PHOTOSYNTHETIC PERFORMANCE OF *CHAMAEDAPHNE CALYCULATA* AFTER TWELVE YEARS OF NUTRIENT FERTILIZATION AT MER BLEUE BOG, ONTARIO, CANADA

By

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A Paper Presented to the Faculty of Mount Holyoke College

in Partial Fulfillment of the Requirements for

the Degree of Bachelors of Arts with Honor

Department of Environmental Studies

South Hadley, MA

April 2013

This paper was prepared under the direction of Professor Jill Bubier
for eight credits

ACKNOWLEDGEMENT

I would like to extend my greatest thanks to my thesis advisor, Professor Jill Bubier, who not only introduced me to the exciting world of environmental science and peatland research, offered me the opportunity to delve into the topic I am interested in, but also supported and guided me along the way, making this thesis possible. It is her encouragement and inspiration that awakened my scientific curiosity, motivated me through challenges, and empowered my future scientific endeavors. Doctor Tuula Larmola has been the most patient and supportive mentor, who struggled with me through every challenge in two field seasons, instructed every leaf I measured, and made detailed comments on every draft I wrote. She taught me from the most basic steps of using a new piece of equipment to the importance of independent and critical thinking in science with continued challenges and encouragement.

I would also like to extend my gratitude to Meng Wang and Tim Moore at McGill University, who analyzed the chlorophyll content data for my 90 leaves, and Cori Magnuson for all the help over the field season in fertilization and leaf measurements. Elyn Humphreys at Carleton University has been a savior during the Li-Cor equipment failure, as well as in equipment calibration. Meaghan Murphy at McGill University not only was of great help in the field, but her knowledge and dedication to science also represents the scientist I would like to become.

This thesis would not have been possible without the statistical assistance from Professor Janice Gifford, who has been not only a patient and supportive guide but also an inspiration for my interest in statistics. My peer reviewers, Paula Mugnani and Emily Eshleman, offered not only helpful feedback but also valuable companionship along the whole process.

My family has taught me the power of determination and dedication, shown unwavering faith in me, and offered the strongest support. All my friends at Mount Holyoke, in Ottawa, and all over the world are the solid anchor that I can always rely on, who have always been there for me in my toughest times.

Last but not least, this project would not have been possible without the funding from the National Science Foundation Grant to Professor Jill Bubier (DEB 0346625), as well as the Howard Hughes Medical Institute Research Program for my summer research. I also appreciate the National Capital

Commission of Canada for granting us access to the Mer Bleue Conservation Area. The Environmental Studies Department has also been supporting this project both financially and logistically.

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ABSTRACT

Peatlands are important ecosystems in the global carbon cycle, as they store 30% of the world's soil carbon. However, combustion of fossil fuels and fertilizer manufacture have increased reactive nitrogen in the form of nitrate and ammonium, which are limiting nutrients in many ecosystems including peatlands, and can cause cascading effects on these ecosystems. The fertilization experiment at Mer Bleue Bog in Ontario was set up in 2000 to investigate the impact of nutrient addition, including nitrogen (N), phosphorus (P) and potassium (K), on the carbon (C) sink function of this peatland.

This study examined the effect of nutrient addition on the dominant ericaceous shrub at the bog, leatherleaf (*Chamaedaphne calyculata*), after twelve years of exposure to five and twenty times the growing season ambient wet N deposition rates with and without P and K. Leaf-level CO_2 gas exchange and chlorophyll fluorescence, an indicator of plant stress in terms of light harvesting capacity, of both current-year and previous-year leaves were measured using a leaf chamber and Licor 6400 infrared gas analyzer to investigate the photosynthetic performance of this shrub. Additionally, from each leaf, maximum electron transport rate (Jmax) and Rubisco carboxylation rate (Vcmax) were derived from the CO_2 response curve, and chlorophyll content analyzed.

After twelve years of fertilization, nutrients were still being invested in chlorophyll and Rubisco, which are the main sinks of nutrients, especially N. Chlorophyll content also reflected differences between old and new leaves due to nutrient transport and light availability. Chlorophyll fluorescence ratios of all measured leaves were within the range of healthy leaves (0.75 to 0.83); therefore, there is no sign of plant stress in terms of maximum light harvesting capacity. New leaves had a significantly lower light harvesting capacity than old leaves due to physiological immaturity. Investment of nutrients both in light and dark reactions of photosynthesis did not translate into higher maximum gross photosynthetic rate (Pmax); in fact, net assimilation rates (Amax) were lowest in the highest nutrient treatments. The decreasing trend in Amax with increased nutrients was due to increased dark respiration along the treatment gradient, and this trend mirrored the response pattern of net ecosystem CO_2 exchange, ecosystem respiration and gross ecosystem photosynthesis to nutrient addition. Thus, the shrub, in response to fertilization, contributes to a weaker C sink in the bog through increased respiration and unchanged photosynthesis.

x

INTRODUCTION

Nitrogen cycle

Nitrogen (N), together with carbon (C), hydrogen (H), oxygen (O), sulfur (S) and phosphorus (P), are vital elements for life. Two of the integral N-containing compounds are amino acids and nucleic acids, which are the building blocks of protein and DNA, two fundamental macromolecules of all living cells. Although crucial to all life forms, N is not always in the forms that are available for plant uptake and usage. Its critical role in the biogeochemistry ecosystems make N the limiting factor of primary production in most terrestrial and many marine ecosystems (Vitousek and Howarth 1991).

The atmosphere is the largest pool of N, with 3.9 Tg N (Tg = 10^{21} g) (78.08% of the atmosphere's volume) in the form of molecular N (N_2) (Schlesinger 1997). This is the most abundant, yet least reactive form of N due to the strong triple bond between two N molecules that requires high energy or specific bacteria to break. Therefore, N_2 is unavailable to most living organisms and often known as non-reactive N (Galloway et al. 2003). Much smaller amounts of N exist in terrestrial and marine ecosystems in organic life forms and soil organic matter. Inorganic N, existing predominantly as NH_4^+ and NO_3^- and in smaller amounts as NO_x and N_2O, constitutes a small but crucial role in the nitrogen cycle (Schlesinger 1997).

The nitrogen cycle represents the movement of N molecules among the atmosphere, land and sea. N in the gaseous phase is fixed into more reactive

forms either by lightning or N-fixing bacteria. Lightning provides high temperature and pressure, which breaks the strong triple bond in N_2 and allows it to combine with O_2. However, only a relatively small amount of N is fixed in this way. Biological nitrogen fixation, on the other hand, fixes up to 140 Tg N per year, and therefore is the main source of fixed N in terrestrial ecosystems (Schlesinger 1997). Once N_2 is converted into more reactive forms, an internal nitrogen cycle takes place when plants take up N in the soil (as NH_4^+, NO_3^- or organic N), assimilate it into organic compounds and return N into the soil when dead organic matter decomposes. A fair amount of N also leaches into groundwater, is washed into the oceans as runoff, or returns to the atmosphere through biological denitrification. In longer terms, in the absence of human activities, the amount of N_2 released back into the atmosphere through denitrification balances out the amount fixed biotically and abiotically, thereby closing the global N cycle (Schlesinger 1997).

Human activities have dramatic impacts on the global N cycle through various agricultural and industrial practices. Since before the industrial period, the selective cultivation of N-fixing crops (legumes) in farming has enhanced the amount of biologically fixed N and produced an additional 40 Tg N per year (Galloway et al. 1995). Additionally, fertilizer manufacture through the Haber-Bosch process, in which N_2 is burned with natural gas to produce NH_3, adds over 80 Tg N/year to agricultural systems (Galloway et al. 1995; Schlesinger 1997). The expanding world population demands more food production, therefore agricultural practices increase the amount of N added to agrosystems through both

N-fixing crops (30%) and fertilizers (70%) (Galloway et al. 2003). However, crops can only take up approximately half of the 120 Tg N added to agrosystems per year; the rest is lost through air or water pathways (Smil 1999). These N losses are a cause for concern, as the extra amounts of reactive N enter natural ecosystems through the air and water and alter their nutrient dynamics wherever they reach (Galloway et al. 2003).

Another anthropogenic source of reactive N is the combustion of fossil fuel. Hameed and Dignon (1989) estimated an average of 3.4 percent annual increase in N emission through fossil fuel combustion from 1860 to 1980. Burning fossil fuels releases about 20 Tg N in the form of NO_x gas (Schlesinger 1997). These gases do not persist long in the atmosphere, but instead are deposited through precipitation or dry deposition (as NH_4^+, NO_3^-) onto terrestrial and aquatic ecosystems, creating more reactive N that has major ecological impacts (Galloway et al. 2003).

Atmospheric N deposition and its impacts

The surplus amount of reactive N from human activities accounts for up to 60% of annual N deposited on the Earth's surface (Schlesinger 1997). This extra amount of reactive N cascades through different ecosystems, most of which are nitrogen-limited, and alters their nutrient dynamics in unexpected ways (Galloway et al. 2003). The volatility of reactive N allows it to transfer from one system to the next and causes significant influence on ecosystems further from the source. As N limits net primary production in most terrestrial ecosystems, changes in the

supply of N can affect the cycling of other elements such as carbon or phosphorus, and consequently ecosystem function.

Aber et al. (1989) emphasized the impact of atmospheric deposition in particular, as fertilizations are one-time applications whose effects could wear off after several years, while elevated atmospheric N deposition is chronic and has more prevalent influences. A large number of studies have been conducted to investigate the impacts of elevated N deposition at the ecosystem level.

A well-studied impact of chronic N deposition on forests is nitrogen saturation, which happens when the reactive N input exceeds the plant or microbial nutritional demand, and leads to failing ecosystem functions. At background levels, most ecosystems are N-limited; therefore, N addition generally leads to an increase in primary production, and initially stimulates carbon uptake and storage (Aber et al. 1989; Vitousek et al. 1997). Elevated N deposition first results in an increase in foliar N content, followed by a rise in foliar biomass. When the input exceeds the N uptake capacity of the system, the system is N-saturated. Most frequently, other essential elements such as phosphorus or water becomes the main limiting factor. Another impact includes increased nitrification, which leads to nitrate leaching from soil and increased emission of N_2O. Both processes mean a change in forest function from an N sink into a source and an increase in greenhouse gas emission into the atmosphere (Aber et al. 1989; Vitousek et al. 1997). N saturation also causes chemical imbalance in trees, such as changes in Ca:Al and Mg:N ratios. The losses of important cations such as Ca and Mg via leaching result in reduced photosynthetic capacity, N use efficiency and general

physiological processes (Magill et al. 1997; Elvir et al. 2006). Alterations in species composition favoring more N-demanding species can also occur, which result in biodiversity loss (Vitousek et al. 1997; Stevens et al. 2010; Ceulemens et al. 2011). Finally, a decline in productivity can lead to disruption of ecosystem functions and tree death. One cause for mortality that recently gained attention from scientists is ammonium toxicity, where too high exposure to NH_4^+ can have detrimental effects on trees (Nihlgard 1985).

Tolerance of high N loads varies among ecosystems. Bobbink and Roelofs (1995) established an empirical approach to examine the threshold level of N deposition above which ecosystem functions are disrupted, known as critical load. They showed that although most ecosystems (grasslands, heathlands, coniferous and deciduous forests) have an average critical load of around 15-20 kg N/ha/year, the most sensitive ecosystems, which are softwater lakes and ombrotrophic bogs, reach the threshold at about 5-10 kg N/ha/year (Bobbink and Roelofs 1995). Their findings were confirmed by many more recent studies by Bowman et al. 2006 and Pardo et al. 2011, and warrant more research into the responses of these nutrient-poor ecosystems to high levels of N deposition.

Northern peatlands

Peatlands are characterized by low plant production and decomposition, due to the low nutrient input, low temperature and water-logged anaerobic soils. Slower decomposition than production leads to peat accumulation. Peat is partially decomposed organic matter that accumulates and forms after a long period of time under waterlogged, cold and anoxic conditions (Rydin and Jeglum 2006).

Peatlands are therefore large pools of carbon sequestered through primary production but not yet released into the atmosphere through decomposition (Gorham 1991). This intricate balance between primary production and decomposition is crucial to all life forms in peatlands, and is controlled by temperature, pH, the fluctuation of water table, and nutrient availability (Rydin and Jeglum 2006). Gorham (1991) estimated a carbon pool of 455 Pg (Pg = 10^{15} g) in boreal and subarctic peatlands, which are located mostly in Russia, United States, Canada, Norway, Sweden and Finland. This amounts to one-third of the total world pool of soil carbon; therefore, peatlands play a critical role as carbon storage, especially when human-induced climate change is altering the global carbon budget (Gorham 1991).

Peatland areas with over 40 cm of peat are classified as fens or bogs. The fundamental difference between the two types of ecosystems lies in the source of nutrients and hydrology. While fens take their nutrients and water from external sources (minerotrophic), bogs maintain their ecosystem functions purely through water and nutrients from precipitation (ombrotrophic) (Rydin and Jeglum 2006). Bogs, therefore, are more nutrient-poor and susceptible to any changes in the atmospheric chemical composition. Although there is a clear scientific distinction, on most landscapes, one type of ecosystem gradually transitions to the next without a fine boundary.

One of the unique features of boreal peatlands, especially ombrotrophic bogs, is the dominance of *Sphagnum* mosses, a bryophyte that is well adapted to the acidic, waterlogged and nutrient poor conditions. In addition, they alter their

environment and minimize competition by making it more acidic and wet. Hyaline cells in *Sphagnum* have very high water-holding capacity, and the cation channels actively take up cations in the water and replace them with protons, thereby acidifying the interstitial water. The phenolic compounds and cell wall polysaccharides in *Sphagnum* are also extremely decay-resistant, which significantly contributes to the peat accumulation process that defines peatlands (Rydin and Jeglum 2006; Hajek et al. 2011).

Besides *Sphagnum*, bogs are also dominated by other bryophytes, sedges (Cyperaceae), rushes (Juncaceae), aquatic plants, evergreen dwarf shrubs (Ericaceae) and occasional trees. The vegetation community in peatlands develops very specific morphologic and functional adaptations to succeed in the wet, anoxic and nutrient-poor conditions. For example, many grasses and sedges have aerenchyma, or open spaces in-between cells that conduct oxygen from the leaves to rhizomes and roots. Sedges (*Carex* and *Eriophorum* spp.) also develop tolerance for inundation by growing in high tussocks that elevate them from the water table. Larger trees in peatlands are usually flood tolerant, but they also develop shallow root systems that stay in the aerated zone (Rydin and Jeglum 2006).

Vegetation also adapts to the low temperature and nutrient-poor conditions by being very conservative of nutrients. Various life strategies adopted by bog plants include perenniality, nutrient resorption, high nutrient use efficiency, etc. (Rydin and Jeglum 2006). For example, the majority of vascular plants are evergreen shrubs of the Ericaceae family, which includes plants with thick leaves to

conserve nutrients. They retain their leaves over more than one growing season to minimize the nutrients and energy required to produce new leaves every year, despite the trade-off of lower photosynthetic rate and less efficient light harvest and CO_2 uptake. Before shedding their leaves, they reabsorb nutrients from old leaves and transfer them to new leaves in the spring (Small 1972; Rydin and Jeglum 2006). Therefore, although peatland plants have low nutrient input and photosynthetic rate, their nutrient use efficiency is very high (Iversen et al. 2010).

Human activities have been disrupting the carbon storage function of peatlands in various ways. Drainage of wetlands into agricultural land and burning of peat as fuel reduce or eliminate many carbon sinks and cause a shift in carbon balance (Armentano and Menges 1986). Human-induced climate change, the most immediate outcome of which is the rise in global temperature, has major ecological implications on the carbon storage function of peatlands in complicated ways, as it enhances primary production yet also accelerates decomposition, both maintained by the low temperature (Bragazza et al. 2006). The increase in temperature also causes the permafrost layer under the peat surface to thaw, which exposes the peat layer to decomposition and leads to net C loss (Schuur et al. 2008). Additionally, because peatlands also frequently release methane (CH_4), a more potent greenhouse gas than CO_2, the balance between CH_4 and CO_2 production also alters as temperature rises (Moore and Knowles 1987; Gorham 1991).

N deposition on bogs

The aforementioned alterations in the global nitrogen cycle do not spare its impacts on northern peatlands. In fact, these extremely nutrient-poor environments are even more vulnerable to atmospheric N deposition (Bobbink and Roelofs 1995). Similar to research into forest health under chronic N deposition, experimental sites have been set up to investigate the long-term impacts of N deposition on bogs, as well as studies that examine natural loads in areas with high deposition rates. Different aspects of the ecosystem have been studied, for example, changes in net CO_2 ecosystem exchange and carbon storage function, vegetation composition, and morphological, distributional or physiological changes in specific functional groups such as *Sphagnum*, shrubs, grasses and sedges, or microbial communities.

Effects on Sphagnum mosses

As a building block of peatlands in general and bogs in particular, *Sphagnum* mosses are one of the well-studied subjects in terms of their responses to N deposition, as they can inform scientists about the changes happening to the ecosystem as a whole. In addition, *Sphagnum* mosses are the first to receive nutrients deposited from the atmosphere, as they form a thick layer between the vascular plant roots and the air and take up any nutrients available before they reach the vascular plants (Malmer et al. 1994). This competition strategy also places *Sphagnum* mosses at a vulnerable position as they are the first to react to any negative effects of atmospheric N deposition.

Fairly similar to the response to N addition in forests, *Sphagnum* also initially reacts positively to N deposition in both fertilization experiments (Aerts et al. 1992; Limpens et al. 2003; Limpens et al. 2011) and under natural N loads (Vitt et al. 2003). In both situations, *Sphagnum* productivity increases without altering the N levels in the tissue or the surrounding peat environment. A meta-analysis by Lamers et al. (2000) indicated that this condition of non-saturated *Sphagnum* persists until N deposition rates reach 12 kg N/ha/year, which is close to the critical load estimate for ombrotrophic bogs made by Bobbink and Roelofs (1995) of 5-10 kg N/ha/year. Above this level, N deposition stops benefiting *Sphagnum* growth, with no increase or even a reduction in productivity in any *Sphagnum* species (Aerts et al. 1992; Gunnarsson and Rydin 2000; Limpens et al. 2011). At deposition rates between 12 and 18 kg N/ha/year, usually caused by anthropogenic N emissions, N is no longer the limiting factor and therefore is stored in organic compounds, such as amino acids, and subsequently increases the N tissue concentration (Lamers et al. 2000). The major storage compounds are identified as asparagine and arginine by Limpens and Berendse (2003). The meta-analysis by Limpens et al. (2011) showed a similar trend of saturating N concentration and diminished productivity with increasing N inputs, which indicates a threshold after which N addition has adverse effects on *Sphagnum*. They suggested physiological stress due to nutrient imbalance as the cause for the decline. For example, fairly similar to forest N saturation, a different soil element, most frequently phosphorus or potassium, might become limiting and influence productivity. As a result, many fertilization experiments include treatments with

both N and P to investigate further the role of P. At very high N loads, *Sphagnum* tissues are no longer able to store the excess N, so atmospheric N will bypass the *Sphagnum* filter and become available in the peat underneath the moss layer (Lamers et al. 2000). This creates the opportunity for more N-demanding species to enter the bog, for example deciduous species such as *Molinea caerulea* (Purple Moorgrass) or *Betula* spp. (Birch) (Lamers et al. 2000).

While elevated N deposition has negative or no effect on *Sphagnum* productivity after the initial increase, it enhances decomposition. Higher N inputs were shown by Bragazza et al. (2006) to lower C:N ratios in plant litter and peat, which favors microbial decomposition. The combination of unchanged productivity and enhanced decomposition results in decreased C sequestration function (Bragazza et al. 2006; Gerdol et al. 2007; Larmola et al. 2013, in prep).

Effects on species composition of bog communities

The failure of the *Sphagnum* filter due to high atmospheric N deposition has been shown to cause a shift in species composition in bogs (Heijman et al. 2001). The nutrients previously filtered by *Sphagnum* are now available to vascular plants. Similar to forest trees or *Sphagnum* mosses, vascular plant productivity initially increases (Limpens et al. 2003). More vascular plant growth blocks sunlight from *Sphagnum* and other mosses, both because of more living biomass shading the understory and more litter accumulation caused by increased biomass turnover (Berendse et al. 2001). Therefore, at high N loads, peatlands and arctic ecosystems observe a decline in *Sphagnum* biomass and an increase in abundance of vascular plants (Hoggs et al. 1995; Berendse et al. 2001; Heijman et al. 2001;

Limpens et al. 2003; Bubier et al. 2007; Juutinen et al. 2010). Shading might not be the only cause of *Sphagnum* decline, as high N loads have been shown to decrease *Sphagnum* growth due to the exposure of high concentration of ammonium (Gunnarson and Rydin 2000; Baxter et al. 2002; Limpens and Berendse 2003). In some ecosystems, available N leads to the dominance of more N-demanding species such as *Betula nana* (Shaver et al. 2001) or *Molinea caerulea* (Hoggs et al. 1995).

Effects on foliar chemistry in vascular plants

The most well-documented impact of N fertilization on vascular plants in peatlands is, similar to forest trees and *Sphagnum* mosses N, an increase in foliar N content. This change in foliar chemistry has been investigated and shown in many functional groups, and is more pronounced in deciduous than evergreen shrubs (Baddeley et al. 1994). A survey by Pitcairn et al. (1995) showed a positive relationship between natural N inputs and tissue N content in *Calluna vulgaris*, an ericaceous shrub, with a change of 0.045 mg/g N dry weight for every additional kg/ha/year of N input. Foliar N is also suggested as an indicator of N deposition in the natural environment at the regional scale (Pitcairn et al. 2001). As these shrubs are very well-adapted to a nutrient-poor environment, the extra amounts of N in the leaves can have a wide range of effects on the physiology of the plants. For example, higher N content makes many dwarf shrubs more appealing to insects, which is shown in the marked increase in herbivory in fertilized areas (Richardson et al. 2002).

Few studies have examined the effect of N fertilization and the subsequent increase in foliar N content on the physiology of shrubs in peatlands. The specific adaptations that bog shrubs develop to the nutrient-poor conditions make the understanding of their responses to N deposition much more fascinating. By directly altering foliar N content, atmospheric N deposition can cause various photosynthetic and physiological responses in shrubs as they try to allocate the new source of N.

Leaf-level CO_2 exchange

Photosynthesis is the process of synthesizing chemical energy in sugar from CO_2, water and sunlight and releasing oxygen as the byproduct. The process consists of two phases, the light-dependent phase that harnesses sunlight and produces energy for the second phase, carbon fixation. Molecules of chlorophyll, a pigment critical to the light harvesting process, are arranged into two complexes, known as photosystem I and II (PS I and PS II). Chlorophyll exists as either chlorophyll a or b, each type responsible for absorbing different light wavelengths. When sunlight hits any molecule, the energy is transferred across the complex to one reaction center where the energy is transported across an electron transport chain to finally generate ATP and NADPH, the energy storing molecules. Concurrently with the energy production, water is split into oxygen and protons to act as an electron acceptor at the end of the electron transport chain (Hopkins and Huner 2009).

Energy from ATP and NADPH is used in the carbon fixation process, which is a completely light-independent cycle. CO_2 molecules diffused from the

atmosphere into the space in mesophyll tissues enter the cycle. Here, Rubisco, an enzyme that plays fundamental role in this cycle, fixes CO_2 to a five-carbon substance called ribulose 1,5-biphosphate (RuBP), to produce two three-carbon molecules, 3-phosphoglyceric acids (PGA). This resulting molecule is the reason that this common pathway of carbon fixation is also known as 3C cycle. With the amount of C assimilation occurring, Rubisco is arguably the most abundant enzyme on earth. PGA is then reduced into an intermediate molecule, glyceraldehydee 3-phosphate (PGAL), from which sugar is made and RuBP is regenerated, ready to bond with new CO_2 molecules (Hopkins and Huner 2009).

Photosynthesis is an N-demanding process, mainly due to the N-rich compounds that are involved in the process. Nitrogen in leaves is invested in various forms of proteins involved in photosynthesis. Evans (1989) divided them into two main sources, proteins in the thylakoid membranes and soluble leaf proteins. The former is comprised of chlorophyll pigment and the proteins associated with the electron transport, therefore nicely corresponds to the light reaction of photosynthesis. Soluble proteins, on the other hand, consist of proteins involved in the Calvin cycle in the dark reaction, most prominently the enzyme Rubisco.

The rate of carbon assimilation is controlled externally by light intensity, temperature, humidity, CO_2 and O_2 concentration and internally by the availability of Rubisco, rate of electron transport and rate of RuBP regeneration (Farquhar et al. 1980; Sharkey et al. 2007). A frequent measurement of photosynthetic performance is net assimilation rate (Amax), which takes into

account the changes in CO_2 concentration measured at constant and ideal conditions (light intensity and quality, temperature, humidity and CO_2 concentration). This parameter provides one perspective into the maximum photosynthetic capacity and the photosynthetic responses of plants at the leaf level to variations in the environment, therefore often referred to as photosynthetic capacity.

Further investigations into the carbon assimilation process are possible using CO_2 response curves, where at constant saturating light and ideal temperature and humidity, the plant (or leaf) is exposed to a wide range of CO_2 concentrations, and the assimilation rate is recorded at each point. The CO_2 response curve is later constructed from the internal CO_2 concentration (Ci) and assimilation rates (A), and has a curvilinear form, according to the model constructed by Farquhar et al. (1980) (Figure 1, Table 1). The curve demonstrates three steps in the assimilation process and the related derivable parameters. At low CO_2 concentration, rate of CO_2 assimilation is limited by Rubisco, and the relationship between Ci and A is linear. The slope of this relationship is the maximum rate of Rubisco carboxylation (Vcmax) and can be calculated from the curve. Some studies consider Vcmax the photosynthetic capacity, while others take the assimilation rate at ambient conditions. Increasing CO_2 concentration leads to a limitation in the rate of RuBP regeneration, where the rate of electron transport (J) controls the amount of NADPH available to regenerate RuBP, and a maximum rate (Jmax) can be derived from the curve. At very high CO_2 concentration, an increase in CO_2 no longer has any effect on A, as the leaf reached triose phosphate limitation.

This state happens when the chloroplasts produce more PGAL than the cell can use, and is characterized by a parameter called triose phosphate use (TPU) (Farquhar et al. 1980; Sharkey et al. 2007). Vcmax, Jmax and TPU are referred to as photosynthetic potentials, as they address the maximum measurements at each stage of limitation.

Because N is integral to the photosynthetic machinery, especially to Rubisco, studying impacts of N deposition on vascular plants includes determining where the newly added N is allocated. In that case, photosynthetic capacity serves as the big picture informing photosynthetic performance, while Vcmax, Jmax, and TPU provide more in-depth information about the assimilation process and N use. The global relationship shows a positive relationship between N inputs and assimilation rates at natural levels (Wright et al. 2004). In many forest trees, most N is invested in Rubisco carboxylation, RuBP regeneration, and use of triose phosphate compounds, and these parameters increase as foliar N content increases (Bauer et al. 2001). However, there are signs that increasing N inputs can exceed the amount required by Rubisco, and are instead stored as amino acids (Magill et al. 1997; Minocha et al. 2000; Warren et al. 2003). A few studies have examined the responses of evergreen shrubs to N addition in terms of foliar chemistry, photosynthetic performance and other physiological changes, and found similar responses (Bowman et al. 1995; Bubier et al. 2011; Heskel et al. 2012). The excess N in shrubs is stored as amino acids, causing foliar N content to increase (Bubier et al. 2011), but not resulting in an increase in photosynthetic capacity. Some chemical changes have also been observed, including the decrease in Ca

and Mg cations, a sign of stress in many plant species, in both forest trees and bog shrubs (Minocha et al. 2000; Bubier et al. 2011).

Chlorophyll fluorescence

Plant stress has been a very interesting topic in plant physiology, and a variety of methods are used to evaluate plant health in different conditions. One of the more recent and easy-to-perform methods of measuring plant stress is chlorophyll fluorescence (Maxwell and Johnson 2000). This method evaluates the maximum capacity to harvest sunlight at the leaf level. It is based on three competing processes when a photon hits the two photosystems (PS): photosynthesis (also called photochemistry), heat dissipation and chlorophyll fluorescence. Light energy of specific wavelengths is absorbed by the corresponding photosystem (680 nm for PS II and 700nm for PS I) to be converted to chemical energy through photosynthesis, and is usually the measurement of interest. This parameter can be inferred indirectly through chlorophyll fluorescence. As any object, leaves reflect light of certain wavelengths off the surface, absorb some other (in the case of photosynthesis, red and blue light at 680 and 700nm) and transmit the rest. The green color we observe in most leaves is the light that chlorophyll molecules cannot absorb and instead reflect back to our eyes. The amount of light reflected from leaves is measured as chlorophyll fluorescence to provide information about light absorption for photosynthesis while accounting for heat dissipation (Maxwell and Johnson 2000; Hopkins and Huner 2009).

The most commonly used and simple form of chlorophyll fluorescence measurement is the dark-adapted measurement that evaluates the maximum light-

harvesting capacity through the potential PS II efficiency. In the dark adaptation measurements, leaves are kept in the dark for a long enough period of time to open all their reaction centers, and the chlorophyll fluorescence level will reach its maximum (Fm), assuming no heat dissipation. After dark adaptation, the leaf is then exposed to a high intensity source of light that will induce all reaction centers to close, giving us the lowest value of chlorophyll fluorescence. The ratio between variable (Fv), the difference between Fm and Fo, and maximum (Fm) photosynthesis, derived from the two above measurements, is often referred to as the maximum dark-adapted chlorophyll fluorescence. Scientists over the world have obtained a global standard Fv/Fm ratio of approximately 0.8 for normally functional plants. Lower ratios indicate physiological stress, including damages to the sensitive PS II and decreased photosynthetic capacity caused by photoinhibition (Maxwell and Johnson 2000).

Many plant scientists have used chlorophyll fluorescence to study physiological health of many types of vegetation under the effect of nutrient addition, yet the results so far have been inconsistent. In forests, while spruce's chlorophyll fluorescence parameters react positively to N addition in some studies (Tomaszewski and Sievering 2007), they behave in the opposite direction in others (Tripodi and Sievering 2010). The same inconsistent pattern appears among peatland vegetation, with more focus on *Sphagnum*. Granath et al. (2009)'s study on *Sphagnum* found a decrease in photosynthetic rates at high N deposition, but could not explain it by the toxic effects of N on the photosystem, as there was no significant effect of N addition on PS II efficiency. Manninen et

al. (2011), on the other hand, found decreased Fv/Fm ratios in red *Sphagnum capillifolium*, especially in response to N additions in the form of ammonium. Fertilization in grassland bryophytes also results in lowered PS II efficiency despite the increase in chlorophyll content, suggesting impaired photosynthetic capacity and photoinhibition (Arroniz-Crespo et al. 2008). In both cases, ammonium has a more negative impact than nitrate (Arroniz-Crespo et al. 2008; Manninen et al. 2011). Besides N deposition, water table depth can also alter chlorophyll fluorescence parameters (Carfrae et al. 2007; Gaalen et al. 2007).

The observed conflicting outcomes in PS II efficiency under enhanced N deposition both demonstrate the complicated nature of N deposition responses in vegetation, and demands for more research into the subject. For example, few studies examine the physiological response of vascular plants in peatlands to N fertilization, especially evergreen shrubs, whose reactions are complex due to their intermediate morphological and physiological structure and function between forest trees and *Sphagnum* mosses. In particular, a study into both the light harvesting efficiency and CO_2 assimilation performance of shrubs under chronic N deposition would allow us to better understand the full impact N has on the whole photosynthetic machinery and as a result, changes in gross and net primary production.

Research at Mer Bleue

Mer Bleue Bog is a conservation area located near Ottawa, Ontario, Canada ($45^{o}40'$N, $75^{o}50'$W), and managed and owned by the National Capital Commission. Many studies on hydrology, biogeochemistry and the effects of

climate change on carbon cycling have been going on at the bog. For example, net CO_2 ecosystem exchange and other environmental variables are constantly measured at an eddy covariance tower (Lafleur et al. 2003; Humphreys et al. 2006; Roulet et al. 2007). A fertilization experiment was established at Mer Bleue in 2000 to study the effects of atmospheric N deposition on ecosystem processes (Juutinen et al. 2010). Triplicates plots have been fertilized with N only (as NO_3^- and NH_4^+ combined) or N, P and K at different levels, to understand the impacts of N deposition as well as nutrient stoichiometry on this sensitive ecosystem. A wide range of studies have been conducted to investigate responses of different elements of the ecosystem to the elevated N levels. At the ecosystem level, Bubier et al. (2007) and Juutinen et al. (2010) conducted frequent NEE chamber gas exchange measurements and found that elevated N deposition reduced the carbon sink function of the bog, especially at high N levels. In an effort to explain this sink reduction at the whole plant and leaf- level, a study conducted in 2008 on leaf-level photosynthesis and leaf morphology in response to N fertilization found that the increased N load did not increase the bog shrubs' maximum photosynthetic capacity (Bubier et al. 2011). As N is the limiting nutrient in most ecosystems, it is expected that an increase in N input at naturally occurring levels would benefit the photosynthetic machinery and hence, increase plant production (Wright et al. 2004). For example, Field and Mooney (1986) found a strongly positive relationship between foliar N content and Amax in a meta-analysis across all ecosystems. The lack of this relationship, therefore, can be an indication of N saturation. The study by Bubier et al. (2011) suggested that the fertilized N was

not used to improve the photosynthetic capacity but instead stored as amino acids to minimize ammonium toxicity. The study also found signs of stress in the three studied bog shrubs through decreased Ca concentration and enhanced storage of N as amino acids, in particular those compounds that are indicators of physiological stress in forest trees under chronic N saturation (e.g. Bauer et al. 2001).

Based on those past studies, I continued investigating leaf level CO_2 exchange in bog shrubs at Mer Bleue Bog, specifically focusing on the dominant evergreen shrub species, *Chamaedaphne calyculata* Moench. (leatherleaf), a perennial shrub of the Ericaceae family. It is a fairly common bog species that exhibits many adaptations of bog shrubs to low nutrient conditions, including perennial life strategy and low photosynthetic capacity. I expected that after twelve years, the shrubs in the high nutrient treatments would be N-saturated and may be suffering from some form of nutrient stress. I evaluated the state of stress using the efficiency of photosystem II (PS II) measured by chlorophyll fluorescence, while at the same time re-evaluated the CO_2 exchange performance through parameters such as maximum rate of net assimilation (Amax) and maximum Rubisco carboxylation rates (Vcmax).

My study set out to investigate the photosynthetic performance of *C. calyculata* under elevated N deposition. My hypotheses are:

1) Older *C. calyculata* leaves (2-3 years old) will be more stressed than current year leaves, as the shrub follows the life strategy of evergreen shrubs in

the Ericaceae family to store nutrients in old leaves instead of shedding them every year like deciduous ones. Therefore, any effects of excess nutrients on the shrubs will show up in old leaves first.

2) Treatments with higher level of nutrients will be under nutrient stress, and therefore have lower chlorophyll fluorescence (Fv/Fm) than the controls. Chlorophyll fluorescence will also be higher in treatments with P and K compared to those without, which indicates that P and K help alleviate nutrient stress by enhancing N use.

3) (Amax) and (Vcmax) will also be affected, and hence reduced, by N saturation in plots with high N levels. The presence of P and K will enhance the use of N, therefore slightly increase the rates compared to treatments with same N levels without P and K.

4) Chlorophyll content is directly affected by fertilization, and increases with higher amount of nutrient input.

5) There is a positive correlation between chlorophyll content and chlorophyll fluorescence, Amax, and Vcmax, as chlorophyll plays a crucial role in photosynthesis.

6) Amax and Vcmax positively correlate with chlorophyll fluorescence, which indicates that the decrease in photosynthetic efficiency leads to the decline in maximum photosynthetic capacity.

METHODS

Site description

The study was conducted at Mer Bleue Bog, Ottawa, Ontario, Canada. The annual temperature is 6.0^{o}C, mean annual precipitation 944 mm (Canadian Climate Normals 1971-2000). The bog is ombrotrophic, and dominated by ericaceous shrubs such as *C. calyculata*, *Ledum groenlandicum*, *Vaccinium myrtilloides*, *Kalmia angustifolia* and *K. polifolia*, sedge *Eriophorum vaginatum*, and *Sphagnum capillifolium*, *S. magellanicum* and *Polytrichum strictum* in the ground layer. The background atmospheric wet N deposition rate is 0.8 g N m^{-2} $year^{-1}$.

The fertilization experiment at Mer Bleue was set up in 2000, with more treatments added in 2005, to study the effect of nutrient addition on the carbon cycling of the bog. Each 3m x 3m triplicate plot was established and fertilized every three weeks from early May to early September. Distilled water was added to control plots, while fertilized treatments include nitrogen (as NH_4NO_3) five (1.6 g N m^{-2} $year^{-1}$) and twenty times (6.4 g N m^{-2} $year^{-1}$) the ambient growing season N deposition rate, with or without phosphorus and potassium (as KH_2PO_4) (Table 2). While the control, 5N, 5NPK and 20NPK treatments were established in 2000, 20N plots were added in 2005 (Juutinen et al. 2010).

Field methods

In summer 2012, chlorophyll fluorescence, leaf-level CO_2 exchange and chlorophyll content of the dominant shrub, *C. calyculata*, were measured at five treatments: Control, 5N, 5NPK, 20N and 20NPK. In each plot, three new and three old top canopy leaves were measured for all three components, giving a total of nine leaves per treatment per age. New leaves were selected and tagged at the beginning of the growing season to distinguish from old leaves as the summer progressed and the new shoots turned woody. Measurements were conducted from June 26th to August 15th using a LI-6400 portable photosynthesis system, which includes an infrared gas analyzer and a leaf fluorometer chamber (Li-Cor, Lincoln, NE, USA). Water table depth was also measured weekly from PVC tubes installed at each plot.

For chlorophyll fluorescence, leaves were wrapped in aluminum foil for at least 24 hours to allow adaptation to complete darkness before measurement. At constant chamber conditions of 25^{o}C, flow of 150 μmol s^{-1}, no light and humidity between 30 and 60%, leaves were clamped into the 2-cm^2 leaf chamber. After fluorescence value stabilized after 2 minutes, the measuring light obtained minimum fluorescence (Fo). Then, a high-intensity saturating flash was induced in 0.8 seconds to cause all photon-receptors to close, and produce maximum fluorescence (Fm). Variable fluorescence (Fv), Fm – Fo, was then used to calculate maximum light harvesting/ photochemical potential, Fv/Fm. Before the flash, assimilation rate was also recorded and used as dark respiration (Adark) (Table 1).

To measure CO_2 gas exchange, leaves were again placed in the leaf chamber with similar flow, temperature and humidity settings but with saturating photosynthetic photon flux density (PPFD) of 1300 μmol m^{-2} s^{-1}. Assimilation rate (A), leaf temperatures and stomatal conductance were recorded for 13 concentrations of CO_2 between 50 and 2200ppm, with 2 minutes of stabilizing at each concentration. All measurements started with the ambient CO_2 concentration (400 ppm), decreasing to 50 ppm, then increasing up to 2200 ppm. Assimilation rate at 400ppm was therefore measured three times for each leaf, and the average of the three values was used as Amax, disregarding negative values. Internal CO_2 concentration was calculated from sample CO_2 concentration and stomatal conductance (LiCor 6400 Manual, 2004). All 13 values were later used to construct CO_2 response curves with assimilation rate (A) and internal CO_2 concentration (Ci), from which parameters such as Vcmax and Jmax could be derived.

At the end of each measurement, leaves were detached from the stem, and the leaf area inside the chamber marked. Their width, length and thickness were measured, and a photo of each leaf was taken to later analyze leaf area. After each field measurement, leaves were wrapped in aluminum foil and transported on ice and frozen after 4 hours.

All leaves were analyzed for chlorophyll content at McGill University using the dimethyl sulphoxide (DMSO) chlorophyll extraction technique (Hiscox and Israelstam 1970). Chlorophyll content was the sum of chlorophyll a and b, calculated on a fresh weight basis.

Data processing

ImageJ (Rasband, National Institute of Health, MD, USA) was used to calculate leaf area inside the chamber. Then, assimilation rates downloaded from the Li-Cor 6400 were recalculated based on the correct leaf area to obtain Amax and Adark. For each leaf, Pmax was calculated as the difference between Amax and Adark (Table 1).

From the assimilation rates at thirteen CO_2 concentrations, a CO_2 response curve (A – Ci) was created for each individual leaf. Maximum Rubisco carboxylation rate (Vcmax, μmol C m^{-2} s^{-1}) and maximum electron transport rates (Jmax, μmol electrons m^{-2} s^{-1}), triose phosphate use (TPU, μmol m^{-2} s^{-1}) were derived from the curve. This was done using the curve-fitting model first introduced theoretically by Farquhar et al. (1980) and developed by Sharkey et al. (2007), in which parameters like Vcmax, Jmax, and TPU are the slopes of the CO_2 response curve at the three stages of Rubisco limitation, RuBP limitation and TPU limitation in photosynthesis, respectively (Figure 1, Table 1). Internal CO_2 concentrations were converted from a ppm basis to Pa basis (known as Cc). All parameters were estimated first at measuring conditions, then converted to ambient (25^{o}C, 100 kPa). To determine which stage of limitation each of the thirteen points on the curve belonged to, I used the relationship between Ci and electron transport rate (J) recorded at each CO_2 concentration. Usually at low CO_2 concentration, J increased with Ci and indicated leaf in the Rubisco-limited stage, from which Vcmax could be estimated; constant J against Ci signified the RuBp-limited stage where Jmax could be derived, and at very high CO_2 concentration,

decreasing J determined TPU-limited stage and corresponded with TPU rate. Sums of squares of unclear trends in the fitted models were compared to select the lowest. Unreasonable values of A or Ci were also eliminated from the curve-fitting model.

STATISTICAL ANALYSES

To initially address the hypotheses, three-way nested ANOVAs were run on the four dependent variables: Fv/Fm, Amax, Vcmax and total chlorophyll content. The three factors of ANOVA were identified as treatment, age, and plot nested within treatment. Treatment and age were the two factors of interest, but plot was included as another variable nested within treatment to reduce variability and to capture unexpected patterns from microtopographic spatial variability. The interaction between two main factors, treatment and age, was also investigated to see if the differences in new and old leaves were the same in all treatments, or if new and old leaves reacted differently to nutrient addition.

An investigation of seasonal patterns revealed that over the course of the measurement period (from June 28th to August 15th), depth to water table generally increased, while leaf temperature decreased (Figure 2a and b). This seasonal pattern appeared to influence the rates of Vcmax, with significantly higher rates in leaves measured in July than in August (Appendix 1, $F_{1, 89}$ = 24.048; P-value <0.001). The addition of July/August as a factor was considered, and different trends were observed when analyzing separate July and August data. For example, treatment effect was significant in July but not in August (Appendix 1). However, the separation of July and August data rendered the dataset unbalanced, and possibly left out important patterns as the transition from one month to another could be continuous. The addition of a covariate to capture the seasonal pattern was necessary, and Julian day, leaf temperature, depth to water

table and chlorophyll content were considered. Chlorophyll content was not chosen as it was also affected by the treatment and age, but instead incorporated later in the form of linear regression. Among the other three abiotic factors, depth to water table was the covariate that reduced the sum of squares of error the most, and was able to capture both seasonal variability and some of the spatial variability partially addressed by the inclusion of plot as a factor. Therefore, it was chosen as the covariate in all ANOVAs. The process of data exploration and analysis resulted in three-way nested ANCOVAs with water level as the covariate, treatment, plot within treatment and age as the three factors. If the ANCOVA yielded significant results, Bonferroni post hoc tests were conducted to find pair-wise differences based on marginal means, taking into account the covariate.

The hypothesis and original plan included four dependent variables: Fv/Fm, Amax, Vcmax and chlorophyll content. After preliminary data analysis and interpretation, I realized it is necessary to also include maximum gross photosynthesis (Pmax), dark respiration (Adark), maximum electron transport rate (Jmax) and chlorophyll a/b ratio. Therefore, similar ANCOVAs were run with these variables as well.

Linear regressions were run between chlorophyll content and the other light-related dependent variables (Fv/Fm, Amax, Pmax, Vcmax and Jmax). Correlations between Fv/Fm and Amax, Pmax, Vcmax and Jmax were also investigated using linear regression.

RESULTS

Research questions were investigated mainly based on four variables: chlorophyll fluorescence ratio Fv/Fm, maximum net assimilation rate (Amax), maximum Rubisco carboxylation rate (Vcmax) and total chlorophyll content collected from 90 leaves from 5 treatments in 2 age groups (n = 90). Chlorophyll fluorescence had a mean of 0.79 (SE 0.04) and ranged from 0.66 to 0.86. Amax ranged from 0.28 to 11.1 μmol CO_2 m^{-2} s^{-1}, with an average of 4.31 μmol CO_2 m^{-2} s^{-1} (SE 0.29). Vcmax had a range between 6 and 179 μmol C m^{-2} s^{-1}, mean 55.54 μmol C m^{-2} s^{-1} (SE 3.66). Chlorophyll content ranged from 0.80 to 4.10 mg/g dry weight, and had a mean of 2.11 mg/g (SE 0.08). Additionally, maximum electron transport rate (Jmax), dark respiration rate (Adark), maximum gross photosynthetic rate (Pmax) and chlorophyll a/b ratio were also analyzed. Further description of each variable in treatment and age group is summarized in Tables 3 and 4.

A general investigation of the seasonal patterns revealed that over the course of the summer, depth to water level increased and strongly correlated with Julian Day (Figure 2a), except for the last week in which water table rose after a precipitation event. Leaf temperature generally decreased over the season (Figure 2b). A linear regression between Julian Day and all observations of each of the aforementioned dependent variables shows that chlorophyll fluorescence and Amax did not show large seasonal variation (Figure 3a and 3b), while Adark, Pmax, Vcmax, Jmax, chlorophyll content and chlorophyll a/b ratio all significantly decreased as the summer progressed (Figure 3c-h).

The first hypothesis examined the stress level between old and new *C. calyculata* leaves in terms of light harvesting capacity, measured by chlorophyll fluorescence ratio Fv/Fm. The three-way nested ANCOVA test taking into account the water level shows a significant difference in chlorophyll fluorescence between old and new leaves (Table 5). Old leaves' mean ratio was higher than that of new leaves by 0.02, a small but statistically significant difference (Figure 5a), which is the opposite trend than expected in the first hypothesis.

The same statistical test was used to address the second hypothesis regarding treatment effects on chlorophyll fluorescence. The hypothesis was not supported, as no significant difference was found in chlorophyll fluorescence among treatments (Table 5, Figure 5b). When old and new leaves were considered separately, neither group had significant treatment effects (Table 5). However, the mean ratios among treatments in new leaves were fairly similar to one another, while that among old leaves were less comparable (p-value 0.066). Figure 5c demonstrates a trend among old leaves of highest mean chlorophyll fluorescence in 5N and lowest in Control and 5NPK.

A similar nested ANCOVA was used to test hypothesis three, comparing net Amax and Vcmax among treatments and leaf age groups. There was neither a significant treatment effect nor a significant difference between old and new leaves in net assimilation rates (Table 5, Figure 6a and 6b). However, Figure 6b shows the lowest mean Amax in 20NPK, the plot with highest amount of nutrients. Amax was also lower in 5N than 5NPK, which is the trend between N-only and NPK treatments that the hypothesis predicted. Fertilization did not have

differential effect on Amax in new and old leaves (Figure 6c), as there was no significant interaction between treatment and age (Table 5).

To investigate further the trend in Amax, similar ANCOVA analyses were done on dark respiration (Adark) and maximum gross photosynthetic rate (Pmax). Dark respiration differed significantly among treatments (Table 5), with 20NPK plot having the highest rate (3.07 μmol CO_2 m^{-2} s^{-1}) (Figure 6b). Both Adark and Pmax did not differ significantly between old and new leaves (Table 5, Figure 6a). Treatment effect in Pmax had a p-value of 0.065 (Table 5). The rates were higher in the control plot (7.56 ± 0.74 μmol CO_2 m^{-2} s^{-1}) compared to all fertilized treatments, whose mean gross photosynthetic rates ranged from 5.88 to 6.31 μmol CO_2 m^{-2} s^{-1} (Table 4, Figure 6b).

Vcmax rates, on the other hand, are significantly different among treatments (Table 5). The maximum enzyme activity was significantly higher in 20N and 20NPK than in Control, 5N and 5NPK (Figure 7b), yet there was no significant difference in Vcmax between old and new leaves (Figure 7a). These results were, therefore, opposite to my expectation indicated in hypothesis three.

Additional analysis was done on maximum electron transport rate (Jmax). No significant difference between old and new leaves were found (Table 5, Figure 8a), while there was a significant treatment effect (Table 5). 20N treatment had significantly higher Jmax compared to 5N and 5NPK, and the general trend showed highest rates in the highest nutrient treatments (Figure 8b).

Hypothesis four was addressed using ANCOVA analysis of the total chlorophyll content in Table 5. There was a significant treatment effect on chlorophyll content, with significantly higher concentrations in Control, 20N and 20NPK than in 5N and 5NPK (Figure 9b). Hence, the hypothesis was supported. Chlorophyll content was also significantly higher in new than old *C. calyculata* leaves (Figure 9a). Chlorophyll content was comparable in the control between old and new leaves, yet all fertilized plots indicated higher content in new than old leaves (Figure 9c). This observation was supported by the statistical results, as there was a trend toward an interaction between treatment and age effect on chlorophyll content ($p = 0.09$, Table 5). Chlorophyll a/b ratio, when compared using the same analysis, was also significantly higher in new than old leaves (Table 5, Figure 10a). Treatment effect in chlorophyll a/b ratio was also significant (Table 5), with a similar pattern to chlorophyll content of higher ratios in higher nutrient treatments (Figure 10b).

Addressing hypothesis five, no correlation between chlorophyll content and either chlorophyll fluorescence ratios or Amax was found (Figure 11a and b). The correlation between chlorophyll content and Vcmax, however, was positive and statistically significant (Figure 11d), as was that between chlorophyll content and Pmax (Figure 11c), Jmax (Figure 11e) and chlorophyll a/b ratio (Figure 11f). Chlorophyll content generally decreased as the summer progressed (Figure 3g), and the treatment effect in both Amax and Vcmax was more apparent when chlorophyll content was high, with significantly lower rates in Control than 20N (Appendix 1).

Chlorophyll fluorescence did not correlate with Amax, Pmax, Vcmax or Jmax (Figure 12a-d). Hypothesis six, therefore, was not supported.

Residual plots of the eight ANCOVAs and ten linear regressions (Appendix 2-4) indicate that the assumption of equal variance in both ANCOVA and regression was generally met.

DISCUSSION

Nitrogen deposition has been causing extensive changes in many nutrient-poor ecosystems, especially those so important to the global carbon cycle such as peatlands. Studying the responses of shrubs to nutrient addition not only contributes to the understanding of the ecosystem-level response, but also provides the background to further understand the physiology of plants adapted to nutrient-poor conditions. Past studies have shown inconsistent patterns of response in terms of photosynthetic performance to nutrient addition, so a long-term experiment like the one at Mer Bleue would be a great contribution to this section of the literature.

The study set out to investigate the photosynthetic performance of *C.calyculata* in response to nutrient addition as well as differences between leaf age groups. Although measurements were conducted in the field, light, CO_2 concentration, temperature and humidity were controlled for in the leaf chamber, while variation in water table was incorporated into most statistical analyses.

Nutrients such as N, P and K provide fundamental resources for photosynthesis, yet are only available through recycling of organic matter. Being the limiting factors of growth, these nutrients are carefully partitioned among various sinks for photosynthesis, for example pigments such as chlorophyll, enzymes such as Rubisco, or proteins such as ATPase. With *C. calyculata*, which has adapted to the nutrient-poor environment of bogs, nutrients are even more

limited, hence nutrient use efficiency more essential. Bartsch (1994) demonstrated that *C. calyculata* is extremely responsive to nutrient addition, with doubled biomass growth after one year of nutrient addition, and further increase with the length of her experiment. At Mer Bleue Bog, the addition of N, P and K at 5 and 20 times the growing season ambient wet N deposition rate significantly altered the total nutrient availability, therefore was expected to influence the amount in each sink and their relative partitioning, which would be reflected in various leaf-level photosynthetic parameters. Twelve years of exposure to nutrients may also indicate the long-term response of the shrub.

Chamaedaphne calyculata adopts the perennial life strategy, a common adaptation of bog shrubs to low nutrient conditions (Rydin and Jeglum 2006). Some of the leaves stayed on the stem over the winter to provide the initial photosynthate for the development of new leaves in the growing season. Not only are nutrients partitioned between components of photosynthesis, they are also optimally distributed among old and new leaves. Therefore, I anticipated that the morphological and physiological differences between current-year (new) leaves and previous-year (old) leaves will lead to differences in their photosynthetic performance.

Chlorophyll

Chlorophyll is the main pigment responsible for light harvest, and has a strong association with the availability in nutrients due to its nitrogen-rich molecular structure. The investigation of chlorophyll response to nutrient addition, particularly chlorophyll content, therefore, is a good indication of where added

nutrients, especially nitrogen, are being invested. Additionally, chlorophyll also reacts to light availability as well as leaf age, so studying the differences in leaf age and age-specific response to nutrient addition will provide more insights into the physiology of the species. Chlorophyll a/b ratio, in this case, is a common indicator of leaf partitioning of resources in response to different light availability (Lambers et al. 1998).

In this study, chlorophyll content and chlorophyll a/b ratio showed a generally similar pattern of increase along nutrient addition gradient (Figure 9b and 10b). As Evans (1989) identified chlorophyll as a major source of nitrogen, this increasing trend indicates investment of added nutrients to this photosynthetic pigment, and is observed in many C3 species (Evans 1989). Chlorophyll content did not differ between N and NPK treatments, indicating that P and K have a less important role in chlorophyll compared to N. The strong linear relationship between chlorophyll and leaf N has long been established across species (Evans 1989, Lambers et al. 1998), so the observed increase in chlorophyll content along treatment reflects elevated foliar N content, and demonstrates the uptake of increased nutrient availability into leaf tissues.

When leaves of all treatments were pooled together, new leaves had significantly higher chlorophyll content and chlorophyll a/b ratio than old ones. There was a small interaction between leaf age and treatment effect, as in the control, chlorophyll content was fairly comparable between new and old leaves, yet new leaves consistently had more chlorophyll in all fertilized treatments (Figure 9c). Chlorophyll a/b ratio, however, was consistently higher in new than

old leaves across treatments (Figure 10c). These differences in pigment composition can be attributed to various factors including nutrient addition, the position of leaves in the canopy and their differential light availability.

The relationship between chlorophyll and nitrogen has been so well-established (Evans 1989) that the difference in chlorophyll content between old and new foliage of fertilized plants could be inferred to nutrient addition response. New leaves tend to be more nutrient-demanding as they need more photosynthate for the new physiological development (Lambers et al. 1998); as a result, it is often found in evergreen species that N content decreases with leaf age (Niinemets et al. 2005). Additionally, nutrient resorption, a common nutrient-conservation strategy of plants grown in nutrient-poor environments, could be an additional explanation. Before plants shed their old leaves, limiting nutrients like N or P are retranslocated to new leaves, or stored in other tissues over the winter and released during the growing season (DeFoliart et al. 1988). For example, in *Eriophorum vaginatum*, a perennial sedge species commonly found in peatlands and other nutrient-poor ecosystems, 85% N and 90% P in new leaves are in fact translocated nutrients from older foliage (Jonasson and Chapin 1985). Nutrients, under higher demand in new leaves, are therefore invested first in new leaves under the form of chlorophyll, resulting in higher chlorophyll content in new than old leaves.

Besides the indirect impact on nutrient distribution, such as the positive correlation between foliar N content and light intensity (Schoettle and Smith 1998), light availability can have a direct impact on chlorophyll and partly explain

the difference in chlorophyll content among leaf ages. As new leaves develop, they are positioned at the top of the canopy, while older overwintering leaves are lower down the canopy. As new leaves form, older, non-senescing ones keep receiving less and less light, thereby slowly acclimate to a lower irradiance, a phenomenon known as self-shading (Brooks et al. 1994; Niinemets et al. 2006). As chlorophyll is a molecule highly sensitive to light, it is also undergoing biochemical, morphological and physiological changes as the leaves acclimate to a different light intensity, resulting in mature leaves that have similar characteristics as shade leaves and new ones as sun leaves. Lambers et al. (1998) documented higher chlorophyll content per unit fresh mass as one of the differences between sun and shade leaves in most species, mainly due to higher number of mesophyll cells, although the responses vary among species (Lambers et al. 1998; Hallik et al. 2009). This discrepancy is compensated for by thicker shade leaves so that chlorophyll content per unit leaf area is the same between two leaf types.

Additionally, the acclimation to different irradiance influences its partitioning between chlorophyll a and b, reflected in the chlorophyll a/b ratio. A higher ratio indicates an investment in light harvesting complex and a divestment from the reaction centers (Evans 1989). Leal and Thomas (2003) also found higher chlorophyll a/b ratio in top-canopy *Pinus strobus* needles compared to lower ones. The difference in chlorophyll content may vary among species, but chlorophyll a/b ratio is fairly indicative of responses to light availability. Therefore, based on

their position on the stem and biochemistry, new and old *C. calyculata* leaves in our experiment appeared to behave similarly to sun and shade leaves.

Besides differential light availability, nutrient addition also interacts with the leaf age and intensifies the difference between control and fertilized plots in terms of chlorophyll content and a/b ratio. Vegetation surveys on the same fertilization experiment showed that vascular plants are growing denser and taller in response to nutrient addition (Juutinen et al. 2010). A denser shrub layer might cause the vertical gradient of light to be more extreme in fertilized plots, resulting in higher chlorophyll content in 5N, 5NPK, 20N and 20NPK compared to control, in addition to the higher chlorophyll content in new compared to old leaves. This deduction is supported by the increase in chlorophyll a/b ratio between control and four fertilized treatments.

Light harvesting capacity

More resources from chlorophyll content did not, however, translate into higher chlorophyll fluorescence due to the lack of a relationship between chlorophyll content and chlorophyll fluorescence (Figure 11a). Fv/Fm did not differ across treatments, and its treatment averages all stayed in the range for healthy leaves (0.75 to 0.83), which indicates that leaves in all treatments are not under stress, at least in terms of light harvesting capacity (Figure 5b).

Fv/Fm ratios of both new and old leaves, when pooled across treatments, were within the range of healthy leaves, so the small but statistically significant difference (0.02) indicates a slightly lower efficiency of new leaves in harvesting

light and transferring it into photochemistry than old leaves. Similar trends of minimally but significantly lower ratios in young, new leaves can be seen in other evergreen species of various ecosystems: rainforest tree *Dryobalanops aromatic* (Ishida et al. 1999), California chaparral shrub *Heteromeles arbutifolia* (Valladares and Pearcy 1999), tropical shrub *Prosopis juliflora* (Shirke 2001). Additionally, upper-canopy leaves are also exposed to higher irradiance and therefore higher risk of photoinhibition (Valladares and Pearcy 1999), so the lower photochemical efficiency can be a combined effect of immaturity and canopy position. However, the association of new and old leaves to sun and shade leaves by itself cannot explain the observed trend, as sun leaves have been shown to have higher Fv/Fm ratios than shade ones (Sarijeva et al. 2007) and lowered ratios due to shading effect (Khan et al. 2000).

Age specific response to nutrient addition, however, exhibited a trend of increased Fv/Fm along treatment in old leaves but not new ones (Figure 5c). This trend is parallel with the lower Fv/Fm in pooled new than old leaves due to physiological immaturity. Current year leaves might have not perfected their photosynthetic apparatus, therefore responded less actively to nutrient addition compared to old leaves. Additionally, due to the extended period of drought in summer 2012 and the instantaneous nature of chlorophyll fluorescence measurement, Fv/Fm in new leaves might have reflected the lower tolerance of new leaves to low water table. On the other hand, old, mature leaves might have acclimated to adverse environmental conditions in the bog and been exposed to high level of nutrients for longer, therefore exhibited increased Fv/Fm as do

conifers in various fertilization experiments. Many authors have shown significantly increased Fv/Fm ratios, especially to N addition and to a lesser extent to P addition in *Pinus radiate* (Bown et al. 2009), *Pinus taeda* (Gough et al. 2004), *Picea abies* (Strand 1997) or *Pinus sylvestris* (Wang and Kellomaki 1997).

Photosynthetic potentials

The two variables addressing photosynthetic potentials in this study are Jmax and Vcmax, each corresponding nicely with the light and dark reactions of photosynthesis as well as the two main sinks of nitrogen. These two photosynthetic parameters are heavily dependent on nitrogen-rich molecules, chlorophyll and Rubisco enzyme. Jmax indicates how fast the products of the light reaction, ATP and NADPH, are used in regenerating RuBP, while Vcmax signifies the maximum rate at which enzyme Rubisco fixes CO_2 to RuBP.

The two parameters' increase with fertilization even after twelve years of fertilization indicates the positive effect of N, P and K addition (Figure 7b and 8b). The effect was more pronounced between different levels of N, while addition of P and K resulted in insignificant and inconsistent differences among the rates. It makes physiological sense that higher inputs of nutrients, particularly N, improve the photosynthetic potentials as it is invested in these compounds (Evans 1989). Past studies have also reported similar trends in other long-term fertilization experiments in other evergreen species (Maroco et al. 2002; Ainsworth et al. 2003). The correlation between the resource-indicative variable, chlorophyll content, and both Jmax and Vcmax further confirms the active investment of added nutrients into both light and dark components of the

photosynthetic machinery, and the plastic responses of photosynthetic potentials to increased available nutrients.

In both control and fertilized treatments, both Jmax (Figure 8a, c) and Vcmax (Figure 7a, c) did not differ significantly between leaf ages, regardless of treatments. The similar Jmax rates between old and new leaves do not agree with a study by Niinemets et al. (2006), which examined old and new leaves of *Quercus ilex* in terms of light availability to compare their photosynthetic potentials. Jmax in their study increased significantly along both light gradient condition during measurement and growth, and potentials tend to decrease with increasing leaf age at the same light intensity. The observed difference in leaf age, therefore, was not attributable to different light availabilities. Vcmax rates' independence of leaf age (Figure 7a, c), however, is generally supported by literature. Niinemets et al. (2006) showed a relationship between Vcmax and light intensity with similar slope among various-aged leaves. They also demonstrated a stronger effect of current light availability in the chamber rather than previous conditions on the photosynthetic potentials, and the association with the partitioning of N and other nutrients to different leaf ages. In my study, both parameters were estimated based on various photosynthetic rates in constant, saturating light. Therefore, it is likely that similar rates in Jmax and Vcmax between new and old leaves are due to the same measurement irradiance rather than the different growing irradiance.

Leaf-level CO_2 gas exchange

Given the improved capacity of all nitrogen-demanding components of the photosynthetic machinery in response to nutrient addition, we could expect an increase in foliar N content and therefore an increase in Amax. Additionally, at the ecosystem level, after the *Sphagnum* layer died, the shrubs grew significantly taller (Juutinen et al. 2010). This increase in biomass requires more photosynthetic products, which prompts us to expect more photosynthetic activities, or higher Amax and Pmax. However, no change in net assimilation rate was observed, and Amax was lowest in the highest treatment, 20NPK (Figure 5b). Literature shows inconsistent responses of Amax to nutrient addition in nutrient-poor ecosystems: Lajtha and Whitford (1989), Bowman et al. (1995), Gerdol et al. (2002), Arens et al. (2008) and St. Clair et al. (2009) reported an increase in Amax with nutrient addition, or increasing foliar N content; Karlsson (1985), Bubier et al. (2011) and Heskel et al. (2012) found no change, while Bigger and Oechel (1982) and Bauer et al. (2004) found a decline in Amax. While increased Amax is expected, the decoupled photosynthetic capacity was attributed to nitrogen saturation (Aber et al. 1998) where nitrogen is channeled into nonphotosynthetic proteins, for example amino acids, instead of invested in photosynthetic machinery (Bubier et al. 2011). This possibility, however, could not explain the enhanced photosynthetic potentials, Jmax and Vcmax, under high nutrient loads in our leaves, as these increases indicate that nitrogen is still being used in photosynthetic apparatus.

Net assimilation rates (Amax) were broken down into maximum gross photosynthetic rate (Pmax) and dark respiration (Adark). Respiration increased along the treatment gradient, and the highest dark respiration rate in 20NPK accounted for the lowest Amax in this treatment. Pmax varied much less than Amax (Figure 6b). The variation in net leaf level gas exchange is, therefore, mainly the result of nutrient addition on leaf respiration, and fertilization had no or slightly negative effect on gross photosynthesis.

The increased dark respiration with nutrient addition was quite consistent with general literature that finds a global linear relationship between leaf N content and leaf-level dark respiration rates, despite interspecific differences (Ryan 1995; Reich et al. 1996; Reich et al. 1998; Maroco et al. 2002; Heskel et al. 2012). Leaf respiration is another protein-demanding process that is limited under natural nutrient availability, especially N. Nutrient addition increases quantity of N-rich proteins like Rubisco and chlorophyll and their photosynthetic potentials (Jmax and Vcmax); therefore it is reasonable to expect elevated respiration to mobilize newly available nutrients and maintain these increasingly active proteins (Ryan 1995). Additionally, added nutrients might also benefit enzymes in the respiration process itself. Heskel et al. (2012) also studied the organelle structure of fertilized tundra species and found expanded volume of mitochondria that can partly account for the increased respiration rate.

Pmax was calculated from Amax and Adark, and a marginally significant trend was found where control has higher Pmax than all other fertilized treatments. It is quite unexpected that although there were increased photosynthetic potentials in

both light and dark reactions, they do not translate into higher photosynthetic rates. Pmax was derived from the sum of Amax and Adark rates under the assumption that maximal respiration potential is reached in the dark. However, this may not be the best method to measure maximum gross photosynthetic rates. Some studies instead use the ^{14}C labeling technique (Karlsson 1985; Bigger and Oechel 1982) to measure Pmax. This method is preferable because it eliminates the probability of photorespiration. At high temperature, Rubisco could fix oxygen rather than CO_2 in the dark using energy from the light reaction, and produce molecules toxic to the leaves as well as CO_2, a process known as photorespiration. By specifically labeling the CO_2 molecules, the technique distinguishes CO_2 released in respiration from that of photorespiration. Estimates of Pmax in the current study might include photorespiration, which could also respond to nutrient addition and add to the variation of the estimate.

Foliar Amax, Pmax and Adark can be associated with net CO_2 ecosystem exchange (NEE), gross ecosystem photosynthesis (GEP) and ecosystem respiration (ER). Gas exchange responses to fertilization at the leaf level mirror the responses at the ecosystem scale. Larmola et al. (2013, in prep.) reported the most recent ecosystem gas exchange measurements using chamber on the same fertilization plots, and found no change in GEP, a significant increase in ER, and an trend of declining NEE making the bog a weaker carbon sink. The similar pattern means that *C. calyculata* leaves are contributing to the ecosystem-level response, and although responses of components such as moss photosynthesis and respiration as well as microbial respiration and decomposition of organic matter

might be different, the dominant shrub's response is somewhat indicative of the ecosystem level.

Comparing across leaf age, Amax, Adark and Pmax did not differ between old and new leaves either within treatment or when all leaves are pooled regardless of treatment. Considering the connection between leaf age and light availability, we would expect some differences in Amax, as different photosynthetic capacity is one of the distinguishing characteristics of leaves grown in different irradiance (Lambers et al. 1998). It is expected that shade leaves, in the process of maximizing light harvest, compromise their photosynthetic capacity (Lambers et al. 1998). However, Niinemets and Sack (2006) outlined Amax as a component of a complex network of interactions between area-based and mass-based leaf traits where leaf dry mass per unit area (M_A) is the structural trait linking them all. The difference in light availability and developmental maturity between old and new leaves result in thicker leaves, and higher Amax on an area basis, although the result can be different when photosynthetic capacity is investigated per unit mass (Ackerly and Bazzaz 1995; Kitajima et al. 1997). The measurement of Amax on an area basis in my study, therefore, might not be the best demonstration of photosynthetic capacity. In addition, nutrient addition might affect nutrient use strategy of the shrub and have an impact on M_A, a response not captured in this study.

Many studies show a gradient of decreasing Amax with increasing leaf age attributable to both the reallocation of nutrients, especially nitrogen among new and old leaves (Hikosaka et al. 1994) and self-shading effect (Kitajima et al.

1997). The fact that there was no significant difference in Amax between my old and new *C. calyculata* leaves might be the results of various interactions among leaf age, shading effect, and nutrient addition effect. Hikosaka et al. (1994) found that the N content gradient across increasing-aged leaves observed under low nutrient conditions disappeared when the vine *Ipomoea tricolor* was exposed to high nitrate concentration. Similar to Pmax and Amax, no significant differences were found in Vcmax and Jmax between my old and new leaves, suggesting some common underlying mechanism.

Interannual variation – Comparison to 2008 study at Mer Bleue

In 2008, a fairly similar set of measurements were conducted on the same fertilization experiment on three dominant shrub species: *Chamaedaphne calyculata*, *Ledum groenlandicum* and *Vaccinium myrtilloides*. These data, which had in common with my current study chlorophyll content, Amax, Vcmax and Jmax, were published by Bubier et al. (2011). Comparison of values and trends between this study and Bubier et al. 2011 offers more insights into the photosynthetic performance of *C. calyculata* and the significance of environmental conditions and interannual variability, particularly with water table depth.

In terms of foliar chemistry, chlorophyll content in 2008 was reported on a dry weight basis, compared to the fresh weight in my study. Unavailable moisture content makes comparison more difficult, but treatment effect was consistent after four years. The fact that foliar N content increased with treatment and strongly

correlated with chlorophyll content (Bubier et al. 2011) provided more foundation for the assumption of increased leaf N along the treatment gradient in our study.

Bubier et al. (2011) also did not find any significant treatment effect on Amax, but measured average Amax rates ranging from 8.6 to 12.9 μmol CO_2 m^{-2} s^{-1}, while this study's average rates are between 2.82 and 5.70 μmol CO_2 m^{-2} s^{-1} (Table 4). The discrepancy is quite large, but when net CO_2 ecosystem exchange of the entire bog using eddy covariance tower was compared between the two years, a similar difference was detected. Cumulative NEE in 2008 was 138 g C m^{-2}, twice as high as that in 2012, 69 g C m^{-2}. Although cumulative NEE also includes photosynthesis of mosses and vascular plants other than *C. calyculata*, respiration of plants, animals and microbial communities and decomposition, the two-fold differences in gas exchange at both ecosystem and leaf level suggests differences in environmental conditions. The Li-Cor 6400 was able to control for light, temperature, humidity and CO_2 concentration, but environmental variables such as soil moisture were not accounted for. In fact, summer 2012 was an exceptionally dry year, with average June-to-August air temperature at 18.4^{o}C (compared to 17.5^{o}C in 2008) and lowest water table depth at 80cm, (compared to 50cm in 2008) (E. Humphreys, personal communication, March 7, 2013). Lowering water table has been seen to lower the carbon sink function of peatlands, or even shift them into carbon sources (Bubier et al. 1999). The lower Amax in 2012 could be mainly attributed to the drought and subsequent water table drop, although other factors that we are unaware of might also be contributing. A two-fold variation in values between years is also found in

Vcmax, and could be accounted for by the same interannual variability, because Vcmax was derived from the CO_2 response curve. However, the fairly similar Jmax values between two years could not be explained.

Bubier et al. (2011) found no significant treatment effect in Amax, Jmax or Vcmax, but instead an increase in concentrations of certain storage amino acids such as alanine and GABA (Bauer et al. 2004; Limpens and Berendse 2003). Therefore, the lack of increase in photosynthetic parameters was accounted for by the fact that leaves stop investing foliar N into photosynthetic machinery but instead store them as amino acids, a common physiological response to N saturation (Magill et al. 1997; Minocha et al. 2000). However, the current study found an increase in both components of photosynthesis, Jmax and Vcmax, yet no change in Amax. Discrepancies in the photosynthetic potentials question the actual potential of the light and dark reactions and environmental controls of the method of measurement. It is likely that the extreme drop in water table in 2012 caused the shrub to react differently to nutrient addition. For example, dry conditions and increased respiration exert additional pressure on the shrub and significantly lowered its photosynthetic capacity. Under more constrained conditions, there might be higher demands for nutrients, allowing higher nutrient treatments to utilize their extra source of N, P and K to maximize both light harvest and carbon assimilation.

Bubier et al. (2011) also investigated specific leaf area (SLA), the inverse of leaf dry mass per unit area (M_A), and found no change across treatments. Photosynthetic parameters, therefore, did not differ when considered on either

mass or area basis. Considering the importance of SLA and the use of both mass and area base photosynthetic parameters in the literature, the lack of measurements SLA in my study prevents further comparison and understanding of photosynthetic performance in response to both nutrient addition and leaf age.

Study limitations

Besides the insights into photosynthetic performance of *C. calyculata* that the data provided, it is necessary to understand other sources of errors and limitations of the study. Although the leaf chamber should be able to control most environmental variables, in a few hot days during the growing season where air temperature exceeded 35^{o}C, temperature control of the chamber could not work as efficiently. Additionally, temporal variations were only accounted for in the statistical analyses to reduce variability. This factor is almost always significant in most analyses, indicating high level of variability. Microtopography, which also has a big impact on each individual plant, could not be taken into account. Although water level was incorporated in most analyses, it did not fully account for seasonal changes in environmental conditions that the leaf chamber could not control.

Future study

Currently, all estimates of treatment effect were done under the assumption that foliar N content increased along the treatment gradient. Considering the universal relationship between N content and many photosynthetic parameters, measurements of this leaf biochemical variable will complete the story and

possibly lend more insights into the specific response of many variables. Specific investigation of N partitioning among major sinks, including measurements of Rubisco content and storage amino acids, would also clarify the observed trends and track down the main source of leaf nitrogen after long-term exposure to nutrients.

The increase in photosynthetic potentials, Jmax and Vcmax, but unchanged photosynthetic capacities, Amax and Pmax, warrant more studies into potential leakages of photosynthetic products, for example photorespiration, in response to nutrient addition. Currently, the stress response was only studied in the form of maximal light harvesting capacity. Further investigation of light-adapted chlorophyll fluorescence and its connection to gas exchange measurements can improve our understanding of the stress response not only in its potential but also during active photosynthesis. Measurements of non-photosynthetic quenching, or quantum yield of photosystem II, for example, can reveal more physiological understanding of light harvest under normal conditions as well as potential for recovery from photoinhibition.

With the connection between leaf-level and ecosystem gas exchange measurements, studies attempting to scale up the leaf-level to canopy-level responses might reveal interesting results. Determining how much of the ecosystem-level gas exchange rates are accounted for by shrubs can direct future studies to focus on other components of the ecosystem and address the main source of the observed decline in sink function of the peatland.

CONCLUSIONS

The study examined the photosynthetic performance of *Chamaedaphne calyculata* after exposure to twelve years of nutrient addition. The shrubs were evaluated in terms of leaf chemistry, chlorophyll fluorescence, photosynthetic potentials, and leaf-level gas exchange in old and new leaves experiencing five and twenty times the growing season ambient wet N deposition, with and without P and K. Increase in chlorophyll content in response to fertilization indicated an investment of added nutrients to this photosynthetic pigment, and chlorophyll fluorescence showed that fertilization had no negative effects on light harvesting capacity. Parameters related to the light reaction of photosynthesis such as chlorophyll content, chlorophyll a/b ratio and fluorescence showed age differences and age-specific treatment effects, which can be attributed either to the direct influence of nutrient addition, or to the indirect effects of light availability and differential physiological maturity.

This study invested photosynthetic potentials by evaluating age and treatment effect of Vcmax and Jmax derived from the CO_2 response curve. The two parameters represent the light and dark reactions of photosynthesis; therefore their increasing trend along the treatment gradient indicated investment of nutrients into both of these processes, which are quite nutrient-demanding. The increased potentials, however, did not translate to increased photosynthesis when leaf-level gas exchange was examined. Amax did not differ across treatments, with a trend

of decreasing Amax at the highest nutrient treatment, which could be explained by increased leaf-level respiration (Adark) and unchanged Pmax. The leaf-level trend mirrored the ecosystem scale, with the bog becoming a weaker carbon sink in response to fertilization due to unchanged ecosystem photosynthesis and increased ecosystem respiration. The results extend our understanding of the leaf-level contribution of the dominant shrub to the ecosystem-level responses in terms of CO_2 gas exchange, and lend more insights into the performance of specific components of the ecosystem in response to altered nutrient balance. The study can, therefore, contribute to better predictions of ecosystem responses to long-term nutrient addition by providing detailed understanding of the shrub layer. For example, modelers can have a better sense of how the carbon budget shifts when nutrient availability and vegetation composition changes.

The results also demonstrate the extensive impact of nutrient addition on every component of the photosynthetic apparatus. Nitrogen, and in a lesser extent P and K, have far-reaching impacts on these shrubs that have been well-adapted to a nutrient-poor environment. The mismatch between photosynthetic potentials and the gas exchange measurements at ambient conditions warrants more research into the physiological responses of the shrub to nutrient addition. In particular, physiological stress to other components of photosynthesis should be investigated, while considering interannual variability and its impacts on photosynthetic performance.

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FIGURES

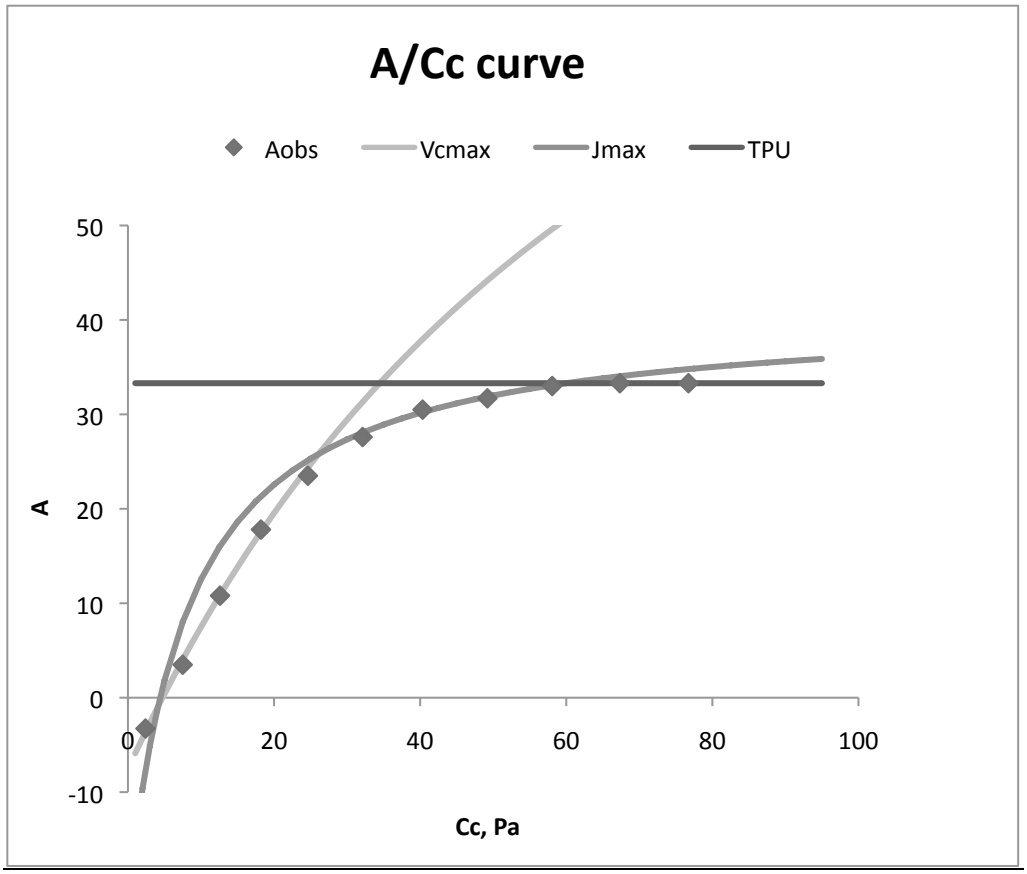

Figure 1. Sample CO_2 response curve and designated estimate of Vcmax, Jmax and TPU. Adapted from Sharkey et al. (2007)

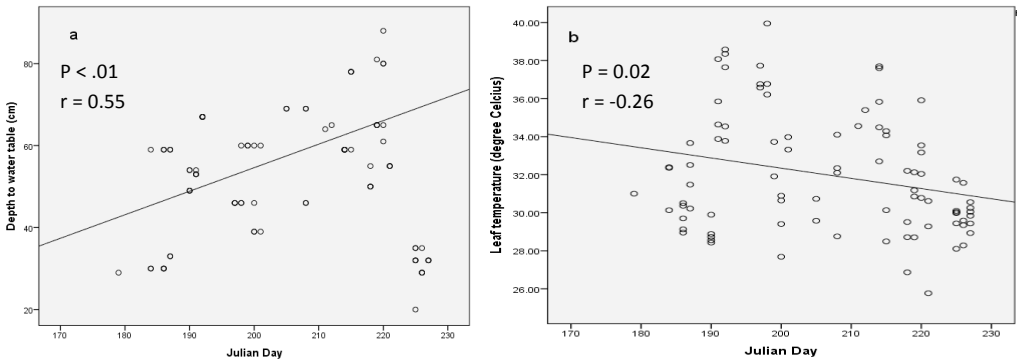

Figure 2. Relationship between Julian Day and **a)** depth to water table before the precipitation event on August 10th; **b)** leaf temperature. Line-of-best-fit and correlation coefficient (r) are added for significant relationships.

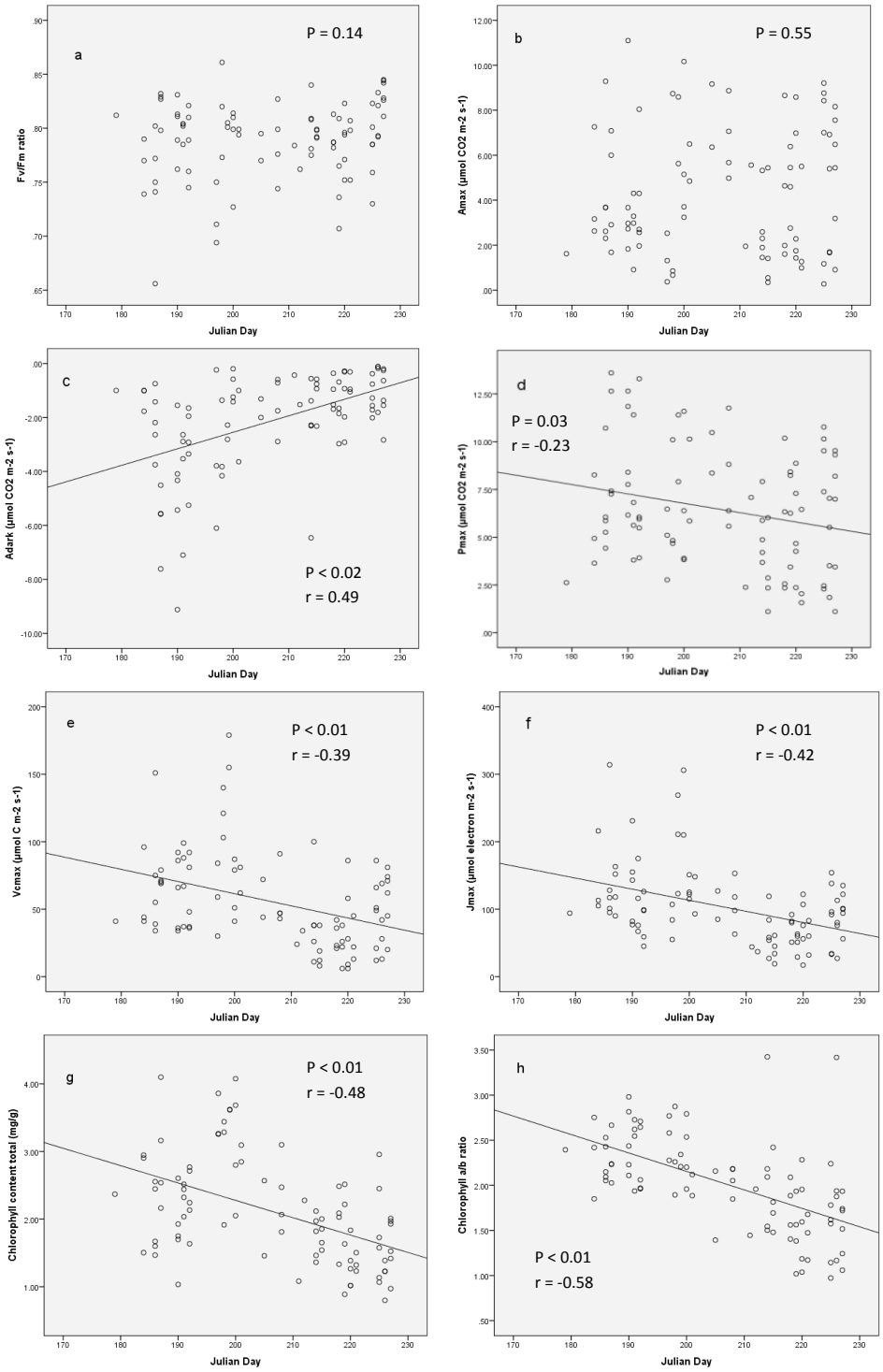

Figure 3. Scatterplot showing seasonal patterns in **a)** chlorophyll fluorescence (Fv/Fm); **b)** net assimilation rate (Amax); **c)** dark respiration (Adark); **d)** maximum gross photosynthetic rate (Pmax); **e)** maximum Rubisco carboxylation rate (Vcmax); **f)** maximum electron transport rate (Jmax); **g)** chlorophyll content;

h) chlorophyll a/b ratio. Line-of-best-fit and correlation coefficient (r) are added for significant relationships.

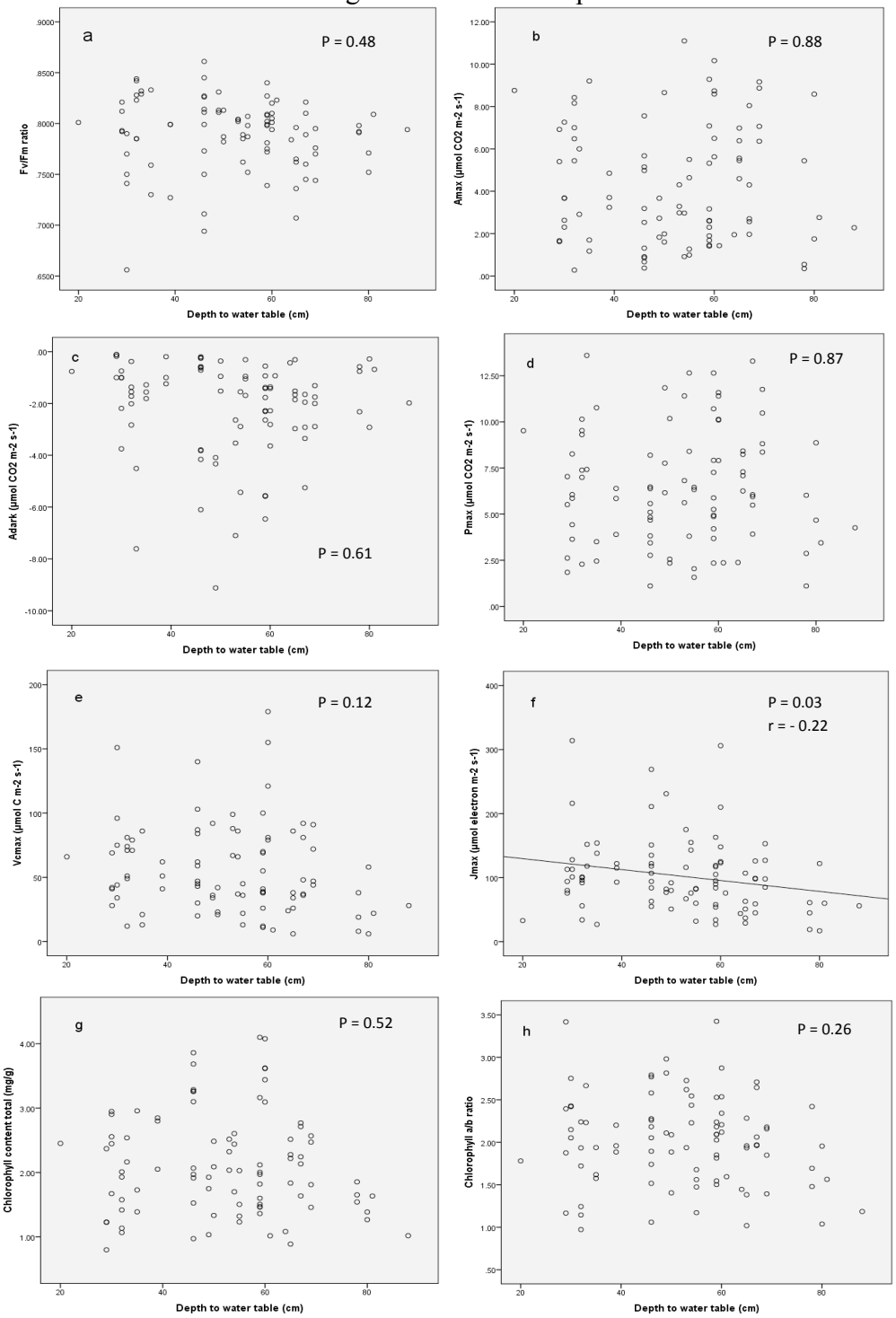

Figure 4. Scatter plots showing the relationship between depth to water table and **a)** chlorophyll fluorescence (Fv/Fm); **b)** net assimilation rate (Amax); **c)** dark respiration (Adark); **d)** maximum gross photosynthetic rate (Pmax); **e)** maximum Rubisco carboxylation rate (Vcmax); **f)** maximum electron transport rate (Jmax);

g) chlorophyll content; **h)** chlorophyll a/b ratio. Line-of-best-fit and correlation coefficient (r) are added for significant relationships.

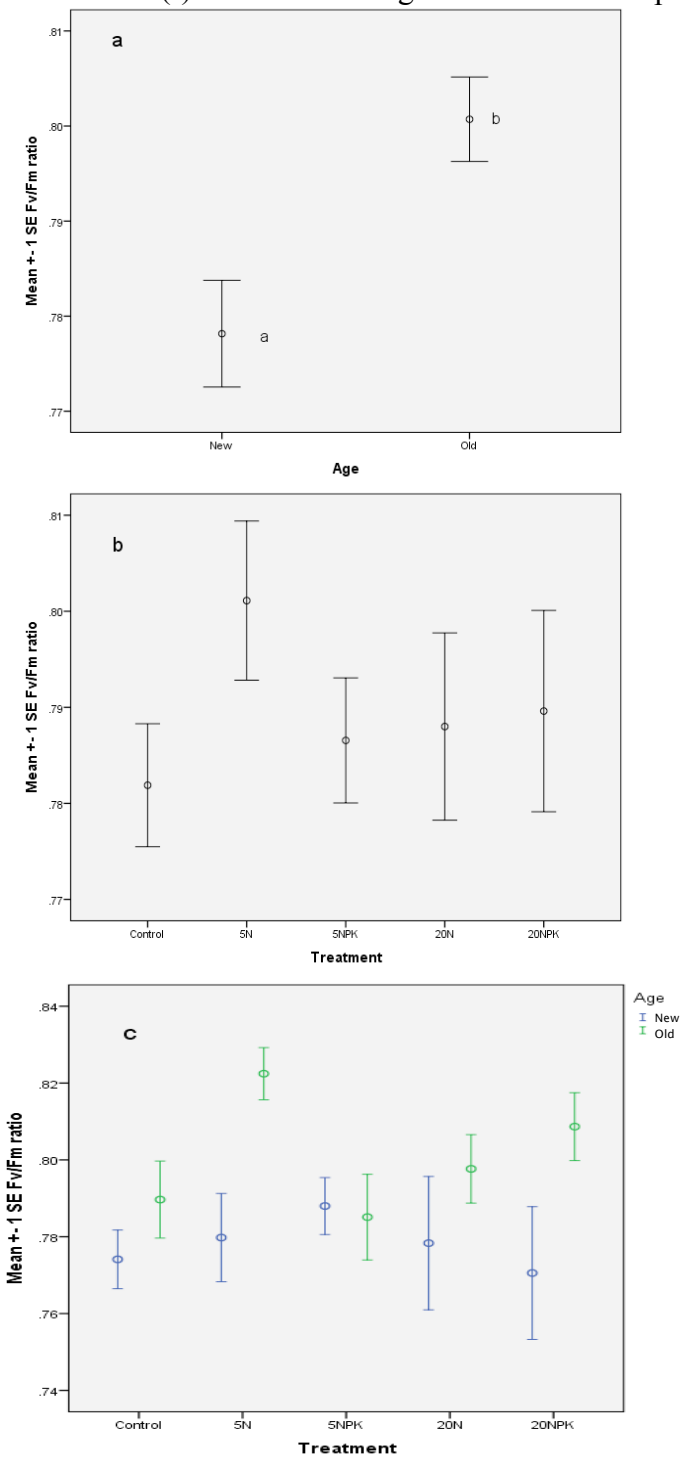

Figure 5. Average chlorophyll fluorescence ratios (Fv/Fm) **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in new and old leaves. Error bars indicate ±1SE. Groups that share letters are not significantly different.

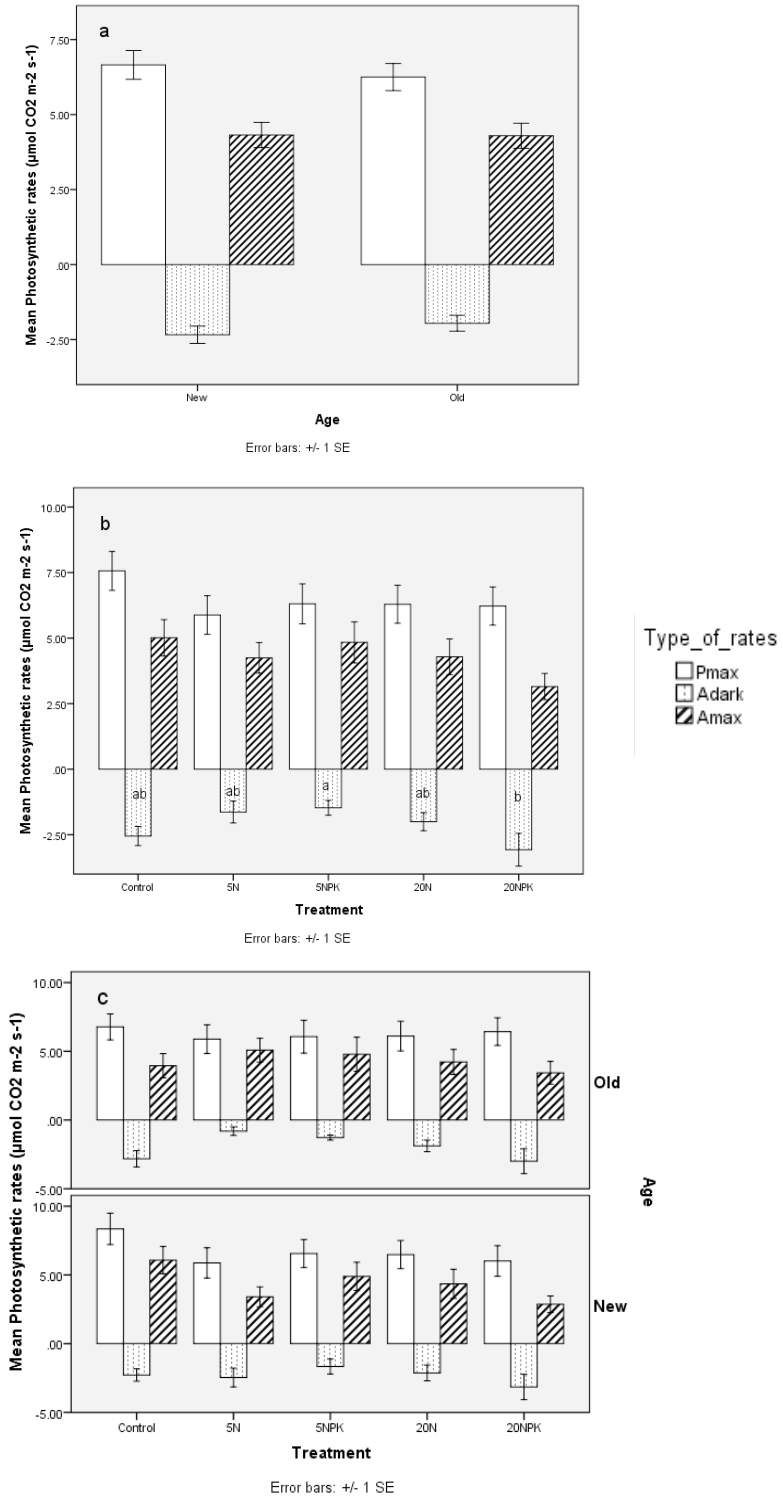

Figure 6. Average maximum gross photosynthetic rate (Pmax), dark respiration (Adark), and net assimilation rate (Amax) **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in new and old

leaves. Error bars indicate ±1SE. Groups that share letters are not significantly different.

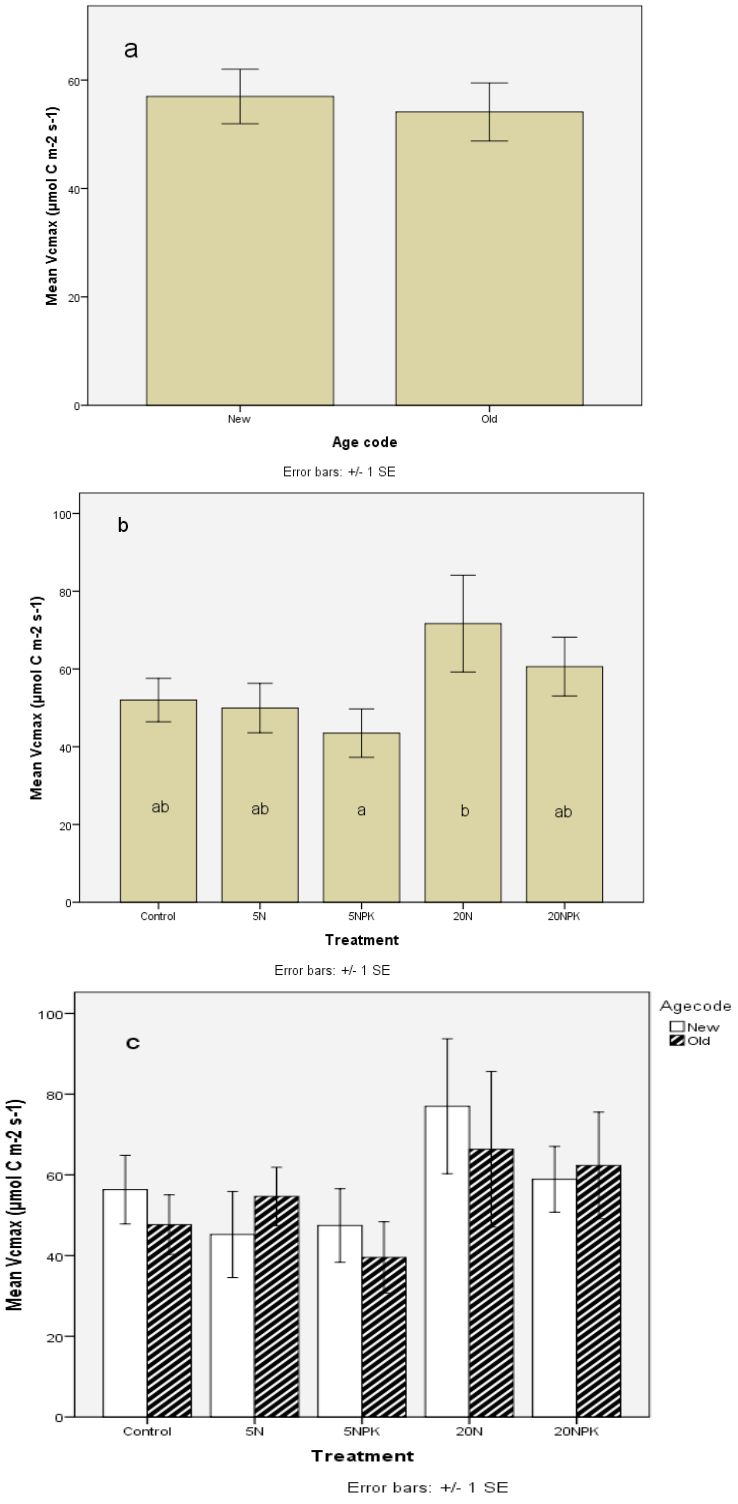

Figure 7. Average maximum Rubisco carboxylation rates (Vcmax) **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in

new and old leaves. Error bars indicate ±1SE. Groups that share letters are not significantly different.

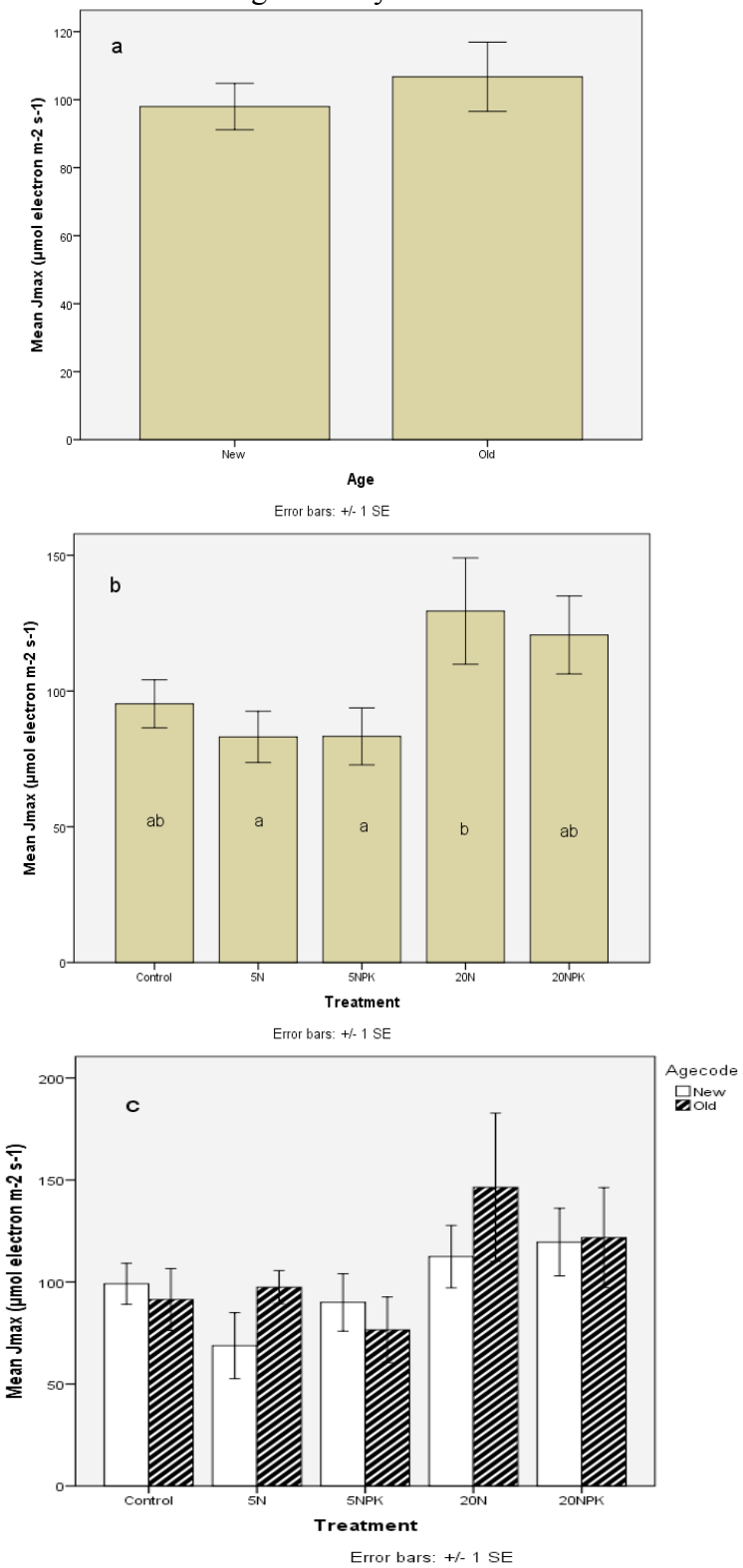

Figure 8. Average maximum electron transport rate (Jmax) **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in new and

old leaves. Error bars indicate ± 1SE. Groups that share letters are not significantly different.

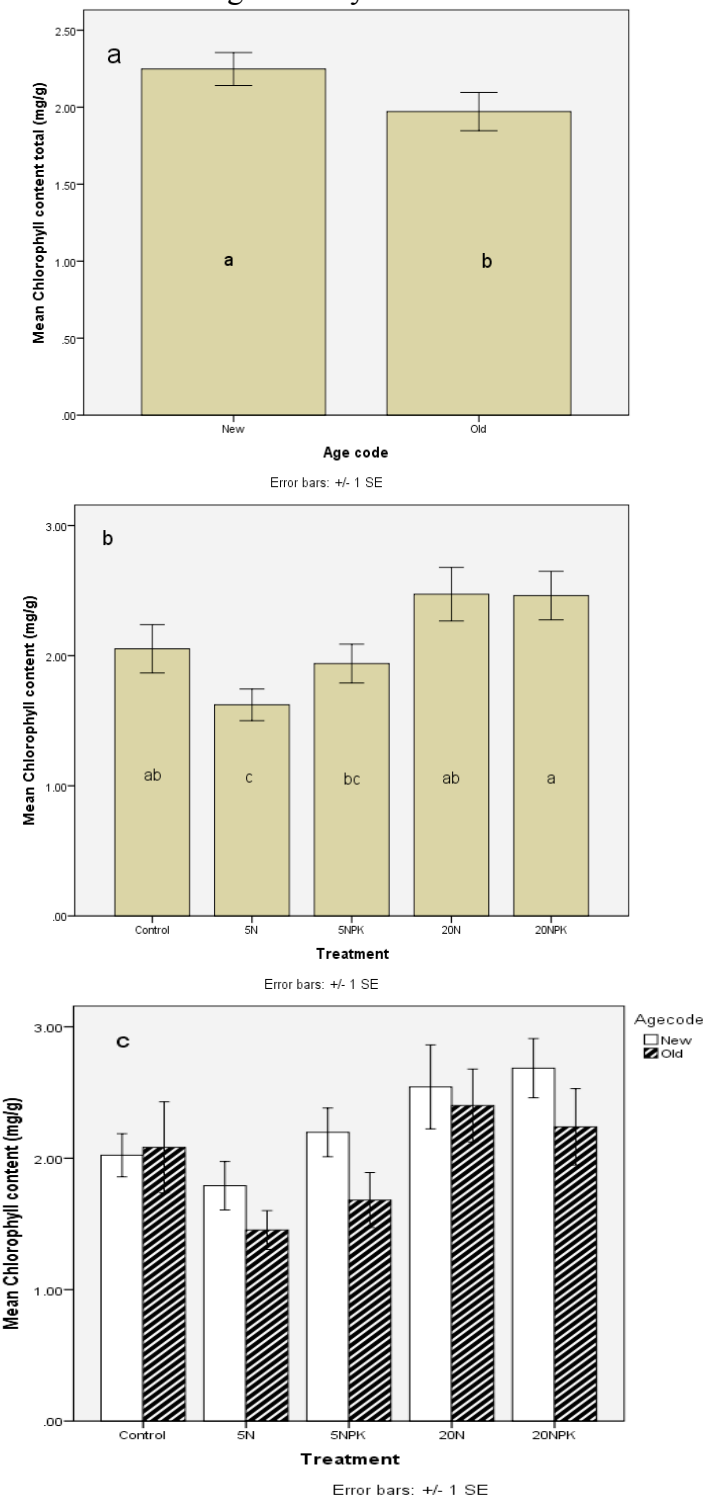

Figure 9. Average total chlorophyll content **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in new and old

leaves. Error bars indicate ± 1SE. Groups that share letters are not significantly different.

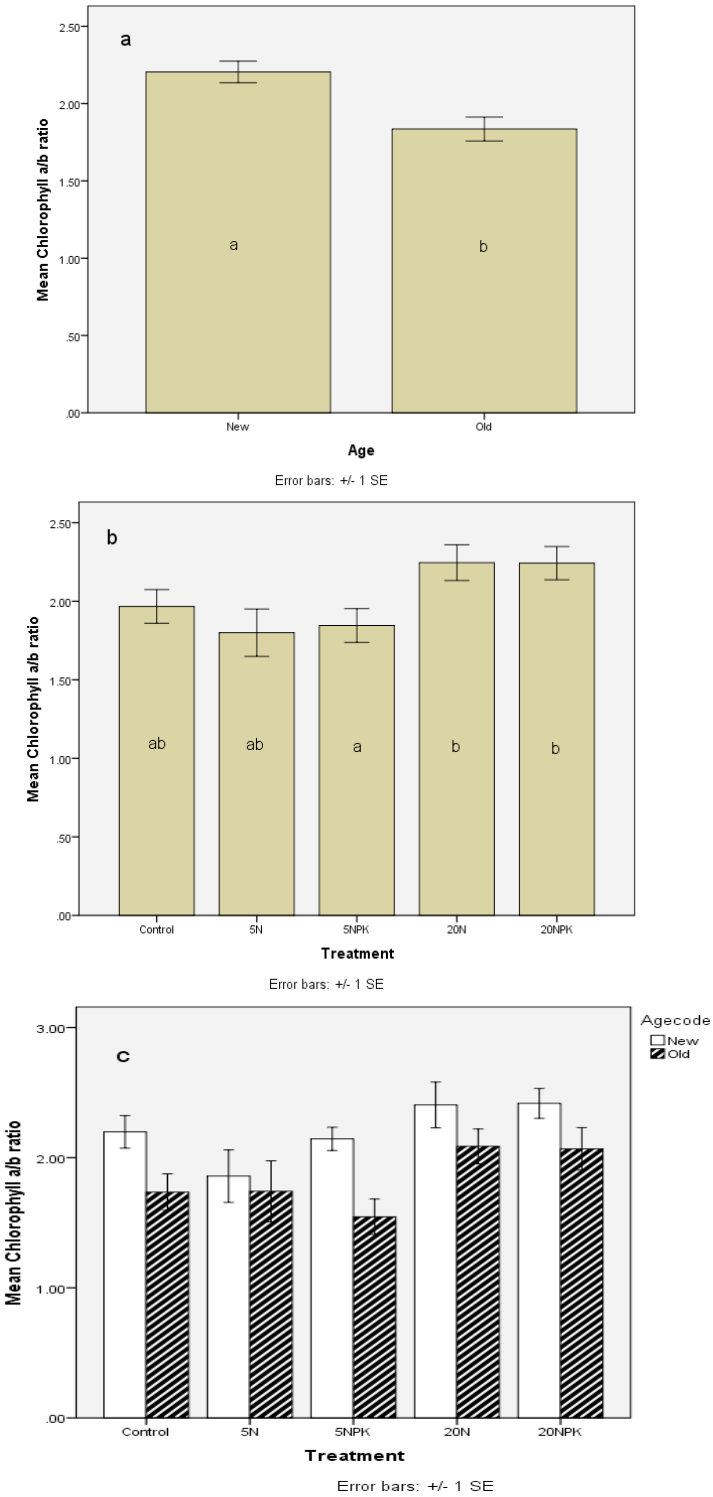

Figure 10. Average total chlorophyll a/b ratio **a)** between new and old *Chamaedaphne* leaves; **b)** among treatments; **c)** among treatments in new and old

leaves. Error bars indicate ±1SE. Groups that share letters are not significantly different.

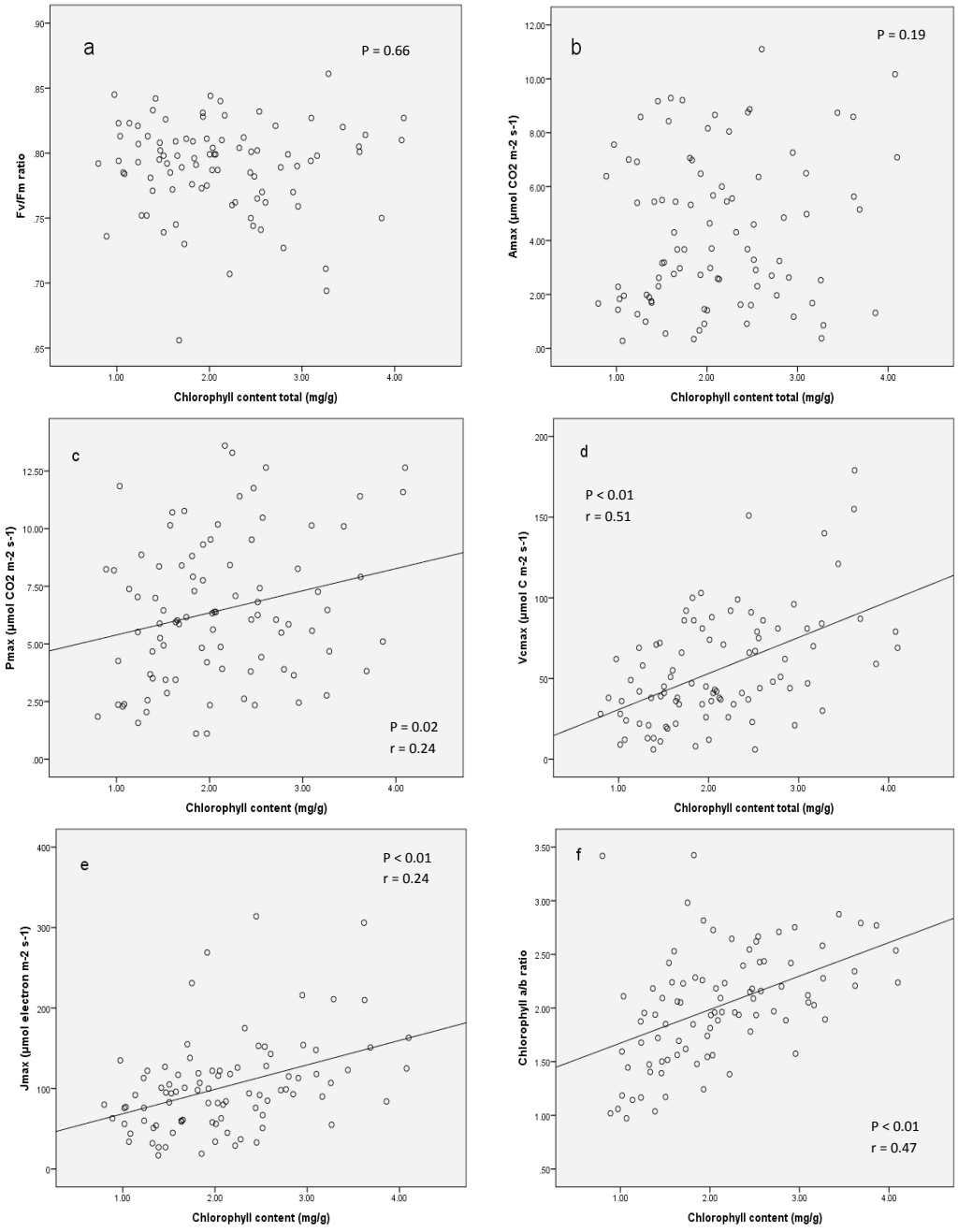

Figure 11. Scatterplot indicating the relationship between chlorophyll content and **a)** chlorophyll fluorescence (Fv/Fm); **b)** net assimilation rate (Amax); **c)** maximum gross photosynthetic rate (Pmax), **d)** maximum Rubisco carboxylation rate (Vcmax); **e)** maximum electron transport rate (Jmax); **f)** chlorophyll a/b ratio.

Line-of-best-fit and correlation coefficient (r) are added for significant relationships.

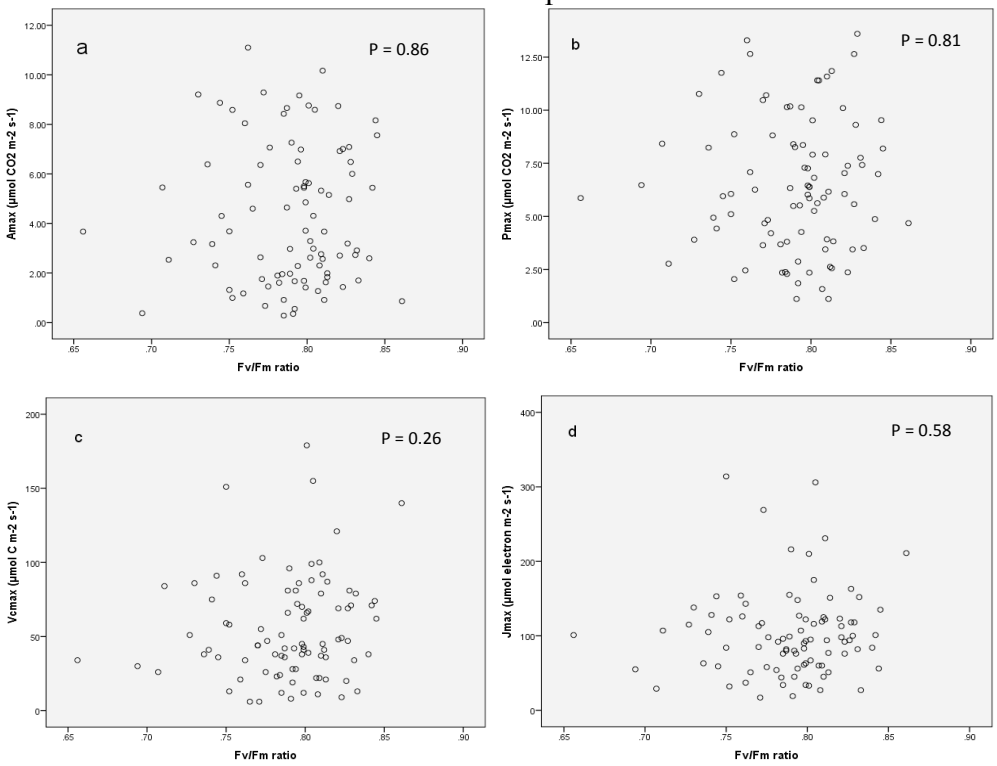

Figure 12. Scatterplot indicating the relationship between chlorophyll fluorescence and **a)** net assimilation rate (Amax); **b)** maximum gross photosynthetic rate (Pmax); **c)** maximum Rubisco carboxylation rate (Vcmax); **d)** maximum electron transport rate (Jmax). Line-of-best-fit and correlation coefficient (r) are added for significant relationships.

TABLES

Table 1. Glossary of terms/ variables used in the study

Term	Variable name	Measurement	Unit
A	Assimilation rate	Calculated from change in CO_2 concentration in chamber	µmol CO_2 m^{-2} s^{-1}
Amax	Net assimilation rate / Photosynthetic capacity	Photosynthetic rate at saturating light, ambient temperature, CO_2 and humidity	µmol CO_2 m^{-2} s^{-1}
Adark	Dark respiration rate	Photosynthetic rate at no light, ambient temperature, CO_2 and humidity	µmol CO_2 m^{-2} s^{-1}
Ci	Internal CO_2 concentration	Calculated from CO_2 concentration in chamber and stomatal conductance	ppm
Ca	CO_2 partial pressure at Rubisco	Calculated from Ci	Pa
Fv/Fm	Chlorophyll fluorescence/ maximum light harvesting capacity	□□–□□□□	
Fo	Minimum fluorescence	Fluorescence of dark-adapted leaf	Fluorescence unit (FU)
Fm	Maximum fluorescence	Fluorescence after flashing dark-adapted leaf with high-intensity light	FU
Jmax	Maximum electron transport rate	Derive from CO_2 response curve at high internal CO_2 concentration	µmol electron m^{-2} s^{-1}
Pmax	Maximum gross photosynthetic rate	Amax – Adark	µmol CO_2 m^{-2} s^{-1}
Vcmax	Maximum Rubisco carboxylation rate	Derive from CO_2 response curve at low internal CO_2 concentration	µmol C m^{-2} s^{-1}

Table 2. Experimental set-up with NPK fertilization levels equal to 5 and 20 times the ambient growing season wet N deposition (0.8 g N m^{-2}). Fertilization began in Controls, 5Ns, 5NPKs and 20NPKs in 2000-2001, and 20Ns in 2005. Nitrogen was provided as both NO_3^- and NH_4^+.

Treatment	**N (g m^{-2} a^{-1})**		**P (g m^{-2} a^{-1})**		**K (g m^{-2} a^{-1})**	
	Annual	**Cumulative**	**Annual**	**Cumulative**	**Annual**	**Cumulative**
Control	0	0	0	0	0	0
5N	1.6	19.2	0	0	0	0
5NPK	1.6	19.2	6.3	75.6	5	60
20N	6.4	44.8	0	0	0	0
20NPK	6.4	76.8	6.3	75.6	5	60

Table 3. Summary of mean ± SE of chlorophyll fluorescence (Fv/Fm), chlorophyll content and chlorophyll a/b ratio in new and old leaves.

Age	Treatment	Fv/Fm	Chlorophyll content (mg/g)	Chlorophyll a/b ratio
New	Control	0.77 ±0 .008	2.02 ± 0.16	2.20 ± 0.13
	5N	0.78 ± 0.010	1.79 ± 0.18	1.86 ± 0.20
	5NPK	0.79 ± 0.007	2.20 ± 0.19	2.14 ± 0.09
	20N	0.78 ± 0.020	2.54 ± 0.32	2.40 ± 0.18
	20NPK	0.77 ± 0.020	2.69 ± 0.23	2.42 ± 0.11
Old	Control	0.79 ± 0.010	2.08 ± 0.35	1.74 ± 0.14
	5N	0.82 ± 0.007	1.45 ± 0.15	1.74 ± 0.23
	5NPK	0.79 ± 0.010	1.68 ± 0.21	1.55 ± 0.14
	20N	0.80 ± 0.009	2.40 ± 0.28	3.09 ± 0.13
	20NPK	0.81 ± 0.009	2.23 ± 0.29	2.07 ± 0.16
Both	Control	0.78 ± 0.006	2.05 ± 0.19	1.97 ± 0.11
	5N	0.80 ± 0.008	1.62 ± 0.12	1.80 ± 0.15
	5NPK	0.79 ± 0.007	1.94 ± 0.15	1.84 ± 0.11
	20N	0.79 ± 0.010	2.47 ± 0.21	2.24 ± 0.11
	20NPK	0.79 ± 0.010	2.46 ± 0.19	2.24 ± 0.11