Chlorophyll concentration (mg g-1)
0
2
4
6
8
B
B
B
B
B
A
May
Jun
Jul
Aug
Sep
Oct

Figure 3.4 The seasonal course of C:N, C:P, N:P, N:K and K:P ratios (means ± SE) in moss, evergreen, deciduous, graminoids and forb. Linear mixed model with repeated measures analyzing the difference among PFTs, sampling months (M) and the interaction (PFT × M) in stoichiometric ratios are shown. NS, $P < 0.05$; *, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$. Standard error is not calculated for graminoid in May and forb, as they contain only one species respectively. Different upper case letters indicate significant difference between sampling months for each PFT. Different lower case letters indicate significant difference among PFTs for each sampling month.




(c)
PFT 1.6^{NS}
M 20.0***
PFT × M 7.2***
N:P ratio (mg mg^{-1})
0
10
20
30
b
b
A
a
B
B
B
B
B
(d)
PFT 17.5***
M 6.1***
PFT × M 2.1*
N:K ratio (mg mg^{-1})
0
2
4
6
8
B
b
b
a
AB
b
ab
a
ab
A
b
b
a
ab
AB
b
b
a
ab
AB
b
b
a
b
B
b
a
ab
(e)
PFT 14.1**
M 16.6***
PFT × M 3.7**
K:P ratio (mg mg^{-1})
0
5
10
15
20
b
A
a
a
b
AB
a
a
a
b
B
ab
a
a
b
AB
ab
a
ab
b
AB
a
a
a
b
A
a
b
May
Jun
Jul
Aug
Sep
Oct

Chapter 4. Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog

(Published on *Oecologia*, 2014, 174, 365-377)

Context within the thesis

Because of the substantial contribution of nutrient resorption to ecosystem nutrient cycling, considerable attention has been given to this process, especially in nutrient-deficient ecosystems, such as, boreal forest, heathland, tundra, subarctic peatland and wet meadow. In Chapter 3, we have observed the essential role of nutrient resorption in controlling seasonal variation in leaf stoichiometry of deciduous shrubs. In contrast, as the dominant vascular species in ombrotrophic peatlands, evergreen shrubs did not vary substantially throughout the growing season with regard to leaf stoichiometry. This discrepancy brings forward an important scientific question: what role does nutrient resorption play in evergreen species? In this chapter, I investigate the magnitude of nutrient resorption by quantifying resorption efficiency (i.e. the proportion of the nutrient that is resorbed during leaf senescence) and proficiency (i.e. the level to which the nutrients are reduced in the senesced leaves) of C, N, P, K, Ca and Mg in two dominant evergreen shrubs at Mer Bleue Bog. Furthermore, in the context of substantially increased atmospheric N and probably P deposition over the past decades, it is critical to understand how ombrotrophic ecosystems respond to these changes. In this chapter, I also examine the effects of long-term N, P and K fertilization and their interactions on nutrient resorption and the homeostatic regulation of different species to nutrient enrichment will be evaluated in Chapter 5. Finally, this chapter complements the lack of data of nutrient resorption in bog vegetation, especially for two essential cations, Ca and Mg.

4.1 Summary

Plant resorption of multiple nutrients during leaf senescence is established but stoichiometric changes among N, P and K during resorption and after fertilization are poorly understood. We anticipated that increased N supply would lead to further P-limitation or co-limitation with N or K, (i.e. P-(co)limitation), decrease N resorption and increase P and K resorption, while P and K addition would decrease P and K resorption and increase N resorption. Furthermore, Ca would accumulate while Mg would be resorbed during leaf senescence, irrespective of fertilization. We investigated the effect of N, P and K addition on resorption in two evergreen shrubs (*Chamaedaphne calyculata* and *Rhododendron groenlandicum*) in a long-term fertilization experiment at Mer Bleue Bog, Ontario, Canada. In general, N addition caused further P-(co)limitation, increased P and K resorption efficiency but did not affect N resorption. Phosphorus and K addition did not shift the system to N-limitation and affect K resorption, but reduced P resorption proficiency. *C. calyculata* resorbed both Ca and Mg while *R. groenlandicum* resorbed neither. *C. calyculata* showed a higher resorption than *R. groenlandicum*, suggesting it is better adapted to nutrient-deficiency than *R. groenlandicum*. Resorption during leaf senescence decreased N:P, N:K and K:P ratios. The limited response of N and K and response of P resorption to fertilization reflects the stoichiometric coupling of nutrient cycling which varies among two shrub species; changes in species composition may affect nutrient cycling in bogs.

4.2 Introduction

Plants may develop two advantageous strategies to adapt to nutrient-deficiency: optimizing nutrient uptake and promoting nutrient conservation (Aerts & Chapin 2000). The withdrawal of nutrients from senescing leaves and the subsequent transportation to storage sites as well as new and growing organs, i.e. resorption (Killingbeck 1986), is an important nutrient conservation strategy, ensuring nutrients are available for future plant growth and reducing the dependence on external nutrient availability (Aerts 1996; Aerts & Chapin 2000). Nutrient resorption efficiency, defined as the proportion of the nutrient that is resorbed during leaf senescence, has global averages of ~65% for N and P and ~70% for K (Vergutz *et al*. 2012). These values for N and P are substantially higher than the widely used global value of ~50% (Aerts 1996; Killingbeck 1996; Aerts & Chapin 2000).

In general, resorption is in a balance with the acquisition of nutrients from the soil and this balance depends on the relative energy consumption of these two processes: which process is energetically ‘cheaper’ (Wright & Westoby 2003). One may anticipate that a high resorption plays an essential role in nutrient-poor environments, where the acquisition of nutrients from the soil is usually more energetically costly relative to resorption (Wright & Westoby 2003). A negative relationship should occur between resorption and nutrient concentration in mature leaves, which is often considered as a good indicator of soil nutrient availability (van den Driessche & Rieche 1974), but this pattern may not occur, for a variety of reasons. First, besides the nutrient concentration in mature leaves (i.e. nutrient availability), other factors can control the resorption process, such as phloem transportation rates and loadings, the extent to which the organic N and P compounds are hydrolyzed (Loneragan *et al*. 1976), the sink strength of different plant organs (Nambiar & Fife 1991) and soil moisture status (Escudero *et al*. 1992; Pugnaire & Chapin 1993). Second, in addition to employing nutrient resorption from leaves to satisfy nutrient demands, symbiotic relationships with ericoid mycorrhizae can mobilize and/or utilize organic forms of limiting nutrients, especially N (e.g. glycine, Näsholm *et al*. 1998; Moore *et al*. unpublished data) and P, reducing plant dependence on nutrient resorption, even in nutrient-poor environments (Straker 1996). The unique function of the ericoid mycorrhizae relies on unique enzymology, which can effectively out-compete other nutrient acquisition mechanisms. Therefore, the relationship between resorption efficiency and nutrient availability is variable, with resorption efficiency being reduced (Kobe *et al*. 2005; Li *et al*. 2010; Vergutz *et al*. 2012), enhanced (Chapin & Kedrowski 1983; Birk & Vitousek 1986; Yuan *et al*. 2005) or unaffected (Chapin & Moilanen 1991; Aerts 1996; Knops *et al*. 1997; Vitousek 1998; Aerts & Chapin 2000; Yuan & Chen 2009a) by increasing nutrient availability.

Resorption proficiency, defined as the level to which the nutrients are reduced in the senesced leaves, has also been used to quantify resorption, with low nutrient concentration in senesced leaves indicating high resorption proficiency (Killingbeck 1996). Resorption proficiency identifies the nutrients returned to the soil in litter and is generally less proficient (i.e. higher nutrient concentration in senesced leaves and litter) under more fertile conditions (Chapin & Moilanen 1991; Vitousek 1998; Diehl *et al*. 2003; Wright & Westoby 2003; Li *et al*. 2010), leaving the litter more rapidly decomposed. The efficiency of resorption reflects plant physiology and metabolic processes, whereas proficiency is directly linked with decomposition processes and hence overall nutrient cycling.

As nutrient-poor ecosystems with slow rates of decomposition, northern peatlands store 547 Gt of C, which is ~30% of global total soil C (Yu *et al*. 2010b), making them important C pools and sinks. Increasing atmospheric N deposition worldwide due to anthropogenic activities has evoked substantial concerns over the functioning of northern peatland ecosystems (Bobbink *et al*. 1998). The availability of N in boreal ombrotrophic (rain-fed) peatlands (i.e. bogs) is often limited, relying on N_2 fixation and atmospheric deposition and the very slow internal decomposition and mineralization processes lead to a substantial accumulation of N in the peat profile. For example, the N accumulation rate in the Mer Bleue Bog over the past 8,000 years averages 0.7 g N m^{-2} yr^{-1} (Wang *et al*. 2014a) and 1.8 g N m^{-2} yr^{-1} in the past 150 years (Moore *et al*. 2004). Enhanced N input may not only increase the primary production of ecosystems but also change the relative significance of nutrients that limit productivity (Verhoeven *et al*. 1996; Aerts & Bobbink 1999). In ombrotrophic peatlands, we anticipate that nutrient resorption is important in regulating nutrient cycling, because plants should be obligated to retain nutrients and reuse them as efficiently as possible. However, recent reviews of nutrient resorption mainly covered forests and grasslands, so peatlands have been overlooked (Yuan & Chen 2009a, b; Vergutz *et al*. 2012).

Plant growth in bogs is often N-limited under low atmospheric N deposition (Aerts *et al*. 1992; Gunnarsson & Rydin 2000), but increasing N deposition may shift to P-(co)limitation (Aerts *et al*. 1992; Bobbink *et al*. 1998; Gunnarsson & Rydin 2000; Bragazza *et al*. 2004). Potassium limitation is rarely studied but can also occur in bogs, especially after CO_2 and N enrichments (Hoosbeek *et al*. 2002). The possible shift in N, P and K interactions highlights that it is the relative balance among multiple nutrients, or ecological stoichiometry (Sterner & Elser 2002), in addition to their absolute amount, that governs metabolic functioning, plant growth and ecosystem processes. Reed *et al*. (2012) examined global stoichiometric patterns in foliar N:P resorption ratios in response to plant nutrient limitation and showed a trend of increasing ratios with latitude and decreasing with mean annual temperature, providing implications on nutrient cycling and limitation.

While N, P and K are acknowledged to be the predominant limiting nutrients in peatlands, Ca and Mg are important in ecological processes in peatlands but frequently overlooked. Calcium and Mg have an extremely low abundance in ombrotrophic bogs compared to minerotrophic fens or upland mineral soils (Rydin & Jeglum 2006). Bubier *et al*. (2011) reported a decline in leaf Ca and Mg concentration in response to fertilization in three dominant bog shrubs, *Chamaedaphne calyculata*, *Rhododendron groenlandicum* and *Vaccinium myrtilloides*, indicating physiological stress under conditions of high N accumulation. The resorption of Ca and Mg differs substantially, with Ca generally accumulating continuously during leaf development, being immobile in the phloem and hence not being resorbed during leaf senescence (Clarkson & Hanson 1980; Negi & Singh 1993; Lambers *et al*. 1998). In contrast, Mg is tightly bound to polyphosphates, readily mobile (Clarkson & Hanson 1980) and thus more likely to be resorbed than Ca. Most resorption studies have mainly focused on N and P and sometimes K, with little attention paid to Ca and Mg, although the interactions between N, P and K affect resorption in forest ecosystems (Chapin & Moilanen 1991; Knops *et al*. 1997; Vitousek 1998; Diehl *et al*. 2003). In bogs where the availability of three potentially limiting macronutrients (i.e. N, P and K) is very small, substantial resorption during senescence is expected to regulate nutrient cycling, and stoichiometric responses to changing nutrient availability from for example nutrient deposition and global warming is important.

Our objective was to investigate the effect of N, P and K addition on resorption efficiencies and proficiencies of N, P, K, C, Ca and Mg in two dominant evergreen shrubs (*C. calyculata* and *R. groenlandicum*) in a long-term fertilization experiment established a decade ago in Mer Bleue Bog in eastern Ontario, Canada. After decades of elevated atmospheric N deposition in eastern Canada (Turunen *et al*. 2004), the leaf-level N, P and K stoichiometric relationship derived from the ternary diagram (Olde Venterink *et al*. 2003) of these two species over the growing season (June to August) indicates that plant growth in this bog is co-limited by P and N, and there is no evidence showing K limitation (Wang & Moore 2014). After a decade of fertilization with N, PK, or their combination, we assumed the addition of N has stimulated further P-(co)limitation and reduced N resorption, which has been shown in a sub-arctic bog (van Heerwaarden *et al*. 2003b). Nitrogen enrichment should stimulate the resorption of P and K to maintain N:P:K stoichiometric homeostasis. Long-term fertilization with P and K should shift the ecosystem to N limitation with a decline in the resorption of P and K while the resorption of N should be enhanced to maintain the N:P:K stoichiometric homeostasis.

Therefore, we hypothesized: (1) the addition of N would reduce the resorption of N, while the addition of PK would reduce the resorption of P and K (i.e. a direct fertilization effect); (2) N addition would stimulate the resorption of P and K, while P and K addition will increase the resorption of N (i.e. stoichiometric interactions); (3) Ca would accumulate while Mg would be resorbed during leaf senescence irrespective of fertilization.

4.3 Materials and Methods

4.3.1 Study site and species description

The experiment was conducted at the Mer Bleue peatland, mainly a bog covering 28 km^2 located 10 km east of Ottawa, Ontario, Canada (45.40°N, 75.50°W). Mean annual temperature is 6.0 °C ranging from -10.8 °C in January to 20.9 °C in July (Bubier *et al*. 2007). Mean annual precipitation is 943 mm (76% as rainfall), 350 mm of which falls from May to August (Canadian Climate Normals 1981-2010). The estimated atmospheric inorganic wet N deposition in this site was ~0.8 g N m^{-2} yr^{-1} (Turunen *et al*. 2004) and total wet P deposition ranges from 6 to 26 mg P m^{-2} yr^{-1} (R. Vet personal communication).

There are two sets of fertilization experiments each with triplicate 3 × 3 m plots, set up on the hummock microtopography at Mer Bleue Bog (see Bubier *et al*. 2007). Experiment 1 was established in 2000 and 2001, and experiment 2 started in 2005 (Table 4.1), with 27 plots in total and a spatial extent of ~600 m^2 (20 × 30 m). The fertilization is conducted every three weeks from May 1 to August 31 each year, with N added as NH_4NO_3 and P and K as KH_2PO4. The dominant vascular species are *C. calyculata* (L.) Moench and *R. groenlandicum* (Oeder) Kron & Judd (formerly *Ledum groenlandicum* Oeder), which are the dominant evergreen shrubs in the boreal bogs of North America (Glaser 1992). *Sphagnum* mosses survive only in some of the plots with low levels of fertilization. *Vaccinium myrtilloides* Michx. and *Kalmia angustifolia* L. were distributed sparsely in some of the plots, but not sampled.

The leaf longevity of both species is two growing seasons (Reader 1980) and to sample leaves that would senesce the coming fall, the previous season's leaves were identified. To guarantee the leaves were sampled from the same cohort with the same age, individual leaves of *C. calyculata* formed in 2010 were marked at the base of the leaf with a dab of latex paint at the end of June 2011, when it was still easy to differentiate the new from the old leaves. This ensured that only leaves that would senesce the coming fall would be sampled. As *R.*

groenlandicum produces its new leaves and stems by extending from old stem and leaf clusters, it was possible to select old leaves without labelling them.

4.3.2 Sampling and chemical analysis

Two replicates (each ~40 leaves) of sun-exposed and fully expanded mature leaves of both species in each of the 27 plots were collected on July 25 and 28, 2011 and a similar number of recently senesced, but still attached, reddish brown leaves were sampled on October 22, 2011. We scanned the leaves immediately (Epson Expression 10000XL, Epson American Inc., USA) to determine the total projected leaf area per sample using Image J software (version 1. 45s, by Wayne Rasband, National Institute of Health, USA). Leaves were then oven-dried at 60 °C to a constant weight and specific leaf area (SLA) was calculated as the leaf area per unit leaf dry mass (cm^2 g^{-1}) (Appendix 4.1). Samples were ground (Wiley Mini Mill 3383-L10, Thomas Scientific, USA) to fine powder (60-mesh sieve) and total C and N concentrations were determined by dry combustion on an elemental analyzer (Leco CNS 2000, Leco Corporation, St. Joseph, Michigan, USA). Subsamples were digested (Technicon BD-40 block digester, Technicon Instruments Corporation, Tarrytown, New York, USA) in concentrated sulphuric acid and hydrogen peroxide with selenium and lithium sulphate as catalysts (Parkinson & Allen 1975) and passed through 0.45 µm glass fibre filters (MN 85/90, MACHEREY-NAGEL GmbH & Co. KG, Germany). Total P was determined colorimetrically by the ammonium molybdate-ascorbic acid method (Murphy & Riley 1962) on a Lachat Quik-Chem AE flow-injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Potassium, Ca and Mg concentrations were measured on an atomic absorption spectrophotometer (PerkinElmer model 2380, PerkinElmer Inc., USA).

4.3.3 Nutrient resorption proficiency and efficiency calculation

Resorption efficiency may be underestimated because the loss of leaf mass or leaf shrinkage during senescence may be up to 10 and 6%, respectively (van Heerwaarden *et al*. 2003a). The resorption efficiency can be corrected using leaf mass loss and shrinkage of leaf area, or lignin concentration (van Heerwaarden *et al*. 2003a) but as the lignin concentration may be affected by our long-term fertilization (Vitousek 1998), we did not measure it to correct for mass loss. Instead, leaf area was used as the metric to calculate resorption efficiency, given that the tough structure of these two sclerophyllous evergreen shrubs was resistant to leaf shrinkage during senescence. Mass-based nutrient concentration was divided by SLA to derive leaf area based nutrient concentration and the latter was used in the calculation of resorption efficiency (nutrient concentrations in mature leaves are shown in Appendix 4.2). Mass-based nutrient concentrations in mature and senesced leaves are presented in Appendices 4.3 and 4.4, respectively. Nutrient resorption proficiency was based on the nutrient concentration in senesced leaves: the lower the nutrient concentration, the higher resorption proficiency (Killingbeck 1996). Nutrient resorption efficiency (RE) was calculated as:

$$\mathrm{RE} = \left(1 - \frac{[\mathrm{Nutrient}]_{\mathrm{senesced}}}{[\mathrm{Nutrient}]_{\mathrm{mature}}}\right) \times 100\%$$

where $[\mathrm{Nutrient}]_{\mathrm{senesced}}$ and $[\mathrm{Nutrient}]_{\mathrm{mature}}$ are the leaf area based nutrients concentration (mg cm^{-2}) of recently senesced and mature leaves, respectively. N_{RE}, P_{RE}, K_{RE}, C_{RE}, Ca_{RE} and Mg_{RE} were calculated correspondingly.

4.3.4 Statistics

The normality of data was examined by Shapiro-Wilk test and Levene's test was used to check the homogeneity of variance. Because the data transformations have to be done in different ways to meet the assumptions of homogeneity of variance, Kruskal-Wallis non-parametric test was performed separately for experiment 1 and 2 with non-transformed data. Differences between individual treatments and corresponding control were assessed by the Steel Method (a non-parametric version of Dunnett's post-hoc test) for experiments 1 and 2 separately. We used *t* test to examine the interspecific difference between *C. calyculata* and *R. groenlandicum* in mature and senesced leaves' nutrient concentration and nutrient resorption efficiency under the same treatment, and the difference between the resorption efficiency under unfertilized conditions and the global averages of evergreen angiosperms (Vergutz *et al*. 2012), using raw data from different sites prior to averaging from the Oak Ridge National Laboratory Distributed Archive Center (http://daac.ornl.gov). The correlation among nutrient concentration in mature and senesced leaves, resorption efficiency and the stoichiometric ratios were conducted by Spearman's rank correlation analysis. Analyses were conducted in JMP 10 software (SAS Institute Inc., Cary, NC, USA).

4.4 Results

4.4.1 Resorption proficiency in senesced leaves

Under the same treatment, N, P, K, C and Mg concentrations in senesced leaves were all significantly lower in *C. calyculata* ($P < 0.001$), although the Ca concentration was significantly higher than that in *R. groenlandicum* ($P < 0.001$; Figure 4.1). The addition with N, PK or their combinations had little effect on N, K and C concentrations in either species compared to the controls ($P > 0.05$; Figure 4.1a,c,d).

All treatments with PK addition (i.e. PK, 5NPK, 10NPK and 20NPK) significantly increased P concentration compared to the control in both species ($P < 0.05$; Figure 4.1b). N addition did not affect P concentration ($P > 0.05$).

The combination of N and PK addition showed stronger effects on Ca and Mg concentrations than N or PK added separately, but no consistent pattern was observed (Figure 4.1e,f).

4.4.2 Resorption efficiency in senesced leaves

The resorption efficiencies of all nutrients were higher in *C. calyculata* than in *R. groenlandicum* under the same treatment ($P < 0.01$; Figure 4.2). High levels of N addition (i.e. 10N and 20N treatments) showed stronger impact on resorption efficiencies than low level of N addition (i.e. 5N treatment) and PK added alone (i.e. PK treatment). The combinations of N and PK did not affect resorption efficiencies in a consistent way and the effect varied between species.

The N_{RE} was not affected in N-only treatments (i.e. 5N, 10N and 20N) compared to controls in either species ($P > 0.05$; Figure 4.2a). 5NPK and 20NPK significantly increased N_{RE} compared to the controls in *C. calyculata* and *R. groenlandicum*, respectively ($P < 0.05$). The P_{RE} was significantly increased under 10N and 20N treatments compared to the control in both species ($P < 0.05$; Figure 4.2b). However, PK treatment did not affect P_{RE} in either species ($P > 0.05$), and 10NPK significantly reduced P_{RE} in *C. calyculata* compared to the control ($P < 0.05$). N-only treatments did not affect K_{RE} in *C. calyculata* ($P > 0.5$; Figure 4.2c), however, both 10N and 20N doubled K_{RE} in *R. groenlandicum* compared to the control ($P < 0.05$). Similar to the pattern observed for P_{RE}, PK treatment did not affect K_{RE} in either species ($P > 0.1$). C_{RE} in *C. calyculata* did not respond to NPK fertilization ($P > 0.05$; Figure 4.2d). However, 20NPK treatment significantly increased C_{RE} in *R. groenlandicum* compared to the control ($P < 0.01$) and so did the 10N and 20N treatments ($P < 0.05$).

In general, *C. calyculata* showed positive resorption efficiencies of both Ca (4-30%) and Mg (29-41%), while Ca and Mg were accumulated (i.e. negative resorption efficiency) in *R. groenlandicum* (Figure 4.2e,f). The effect of N addition on Ca_{RE} and Mg_{RE} in both species was very weak and no significant effect of PK or NPKs addition was detected ($P > 0.5$).

4.4.3 Correlations among nutrient concentration, resorption efficiency and stoichiometric ratios

For both species, the nutrient concentrations in mature leaves were positively correlated with those in senesced leaves, except for N (Table 4.2). In contrast, resorption efficiencies were negatively correlated with the nutrient concentrations in senesced leaves (i.e. positively with resorption proficiency) in both species. In general, resorption efficiencies were significantly correlated with nutrient concentration in both mature and senesced leaves.

In both species, N_{RE}, Ca_{RE} and Mg_{RE} were weakly correlated with the stoichiometric ratios (i.e. N:P, N:K and K:P) considered indicators of the types of nutrient limitation (Table 4.2). The correlation between nutrient resorption efficiencies and stoichiometric ratios was generally stronger in *C. calyculata* than *R. groenlandicum*: P_{RE} in both species was positively correlated but K_{RE} and C_{RE} were negatively correlated with all three stoichiometric ratios only in *C. calyculata*.

Few significant correlations between resorption efficiencies were observed in *C. calyculata*, whereas in *R. groenlandicum* the resorption efficiencies were significantly correlated with each other, except for Ca_{RE}.

4.4.4 The stoichiometric relationship between N, P and K during leaf senescence

Neither species showed evidence of N being the predominantly limiting nutrient under any treatments, no samples falling in the N-limited section of the ternary diagram, nor were they primarily N-limited even after P and K addition (Figure 4.3). The effect of fertilization on N:P:K stoichiometry was more substantial in senesced leaves than in mature leaves. In both species, the addition of P and K drove the symbols to the bottom left, reflecting an increase in the relative P concentration, a decrease in the relative N concentration and little effect on the relative K concentration, compared to the control (i.e. moving parallel to the P-axis) (Figure 4.3). If N was added alone, no substantial change was observed. For both species, after fertilized with PK (i.e. PK, 5NPK, 10NPK and 20NPK), leaf senescence generally increased the relative P concentration and decreased the relative N concentration, but did not affect the relative K concentration substantially, similar to the effect of P and K addition compared to the control or N-only treatments (Figure 4.3).

4.5 Discussion

4.5.1 Resorption efficiency: boreal bog plants in the global context

We used leaf area-based nutrient concentration to derive nutrient resorption efficiency because mass-based data can underestimate the resorption efficiency by up to 10% (van Heerwaarden *et al*. 2003a). Mass loss during leaf senescence is likely due to the removal of compounds and carbohydrates (Nordell & Karlsson 1995). The leaves of both evergreen shrubs contained ~54% C (Appendix 4.3) and assuming that leaf shrinkage of both sclerophyllous species during senescence is negligible, we can estimate leaf mass loss based on C_{RE}, which are ~27 and ~3% in *C. calyculata* and *R. groenlandicum*, respectively, under unfertilized conditions. The leaf mass loss in *C. calyculata* is similar to the global average of evergreen angiosperms (Vergutz *et al*. 2012), but the small mass loss in *R. groenlandicum* requires further investigation.

Vergutz *et al*. (2012) compiled a global dataset of nutrient resorption efficiency with the averages of N, P, K, C, Ca and Mg for evergreen angiosperms (mainly tree species) being 56, 58, 56, 21, -5 and 12%, respectively. The N_{RE} and P_{RE} are slightly higher than those reported by Aerts & Chapin (2000) for evergreen species (50 and 55%, respectively), probably because their study covered a larger proportion of evergreen species in boreal and subarctic ecosystems. Our study indicated interspecific differences under natural conditions: in *C. calyculata*, the C_{RE} (27%) and Mg_{RE} (29%) were higher ($P < 0.001$) while N_{RE} (50%) and P_{RE} (41%) were lower ($P < 0.001$) than the global average and there was no significant difference for K_{RE} (55%) and Ca_{RE} (4%) ($P > 0.5$). In *R. groenlandicum*, the resorption efficiency of all nutrients (N, P, K, C, Ca and Mg: 24, 10, 28, 3, -26% and -16%, respectively) was significantly lower than the global averages ($P < 0.001$). The N_{RE} and P_{RE} in *R. groenlandicum* were also substantially lower than in a congeneric species, *R. lapponicum*, in which N_{RE} and P_{RE} were 78-79% and 80-85%, respectively (Karlsson 1994). As both species are ericoid mycorrhizal evergreen shrubs, mycorrhizal fungi may mediate uptake, reducing dependence on resorption from senesced leaves.

Small (1972) reported the N, P and K concentration in fresh litters at Mer Bleue were 7.5, 0.3 and 0.3 mg g^{-1} in *C. calyculata* and 4.0, 0.3 and 1.1 mg g^{-1} in *R. groenlandicum* (referred to as *Ledum groenlandicum* in his work), respectively, similar to Killingbeck's (1996) critical values of potential N and P resorption proficiency for evergreen species. Therefore, we consider these N, P and K concentration in fresh litters as the critical values of potential resorption proficiencies under natural condition in *C. calyculata* and *R. groenlandicum*. *C. calyculata* resorbs N and P proficiently by minimizing nutrient loss through litters (7.4 mg N g^{-1} and 0.4 mg P g^{-1} in unfertilized senesced leaves; Appendix 4.4), while *R. groenlandicum* does not (8.4 mg N g^{-1} and 0.6 mg P g^{-1} in unfertilized senesced leaves; Appendix 4.4). In contrast, neither species resorbs K proficiently (1.8 and 3.2 mg K g^{-1} in unfertilized senesced leaves of *C. calyculata* and *R. groenlandicum*, respectively). The general trend of lower nutrient concentration, or higher resorption proficiency, in the senesced leaves of *C. calyculata* than *R. groenlandicum*, apart from Ca, suggests that *C. calyculata* adapts to nutrient-deficiency better than *R. groenlandicum*, by minimizing nutrient loss. The two species did not show differences in SLA of mature leaves (Appendix 4.1) but the difference appeared in the senesced leaves. A larger SLA in senesced leaves contributes little to changes in leaf life span or morphological adjustment (Shipley *et al.* 2005), but reflects mass loss during leaf senescence.

4.5.2 Effects of fertilization on resorption proficiency and efficiency

Resorption proficiency and efficiency complement each other and are often used together to investigate nutrient resorption. We found there was a strong negative correlation between nutrient resorption efficiency and the nutrient concentration in senesced leaves (Table 4.2), indicating that, in general, resorption efficiency and proficiency respond to nutrient addition identically.

We found the addition of N or PK only weakly affected resorption directly (hypothesis 1) and stoichiometric interactions played a more important role, given the significant positive effect of N addition on P and K resorption (hypothesis 2). We found an inconsistent effect of fertilization on Ca and Mg resorption and the two species resorbed Ca and Mg in different ways: *C. calyculata* resorbed both Ca and Mg while *R. groenlandicum* resorbed neither (hypothesis 3).

In general, the response of nutrient resorption to NPK addition depended on the types of nutrient limitation.

As expected, after a decade of N addition combined with atmospheric N deposition (0.8 g N m^{-2} yr^{-1}), P was the predominant limiting nutrient or at least co-limited with N (Figure 4.3). Contrary to our first hypothesis, the addition of N did not affect N resorption, regardless of the levels of addition (1.6, 3.2 or 6.4 g N m^{-2} yr^{-1}), supporting the argument of possible N saturation in this ecosystem (Bubier *et al*. 2011). To maintain a balanced N:P:K stoichiometry, we anticipated an increase in P and K resorption in response to N addition, especially under P-(co)limited condition: we found P_{RE} of both species was increased by high levels of N addition (3.2 and 6.4 g N m^{-2} yr^{-1}) which also increased K_{RE} of *R. groenlandicum*. This positive response of P resorption to N addition follows the general anticipation that in a P-(co)limited ecosystem, the conservation of P will be stimulated to meet the requirement of P when the availability of N is increased (Rejmánková 2005).

A difference in resorption proficiency response to increasing nutrient availability among species affects the role of vegetation in ecosystem nutrient cycling (van Heerwaarden *et al*. 2003b), through a feedback between soil fertility and plant economics. Raising soil fertility usually has positive effects on plant leaf nutrient concentration, increasing the amount of nutrients returning to the soil. Increasing abundance of *C. calyculata* over *R. groenlandicum* after a decade with the highest N level together with PK (i.e. 20NPK) (Bubier *et al*. 2011) suggests further attention to the relationship between nutrient resorption and changes in species composition in bog ecosystems. The interspecific divergence in nutrient resorption can mediate ecosystem nutrient cycling through stoichiometric interactions with consumers and decomposers along the food webs (Sterner & Elser 2002). For example, the higher resorption proficiency in *C. calyculata* than *R. groenlandicum* would further decrease litter decomposition by exacerbating the mismatch of C:element ratios between plants and decomposers.

After the addition of PK alone for 12 years, P-(co)limitation was compensated but not yet shifted into N limitation and the shrubs are on the margin of K limitation (Figure 4.3). This may be related to K leaching through the peat owing to the high mobility of K under acidic conditions in this ecosystem (Rydin & Jeglum 2006) and the lack of response of K resorption to PK addition goes against our first hypothesis. The dominance of co-limitation by N and PK is supported by changing shrub abundance in response to fertilization: the abundance of *C.*

calyculata and *R. groenlandicum* under N- or PK-only treatments are not significantly different from controls, whereas their abundance increased by ~50% under 10NPK and 20NPK treatments (Larmola *et al*. 2013). There was a strong correlation between mature and senesced leaves' P concentration (Spearman's $\rho = 0.9$), and the addition of PK increased the P concentration in mature and senesced leaves to a similar extent, leading to a higher P resorption proficiency but unchanged efficiency. This response of resorption proficiency over efficiency to nutrient addition has been discussed in subarctic bogs species (van Heerwaarden *et al*. 2003b), mangroves (Feller *et al*. 2003; Rejmánková 2005) as well as a perennial herb (Anderson & Eickmeier 2000). Contrary to our second hypothesis, PK addition did not significantly increase N resorption. The weak trend of increasing N resorption efficiency and proficiency in response to PK addition in both species implies the increase in N resorption cannot keep pace with the increasing P concentration, leading to an ultimate shift in nutrient limitation.

There was no general, strong interactive effect among N, P and K resorption, contrary to our expectation and the few significant effects are mainly due to the cumulative rather than the interactive effect of N and PK. For example, although 5N, 20N and PK can increase N_{RE} substantially but insignificantly (from a statistical perspective), their combination showed a significant increase of N_{RE} in 5NPK and 20NPK treatments in *C. calyculata* and *R. groenlandicum* respectively.

The lack of correlation between mature and senesced leaves' N concentration to a certain extent supports the argument that a large portion of N in mature leaves is probably stored as amino acids. These are easy to transport when resorbed (Chapin & Kedrowski 1983) and reduce the risk of toxic effects of ammonium through excess N availability (Bubier *et al*. 2011).

Contrary to our third hypothesis, there was interspecific divergence in Ca and Mg resorption, with Ca accumulation in *R. groenlandicum* and Mg resorption in *C. calyculata* during leaf senescence. However, *C. calyculata* can resorb Ca as reported in grasslands and alpine forests (Ralhan & Singh 1987; Peri & Lasagno 2010), while *R. groenlandicum* did not conserve Mg through resorption (i.e. resorption efficiency ≤ 0). The positive resorption of Ca and Mg in *C. calyculata* is attributable to the conservation of these two important cations in bogs, and might facilitate the growth of *C. calyculata* in acidic ombrotrophic environment where the available Ca and Mg are extremely low and benefit its competitive advantage over *R. groenlandicum*.

4.5.3 Stoichiometric relationships and the implications for bogs

Bogs, covering 760,000 km^2 in Canada (Tarnocai 2006), are generally poor in nutrients, dominated by evergreen shrubs and mosses and nutrient retention and recycling by plants play an important role in the functioning of these peatland ecosystems, one process being the resorption of nutrients from senescent leaves. Elevated rates of atmospheric deposition of nutrients, especially N, and global warming (Rustad *et al*. 2001) have increased nutrient availability. Thus, determining shrub nutrient resorption and the effect of added nutrients are important in the future of these important C-sequestering ecosystems. Our results illustrate that, although *C. calyculata* and *R. groenlandicum* share the same habitat and have a similar leaf life span and N and P concentration in mature leaves, they have different resorption characteristics.

Under natural conditions, a substantial portion of N, P and K were resorbed (10-55%) during leaf senescence in the two shrubs, though Ca and Mg resorption was lower and *R. groenlandicum* showed a gain in Ca and Mg in some treatments during senescence. Fertilization with PK (i.e. PK, 5NPK, 10NPK and 20NPK) drove the trend of increasing relative concentration of P, decreasing N and not affecting K in both mature and senesced leaves in the ternary diagram. A similar pattern was observed during leaf senescence (i.e. difference between mature and senesced leaves) under PK treatments, suggesting that litter decomposition could become more N-limited after P and K fertilization. By resorbing nutrients proficiently, the shrubs maintain a low nutrient concentration in the litter, with likely slower decomposition rates, thereby linking the leaf economy and soil processes and imposing a feedback on nutrient cycling.

The lack of correlation between N_{RE} and the resorption efficiency of other nutrients, especially P, confirms that N and P resorption are not always coupled, as the hydrolysis processes of N and P during leaf senescence are regulated by independent mechanisms (Rejmánková 2005). The N_{RE} showed no correlation with the types of nutrient limitation, as indicated by the stoichiometric ratios (Table 4.2). In contrast, P_{RE} in general positively correlated with N:P and K:P ratios, appearing to be against the argument that there is no relationship between nutrient resorption and the types of nutrient limitation among wetland graminoids (Güsewell 2005a). However, our results indirectly confirmed the conclusion from Rejmánková (2005) that P resorption was significantly higher in the P-limited plots, facilitating adaptation to nutrient limitation. This may be an important strategy of these two shrubs at Mer Bleue Bog, adapting to increased N deposition (from ~0.2 to ~0.8 g m^{-2} yr^{-1}).

C. calyculata and *R. groenlandicum* are two dominant shrubs in boreal peatlands and appear similar in many characteristics including leaf-level photosynthetic capacity, leaf chemistry and morphology (Bubier *et al*. 2011), yet resorbed nutrients in different ways during the long-term fertilization: *C. calyculata* showed a greater ability to resorb nutrients than *R. groenlandicum* by embracing a higher SLA in senesced leaves. Other physiological controls of the interspecific difference in terms of resorption efficiency and proficiency in response to the increased soil N, P and K availability remain unknown, but observed changes in species composition can pose a strong impact on nutrient cycling through nutrient resorption. Recent reviews of nutrient resorption have established broad patterns, mainly in forests and grasslands (Yuan & Chen 2009a, b; Vergutz *et al*. 2012). Our study advances the understanding of the stoichiometric interactions of multiple limiting nutrients in bog ecosystems, showing the large variation between species within PFTs in nutrient resorption (Nordell & Kalsson 1995; Aerts 1996; Eckstein *et al*. 1998; Aerts & Chapin 2000). Changes in species composition through changing nutrient limitation, owing to nutrient enrichment or global warming, could mediate ecosystem nutrient cycling in bog ecosystems.

Table 4.1 The fertilization experimental design at Mer Bleue Bog.

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

N fertilization levels equal 5, 10 and 20 times the estimated summer time atmospheric wet N deposition and treatments are abbreviated accordingly. C1 and C2 are the controls (added distilled water only) for the two sets of experiment, respectively.

Table 4.2 The correlation among leaf area based N, P, K, C, Ca and Mg concentration in mature leaves (subscript ‘m’), senesced leaves (subscript ‘s’), their nutrient resorption efficiencies (subscript ‘RE’) and stoichiometric ratios (N:P, N:K and P:K) of *C. calyculata* (the bottom left) and *R. groenlandicum* (the upper right).

	N_m	P_m	K_m	C_m	Ca_m	Mg_m	N_s	P_s	K_s	C_s	Ca_s	Mg_s	N_{RE}	P_{RE}	K_{RE}	C_{RE}	Ca_{RE}	Mg_{RE}	$N:P_m$	$N:K_m$	$K:P_m$
N_m		-0.14	0.43*	0.77*	0.28*	0.30*	0.23	-0.21	-0.01	0.28*	0.04	-0.24	0.35*	0.19	0.37*	0.38*	0.05	0.48*	0.45*	0.37*	0.27*
P_m	0.06		0.08	-0.11	-0.01	0.33*	-0.42*	0.88*	0.10	-0.40*	-0.20	0.27	0.31*	-0.27*	0.05	0.24	0.25	0.13	-0.93*	-0.22	-0.91*
K_m	0.32*	0.73*		0.43*	-0.05	0.09	-0.02	0.00	0.15	0.02	-0.08	-0.32*	0.27*	-0.04	0.47*	0.35*	-0.14	0.40*	0.05	-0.64*	0.27
C_m	0.37*	-0.27*	-0.12		0.56*	0.51*	0.29*	-0.22	0.02	0.52*	0.27	0.06	0.15	0.13	0.33*	0.31*	0.09	0.44*	0.36*	0.21	0.27*
Ca_m	-0.42*	-0.35*	-0.55*	0.20		0.51*	0.22	-0.11	0.09	0.47*	0.53*	0.33*	-0.10	-0.02	-0.05	-0.04	0.26	0.11	0.13	0.34*	0.02
Mg_m	-0.40*	0.16	-0.18	0.03	0.52*		-0.03	0.22	-0.12	0.13	0.16	0.39*	0.18	-0.01	0.27*	0.28*	0.10	0.55*	-0.18	0.13	-0.26
N_s	0.23	-0.24	-0.22	0.19	0.13	0.06		-0.29*	0.27*	0.69*	0.48	0.18	-0.54*	-0.21	-0.12	-0.31*	-0.27*	-0.13	0.46*	0.27*	0.42*
P_s	-0.10	0.84*	0.65*	-0.50*	-0.28*	0.14	-0.09		0.27*	-0.29*	-0.11	0.30*	0.21	-0.50*	-0.11	0.11	0.09	0.01	-0.88*	-0.19	-0.86*
K_s	0.20	0.28*	0.44*	0.16	-0.28*	-0.26	0.30*	0.32*		0.31*	0.20	0.13	-0.24	-0.40*	-0.51*	-0.25	-0.08	-0.20	-0.10	-0.12	-0.04
C_s	0.43*	-0.46*	-0.40*	0.51*	0.09	-0.17	0.54*	-0.52*	0.08		0.69*	0.45*	-0.44*	-0.11	-0.09	-0.37*	-0.23	-0.22	0.45*	0.23	0.42*
Ca_s	-0.24	-0.42*	-0.63*	0.13	0.54*	0.29*	0.38*	-0.34*	-0.40*	0.49*		0.51*	-0.47*	-0.28*	-0.15	-0.45*	-0.33*	-0.35*	0.21	0.18	0.19
Mg_s	-0.21	-0.05	-0.35*	-0.02	0.30*	0.35*	0.17	-0.10	-0.45*	0.18	0.68*		-0.28*	-0.29*	-0.22	-0.31*	-0.15	-0.40*	-0.30*	0.09	-0.32*
N_{RE}	0.42*	0.27	0.45*	0.07	-0.44*	-0.34*	-0.48*	0.01	-0.05	-0.13	-0.53*	-0.25		0.22	0.42*	0.65*	0.27*	0.48*	-0.17	-0.02	-0.24
P_{RE}	0.24	-0.54*	-0.43*	0.59*	0.22	-0.06	0.06	-0.83*	-0.17	0.54*	0.28*	0.08	0.09		0.34*	0.32*	0.14	0.24	0.32*	0.16	0.29*
K_{RE}	0.22	0.59*	0.57*	-0.29*	-0.23	0.16	-0.19	0.56*	-0.15	-0.42*	-0.30*	0.04	0.26	-0.38*		0.57*	-0.09	0.46*	0.08	-0.22	0.15
C_{RE}	-0.24	0.33*	0.32*	-0.14	-0.04	0.09	-0.39*	0.32*	0.09	-0.67*	-0.40*	-0.20	0.18	-0.21	0.32*		0.25	0.64*	-0.08	-0.08	-0.11
Ca_{RE}	0.06	0.17	0.26	-0.06	-0.09	-0.10	-0.24	0.12	0.34*	-0.34*	-0.48*	-0.42*	0.17	0.05	-0.02	0.42*		0.31*	-0.18	0.19	-0.30*
Mg_{RE}	-0.26	0.19	0.15	-0.02	0.14	0.28*	-0.28*	0.16	0.18	-0.32*	-0.31*	-0.36*	0.08	-0.08	0.04	0.33*	0.32*		0.06	-0.02	0.01
$N:P_m$	0.28*	-0.91*	-0.64*	0.41*	0.27*	-0.23	0.28*	-0.85*	-0.28*	0.59*	0.38*	0.09	-0.10	0.62*	-0.45*	0.40*	-0.15	-0.28*		0.33*	0.91*
$N:K_m$	0.13	-0.78*	-0.87*	0.35*	0.42*	0.03	0.27*	-0.77*	-0.40*	0.60*	0.52*	0.25	-0.22	0.62*	-0.49*	-0.43*	-0.22	-0.23	0.85*		-0.03
$K:P_m$	0.21	-0.84*	-0.34*	0.41*	0.14	-0.36*	0.18	-0.76*	-0.13	0.42*	0.21	-0.07	0.00	0.52*	-0.39*	-0.23	-0.13	-0.26	0.85*	0.52*	

Spearman’s correlation coefficient (*n* = 54) are shown. Asterisks indicate significant results, which are also highlight in italic.

Figure 4.1 N, P, K, C, Ca and Mg concentration (mg cm^{-2}) (a-f) in senesced leaves of *C. calyculata* (left bar) and *R. groenlandicum* (right bar) in response to long-term N, P and K fertilization. Values are mean ± standard error ($n = 6$). Open and pattern filled bars are treatments in experiment 1, and light and dark grey filled bars are treatments in experiment 2. Open and filled stars indicate significant difference between individual treatments and the controls in experiment 1 and 2, respectively ($P < 0.05$). Treatment abbreviations as described in Table 4.1.

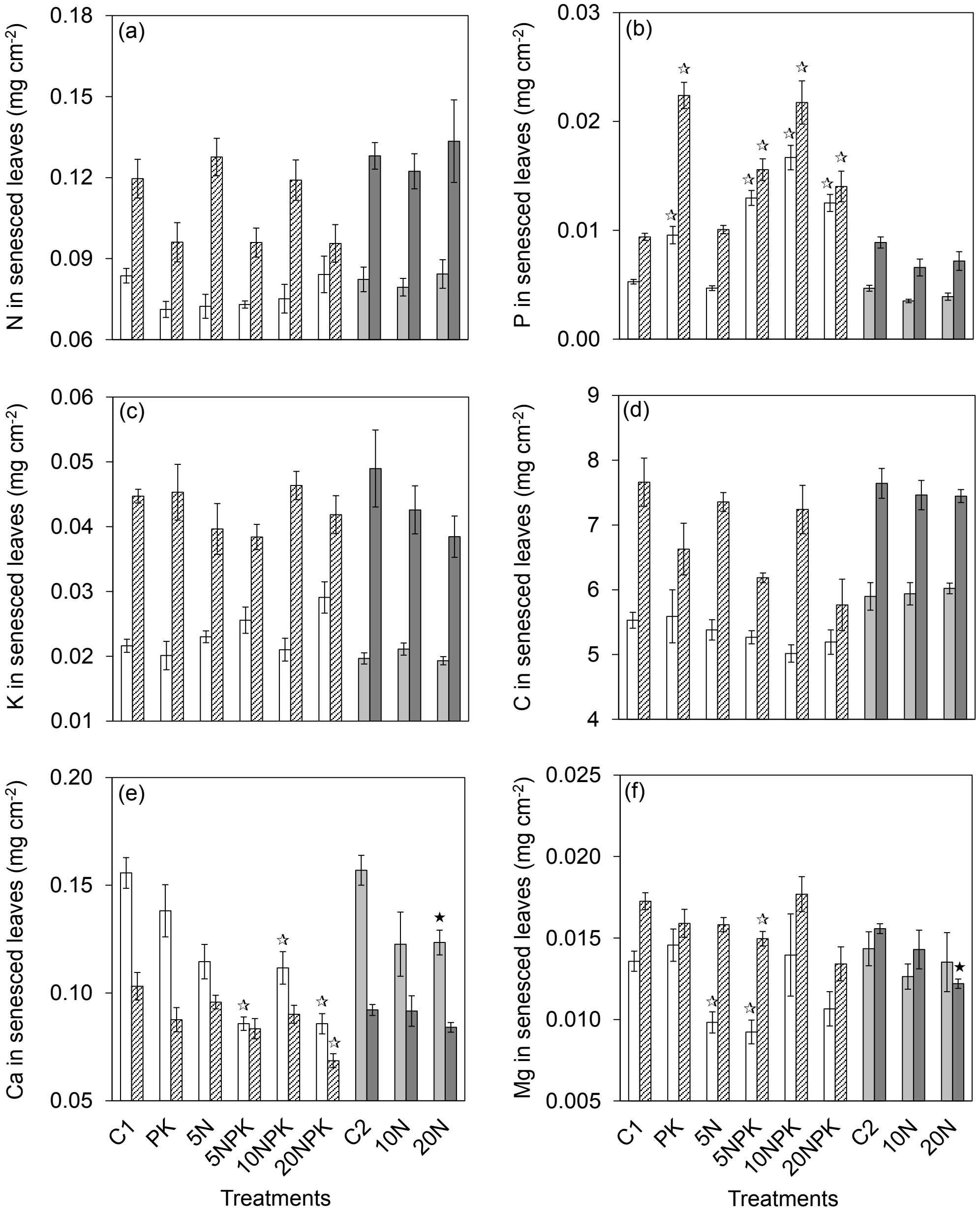


Figure 4.2 Nutrient resorption efficiency of N (N_{RE}), P (P_{RE}), K (K_{RE}), C (C_{RE}), Mg (Mg_{RE}) and Ca (Ca_{RE}) (a-h) of *C. calyculata* (diamonds) and *R. groenlandicum* (triangles) in response to long-term N, P and K fertilization. Values are mean ± standard error ($n = 6$). Open and filled stars above the symbols indicate significant difference between individual treatments and controls in *C. calyculata* in experiment 1 and 2, respectively; open and filled stars below the symbols indicate significant difference between individual treatments and controls in *R. groenlandicum* in experiment 1 and 2, respectively ($P < 0.05$). Treatment abbreviations as described in Table 4.1.

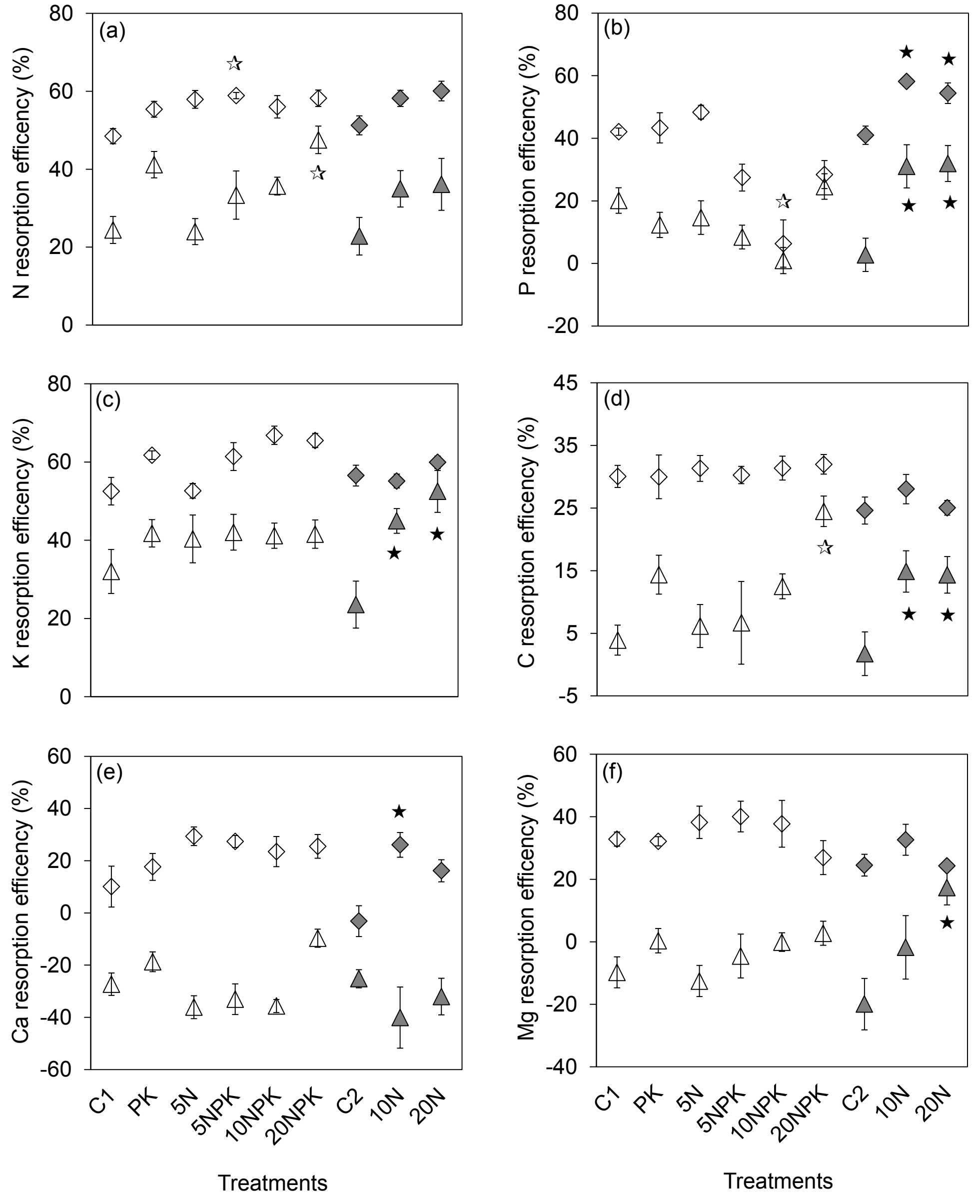
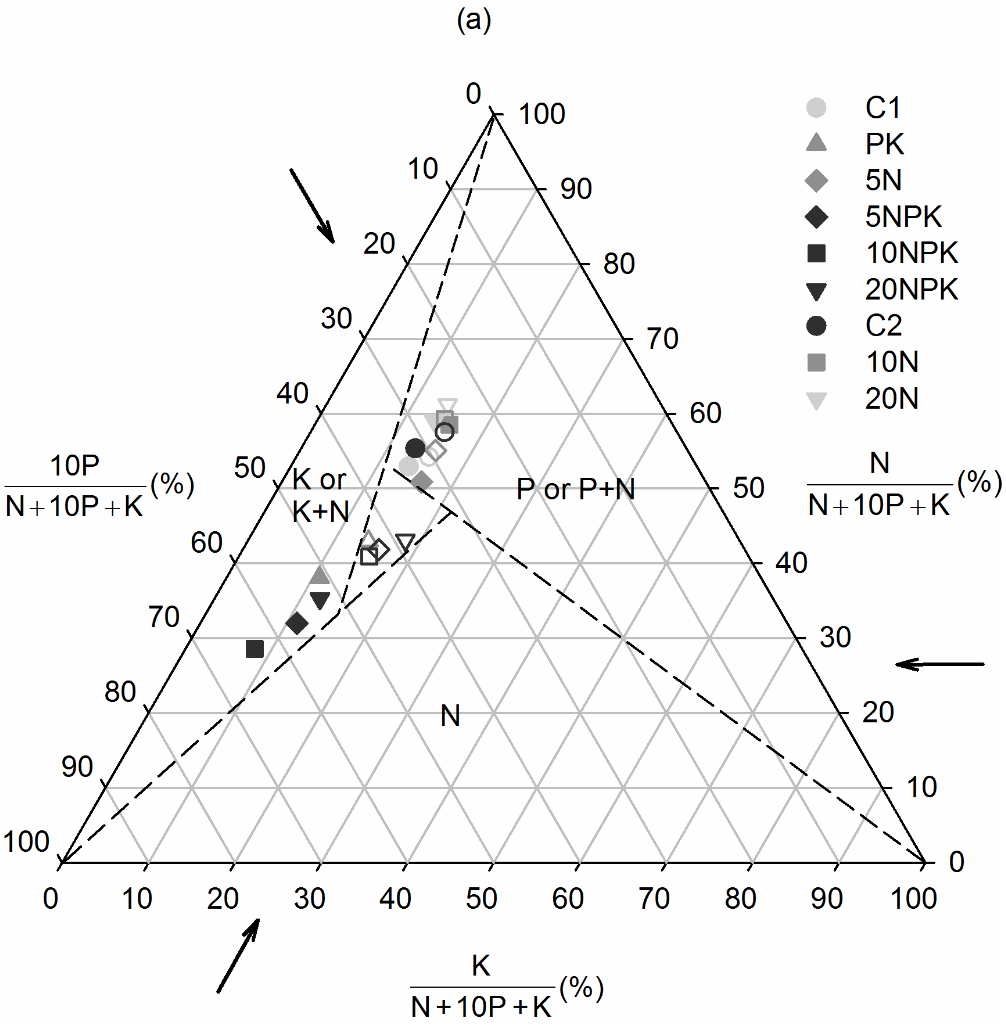
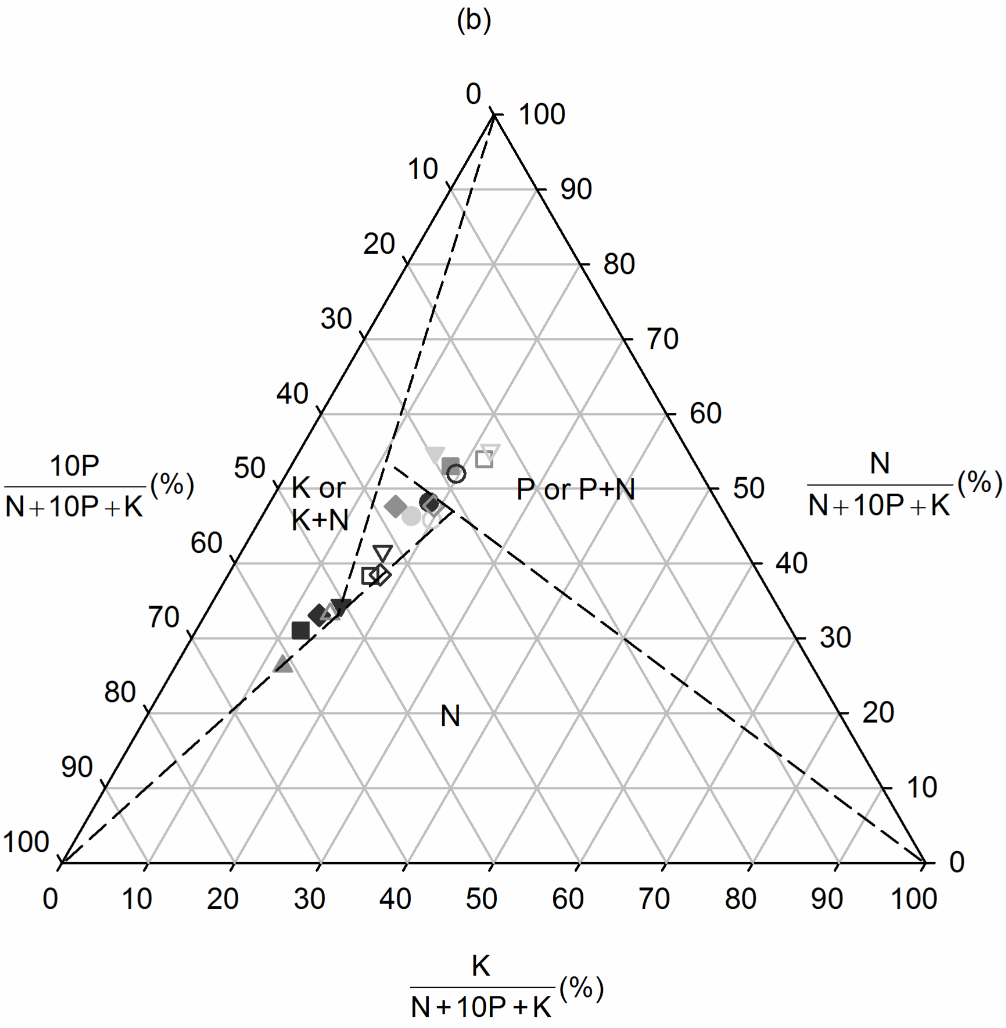


Figure 4.3 Ternary diagram showing the stoichiometric relationship of N, P and K in the mature and senesced leaves of *C. calyculata* (a) and *R. groenlandicum* (b) in response to long-term N, P and K fertilization. Open and filled symbols indicate mature and senesced leaves, respectively. Dashed lines 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 parts, and three of them indicate N-limitation (N section), P or P, N-colimitation (P or P+N section) and K or K, N-colimitation (K or K+N section), respectively. For the central triangle section, the stoichiometric ratio 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 show the direction in which the axes should be read. Treatment abbreviations as described in Table 4.1.




(b)
0
10
20
30
40
50
60
70
80
90
100
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
10P / N + 10P + K (%)
N / N + 10P + K (%)
K / N + 10P + K (%)
K or K+N
P or P+N
N

Chapter 5. Differences in stoichiometric homeostasis between shrubs and bryophytes in an ombrotrophic peatland in response to long-term fertilization

(Submitted to *Plant and Soil*, in revision)

Context within the thesis

In the context of global changes, especially increasing atmospheric deposition due to anthropogenic activities, the stoichiometric response of vegetation in nutrient-deficient bogs to these changes becomes critical. The substantial difference in leaf-level stoichiometry among PFTs (Chapter 3) and the interspecific difference in nutrient resorption characteristics in response to long-term N, P and K fertilization (Chapter 4) provides the rationale to evaluate the impact of long-term fertilization on the foliar stoichiometry in the dominant species/PFTs. The long-term fertilization experiment at Mer Bleue Bog offers a great opportunity to investigate the response of foliar tissue concentration of nutrients and also a test of stoichiometric homeostasis, which has been suggested to regulate the stability and productivity of plant community in grassland ecosystems. This chapter examines whether similar mechanism is applicable to ombrotrophic bog ecosystem.

5.1 Summary

Stoichiometric homeostasis is the degree to which organisms maintain a constant nutrient stoichiometry despite major fluctuation in resource stoichiometry. Homeostasis is a keystone concept of ecological stoichiometry, while its relationship with changing species abundance of shrubs and bryophytes in response to environmental changes in ombrotrophic bogs remains unknown. We examined the changes in foliar stoichiometry and species abundance of shrubs and moss after N, P and K fertilization over a decade at Mer Bleue Bog in eastern Canada. Shrubs exhibited the strongest homeostasis to N with the homeostatic regulation coefficient (H) > 10.0, compared to 1.4 in moss. Shrubs showed weaker homeostasis to P and K, with H of 4.7-6.6 and 4.3-6.9, respectively, whereas moss had stronger homeostasis to P and K than N with H of 2.4 and infinity respectively. Shrub abundance increased and moss cover decreased with heavy NPK fertilizations. Strong homeostasis of shrubs may be a long-term adaption to impoverished soil. Moss has weak homeostasis and only benefits from limited nutrient availabilities and the harsh micro-environment. The dominance of shrubs with strong homeostasis does not increase ecosystem productivity and the loss of moss weakens the C sink potential.

5.2 Introduction

Ecological stoichiometry deals with the balance of multiple elements in ecological interactions and processes, and links the physiological controls at the cellular or organismal level to biogeochemical patterns at the global scale (Sterner & Elser 2002). Stoichiometric homeostasis is the degree to which an organism maintains its body chemical composition constant, despite changes in the chemical composition of the external environment (Kooijman 1995). The degree of stoichiometric homeostasis of an organism is critical to food webs and ecosystem functioning in the context of elemental imbalance between organisms and external conditions (Frost *et al*. 2005). Yu *et al*. (2010a) demonstrated that species with strong homeostasis are dominant in an Inner Mongolia grassland communities and lead to high productivity and stability.

Stoichiometric homeostasis is generally considered to increase from autotrophic to heterotrophic organisms (Sterner & Elser 2002). Autotrophs often show weak homeostasis as their body stoichiometry can vary to a large extent in response to the fluctuations in resource stoichiometry (Rhee 1978; Sterner *et al*. 1992; Loladze *et al*. 2000). In contrast, heterotrophs may be strictly homeostatic with body stoichiometry constant despite major fluctuations in the elemental composition of their resources (Levi & Cowling 1969; Goldman *et al*. 1987; Andersen & Hessen 1991; Sterner *et al*. 1992). Although this generality is often questioned (DeMott & Pape 2005; Jeyasingh *et al*. 2009; Persson *et al*. 2010), it offers important insights to the understanding of consumer-resource interaction, element and energy transportation along the food web and biogeochemical cycling of critical elements in ecosystems (Sterner & Elser 2002).

Sterner & Elser (2002) defined a homeostatic 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 stoichiometry (masses or ratios of substances) of the organism and the external conditions (e.g. food or external environment), respectively, and c is a constant. The lack of stoichiometric homeostasis has been reported in algae with $H = 1$ (Rhee 1978): the body stoichiometry of algae perfectly reflects that of the external substance. In contrast, marine bacteria (Goldman *et al*. 1987), fungi (Levi & Cowling 1969) and zooplankton (Zauke *et al*. 1998) have been reported to show strict homeostasis (i.e. H approaches infinity). Little is known about the stoichiometric homeostasis of terrestrial plants, which are expected to show stronger homeostasis than algae or fungi (Sterner & Elser 2002; Yu *et al*. 2011). For example, Güsewell (2004) reported a range of 1.7 to 4.6 in the H value of N:P ratio ($H_{\mathrm{N:P}}$, the aspect of elements/ratios as subscript and hereafter) in herbaceous plants. Yu *et al*. (2011) reported H_{N}, H_{P} and $H_{\mathrm{N:P}}$ ranging from 1.9 to 14.5 in grass and forb species in a grassland. The association between strong homeostasis and conservative nutrient use suggests an important ecological strategy in other nutrient-deficient ecosystems.

Northern peatlands store 547 Gt (~30%) of global soil organic C (Yu *et al*. 2010b), related to slow rates of plant production and organic matter decomposition (Charman 2002). Covering 760,000 km^2 in Canada (Tarnocai 2006), ombrotrophic (rain-fed) bog peatlands have extremely low nutrient availabilities, especially N and P, and plant growth is often co-limited by N and P (Wang & Moore 2014). Stoichiometric homeostasis in bogs remains unknown, especially for bryophytes such as *Sphagnum* moss which is the ‘ecosystem engineer’ in northern peatlands and contributes to their capacity of C sequestration (van Breemen 1995).

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 capitula of mosses) in response to different levels of fertilization. We examine stoichiometric homeostasis in a fertilized northern ombrotrophic bog with the metric of H and link homeostasis to changes in plant community composition. We measured foliar stoichiometry and species abundance of 4 shrubs and a combination of 3 moss species in response to N, P and K fertilization over a decade at Mer Bleue Bog in eastern Ontario, Canada. The elevated N deposition over the past decades in eastern Canada (Turunen *et al*. 2004) may account for the predominant N and P co-limitation at Mer Bleue Bog. 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. Similar assumption would hold for PK-only *versus* NPK treatments. As a result, we hypothesized:

(1) In N-only plots, plants would show stronger homeostasis to N (i.e. less responsive to N-only fertilization) than fertilized with NPK, that is, $H_{N(+N)} > H_{N(+NPK)}$.

(2) In PK-only plots, plants would show stronger homeostasis to P than in NPK plots, that is, $H_{P(+PK)} > H_{P(+NPK)}$.

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

5.3 Materials and Methods

5.3.1 Study site and species description

This study was conducted at Mer Bleue Bog located 10 km east of Ottawa, eastern Ontario, Canada (45.40°N, 75.50°W). The monthly average air temperature in January and July is -10.8 and 20.9 °C respectively, with the mean annual temperature 6.0 °C (Bubier *et al*. 2007). The average precipitation from May to August is 350 mm with the mean annual precipitation 943 mm (76% as rainfall) (Canadian Climate Normals 1981-2010). The total atmospheric wet N deposition at Mer Blue Bog is ~0.8 g m^{-2} yr^{-1} (Turunen *et al*. 2004) and the total wet P deposition in eastern Canada ranges from 6 to 26 mg 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. Experiment 1 started in 2000 and 2001, and experiment 2 started in 2005 (Table 5.1) with 27 plots in total and a spatial extent of ~600 m^2

(~20 × 30 m). Nitrogen was fertilized as ammonium nitrate (NH_4NO_3), and P and K were applied as monopotassium phosphate (KH_2PO_4) 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 *Sphagnum capillifolium* (Ehrh.) Hedw., *S. magellanicum* Bird. and *Polytrichum strictum* Brid. survive mainly in the control plots and those with a low level of fertilization.

5.3.2 Foliar sampling and chemical analysis

Two samples (~40 leaves each, exclusive of petioles) of fully expanded and sun-exposed current year's leaves of evergreen shrubs and mature leaves of deciduous *V. myrtilloides* from each plot (n = 6) were collected from July 9 to 11, 2012. 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 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 concentration 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 & Riley 1962) on a Larchat Quik-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 & Allen 1975). After digestion, samples were filtered by 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 (PerkinElmer model 2380, PerkinElmer Inc., USA).

5.3.3 Soil nutrient availability

Plant root simulator (PRS^{TM}) 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 (July 1 to 31, 2012). Four pairs of PRS$^{\mathrm{TM}}$ 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 one 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. Probes were 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 (PerkinElmer Optima 3000-DV ICP, PerkinElmer Inc., Shelton, Connecticut, USA). More details about the preparation and analysis of PRS$^{\mathrm{TM}}$ probes can be found in Hangs *et al*. (2004). Nutrient availabilities were reported as mg m^{-2} of the membrane surface area over one month (Appendix 5.1).

5.3.4 Point intercept measurements

Vegetation structure and species composition were measured mid-July 2011 using a point intercept method (Larmola *et al*. 2013). 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. We analyzed the among-treatment differences in abundance (the total hits to leaf and woody stem) of *C. calyculata*, *R. groenlandicum*, *K. angustifolia* and *V. myrtilloides* and in the cover of mosses.

5.3.5 Statistics

All data were log10(x+1) transformed and the raw (untransformed) data were reported in the figures and tables. The overall variation among treatments in nutrient concentrations and stoichiometric ratios was examined using One-way ANOVA for experiment 1 and 2 separately. Levene’s test was conducted to examine the homeogeneity of the variance. Difference between individual treatments and corresponding control were assessed by Dunnett’s post hoc test for experiment 1 and 2 separately. Difference among species under the same treatment was examined by Tukey’s post hoc test.

The homeostatic regulation coefficient (*H*) was estimated by linearizing the power function ($y = cx^{1/H}$) using logarithms (Sterner & 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^{TM} probes and c is a constant. As H was expected to be equal or greater than 1 (Sterner & Elser 2002), the slope ($1/H$) should be between 0 and 1 and we used a one-tailed t test with α=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 relationship was insignificant, we compared the root mean square of error (RMSE) (i.e. the residual error) (Zar 1999) for each nutrient concentration and stoichiometric ratio with their standard deviation. If these two metrics did not differ, 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, H_K, $H_{N:P}$, $H_{N:K}$ and $H_{K:P}$), we examined the difference in H that were derived from different sets of treatments (i.e. +N, +PK and +NPK) for each species by comparing the difference between/among regression slopes (Zar 1999). For each aspect of H, we only included controls (C1 and C2) and the treatments that had direct fertilization effect on the resource stoichiometry. Specifically, for H_N, we included N-only treatments (5N, 10N and 20N), NPK treatments (5NPK, 10NPK and 20NPK) and controls. For H_P, H_K and $H_{K:P}$, we included PK-only treatment, NPK treatments and controls. For $H_{N:P}$ and $H_{N:K}$, we included all treatments. If there was no significant difference in H between/among different sets of treatments, H was reported by combining all data in the regression analysis.

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. myrtillodies* and moss. The result 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*, and among $H_{N:P(+N)}$, $H_{N:P(+PK)}$ and $H_{N:P(+NPK)}$ for *C. calyculata* (Table 5.2). Therefore, we used $H_{P(+PK)}$ of *C. calyculata* and *K. angustifolia*, and $H_{N:P(+NPK)}$ of *C. calyculata* 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).

5.4 Results

5.4.1 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 between fertilized plots and the controls ($P > 0.05$; Figure 5.1a). The N concentration in moss varied from 3.6 to 26.1 mg g^{-1} with different levels of fertilization. Moss species in the 10NPK and 20NPK plots showed a significantly larger N concentration (16.1 ± 0.6 and 26.1 ± 1.6 mg g^{-1}, respectively, mean ± SE) than in C1 plots (3.6 ± 2.1 mg g^{-1}) ($P < 0.05$). Among all species, N concentration in moss was the smallest in C1 plots ($P < 0.01$) and the largest in 20NPK plots ($P < 0.001$).

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 (Figure 5.1b). 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}) ($P < 0.001$). 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}) ($P = 0.007$) and it was significantly larger than that in shrubs in PK and NPK plots by up to 173% ($P < 0.01$), and significantly smaller than shrubs in 10N plots by up to 63% ($P < 0.01$).

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 (Figure 5.2a). In general, K concentration in shrubs was 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}) ($P < 0.05$), except for *V. myrtilloides* in 10NPK and 20NPK plots ($P > 0.1$). There was no significant difference in K concentration between fertilized plots and the controls in moss ($P > 0.5$) which showed significantly smaller K concentration than shrubs in all plots by up to 86% ($P < 0.001$).

The Ca concentration did not vary substantially between the controls (3.7 to 5.6 mg g^{-1}) and fertilized plots (2.4 to 6.0 mg g^{-1}) (Figure 5.2b). Generally, the highest level of N addition alone (20N) or with PK (20NPK) reduced Ca concentration, whereas no identical fertilization effect occurred for Mg (Figure 5.2c). *V. myrtilloides* showed significantly larger Mg concentration than the other shrubs ($P < 0.05$). 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 ($P < 0.001$).

5.4.1 Changes in species abundance in response to fertilization

The abundance of *C. calyculata* significantly increased in 20NPK and 10N plots compared to C1 ($P = 0.04$) and C2 ($P = 0.03$) respectively (Figure 5.3a). The moss cover decreased in PK and NPK plots as well as 20N plots compared to C1 and C2, respectively ($P < 0.05$; Figure 5.3e). 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 (Figure 5.3a), followed by *R. groenlandicum* at 5 to 50% and it dominated in 20N plots (50%) (Figure 5.3c). The total abundance of shrubs was significantly larger in 10NPK and 20NPK plots than in C1 by 87 and 81% respectively ($P < 0.001$; Figure 5.3f).

5.4.2 Stoichiometry and homeostasis

Shrubs showed similar N:P:K stoichiometric characteristics to each other with considerable variation among replicates in response to fertilization in mid-July (Figure 5.4), showing N limitation after P and K addition (i.e. PK, 5NPK, 10NPK and 20NPK), and P or P, N-colimitation after high levels of N addition (i.e. 10N and 20N) (Figure 5.5a). Moss species showed a consistent pattern of K or K, N-co-limitation, even in PK and NPK plots (Figure 5.5b).

In general, all shrubs showed large values of H_N, ranging from 10.0 (*R. groenlandicum*) to infinity (*V. myrtilloides*) and moss showed a significantly smaller H_N of 1.4 ($P < 0.001$; Table 5.2). All shrubs showed weaker homeostasis to P and K compared with N, with values of 4.7 to 6.6 (H_P) and 4.3 to 6.9 (H_K), whereas moss showed a stronger homeostasis to P and K with H of 2.4 (H_P) and infinity (H_K), compared to N. Moss showed a significantly smaller H_P than shrub species ($P < 0.001$). There was no consistent pattern of the homeostatic coefficient of stoichiometric ratios ($H_{N:P}$, $H_{N:K}$ and $H_{K:P}$) compared to H_N, H_P and H_K accordingly.

The interaction between N and PK treatments showed a significant effect on some aspects of the homeostasis coefficient in *C. calyculata* and *K. angustifolia*: H_{P} was significantly larger in PK plots than in NPK plots (i.e. $H_{\mathrm{P(+PK)}} > H_{\mathrm{P(+NPK)}}$) (Table 5.2). In *C. calyculata*, $H_{\mathrm{N:P}}$ was significantly larger in N or PK plots than in NPK plots (i.e. $H_{\mathrm{N:P(+N)}} > H_{\mathrm{N:P(+NPK)}}$ and $H_{\mathrm{N:P(+PK)}} > H_{\mathrm{N:P(+NPK)}}$).

5.5 Discussion

5.5.1 Stoichiometric homeostasis in bog vegetation

Persson *et al*. (2010) suggested that the assumption of flexible stoichiometry in autotrophs and strict homeostasis in heterotrophs was oversimplified and should be utilized with caution. Yu *et al*. (2011) examined the stoichiometric homeostasis of two grass and one forb species in a semiarid grassland and found that it was stronger than in algae or fungi but weaker than in animals. In our study, bog shrubs show strong homeostasis with H ranging from 3.4 to infinity, similar to that observed in dominant grassland species (3.1 to 14.5) (Yu *et al*. 2011) and stronger than those compiled by Güsewell (2004) on herbaceous plants (1.7 to 4.6). $H_{\mathrm{N:P}}$ varied from 4.3 to 9.6 in C3 and C4 grass species and *Carex* sedges in a northern mixed-grass prairie (Dijkstra *et al*. 2012), while Elser *et al*. (2010) found a much weaker homeostasis in two C3 grass species and *Carex* sedge, with $H_{\mathrm{N:P}}$ ranging from 2.2 to 3.7; the smaller variation in soil N:P ratios in the study by Elser *et al*. (2010) than by Dijkstra *et al*. (2012) may account for this. The strong homeostasis in shrub leaves in our study may be related to phenotypic plasticity and nutrient translocations and the allocation of biomass and nutrients to belowground tissues. Yu *et al*. (2011) noted that the strong homeostasis in the 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 ratios of 0.56 ± 0.30 (mean ± SD) (Murphy *et al*. 2009), suggesting a great potential of modulating the elemental composition in belowground biomass to keep foliar elemental composition stable.

In contrast, as less developmentally complex plants, moss showed weak homeostasis in the photosynthetically active tissues (i.e. capitulum) with H ranging from 1.4 to 3.0, except for K. Mosses have powerful capillary networks, especially in *Sphagnum*, which can transport water and metabolites upwards to the growing capitulum (Rydin & Clymo 1989; Aldous 2002; Rydin

& Jeglum 2006). 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 acid (Limpens & Berendse 2003) and account for the weak homeostasis to N in moss. Furthermore, moss continuously creates cation exchange sites which may efficiently capture the nutrients from fertilization without assimilating all of them into living cells (Rydin & Jeglum 2006), and thus exhibit weak homeostasis.

5.5.2 Interaction of multiple elements affects homeostasis

The homeostasis of the N:P ratio is generally considered stronger than that of N and P alone (Yu *et al*. 2010a; Yu *et al*. 2011), as N and P are usually coupled in major biochemical components, such as nucleic acids and adenosine triphosphate (ATP), and the change in the composition in one is often accompanied by a change in the other. However, we did not find any consistent pattern of stronger homeostasis of N:P ratio than N and P, and the homeostasis of N:K and K:P ratios were not consistently stronger than N, P and K. As N is probably saturated in this ecosystem after excessive N input, the stoichiometric ratios are controlled by the variation in P and K concentrations. Therefore, the stoichiometric ratios may vary as much as each individual element and show weaker homeostasis than in other studies.

Contrary to our first 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 two sets of treatments in either shrubs or moss, resulting from N saturation. The second 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 benefits of this species-specific characteristic remain unknown and require further research. Even though we accept our third hypothesis that the degree of homeostasis to K did not differ between PK-only and NPK treatments, it does not seem to be associated with the nature of K limitation. There was no evidence of K limitation based on leaf N:P:K stoichiometry in shrubs, but moss capitulum showed strong K limitation. Intriguingly, K concentration in moss did not increase after PK and NPK fertilization, perhaps because of leaching under acidic conditions or replacement by Mg from the cation exchange sites, which prefer bi-valent cations to uni-valent ones, so nearly all of the K should be replaced by Ca and Mg if these are available (Pakarine & Tolonen 1977).

The decline in leaf Ca concentration under high levels of N and NPK fertilization in most shrub species confirms the pattern reported by Bubier *et al*. (2011), indicating physiological stress under conditions of high N addition. *V. myrtilloides* had higher Mg concentration than the other shrubs, suggesting that deciduous species are better adapted to higher nutrient environment than evergreens. With a significantly larger Mg concentration, moss showed extremely small K concentration that attributes to the predominant K limitation in moss.

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. Mer Bleue Bog is located in a region with moderate atmospheric N deposition of 0.8 g m^{-2} yr^{-1}, which may also have compensated the scarcity of N in unfertilized plots, resulting in the weak response to N addition. Factors other than nutrient availabilities may affect stoichiometric homeostasis, e.g. light, temperature and water availabilities, which we are not able to rule out. We suggest that future work on stoichiometric homeostasis in higher plants should take them into consideration.

5.5.3 Homeostasis and species abundance: shrubs differ from moss

Yu *et al*. (2010a) have suggested that species with strong homeostasis should dominate a plant community. Although *C. calyculata* dominated in all plots without having a stronger homeostasis than the other shrubs, we observed that shrubs showed a stronger homeostasis to N and P than moss and were more abundant in heavily fertilized plots. The shrub growth may further reduce moss growth through the shading by a denser canopy and through a thicker layer of shrub leaf litter (Chong *et al*. 2012; Larmola *et al*. 2013).

In contrast to the Inner Mongolia grassland where plant communities dominated by species with strong homeostasis were more stable and productive (Yu *et al*. 2010a), the increased abundance of shrubs with strong homeostasis at Mer Bleue did not enhance ecosystem productivity. Larmola *et al*. (2013) found the gross photosynthesis rates did not change significantly as the greater shrub biomass compensated for the loss of the *Sphagnum* cover with the highest addition of N and NPK. In these heavily fertilized plots, net CO_2 assimilation per leaf area in the top shrub canopy was largely unaltered (Bubier *et al*. 2011), but the net ecosystem uptake of CO_2 was substantially diminished owing to an increase in ecosystem respiration (Larmola *et al*. 2013). As an 'ecosystem engineer' in northern peatlands, *Sphagnum* mosses establish an acid, wet and anoxic environment that impedes the growth of other species and slows decomposition (Rydin & Jeglum 2006), all of which contribute to the important role of northern peatlands as C sink (Turetsky *et al*. 2012). The dominance of shrubs with strong homeostasis would not increase ecosystem productivity in ombrotrophic bog, as the gain of shrub biomass cannot compensate the loss of moss cover in terms of net C sequestration.

We have demonstrated strong and weak homeostasis in shrubs and moss, respectively, in a bog. The intrinsic nature of strong homeostasis of shrubs may be an evolutionary strategy or a long-term adaption to impoverished soil fertility. Moss has a weak homeostasis and only benefits 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 and weaken the C sink potential.

Table 5.1 The fertilization experimental design at Mer Bleue Bog.

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

N fertilization levels equal 5, 10 and 20 times the estimated summer time atmospheric wet N deposition and treatments are abbreviated accordingly. C1 and C2 are the controls (added distilled water only) for the two sets of experiment, respectively.

Table 5.2 Results of regression analysis between nutrients and stoichiometric ratios in foliar tissues and soil. 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. Different low case letters indicate significant differences in the same aspect of *H* derived from different sets of experiment for the same species. The subscripts of the aspects indicate the fertilization treatments: +N, N-only treatment; +PK, PK-only treatment; +NPK, NPK treatments.

Species	Aspects	Slope	*H*	R^2	*RMSE*	*SD*	*P* value
C. calyculata	N	0.089	11.236^{A}	0.066	0.104	0.106	0.078
	$P_{(+PK)}$	0.151	6.623^{Aa}	0.811	0.065	0.145	<0.001
	$P_{(+NPK)}$	0.211	4.739^{b}	0.903	0.057	0.181	<0.001
	K	0.174	5.747	0.391	0.068	0.086	<0.001
	$N{:}P_{(+N)}$	0.157	6.369^{a}	0.236	0.106	0.119	0.007
	$N{:}P_{(+PK)}$	0.138	7.246^{a}	0.820	0.059	0.134	<0.001
	$N{:}P_{(+NPK)}$	0.239	4.184^{b}	0.816	0.083	0.190	<0.001
	N:K	0.159	6.289	0.256	0.127	0.146	<0.001
	K:P	0.296	3.378^{B}	0.236	0.297	0.337	<0.001
R. groenlandicum	N	0.100	10.000^{A}	0.172	0.094	0.102	0.004
	P	0.214	4.673^{A}	0.893	0.060	0.181	<0.001
	K	0.144	6.944	0.354	0.061	0.075	<0.001
	N:P	0.249	4.016	0.822	0.099	0.233	<0.001
	N:K	0.151	6.523	0.303	0.106	0.126	<0.001
	K:P	0.210	4.762^{A}	0.811	0.061	0.138	<0.001
Moss spp.	N	0.739	1.353^{B}	0.286	0.370	0.428	0.007
	P	0.425	2.353^{B}	0.926	0.100	0.358	<0.001
	K	0.059	∞	0.052	0.082	0.081	0.361
	N:P	0.399	2.506	0.456	0.387	0.514	<0.001
	N:K	0.337	2.967	0.140	0.401	0.424	0.055
	K:P	0.488	2.049^{C}	0.758	0.171	0.337	<0.001
K. angustifolia	N	0.099	10.101^{A}	0.060	0.124	0.127	0.096
	$P_{(+PK)}$	0.168	5.952^{Aa}	0.937	0.039	0.152	<0.001
	$P_{(+NPK)}$	0.223	4.484^{b}	0.905	0.060	0.190	<0.001
	K	0.152	6.579	0.285	0.073	0.085	<0.001
	N:P	0.234	4.274	0.740	0.121	0.236	<0.001
	N:K	0.172	5.814	0.252	0.141	0.162	<0.001
	K:P	0.156	6.410^{AB}	0.745	0.055	0.107	<0.001
V. myrtilloides	N	0.098	∞^{A}	0.042	0.147	0.148	0.180
	P	0.178	5.618^{A}	0.623	0.119	0.191	<0.001
	K	0.234	4.274	0.288	0.112	0.131	0.002
	N:P	0.207	4.831	0.473	0.185	0.252	<0.001
	N:K	0.239	4.184	0.260	0.173	0.199	<0.001
	K:P	0.101	9.901^{AB}	0.200	0.128	0.141	0.010

Figure 5.1 N and 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 = 6 for *C. calyculata*, *K. angustifolia*, *R. groenlandicum* and *V. myrtilloides* and n = 3 for moss). Asterisks indicate a significant difference between individual treatments and C1 in experiment 1 (left panel) and C2 in experiment 2 (right panel) for the same species. Different letters indicate a significant difference among species under the same treatment. The scale of P concentration in panel (b) differs between two sets of experiments. Treatment abbreviations as described in Table 5.1.

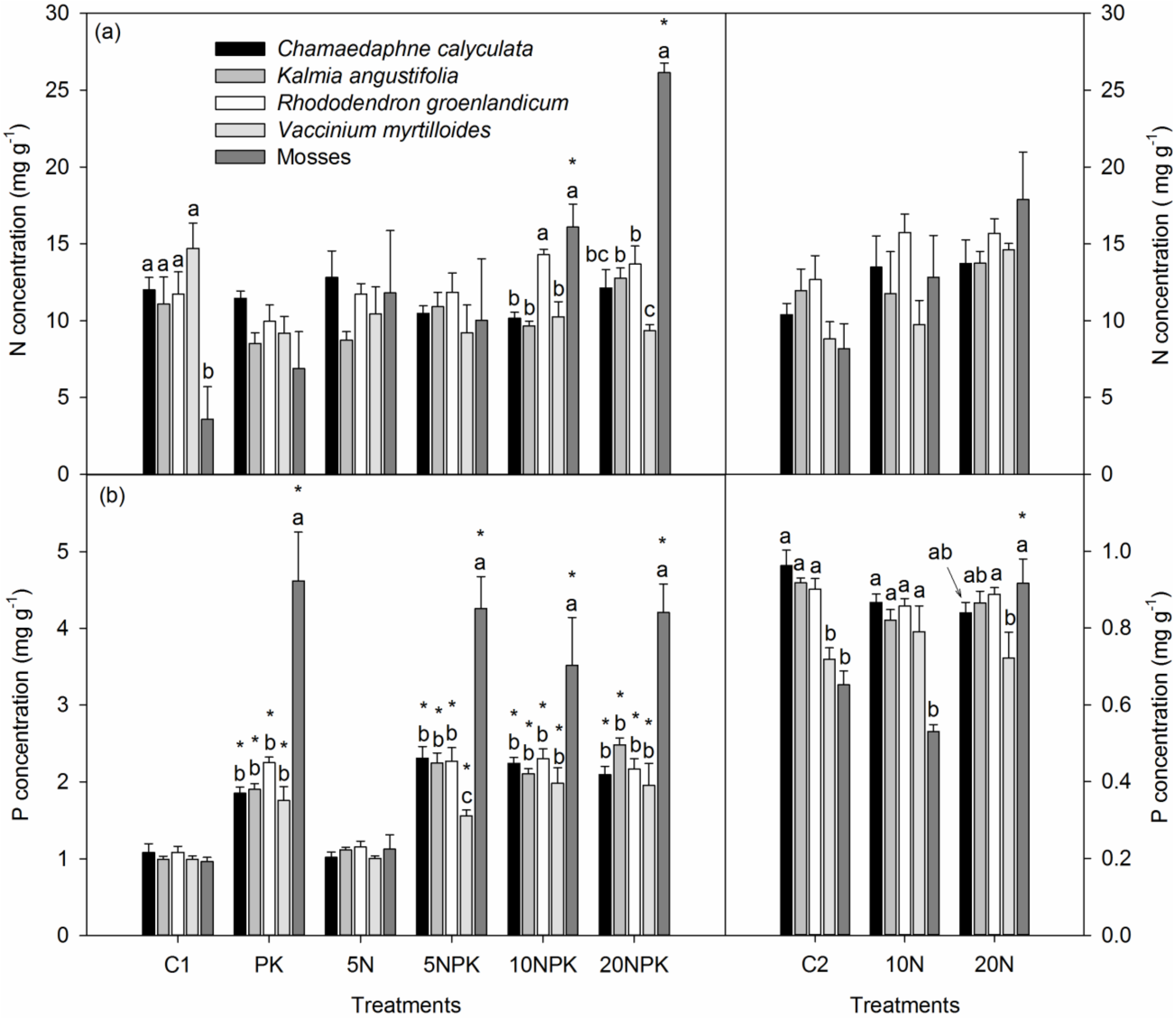


Figure 5.2 K, Ca and Mg concentrations in the foliar tissues of different species in response to long-term N, P and K fertilization. Bars are mean with SE (n = 6 for *C. calyculata*, *K. angustifolia*, *R. groenlandicum* and *V. myrtilloides* and n = 3 for moss). Asterisks indicate a significant difference between individual treatments and C1 in experiment 1 (left panel) and C2 in experiment 2 (right panel) for the same species. Different letters indicate a significant difference among species under the same treatment. The scale of K concentration in panel (a) differs between two sets of experiments. Treatment abbreviations as described in Table 5.1.

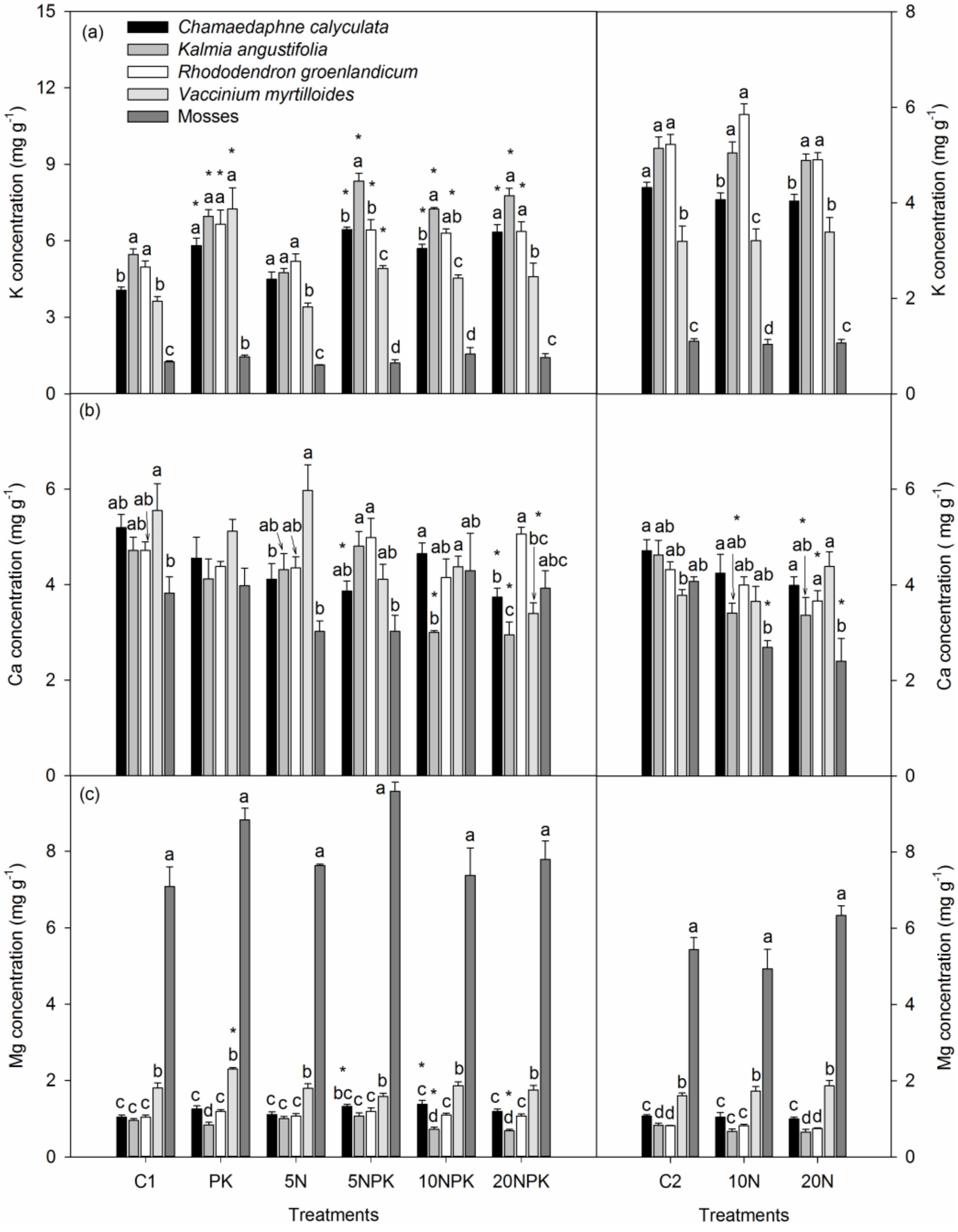


Figure 5.3 Vegetation abundance of *C. calyculata* (a), *K. angustifolia* (b), *R. groenlandicum* (c), *V. myrtilloides* (d), mosses (e) and total vascular plants (f) in response to long-term N, P and K fertilization. Data are expressed on a logarithmic (base$_{10}$) basis. Bars are mean with SE (n = 6 for *C. calyculata*, *K. angustifolia*, *R. groenlandicum* and *V. myrtilloides* and n = 3 for moss). Asterisks indicate a significant difference between individual treatments and C1 in experiment 1 (open bars) and C2 in experiment 2 (filled bars) 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 5.1.

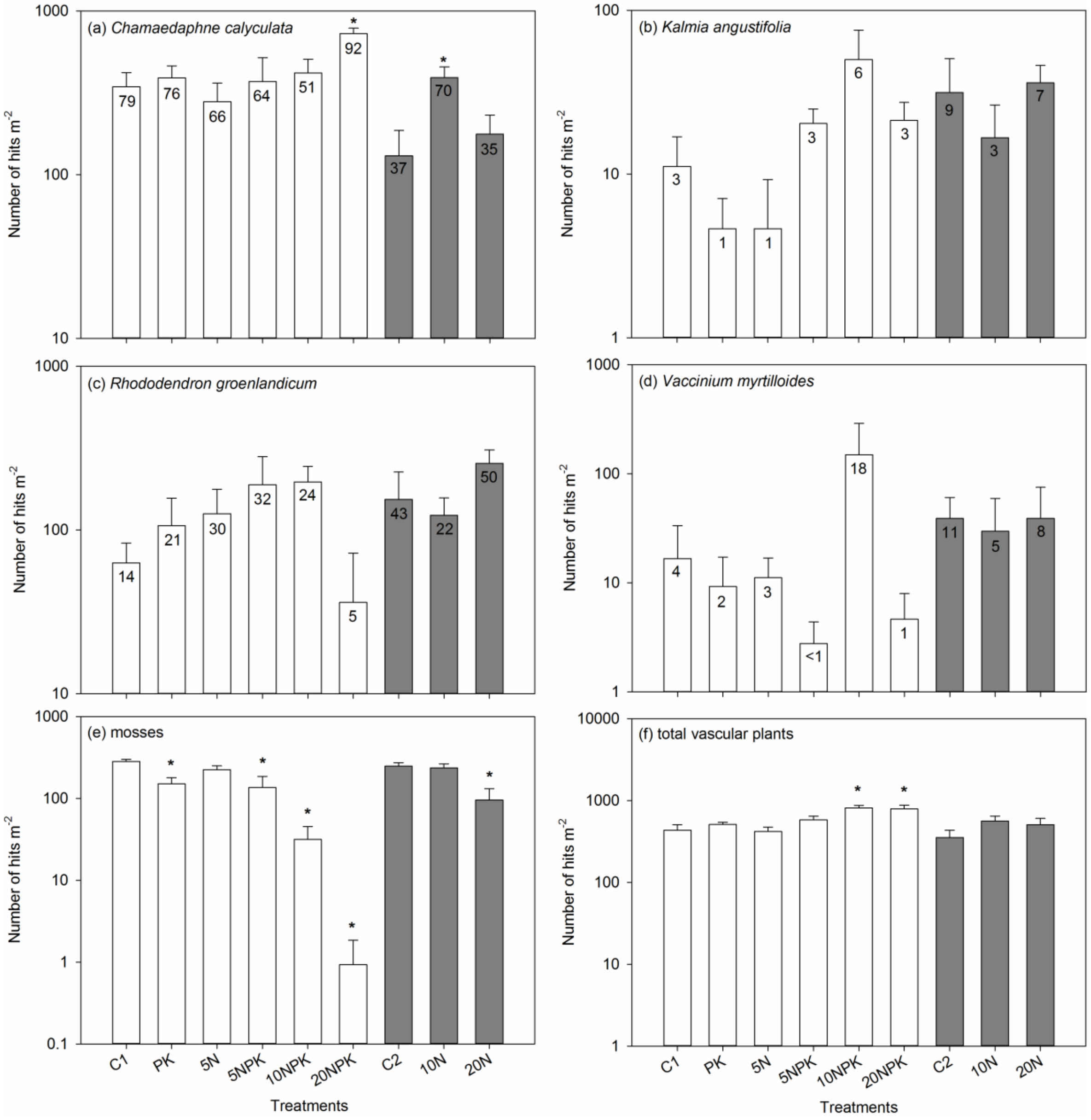


Figure 5.4 Ternary diagram showing the stoichiometric relationship of N, P and K in *C. calyculata* (a), *K. angustifolia* (b), *R. groenlandicum* (c) and *V. myrtilloides* (d) in response to long-term N, P and K fertilization. Open and filled symbols are individual replicate and mean under each treatment, 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 5.1.

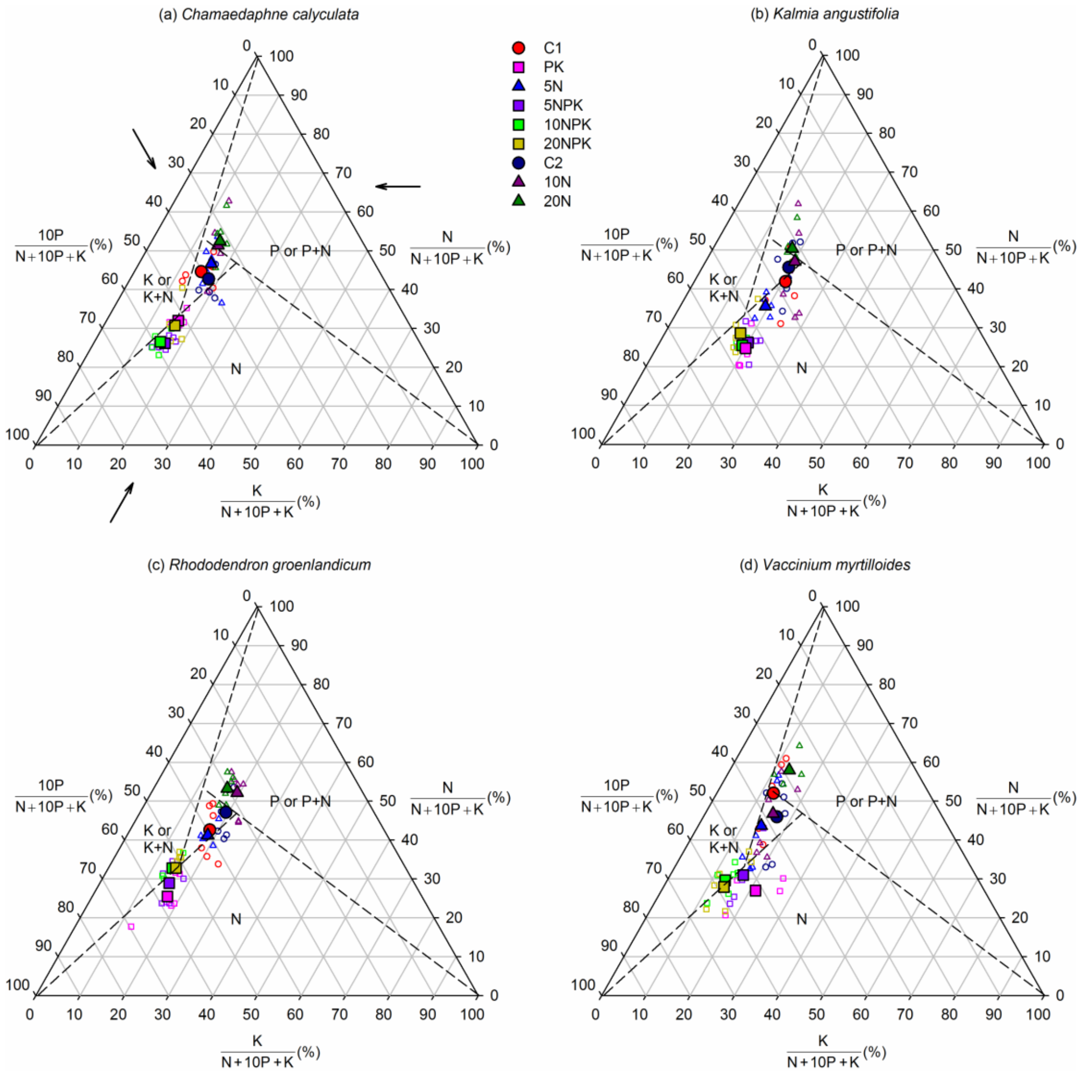
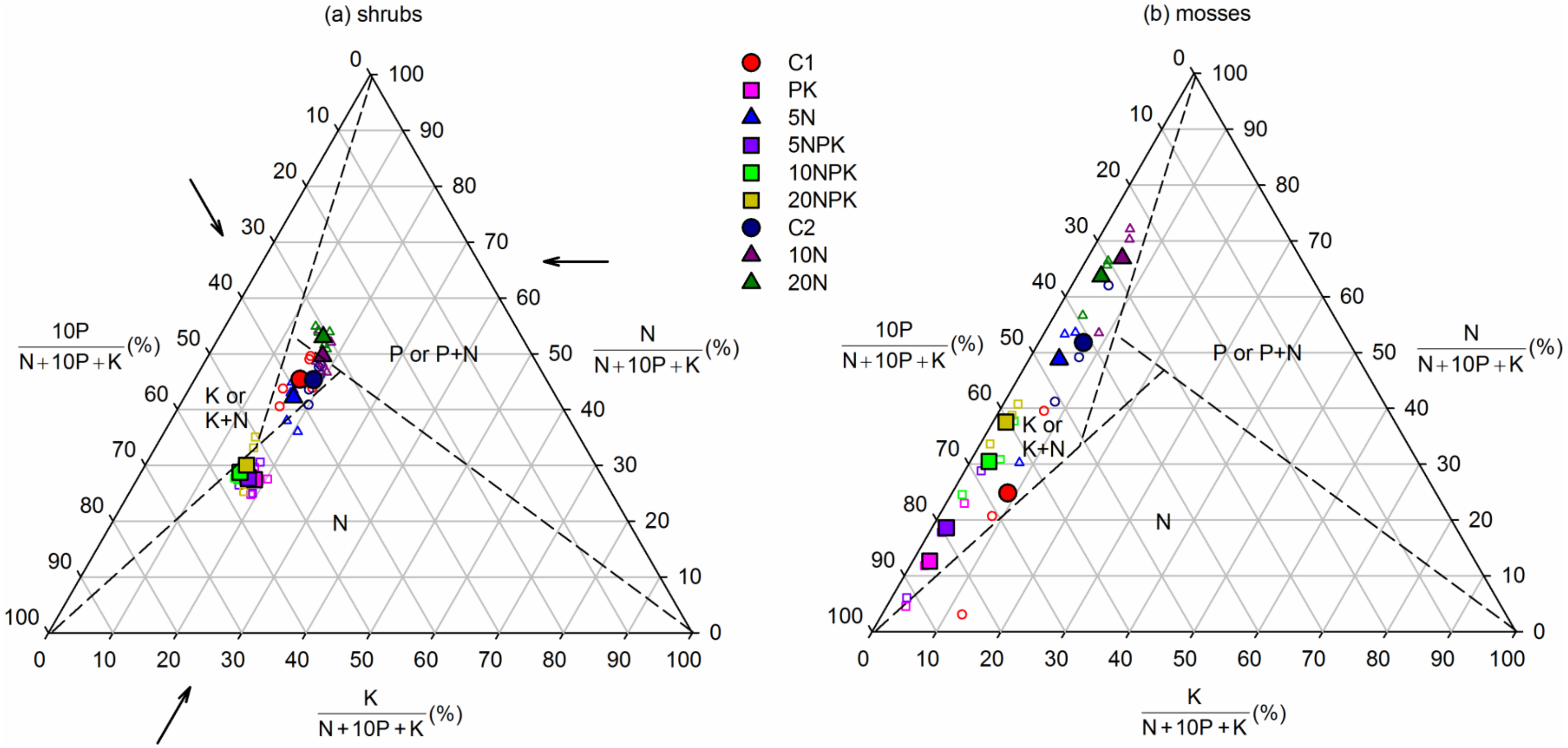


Figure 5.5 Ternary diagram showing the stoichiometric relationship of N, P and K in response to long-term N, P and K fertilization in shrubs (a) and mosses (b). Open and filled symbols are individual replicates and means under each treatment, respectively. Treatment abbreviations as described in Table 5.1.



Chapter 6. The cascade of C:N:P stoichiometry in an ombrotrophic peatland: from plants to peat

(Published on *Environmental Research Letters*, 2014, 9, 024003)

Context within the thesis

As presented in Chapter 2, C:N:P stoichiometry in mature tissues can suggest nutrient limitation, while C:N:P in litters can be a useful indicator of decomposition processes. However, owing to the extremely slow rate of litter and organic matter decomposition, there is no knowledge of long-term decomposition in ombrotrophic peatlands. Yet peat profiles can be regarded as a record of the temporal changes in litter decomposition, over millennia. In this chapter, I demonstrate the decomposition cascades of C:N:P stoichiometry by linking the C:N:P stoichiometry from foliar plant tissues (Chapter 3), through senescent litters (Chapters 3 and 4) to peat, with a supplementary dataset of C:N:P stoichiometry in two peat profiles from Mer Bleue Bog. As an additional scientific contribution to palaoecological studies, I also determine the C, N and P accumulation rates at Mer Bleue Bog.

6.1 Summary

Northern peatlands are important C sinks and while the patterns of C accumulation have been frequently investigated, N and P accumulation are often neglected. Here we link the C:N:P stoichiometry from foliar plant tissues, through senescent litters to peat, and determine C, N and P accumulation rates at Mer Bleue Bog, eastern Canada. Average C:N:P ratios changed from 794:17:1 in the foliar tissues to 911:10:1 in litter and 1285:32:1 in acrotelm peat. The increase in C:N and C:P ratios from mature to senescent tissues is related to nutrient resorption. The increase in C:P and N:P ratios in peat, which was contrary to that observed in Canadian forest soils, may be related to plant/mycorrhizae uptake of P. The long term apparent rates of C, N and P accumulation were 29.5 ± 2.1 (SE) g C, 0.87 ± 0.01 g N and 0.017 ± 0.002 g P m^{-2} yr^{-1}, respectively. The significant correlation between the accumulation rates of N and P and that of C suggests more attention be placed on C:N:P stoichiometry in peatland biogeochemistry, in particular in understanding why C:P ratios are so large in the lower parts of the profile.

6.2 Introduction

Northern peatlands store one-third (547 Gt) of the global total soil C (Yu *et al*. 2010b), arising from the imbalance between primary productivity and litter and peat decomposition (Charman 2002). However, we know little about the accumulation of N and P, two macro-nutrients essential in controlling both primary productivity and litter and peat decomposition. Ecological stoichiometry deals with elemental balances in ecological processes and interactions, providing an integrative nutrient framework linking biogeochemical patterns at scales from the global to the cellular or organismal (Sterner & Elser 2002). Koerselman & Meuleman (1996) suggest plant growth is N- or P-limited when the plant N:P ratio is smaller than 14 or larger than 16, respectively, and graminoid litter decomposition is affected by N and P concentrations and their ratio (Güsewell & Verhoeven 2006). Therefore, examining the decomposition cascade of C:N:P stoichiometry from plant foliage to senescent litters and to peat is of great importance in understanding nutrient cycling in northern peatlands.

There is significant (~50%) resorption of nutrients, especially N and P, into plants during senescence (Aerts 1996; Güsewell 2005a; Wang *et al*. 2014b), resulting in nutrient-depleted litter. The mismatch between the stoichiometry of litter and that of decomposers, primarily bacteria and fungi in northern peatlands (Winsborough & Basiliko 2010), governs ecosystem nutrient cycling and thus C accumulation (Sterner & Elser 2002; Hessen *et al*. 2004). For example, the substantially larger C:N:P mass ratio of 1000:20:1 in subarctic flora than 180:6:1 in soil microbial biomass in tundra (Cleveland & Liptzin 2007) indicates a possible long term net N and P immobilization in soil until a critical litter C:N and C:P ratio is reached.

A 12-year litter decomposition study in Canadian forests (Moore *et al*. 2011), showed a convergence of litter C:N:P ratio of 427:17:1 with 20% of initial C remaining. This study also suggested the critical C:nutrient ratios for net mineralization were ~40:1 for N and ~1000:1 for P. Unlike most forest leaf litters, the litter of peat-forming *Sphagnum* mosses decomposes at much slower rates, with exponential k values of 0.02 to 0.08 yr^{-1} (Moore & Basiliko 2006), making the long-term measurement of nutrient dynamics more difficult. Yet peat profiles can be regarded as a record of the temporal changes in litter decomposition, over millennia. Here we link the C:N:P stoichiometry from mature plant tissues, through senescent litter to peat and determine C, N and P accumulation rates in an ombrotrophic bog located in eastern Canada. We hypothesize that:

(1) the C:N and C:P stoichiometric ratios would increase from mature to senescent tissues due to nutrient resorption; and

(2) the C:N and C:P stoichiometric ratio would decrease from senescent tissues to peat owing to microbial immobilization during decomposition.

6.3 Materials and methods

6.3.1 Study site

The Mer Bleue peatland is located 10 km east of Ottawa, eastern Ontario, Canada (45.40°N, 75.50°W). The central part of the peatland is an ombrotrophic bog with hummock and hollow micro-topography, covered mainly by a continuous layer of *Sphagnum* mosses and evergreen shrubs. The Mer Bleue peatland started accumulating ~8500 yr before present (BP) in an abandoned channel of the Ottawa River. The early sedge-dominated fen phase switched to the current bog phase ~6800 yr BP. The mean annual temperature, and monthly mean temperature in January and July are 6, -10.8 and 20.9 °C, respectively (Bubier *et al*. 2007). The mean annual precipitation is 943 mm (76% as rainfall) and 350 mm of the precipitation falls from May to August (Canadian Climate Normals 1981-2010). The wet atmospheric N deposition is ~0.8 g N

m^{-2} yr^{-1} (Turunen *et al* 2004) and wet P deposition ranges from 6 to 26 mg P m^{-2} yr^{-1} (R. Vet personal communication).

6.3.2 Plant tissues sampling

The capitula of moss (*S. capillifolium* (Ehrh.) Hedw. and *S. magellanicum* Brid.) and the sun-exposed mature leaves from the top canopy of three evergreen shrubs (*Chamaedaphne calyculata* (L.) Moench, *Rhododendron groenlandicum* (Oeder) K.A. Kron & W.S. Judd and *Kalmia angustifolia* L.), one deciduous shrub (*Vaccinium myrtilloides* Michx.), one graminoid (*Eriophorum vaginatum* L.) and one forb (*Maianthemum trifolium* (L.) Sloboda) were randomly sampled (n = 5) in July and August. Senescent tissues of *M. trifolia* and *V. myrtilloides* were collected in September, and in October for *E. vaginatum*. The nutrient concentration in senescent leaves of *C. calyculata* and *R. groenlandicum* were derived from Wang *et al*. (2014b). For *S. capillifolium*, the current year's stem (the upper portion below the capitulum), collected in September 2012, was considered to represent their senescent tissues based on cranked wire growth (Moore *et al*. 2002) and the morphology of *Sphagnum* stem leaves.

Total C and N concentration were determined by dry combustion on an elemental analyzer (Leco CNS 2000, Leco Corporation, St. Joseph, Michigan, USA). Total P was determined colorimetrically by the ammonium molybdate-ascorbic acid method (Murphy & Riley 1962) on a Lachat Quik-Chem AE flow-injection auto-analyzer (Lachat Instruments, Milwaukee, WI), after digestion in concentrated sulphuric acid and hydrogen peroxide with selenium and lithium sulphate as catalysts (Parkinson & Allen 1975). Digested samples were filtered through 0.45 µm glass fibre filters (MN 85/90, MACHEREY-NAGEL GmbH & Co. KG, Germany) before analysis.

6.3.3 Soil microbial biomass C, N and P

The C, N and P concentration in soil microbial biomass were derived from Basiliko (2004) who sampled peat at depth intervals of 0-5, 5-10, 10-20 and 20-30 cm from the surface at Mer Bleue. Samples were extracted by potassium sulphate after a chloroform fumigation treatment for 24 hours.

6.3.4 Peat cores

Two peat cores, MB930 and MB775, were collected in the northwestern portion of the peatland, yielding complete peat profiles of 5.97 m and 3.47 m, respectively. The age models of the cores are based on radiocarbon dates and palynostratigraphical correlations for core MB930 (Frolking *et al*. 2010), and on palynostratigraphical correlation with core MB930 for core MB775. Carbon, N and P concentrations were determined as described above, every 2 cm for the top 2 m of the cores and every 4 cm for the rest. Based on the stratigraphy of the peat cores, the portion of the peat profile corresponding to the acrotelm for this analysis is the upper ~50 cm, a depth at which ~20% of original C remains, based on the profile generated by the Holocene Peatland Model (S. Frolking personal communication).

The C, N and P data from MB930 and MB775 were averaged by time interval (0-100, 100-200, 200-500, 500-1000, 1000-1500, 1500-2000, 2000-3000, 3000-4000, 4000-5000, 5000-6000, 6000-7000, 7000-8000 and >8000 cal. yr BP).

6.3.5 Statistics

The C:N:P stoichiometric mass ratios of living and senescent tissues at Mer Bleue were weighted arithmetic means based on foliar biomass of each species (Bubier *et al*. 2006) and the net primary productivity of *Sphagnum* mosses (Moore *et al*. 2002).

Shapiro-Wilk tests were used to examine the normality of data. Linear regression was used to examine the relationship between the accumulation rates C, N and P. The differences in the slope and intercept of the regression models between the bog and the fen phase were examined by *t* test (Zar 1999). The differences in stoichiometric ratios between bog and fen phase were examined by *t* test. Coefficients of variation (CV) were calculated as the mean divided by standard deviation. All the analyses were conducted in IBM SPSS Statistics Version 20.0 for Windows (IBM Corp., Armonk, New York State, USA).

6.4 Results

6.4.1 C:N:P stoichiometry from plants to peat

The average mass C:N:P ratio was 794:17:1 and 911:10:1 in mature and senescent tissues, with a smaller variation among PFTs in mature tissues than that in senescent tissues in C:N (CV = 31 and 53% respectively) and N:P ratios (CV = 20 and 27% respectively) (Figure 6.1). The C:N:P stoichiometry in graminoid and forb types varied little from mature to senescent tissues, whereas the C:N ratio increased in moss and deciduous species (by 121 and 97% respectively), the C:P ratio increased by 59% in deciduous species and N:P ratio decreased by 53% in moss during tissue senescence.

The C:N:P stoichiometry averaged 31:3:1 and 2073:56:1 in microbes and peat, respectively, with significantly smaller C:P and N:P ratios and larger C:N ratios in the bog than fen phase (C:N:P = 1942:50:1 and 2422:78:1, respectively, $P < 0.001$; Figure 6.2). From senescent tissues to acrotelm bog peat, the C:P and N:P ratios increased by 41 and 220% respectively, and the C:N ratio decreased by 54% (Figure 6.1). An overall trend of decreasing C:N ratio and increasing C:P and N:P ratios with depth is observed from the top (young) to the bottom (old) of the bog phase and all three ratios slightly decrease with time (depth) within the previous fen phase (Figure 6.2).

6.4.2 The accumulation rates of C, N and P

The long term (~8000 yr) apparent rates of C, N and P accumulation (LORCA, LORNA and LORPA, respectively) at Mer Bleue were 29.5 ± 2.1 g C m^{-2} yr^{-1} (mean ± SE), 0.87 ± 0.01 g N m^{-2} yr^{-1} and 0.017 ± 0.002 g P m^{-2} yr^{-1} respectively (Figure 6.3). The rates were significantly smaller in the bog phase (22.7 ± 1.0 g C m^{-2} yr^{-1}, 0.57 ± 0.01 g N m^{-2} yr^{-1} and 0.015 ± 0.003 g P m^{-2} yr^{-1}) than in the fen phase (46.0 ± 3.8 g C m^{-2} yr^{-1}, 1.63 ± 0.04 g N m^{-2} yr^{-1} and 0.023 ± 0.002 g P m^{-2} yr^{-1}). The average recent rate of C, N and P accumulation (RERCA, RERNA and RERPA, respectively) over the past 150 yr were 35.1 ± 2.4 g C m^{-2} yr^{-1}, 0.78 ± 0.05 g N m^{-2} yr^{-1} and 0.033 ± 0.009 g P m^{-2} yr^{-1}.

The accumulation rates of C were positively correlated with N and P accumulation rates in both the bog and fen phases, but there were differences (Figure 6.4). In the bog and fen phases, a similar pattern of C and N accumulation occurs, whereas C and P have different relationships in the bog and fen phases, with C accumulation consistently faster in the fen phase than in the bog phase under the same rate of P accumulation. N and P accumulation rates were also positively correlated in both the bog and fen phases ($R^2 = 0.76$ and 0.96 respectively, $P < 0.001$).

6.5 Discussion

As part of a nutrient-deficient ecosystem, ombrotrophic bog evergreen plants show strong resorption of nutrients during tissue senescence, especially N and P (Wang *et al*. 2014b), that drives the trend of increasing C:N and C:P ratios from mature to senescent tissues (Figure 6.5), supporting our first hypothesis. In senescent tissues at Mer Bleue, the C:N ratio (92:1) was larger and the C:P (911:1) and N:P ratios (10:1) were smaller than the C:N (50:1), C:P (1000:1) and N:P ratios (20:1) from subarctic flora (Aerts *et al*. 2012) respectively. This discrepancy is probably because of the larger resorption efficiency of P (63%) in these high-latitude ecosystems (Freschet *et al*. 2010) and the larger resorption of N of *Sphagnum* moss at Mer Bleue.

Litter decomposition should decrease C:N and C:P ratios to approach that of the decomposers as N and P are immobilized and C released until critical C:N and/or C:P ratios are reached, for example mass C:N and C:P ratios of ~40 and ~1000 in Canadian forest litter (Moore *et al*. 2011). There are no equivalent long-term litter decomposition studies in peatlands, so it is not clear whether these critical ratios may be applicable for ombrotrophic bogs. After 12 years of decomposition in Canadian forests, Moore *et al*. (2011) found a convergence in mass C:N:P to 427:17:1 with 20% of the original C remaining. Compared to the soil surface organic horizons (i.e. LFH), the C:N ratios increased from 25 to 42, the N:P ratio decreased from 17 to 10 and the C:P ratios remained similar at 427 to 420 (Figure 6.5) (Moore *et al*. 2011). In contrast, our results show a decrease in C:N ratio but a substantial increase in C:P and N:P ratios from senescent tissues to acrotelm and deeper peat. These are very different from the ratios in Mer Bleue peat microbes (Figure 6.5), rejecting our second hypothesis. Our *in situ* buried-bag peat incubation showed a weak mineralization of N and immobilization of P after 3 months (M. Wang unpublished data), supporting the hypothesis that the mismatch of the stoichiometry between peat and decomposers plays an important role in regulating nutrient cycling.

In the peat, the movement of the C:P and N:P ratios away from those of the soil microbes may be the result of two mechanisms. First, the formation of cluster roots and the carboxylate exudation pathway have been shown to be an important P-acquisition strategy in nutrient-impoverished soils in Australia (Lambers *et al*. 2012, Lambers *et al*. 2013). However, there is no evidence of cluster roots in the dominant shrub species at Mer Bleue (M. Murphy personal communication) and the low pH (< 5.0) does not favour the carboxylate-releasing strategy (Lambers *et al*. 2012).

Second, associations with ericoid mycorrhizae grant ericaceous shrubs the advantage of taking up N and P, the co-limiting nutrients (Smith & Read 2008). Long-term fertilization at Mer Bleue with N, P and K over a decade showed that shrub growth was only stimulated after fertilization by both N and PK (Larmola *et al*. 2013), supporting N and P co-limitation based on leaf-level N:P:K stoichiometry (Wang & Moore 2014). The absence of significant external P input and elevated atmospheric N deposition in ombrotrophic systems requires efficient internal cycling of P. Consequently, the preference of uptake for P over N may account for the increase in C:P and N:P ratios with depth in the bog phase of the peat. In the fen phase with dominant sedges without mycorrhizae, decreasing C:P and N:P ratios with depth were observed (Figure 6.2). In contrast to bogs, the preferential uptake of N over P in Canadian forests, which are primarily N-deficient ecosystems, may explain the increasing C:N ratio, decreasing N:P ratio and litter change in C:P ratio from litter to soil organic horizon as discussed above. The preferential uptake of limited nutrients in bogs and Canadian forests suggests that plant/mycorrhizae associations can out-compete microbes in taking up limited nutrients and are important in biogeochemical cycling.

The strong correlation between C and N and P accumulation rates at Mer Bleue suggests the indispensable role of N and P in controlling C accumulation. Extrapolating the average C:N (37) and C:P (2073) ratios in peat from Mer Bleue to northern peatlands with a storage of 547 Gt C, we estimate that northern peatlands store 15 Gt N and 0.3 Gt P, respectively, comprising 11-16% of the N in global soil organic matter (95-140 Gt N) and ~2% of the P in labile or organic form in soils to a 50 cm depth (13.8 Gt P) (Schlesinger & Bernhardt 2013). Our LORCA falls within the range from 24 to 37 g C m^{-2} yr^{-1} in North America (Gorham & Janssens 2005) and from 5 to 34 g C m^{-2} yr^{-1} in eastern Canada (Turunen *et al*. 2004), and larger than the time-weighted C accumulation rate of 22.9 ± 2.0 g C m^{-2} yr^{-1} in northern peatlands during the Holocene (J. Loisel in press). However, N and P accumulated faster at Mer Bleue than rates reported by Gorham & Janssens (2005) of 0.36-0.55 g N m^{-2} yr^{-1} and 0.008-0.014 g P m^{-2} yr^{-1}, and the Holocene time-weighted N accumulation rate of 0.5 ± 0.04 g N m^{-2} yr^{-1} in northern peatlands (J. Loisel in press). The short term (~150 years) C and N accumulation rates are lower than those determined by ^{210}Pb dating in eastern Canada, ranging from 40 to 117 g C m^{-2} yr^{-1} and from 1.1 to 2.5 g N m^{-2} yr^{-1} (Turunen *et al*. 2004).

The P (co)limitation at Mer Bleue, as indicated by C:N:P:K stoichiometry in mature tissues and long-term fertilization, suggests the processes which maintain P at co-limitation are important to understanding biogeochemical processes in ombrotrophic bogs. The increase in C:nutrient ratios from mature to senescent tissues is related to efficient nutrient resorption and the increase in C:P and N:P ratios in peat profiles may be related to plant/mycorrhizae uptake of nutrients. The significant correlation between the accumulation rates of N and P and that of C suggests more attention be placed on C:N:P stoichiometry in peatland biogeochemistry, in particular in understanding why C:P ratios are so large in the lower parts of the profile and that P limitation may be a significant control on plant production.

Figure 6.1 Ternary diagram showing the stoichiometric relationship of C, N and P from mature (green triangles) and senescent tissues (brown triangles) in deciduous (D), evergreen (E), graminoid (G), forb (F) and moss species (M), to peat (bog (squares) and fen (diamonds) phases) in comparison with microbes (circle). Dashed lines indicate the critical ratios of C:N (40) and C:P (1000). The thick arrows indicate the direction in which the axes should be read and the increase (↑) or decrease (↓) of the stoichiometric ratios. For visual reasons, the C and P concentration is divided and multiplied by a factor of 100 and 10, respectively.

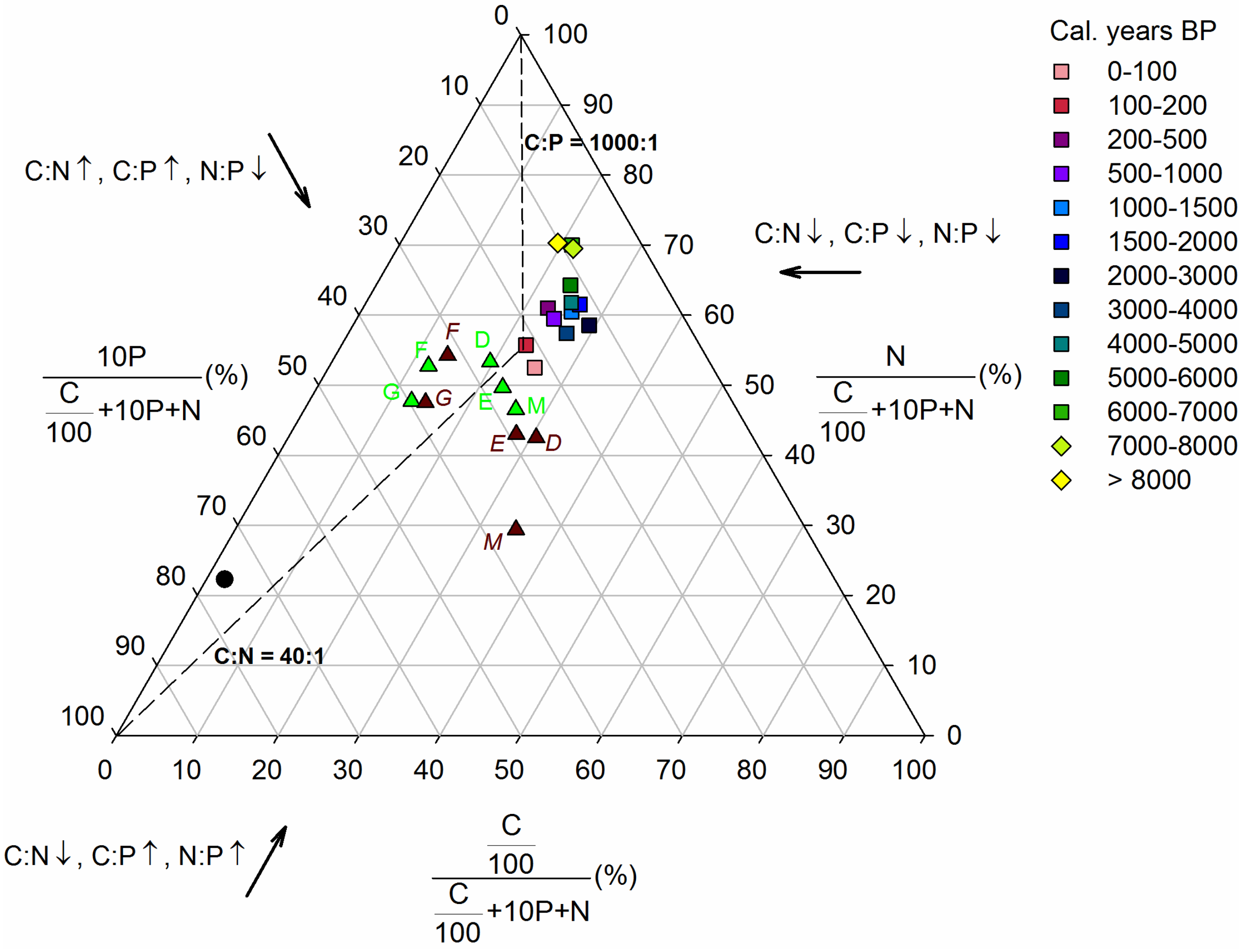


Figure 6.2 The stoichiometric mass ratio (mean ± SE) of C:N, C:P and N:P in peat profiles through time. Open circles are the individual data points from two profiles and filled circles are means based on grouped age intervals (see *Materials and Methods* for details). The horizontal lines indicate the bog and fen phases in the profiles. The vertical dashed lines indicate the critical C:N and C:P ratios.

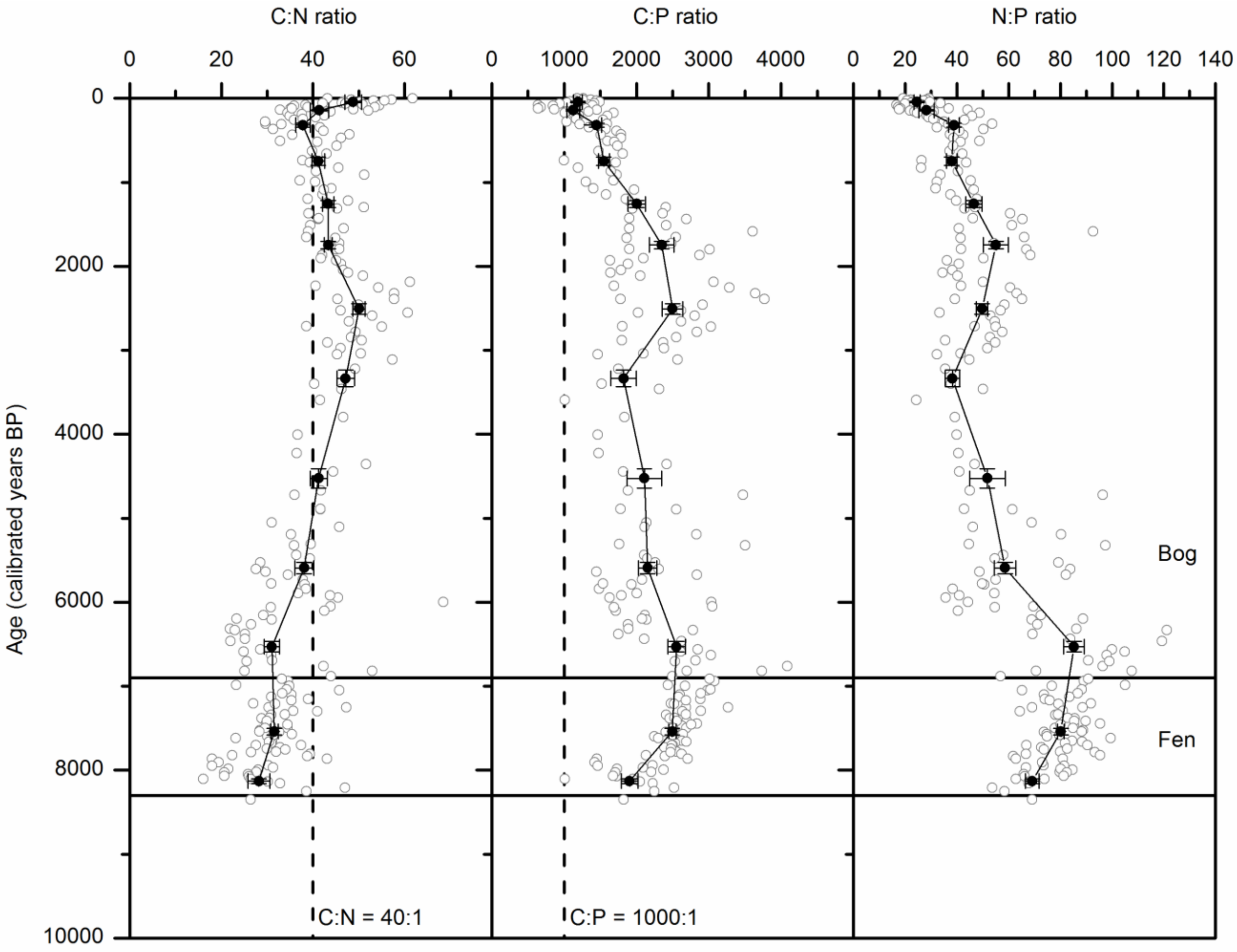


Figure 6.3 The apparent accumulation rates (mean ± SE) of C, N and P in peat profiles through time. Open circles are the individual data points from two profiles and filled circles are means based on grouped age intervals. The horizontal lines indicate the bog and fen phase in the profiles.

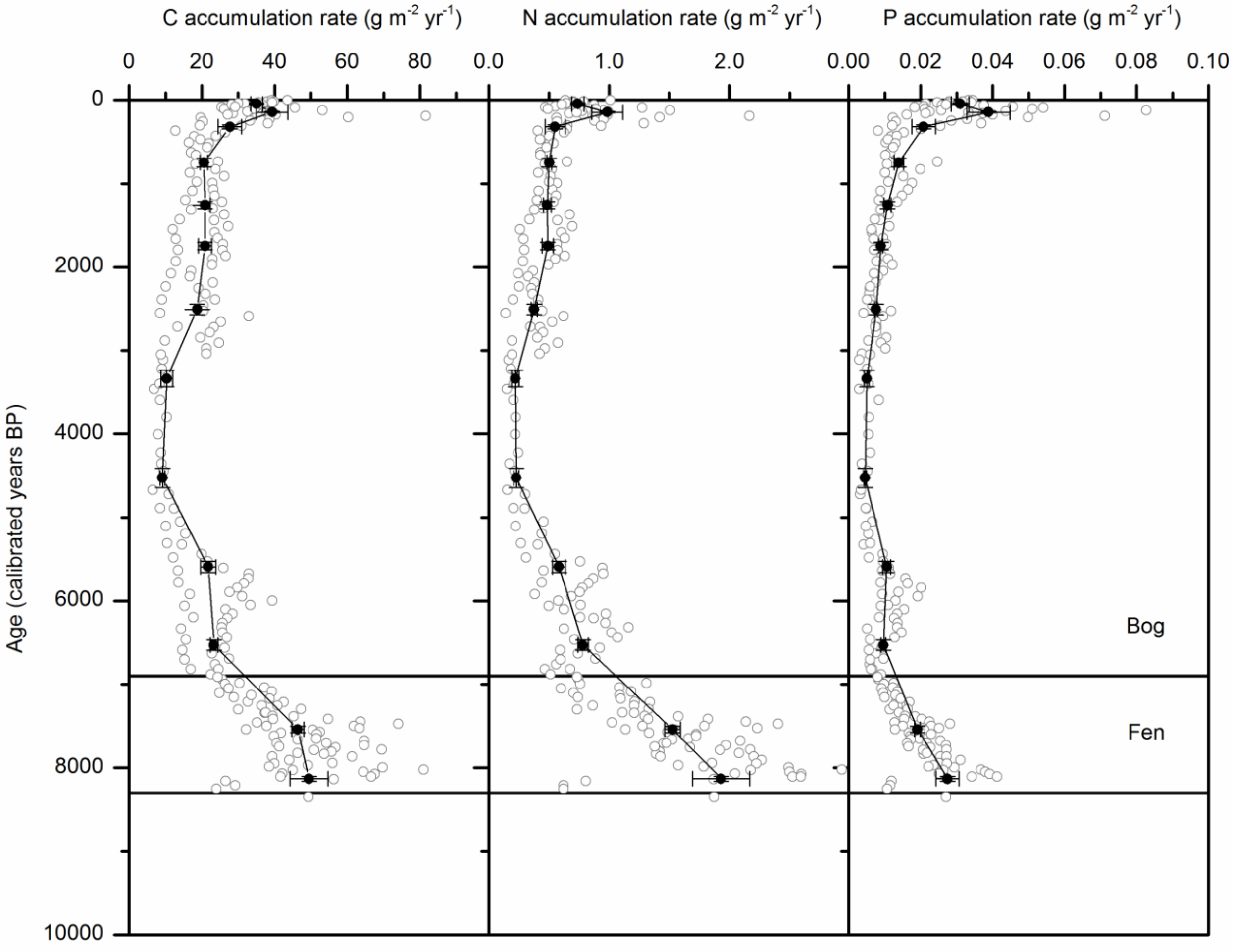


Figure 6.4 Relationship between the apparent accumulation rates of N and P and C accumulation rate in the bog (open triangle, solid lines) and fen (filled triangles, dashed lines) phases of the peat profiles. The regression model is significant ($P < 0.001$) in all cases. For each model, SE_s and SE_i indicate the standard error of the slope and intercept. The differences in the slope and intercept of the regression models between the bog and fen phases are all significant ($P < 0.001$) with t_s and t_i (degree of freedom in the parentheses) indicating the t values of the t test on the difference in slope and intercept, respectively.

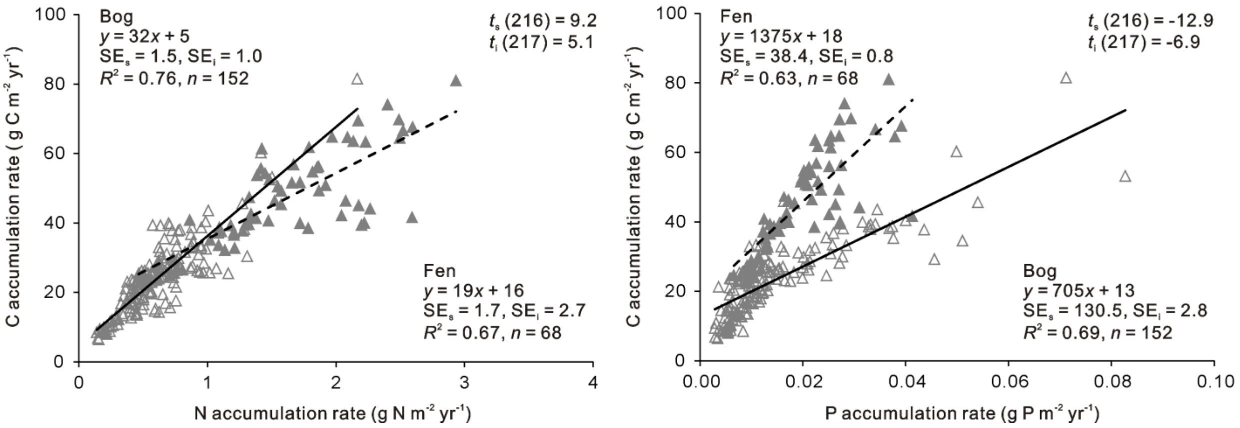
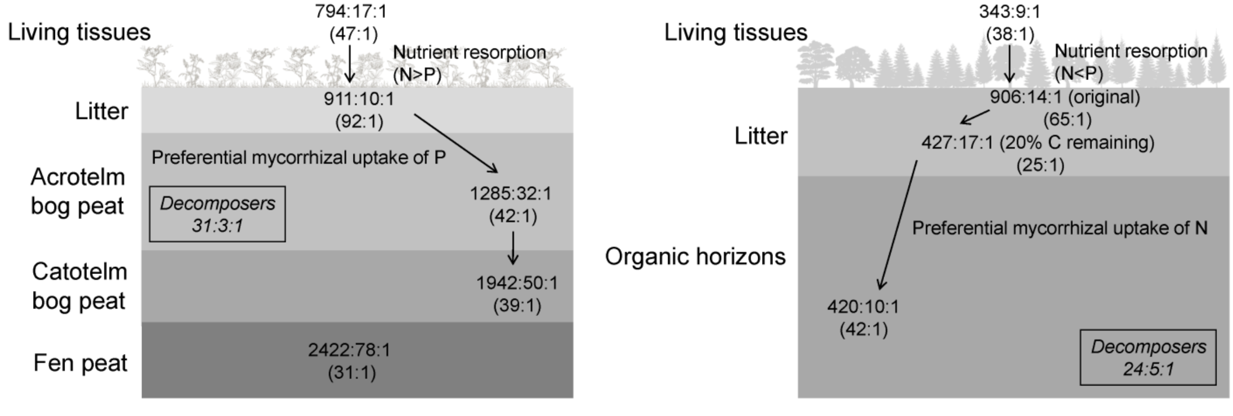


Figure 6.5 The cascade of C:N:P stoichiometry from leaves to peat in Mer Bleue Bog and soil organic horizons in Canadian forests, with C:N ratios in parentheses. The C:N:P stoichiometry in living tissues from Canadian forests are derived from Paré *et al*. (2012), with 8 of the species included in Moore *et al*. (2011) from Canadian sites (*Betula papyrifera*, Fagus grandifolia, *Larix laricina*, *Picea mariana*, *Pinus banksiana, Populus tremuloides*, *Pseudotsuga menziesii* and *Thuja plicata*) and assuming the C content is 50% of the dry mass. The C:N:P stoichiometry in litter (arithmetic mean of 10 litter types) and soil organic horizons (arithmetic mean of 22 sites) from Canadian forests are derived from Moore *et al*. (2011). The C:N:P stoichiometry of soil microbial biomass (decomposers) in forest organic horizons are derived from Basiliko *et al*. (2009). Not drawn to scale.



Mer Bleue Bog

Canadian forests

Chapter 7. Summary, conclusions and future research directions

Northern peatlands are nutrient-deficient ecosystems and significant C sinks. The substantially increased atmospheric deposition compared to the pre-industrial era has generated scientific concerns about the consequences of the changes in biogeochemical characteristics and hence ecosystem functioning in northern peatlands, especially ombrotrophic bogs. The prevalence of N limitation in bogs under low levels of atmospheric N deposition may be shifted to P or K (co)limitation owing to enhanced atmospheric N deposition and accelerated organic matter decomposition. This possible shift in N, P and K interactions highlights the essential role of the relative balance among multiple elements, i.e. ecological stoichiometry, in controlling metabolic functioning, plant growth and ecosystem processes. In a world of elemental imbalance between organisms and the external environments, such as between plants and decomposers, a better understanding of different aspects of ecological stoichiometry in plants and soils and how they are affected by nutrient enrichment becomes critical in these nutrient-deficient ecosystems. In this thesis, I have examined the stoichiometry in photosynthetically active tissues under natural climate variability and increased nutrient deposition, determined the contribution of nutrient resorption and linked the stoichiometry in plants with soil through the decomposition cascade in an ombrotrophic bog.

In Chapter 3, we observed a convergence of C:N:P:K to an average mass ratio of 445:14:1:9 though there were variations in stoichiometry among PFTs at peak growing season, indicating N and P co-limitation. There was little seasonal variation in N, P and K concentrations and stoichiometric ratios in mosses, evergreen and graminoids. But in forb and deciduous species, they were the largest in spring and decreased throughout the growing season. Variations in nutrient concentrations and stoichiometric ratios among PFTs were greater than seasonal variation. Plants show N and P co-limitation and adapt to nutrient-impoverished soil by maintaining small nutrient concentrations in photosynthetically active tissues, especially for evergreen shrubs and *Sphagnum* mosses. Despite strong seasonal variations in nutrient availabilities, few species show strong seasonal variation in nutrient concentrations, suggesting a strong homeostasis at Mer Bleue Bog.

In Chapter 4, N addition generally caused further P-(co)limitation, increased P and K resorption efficiency but did not affect N resorption. We did not observe the shift to N-limitation with the addition of P and K, which reduced P resorption but did not affect K resorption. *C. calyculata* resorbed both Ca and Mg while *R. groenlandicum* resorbed neither. *C. calyculata* showed a stronger resorption than *R. groenlancidum*, suggesting it is better adapted to nutrient-impoverished condition than *R. groenlandicum*. Resorption during leaf senescence decreased N:P, N:K and K:P ratios. The limited response of N and K and active response of P resorption to nutrient addition indicates the stoichiometric coupling of nutrient cycling which varies between two shrubs; changes in species composition may affect nutrient cycling in bogs.

Chapter 5 demonstrated the stability of nutrient concentrations and stoichiometric ratios in shrubs and mosses in response to different levels of nutrient addition, i.e. stoichiometric homeostasis. The results showed that shrubs exhibited the strongest homeostasis to N with the homeostatic regulation coefficient (H) > 10.0, compared to 1.4 in moss. Shrubs showed weaker homeostasis to P and K with H of 4.7-6.6 and 4.3-6.9, respectively, whereas moss had stronger homeostasis to P and K than N with H of 2.4 and infinity, respectively. The abundance of shrubs increased and the cover of moss decreased in heavily NPK fertilized plots. Strong homeostasis of shrubs may be an evolutionary strategy or a long-term adaption to extremely low nutrient availability. Although with weak homeostasis, moss takes advantage of limited nutrient availabilities and the harsh micro-environment it establishes. Because of the increased ecosystem respiration associated with the loss of moss, the dominance of shrubs with strong homeostasis does not increase productivity as observed in other ecosystems.

In Chapter 6, we found a change in average C:N:P ratio from 794:17:1 in foliar tissues to 911:10:1 in litter and 1285:32:1 in acrotelm peat. Nutrient resorption accounted for the increase in C:N and C:P ratios from mature to senescent tissues. The increase in C:P and N:P ratios in peat, which as contrary to that found in Canadian forest soils, may be related to the preferential uptake of P by plant/mycorrhizae. The long-term apparent rates of C, N and P accumulation were 29.5 ± 2.1 (SE) g C m^{-2} yr^{-1}, 0.87 ± 0.01 g N m^{-2} yr^{-1} and 0.017 ± 0.002 g P m^{-2} yr^{-1}, respectively. The significant correlation between the accumulation rates of N and P and that of C requires more attention on C:N:P stoichiometry in peatland biogeochemistry, in particular in understanding why C:P ratios are so large in the lower parts of the profile.

My overall objective was to fill some scientific gaps in nutrient stoichiometry in a bog ecosystem. My results suggested the prevalence of N and P co-limitation at Mer Bleue Bog with moderate atmospheric N deposition. Although with considerable nutrient resorption, there was no substantial seasonal variation in foliar stoichiometry in evergreen and moss species, whereas deciduous species showed a significant variation in nutrient concentration and stoichiometric ratios throughout the growing season. The examination of the effect of long-term fertilization on plant stoichiometry in shrub and moss species confirmed the result of N and P co-limitation. The strong and weak homeostasis in shrubs and mosses, respectively, evoked further attention on the shift in species composition in plant community. The unique adaptation of moss species to a harsh micro-environment is attributed to maintain the stability and productivity at Mer Bleue Bog. By linking stoichiometry from foliar plant tissues through senescent litters to peat, the decomposition cascade revealed a resorption-associated decrease in C:nutrient ratios from mature to senescent tissues, and hypothesized the connection between increase in C:P and N:P ratios in peat with plant/mycorrhizae uptake of P.

This thesis provides insights to understand the stoichiometric characteristics in ombrotrophic bogs. The predominant co-limitation by N and P emphasizes the necessity of incorporating the biogeochemical cycling of P, which is substantially overlooked, into the ecosystem models in ombrotrophic bog peatland. The interspecific variation in the resorption of essential macronutrients and difference in stoichiometric homeostasis between shrubs and mosses suggest the changes in species composition in plant community may affect nutrient cycling and ecosystem stability and productivity in bogs. The potential plant/mycorrhizae uptake of P in bog is probably the key in controlling peatland biogeochemistry and maintaining the system at N and P co-limitation with substantial atmospheric N deposition.

In the end, I propose several research directions that could be pursued in future studies to improve our understanding of peatland biogeochemistry. First, as the experimental design of the long-term fertilization at Mer Bleue Bog does not separate the addition of P from K, the possible interaction between P and K may influence the interpretation of results with regard to the effect of PK addition. A full factorial design with all possible combinations of N, P and K addition will disentangle the possible effect of interactions among N, P and K on plant stoichiometry and contribute to a better estimation of the critical stoichiometric ratios that determine the shift in nutrient limitation.

Second, although the atmospheric deposition in Canada is considerably lower than in much of Europe, Mer Bleue bog is located in a region with the highest atmospheric N deposition in Canada, which may have compensated the scarcity of N even under unfertilized condition, contributing to the weak response to N addition. Besides, as the fertilization experiment at Mer Bleue Bog 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 also influence the homeostasis. A similar set-up of fertilization experiment with full factorial designs and multiple levels of P and K addition in a remote area with lower atmospheric nutrient deposition is desired. A repeated-measure approach with samples from each year after the initiation of fertilization will improve our understanding of stoichiometric homeostasis as a function of plant species adaption to nutrient enrichment over a long temporal scale.

Third, we found the resorption of Ca and Mg differed considerably during leaf senescence. As two of the most important divalent cations in peatlands, the efficient conservation of Ca and Mg through resorption can be a beneficial adaption to an ombrotrophic environment where the available Ca and Mg are extremely low. However, the underlying physiological mechanism attributing to the difference in Ca and Mg resorption remains ambiguous (Clarkson & Hanson 1980; Negi & Singh 1993; Lambers *et al*. 1998). A further exploration in this respect will facilitate the understanding of the discrepancy between Ca and Mg in decomposition cascade and peat burial.

Fourth, the potential role of mycorrhizal fungi in controlling soil organic P acquisition merits further exploration. Mycorrhizae can synthesize phosphatase and involve in the acquisition of soil organic P (Smith & Read 2008). Among the major groups of mycorrhizae, the ectomycorrhizae and ericoid mycorrhizae are well-known to efficiently utilize phosphate esters (Antibus *et al*. 1992) and diesters (Leake & Miles 1996), respectively. Ericoid mycorrhizae are abundant in soils associated with ericaceous shrubs (Turner *et al*. 2004) which are dominant in ombrotrophic bogs. Besides, the arbuscular mycorrhizae are also proved to be able to use organic P (Joner *et al*. 2000; Koide & Kabir 2000). Therefore, it will contribute new knowledge of peatland biogeochemistry by exploring the role of different groups of mycorrhizae in peatlands (1) under natural climate variation, (2) along nutrient gradients, such as from ombrotrophic bogs to minerotrophic poor and rich fens, and (3) in response to extreme nutrient deposition through fertilization experiments.

Last, but not least, to assess whether the stoichiometric patterns observed at Mer Bleue Bog are applicable to a broader spatial extent, such as regional scale, we need to expand current research and conduct parallel studies in other ombrotrophic/minerotrophic systems spanning a larger spatial extent along environmental gradients. To do so, I hope a better understanding of plant and soil stoichiometry and their interactions will contribute new knowledge of peatland biogeochemistry that can be used to improve the predictive ability of large-scale ecosystems models.

References

Aerts, R. (1996). Nutrient resorption from senescing leaves of perennials: are there general patterns? *Journal of Ecology*, 84, 597-608.

Aerts, R. & Berendse, F. (1988). The effect of increased nutrient availability on vegetation dynamics in wet heathlands. *Vegetatio*, 76, 63-69.

Aerts, R. & Bobbink, R. (1999). The impact of atmospheric nitrogen deposition on vegetation processes in terrestrial, non-forest ecosystems. In: *The impact of nitrogen deposition on natural and semi-natural ecosystems* (ed. Langan, SJ). Kluwer Academic Publishers Dordrecht, the Netherlands, pp. 85-122.

Aerts, R. & Chapin, F.S. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*, 30, 1-67.

Aerts, R., van Bodegom, P.M. & Cornelissen, J.H.C. (2012). Litter stoichiometric traits of plant species of high-latitude ecosystems show high responsiveness to global change without causing strong variation in litter decomposition. *New Phytologist*, 196, 181-188.

Aerts, R., Wallén, B. & Malmer, N. (1992). Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *Journal of Ecology*, 80, 131-140.

Ågren, G.I. (2004). The C : N : P stoichiometry of autotrophs – theory and observations. *Ecology Letters*, 7, 185-191.

Ågren, G.I. (2008). Stoichiometry and nutrition of plant growth in natural communities. *Annual Review of Ecology Evolution and Systematics*, 39, 153-170.

Aldous, A.R. (2002b). Nitrogen translocation in *Sphagnum* mosses: effects of atmospheric nitrogen deposition. *New Phytologist*, 156, 241-253.

Andersen, T. & Hessen, D.O. (1991). Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography*, 36, 807-814.

Anderson, W.B. & Eickmeier, W.G. (2000). Nutrient resorption in *Claytonia virginica* L.: implications for deciduous forest nutrient cycling. *Canadian Journal of Botany*, 78, 832-839.

Andrews, J.A. & Siccama, T.G. (1995). Retranslocation of calcium and magnesium at the heartwood-sapwood boundary of Atlantic white cedar. *Ecology*, 76, 659-663.

Arndal, M.F., Illeris, L., Michelsen, A., Albert, K., Tamstorf, M. & Hansen, B.U. (2009). Seasonal variation in gross ecosystem production, plant biomass, and carbon and nitrogen pools in five high arctic vegetation types. *Arctic Antarctic and Alpine Research*, 41, 164-173.

Antibus, R.K., Sinsabaugh, R.L. & Linkins, A.E. (1992). Phosphatase activities and phosphorus uptake from inositol phosphate by ectomycorrhizal fungi. *Canadian Journal of Botany*, 70, 794-801.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1-15.

Basiliko, N. (2004). Nutrient, substrate, and microbial-ecological links to decomposition and greenhouse production in northern peatlands. In: *Geography*. McGill University Montreal, p. 163.

Bedford, B.L., Walbridge, M.R. & Aldous, A. (1999). Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology*, 80, 2151-2169.

Berendse, F., van Breemen, N., Rydin, H., Buttler, A., Heijmans, M., Hoosbeek, M.R. *et al.* (2001). Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in *Sphagnum* bogs. *Global Change Biology*, 7, 591-598.

Birk, E.M. & Vitousek, P.M. (1986). Nitrogen availability and nitrogen use efficiency in loblolly pine stands. *Ecology*, 67, 69-79.

Bobbink, R., Hornung, M. & Roelofs, J.G.M. (1998). The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, 86, 717-738.

Bragazza, L., Freeman, C., Jones, T., Rydin, H., Limpens, J., Fenner, N. *et al*. (2006). Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences*, 103, 19386-19389.

Bragazza, L., Tahvanainen, T., Kutnar, L., Rydin, H., Limpens, J., Hájek, M. *et al*. (2004). Nutritional constraints in ombrotrophic *Sphagnum* plants under increasing atmospheric nitrogen deposition in Europe. *New Phytologist*, 163, 609-616.

Breeuwer, A., Heijmans, M., Gleichman, M., Robroek, B. & Berendse, F. (2009). Response of *Sphagnum* species mixtures to increased temperature and nitrogen availability. *Plant Ecology*, 204, 97-111.

Bubier, J.L., Moore, T.R. & Bledzki, L.A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, 13, 1168-1186.

Bubier, J.L., Moore, T.R. & Crosby, G. (2006). Fine-scale vegetation distribution in a cool temperate peatland. *Canadian Journal of Botany*, 84, 910-923.

Bubier, J.L., Smith, R., Juutinen, S., Moore, T.R., Minocha, R., Long, S. *et al*. (2011). Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs. *Oecologia*, 167, 355-368.

Canadian Climate Normals. (1981-2010). National climate data and information archive. Available at: http://climate.weather.gc.ca/climate_normals Last accessed February 4 2014.

Chapin, F.S., Johnson, D.A. & McKendrick, J.D. (1980). Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: implications for herbivory. *Journal of Ecology*, 68, 189-209.

Chapin, F.S. & Kedrowski, R.A. (1983). Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous taiga trees. *Ecology*, 64, 376-391.

Chapin, F.S. & Moilanen, L. (1991). Nutritional controls over nitrogen and phosphorus resorption from Alaskan birch leaves. *Ecology*, 72, 709-715.

Charman, D. (2002). *Peatlands and Environmental Change*. Wiley, Chichester.

Chong, M., Humphreys, E. & Moore, T.R. (2012). Microclimatic response to increasing shrub cover and its effect on *Sphagnum* CO_2 exchange in a bog. *Écoscience*, 19, 89-97.

Clarkson, D.T. & Hanson, J.B. (1980). The mineral nutrition of higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 31, 239-298.

Clymo, R.S. (1984). The limits to peat bog growth. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 303, 605-654.

Cleveland, C.C. & Liptzin, D. (2007). C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry*, 85, 235-252.

DeMott, W.R. & Pape, B.J. (2005). Stoichiometry in an ecological context: testing for links between *Daphnia* P-content, growth rate and habitat preference. *Oecologia*, 142, 20-27.

Diehl, P., Mazzarino, M.J., Funes, F., Fontenla, S., Gobbi, M. & Ferrari, J. (2003). Nutrient conservation strategies in native Andean-Patagonian forests. *Journal of Vegetation Science*, 14, 63-70.

Downing, J.A. (1997). Marine nitrogen: phosphorus stoichiometry and the global N:P cycle. *Biogeochemistry*, 37, 237-252.

Eckstein, R.L., Karlsson, P.S. & Weih, M. (1998). The significance of resorption of leaf resources for shoot growth in evergreen and deciduous woody plants from a subarctic environment. *Oikos*, 81, 567-575.

Ellison, A.M. (2006). Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology*, 8, 740-747.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H. *et al.* (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135-1142.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996). Organism size, life history, and N:P stoichiometry. *Bioscience*, 46, 674-684.

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A. *et al*. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408, 578-580.

Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G. & Enquist, B.J. (2010). Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist*, 186, 593-608.

Escudero, A., Del Arco, J.M., Sanz, I.C. & Ayala, J. (1992). Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. *Oecologia*, 90, 80-87.

Feller, I.C., McKee, K.L., Whigham, D.F. & O'Neill, J.P. (2003). Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry*, 62, 145-175.

Filippelli, G.M. (2008). The global phosphorus cycle: past, present, and future. *Elements*, 4, 89-95.

Freschet, G.T., Cornelissen, J.H.C., van Logtestijn, R.S.P. & Aerts, R. (2010). Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? *New Phytologist*, 186, 879-889.

Frolking, S., Roulet, N.T., Tuittila, E., Bubier, J.L., Quillet, A., Talbot, J. *et al*. (2010). A new model of Holocene peatland net primary production, decomposition, water balance, and peat accumulation. *Earth System Dynamics*, 1, 1-21.

Frost, P.C., Evans-White, M.A., Finkel, Z.V., Jensen, T.C. & Matzek, V. (2005). Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos*, 109, 18-28.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z.C., Freney, J.R. *et al.* (2008). Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, 320, 889-892.

Givnish, T.J., Burkhardt, E.L., Happel, R.E. & Weintraub, J.D. (1984). Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist*, 124, 479-497.

Glaser, P.H. (1992). Raised bogs in eastern North America - regional controls for species richness and floristic assemblages. *Journal of Ecology*, 80, 535-554.

Goldman, J.C., Caron, D.A. & Dennett, M.R. (1987). Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. *Limnology and Oceanography*, 32, 1239-1252.

Gorham, E. (1991). Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications*, 1, 182-195.

Gorham, E. & Janssens, J.A. (2005). The distribution and accumulation of chemical elements in five peat cores from the mid-continent to the eastern coast of North America. *Wetlands*, 25, 259-278.

Gotelli, N.J., Mouser, P.J., Hudman, S.P., Morales, S.E., Ross, D.S. & Ellison, A.M. (2008). Geographic variation in nutrient availability, stoichiometry, and metal concentrations of plants and pore-water in ombrotrophic bogs in New England, USA. *Wetlands*, 28, 827-840.

Gruber, N. & Galloway, J.N. (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, 451, 293-296.

Gunnarsson, U. & Rydin, H. (2000). Nitrogen fertilization reduces *Sphagnum* production in bog communities. *New Phytologist*, 147, 527-537.

Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, 164, 243-266.

Güsewell, S. (2005a). Nutrient resorption of wetland graminoids is related to the type of nutrient limitation. *Functional Ecology*, 19, 344-354.

Güsewell, S. (2005b). Responses of wetland graminoids to the relative supply of nitrogen and phosphorus. *Plant Ecology*, 176, 35-55.

Güsewell, S. & Bollens, U. (2003). Composition of plant species mixtures grown at various N : P ratios and levels of nutrient supply. *Basic Applied Ecology*, 4, 453-466.

Güsewell, S. & Koerselman, M. (2002). Variation in nitrogen and phosphorus concentrations of wetland plants. *Perspectives in Plant Ecology Evolution and Systematics*, 5, 37-61.

Güsewell, S. & Verhoeven, J.T.A. (2006). Litter N : P ratios indicate whether N or P limits the decomposability of graminoid leaf litter. *Plant and Soil*, 287, 131-143.

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J. & de Ruiter, P.C. (2004). Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology*, 85, 1179-1192.

Hiscox, J.D. & Israelstam, G.F. (1979). Method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, 57, 1332-1334.

Hoosbeek, M.R., van Breemen, N., Vasander, H., Buttler, A. & Berendse, F. (2002). Potassium limits potential growth of bog vegetation under elevated atmospheric CO_2 and N deposition. *Global Change Biology*, 8, 1130-1138.

Jeyasingh, P.D., Weider, L.J. & Sterner, R.W. (2009). Genetically-based trade-offs in response to stoichiometric food quality influence competition in a keystone aquatic herbivore. *Ecology Letters*, 12, 1229-1237.

Jiroušek, M., Hájek, M. & Bragazza, L. (2011). Nutrient stoichiometry in *Sphagnum* along a nitrogen deposition gradient in highly polluted region of Central-East Europe. *Environmental Pollution*, 159, 585-590.

Joner, E.J., Ravnskov, S. & Jakobsen, I. (2000). Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. *Biotechnology Letters*, 22, 1705-1708.

Joosten, H. & Clarke, D. (2002). Wise use of mires and peatlands - background and principles including a framework for decision-making. Available at: http://www.mirewiseuse.com Last accessed January 14 2014.

Karimi, R. & Folt, C.L. (2006). Beyond macronutrients: element variability and multielement stoichiometry in freshwater invertebrates. *Ecology Letters*, 9, 1273-1283.

Karlsson, P.F. (1994). The significance of internal nutrient cycling in branches for growth and reproduction of *Rhododendron lapponicum*. *Oikos*, 70, 191-200.

Kercher, S.M. & Zedler, J.B. (2004). Flood tolerance in wetland angiosperms: a comparison of invasive and noninvasive species. *Aquatic Botany*, 80, 89-102.

Kerkhoff, A.J. & Enquist, B.J. (2006). Ecosystem allometry: the scaling of nutrient stocks and primary productivity across plant communities. *Ecology Letters*, 9, 419-427.

Killingbeck, K.T. (1986). The terminological jungle revisited: making a case for use of the term resorption. *Oikos*, 46, 263-264.

Killingbeck, K.T. (1996). Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology*, 77, 1716-1727.

Knops, J.M.H., Koenig, W.D. & Nash, T.H. III (1997). On the relationship between nutrient use efficiency and fertility in forest ecosystems. *Oecologia*, 110, 550-556.

Kobe, R.K., Lepczyk, C.A. & Iyer, M. (2005). Resorption efficiency decreases with increasing green leaf nutrients in a global data set. *Ecology*, 86, 2780-2792.

Koerselman, W. & Meuleman, A.F.M. (1996). The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, 33, 1441-1450.

Koide, R.T. & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist*, 148, 511-517.

Končalová, H. (1990). Anatomical adaptations to waterlogging in roots of wetland graminoids: limitations and drawbacks. *Aquatic Botany*, 38, 127-134.

Konings, H., Verhoeven, J.T.A. & Groot, R. (1992). Growth characteristics and seasonal allocation patterns of biomass and nutrients in *Carex* species growing in floating fens. *Plant and Soil*, 147, 183-196.

Kooijman, S.A.L.M. (1995). The stoichiometry of animal energetics. *Journal of Theoretical Biology*, 177, 139-149.

Lambers, H., Bishop, J.G., Hopper, S.D., Laliberté, E. & Zúñiga-Feest, A. (2012). Phosphorus-mobilization ecosystem engineering: the roles of cluster roots and carboxylate exudation in young P-limited ecosystems. *Annals of Botany*, 110, 329-348.

Lambers, H., Chapin, F.S.I. & Pons, T.L. (1998). *Plant physiological ecology*. Springer-Verlag, New York, USA.

Lambers, H., Clements, J.C. & Nelson, M.N. (2013). How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus, Fabaceae*). *American Journal of Botany*, 100, 263-288.

Lamers, L.P.M., Bobbink, R. & Roelofs, J.G.M. (2000). Natural nitrogen filter fails in polluted raised bogs. *Global Change Biology*, 6, 583-586.

Larmola, T., Bubier, J.L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E. *et al*. (2013). Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global Change Biology*, 19, 3729-3739.

LeBauer, D.S. & Treseder, K.K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371-379.

Levi, M.P. & Cowling, E.B. (1969). Role of nitrogen in wood deterioration. VII. Physiological adaptation of wood-destroying and other fungi to substrates deficient in nitrogen. *Phytopathology*, 59, 460-468.

Li, X.F., Zheng, X.B., Han, S.J., Zheng, J.Q. & Li, T.G. (2010). Effects of nitrogen additions on nitrogen resorption and use efficiencies and foliar litterfall of six tree species in a mixed birch and poplar forest, northeastern China. *Canadian Journal of Forest Research*, 40, 2256-2261.

Limpens, J. & Berendse, F. (2003). Growth reduction of *Sphagnum magellanicum* subjected to high nitrogen deposition: the role of amino acid nitrogen concentration. *Oecologia*, 135, 339-345.

Loisel, J., Yu, Z., Beilman, D.W., Camill, P., Alm, J., Anderson, D. *et al*. (2014). A circum-Arctic database and synthesis of peatland soil properties and Holocene carbon and nitrogen accumulation. *The Holocene*, in press (accepted).

Loladze, I. & Elser, J.J. (2011). The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio. *Ecology Letters*, 14, 244-250.

Loladze, I., Kuang, Y. & Elser, J.J. (2000). Stoichiometry in producer-grazer systems: linking energy flow with element cycling. *Bulletin of Mathematical Biology*, 62, 1137-1162.

Loneragan, J.F., Snowball, K. & Robson, A.D. (1976). Remobilization of nutrients and its significance in plant nutrition. In: *Transport and transfer processes in plants* (eds. Wardlaw, IF & Passioura, JB). Academic Press New York, USA, pp. 463-469.

Makino, W., Cotner, J.B., Sterner, R.W. & Elser, J.J. (2003). Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. *Functional Ecology*, 17, 121-130.

Malmer, N., Horton, D.G. & Vitt, D.H. (1992). Element concentrations in mosses and surface waters of western Canadian mires relative to precipitation chemistry and hydrology. *Ecography*, 15, 114-128.

Malmer, N. & Wallén, B. (2005). Nitrogen and phosphorus in mire plants: variation during 50 years in relation to supply rate and vegetation type. *Oikos*, 109, 539-554.

Marschner, H. (1995). *Mineral nutrition of higher plants*. Academic Press, Cambridge, UK.

McGroddy, M.E., Daufresne, T. & Hedin, L.O. (2004). Scaling of C:N:P stoichiometry in forests worldwide: implications of terrestrial Redfield-type ratios. *Ecology*, 85, 2390-2401.

Mitsch, W.J. & Gosselink, J.G. (2007). *Wetlands, 4th edition*. John Wiley & Sons, Hoboken, New Jerwey.

Moore, T., Blodau, C., Turunen, J., Roulet, N. & Richard, P.J.H. (2004). Patterns of nitrogen and sulfur accumulation and retention in ombrotrophic bogs, eastern Canada. *Global Change Biology*, 11, 356-367.

Moore, T.R. & Basiliko, N. (2006). Decomposition in boreal peatlands. In: *Boreal peatland ecosystems* (eds. Wieder, RK & Vitt, DH). Springer-Verlag Heidelberg, Germany, pp. 125-143.

Moore, T.R., Bubier, J.L., Frolking, S.E., Lafleur, P.M. & Roulet, N.T. (2002). Plant biomass and production and CO_2 exchange in an ombrotrophic bog. *Journal of Ecology*, 90, 25-36.

Moore, T.R., Trofymow, J.A., Prescott, C.E. & Titus, B.D. (2011). Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. *Plant and Soil*, 339, 163-175.

Moore, T.R., Trofymow, J.A., Siltanen, M. & Kozak, L.M. (2008). Litter decomposition and nitrogen and phosphorus dynamics in peatlands and uplands over 12 years in central Canada. *Oecologia*, 157, 317-325.

Murphy, M.T., McKinley, A. & Moore, T.R. (2009). Variations in above- and below-ground vascular plant biomass and water table on a temperate ombrotrophic peatland. *Botany*, 87, 845-853.

Murphy, J. & Riley, J.P. (1962). A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta*, 26, 31-36.

Nambiar, E.K.S. & Fife, D.N. (1991). Nutrient retranslocation in temperate conifers. *Tree Physiology*, 9, 185-207.

National Wetlands Working Group (1997). *The Canadian wetland classification system, 2nd edition*. University of Waterloo, Canada.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M. & Högberg, P. (1998). Boreal forest plants take up organic nitrogen. *Nature*, 392, 914-916.

Negi, G.C.S. & Singh, S.P. (1993). Leaf nitrogen dynamics with particular reference to retranslocation in evergreen and deciduous tree species of Kumaun Himalaya. *Canadian Journal of Forest Research*, 23, 349-357.

Nordell, K.O. & Karlsson, P.S. (1995). Resorption of nitrogen and dry matter prior to leaf abscission: variation among individuals, sites and years in the mountain birch. *Functional Ecology*, 9, 326-333.

Nordin, A., Strengbom, J., Witzell, J., Näsholm, T. & Ericson, L. (2005). Nitrogen deposition and the biodiversity of boreal forests: implications for the nitrogen critical load. *Ambio*, 34, 20-24.

Olde Venterink, H., Wassen, M.J., Verkroost, A.W.M. & de Ruiter, P.C. (2003). Species richness-productivity patterns differ between N-, P-, and K-limited wetlands. *Ecology*, 84, 2191-2199.

Pakarinen, P. & Gorham, E. (1983). Mineral element composition of *Sphagnum fuscum* peats collected from Minnesota, Manitoba and Ontario. In: *Proceedings of the international peat symposium* (ed. Spigarelli, S). Bemidji State University Bemidji, pp. 471-479.

Pakarinen, P. & Tolonen, K. (1977). Nutrient contents of *Sphagnum* mosses in relation to bog water chemistry in northern Finland. *Lindbergia*, 4, 27-33.

Paré, D., Titus, B.D., Lafleur, B., Maynard, D.G. & Thiffault, E. (2012). *Canadian tree species nutrient database*. Available at: https://apps-scf-cfs.rncan.gc.ca/calc/bundles/biomasse/files/data/Canadian%20Tree%20Species%20Nutrient%20Database.zip Last accessed January 25 2013.

Parkinson, J.A. & Allen, S.E. (1975). Wet oxidation procedure suitable for determination of nitrogen and mineral nutrients in biological material. *Communications in Soil Science and Plant Analysis*, 6, 1-11.

Peri, P.L. & Lasagno, R.G. (2010). Biomass, carbon and nutrient storage for dominant grasses of cold temperate steppe grasslands in southern Patagonia, Argentina. *Journal of Arid Environments*, 74, 23-34.

Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J. & Kato, S. (2010). To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos*, 119, 741-751.

Pugnaire, F.I. & Chapin, F.S. (1993). Controls over nutrient resorption from leaves of evergreen Mediterranean species. *Ecology*, 74, 124-129.

Ralhan, P.K. & Singh, S.P. (1987). Dynamics of nutrients and leaf mass in central Himalayan forest trees and shrubs. *Ecology*, 68, 1974-1983.

Ramsar (1987). *Convention on wetlands of international importance especially as waterfowl habitat*. Ramsar (Iran), 2 February 1971. UN treaty series No. 14583. As amended by the Paris Protocol, 3 December 1982, and Regina Amendments, 28 May 1987.

Reader, R.J. (1980). Effects of nitrogen fertilizer, shade, and the removal of new growth on longevity of overwintering bog ericad leaves. *Canadian Journal of Botany*, 58, 1737-1743.

Redfield, A.C. (1958). The biological control of chemical factors in the environment. *American Scientist*, 46, 205-221.

Reed, S.C., Townsend, A.R., Davidson, E.A. & Cleveland, C.C. (2012). Stoichiometric patterns in foliar nutrient resorption across multiple scales. *New Phytologist*, 196, 173-180.

Rejmánková, E. (2005). Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status. *New Phytologist*, 167, 471-482.

Rhee, G.Y. (1978). Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnology and Oceanography*, 23, 10-25.

Richardson, C.J. & Marshall, P.E. (1986). Processes controlling movement, storage, and export of phosphorus in a fen peatland. *Ecological Monographs*, 56, 279-302.

Robert, B., Caritat, A., Bertoni, G., Vilar, L. & Molinas, M. (1996). Nutrient content and seasonal fluctuations in the leaf component of coark-oak (*Quercus suber* L.) litterfall. *Vegetatio*, 122, 29-35.

Roulet, N.T., Lafleur, P.M., Richard, P.J.H., Moore, T.R., Humphreys, E.R. & Bubier, J. (2007). Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Global Change Biology*, 13, 397-411.

Rozbrojová, Z. & Hájek, M. (2010). Can tissue element concentration patterns at the individual species level indicate the factors underlying vegetation gradients in wetlands? *Journal of Vegetation Science*, 21, 355-363.

Rustad, L.E., Campbell, J.L., Marion, G.M., Norby, R.J., Mitchell, M.J., Hartley, A.E. *et al*. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126, 543-562.

Rydin, H. & Clymo, R.S. (1989). Transport of carbon and phosphorus compounds about *Sphagnum*. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 237, 63-84.

Rydin, H. & Jeglum, J.K. (2006). *The biology of peatlands*. Oxford University Press, New York, USA.

Ryser, P. & Lambers, H. (1995). Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil*, 170, 251-265.

Sardans, J., Rivas-Ubach, A. & Peñuelas, J. (2012). The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. *Biogeochemistry*, 111, 1-39.

Schindler, D.W. (1977). Evolution of phosphorus limitation in lakes. *Science*, 195, 260-262.

Schlesinger, W.H. & Bernhardt, E.S. (2013). *Biogeochemistry: an analysis of global change*. Academic Press, Waltham, MA, USA.

Shaver, G.R. & Melillo, J.M. (1984). Nutrient budgets of marsh plants: efficiency concepts and relation to availability. *Ecology*, 65, 1491-1510.

Shipley, B., Vile, D., Garnier, E., Wright, I.J. & Poorter, H. (2005). Functional linkages between leaf traits and net photosynthetic rate: reconciling empirical and mechanistic models. *Functional Ecology*, 19, 602-615.

Small, E. (1972). Photosynthetic rates in relation to nitrogen recycling as an adaptation to nutrient deficiency in peat bog plants. *Canadian Journal of Botany*, 50, 2227-2233.

Smil, V. (2000). Phosphorus in the environment: natural flows and human interferences. *Annual Review of Energy and the Environment*, 25, 53-88.

Smith, S.E. & Read, D.J. (2008). *Mycorrhizal symbiosis*. 3rd edn. Academic Press, New York, NY.

Sterner, R.W. & Elser, J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, New Jersey, USA.

Sterner, R.W., Elser, J.J. & Hessen, D.O. (1992). Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry*, 17, 49-67.

Straker, C.J. (1996). Ericoid mycorrhiza: ecological and host specificity. *Mycorrhiza*, 6, 215-225.

Tarnocai, C. (2006). The effect of climate change on carbon in Canadian peatlands. *Global Planet Change*, 53, 222-232.

Tarnocai, C., Kettles, I.M. & Lacelle, B. (2002). *Peatlands of Canada database*. Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada.

Thormann, M.N. & Bayley, S.E. (1997). Aboveground plant production and nutrient content of the vegetation in six peatlands in Alberta, Canada. *Plant Ecology*, 131, 1-16.

Tilman, D. (1996). Biodiversity: population versus ecosystem stability. *Ecology*, 77, 350-363.

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 Phytologist*, 196, 49-67.

Turner, B.L., Baxter, R., Mahieu, N., Sjögersten, S. & Whitton, B.A. (2004). Phosphorus compounds in subarctic Fennoscandian soils at the mountain birch (*Betula pubescens*)–tundra ecotone. *Soil Biology & Biochemistry*, 36, 815-823.

Turunen, J., Roulet, N.T., Moore, T.R. & Richard, P.J.H. (2004). Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in eastern Canada. *Global Biogeochemical Cycles*, 18, GB3002.

Tyrrell, T. (1999). The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature*, 400, 525-531.

van Breemen, N. (1995). How *Sphagnum* bogs down other plants. *Trends in Ecology & Evolution*, 10, 270-275.

van den Driessche, R. & Rieche, K. (1974). Prediction of mineral nutrient status of trees by foliar analysis. *Botanical Review*, 40, 347-394.

van Heerwaarden, L.M., Toet, S. & Aerts, R. (2003a). Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. *Oikos*, 101, 664-669.

van Heerwaarden, L.M., Toet, S. & Aerts, R. (2003b). Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *Journal of Ecology*, 91, 1060-1070.

Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. & Jackson, R.B. (2012). Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecological Monographs*, 82, 205-220.

Verhoeven, J.T.A., Koerselman, W. & Meuleman, A.F.M. (1996). Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology & Evolution*, 11, 494-497.

Vernescu, C., Coulas, J. & Ryser, P. (2005). Leaf mass loss in wetland graminoids during senescence. *Oikos*, 109, 187-195.

Vicars, W.C., Sickman, J.O. & Ziemann, P.J. (2010). Atmospheric phosphorus deposition at a montane site: size distribution, effects of wildfire, and ecological implications. *Atmospheric Environment*, 44, 2813-2821.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W. *et al.* (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, 7, 737-750.

Vitousek, P.M. & Howarth, R.W. (1991). Nitrogen limitation on land and in the sea - How can it occur? *Biogeochemistry*, 13, 87-115.

Vitousek, P.M. (1998). Foliar and litter nutrients, nutrient resorption, and decomposition in Hawaiian *Metrosideros polymorpha*. *Ecosystems*, 1, 401-407.

Vitt, D.H. (2006). Functional characteristics and indicators of boreal peatlands. In: *Boreal peatland ecosystems* (eds. Wieder, RK & Vitt, DH). Springer Heidelberg, Germany, pp. 9-24.

von Oheimb, G., Power, S., Falk, K., Friedrich, U., Mohamed, A., Krug, A. *et al*. (2010). N:P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands. *Ecosystems*, 13, 317-327.

Wakefield, A.E., Gotelli, N.J., Wittman, S.E. & Ellison, A.M. (2005). Prey addition alters nutrient stoichiometry of the carnivorous plant *Sarracenia purpurea*. *Ecology*, 86, 2553-2553.

Walbridge, M.R. (1991). Phosphorus availability in acid organic soils of the lower North Carolina coastal plain. *Ecology*, 72, 2083-2100.

Walbridge, M.R. & Navaratnnam, J.A. (2006). Phosphorus in boreal peatlands. In: *Boreal peatland ecosystems* (eds. Wieder, RK & Vitt, DH). Springer Heidelberg, Germany, pp. 231-258.

Walker, T.W. & Syers, J.K. (1976). Fate of phosphorus during pedogenesis. *Geoderma*, 15, 1-19.

Wang, M. & Moore, T.R. (2014). Carbon, nitrogen, phosphorus and potassium stoichiometry in an ombrotrophic peatland reflects plant functional type. *Ecosystems*, in press (accepted).

Wang, M., Talbot, J., Moore, T.R. & Richard, P.J.H. (2014a). The cascade of C:N:P stoichiometry in an ombrotrophic peatland: from plants to peat. *Environmental Research Letters*, in press (accepted).

Wang, M., Murphy, M. & Moore, T.R. (2014b). Nutrient resorption of two evergreen shrubs in response to long-term fertilization in a bog. *Oecologia*, 174, 365-377.

Winsborough, C. & Basiliko, N. (2010). Fungal and bacterial activity in northern peatlands. *Geomicrobiology Journal*, 27, 315-320.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Garnier, E., Hikosaka, K. *et al.* (2005). Assessing the generality of global leaf trait relationships. *New Phytologist*, 166, 485-496.

Wright, I.J. & Westoby, M. (2003). Nutrient concentration, resorption and lifespan: leaf traits of Australian sclerophyll species. *Functional Ecology*, 17, 10-19.

Yu, Q., Chen, Q.S., Elser, J.J., He, N.P., Wu, H.H., Zhang, G.M. *et al*. (2010a). Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability. *Ecology Letters*, 13, 1390-1399.

Yu, Q., Elser, J., He, N., Wu, H., Chen, Q., Zhang, G. *et al*. (2011). Stoichiometric homeostasis of vascular plants in the Inner Mongolia grassland. *Oecologia*, 166, 1-10.

Yu, Z., Loisel, J., Brosseau, D.P., Beilman, D.W. & Hunt, S.J. (2010b). Global peatland dynamics since the Last Glacial Maximum. *Geophysical Research Letters*, 37, L13402.

Yuan, Z.Y. & Chen, H.Y.H. (2009a). Global-scale patterns of nutrient resorption associated with latitude, temperature and precipitation. *Global Ecology and Biogeography*, 18, 11-18.

Yuan, Z.Y. & Chen, H.Y.H. (2009b). Global trends in senesced-leaf nitrogen and phosphorus. *Global Ecology and Biogeography*, 18, 532-542.

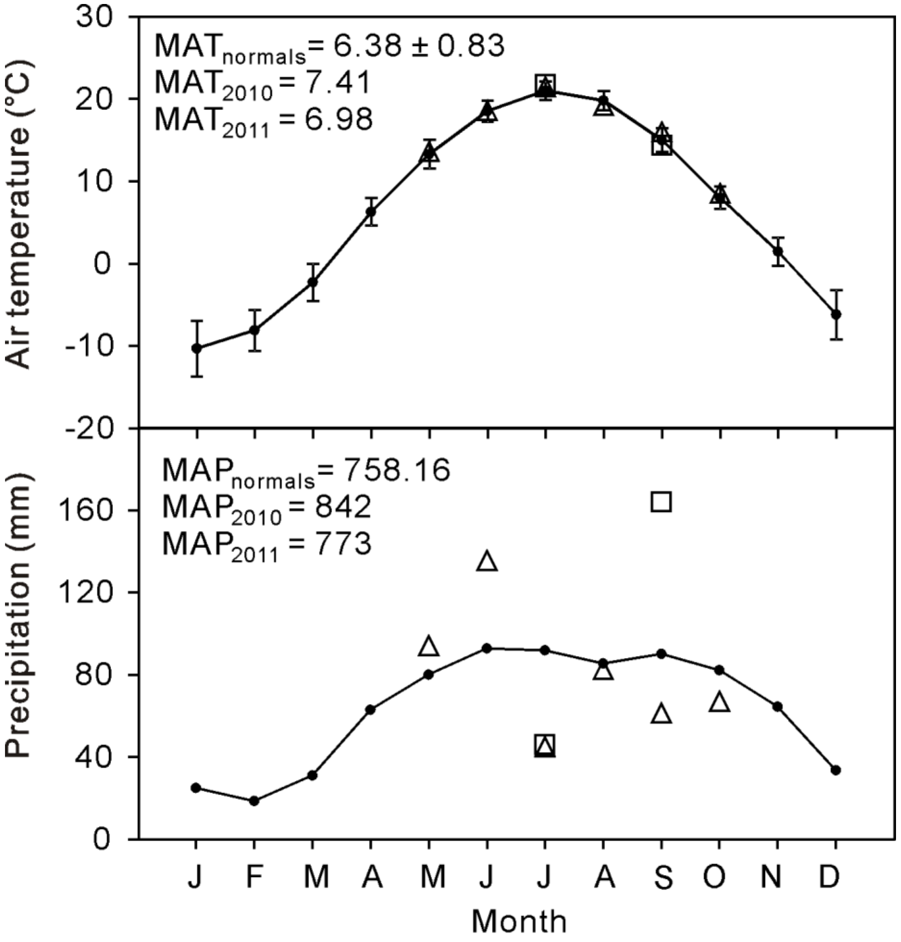
Yuan, Z.Y., Li, L.H., Han, X.G., Huang, J.H., Jiang, G.M., Wan, S.Q. *et al*. (2005). Nitrogen resorption from senescing leaves in 28 plant species in a semi-arid region of northern China. *Journal of Arid Environments*, 63, 191-202.

Zar, J.H. (1999). *Biostatistical analysis, 4th edition*. Prentice Hall, Upper Saddle River, New Jersey, US.

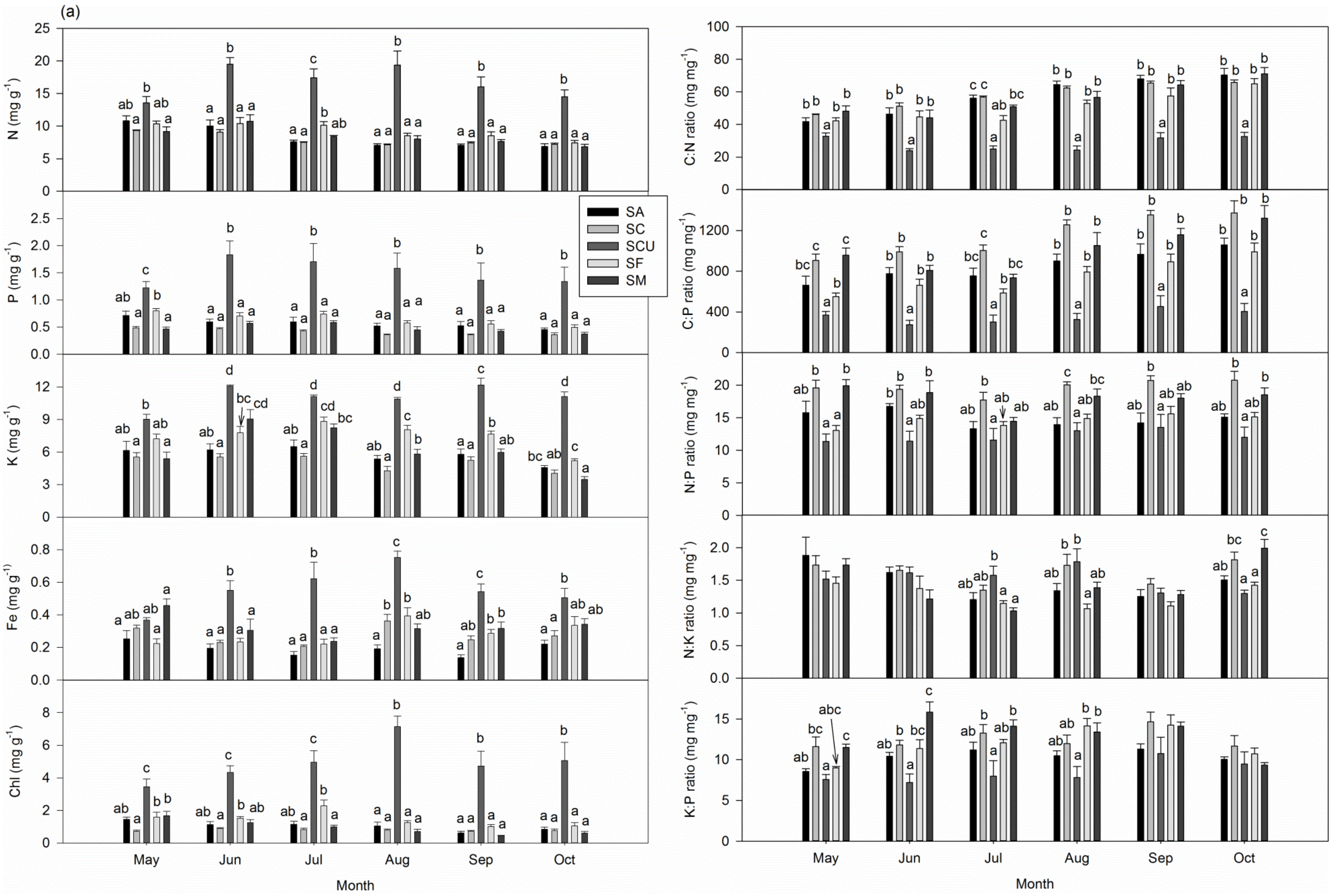
Zauke, G.-P., Bohlke, J., Żytkowicz, R., Napiórkowski, P. & Giziński, A. (1998). Trace metals in tripton, zooplankton, zoobenthos, reeds and sediments of selected lakes in north-central Poland. *International Review of Hydrobiology*, 83, 501-526.

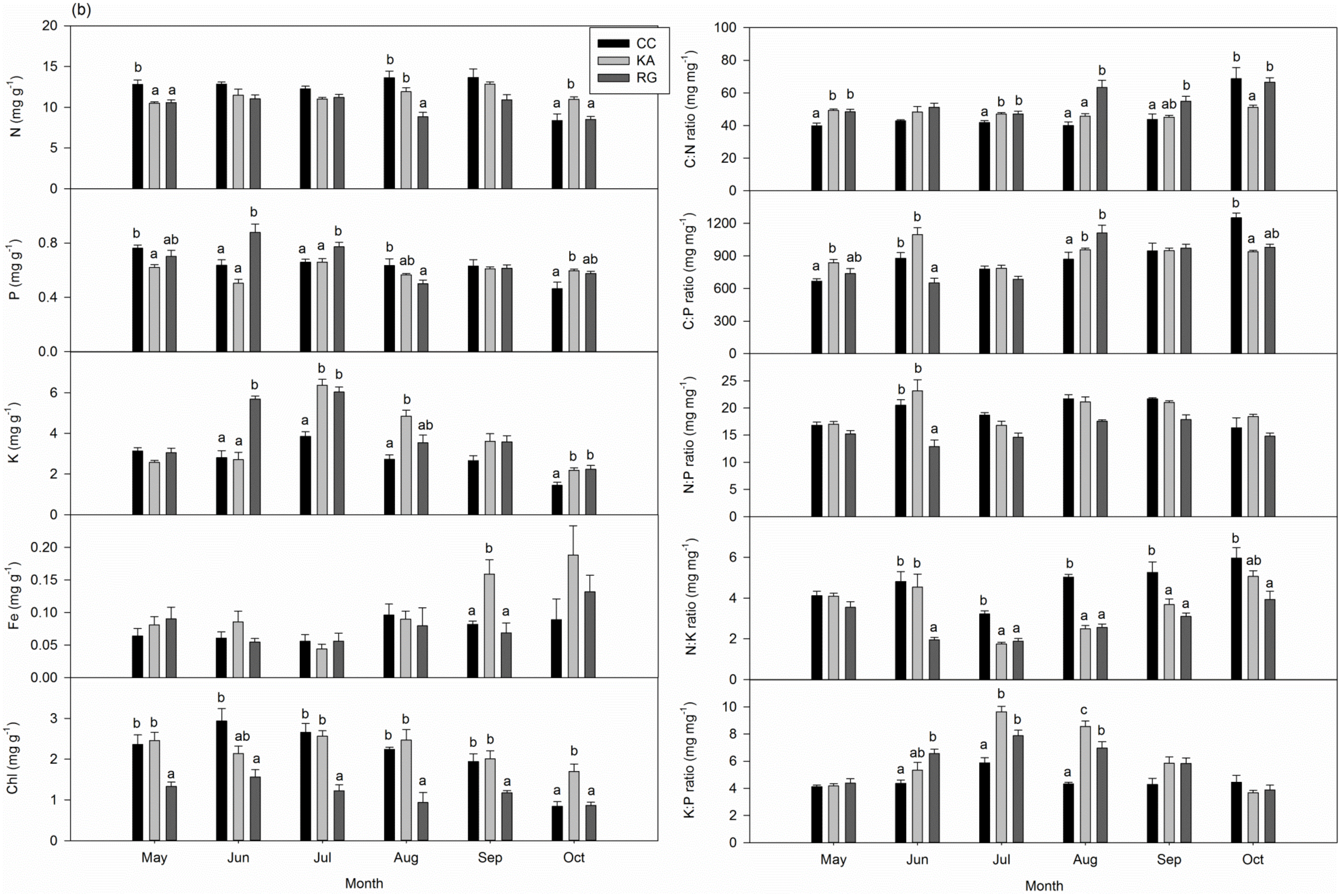
Appendices

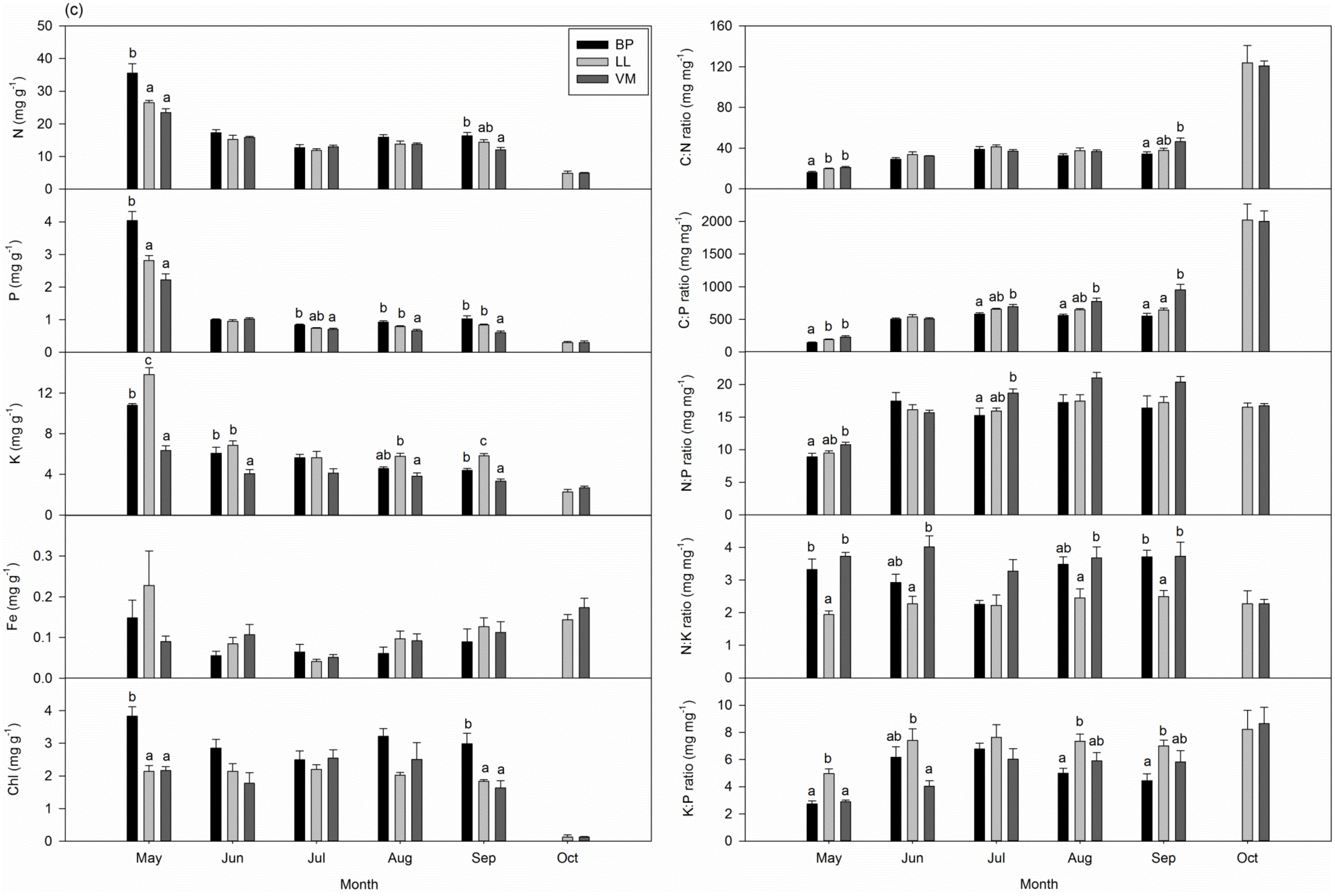
Appendix 3.1 The monthly average air temperature and precipitation at MacDonald-Cartier International Airport (45.32°N, 75.67°W) and Mer Bleue (45.41°N, 75.52°W). Filled circles with lines represent the averages over 30 years at MacDonald-Cartier International Airport (Canadian Climate Normals 1981-2010). Open squares and triangles represent 2010 and 2011 respectively at Mer Bleue (E. Humphreys personal communication). Mean annual temperature (MAT) and precipitation (MAP) (rain fall only). Mean ± standard deviation.

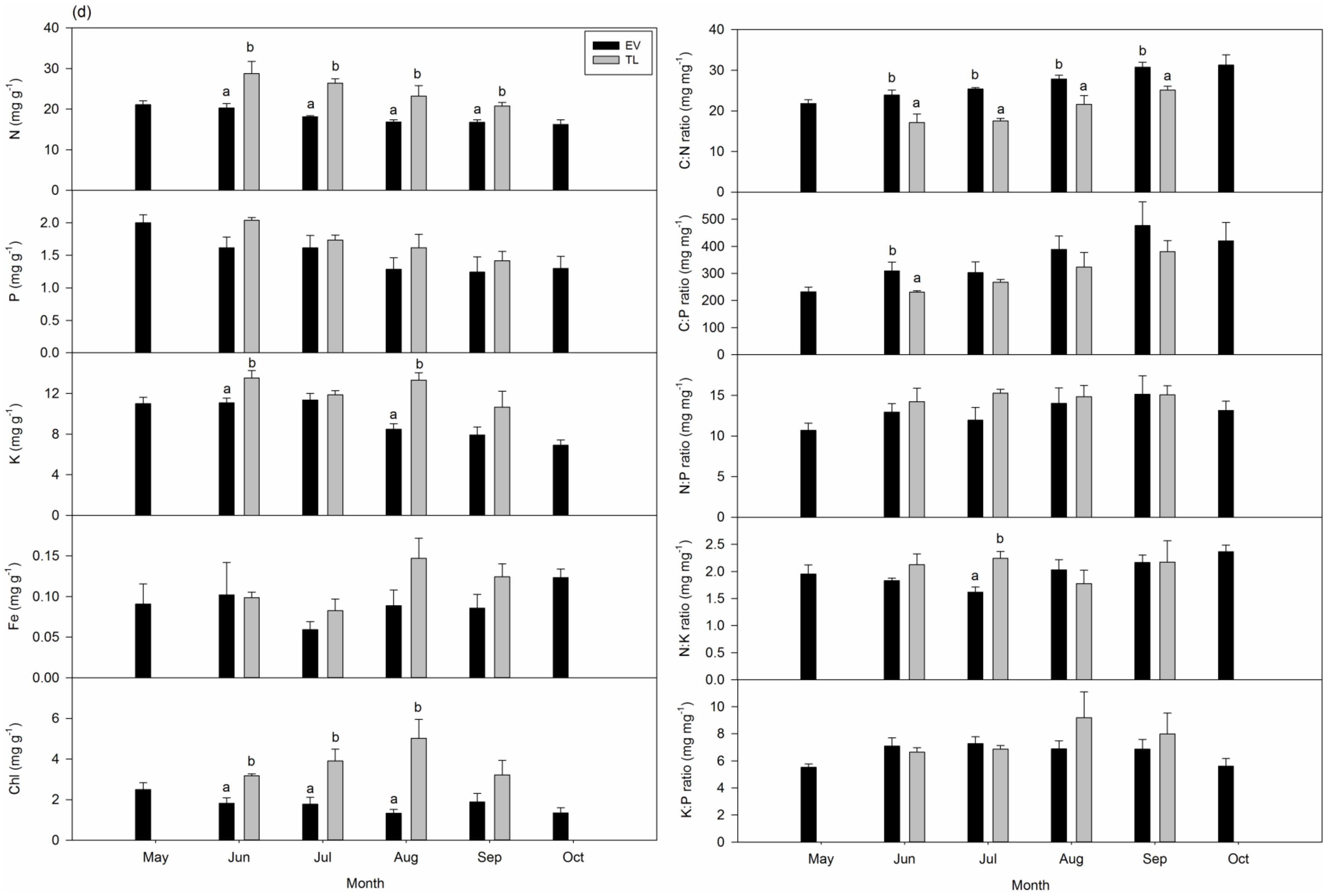


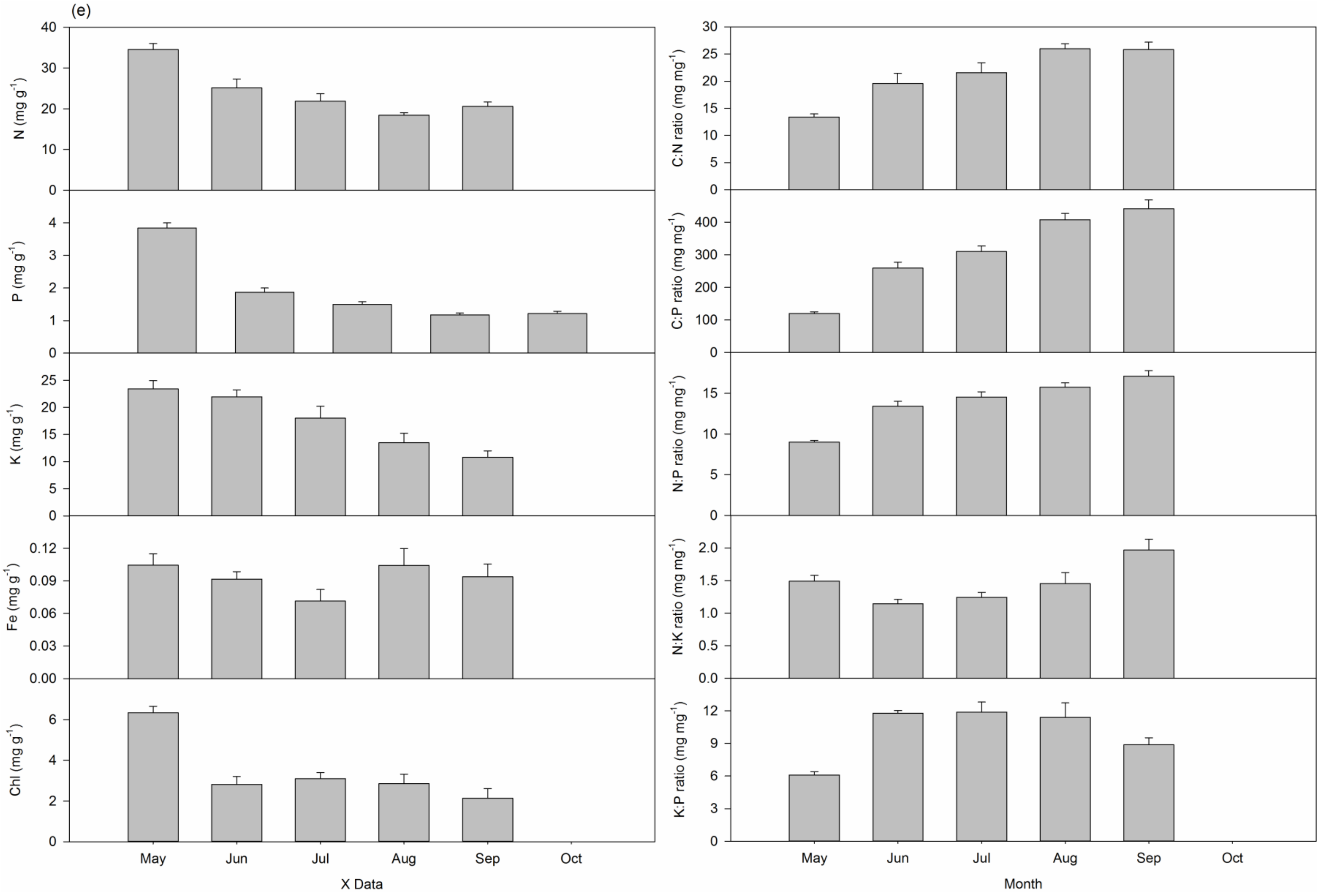
Appendix 3.2 The seasonal course of C, N, P, K, Fe and chlorophyll concentration, and C:N, C:P, N:P, N:K and K:P ratios (mean ± 1 SE) in moss (a), evergreen (b), deciduous (c), graminoid (d) and forb (e) types. Species abbreviations as shown in Figure 3.1.


(a)
N (mg g-1)
0
5
10
15
20
25
P (mg g-1)
0.0
0.5
1.0
1.5
2.0
2.5
K (mg g-1)
0
3
6
9
12
Fe (mg g-1)
0.0
0.2
0.4
0.6
0.8
Chl (mg g-1)
0
2
4
6
8
SA
SC
SCU
SF
SM
C:N ratio (mg mg-1)
0
20
40
60
80
100
C:P ratio (mg mg-1)
0
400
800
1200
N:P ratio (mg mg-1)
0
5
10
15
20
N:K ratio (mg mg-1)
0.0
0.5
1.0
1.5
2.0
K:P ratio (mg mg-1)
0
5
10
15
May
Jun
Jul
Aug
Sep
Oct
Month


(b)
CC
KA
RG
N (mg g^{-1})
P (mg g^{-1})
K (mg g^{-1})
Fe (mg g^{-1})
Chl (mg g^{-1})
C:N ratio (mg mg^{-1})
C:P ratio (mg mg^{-1})
N:P ratio (mg mg^{-1})
N:K ratio (mg mg^{-1})
K:P ratio (mg mg^{-1})
May
Jun
Jul
Aug
Sep
Oct
Month


(c)
BP
LL
VM
N (mg g-1)
P (mg g-1)
K (mg g-1)
Fe (mg g-1)
Chl (mg g-1)
C:N ratio (mg mg-1)
C:P ratio (mg mg-1)
N:P ratio (mg mg-1)
N:K ratio (mg mg-1)
K:P ratio (mg mg-1)
0
10
20
30
40
50
1
2
3
4
8
12
0.0
0.1
0.2
0.3
40
80
120
160
500
1000
1500
2000
5
15
20
6
10
May
Jun
Jul
Aug
Sep
Oct
Month
a
b
c
ab


(d)
EV
TL
N (mg g^{-1})
0
10
20
30
40
P (mg g^{-1})
0.0
0.5
1.0
1.5
2.0
K (mg g^{-1})
0
4
8
12
Fe (mg g^{-1})
0.00
0.05
0.10
0.15
Chl (mg g^{-1})
0
2
4
6
C:N ratio (mg mg^{-1})
0
10
20
30
40
C:P ratio (mg mg^{-1})
0
100
200
300
400
500
N:P ratio (mg mg^{-1})
0
5
10
15
N:K ratio (mg mg^{-1})
0.0
0.5
1.0
1.5
2.0
2.5
K:P ratio (mg mg^{-1})
0
2
4
6
8
10
a
b
May
Jun
Jul
Aug
Sep
Oct
Month



(e)
N (mg g^{-1})
P (mg g^{-1})
K (mg g^{-1})
Fe (mg g^{-1})
Chl (mg g^{-1})
C:N ratio (mg mg^{-1})
C:P ratio (mg mg^{-1})
N:P ratio (mg mg^{-1})
N:K ratio (mg mg^{-1})
K:P ratio (mg mg^{-1})
May
Jun
Jul
Aug
Sep
Oct
X Data
Month

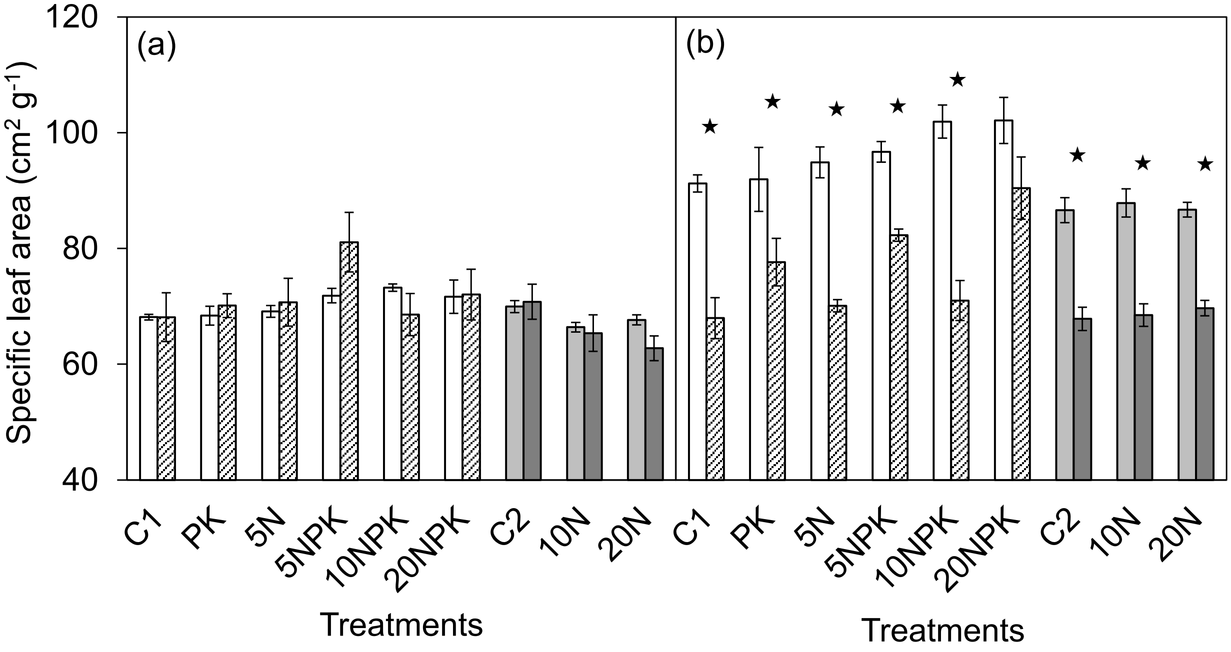
Appendix 3.3 Coefficients of variation (%) among species within each PFT for C, N, P, K, Fe and chlorophyll (Chl) concentration and C:N, C:P, N:P, N:K and K:P ratios. Different letters indicate significant difference in mean coefficient of variation of all nutrient concentrations and stoichiometric ratios among PFTs at $P = 0.05$. See *Materials and Methods* for details of calculations.

Coefficients of variation (%)	C	N	P	K	Fe	Chl	C:N	C:P	N:P	N:K	K:P	Mean	SE
mosses	0.2	30.1	23.7	27.0	11.3	50.8	31.3	49.6	18.6	4.5	17.9	25.0^{a}	4.6
evergreen species	0.5	11.8	3.2	6.7	1.4	25.7	13.1	7.6	10.4	15.5	12.0	10.1^{b}	2.0
deciduous species	1.3	23.8	14.4	19.8	1.2	25.7	24.4	30.2	6.7	14.5	15.2	16.5^{ab}	2.7
graminoids	1.1	14.7	4.4	15.2	2.1	17.0	14.9	8.6	7.6	2.0	7.7	9.0^{b}	1.7

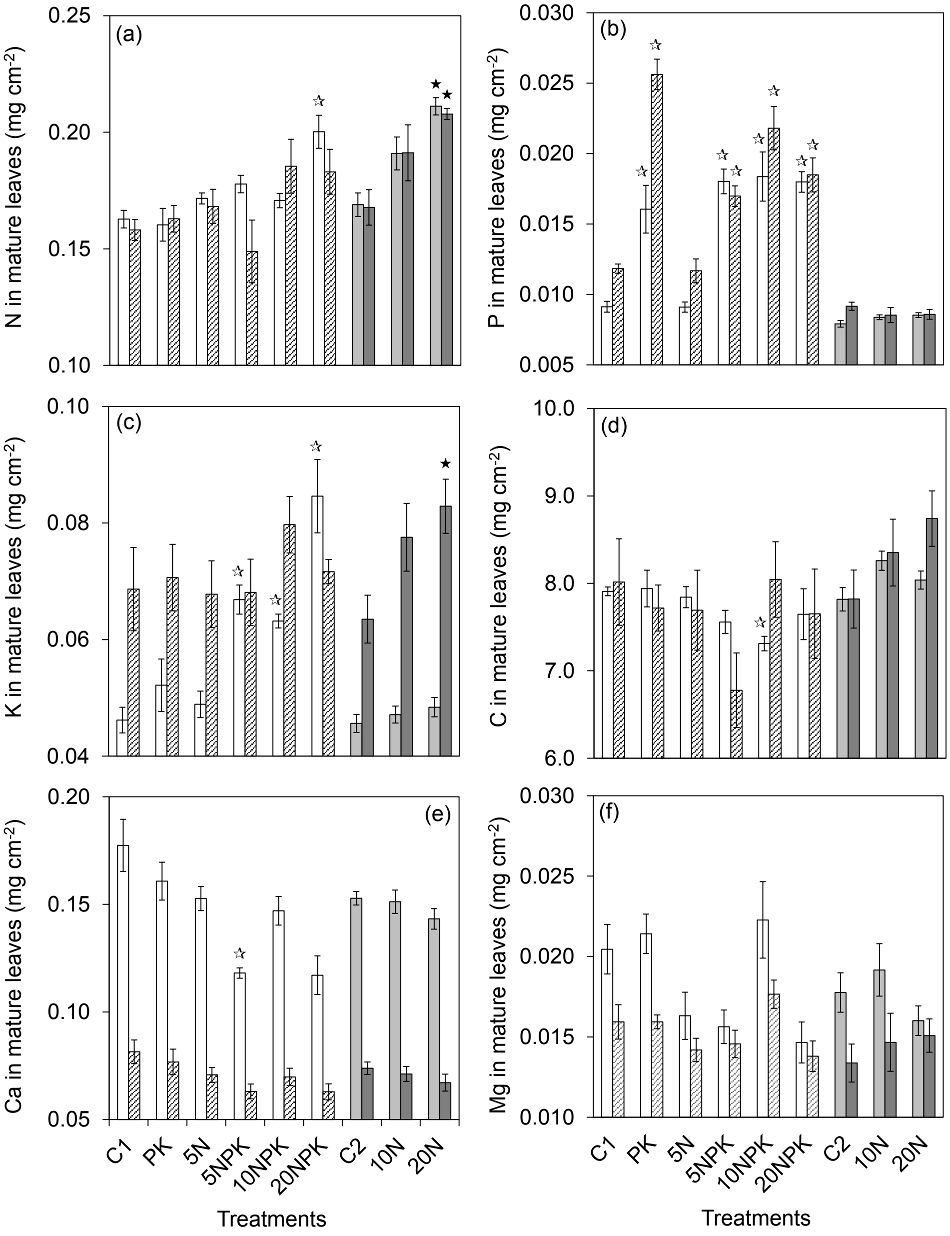
Appendix 3.4 N (ammonium and nitrate), P (orthophosphate) and K supply rates (mg m^{-2} $month^{-1}$) in peat soil at 10 cm depth as measured by PRS^{TM} ion-exchange resin membrane for a 30-day time period. Values are mean with standard error ($n = 3$).

	N	P	K
May	4.79 ± 0.92	1.41 ± 0.38	48.43 ± 15.01
July	9.43 ± 0.11	1.49 ± 0.13	68.31 ± 4.87
September	12.08 ± 1.07	1.51 ± 0.41	85.98 ± 31.04

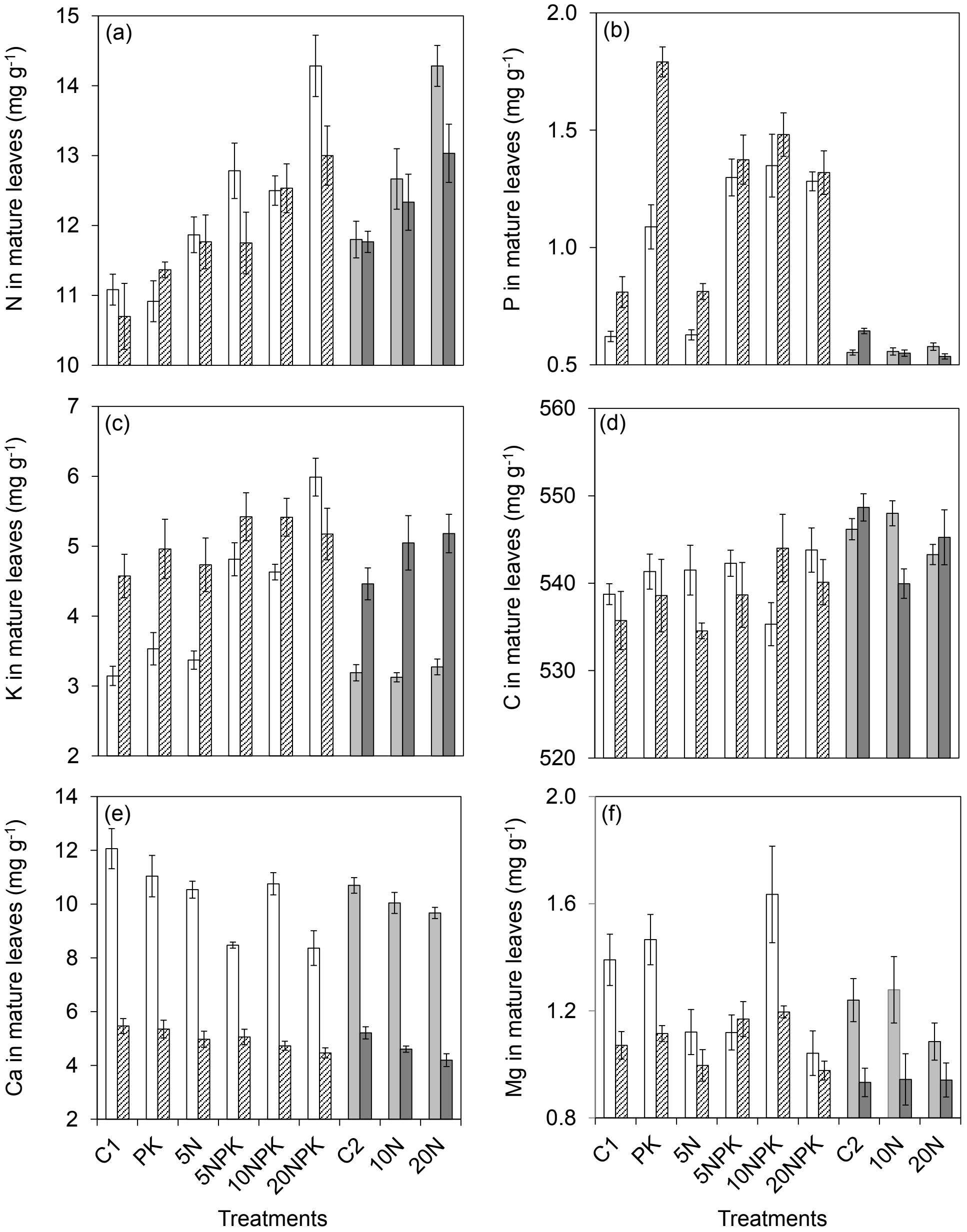
Appendix 4.1 Specific leaf area in *C. calyculata* (left bars) and *R. groenlandicum* (right bars) in mature (a) and senesced leaves (b) in response to long-term N, P and K fertilization. Open and pattern filled bars are treatments in experiment 1, and light and dark grey filled bars are treatments in experiment 2. The stars indicate significant difference between two species ($P < 0.05$). Treatment abbreviations as described in Table 4.1.



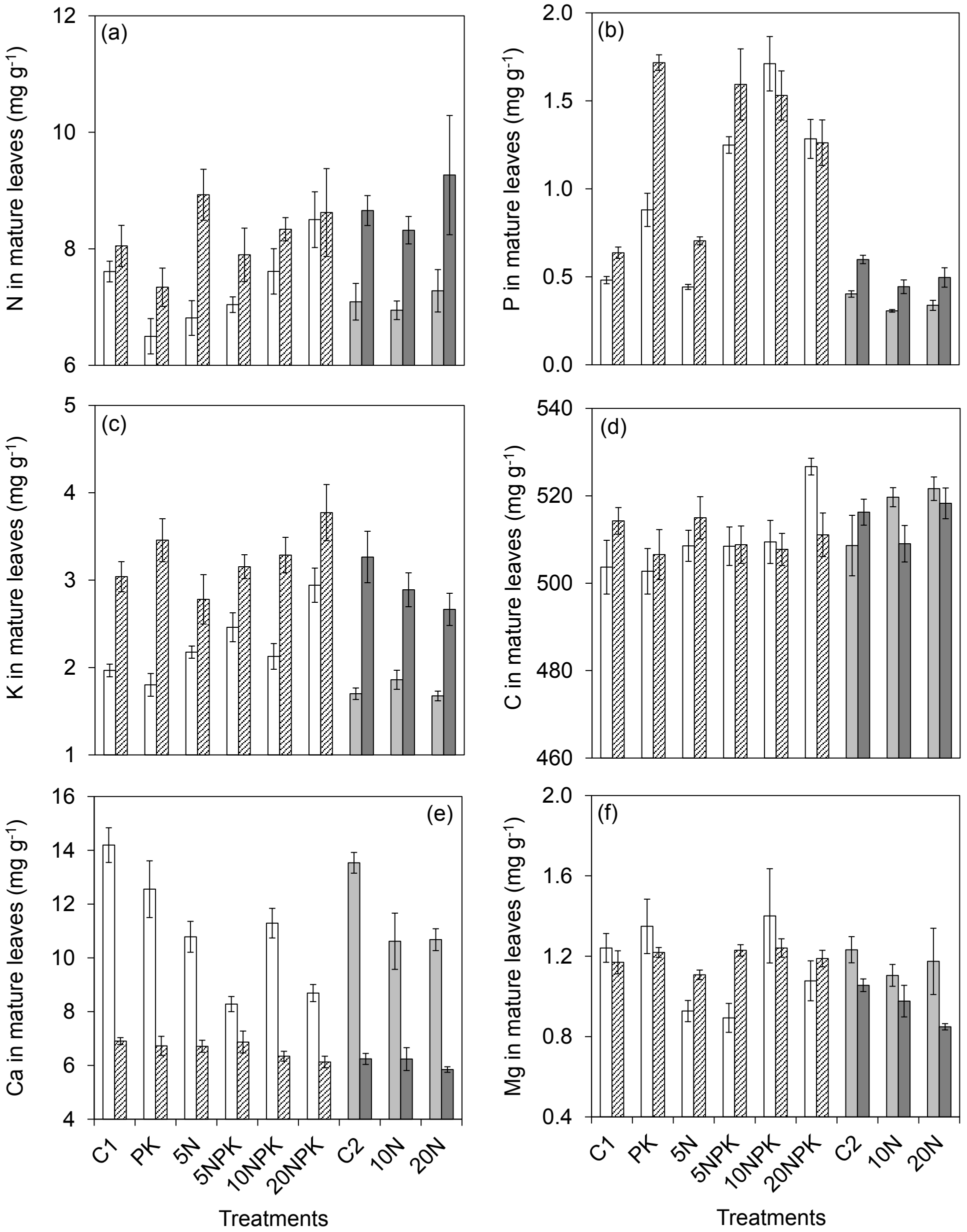
Appendix 4.2 N, P, K, C, Ca and Mg concentration on an area basis (mg cm^{-2}) (a-f) in mature leaves of *C. calyculata* (left bars) and *R. groenlandicum* (right bars) in response to long-term N, P and K fertilization. Values are mean ± standard error (n = 6). Open and pattern filled bars are treatments in experiment 1, and light and dark grey filled bars are treatments in experiment 2. Open and filled stars indicate significant difference between individual treatments and the controls in experiment 1 and 2, respectively (P < 0.05). Treatment abbreviations as described in Table 4.1.



Appendix 4.3 N, P, K, C, Ca and Mg concentration on a mass basis (mg g^{-1}) (a-f) in mature leaves of *C. calyculata* (left bars) and *R. groenlandicum* (right bars) in response to long-term N, P and K fertilization. Values are mean ± standard error ($n = 6$). Open and pattern filled bars are treatments in experiment 1, and light and dark grey filled are treatments in experiment 2. Treatment abbreviations as described in Table 4.1.



Appendix 4.4 N, P, K, C, Ca and Mg concentration on a mass basis (mg g^{-1}) (a-f) in senesced leaves of *C. calyculata* (left bars) and *R. groenlandicum* (right bars) in response to long-term N, P and K fertilization. Values are mean ± standard error (n = 6). Open and pattern filled bars are treatments in experiment 1, and light and dark grey filled bars are treatments in experiment 2. Treatment abbreviations as described in Table 4.1.



Appendix 5.1 N, P, K, Ca and Mg supply rates (mg m^{-2} $month^{-1}$, mean ± SE) measured by PRS^{TM} probes in response to long-term fertilization.

Treatments	N	P	K	Ca	Mg
C1	9.4 ± 0.1	1.5 ± 0.1	68.3 ± 4.9	108.3 ± 14.6	26.2 ± 2.4
PK	8.7 ± 2.2	54.8 ± 6.1	412.7 ± 59.7	186.6 ± 25.2	37.1 ± 6.3
5N	11.9 ± 2.9	2.7 ± 0.7	63.9 ± 11.3	117.5 ± 20.8	27.4 ± 5.7
5NPK	7.5 ± 1.0	51.7 ± 21.3	362.0 ± 101.9	130.8 ± 12.5	28.9 ± 4.1
10NPK	24.1 ± 6.0	44.1 ± 4.0	253.8 ± 21.3	100.9 ± 4.1	27.2 ± 2.5
20NPK	38.8 ± 10.5	36.4 ± 11.4	132.8 ± 26.9	118.6 ± 28.2	47.3 ± 19.5
C2	8.8 ± 1.1	0.8 ± 0.2	112.7 ± 25.8	148.1 ± 28.2	31.8 ± 7.7
10N	21.8 ± 1.3	1.1 ± 0.1	114.8 ± 10.1	119.9 ± 11.0	29.2 ± 4.6
20N	45.8 ± 10.0	1.2 ± 0.1	90.7 ± 13.3	114.0 ± 9.2	25.3 ± 0.8