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Biogeochemical Implications of Plant-Soil Interactions in Peatland Ecosystems

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Biogeochemical Implications of Plant-Soil Interactions in Peatland Ecosystems

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Summary

Models of global environmental change are dependent on accurate predictions of the processes regulating carbon (C) and nutrient cycling in terrestrial ecosystems. Currently much research is focused on peatlands since they contain one-third of the world's soil C stock and therefore alteration to the processes regulating C and nutrient cycling have the potential to feedback on predicted global climate change via carbon dioxide (CO_2) loss to the atmosphere.

This study examined the climatic/physicochemical factors that regulate biogeochemical cycling in *Sphagnum* dominated peatlands and whether *Sphagnum* productivity affects soil decomposition processes under current and future climate change scenarios. Field surveys of a riparian peatland and a blanket bog identified a number of climatic and physicochemical factors that regulate CO_2 fluxes and soil enzyme activities. Peatland mesocosm experiments identified the effect of *Sphagnum* productivity on net CO_2 ecosystem exchange and soil enzyme activities under current and future climate change scenarios.

From this thesis, the following conclusions could be drawn regarding plant and soil processes in peatland ecosystems: (1) temperature is the major control on CO_2 fluxes in peatland ecosystems although shifts in the water table can modify the effect; (2) *Sphagnum* and soil respiration represent approximately 50 % of ecosystem respiration and thus both are important for predicting respiratory losses from peatlands by global warming; (3) ecosystem respiration showed a greater sensitivity to temperature (Q_{10}) than estimated gross *Sphagnum* photosynthesis suggesting that global warming may lead to the release of CO_2 from blanket bogs; (4) elevated CO_2 and temperature forced net CO_2 sequestration in the short-term that may lead to an increase in the growing season; (5) the CO_2 fertilization effect may be limited by climatic and physicochemical factors particularly nutrient availability suggesting that in the long-term global warming may result in net CO_2 emission from peatlands; and (6) under current environmental conditions, *Sphagnum* productivity did not appear to affect *ex situ* measurements of soil decomposition but may affect *in situ* soil CO_2 respiration. However, an increase in *Sphagnum* CO_2 fixation by elevated CO_2 may increase soil enzyme activities that may feedback on plant and microbial growth by increasing nutrient availability and affect the C balance of northern peatland ecosystems in the future.

Overall, the results from this thesis suggest that multiple, interacting factors will determine the effect of climate change on the C balance of peatlands in the future. Positive feedback mechanisms such as the CO_2-plant-enzyme-nutrient interaction may have important implications for future C storage in these systems. The magnitude and duration of this indirect enzymic response to elevated CO_2 may be the key to the C balance of peatland ecosystems in the future. Without this mechanism, global warming will probably lead to the release of stored soil C to the atmosphere as CO_2 or DOC from northern peatlands.

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Finally, I would not have reached this far in higher education had it not been for the love and financial support of my parents Ros and Roger. This thesis is yours as much as it is mine.

Abbreviations

ANOVA	Analysis of variance
C	Carbon
Ca^{2+}	Calcium
CH_4	Methane
CO_2	Carbon dioxide
diqc	2, 3-dihydroindole-5,6-quinone-2-carboxylate
DOC	Dissolved organic carbon
GPP	Gross primary production
IR-GCMS	Isotope Ratio Gas Chromatography Mass Spectrometry
K^+	Potassium
L-DOPA	L-dihydroxy phenyalanine
Mg^{2+}	Magnesium
MUF	Methylumbelliferyl
N	Nitrogen
N_2O	Nitrous oxide
NEE	Net CO_2 ecosystem exchange
NEP	Net ecosystem production
NH_4^+	Ammonium
NO_3^-	Nitrate
NPP	Net primary production
O_2	Oxygen
P_g	Gross photosynthesis
P_n	Net photosynthesis
PO_4^{3+}	Phosphate
PPFD	Photosynthetic photon flux density
R_e	Ecosystem respiration
R_p	Plant respiration
R_s	Soil respiration
SO_4^{2-}	Sulphate
SOM	Soil organic matter

Chapter 1

Introduction

1.1 Peatland ecosystems

1.1.1 Preamble

Peatlands are unbalanced wetland ecosystems where plant production has historically exceeded decomposition of organic material (Shaw and Goffinet, 2000). As a result, organic material accumulates as peat. Most northern hemisphere peatland plant communities are composed of a high percentage of bryophytes (Vitt, 1990) and the large amount of this biomass is composed of structural cell-wall compounds (e.g. celluloses, hemi-celluloses and phenolics) that decompose slowly (Verhoeven & Liefveld, 1997; Berendse *et al.*, 2001). Decomposition of plant derived organic matter is further reduced by water saturated, anaerobic conditions, cool climates, and/or short growing seasons that allow organic matter to accumulate over time across large areas. Most peatlands contain less than 20 % inorganic material and more than 80 % organic matter of which 40 % is carbon.

Hydrology is probably the most important determinant of the establishment and maintenance of peat forming processes (Mitsch and Gosselink, 2000). Peat is composed primarily of the remains of plants in various stages of decomposition that accumulate as a result of the anaerobic conditions created by standing water or poorly drained conditions. The unique hydrology of a peatland contributes to the creation of the physicochemical conditions that make such an ecosystem different from both well-drained terrestrial systems and deepwater aquatic systems. Hydrological pathways such as precipitation, surface runoff, groundwater, and flooding rivers transport organic matter nutrients to and from peatlands (Schlesinger, 1997; Emmett *et al.*, 1994) while water table depth, flow patterns, and duration and frequency of waterlogging influence the biochemistry of peat decomposition processes (Schlesinger, 1997; Maier *et al.*, 1999). In particular anaerobic conditions lead to the accumulation of organic matter as peat

due to the inhibition of microbial activities that require molecular oxygen (e.g. oxidative respiration). Other features of organic soils and peat include: low bulk density, high porosity, low hydraulic conductivity, reduced nutrient availability and a strong cation exchange capacity.

Biological activity in peatland ecosystems is constrained by the absence of molecular oxygen (O_2) that prevents organisms from respiring through normal aerobic metabolic pathways. When respiratory demand exceeds the O_2 supply, dissolved oxygen is depleted, the redox potential in the soil drops rapidly so that other ions (nitrate, manganese, iron, sulphate and carbon dioxide) are progressively reduced (Maier *et al.*, 1999). These anaerobic conditions thus modify the supply of nutrients available to plants and microbiota. Hydrophytic plants posses a range of adaptations that enable them either to tolerate these stresses or to circumvent them. For example some peatland plants have structural (e.g. aerenchyma in *Eriophorum* species) as well as physiological (e.g. anaerobic respiration) mechanisms to tolerate root anoxia and low oxygen supply (Hook and Crawford, 1978). Peatland plants are also adapted to low nutrient availability by adaptations such as efficient use of nutrients (e.g. bog Ericoids), nutrient accumulation (e.g. cation exchange of *Sphagnum*) and nitrogen fixation (e.g. *Alnus spp.* with *Frankia spp* in riparian systems.) (Moore and Bellamy, 1974).

It is the role of the interaction between plant and soil processes that regulate peatland biogeochemical cycling that is of particular relevance to this thesis. Waddington and Roulet (2000) found that non-CO_2 fluxes accounted for less than 1 % of the carbon exchange of a boreal peatland. Thus climatic and physicochemical factors that affect plant and soil processes and interactions between plant productivity and soil decomposition are critical to net C sequestration in peatland ecosystems. These interactions may have implications for predicting the C balance of peatland ecosystems under future climate change scenarios.

1.1.2 Forms and classification

In wetlands, physical and biotic characteristics grade continuously from one wetland type to another (table 1-1). Fens can be further classified as poor,

moderate-rich and extreme-rich, and marshes as tidal, freshwater and saline wetlands. Therefore any classification based on common terms is, to an extent arbitrary. Also, the same term may refer to different systems in different regions. The terminology used to define wetland types is confused as a result of different regional uses of terms for similar types of wetlands. For example, several terms are used to denote peat-accumulating systems. The most general term is peatland, which is generally synonymous with moor. However there are many types of peatlands, the most general being fens and bogs. Table 1-1 illustrates the confusion in terminology that occurs because of different regional or continental uses of terms for similar wetlands.

Table 1-1 Comparison of terms used to describe wetlands (Mitsch and Gosselink, 2000)

North American	← Marsh or fen → ← Bog →
European	← Swamp → ← Marsh → ← Fen → ← Bog →
Characteristics	
Vegetation	← Reeds → ← Grasses and sedges → ← Mosses →
Hydrology	← Rheotrophic → ← Ombrotrophic →
Soil	← Mineral → ← Peat →
pH	← Roughly neutral → ← Acid →
Trophic state	← Eutrophic → ← Mesotrophic → ← Oligotrophic →

Although many types of peatlands are identifiable, classification usually defines two main types: (1) minerotrophic (fens) and (2) ombrotrophic (bogs):

Minerotrophic — A peat-accumulating wetland that receives water rich in dissolved minerals with vegetation cover composed of graminoid species and brown mosses.

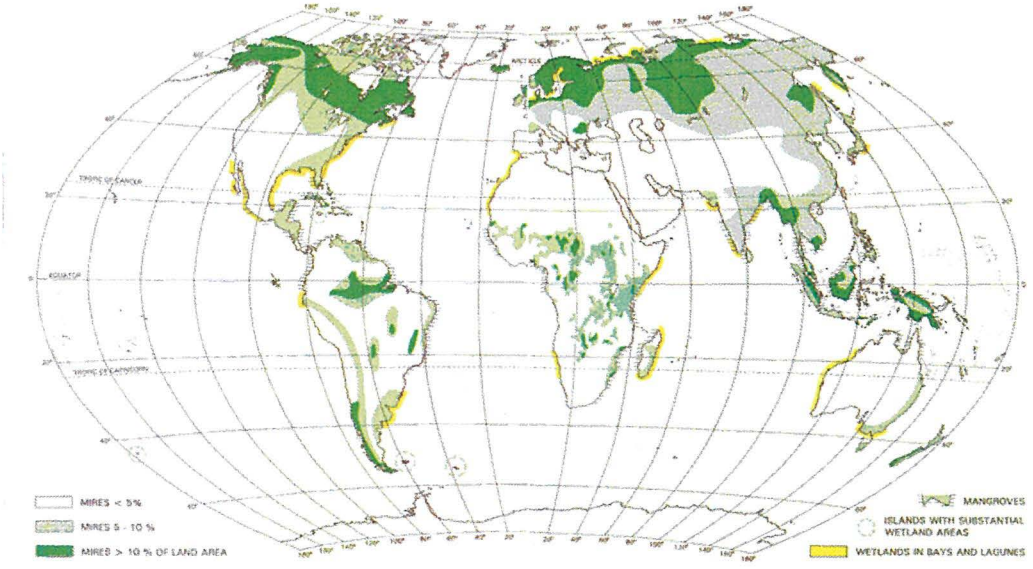
Ombrotrophic — A peat-accumulating wetland that receives water exclusively from precipitation and supports acidophilic mosses, particularly *Sphagnum.*

Ombrotrophic bogs are further classified according to altitude and total annual rainfall. Raised bogs are lowland peatlands that receive approximately 800mm of rainfall annually whilst blanket bogs are upland peatlands receiving approximately 1200mm of rain annually.

1.1.3 Global distribution

The extent of the world's wetlands is thought to be from 7 to 9 million km^2, or about 4 to 6 percent of the land surface of the Earth (Mitsch & Gosselink, 2000). Aselmann and Crutzen (1989, 1990) categorized wetlands as: (1) bogs, (2) fens, (3) swamps, (4) marshes, and (5) floodplains. They estimated that bogs and fens accounted for 60 percent of the world's wetlands (3.35 million km^2) that is very close to the 3.46 million km^2 of northern boreal and subarctic peatlands given by Gorham (1991) (fig. 1-1). Extensive areas of bogs and fens occur in Scandinavia, eastern Europe, western Siberia, Alaska, and Canada. Major areas where a very large percentage of the landscape is peatland include the Hudson Bay lowlands in Canada, the Fennoscandian Shield in northern Europe, and the western Siberian lowland around the Ob and Irtysh Rivers (Mitsch and Gosselink, 2000).

Figure 1-1 Global distribution of the world's peatlands (http://www.peatlandsni.gov.uk/formation/global.htm)



1.2 Peatland biogeochemistry

The transport and transformation of elements and compounds in ecosystems, known as biogeochemical cycling, involve a great number of interrelated physical, chemical, and biological processes. The biogeochemistry of peatlands can be divided into: (1) intrasystem cycling through various transformation processes, and (2) the exchange of chemicals cycling between the peatland and its environment (Maier *et al.*, 1999). Key elements in peatland ecosystem biogeochemistry are carbon (CO_2, CH_4, DOC), nitrogen (NH_4^+, NO_3^-), phosphorus (PO_4^{3+}), sulphur (SO_4^{2-}), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}). The hydrology of a peatland directly modifies and changes its physicochemical environment, particularly oxygen availability and related chemistry such as nutrient availability, pH and toxicity. The diffusion coefficient of molecular oxygen through water-filled pores is 10^4 times less than through air, and cannot supply the metabolic demands of submerged soil organisms (Drew and Lynch, 1980). Under anaerobic conditions, alternative electron acceptors, either organic compounds for fermentation or one of a series of inorganic electron acceptors are utilised for anaerobic respiration (see table 1-2). The importance of these electron acceptors depends on the redox potential of the soil.

Table 1-2 Relationship between respiration, redox potential and typical electron acceptors (from Maier *et al.*, 1999)

Type of respiration	Reduction reaction	Reduction potential (V)	Oxidation reaction	Oxidation potential (V)	Difference (V)
Aerobic	$O_2 - H_2O$	+0.81	$CH_2O - CO_2$	-0.47	-1.28
Denitrification	$NO_3^- - N_2$	+0.75	$CH_2O - CO_2$	-0.47	-1.22
Manganese reduction	$Mn^{4+} - Mn^{2+}$	+0.55	$CH_2O - CO_2$	-0.47	-1.02
Nitrate reduction	$NO_3^- - NH_4^+$	+036	$CH_2O - CO_2$	-0.47	-0.83
Sulphate reduction	$SO_4^{2-} - HS^-$, H_2S	-0.22	$CH_2O - CO_2$	-0.47	-0.25
Methanogenesis	$CO_2 - CH_4$	-0.25	$CH_2O - CO_2$	-0.47	-0.22

The terminal electron acceptor used depends on availability, and follows a sequence that corresponds to the electron affinity of the electron acceptors (e.g. $NO_3^{--} > Mn^{4+} > Fe^{3+} > SO_4^{2-} > CO_2$) (Maier *et al.*, 1999). Oxidized ions such as NO_3^-, Mn^{4+}, Fe^{3+}, and SO_4^{2-} are found in a thin oxidized surface layer that is particularly important in the chemical transformations and nutrient cycling of anaerobic peatlands. The lower anaerobic layer is dominated by reduced forms such as ferrous and manganous salts, ammonia and sulphides. The products or by-products of the reactions shown in table 1-2 include the greenhouse gases CO_2 (aerobic / methanotrophic respiration), CH_4 (methanogenesis) and N_2O (denitrification). Although these reactions occur in pasture, forest and aquatic ecosystems, the unique properties of peat allows these reactions to operate in close proximity to one another over short time periods. In effect, the reduced, anaerobic conditions and low temperature, low pH and / or low nutrient availability permit the accumulation of organic matter as peat and 'lock-up' other nutrients such as nitrogen (i.e. NH_4^+, NO_3^-), phosphorus (i.e. PO_4^{3-}), sulphur (i.e. SO_4^{2-}), potassium (K^+) magnesium (Mg^{2+}) and calcium (Ca^{2+}) by complexation with organic molecules. The low availability of these nutrients (particularly nitrogen and phosphorus) to plants and soil microorganisms feeds-back to further limit the cycling of carbon in peat.

1.2.1 The carbon cycle

The major biological carbon (C) transformations in peatlands are shown in fig. 1-2. Carbon dioxide (CO_2) is produced by ecosystem respiration (plant + soil respiration) and by the oxidation of methane (CH_4) in oxic zones of the soil. Waddington and Roulet (2000) found that CO_2 fluxes accounted for more than 99 % of the effluxes from a boreal peatland. Soil climate including temperature/ aeration and nutrient availability are thus important factors regulating CO_2 production (Silvola *et al.*, 1996). However, in summer, photosynthesis can be relatively higher per gram of CO_2 fixed / produced per unit area of peatland resulting in a negative net C flux. Thus factors affecting plant growth and productivity such as light intensity and temperature will have a significant effect on the net CO_2 exchange in peatlands. Determining the principal factors

regulating ecosystem respiration, photosynthesis, and dissolved organic carbon (DOC) production is important for predicting the C balance of peatlands under IPCC scenarios (IPCC, 2001).

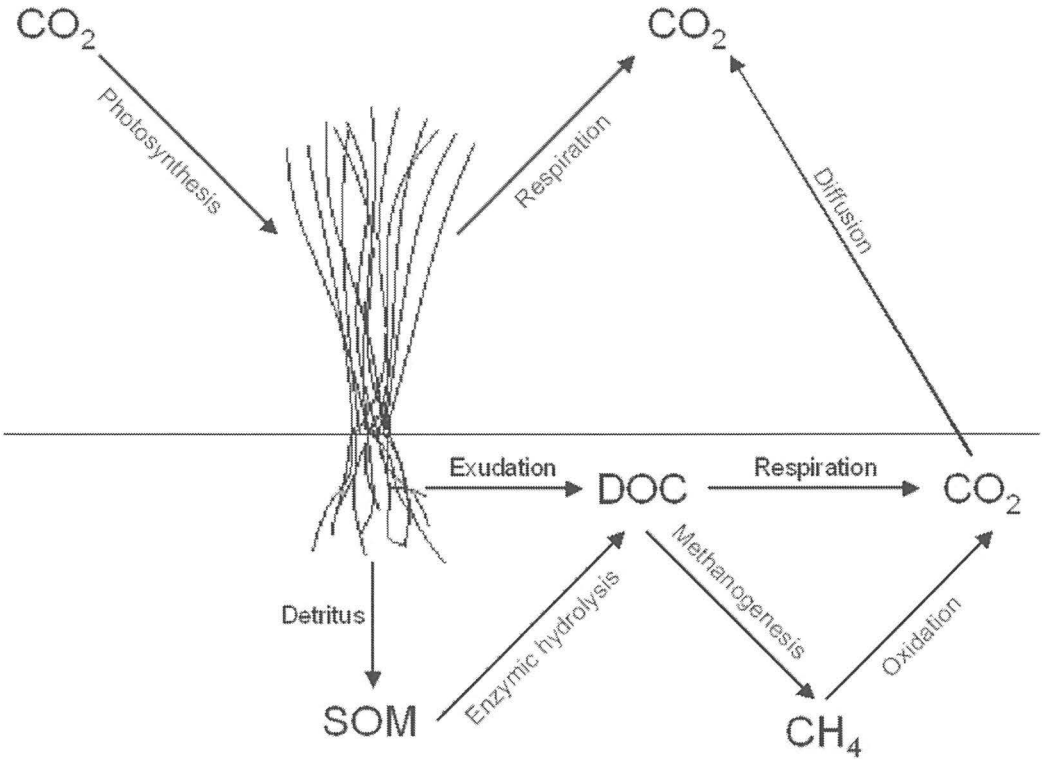


Figure 1-2 The major biological carbon transformations in peatlands (adapted from Mitsch and Gosselink, 2000).

The input of organic C is mainly from plants as detritus and exudates and is generally of high molecular weight (e.g. cellulose, hemicellulose, lignin). Therefore the degradation of these materials into utilisable monomers by extracellular soil enzymes is often the rate – limiting step in the C cycle (Chróst, 1991). Soil microbes utilize low molecular weight DOC from enzymic hydrolysis as a source of C that returns the plant derived C to the atmosphere as CO_2 or CH_4 and completes the C cycle. Photosynthesis and aerobic respiration dominate the aerobic horizons but occur in close proximity to anaerobic processes in the soil due to the anoxic nature of peatlands (Mitsch and Gosselink, 2000).

Aerobic respiration of organic matter to CO_2 (Eq. 1) is efficient in terms of energy transfer compared to anaerobic respiration with O_2 as the terminal electron acceptor:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_20 + 12e^- + \text{energy} \qquad (1)$$

However, because of the anoxic nature of peatlands, anaerobic processes, less efficient in terms of energy transfer, occur in close proximity to aerobic processes. The two major anaerobic microbial processes in peatland soils are fermentation and methanogenesis. In fermentation (Eq. 2), the organic matter itself is the terminal electron acceptor for facultative or obligate anaerobes and forms various low molecular weight acids (e.g. lactic acid), alcohols (e.g. ethanol) and CO_2 (Maier *et al.*, 1999):

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + CO_2 \qquad (2)$$

It represents one of the major ways in which high molecular weight carbohydrates are broken down to low molecular weight compounds, usually as dissolved organic carbon (DOC), which are available to other microbes (Valiela, 1984).

Net ecosystem production

Gorham (1991) stated that in peatlands 'there is far more carbon beneath the surface than in the vegetation currently growing on that surface.' Of the total carbon stock in global peatlands (455 Pg), 98.5% occurs in the form of peat against 1.5% in the vegetation. The accumulation-sequestration of carbon or net ecosystem production is due more to slow decomposition processes than to net primary productivity. Besides leading to peat accumulation, slow decomposition leads to slower nutrient cycling in an already nutrient-limited system.

In order to understand why low decomposition rates and high primary productivity lead to the accumulation of organic matter as peat, it is necessary to understand the fluxes of carbon between the atmospheric, plant and soil carbon pools. As communities of long-lived plants develop on land, a certain fraction of net primary production is allocated to living tissue that accumulates as biomass

through time. Plant communities achieve a steady state in living biomass when the allocation to living tissue is balanced by the death and loss of older parts. At that point, there is no true increment in plant biomass, although dead organic matter may still be accumulating in the soil (Schlesinger, 1997).

The rate of net primary production (NPP) is analogous to the rate of net photosynthetic activity (P_n):

$$NPP = GPP - R_p$$
$$P_n = P_g - R_p$$

where GPP = Gross primary production
R_p = plant respiration
P_g = gross photosynthetic activity
and P_n = net photosynthetic activity

If we assume that herbivore respiration is minimal in most peatlands, then increasing fractions of gross primary production are lost to plant respiration and decomposition through time (Odum, 1969). Then net ecosystem production (NEP) is defined as

$$NEP = NPP - R_s$$

where R_s is respiration of soil decomposers. Thus

$$NEP = GPP - R_e$$

where R_e is ecosystem respiration (plant and microbial respiration). This shows that if the relative amount of carbon lost to the atmosphere per unit area of soil by ecosystem respiration is lower than the amount of carbon fixed per unit area of soil by photosynthesis, then the net ecosystem production will be positive and thus the peatland will be a sink for atmospheric CO_2. However, if ecosystem respiration exceeds gross photosynthesis or GPP then the peatland will be a source of atmospheric CO_2. Thus the carbon sink capacity at anyone time is dependent on plant photosynthesis, plant respiration and soil respiration, the

interaction of these processes, and climatic/physicochemical factors that affect each process independently. NEP can be determined non-destructively in the field by measuring the net flux of CO_2 between the soil and atmosphere. This flux is known as net CO_2 ecosystem exchange (NEE) and is equivalent to NEP because it is the net sum of gross photosynthesis and ecosystem respiration.

Carbon storage

Northern peatlands are considered to be net carbon sinks and contain approximately one-third of the world's soil carbon stock (455 Pg); equivalent to over 60% of the atmospheric pool. Carbon is currently accumulating in northern peatlands at a rate of between 0.076-0.096 Pg yr (23-29 g m^{-2} yr^{-1}) (see Gorham, 1991). The sequestration of carbon by primary production accumulates as peat due to exceptionally low decomposition rates (Clymo, 1983). In bogs, the rate of peat accumulation is determined by the rate of decomposition in the aerobic upper levels (the acrotelm) and in the lower levels (the carotelm) of the deposit. Clymo (1984) proposed a model for peat accumulation, which predicts that peatlands will eventually attain a steady state when the inputs of detritus from primary production at the peat surface is balanced by the loss of organic matter by decomposition throughout the peat profile. Many peatlands particularly tundra have accumulated soil carbon since the retreat of the last continental glacier (Harden *et al.*, 1992). If these areas are subject to drainage and warmer climatic conditions, the rate of carbon storage will decline, decomposition will increase, and peatlands could become a significant source of CO_2 for the atmosphere (Tate, 1980; Hutchinson, 1980; Armento and Menges, 1986; Gorham, 1991; Silvola *et al.*, 1996; Schlesinger, 1997). Thus peatlands are of global, economical and ecological significance as atmospheric CO_2 is currently rising at a rate of 1.5 ppm per year on average (IPCC, 2001). The Intergovernmental Panel on Climate Change (IPCC) predicts a 1.4 – 5.8 °C average increase in the global surface temperature over the period 1990 to 2100 (IPPC, 2001). High-latitude ecosystems are expected to undergo the greatest increases in surface temperature with a doubling of atmospheric CO_2 by 2100 (Oechel *et al.*, 1993). Thus predicting the future of peatlands as global C sinks under IPCC climate change scenarios is of considerable importance since not only may global warming reduce the C sink capacity of peatlands but may also result in a positive feedback

to global warming *via* the release of the greenhouse gas CO_2 to the atmosphere as a result of increased organic matter decomposition.

1.2.2 Nutrient cycling in peatlands

Peatlands are generally deficient in nutrients available to plants and microorganims due to the inhibition of decomposition and mineralization of organic matter. At the same time the deficiency of elements such as N, P, S, and K is in part responsible for the limited rate of organic matter decomposition in peat. Nitrogen (N) and phosphorus (P) are often the most limiting nutrients in peatland ecosystems. The main inputs of N into peatlands include wet / dry deposition, N fixation by certain microorganisms, and / or inflow from the surrounding hydrological catchment (Hemond, 1983). N appears in a number of oxidation states in peatlands but because of the anoxic conditions, microbial denitrification of nitrates to gaseous forms (i.e. N_2, N_2O) is one of the most significant losses of N to the atmosphere (Mitsch and Gosselink, 2000). Nitrates serve as one of the first terminal electron acceptors in peatlands after the disappearance of oxygen, making them an important chemical in the oxidation of organic matter. Ammonium (NH_4^+) is the primary form of mineralised N although much N can be tied up in organic forms and must be cleaved prior to uptake (Updegraff *et al.*,1995).

Once the ammonium ion is formed, it can be taken up by plants and/or immobilised by microorganims and thus converted back to organic matter; complexed through ion exchange onto negative organic molecules; or reduced to ammonia (NH_3) and released to the atmosphere by volatilization (Mitsch and Gosselink, 2000). Normally, NH_4^+ builds up in the soil because of the anaerobic conditions that restrict further oxidation. However, in peat a concentration gradient develops between the lower reduced layer and the thin oxidised surface layer leading to the upward diffusion of NH_4^+ to the surface. In the surface layer, NH_4^+ can be oxidised through the process of nitrification in two steps by *Nitrosomonas* species to give nitrate (NO_3^-). Nitrate is a mobile ion and can be incorporated into plant and microbial tissues or lost through leaching. The most prevalent nitrate reduction pathway in anaerobic peat is denitrification in which

NO_3^- acts as a terminal electron acceptor and is converted to gaseous nitrous oxide (N_2O) and molecular nitrogen (N_2). Denitrification is inhibited by low pH and is therefore thought to be of less consequence in northern peatlands than in less acid salt marshes, forested wetlands and rice paddies (Etherington, 1983). Also, since denitrification is dependent on the supply of nitrate, it follows that denitrification will be limited by ammonium diffusion, nitrification and nitrate diffusion (Reddy and Patrick, 1984).

Phosphorus is one of the most limiting elements in peatlands particularly northern hemisphere ombrotrophic bogs. Unlike the other major nutrient cycles, phosphorus occurs in a sedimentary cycle rather than a gaseous cycle. Phosphorus occurs as soluble and insoluble complexes in both organic and inorganic (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$) forms depending on the pH. Although phosphorus is not directly altered by changes in redox potential, its association with several elements, especially iron, indirectly render it relatively unavailable to plants and microorganisms by: (1) precipitation of insoluble phosphates with ferric iron, calcium, and aluminium under aerobic conditions; (2) adsorption of phosphate onto clay particles, organic peat, and ferric and aluminium hydroxides, and (3) binding of phosphorus in organic matter as a result of its incorporation into living organisms (Mitsch and Gosselink, 2000).

1.2.3 *Sphagnum* physiology

Non-peat forming wetlands are dominated by either a well-developed tree or shrub layer (swamps), by sedges (*Carex* sp.) and rushes (*Juncus* sp.) without trees and shrubs (marshes), or contain emergent vegetation in less than a metre of water (shallow open water) (Zoltai and Vitt, 1995). Nitrogen mineralization rates are high (Bridgham *et al.*, 1998), surface inflow often contains abundant nutrients, and these wetlands are often eutrophic (Mitsch and Gosselink, 2000). The lack of a well-developed bryophyte-dominated ground layer, coupled with abundant vascular plant litter, allows relatively rapid decomposition. Physiognomically, peatlands vary considerably. Bogs almost exclusively lack a sedge component, often have an abundance of ericaceous shrubs, and *Sphagnum* moss (plate 1-1), feather mosses or lichens are always present (Glaser, 1992).

Fens are usually sedge-dominated, and they may be wooded, shrubby or open (Halsey *et al.*, 1997). Poor fens are dominated by *Sphagnum* moss and ericaceous shrubs may be present; moderate- and extreme-rich fens are dominated by 'brown mosses' and ericaceous shrubs are lacking (Vitt, 1990). Bogs and poor fens are generally more closely related than poor fens and rich fens.

Plate 1-1 *Sphagnum capillifolium*

Bryophytes are non-vascular plants that receive nutrients predominantly from precipitation. Clymo (1970) has estimated that there is more biomass in *Sphagnum* than in any other plant in the world. *Sphagnum* create waterlogged, nutrient-poor and acidic conditions that strongly favour carbon sequestration (Van Breeman, 1995; Hoosbeek *et al.*, 2001). The dominance of mosses, particularly *Sphagnum*, in poor fens and bogs and lack of it in rich fens correlates well with acidity. The ground layer in peatlands is frequently dominated by a 90-100 % cover of bryophytes. Functioning of the peatland ecosystem is highly dependent on this bryophyte layer including production and decomposition, as well as community development. In particular, the moss layer influences peatland function in four ways:

1) Nutrient sequestration (oligotrophication)

Increasing nitrogen input to bogs (Rochefort *et al.*, 1990) and rich fens (Rochefort and Vitt, 1988) results in increased bryophyte growth, suggesting that

nitrogen is limiting in most cases. However, in a few experiments, other factors such as precipitation (Bayley, 1993) and phosphorus (Aerts *et al.,* 1992) may influence plant growth as well. With poorly developed conduction systems, water and solutes are taken up over the entire plant surface (Turetsky, 2003). Lack of both gametophyte stomata and effective cuticles in many species allows free exchange of solutions and gases across cell surfaces. Thus bryophytes often serve as effective traps for water and nutrients. This also makes them more sensitive to atmospheric chemical deposition than vascular plants (Turetsky, 2003). Through release and mineralization, the moss layer effectively controls subsequent nutrient movement, and hence plant production, in peatlands (Rochefort *et al.*, 1990).

2) Water-holding abilities

In comparison to almost all vascular plants that are drought-avoidant, mosses are drought-tolerant (poikilohydric) that allows bryophytes to tolerate longer periods of water stress, and to recover quickly with rehydration (Turetsky, 2003). They are active when they are wet, but they also have the ability to become inactive when dry and can revitalize when re-wetted (Bewley, 1979). Peatland mosses have developed morphological adaptations to remain moist and active as long as possible, allowing longer photosynthetically active periods and thus greater growth (Stålfelt, 1937). The leaves of *Sphagnum* and other mosses hold large amounts of water in dead hyaline cells that enclose smaller, living, green cells, in a 1:2 ratio (Shaw and Goffinet, 2000). These hyaline cells have lost their living cell contents and as a result, the ratio of carbon to nitrogen increases that influences decomposition rates. Hyaline cell modifications allow *Sphagnum* species to retain water, and cope with temperature and evaporative stress in order to increase photosynthetic active periods and hence growth. In addition, hyaline cells allow *Sphagnum* species to move away from the water table, thus increasing water height, creating greater amounts of anaerobic conditions; and through complex canopy development allow *Sphagnum* species to expand habitat space (Shaw and Goffinet, 2000).

3) Decomposition

Bryophytes appear to produce litter with poor organic matter quality. Bryophytes are not able to synthesize lignin and therefore it would seem reasonable that bryophyte litter would decay more rapidly than vascular material. However, the slow rates of bryophyte decomposition could be caused by low N concentrations, particularly in *Sphagnum* material that has an abundance of hyaline cells (Turetsky, 2003). Also, the complex cell walls of *Sphagnum* tissues contain cellulose with a high proportion of xylan units, pectin content, and polyphenolic network polymers. This tissue is far more resistant to microbial decomposition than litter of vascular plants (Berendse *et al.*, 2001; Verhoeven & Liefveld, 1997). Water-soluble phenolic compounds released by living *Sphagnum* (Rassmussen *et al.*, 1994) may have an immediate impact on decomposition rates by generating acidity and inhibiting microbial activity (Verhoven & Toth, 1995). Peat accumulation may be promoted by the recalcitrant nature of bryophyte material and thus bryophytes are important to the sequestration of carbon in peat-accumulating ecosystems (Turetsky, 2003).

4) Acidification

Clymo (1963) argued that peatland acidity is produced by *Sphagnum* cell walls where hydrogen ions produced by uronic acid molecules held on the cell wall are exchanged for base cations contained in pore waters. However, in 1980, Harry Hemond argued that although this process of cation exchange by *Sphagnum* undoubtedly occurs, it is not sufficient to produce the acidity that is present in bogs (i.e. pH 3.0-3.7). He concluded that bog acidity is largely due to decomposition and the production of humic acids present in pore water as dissolved organic carbon (DOC), and that acidity in bogs is a result of hydrogen release through decomposition of organic molecules that in turn are dissolved in the pore water as organic carbon.

1.2.4 Soil microorganisms and decomposition

Most detritus is delivered to the upper layers of the soil where it is subject to decomposition by microfauna, bacteria and fungi (Swift *et al.*, 1979).

Decomposition leads to the release of CO_2, CH_4, H_2O and nutrient elements, and to the microbial production of highly recalcitrant organic compounds known as humus (Schlesinger, 1997). Humus compounds accumulate in the lower soil profile and compose the bulk of soil organic matter (Schlesinger and Peterjohn, 1988). The dynamics of the pool of carbon in soils is best viewed in two stages: (1) processes leading to the turnover of the majority of litter at the surface and (2) processes leading to the slower production, accumulation, and turnover of humus at depth. Decomposition rates vary as a function of temperature, moisture, aeration and the chemical composition of the litter material. Microbial activity increases exponentially with increasing temperature and within the range 10 - 30 °C, Q_{10} values for soil respiration have been reported to be about 2-2.5, that is a doubling of respiration per 10 °C increase in temperature (Raich and Schlesinger, 1992; Peterjohn *et al.,* 1994). However, many studies that have included temperatures below 10 °C have shown that the Q_{10} increases as the soil temperature decreases (Lloyd and Taylor, 1994; Chapman and Thurlow, 1998).

Plant litter and soil microbes constitute the cellular fraction of soil organic matter (Schlesinger, 1997). Although soil organic carbon (SOC) exists in a variety of forms, it can most simply be considered as three main pools (1) insoluble, (2) soluble, and (3) biomass carbon (Killham, 2001). Insoluble soil organic carbon comprises over 90 % of total soil organic carbon and includes the main components of many plant cell walls – cellulose and lignin – and fungal walls – chitin. The insoluble soil carbon pool also includes decomposed material in the form of soil humus. Soluble carbon provides an immediate substrate for a wide range of soil microbes and is largely produced by plant roots as root exudates, by other living soil organisms that produce exudates, and by the enzymatic decomposition of insoluble and biomass carbon (Killham, 2001). The biomass carbon pool of soil consists of plant roots, microbes and microfauna (e.g. collembola, enchytraids). It is the activity of decomposer (saprophytic) soil microbes that is most responsible for the turnover of carbon in soil. This biomass carbon pool represents the driving force of the soil carbon cycle and, hence, of other nutrient cycles since all organic matter passes through the microbial pool before being redistributed to other pools.

The main forms of plant organic carbon entering the soil are cellulose, hemicellulose and lignin. Cellulose is a polymer of glucose units joined together

by 1-4 linkages, and decomposition is catalysed by the enzyme system cellulase (see Section 1.2.5). Cellulose often represents more than half of plant carbon and is decomposed in the soil by fungi (e.g. *Fusarium, Aspergillus, Penicillium*) and bacteria (e.g. *Bacillus, Pseudomonas*) by either aerobic or anaerobic pathways. Decomposition in peatlands will primarily be by obligate / facultative anaerobes that are not as efficient as obligate aerobes and thus reduce the potential rate of soil organic carbon decomposition (Killham, 2001). Hemicelluloses (polymers of hexoses, pentoses and uronic acids) can constitute up to one third of plant carbon. The decomposition of the hemicellulose compound pectin is carried out by a three stage enzyme system involving pectinase. Pectinolytic bacteria in soil include species of *Arthrobacter, Pseudomonas* and *Bacillus* (Killham, 2001). Lignin is the most resistant component of plant carbon and is the third most abundant component after cellulose and hemicellulose. It is a complex non-uniform polymer of aromatic nuclei, with the basic building unit being a phenyl-propane (C_6-C_3) type structure. The 'random' structure of lignin and the strong linkages make lignin very resistant to microbial decomposition and thus lignin accumulates with time in the soil. The decomposition of lignin in soil is a three-stage process (Section 1.2.5). Many soil microbes are known to be able to degrade some of the individual aromatic molecules that occur in lignin polymers. Only a very limited number of soil microbes, however, can degrade the entire lignin structure, and these microbes are thought to be largely fungi of the Basidiomycotina.

As decomposition proceeds, there is an increasing content of humus that appears to result from microbial activity. The structure of humus is poorly understood, but it contains numerous aromatic rings with phenolic (-OH) and organic acid (-COOH) groups (Stevenson, 1986). The phenolic and lignin degradation products and some of the other products of residue decomposition are considerably modified by microbial reaction and/or through chemical, oxidative condensations to form the humic acids, fulvic acids and humin, which together make up the soil humus (Killham, 2001). This physically and chemically stabilised organic carbon is decomposed but at much slower rates than other carbon fractions such as cellulose, hemicellulose and glucose dimmers. Turnover times involving hundreds or even thousands of years are more realistic for the recalcitrant fractions of soil organic matter. Also, humic

compounds such as dissolved phenolics are known to be potent inhibitors of extracellular hydrolase enzymes that further increases the residence time of soil organic carbon in peatlands (Freeman *et al.,* 2001a; Wetzel, 1992). The relatively rapid turnover of plant / microbial residues is carried out largely by zymogenous soil microbes (proliferate only under high substrate additions) and the much slower turnover of 'stable' organic carbon largely involves the autochthonous soil microbes (competitive under low substrate concentrations) (Killham, 2001).

1.2.5 Soil enzyme activities

Definition

Soil enzymes are biological catalysts which function in the decomposition of organic matter in soil ecosystems (Dick and Tabatabai, 1993). Like all catalysts, enzymes increase the rate of a chemical reaction without themselves undergoing permanent alteration. In the decomposition of organic matter and nutrient cycling in peatlands, extracellular hydrolysis has been reported as a critical step for three reasons. First, other sources of nutrients (i.e. weathering, atmospheric deposition) are generally insignificant for the nutrient demand of plants and microbes. Second, almost all microorganisms and plants in wetlands are not permeable to high molecular weight compounds (Paul and Clark, 1989). Therefore, extracellular hydrolysis of organic matter is a crucial step prior to nutrient uptake (Chróst, 1991). Finally, the extracellular hydrolysis rate is much lower than the rate of uptake of low molecular weight substrates, which implies the importance of extracellular hydrolysis as a rate limiting step in nutrient cycles (Hoppe *et al.*, 1988).

Origin and location

At any one time the total activity of an enzyme in soil is comprised of activities associated with different soil constituents (e.g. viable microorganisms, cell debris, clay and humic colloids) (Burns, 1982). Soil enzymes are largely of microbial origin, but also include contributions from plants and animals (Ladd, 1978). The term soil enzymes in this study refers to both enzymes associated with metabolising and proliferating microbial cells (intracellular/cell associated)

and truly extracellular (abiontic) enzymes that are freely dissolved in pore water or stabilised by humus or clay colloids. Burns (1982) characterised ten soil enzymes according to their location in soils. These can be characterised into five principle groups of enzymes according to their origin and location:

(1) Living cells: (a) cytoplasmic, (b) periplasmic, or (c) cell-attached enzymes.
(2) Dead cells: (a) leaked, or (b) cell-attached enzymes.
(3) Non-proliferating cells
(4) Dissolved enzymes: (a) free, or (b) enzyme-substrate complex.
(5) Immobilised enzymes: (a) clay absorbed, or (b) humic bound.

The activity in each category changes with time and from enzyme to enzyme. Thus these categories represent various stages in the life of an enzyme. However, category (5) can be considered to be distinct from the remaining categories due to their immobilised nature in soil. Burns (1982) further hypothesised that immobilised enzymes play a role in substrate catalysis by signalling microorganisms to initiate or repress production of extracellular enzymes. Also, constitutive and inducible enzymes are both present in the soil. Constitutive enzymes are those which are present in nearly constant amounts in a cell and their activity is not affected by the addition of any particular substrate. Inducible enzymes are those present only in trace amounts in a cell but which quickly increase in concentration when its substrate is present.

Types of enzymes

Decomposition in soil is controlled by a range of enzyme systems that transform and mineralize organic compounds by a combination of intra- and extracellular enzymes operating in synergistic and / or competitive interactions. Examples are the enzymes that decompose cellulose and lignin. Cellulase's are a suite of enzymes that are responsible for the decomposition of cellulose. Cellulase activity involves the loss of the crystalline structure of cellulose as well as the subsequent depolymerisation. After depolymerisation, resulting short chains of glucose units are hydrolysed to single glucose units by cellobiase and cellotriase. The decomposition of cellulose is representative of many large organic polymers

in soil, with initial depolymerisation by microbial enzyme systems, and subsequent release of simpler units serving as substrate for a wider group of microbes. Ultimately, the release of low molecular weight compounds such as glucose into the soluble carbon pool provides a substrate for all heterotrophic soil microbes possessing β-glucosidase enzymes (Killham, 2001). The decomposition of lignin in soil can be considered as a three-stage process. Stage one involves the esterification of the exposed methoxyl groups. Stage two is a depolymerisation process that involves extracellular enzymatic cleavage of the inter-unit bonds, while stage three involves the splitting of the phenolic ring after initial side chain removal. The enzymes involved in the complex breakdown of lignin include esterases, phenolases and peroxidases (Killham, 2001). Recalcitrant phenolic compounds are broken down by phenol oxidases in the presence of oxygen (Freeman *et al.*, 2001a). Chitin is a polymer of β-(1,4)-*N*-acetyl-D-glucosamine (NAG) units, and the second most abundant polysaccharide in nature after cellulose (Chen *et al.*, 1994). Chitin is recognized as a significant fraction of humus-bound nitrogen in soil (Aber and Mellilo, 1991). Chitin is decomposed in the soil by the enzyme *N*-acetylglucosamidase (NAGase). A large range of enzymes are also involved in the mineralization of organic matter to phosphate (phosphatases) and sulphate (sulphatases).

Factors affecting activity

A large amount of enzymes in soil are stabilised by clays or colloidal organic matter. Enzymes are associated with clay particles through ionic bond or salt bridges (Stotzky and Burns, 1982). In contrast, the associations between enzymes and humic acids are more complicated, and often involve covalent and hydrogen bonding mechanisms as well as ionic association (Tate, 1995). Generally, binding of enzymes to clay or colloidal organic matter affects enzyme activities in such a way that the half-life of enzymes increases. However, the binding often decreases enzyme-substrate affinity (i.e. increase in K_m) and / or decreases the maximum reaction rate (V_{max}) that alters the velocity of the reaction following the Michaelis-Menten equation:

$$V_i = V_{max} (S) / K_m + (S)$$

where V is the velocity of the reaction and S is the substrate concentration.

Apart from factors that affect the origin, location, production and stabilisation of enzymes in the soil, subsequent enzyme activity in the soil is controlled by climatic and physicochemical factors in the soil that interact over a wide range of spatio-temporal scales. Enzyme production is determined by microbial activity that is regulated by temperature, water content, nutrient availability and / or vegetation type (Insam, 1990). Thereafter, enzyme activities are determined by factors such as temperature, pH, redox potential, inhibitors (e.g. phenolics, iron) and activators (eg. Mg^{2+}, Ca^{2+}).

Enzyme activities in soil increase with temperature. The Q_{10} for a number of enzymes ranges from between 1.5-2.5 (Atlas and Bartha, 1987). Soil pH affects enzyme activities since the active sites of enzymes are frequently composed of ionisable groups that must be in the proper ionic form in order to maintain the conformation and to catalyse the reaction (Lehninger, 1982). Soil pH may also affect enzyme activities indirectly by changing the interaction between immobilised enzymes and the soil matrix, or via affects on microbial activity (Frankenberger and Johanson, 1982). Redox potential can directly affect enzyme activities, particularly oxidoreductase. Decrease of redox potential indirectly influences enzyme activities by changes in the microbial population (i.e. shift to anaerobes), inhibition of *de novo* enzyme synthesis, or mobilising of reduced metal ions which have inhibitory effects on the enzymes (Pulford and Tabatabai, 1988). Many substances inhibit or activate specific enzymes in the soil. Various trace metal ions have been reported to inhibit soil enzyme activities by complexing with the protein active group of the enzymes (Eivasi and Tabatabai, 1990). Phenolic materials have also been reported to have inhibitory effects on enzyme activities, since they can bind to the protein surface and precipitate with it (Wetzel, 1992). Phenolic inhibition may be of considerable importance in peatlands since phenolic compounds frequently constitute 80 % of the total dissolved organic matter (Wetzel, 1992). In contrast, some ions such as Mg^{2+} and Ca^{2+} have been reported to activate soil enzyme activities. These ions are cofactors of some enzymes (Lehninger, 1982), and they could reduce the inhibitory effect of phenolics by reacting with phenolics and hence prevent complexation between enzymes and phenolics (Wetzel, 1992; Freeman *et al.*, 1996).

1.3 Importance of plant-soil processes in peatland biogeochemistry

Models of global environmental change are dependent on accurate predictions of the processes regulating C and nutrient cycling in terrestrial ecosystems. Currently much research is focused on the biogeochemical cycles of peatlands due to their involvement in the terrestrial sequestration of atmospheric carbon. Since the beginning of industrialisation the global concentration of atmospheric carbon dioxide (CO_2) has risen from about 280 to 370 ppm, mainly due to fossil fuel burning and land-use change (Ebersberger *et al.*, 2003). Currently it is rising at 1.5 ppm per year on average and models have predicted that from the present-day level of about 370 ppm, atmospheric CO_2 concentrations will reach 550 ppm in only 30 years (IPCC, 2001). The IPCC predicts a 1.4 – 5.8 °C average increase in the global surface temperature over the period 1990 to 2100 (IPCC, 2001).

The ability of peatlands to sequester atmospheric CO_2 under current climatic conditions and future global change scenarios will be dependent on the response of plants and microorganisms (Ovenden, 1989; Van der Heijden *et al.*, 2000). Approximately 60 Gt of CO_2-C is cycled annually along the terrestrial photosynthesis-decomposition pathway, 20 times more than the annual net addition to the atmosphere (Goudriaan, 1992). Thus small changes to net primary productivity or decomposition of soil organic carbon could significantly influence the net increase of atmospheric CO_2 (Hoosbeek *et al.*, 2001). Because elevated CO_2 may strongly influence net primary productivity, species abundance and community composition (Mooney *et al.*, 1999) and the chemical and physical composition of plant material, and therefore the decomposability of plant litter, strong feedbacks to the soil carbon pool are expected (Hoosbeek *et al.*, 2001).

With the potential for increased microbial activity in peatlands due to a rise in temperature and / or soil aeration, there is the potential for increased rates of organic matter decomposition. As mentioned, northern peatlands contain approximately one-third of the world's soil carbon stock (Gorham, 1991) and therefore alteration to the processes regulating C and nutrient cycling have the potential to feedback on predicted global climate change (Updegraff *et al.*, 1995; Zak *et al.*, 1993). However, plants may respond positively to climate change (i.e. elevated CO_2) that may 'offset' the increase in soil respiration and maintain the

net exchange of CO_2 between the peatland and the atmosphere. Thus there remains a requirement to understand the climatic/physicochemical factors that regulate plant and soil processes in peatlands. Table 1-3 and 1-4 summarize our current understanding of the major climatic and physicochemical factors affecting plant productivity and soil decomposition in peatland ecosystems.

Table 1-3 Summary of the climatic factors affecting plant productivity and soil decomposition in peatland ecosystems. (+ = positive effect, - = negative effect, n = no effect).

Factor	**Plant productivity**			**Soil decomposition**		
	Effect	Plant type	Reference	Effect	Process	Reference
Temperature	+	Vascular	Mack *et al.* (2004)	+	CO_2 respiration	Mack *et al.* (2004)
				+	CO_2 respiration	Neff and Hooper (2002)
				+	CO_2 respiration	Oechel *et al.* (1993)
				n	Soil enzymes	Kang and Freeman (1999)
Waterlogging	+ / -	*Sphagnum*	Shaw and Goffinet (2000)	+	CO_2 respiration	Keller *et al.* (2004)
				-	CO_2 respiration	Blodau and Moore (2003)
				-	Soil enzymes	Kang and Freeman (1999)
				+ / -	Soil enzymes	Pulford and Tabatabai (1987)
				-	Soil enzymes	Freeman *et al.* (1996)

Table 1-4 Summary of the physicochemical factors affecting plant productivity and soil decomposition in peatland ecosystems. (+ = positive effect, - = negative effect, n = no effect).

Factor	**Plant productivity**			**Soil decomposition**		
	Effect	Plant type	Reference	Effect	Process	Reference
Nitrogen	+	Vascular	Mack *et al.* (2004)	+	CO_2 respiration	Mack *et al.* (2004)
	+	*Sphagnum*	Aerts *et al.* (1992)			
	n	*Sphagnum*	Van der Heijden *et al.* (2000)			
	-	*Sphagnum*	Heijmans *et al.* (2002)			
	-	*Sphagnum*	Hoosbeck *et al.* (2002)			
	-	*Sphagnum*	Berendse *et al.* (2001)			
CO_2	n	Vascular	Oechel *et al.* (1993)	+	Soil enzymes	Moorhead and Linkens (1997)
	+	*Sphagnum*	Heijmans *et al.* (2002)	n	Soil enzymes	Kang *et al.* (2001)
	+	*Sphagnum*	Van der Heijden *et al.* (2000)	+	CO_2 respiration	Kang *et al.* (2001)
	n	*Sphagnum*	Hoosbeck *et al.* (2001)			
	n	*Sphagnum*	Berendse *et al.* (2001)			

1.4 Aims and objectives

The main aim of this research thesis was to examine the climatic and physicochemical factors that regulate carbon dioxide fluxes and soil enzyme activities in *Sphagnum* dominated peatlands. Research focused on plant and soil CO_2 processes since Waddington and Roulet (2000) found that CO_2 fluxes accounted for more than 99 % of the carbon exchange of a boreal peatland. Understanding the climatic and physicochemical factors (i.e. temperature, water table depth, CO_2, nutrient availability) that regulate carbon dioxide fluxes and soil enzyme activities is critical for determining the impact of climate change on the C balance of these ecosystems.

The second aim was to determine whether *Sphagnum* productivity affects soil decomposition processes under current and future climate change scenarios. Climatic and physicochemical factors affect plant and soil processes directly but may also affect C cycling indirectly by altering interactions between plant and soil processes. For example, climatic stimulation of plant rhizodeposition of labile C may increase decomposition via stimulatory effects on soil microbial activity. An indirect effect of *Sphagnum* productivity on soil decomposition processes may feedback on productivity via an increase in nutrient availability. Thus, plant-soil interactions in peatlands may have implications for the C balance of *Sphagnum* dominated peatlands.

In Chapter 2, a year long field survey of temperature, physicochemistry, organic C pools and soil decomposition processes was carried out in a valley-bottom riparian peatland. Riparian peatlands differ from upland, ombrotrophic peat-accumulating ecosystems in that soils and soil moisture are influenced by adjacent streams or rivers. The plant communities are generally productive and diverse in comparison to ombrotrophic bogs due to the replenishment of soil nutrients. The objective of this chapter was to examine relationships between temperature, physicochemistry, organic C pools, basal respiration and soil enzyme activities based on hypothetical relationships. Also, the effect of plant types on soil decomposition pools and processes was examined to determine the importance of plant-soil interactions relative to seasonal variation in decomposition processes. It was hypothesized that temperature would regulate decomposition via effects on basal respiration and soil enzyme activities. This

affect in turn would determine subsequent interactions between organic C pools and physicochemistry.

In Chapter 3, a year long field survey of abiotic factors, NEE, ecosystem respiration, and soil enzyme activities were carried out in a blanket bog. As with Chapter 2 the aim was to examine relationships between abiotic factors, carbon dioxide fluxes and soil enzyme activities. In particular, the objectives were to determine the abiotic factors that determine NEE and the relative contribution of *Sphagnum* respiration to ecosystem respiration. The experimental design consisted of control and shaded plots in order to determine the effect of reduced productivity on soil decomposition processes. Shading was used as a means to reduce plant productivity since it does not have a direct effect on soil decomposition processes. It was hypothesized that a reduction in *Sphagnum* productivity would reduce soil decomposition via a reduction in rhizodeposition of labile carbon. An important question was how these interactions may impact the C balance of the peatland.

In Chapter 4, a peatland mesocosm experiment assessed the effect of shading and nitrogen induced changes in *Sphagnum* productivity on soil decomposition processes. The aim was to extend the experiment in Chapter 3 by examining the effect of shading and nitrogen on productivity, *Sphagnum* N and pigment content and the cycling of ^{13}C stable isotopes in *Sphagnum*.

In Chapter 5, a peatland mesocosm experiment assessed the short-term interactive effect of elevated CO_2, temperature and waterlogging on NEE and soil enzyme activities. Of interest was how these factors interactively affected NEE and soil enzyme activities. It was hypothesized that CO_2 would stimulate sequestration, temperature would stimulate emission and waterlogging would stimulate sequestration. Also, it was hypothesized that elevated CO_2 would affect soil enzyme activities via an indirect effect on *Sphagnum* productivity (i.e. rhizodeposition).

In Chapter 6, the results from each chapter are compiled and discussed in reference to the principle aims of the thesis. This chapter summarizes the climatic and physicochemical factors that regulate carbon dioxide processes and soil enzyme activities, and the implications of these findings and plant-soil interactions to the C balance of peatland ecosystems.

Chapter 2

Decomposition processes in a valley-bottom riparian peatland: seasonal variation and effect of plant types

2.1 Introduction

Riparian peatlands differ from upland, ombrotrophic peat-accumulating ecosystems in that soils and soil moisture are influenced by adjacent streams or rivers. Flooding of the riparian zone affects soil chemistry by producing anaerobic conditions, importing and removing organic matter, and replenishing mineral nutrients (Mitsch and Gosselink, 2000). The plant communities are generally productive and diverse in comparison to ombrotrophic bogs due to the replenishment of soil nutrients. However, mineralization of organic matter in both riparian and ombrotrophic peatlands by microorganisms and extracellular enzymes is limited by climatic and physicochemical conditions (e.g. soil temperature, pH, and hydrology) that influence the supply of accessible organic and inorganic nutrients (particularly nitrogen and phosphorous) to plants and microorganisms (Sinsabaugh, 1994; Shackle *et al.*, 2000).

Dissolved organic carbon (DOC) concentrations have increased significantly in rivers draining temperate UK peatlands raising concerns that carbon (C) stores are beginning to destabilize (Freeman *et al.*, 2004; Freeman *et al.*, 2001b). The release of DOC in the soil solution represents a key process for the loss of C from peatlands, since DOC is an important energy source for microbial metabolism (Qualls and Haines, 1992). Peatland DOC is a mixture of organic compounds ranging from simple, short-chain to complex higher molecular substances that are produced by plant tissues and as a result of microbial degradation of soil organic matter (Moore and Dalva, 2001). Studies have shown strong spatial and temporal variations in soil DOC concentrations that are linked with seasonality. Christ and David (1996) determined that increases in temperature and moisture content increased the rate of DOC production from the organic horizons of red spruce Spodosols while Anderson *et al.* (2000) showed that temperature and pH influenced the leaching of DOC from

a forest mor humus in Sweden. Fenner *et al.* (2003) showed that the *Sphagnum* community rapidly contributes recently synthesized C to the peatland DOC pool. DOC contains labile C substrates for microbes and extracellular enzymes, but it can also contain high concentrations of phenolic compounds in peat-forming ecosystems. These compounds are recalcitrant compounds resistant to microbial decomposition (Berendse *et al.*, 2001; Verhoeven & Liefveld, 1997) that accumulate in soil during the decomposition of plant and microbial biomass (Van Breeman, 1995). Phenolic compounds consist of a wide range of substances which posses an aromatic ring bearing a hydroxyl substituent, including their functional derivatives (Thomson, 1964). They are produced by plants as secondary compounds and include phenolic acids, coumarins, flavones, and anthocyanins. Phenolic compounds are of importance since they have been shown to inhibit the activity of extracellular hydrolase enzymes (Wetzel, 1992; Freeman *et al.*, 1996; Freeman *et al.*, 2001a).

Numerous results of field and laboratory experiments confirm that soil decomposition processes increase with temperature and aeration (Peterjohn *et al.*, 1994; Katterer *et al.*, 1998) raising the possibility of significant greenhouse gas emissions from peatland soils under warmer and drier climatic regimes (Neff & Hooper, 2002). However, the microbial response to seasonal soil temperature variation may be limited by other factors. Soil enzymes are primarily responsible for the decomposition of soil organic matter to low molecular weight compounds that can be utilized by microorganisms for growth and respiration producing the greenhouse gas carbon dioxide (CO_2). Extracellular enzyme activity in peat is principally the result of microbial production that is regulated by temperature, hydrology, nutrient availability and vegetation type (Insam, 1990). Thereafter, enzyme activities are determined by climatic and physicochemical factors such as temperature, pH, redox potential, inhibitors (e.g. phenolics) and activators (eg. Mg^{2+}, Ca^{2+}). Various trace metal ions have been reported to inhibit soil enzyme activities by complexing with the protein active group of the enzymes (Eivasi and Tabatabai, 1990). Phenolic materials inhibit enzyme activities (Freeman *et al.*, 1996; Freeman *et al.*, 2001a) since they can bind to the protein surface and precipitate with it (Wetzel, 1992). Phenolic inhibition may be of considerable importance in wetland soils since phenolic compounds frequently constitute 80 % of the total dissolved organic matter (Wetzel, 1992). Freeman *et al.* (2001a)

showed that the low activity of phenol oxidase due to low dissolved O_2 concentrations allows phenolic compounds to accumulate and inhibit pivotal hydrolase enzymes. Factors affecting the concentration of phenolic compounds in peat are therefore expected to alter the activities of hydrolase enzymes and therefore limit the availability of low molecular weight C compounds for microbial respiration. In contrast, some ions such as Mg^{2+} and Ca^{2+} have been reported to activate soil enzyme activities. These ions are cofactors of some enzymes (Lehninger, 1982), and they could reduce the inhibitory effect of phenolics by reacting with phenolics and hence prevent complexation between enzymes and phenolics (Wetzel, 1992; Freeman *et al.*, 1996).

Extracellular enzyme activities have been shown to vary among the litter of different plant species (Linkins *et al.*, 1990; Fioretto *et al.*, 2000), reflecting both their differences in chemical composition and physiological status, as well as edaphic conditions (Kourtev *et al.*, 2002). Non-vascular species such as *Sphagnum* create waterlogged, nutrient-poor and acidic conditions that strongly favor carbon sequestration (Van Breeman, 1995; Hoosbeek *et al.*, 2001). The complex cell walls of *Sphagnum* tissues contain cellulose with a high proportion of xylan units, pectin content, and polyphenolic network polymers (Kang, 1999). This tissue is far more resistant to microbial decomposition than litter of vascular plants (Berendse *et al.*, 2001; Verhoeven & Liefveld, 1997). Water-soluble phenolic compounds released by living *Sphagnum* (Rassmussen *et al.*, 1994) may have an immediate impact on decomposition rates by generating acidity and inhibiting microbial activity and extracellular enzyme activities (Verhoven & Toth, 1995). Thus, turnover of phenolic acids may play a key role in carbon dynamics in *Sphagnum*-dominated peatlands (Williams *et al.*, 2000). In contrast, the vascular plant *Juncus effusus* grows in large tussocks, which contain several thousand leaves, both living and dead. Vascular plants are composed of aerenchymous tissue that provides a pathway for the transport of O_2 to the roots (Roura-Carol & Freeman, 1999; Wassmann & Aulakh, 2000). This will potentially affect the growth of aerobic (positive) and obligate anaerobic (negative) microbes that could affect enzyme activity in the rhizosphere of vascular plants. The growth and physiology of these plant types will potentially impact on the spatial variation of basal CO_2 respiration, DOC and extracellular

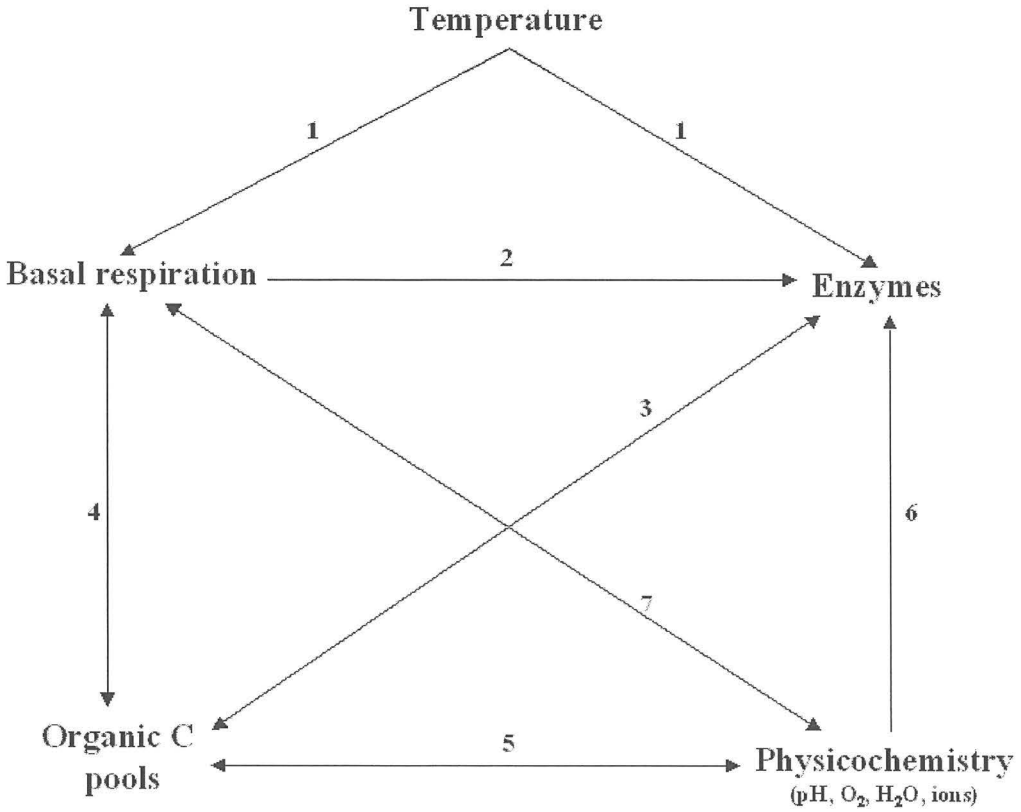
enzyme activities via differences in the physicochemistry of the rhizospheres, and the substrate quality of the decomposing plant matter.

In this study, the aim was to (1) examine seasonal relationships between temperature, physicochemistry, organic C pools and decomposition processes, and (2) determine the role of plant types on decomposition processes (defined as differences in peat beneath *Juncus effusus* and *Sphagnum recurvum*). Decomposition processes consist of extracellular enzyme activities and microbial activity (measured as basal CO_2 respiration). A one-year field survey of temperature, physicochemistry, organic C pools, extracellular enzyme activities, net CO_2 ecosystem exchange (NEE) and basal CO_2 respiration was carried out at a valley-bottom riparian peatland in 2002. Figure 2-1 shows a conceptual model of how decomposition processes, organic C pools and physicochemistry are hypothesized to interact seasonally:

1. Temperature has a direct effect on decomposition by increasing basal CO_2 respiration and soil enzyme activities;
2. Microbial proliferation increases soil enzyme activities;
3. Enzymes breakdown HMW carbon to labile carbon that may feedback on enzyme activities (i.e. soil phenolics);
4. Labile organic C from enzymic hydrolysis is substrate for basal CO_2 respiration that will reduce the organic C pool;
5. Breakdown of organic C via enzymic hydrolysis will affect physicochemistry and increase ionic concentrations that will both feedback on DOC;
6. Physicochemical changes will feedback on soil enzyme activities; and
7. Physicochemical changes will interact with basal CO_2 respiration (i.e. acidity, nutrient uptake, mineralization).

Results from principal component analysis were compared to this conceptual model to identify seasonal relationships between the abiotic and biotic variables.

Figure 2-1 Conceptual model of how temperature, decomposition processes, organic C pools and physicochemistry interact seasonally.



The role of plant types (*J.effusus* and *S. recurvum*) on decomposition processes was studied by measuring physicochemistry and decomposition processes within peat beneath each plant type. It was hypothesized that decomposition processes would be lower in peat beneath *Sphagnum* relative to *Juncus* due to the production of soluble phenolics by rhizodeposition and plant decomposition.

2.2 Materials and methods

2.2.1 Seasonal variation of physicochemistry, organic C pools, decomposition processes and net CO_2 ecosystem exchange

Site description

The field survey was carried out at a valley-bottom riparian peatland site near Nant Ffrancon Pass, Ogwen Valley, Gwynedd, North Wales (UK grid reference SH 615 640) (Plate 2-1). Nant Ffrancon is a U shaped valley carved out by glaciers in the last Ice Age 10 000 ya. The peatland site lies at the base of the valley at an altitude of 200 m (Roura-Carol & Freeman, 1999) and is characterized by the presence of *Sphagnum recurvum, Juncus effusus* and *Polytrichum* species and is under waterlogged conditions for most of the year. The peat is classified as Fibrist (von Post humification scale number H3) (Kang & Freeman, 1998; Clymo, 1983).

Plate 2-1 Ogwen valley, Gwynedd, North Wales in August 2002.

A representative area of ground measuring approximately 16 m^2 was used for the survey. Within the area five plots were used for water and peat sample collection. Water samplers were installed one week prior to the first sampling in January 2002. Soil solution was collected using syringe and water sampler (Freeman *et al.*, 1994) placed at a depth of 10 cm. The water sampler was composed of a tube (3mm in diameter) connected to the tip of a 5 ml syringe packed with glass wool to act as a particulate filter. Five replicate water samples of approximately 5 ml were collected every month. Water samples were filtered with 0.45 μm filters on the day of collection and kept at field temperature (4-15 °C) until analysis. Five peat samples measuring approximately 100 cm^3 each were collected to a depth of 10 cm each month. Peat samples were placed in sealed polythene bags and kept at field temperature until analysis. Prior to analysis peat samples were homogenized by hand and large roots were removed.

Physicochemistry

Soil temperature was measured using a digital thermometer in the field. Soil pH and dissolved oxygen were measured with an electrode in the lab using soil solution. Bulk density and the gravimetric water content were determined by drying 1 cm^3 of each peat replicate at 105 °C for 24 hours.

Ion concentrations were determined with a Dionex 2000i ion chromatograph. Anions (nitrate, phosphate and sulphate) were measured by an AS4A anion column with $NaHCO_3/NaCO_3$ eluent solution and H_2SO_4 regenerant solution, whilst cations (sodium, ammonium, potassium, magnesium and calcium) were measured with a CS10 cation column with HCl/DAP (Diaminopropionic acid monohydrochloride) eluent solution and TBAOH (Tetrabutylammonium hydroxide) regenerant solution (Freeman *et al.*, 1993).

Organic C pools

Soil organic matter (SOM) was determined by loss on ignition where the dried soil samples of known weight were placed in an oven at 550 °C for 24 hours and reweighted. SOM was expressed as (Oven dried soil weight – Ignited soil weight / oven dried soil weight) x 100 %.

Total carbon (TC) in peat water samples and inorganic carbon (IC) were measured with a TOC analyzer (Shimadzu TOC-5000) by injecting 30 μl of 0.45 μm filtered peat solution. The analyser was calibrated with 100 mg l^{-1} of potassium hydrogen phthalate solution (for TC) and sodium hydrogen carbonate / sodium carbonate solution (for IC). Dissolved organic carbon (DOC) was calculated as the difference between TC and IC.

Dissolved soil phenolic concentration was assayed on the same samples using Folin-Ciocalteau reagent (Box, 1983). Using a spectrophotometer plate, 250 μl of filtered soil solution was added with 37.5 μl of Na_2CO_3 solution (200 g l^{-1}). To each replicate, 12.5 μl of Folin-Ciocalteau solution were added underneath a fume hood and maintained for 2 hours at room temperature. A standard curve was prepared by applying the same chemicals to 0 – 2 mg l^{-1} phenol solution. The change in colour was measured with a spectrophotometer at 750 nm. Samples out of range were diluted with deionised water.

Decomposition processes

Basal respiration was measured in the laboratory by placing 1 cm^3 peat samples in 66 ml glass bottles with rubber septa. After 1 hour incubation at field temperature, approximately 10 ml of gas were removed and analysed by gas chromatography. The gas samples were injected into an Ai Cambridge model 92 Gas Chromatograph equipped with a Porapak QS column and flame ionization detector (Freeman *et al.*, 1994). The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1}. Soil respiration rates were calculated by subtracting background gas - determined by incubating an empty glass bottle for one hour. Basal respiration was expressed as μg CO_2-C g^{-1} dry soil weight h^{-1}.

The activity of three extracellular hydrolase enzymes (β-glucosidase, phosphatase and sulphatase) were determined using fluorogenic methylumbelliferyl (MUF)-substrates (Freeman *et al.*, 1995). MUF substrates were pre-dissolved in 2 ml cellosolve (ethylene glycol monomethyl ether) using an ultrasonic bath. For each of the five replicate peat samples, 1 cm^3 of peat was placed in a stomacher bag for each enzyme (3 x 5 samples) with 7 ml of MUF substrate (400 μM for MUF-β-glucoside and MUF-sulphate; 200 μM MUF-phosphate). MUF-substrate concentrations had previously been determined to be

optimal (V_{max}) for the site (Kang, 1999). Peat slurries were homogenised for 2 min in a stomacher (Seward Colworth model 400) and incubated at field temperature for 60 min (except MUF-phosphate for 45 min) (Kang, 1999) without addition of buffer in order to determine potential seasonal variation in the enzymes. A stock solution of 1000 μM MUF-free acid standard solution was prepared by pre-dissolving in cellosolve using an ultrasonic bath. Six standard solutions were prepared from this stock by sequential dilution (0 – 1000 μM). To account for quenching, 1 cm^3 peat samples from each replicate were placed in stomacher bags, 7 ml of deionised water added and homogenised for 2 min. Three ml of slurry from each of the five replicates were extracted into 2 eppendorf vials (1.5 ml each; ten vials). These were centrifuged at 10 000 rev min^{-1} (7 200 g) for 5 min and the supernatant transferred to new vials. 250 μl of each sample was pipetted into five wells on a plate and 50 μl of each MUF-free acid standard added to each well. After the substrate incubation period, 1.5 ml of reactant was pipetted into a centrifuge vial and centrifuged at 10 000 rev min^{-1} for 5 min. 300 μl of supernatant was placed in a well next to the standards and fluorescence determined at 450 nm emission and 330 nm excitation wave-length with a slit setting of 2.5 using a BMG Fluostar Galaxy fluorimeter. The enzyme concentrations were determined automatically by a macro regression program using the standards. Enzyme activity is expressed as MUF produced (nmol MUF g^{-1} soil dry weight min^{-1}).

Phenol oxidase was measured using the method of Pind *et al.* (1994). 1 cm^3 of peat was placed in a stomacher bag with 9 ml deionised water and equilibrated to field temperature for each sample. The peat slurries were homogenised for 1 min in a stomacher (Seward Colworth model 400). 0.75 ml of each solution was pipetted into two eppendorf vials. Then, 0.75 ml of 10 mM L-DOPA solution was pipetted into one vial and a timer started. After 8 min, 0.75 ml was pipetted into the second vial. The reactions were terminated after a further minute by boiling both vials for 2 min and then centrifuged at 10000 rev min^{-1} for 5 min. This gave incubation periods of 1 and 9 minutes for each replicate. 300 μl of supernatant from each vial was pipetted onto a plate and the absorbance was measured at 460 nm. The activity was expressed in terms of nmol 2, 3-dihydroindole-5,6-quinone-2-carboxylate (diqc) per min per g dry

weight, by using Beers Law and the difference in absorbance between incubation times of 1 and 9 min and a molar absorbancy of 3.7×10^4.

Net CO_2-C ecosystem exchange

Net CO_2-C ecosystem exchange was determined using a closed-chamber technique (Freeman *et al.*, 1994). Five chambers (4.5 litre) were constructed from polyethylene bottles (Fisons Scientific Apparatus) with the bases removed and placed over *Juncus* and *Sphagnum* plants randomly. These were inserted to a depth of 5 cm and each chamber was sealed with a urea cap containing a septum at midday on the day of sampling. Gas accumulation had previously been shown to be linear up to two hours incubation (Kang, 1999; Freeman *et al.*, 1994). After one hour, 20 ml gas samples were collected from the head space into gas-tight syringes. Background gas samples were also collected. The gas samples were injected into an Ai Cambridge model 92 Gas Chromatograph equipped with a Porapak QS column and flame ionization detector (Freeman *et al.*, 1994). The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1} for CO_2. Background gas concentrations were subtracted from chamber concentrations in the headspace after one hour incubation to give an estimate of flux and converted to mg CO_2-C m^2 h^{-1}.

Statistical Analysis

All statistical analyses were performed with Minitab Release 13 (Minitab Inc.). Differences between all months and min/max months were determined using General Linear Model and one-way ANOVA analysis. The residuals from ANOVA analysis were tested for normality using Anderson-Darling Normality Test. For non-normal distributions the data was ranked and Kruskal-Wallis analysis performed.

Principal component analysis (PCA) was performed on mean monthly data to detect relationships between variables. Net CO_2 ecosystem exchange was not included in PCA since it is the net result of gross photosynthesis and ecosystem respiration. Using principal component coefficients, the variables were grouped to allow comparison of minimum and maximum monthly means. Spearman rank correlation coefficients are presented as supporting evidence where it is reasonable to assume the dependent variable (i.e temperature).

Stepwise multiple regression analysis was performed for basal respiration, organic C pools and soil enzyme activities by adding/removing independent factors at $P < 0.15$ from the initial model.

The temperature sensitivity (Q_{10}) of basal respiration, DOC and soil enzyme activities were assessed by first fitting an exponential function to the mean monthly data as by Fang *et al.* 2005 (Eq.1):

$$D = a\, e^{bT} \quad (1)$$

where D = decomposition pool or process, a and b are fitted parameters, and T is the soil temperature in °C. By definition, the value of Q_{10} is the factor by which the rate differs for a temperature interval of 10 °C (Eq.2):

$$Q_{10} = D_{T+10} / D_T \quad (2)$$

where R_T and R_{T+10} are respiration rates at temperature of T and T+10. Combining equation 1 and 2, Q_{10} was estimated by Eq.3:

$$Q_{10} = e^{10b} \quad (3)$$

2.2.2 Effect of plant types on decomposition processes

Site description

Peat and plant samples were collected from the same peatland site in Nant Ffrancon Pass, Ogwen Valley, Gwynedd, North Wales (UK grid reference SH 615 640) in August 2002. Plant samples were collected by placing a circular disc of 20 cm diameter over *Juncus effusus* or *Sphagnum recurvum*, removing the aboveground vegetation and placing in paper bags. 100 cm^3 peat samples were collected within each area where plant vegetation had been removed. Ten replicate samples were collected for both *J. effusus* and *S. recurvum*.

Plant material

Aboveground plant fresh biomass was measured for each replicate and dried at 60 °C for two days. The dry material was reweighed and then ground using a ball mill and 1 gram dissolved in 20 ml of 80 % methanol for 1.5 hour at room temperature (Scheffer *et al.*, 2001). The solution was then filtered through 0.45 μm filter paper, diluted by 1/2 and analysed for soluble phenolics by the Box (1983) method described above.

Biogeochemical analyses

DOC and phenolics were determined by extraction with deionised water where 1 cm^3 of peat was homogenised with 9 ml deionised water. Two 1.5 ml samples were then placed in vials and centrifuged at 10 000 rev min^{-1} for 10 min. The supernatant was collected and filtered through 0.45 μm filters prior to analysis. DOC and phenolics were assayed using the methods described in Section 2.2.1.

Bulk density, gravimetric water content, hydrolase enzyme activities, phenol oxidase activity and basal CO_2 respiration were determined using the methods described in Section 2.2.1.

Statistical analysis

All statistical analyses were performed with Minitab Release 13 (Minitab Inc.). Differences between plant types were determined using one-way ANOVA analysis. The data was tested for normality using Anderson-Darling's normality test. Regression analysis was performed using all data points (n = 20).

2.3 Results

2.3.1 Seasonal variation of physicochemistry, organic C pools, decomposition processes and net CO_2 ecosystem exchange

Table 2-1 shows eigenanalysis of the correlation matrix. The first principal component (PC1) explained 36.2 % of the variation, PC2 explained 27.5 % (cumulative 63.7 %) and PC3 explained 12.6 % (cumulative 76.3 %). The first five principal components explained 89.2 % cumulatively although PC4 and PC5 each explained only 7.3 % and 5.6 % respectively. Figure 2-2 shows a plot of the first two principal components showing four distinct groups. Each group was then further characterized by the coefficient of PC3 (Table 2-1).

The results suggest that soil temperature (fig. 2-3a) and pH (fig. 2-3b) were related to DOC (fig. 2-4a), soil phenolics (fig. 2-4b) and basal CO_2 respiration (fig. 2-5) along with the enzymes sulphatase (fig. 2-6c) and phenol oxidase (fig. 2-6d). Table 2-2 shows that these variables were lowest in cold months (December/March) and highest in summer (June, July or August). However, the minimum and maximum monthly mean activities of phenol oxidase and sulphatase were not consistent and appear negatively correlated. Also, the first PC coefficients for phenol oxidase and sulphatase were close to zero (Table 2-1). Dissolved oxygen (fig. 2-7a), magnesium (fig. 2-8c) and calcium (fig. 2-8d) were related to this group by two principal components (fig. 2-2) and show a similar trend of lowest concentrations in cold months (January/February) and highest concentrations in summer (July) (Table 2-2). All of these variables varied significantly through out the year.

Table 2-1 Eigenanalysis of the correlation matrix. Variables are grouped according to coefficients within the first two principal components (solid lines) and first three principal components (dashed lines).

Variable	PC1	PC2	PC3	PC4	PC5
Temperature	-0.345	0.089	-0.187	-0.099	-0.004
Phenolics	-0.327	0.151	-0.088	-0.284	0.069
DOC	-0.340	0.012	-0.119	0.200	0.243
pH	-0.254	0.207	-0.042	0.038	-0.309
Basal respiration	-0.263	0.236	-0.116	0.132	-0.253
Phenol Oxidase	-0.052	0.358	-0.226	0.148	0.109
Sulphatase	-0.037	0.326	-0.324	-0.144	-0.007
O_2	-0.217	0.306	0.145	-0.020	0.008
Mg^{2+}	-0.164	0.175	0.484	0.161	0.176
Ca^{2+}	-0.342	0.061	0.076	0.214	0.262
SOM	-0.048	-0.148	-0.409	0.283	-0.224
K^+	-0.242	-0.248	-0.107	0.191	-0.352
H_2O	0.298	0.154	-0.198	0.100	-0.284
Phosphatase	0.264	0.264	-0.212	0.058	0.031
B-glucosidase	0.177	0.193	-0.313	-0.237	0.418
NO_3^-	0.174	0.336	0.067	0.138	-0.039
NH_4^+	0.111	0.276	0.249	0.181	-0.337
PO_4^{3-}	0.163	0.305	0.240	0.163	0.002
SO_4^{2-}	0.101	-0.105	-0.156	0.686	0.346
Eigenvalue	6.87	5.22	2.39	1.39	1.056
Proportion	36.2	27.5	12.6	7.3	5.6
Cumulative	36.2	63.7	76.3	83.6	89.2

Figure 2-2 Plot of the first two PCA coefficients. Variables in circles were related by two principal components that explained 64 % of the variation.

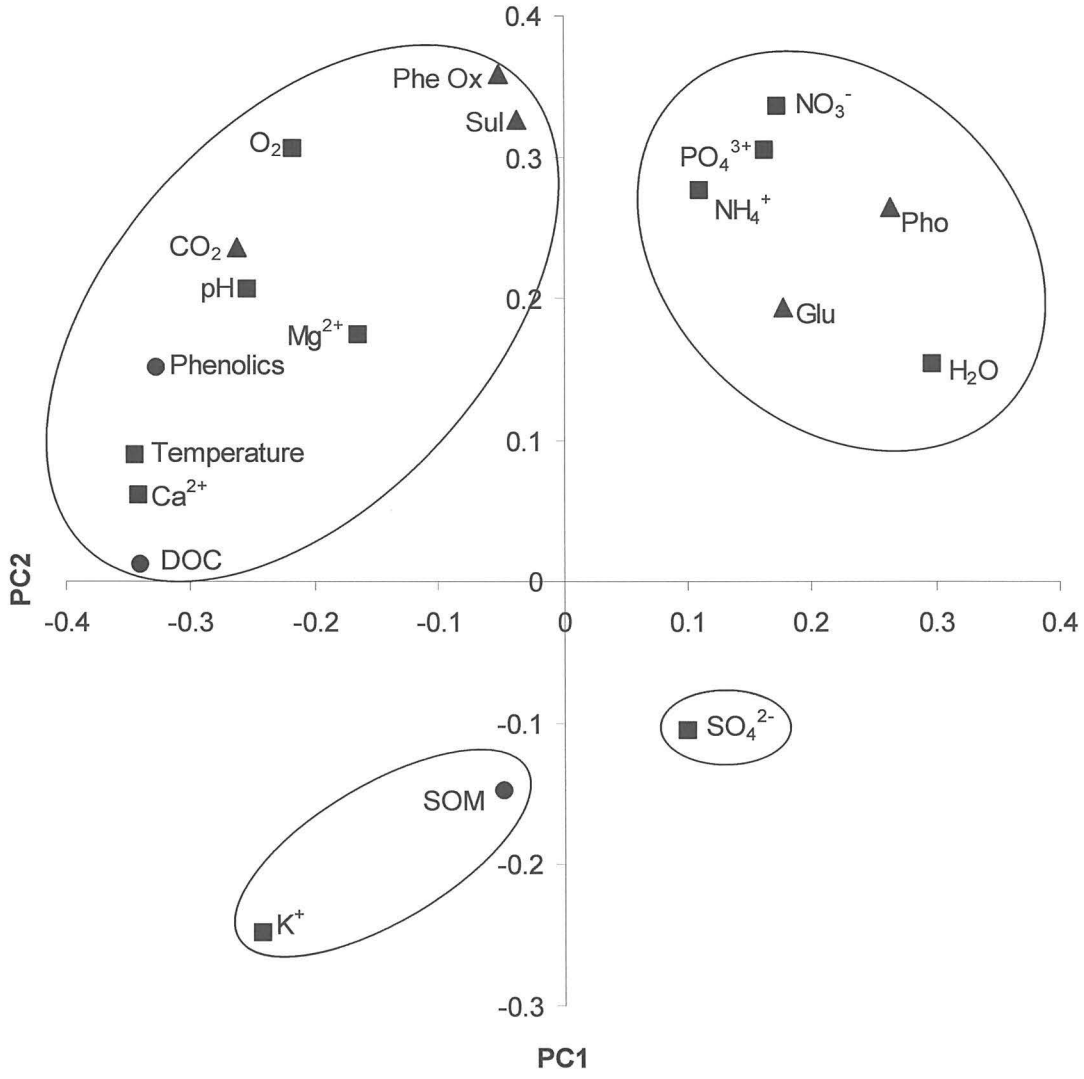


Table 2-2 Comparison of minimum and maximum monthly means for each variable (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Units are given in the methodology section. Variables are grouped according to PCA coefficients (see table 2-1) within the first two principal components (solid lines) and first three principal components (dashed lines). *a* Feb, Mar, Jun and Aug.

Variable	Min	Month	Maxi	Month	*P*-value (*F* value)
Temperature	4.6	Dec	14.4	Aug	*** (1249.14)
Phenolics	0.26	Mar	3.97	Aug	** (11.67)
DOC	4.3	Mar	34.6	Jun	**(19.17)
pH	3.96	Mar	5.26	Jul	*** (56.07)
Basal respiration	18.1	Dec	185.9	Jul (Oct)	* (9.75)
Phenol oxidase	10.8	Mar	27.9	Oct	*** (51.23)
Sulphatase	4.8	Jul	17.5	Jan/Oct	*** (56.2)
O_2	2.39	Jan	3.66	Jul	** (13.63)
Mg^{2+}	0.25	Feb	2.19	Jul	* (9.68)
Ca^{2+}	0.89	Feb	3.34	Jul	* (8.18)
SOM	86.3	Sep	93.1	May	ns
K^+	0	Oct	0.56	Apr	* (6.11)
H_2O	5.68	Jul	8.4	Oct	** (10.55)
Phosphatase	25	Aug	64.4	Feb/Oct	ns
B-glucosidase	27.9	Jul	75.6	Feb/Oct	** (30.2)
NO_3^-	0.02	Jun	0.47	Oct	** (11.24)
NH_4^+	0	*a*	0.05	Dec	ns
PO_4^{3-}	0	Mar	0.6	Dec	** (22.19)
SO_4^{2-}	0.34	Aug	22.5	Jun	*** (121.25)

Figure 2-3 Seasonal variation of soil and air temperature (a) and soil pH (b). Mean ± standard error (n=5).

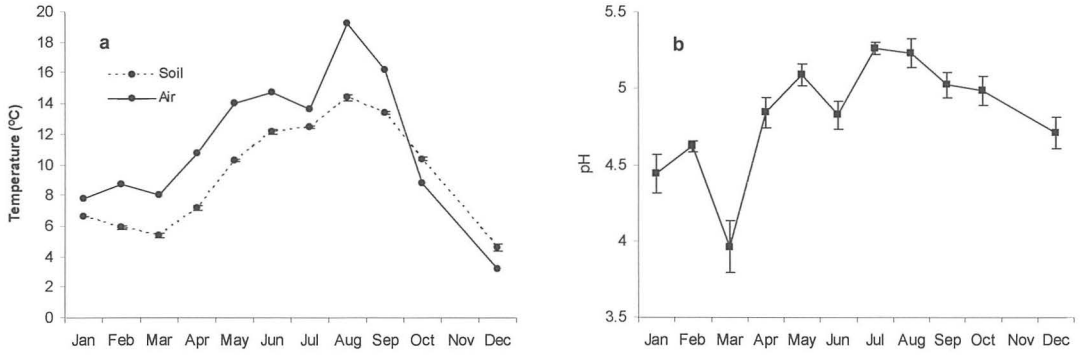


Figure 2-4 Seasonal variation of dissolved organic carbon (a) and soil phenolics (b). Mean ± standard error (n=5).

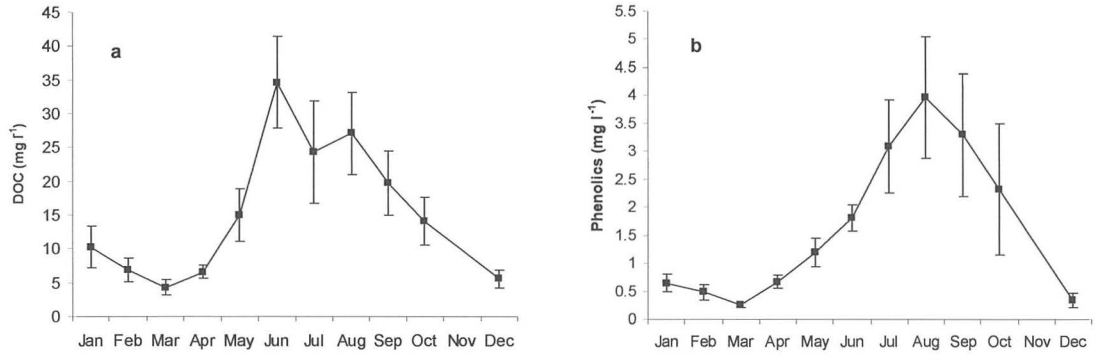


Figure 2-5 Seasonal variation of basal CO_2 respiration. Mean ± standard error (n=5).

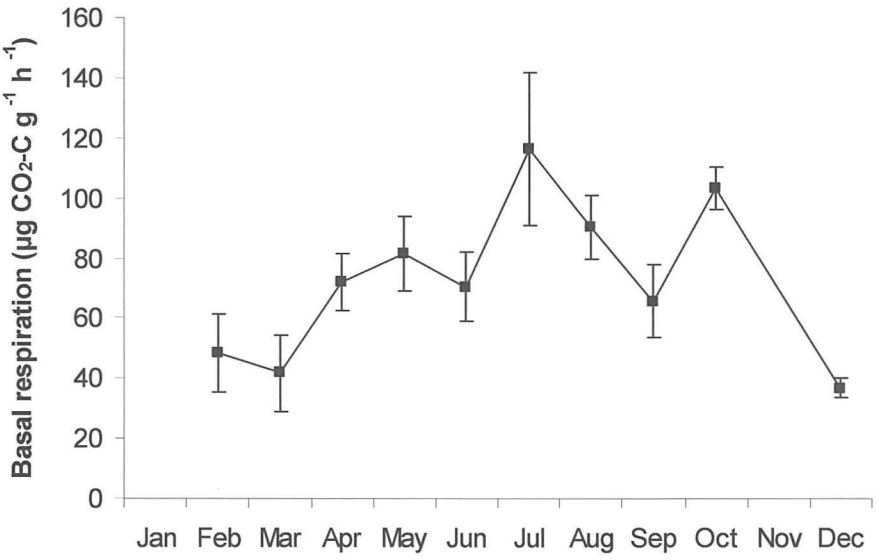


Figure 2-6 Seasonal variation of β-glucosidase (a), phosphatase (b), sulphatase (c), and phenol oxidase (d) activities. Mean ± standard error (n=5).

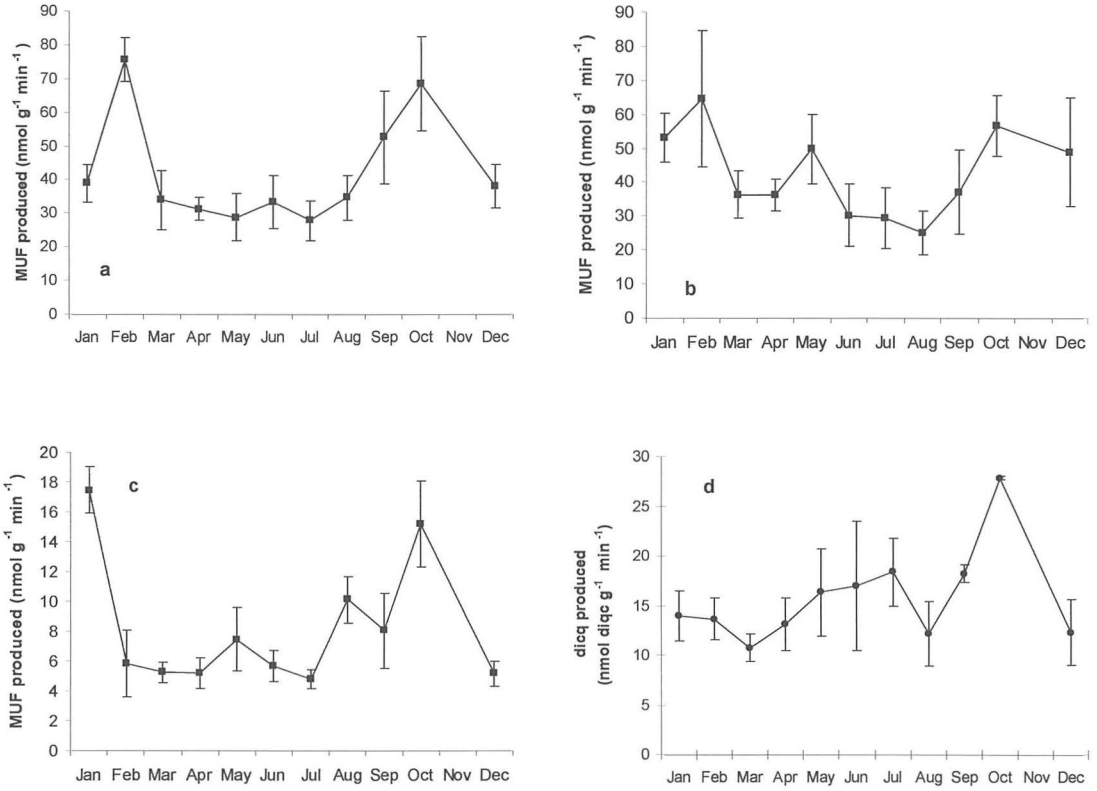


Figure 2-7 Seasonal variation of dissolved oxygen (a) and gravimetric water content (b). Mean ± standard error (n=5).

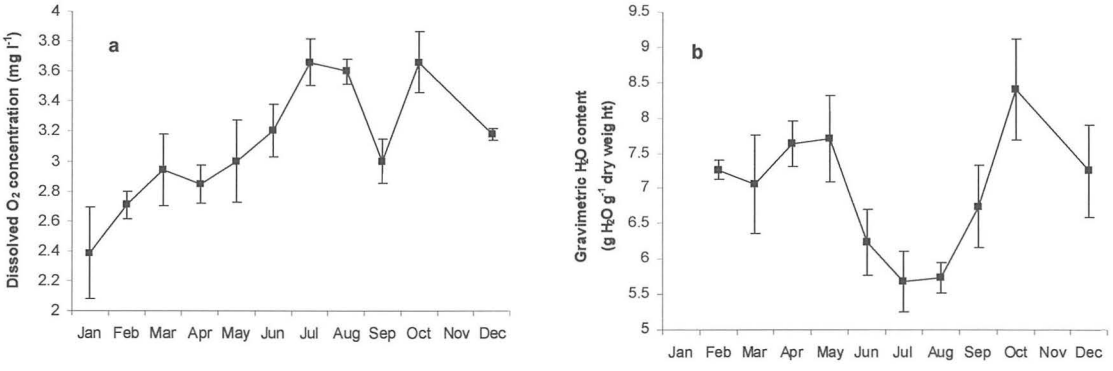


Figure 2-8 Seasonal variation of ammonium (a), potassium (b), magnesium (c), and calcium (d). Mean ± standard error (n=5).

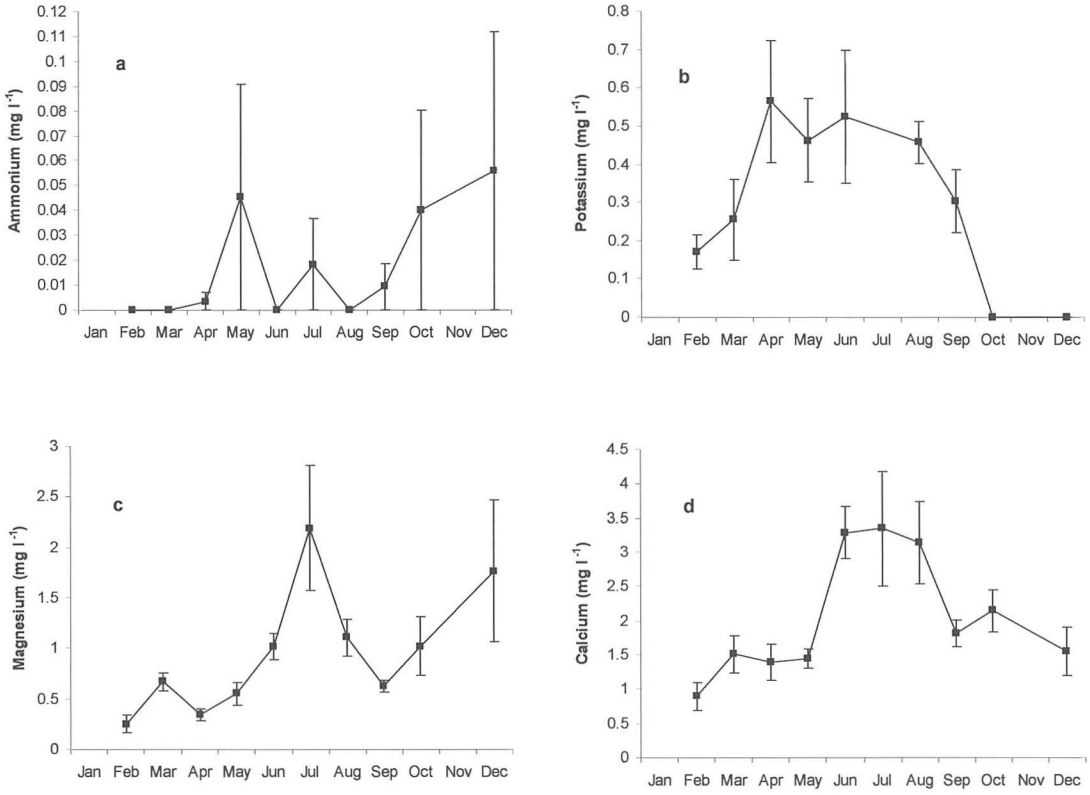
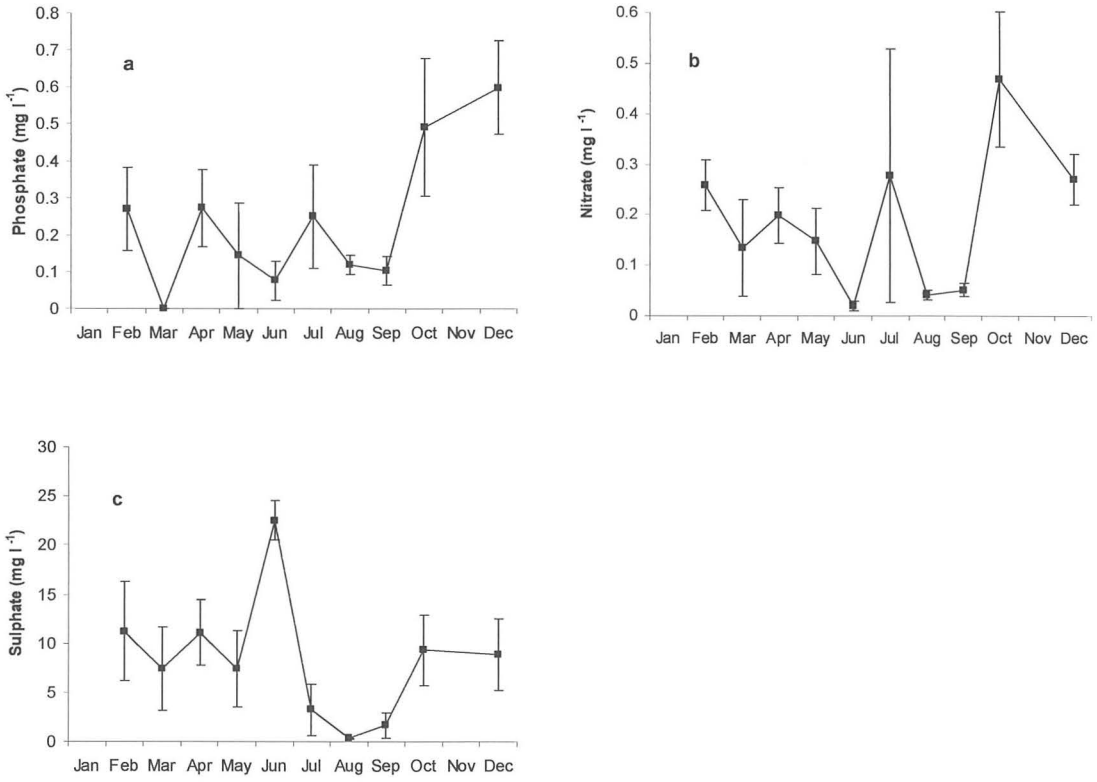


Table 2-1 shows that SOM and potassium (fig. 2-8b) were separated from the other variables by three principal components. Both were lowest in autumn (September/October) and highest in spring (April/May) (table 2-1). However, SOM did not vary significantly throughout the year.

Figure 2-2 shows that the enzymes β-glucosidase (fig. 2-6a) and phosphatase (fig. 2-6b) were related to the gravimetric water content (fig. 2-7b) and the ions ammonium (fig. 2-8a), phosphate (fig. 2-9a) and nitrate (fig. 2-9b) by two principal components. Tables 2-1 and 2-2 suggest however that the enzymes are closely related to the gravimetric water content with lowest means occurring in warm months (Jul/Aug) and highest means in colder months (Feb/Oct). However, phosphatase activity did not vary significantly throughout the year. Seasonal variation in the ions nitrate, ammonium and phosphate appear closely related with highest means in colder months (Oct/Dec) although the lowest means were not consistent. Also, ammonium did not vary significantly

throughout the year. Sulphate (fig. 2-9c) appears distinct from the main groups by two principal components since the lowest mean was in August and the highest in June. All of the enzymes (fig. 2-6) showed peaks in October that corresponded to a secondary peak in basal CO_2 respiration (fig. 2-5) and an increase in anionic concentrations in October (Table 2-2 and fig. 2-9).

Figure 2-9 Seasonal variation of phosphate (a), nitrate (b) and sulphate (c). Mean ± standard error (n=5).



Stepwise multiple regression analysis

Stepwise multiple regression analyses were performed to determine the best abiotic predictors of basal CO_2 respiration, DOC, soil phenolics and soil enzyme activities (Table 2-3). Factors added and removed from the model at $P < 0.15$ were soil temperature, dissolved oxygen, pH, soil water content, cations and anions. The best predictors of basal respiration were sulphatase, potassium and phosphate that all had a positive relationship with respiration. The residuals were normally distributed and VIFs were less than 2.

Table 2-3 Stepwise multiple regression analyses showing the best predictors of basal respiration (R_s), DOC, phenolics, β-glucosidase activity (Glu), phosphatase (Pho), sulphatase activity (Sul) and phenol oxidase activity (Phe Ox). (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (n=12).

y	***Linear regression y = ax + b***	***P*-value**	**R^2**
R_s	$(0.1\ PO_4^{3+} + 0.289\ K^+ + 0.0247\ Sul) - 0.0485$	***	98.2
DOC	$(6.08\ Ca^{2+} + 1.42\ temp) - 10.2$	***	85.6
Phenolics	$(0.357\ temp) - 1.68$	***	87.2
Glu	$(0.969\ DOC - 32.7\ Mg^{2+} - 104\ K^+) + 87.9$	**	81.2
Pho	$(88.1\ NO_3^-) + 26.7$	**	52.4
Sul	$(-5.26\ Mg^{2+} + 11.8\ O_2) - 24.9$	**	73.5
Phe Ox	$(29.3\ NO_3^- + 0.989\ temp) + 1.03$	***	67

Multiple regression analysis showed the best predictors of DOC to be soil temperature and calcium. The residuals were normally distributed and VIFs were less than 2.3. Multiple regression analysis showed that soil temperature was the only significant predictor of soil phenolic concentration.

The best predictors of β-glucosidase activity were DOC, magnesium and potassium. The residuals were normally distributed and VIFs were less than 2. The only predictor of phosphatase activity was nitrate. The best predictors of sulphatase activity were dissolved oxygen and magnesium. The residuals were normally distributed and VIFs were less than 2. Phenol oxidase activity was

predicted best by soil temperature and nitrate that had positive effects on phenol oxidase activity. The residuals were normally distributed and VIFs were less than 1.2.

Q_{10}s

The hydrolase enzymes were not related either linearly or exponentially with seasonal temperature variation. Basal CO_2 respiration showed a significant exponential relationship with seasonal soil temperature variation ($P < 0.01$; $F = 12.25$; $R^2 = 60$ %) with a Q_{10} of 2.29 (fig. 2-10). DOC showed an exponential response to soil temperature ($P < 0.001$; $F = 59.8$; $R^2 = 87$ %) with a Q_{10} of 6.42 (fig. 2-11). Soil phenolics showed an exponential response to soil temperature ($P < 0.001$; $F = 141.27$; $R^2 = 94$ %) with a Q_{10} of 14.42 (fig. 2-11).

Figure 2-10 Mean exponential response of basal CO_2 respiration to seasonal soil temperature variation (n=11).

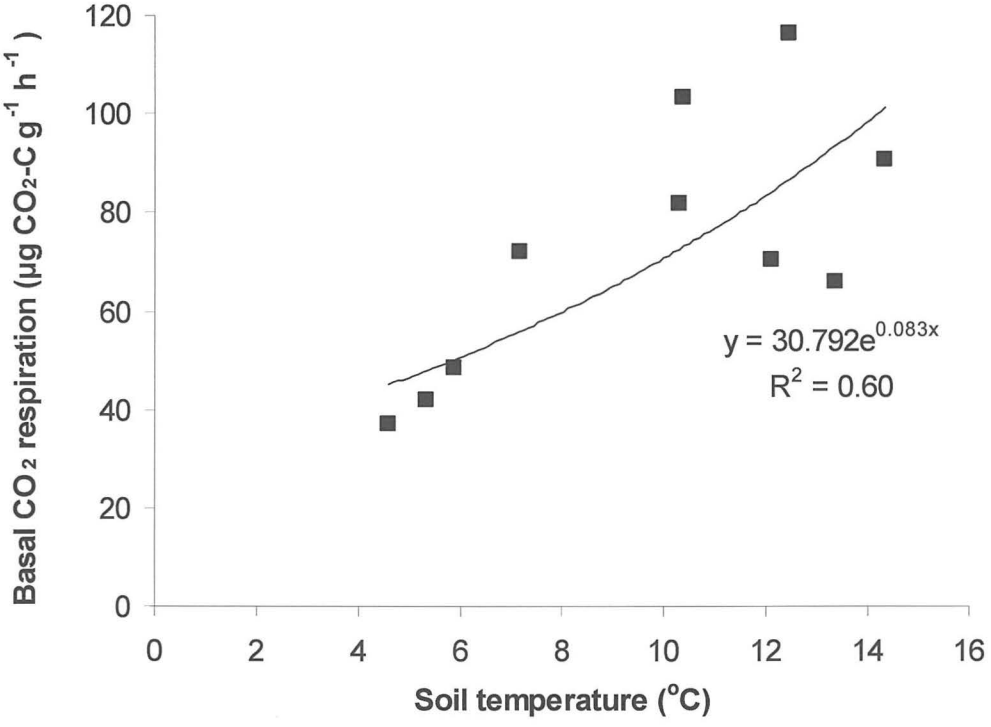
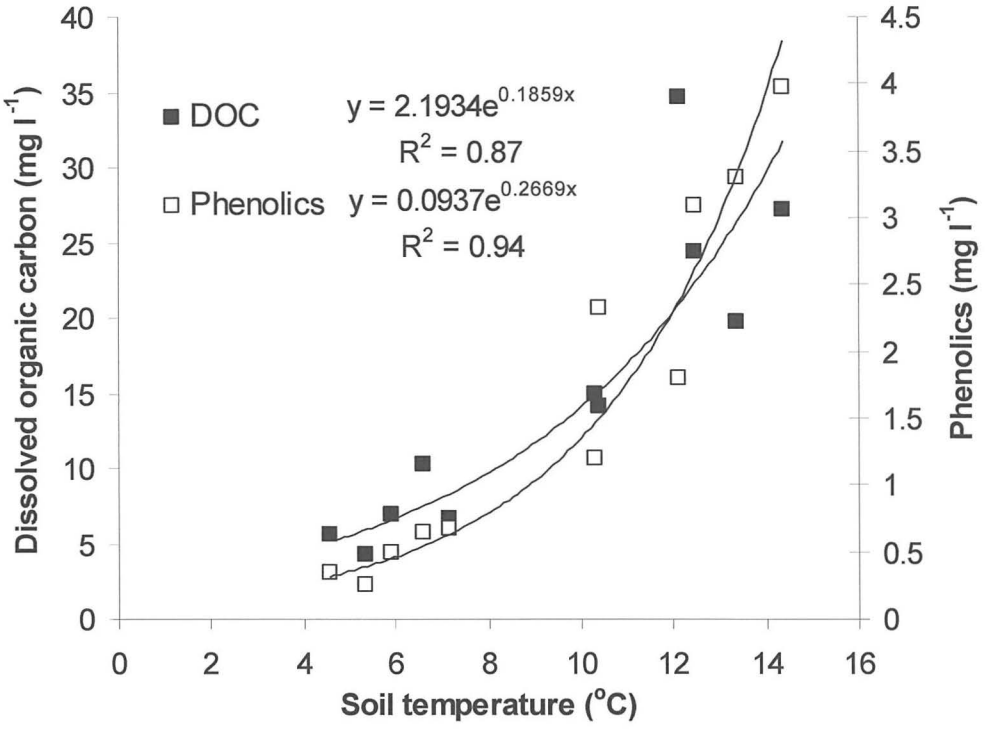


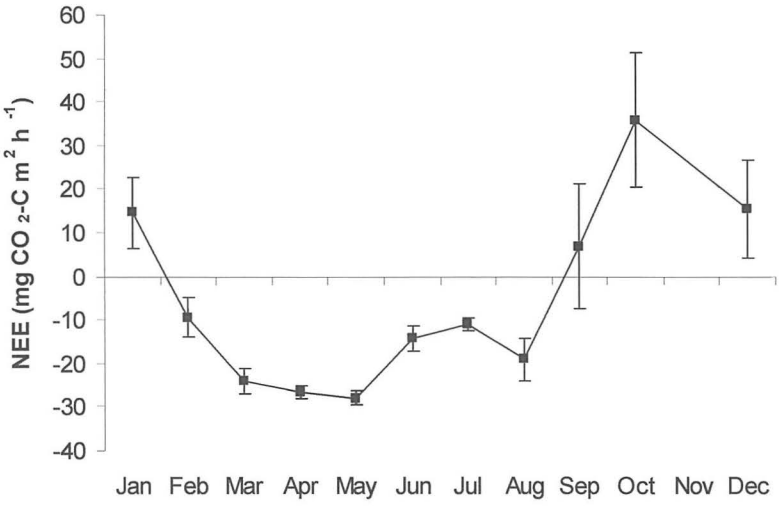
Figure 2-11 Mean exponential response of DOC and phenolics to seasonal soil temperature variation (n=11).



Net CO_2 ecosystem exchange

The peatland was a sink for atmospheric CO_2 in early spring / summer and a source in autumn / winter ($P < 0.001$). Net CO_2-C ecosystem exchange ranged from -32.4 (May) to 81.1 (October) mg CO_2-C m^{-2} h^{-1} (fig. 2-12).

Figure 2-12 Seasonal variation of net CO_2-C ecosystem exchange. Mean ± standard error (n=5).



2.3.2 Effect of plant types on decomposition processes

Mean values for variables in *J. effusus* and *S. recurvum* derived peat are shown in table 2-4. The water content of *S. recurvum* tissue was significantly higher than *J. effusus* aboveground tissue ($P < 0.001$; $F = 38.64$). *J. effusus* had a significantly higher concentration ($P < 0.005$; $F = 16.17$) of soluble phenolics per gram of dry shoot tissue than *S. recurvum*. The gravimetric soil water content was significantly higher in peat beneath *S. recurvum* than *J. effusus* derived peat ($P < 0.05$; $F = 7.4$). Soil phenolics beneath *J. effusus* were significantly higher ($P < 0.001$; $F = 18.44$) than in peat beneath *S. recurvum*. There were no significant differences in DOC or basal CO_2 respiration between peat beneath *J. effusus* and *S. recurvum*. All soil enzyme activities were lower in peat beneath *J. effusus* relative to peat beneath *S. recurvum*. However, only phosphatase activity was significantly lower ($P < 0.05$; $F = 7.41$). Linear regression analysis (fig. 2-13) showed a weak negative relationship between soil phenolics and phosphatase activity ($P = 0.05$; $R^2 = 0.32$). A significant positive correlation was found between DOC and basal CO_2 respiration ($P < 0.001$; $R^2 = 0.916$) (fig. 2-14).

Table 2-4 Differences in physicochemitry, soil carbon pools, basal respiration and soil enzyme activities in peat beneath *J. effusus* and *S. recurvum* with mean ± standard deviation and one-way ANOVA *P*-values (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Variable	***J. effusus***	***S. recurvum***	***P*-values**
Plant aboveground dry weight (kg dry weight m^2)	2.14 ± 1.35	0.87 ± 0.27	ns
Plant H_2O content (g H_2O g^{-1} dry weight)	3.21 ± 0.21	14.18 ± 3.94	***
Plant soluble phenolics (mg g^{-1} dry weight)	1.93 ± 0.52	0.61 ± 0.52	**
Gravimetric H_2O content (g H_2O g^{-1} dry weight)	5.73 ± 0.54	6.79 ± 1.1	*
Volumetric H_2O content (ml H_2O cm^3 dry weight)	0.97 ± 0.097	1.01 ± 0.16	ns
Basal CO_2 respiration (mg g^{-1} h^{-1})	0.54 ± 0.1	0.53 ± 0.07	ns
DOC (mg l^{-1})	42.06 ± 12.1	39.48 ± 11.73	ns
Soil phenolics (mg l^{-1})	39.66 ± 10.56	23.14 ± 6.04	***
Phosphatase (nmol g^{-1} min^{-1})	38.72 ± 18.42	64.02 ± 22.9	*
β-glucosidase (nmol g^{-1} min^{-1})	66.94 ± 24.01	71.47 ± 21.22	ns
Sulphatase (nmol g^{-1} min^{-1})	13 ± 6.03	16.36 ± 8.46	ns
Phenol oxidase (nmol g^{-1} min^{-1})	10.91 ± 3.8	11.13 ± 7.93	ns

Figure 2-13 Relationship between soil phenolic concentrations and phosphatase activity in peat beneath *J. effusus* and *S. recurvum*. Results are given for regression analysis where $n = 20$.

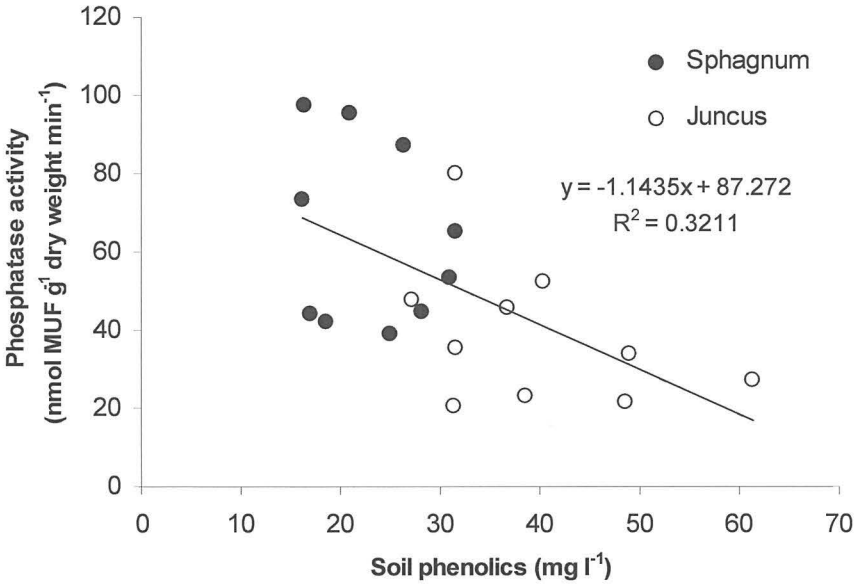
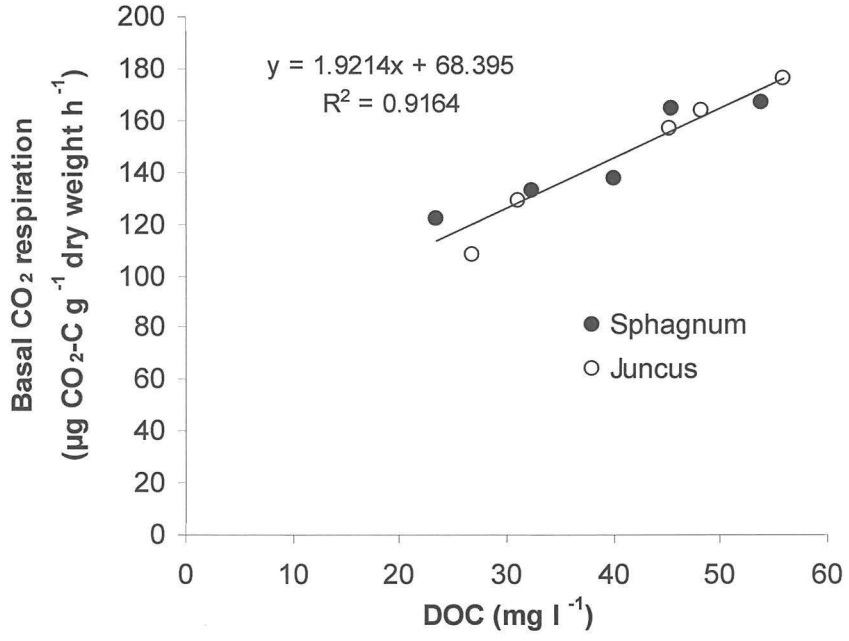


Figure 2-14 Relationship between DOC and basal CO_2 respiration in peat beneath *J. effusus* and *S. recurvum*. Results are given for regression analysis where $n = 10$.



2.4 Discussion

2.4.1 Seasonal variation of physicochemistry, organic C pools, decomposition processes and net CO_2 ecosystem exchange

Temperature and physicochemistry

Principal component analysis and comparison of the minimum and maximum monthly means showed that soil temperature and pH were closely related to DOC, soil phenolics and basal CO_2 respiration and possibly the enzymes sulphatase and phenol oxidase. It is reasonable to assume that soil temperature was the only independent variable in this group and thus the other variables were probably interacting. Soil temperature and pH were highest in summer time and correlated positively ($P < 0.001$; $r = 0.678$). Explanations for the change in soil pH include plant growth in spring (Conyers *et al.*, 1995), microbial redox reactions (e.g. nitrification), increase of humus content, weathering of minerals and cation exchange capacity. Clymo (1963) and Clymo and Hayward (1982) stated that cation exchange by *Sphagnum* is the most important mechanism for the generation of acidity in peatlands. Soil calcium is one of the major determinants of soil pH since calcium ions occupy the exchange sites on soil minerals and act as a buffer system. Calcium, magnesium and soil pH were related by two principal components and this is supported by a positive correlation between calcium and soil pH ($P < 0.001$; $r = 0.535$) (low cation concentration; high H^+ concentration). Organic soils have a greater cation exchange capacity (CEC) than mineral soils (Mitsch and Gosselink, 2000) since organic matter contributes to cation exchange through ionization of carboxyl and hydroxide functional groups, a process which is dependent on the soil pH (Maier *et al.*, 1999). At low pH and low soil temperatures, microbial activity is inhibited, so decomposition and the release of ions from organic matter are slowed. Indeed magnesium and calcium showed significant peaks in summer that may have been the result of cation exchange and increased microbial activity in spring and increased plant uptake in late summer.

Organic C pools

Dissolved organic carbon (DOC) is a poorly defined pool of organic C compounds in various states of decomposition and contains both substrates and end products of enzymic reactions of varying molecular weight. DOC and soil phenolics were related to soil temperature by three principal components. Litter leachates, root exudates and microbial degradation products are regarded as the most important DOC-sources (Zsolnay, 1996). The increase in these soil organic carbon pools during June – August may be explained by temperature effects on decomposition of organic matter by microbial activity. DOC concentrations in soil solutions are often elevated during summer when microbial activity is high (Guggenberger & Zech, 1993; Kaiser *et al.*, 2001). Kaiser *et al.* (2001) found a relative increase of the more recalcitrant aromatic DOC-compounds in forest soil solutions during summer while concentrations of the easily degradable low molecular organic acid were higher during winter when biological activity was low. Microbial activity as basal CO_2 respiration was related by three principal components to DOC and correlated with DOC ($P < 0.001$; $r = 0.451$) and soil phenolics ($P < 0.001$; $r = 0.487$) suggesting that these organic C pools were related to the aerobic / fermentative microbial communities. Ponnamperuma (1972) stated that under anaerobic conditions, fermentative metabolism is dominant, resulting in the production of DOC compounds, rather than the rapid production of CO_2. Regression analysis showed that DOC and soil phenolics were actually exponentially related to soil temperature. The Q_{10} between 4 and 14 °C for DOC was 6.42, and 14.42 for soil phenolics. Thus it is apparent that within the natural temperature range of this site, soil phenolic production was greatly enhanced by temperature increase than DOC production. Freeman *et al.* (2001b) subjected peat soil to a thermal gradient of 2-20 °C and found that an increase of 10 °C led to a 33 % increase in DOC release ($Q_{10} = 1.33$) and an even greater increase in release of phenolic compounds ($Q_{10} = 1.72$) from the soil matrix. Freeman *et al.* (2001b) stated that under warmer conditions, this selective enrichment should impair the metabolism of the remaining DOC (Wetzel, 1992), allowing DOC to reach the oceans and thus leading to the loss of the organic C store in peatlands.

Alternatively, the DOC and phenolic peaks in summer may be explained by plant rhizodeposition. Freeman *et al.* (2004) showed that atmospheric CO_2

caused significant DOC release from bog (14 %), fen (49 %) and riparian (61 %) wetlands that is so ranked because of nutritional grounds. They proposed that the nutrient dependence of this response to CO_2 indicates that the DOC increases were induced by increased primary production and DOC exudation from plants. Although plants are CO_2 limited, temperature and light intensity effects on photosynthesis in summer may have led to rhizodeposition of DOC compounds due to nutrient limitation. Fenner *et al.* (2003) showed that the *Sphagnum* community rapidly contributes recently synthesized carbon to the peatland DOC pool. DOC may also have accumulated as products of microbial detritivore organisms, especially enchytraeid worms. Cole *et al.* (2002) found positive relationships between the abundance of enchytraeids and soil temperature, as well as with concentrations of DOC in a blanket peat soil. They calculated that enchytraeids accounted for 26 % of the DOC produced. Thus microbial activity as basal respiration may have increased in response to warmer conditions and an increased supply of labile C substrate from DOC leaching and plant rhizodeposition.

The results also suggest that both DOC and soil phenolics were closely related to soil pH and this was supported by a positive correlation ($P < 0.001$; r = 0.533). Liming of soils increases leaching of DOC and dissolved organic nitrogen (Anderson *et al.*, 1994; Anderson *et al.*, 2000). Anderson *et al.* (2000) suggested that pH could sometimes be more important for DOC leaching than the microbial activity. An increase in pH increases the amount of negatively-charged groups on the humus colloids and the solubility should therefore increase. Thus the increase in soil pH may partially explain the increase in DOC via increased leaching that would explain the effect of soil temperature and Ca^{2+} on DOC. However, Cronan *et al.* (1992) found no effect of liming on DOC and suggested that Ca^{2+} stabilizes DOC (Römkens *et* al., 1996) like the stabilizing effects of Al and Fe.

Soil organic matter (SOM) did not vary significantly throughout the year and remained at about 90 % of soil dry weight. This suggests that decomposition of SOM is constrained by soil anaerobiosis, limited oxygen availability, acidity and nutrient availability that suppress decomposition under warmer summer conditions (Mitsch and Gosselink, 200). Whilst there was significant seasonal variation in soil pH, dissolved O_2 concentration and soil water content in

directions required for SOM decomposition (i.e. increased pH, increased O_2, reduced water content), the levels reached in summer remained constraints on SOM decomposition. The close relationship between SOM and potassium may partially be explained by cation exchange capacity.

Decomposition processes

Basal CO_2 respiration was related by three principal components to soil pH and temperature. Thus it is apparent that soil acidity and temperature are major factors affecting microbial activity although temperature effects on microbial activity may also have affected soil pH via microbial redox reactions (i.e. mineralization, uptake). It has been estimated that soil heterotrophic respiration and CO_2 production doubles with every 10 °C increase in temperature (Sarmiento, 2000). Within the range 10-30 °C, soil respiratory quotients (Q_{10} values) for soil respiration have been reported to be about 2 to 2.5 (Raich and Schlesinger, 1992; Peterjohn *et al.*, 1994; Thomas *et al.*, 1996; Chapman and Thurlow, 1998; Briones *et al.*, 2004). Basal CO_2 respiration between 4 and 14 °C at this site showed a Q_{10} of 2.29 that suggests that within the current temperature range CO_2 production is in agreement with these studies even though other factors such as soil anaerobiosis, pH, labile DOC substrate availability and limited nutrient availability may have affected microbial activity. Multiple linear regression analysis suggested that basal CO_2 respiration was limited by the availability of PO_4^{3-} and Mg^{2+} and possibly affected sulphatase activity. Thus both an exponential response to soil temperature and linear effects of nutrient availability may determine basal respiration.

Despite the effect of soil temperature and pH on basal CO_2 respiration, enzyme activities showed no apparent response. Phenol oxidase and sulphatase activities were related to soil temperature, pH, DOC and basal CO_2 respiration by three principal components although evaluation of PC1 and the minimum and maximum monthly means shows that activities were not lower in winter and/or higher in summer as with the other variables. Soil temperature affects enzyme activities indirectly through influencing microbial proliferation and also directly by modifying enzyme kinetics (Freeman *et al.*, 1999). As in this study, Kang and Freeman (1999) and Kang (1999) also found a limited response of extracellular enzyme activities to soil temperature in a bog in Migneint, North Wales (UK grid

SH 805 458). It was suggested that factors such as low pH, low ion concentrations, low oxygen content and low microbial proliferation (Brock and Bregman, 1989) may have limited enzyme activities. For determining the seasonal variation of extracellular enzyme activities in this survey, assays were performed using V_{max} substrate concentrations, at field temperature and without the addition of buffer in order to determine seasonal change within laboratory conditions. Thus it is hypothesized that the majority of soil enzymes are extracellular and that microbial proliferation probably has a limited effect on the soil enzyme pool due to physicochemical constraints on microbial metabolism.

The activities of the enzymes β-glucosidase and phosphatase were closely related to the gravimetric water content suggesting higher activities under higher soil water content. Freeman *et al.* (1996) showed that β-glucosidase, sulphatase and phosphatase activities increased under drier conditions due to a reduction in inhibitory compounds such as iron and soil phenolics. Thus these results are contradictory to the known response of soil enzymes in wetlands to drought. However, the measurements of drought were based on water table depth and not soil water content as in this study. In this study, soil phenolics were higher under reduced soil water content and may therefore partially explain the reduction in soil enzyme activities. However, only phosphatase was negatively correlated with the soil phenolic concentration.

Phosphate, nitrate and ammonium were related to one another but were not related to soil temperature or pH most likely because of their limited availability since the majority of inorganic N and P are locked up in soil organic matter (Mitsch and Gosselink, 2000). This may be especially true for ammonium that did not vary significantly throughout the year. However, these nutrients were unsurprisingly related to the enzymes β-glucosidase, phosphatase and possibly phenol oxidase. Enzyme production is nitrogen and energy intensive and thus microbes should only produce enzymes at the expense of growth and metabolism if available nutrients are scarce (Koch, 1985). Allison and Vitousek (2005) found that β-glucosidase and phosphatase activities increased upon addition of C and N. They suggested that enzyme allocation patterns reflect microbial nutrient demands and may allow microbes to acquire limiting nutrients from complex substrates available in the soil. Multiple regression analysis suggested that DOC had a positive effect on β-glucosidase, and Mg^{2+} and K^{+} had negative effects.

The majority of DOC is composed of polymeric compounds, which need to be enzymatically depolymerised before utilization by heterotrophic microorganisms (Chróst, 1991). Thus β-glucosidase production may have been induced by high concentrations of high molecular weight DOC or the enzyme reaction caused an accumulation of low molecular weight DOC. Freeman *et al.* (1998a) reported a positive correlation between β-glucosidase and DOC in a wetland soil. However, the multiple regressions suggest that other factors such as NO_3^-, Mg^{2+}, K^+ and phenolics may be involved in the regulation of soil enzymes. The inhibitory effects of phenolics are alleviated by Mg^{2+} and Ca^{2+} (Wetzel, 1992) and both cations increased significantly during lower soil water content when soil phenolics were highest. Kang (1999) found that phenolics inhibited β-xylosidase and N-acetylglucosaminidase and showed that the enzymes are strongly inhibited by phenolics and low pH. It is hypothesized that the soil water content in summer may have remained a limit on microbial activity (and hence enzyme production) in summer, and the interactive effect of soil phenolics and other factors (i.e. ions) accounted for the seasonal variation of soil enzyme activities.

Alternatively, the substrate quality in autumn may explain the variation in soil enzyme activities. In autumn, when plants become senescent, the substrate quality and quantity may be optimal for the proliferation of some microorganisms including cellulose degrading fungi and bacteria (Eriksson *et al.*, 1990), which in turn yield high enzyme activities. Kourtev *et al.* (2002) suggest that enzyme expression is a labile property of microbial communities, changing in response to the specific concentration of particular litter substrates independently from the surrounding environment. Early in the decomposition process, cellulolytic enzymes show an increase in activity, which later decreases, as cellulose is being decomposed and used by microorganisms. At the end of the decomposition process, oxidative enzyme (phenol oxidase) activities become more important, since the recalcitrant materials from the litter (e.g. phenolics) are the only sources of C that are left (Kourtev *et al.*, 2002). Seasonally, phenol oxidase activity and hydrolase activities were highest in autumn (October) and lowest in summer when soil phenolics were highest. These results agree with results that show higher enzyme activities occurring in autumn months (Criquet *et al.*, 2000; Criquet *et al.,* 2002). Also, the peaks in October corresponded to an increase in anion concentrations and basal CO_2 respiration that may represent a

short period of intense decomposition. Phenol oxidase is one of a few enzymes that are able to degrade soil phenolic compounds (Freeman *et al.*, 2001a). The low activity of this enzyme and hence its inability to degrade phenolics in this system may go some way to explaining part of the variation in hydrolytic enzyme activity (Freeman *et al.*, 2001a). The enzyme requires bimolecular oxygen, even though it exists in an essentially anaerobic environment (Freeman *et al.*, 2001a). It is hypothesized that as microbial activity declined with seasonal temperature change, the production of soil phenolic compounds was reduced and potentially the remaining pool was oxidized by phenol oxidase in autumn leading to an increase in hydrolase activity and mineralization that may have increased microbial activity.

Net CO_2 ecosystem exchange

Net CO_2 ecosystem exchange (NEE) showed that the peatland was a sink for atmospheric CO_2 between February and August and a source between September and January. Overall the results suggest that the site was a sink for atmospheric CO_2 due in particular to the productivity of vascular species such as *Juncus*. NEE is the result of gross photosynthetic activity and ecosystem respiration and thus photosynthesis was relatively higher per unit soil area than respiration between February and August. The sink strength was highest in May since basal CO_2 respiration did not peak until July that would have reduced the sink strength by photosynthetic activity at peak summer time. Thus gross photosynthetic activity may have been highest between June and August when temperature and light intensity would have been highest.

2.4.2 Effect of plant types on decomposition processes

Despite the relative individual size of *Sphagnum* compared to higher plants such as *Juncus effussus*, *Sphagnum* biomass forms a significant fraction of total plant biomass in peat accumulating ecosystems as evident by the insignificant difference in dry plant biomass per unit area of soil. *S. recurvum* tissue had a significantly higher water content than *J. effussus* aboveground tissue. This is due to hyaline cells that retain water and allow *Sphagnum* species to move away

from the water table, thus increasing water height (Shaw and Goffinet, 2000). These conditions create greater amounts of anaerobic conditions and through complex canopy development allow *Sphagnum* species to expand habitat space (Shaw and Goffinet, 2000). Indeed, soil water content beneath *S. recurvum* was significantly higher than beneath *J. effussus* that is most likely due to decaying *Sphagnum* plants that can still retain water in the dead hyaline cells.

Soluble phenolic concentrations were higher in *J. effussus* aboveground dry tissue relative to *S. recurvum* tissue even though *Sphagnum* tissues are known to contain high concentrations of polyphenolic compounds (Berendse *et al.*, 2001; Verhoeven & Liefveld, 1997; Kang, 1999) relative to certain vascular plants (Williams *et al.*, 2000). Soil phenolic concentrations were also higher in the rhizosphere of *J. effusus* relative to *S. recurvum* derived peat. This suggests that soluble phenolics may accumulate in the peat via vascular plant rhizodeposition. However, soluble phenolics constitute at most 25 % of total soluble material and other compounds such as soluble sugars and proteins form the largest part of the leachable pool (Scheffer *et al.* 2001). Scheffer *et al.* (2001) found that soluble phenolics were higher in *Carex* than in *Sphagnum* species. However, *Sphagnum* species produce secondary metabolites, such as *Sphagnum* acid, which may inhibit the activity of microorganisms involved in the decomposition process (Verhoeven and Toth, 1995; Verhoeven and Liefveld, 1997).

This spatial variation of soil phenolics potentially had a significant negative effect on phosphatase activity in peat directly beneath *J. effusus* relative to *S. recurvum*. Williams *et al.* (2000) compared phenol oxidase activity between *Sphagnum* and *Carex* dominated wetlands. They found that *Carex*-derived peat supported substantially higher phenol oxidase activity than *Sphagnum* peat, and significantly higher concentrations of soluble phenolic compounds were found in *Sphagnum*-derived peat than in *Carex*-derived peat. They stated that the low phenol oxidase activity in the *Sphagnum*-derived peat suggests an enzyme inhibitory effect by compounds derived from the vegetation. They also showed that vascular plant roots are a potential source of phenol oxidase activity. Whilst this is contradictory to the data presented here, in terms of soluble phenolic concentrations between vascular and non-vascular plants, it does support the hypothesis that certain extracellular enzymes can be inhibited by compounds

derived from specific plant species. The interactive effect of soil phenolics and other factors on phosphatase activity in the seasonal survey suggests that phosphatase may be susceptible to phenolic inhibition. However, only 32 % of phosphatase activity was explained by soil phenolics and factors not measured my account for the remaining variation. Other factors that could affect phosphatase activity include PO_4^{3-} availability. Press and Lee (1983) found that intracellular acid phosphatase activity in 11 species of *Sphagnum* was negatively correlated with the total phosphorus concentration and, in experiments, increased under conditions of phosphate starvation. Also, *Sphagnum* species do not possess true roots and thus zones of depletion may have occurred beneath *J. effusus* that could have stimulated the production of phosphatase enzymes.

Regression analysis between DOC and basal CO_2 respiration in peat beneath both species showed that DOC is a source of substrate for aerobic respiration and that microbial populations within each rhizosphere show the same response to basal DOC concentrations. This positive response of basal CO_2 respiration to DOC also implies that aerobic/fermentative microbial activity maybe limited by the availability of low molecular weight organic compounds from enzymic decomposition.

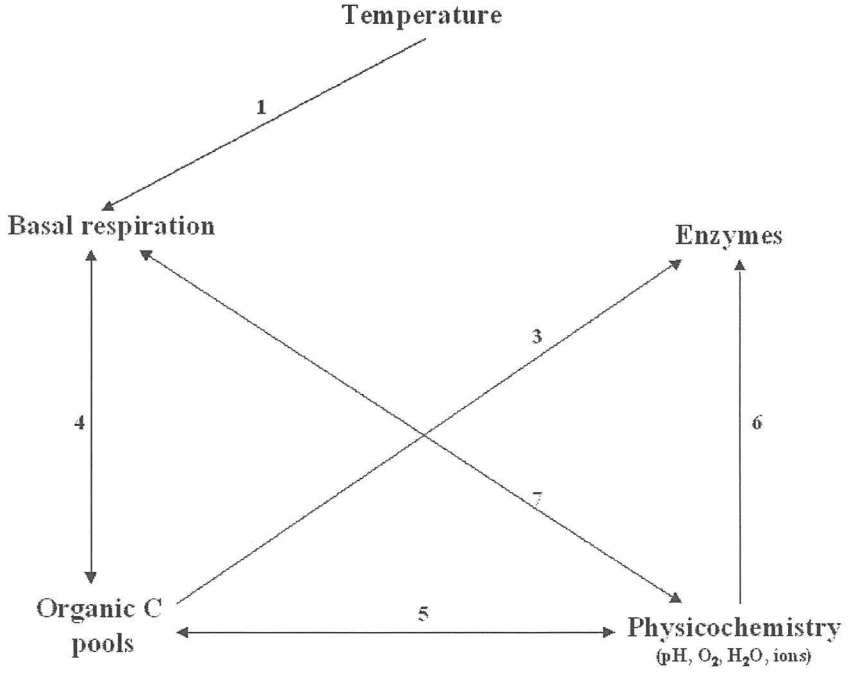
2.4.3 Conclusions

The results from this study allow comparison to the hypotheses stated in the introduction. A revised conceptual model of the relationships between decomposition processes, organic C pools and physicochemistry is shown in figure 2-14: (1) seasonal temperature variation only affected decomposition via a positive effect on microbial activity suggesting that soil enzyme activity maybe inhibited by other factors (i.e. soil phenolics); (2) no apparent relationship was found between basal CO_2 respiration and all of the enzymes studied suggesting that microbial enzyme production is limited; (3) the limited response of soil enzyme activities to seasonal temperature variation suggests that the breakdown of HMW carbon to labile carbon is also limited but that soil phenolics may feedback by inhibiting soil enzyme activities; (4) DOC is potentially both a substrate and end-product of microbial activity; (5) soil pH was related to DOC

concentrations and possibly the cations Mg^{2+} and Ca^{2+} via cation exchange; (6) ionic concentrations and soil water content were related to soil enzyme activities; and (7) soil pH was related to basal CO_2 respiration.

These results imply that the limited response of extracellular enzymes to soil temperature may limit the loss of soil carbon as CO_2 by global warming since microbial activity is limited by the supply of labile carbon and minerals from enzymic hydrolysis of SOM. However, approximately 87 % of the seasonal variation of DOC was related to soil temperature suggesting that global warming may stimulate HMW DOC release. Also, the enhanced production of phenolics relative to DOC may inhibit hydrolase enzymes that are responsible for the breakdown of HMW DOC that may permit leaching of DOC to rivers draining peatlands. The effect of plant types on soil decomposition processes spatially is probably of less importance than temporal variations due to changes in climate. Further experimental analysis is required to determine the factors that regulate soil enzyme activities and whether increased microbial activity and DOC may feedback on soil enzyme activities under elevated temperature.

Figure 2-14 A revised conceptual model of the relationship between temperature, decomposition processes, organic C pools and physicochemistry. Numbers refer to hypotheses.



Chapter 3

Effect of reduced *Sphagnum* productivity and abiotic factors on soil decomposition processes in a blanket bog

3.1 Introduction

Blanket bogs are typically ombrotrophic, oligotrophic and strongly acidic peatlands (Mitsch and Gosselink, 2000). Unlike riparian systems, bogs have no significant inflow from adjacent mineral soils except via precipitation and thus are characterized by low nutrient concentrations. The limited availability of nutrients, high water table and low temperatures account for the dominance of *Sphagnum* species and the low rates of organic matter decomposition. More than half of the world's peat originated from *Sphagnum*, representing 10-15 % of the terrestrial carbon stock, with more carbon held in dead and living *Sphagnum* than is fixed annually by all terrestrial vegetation (Clymo and Hayward, 1982). *Sphagnum* species affect soil decomposition by creating waterlogged, nutrient-poor and acidic conditions that strongly favour carbon sequestration (Van Breeman, 1995; Hoosbeek *et al.*, 2001; Clymo, 1963). However, little information is available regarding the effect of living *Sphagnum* productivity on decomposition processes via rhizodeposition. Interactions between *Sphagnum* productivity and soil decomposition processes may have important implications for the sequestration of C in peatlands under future IPCC scenarios (IPCC, 2001). Thus there is a requirement to determine whether simple interactions exist between *Sphagnum* productivity and soil decomposition processes in these systems and how this may affect net ecosystem CO_2-C exchange (NEE).

Net photosynthetic CO_2 fixation is the sum of gross photosynthetic activity and respiration, resulting in the production of organic biomass. Plants attain a steady-state in which net CO_2 fixation is balanced by the loss of organic C by respiration, rhizodeposition, and death and decay. Bryophytes are generally considered to be shade adapted plants, reaching photosynthetic light saturation at low irradiances (Shaw and Goffinet, 2000; Davey *et al.*, 1997). Typically, photosynthesis of bryophytes saturate between 30 – 300 μmol m^{-2} s^{-1} (i.e. 5-10 %

of full sunlight) (Shaw and Goffinet, 2000; Davey *et al.*, 1997). A reduction in photosynthetic photon flux density (PPFD) affects photosynthetic CO_2 fixation by reducing ribulose bisphosphate (RuBP) regeneration via effects on electron transport and ATP synthesis (Taiz and Zeiger, 1998). In order to maintain a positive C balance, some plants can reduce their respiratory rate to lower the compensation point - the PPFD value at which a positive C balance is reached (Fitter and Hay, 1987). Ultimately this causes a reduction in growth or NPP and thus is only of value to severely shaded plants.

Plants and microorganisms have a mutualistic relationship that is driven in part by climatic factors via the supply of labile C and nutrients (Schmidt *et al.*, 1997; Van Veen *et al.*, 1989). Rhizodeposition is considered an important pathway of carbon incorporation into the rhizosphere of grassland and arable ecosystems (Reynolds *et al.*, 2003; Ostle *et al.*, 2003). Vascular plants export approximately 30 – 90 % of net photosynthate to the roots (Whipps, 1990). Wallén (1986) showed that up to 98 % of carbon assimilated by a subarctic mire ecosystem could enter below-ground structures. Fenner *et al.* (2003) showed that *Sphagnum* photosynthate rapidly contributes to the soil DOC pool. This dynamic carbon pool is mobile, biologically reactive and can account for important losses of C from peatland ecosystems (Freeman *et al.*, 2001b; Fenner *et al.*, 2003). DOC contains carbohydrates, amino acids and organic acids that have been shown to increase the abundance and activity of soil microbes in the rhizosphere (Newman, 1985; Lynch and Whipps, 1990). Thus soil microorganisms are dependent on plant derived DOC and organic matter as a source of substrate, and are therefore potentially affected by climate both directly and indirectly via plant productivity. Change in the supply of *Sphagnum* derived labile C substrates to soil microorganisms may therefore significantly affect decomposition and the CO_2-C balance of peatland ecosystems.

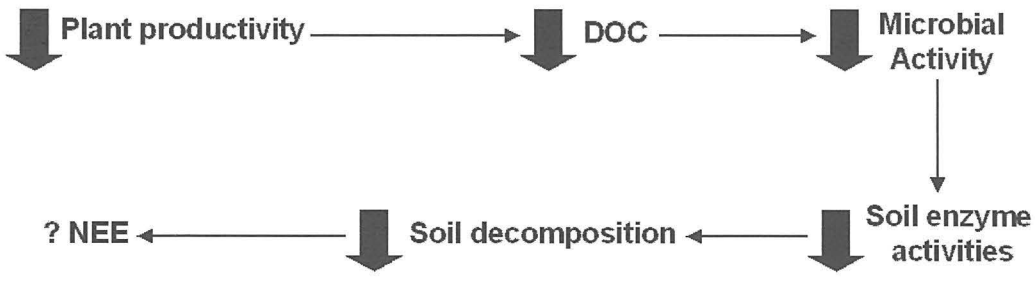
The aim of this research was to evaluate the interaction between *Sphagnum* productivity and soil decomposition processes under *in situ* climatic conditions. In this experiment, *Sphagnum* productivity was reduced by shading since shading does not have a direct effect on soil decomposition processes. It was hypothesized that a reduction in *Sphagnum* productivity by shading would reduce soil decomposition by reducing the allocation of fixed C to rhizodeposition (fig. 3-1). Of interest was how these potential alterations to

Sphagnum productivity and soil decomposition may impact on the C balance (NEE). Also, the experimental design was utilized to estimate the proportion of ecosystem respiration that is derived from *Sphagnum* respiration. This is of importance since climatic factors may affect plant and soil respiration differently and thus complicate model predictions of ecosystem respiration.

At monthly intervals over the course of one-year, abiotic factors, organic C pools, net CO_2 ecosystem exchange (NEE), ecosystem respiration, basal CO_2 respiration and soil enzyme activities were measured within shaded and control treatment plots. NEE of the shaded treatment was determined under natural PPFD conditions (as in the control) since it allowed non-destructive estimation of *Sphagnum* productivity (under natural PPFD conditions, gross photosynthetic fixation of atmospheric CO_2 per unit biomass in the shaded treatment would potentially operate at the same rate as the control treatment and thus a reduction in estimated gross photosynthesis per unit area would indicate a reduction in productivity by shading).

The seasonal data of the control (non-shaded) treatment were then analysed by principal component analysis (PCA) and multiple regression analyses to determine relationships between abiotic factors, organic C pools, CO_2 fluxes and decomposition processes. Since a destructive estimation of *Sphagnum* productivity in the shaded treatment could not be determined each month throughout the year, the affect of shading on *Sphagnum* productivity and CO_2 fluxes after 14 months is presented first in the methodology and results sections prior to the seasonal variation of abiotic and biotic variables.

Figure 3-1 Conceptual hypothesis of the effect of *Sphagnum* productivity on soil decomposition processes



3.2 Materials and methods

Site description

The study was conducted at Moorhouse National Nature Reserve, Upper Pennines, UK (plate 3-1) because it is considered typical of British moorland (Heal *et al.*, 1975) or blanket bog. It has a strong history of ecological research (Heal and Perkins, 1978; Ineson *et al.*, 1998) and since 1992 has been a site of the UK Environmental Change Network (ECN), which aims to collect, store, analyse and interpret long-term data on the factors that drive and respond to environmental change (Burt, 1994). Physicochemical data collected by ECN in 2003-2004 was agreed to be used as additional biogeochemical data for this study. However, due to unexpected issues the data was not available for the period of study. The peat at Moorhouse belongs to the Winter Hill soil series (Carroll *et al.*, 1979) which Avery (1980) classified as raw oligo-fibrous peat. The site (UK grid reference NY 755 336) is at an altitude of 500m and is dominated by ling heather (*Calluna vulgaris* L.), cotton grass (*Eriophorum* spp.), and bryophytes (*Sphagnum* spp.), and is described within the National Vegetation Classification (Rodwell, 1991) as *C. vulgaris-Eriophorum vaginatum* blanket mire with *Empetrum nigrum* sub-community (M19b).

Plate 3-1 Moorhouse NNR in December 2003 looking west from the field site.

The experimental design consisted of twenty 4 m^2 plots that were distributed over the slope of a hill with 100 % ground cover of *Sphagnum* species in the base of a central gas chamber that remained open during non-sampling periods. Since the slope faced south, this maximised the duration and interception of sunlight. Ten plots were randomly chosen for the shaded treatment and covered with 3 mm mesh above the *Calluna vulgaris* canopy that reduced PPFD to 15 % of natural PPFD. The mesh was elevated in the centre using wooden poles to prevent excessive rainwater accumulation in the gas chambers. Each plot consisted of a central gas chamber base, two water samplers, and an area for random peat sample collection from beneath *Sphagnum* species only (fig. 3-2 and plate 3-2). The mesh, water samplers and gas chamber bases were placed in position one month prior to the first seasonal collection data in April 2003.

Figure 3-2 Experimental design of control and shaded plots

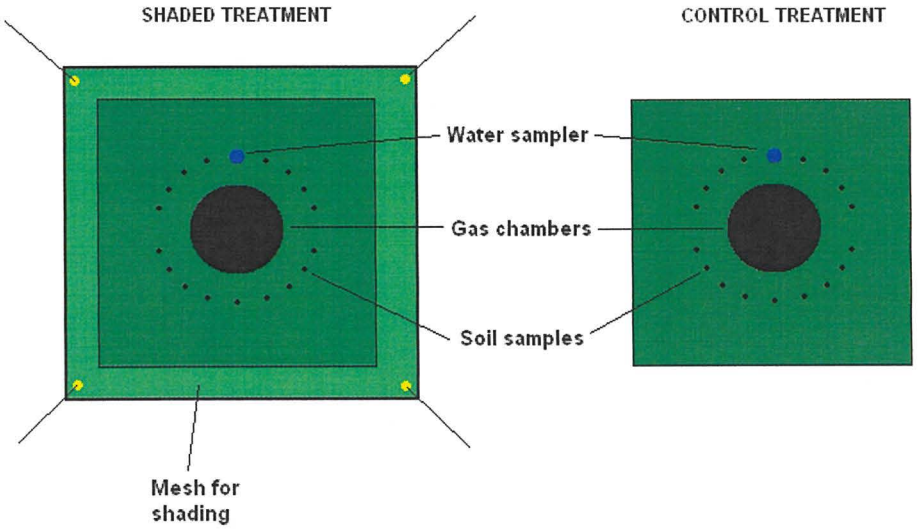


Plate 3-2 Experimental design showing the canopy shading mesh (a) and (b), gas chambers (c) and water samplers (d).

3.2.1 Effect of shading on vegetation composition and *Sphagnum* biomass

After 14 months growth, in June 2004, *Sphagnum* vegetation was removed from within each gas chamber following measurement of carbon dioxide fluxes to determine the effect of shading on *Sphagnum* productivity. The vegetation was weighed and separated into *Sphagnum* species. Samples were then dried at 70 °C for 3 days and dry weights of each species determined and totaled.

3.2.2 Effect of shading on carbon dioxide fluxes

Gas chambers were of two parts (base and lid) made of PVC pipe measuring 24 cm diameter. The base measuring 20 cm depth were placed in the soil at the centre of each plot in April 2003 to a soil depth of 10 cm and remained in position throughout the experiment. The lid measured 20 cm depth with a transparent acrylic cover sealed with glue and silicon sealant to allow light

penetration with a septum for gas collection. The top gas chamber was sealed to the base soil chamber using rubberised tyre inner-tube to prevent gaseous release during a two-hour gas sampling incubation period.

In April 2003, gas chambers were attached and all of the plots were covered with black-out covers in order to measure ecosystem respiration by inhibiting gross photosynthesis. A 12 ml gas tight exetainer was extracted of air in the field using a 20 ml syringe and 20 ml of gas was extracted from the chamber headspace and placed in the exetainer. Each hour a sample was collected. Gas samples were injected into a Perkin Elmer Gas Chromatograph equipped with a Porapak QS column with flame ionization detector. The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1}. Background gas concentrations were subtracted from incubated chamber concentrations to give an estimate of flux and converted to mg CO_2-C m^{-2} h^{-1}. Preliminary data showed that gas concentrations increased linearly for up to 3 hours (Appendix 1; fig. A1-1). An incubation of 2 hours was used for all further analyses. Plots within each treatment (n=10) were paired according to ecosystem respiration rates so that NEE measurements (n=5 per treatment) could be paired with ecosystem respiration measurements (n=5 per treatment) to estimate gross photosynthesis by subtraction where Estimated gross photosynthetic activity (negative sign) = NEE – ecosystem respiration. NEE values had a negative sign to represent CO_2-C sink and a positive sign to represent CO_2-C source. Gross photosynthesis was converted to a positive sign when not comparing photosynthesis with NEE.

After 14 months, in June 2004, net CO_2 ecosystem exchange and ecosystem respiration of the control and shaded treatments were determined simultaneously. Unlike the seasonal assessment of NEE (see below), the shaded plots remained shaded throughout the gas incubation period. Estimated gross photosynthesis was determined as NEE – ecosystem respiration. A week later, ecosystem respiration and soil respiration were determined simultaneously for each treatment using paired replicates. Five paired plots within each treatment were covered with black-out cover in order to measure ecosystem respiration whilst in the remaining five paired plots *Sphagnum* tissue was removed and respiration was determined as above. *Sphagnum* respiration was determined as ecosystem respiration – soil respiration (no *Sphagnum*).

3.2.3 Seasonal variation of abiotic factors, carbon dioxide fluxes, organic C pools and soil enzyme activities

Prior to the destructive sampling above, a one-year field survey was carried out between May 2003 and April 2004 to examine the effect of shading (reduced productivity) on decomposition processes and relationships between variables.

Abiotic factors

Soil solution was collected using a syringe and water sampler (Freeman *et al.*, 1994) placed at a depth of 15 cm (see Chapter 2.2). Water samples were filtered with 0.45 μm filters on the day of collection and kept at field temperature (4-15 °C) until analysis. A peat sample measuring approximately 100 cm^3 was collected from each plot to a depth of 10 cm each month from beneath *Sphagnum* species only. Peat samples were placed in sealed polythene bags and kept at field temperature until analysis. Prior to analysis peat samples were homogenised by hand and large roots were removed. Soil and air temperature were measured using a digital thermometer with a Tinytalk recorder. Photosynthetic photon flux density (PPFD) was measured with a photosynthetic active raditation (PAR) sensor. Bulk density and gravimetric water content were determined by drying 1 cm^3 of each peat replicate at 105 °C for 24 hours. Rainfall and water table depth data were taken from automatic weather stations (ECN) where water table depth was determined as centimetres below the soil surface.

Carbon dioxide fluxes

The reduction in *Sphagnum* productivity was determined each month by removing the canopy shading so as to measure net CO_2 ecosystem exchange (NEE) of both treatments under natural PPFD conditions. Measurements of NEE, ecosystem respiration and estimation of gross photosynthesis were made periodically in the middle of each month as by the method above using paired replicates (Section 3.2.2).

Basal CO_2-C respiration was measured in the laboratory by placing 1 cm^3 peat samples for each of the peat replicates in 100 ml plastic bottles with rubber septa. Bottles were sealed and 10 ml of gas taken and injected into a pre-evacuated, gas-tight 5 ml exetainer. Ten ml of N_2 was added to each pot to

replace the extracted gas volume. After 2 hours incubation at field temperature, approximately 10 ml of gas was removed and injected into a pre-evacuated, gas-tight 5 ml exetainer. The gas samples were injected into a Perkin Elmer Gas Chromatograph equipped with a Porapak QS column with flame ionization detector. The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1}. Background gas concentrations were subtracted from incubated concentrations to give an estimate of flux and expressed as μg CO_2-C g^{-1} dry soil weight h^{-1}.

Organic C pools

Water samples were filtered with 0.45 μm filters on the day of collection and kept at field temperature (4-15 °C) until analysis. Dissolved organic carbon (DOC) was analysed by the chemistry department at CEH Merlewood/Lancaster. Samples were analysed by the standard Skalar method as follows. Inorganic C was removed by acid addition, then organic C was oxidized using a UV / persulphate digest. The acidic solution was then used to decolourise a phenolphthalein buffer and the colour change was measured at 550 nm. Samples giving results greater than the top calibration standard were re-analysed following a two-fold dilution in water. Dissolved soil phenolic concentration was assayed on the same samples using Folin-Ciocalteau reagent (Box, 1983). In a 2 ml centrifuge vial, 1 ml of filtered soil solution was added with 150 μl of Na_2CO_3 solution (200 g l^{-1}). To each replicate, 50 μl of Folin-Ciocalteau solution was added underneath a fume hood and maintained for 2 hours at room temperature. A standard curve was prepared by applying the same chemicals to 0 – 2 mg l^{-1} phenol solution. The change in colour was measured with a spectrophotometer at 750 nm. Samples out of range were diluted with deionised water.

Soil enzyme activities

The activity of three extracellular hydrolase enzymes (β-glucosidase, phosphatase and sulphatase) were determined using fluorogenic methylumbelliferyl (MUF)-substrates (Freeman *et al.,* 1995). MUF substrates were pre-dissolved in 2 ml cellosolve (ethylene glycol monomethyl ether) using an ultrasonic bath. For each of the twenty peat samples, 10 cm^3 of peat was placed in 50 ml vial with 40 ml of deionised water. Vials were homogenised for 2 minutes using a vortex and passed through a 2 mm filter to remove roots. 1 ml of slurry was placed in a 2 ml centrifuge vial with 1 ml of MUF substrate (400 μM for MUF-β-glucoside and 1000 μM for MUF-sulphate and MUF-phosphate). MUF-substrate concentrations had previously been determined to be optimal (V_{max}) for the site (Appendix 1; fig. A1-2, A1-3 and A1-4). Samples were incubated at field temperature for 60 minutes without addition of buffer in order to determine potential seasonal variation in the enzymes. After the incubation period, the vials were centrifuged at 10 000 rev min^{-1} (7 200 g) for 5 min and the fluorescence of the supernatant determined at 450 nm emission and 330 nm excitation wave-length with a slit setting of 2.5 using a fluorimeter (LKB Biochrom 4460 Fluorescence Detector Model 420-C). Standards were prepared from a stock solution of 1000 μM MUF-free acid solution that was prepared by pre-dissolving in cellosolve using an ultrasonic bath. Six standard solutions were prepared from this stock by sequential dilution (0 – 1000 μM). To account for quenching, 1 ml of peat slurry from each of three replicates were placed in 2 ml vials with 1ml of each standard solution. Solutions were vortexed and centrifuged at 10 000 rev min^{-1} (7 200 g) for 5 min and the fluorescence of the supernatant determined at 450 nm emission and 330 nm excitation wave-length with a slit setting of 2.5 using a fluorimeter. Flouresence of the sample solutions was converted to MUF concentrations by linear regression analysis. Enzyme activity was expressed as MUF produced (nmol MUF g^{-1} soil dry weight min^{-1}). Phenol oxidase activity was not measured due to methodological problems with the assay.

3.2.4 Statistical analysis

All statistical analyses were performed with Minitab Release 13 (Minitab Inc.). Differences between treatments at specific time intervals were determined using one-way ANOVA analysis. Differences between months were determined using General Linear Model analysis. The residuals were tested for normality using Anderson-Darling Normality Test. Variables that were not normally distributed were ranked and compared using Mann-Whitney or Kruskal-Wallis tests.

Principal component analysis (PCA) was performed on mean monthly data (n=12) of the control treatment to detect relationships between variables. Net CO_2 ecosystem exchange was not included in PCA since it is the net result of photosynthesis and ecosystem respiration. The depth of the water table on the day of sampling was used in the analysis along with total monthly rainfall. Using principal component coefficients, the variables were grouped to allow comparison of minimum and maximum monthly means that were tested for significant difference using one-way ANOVA analysis. Stepwise multiple regression analyses of the mean monthly data are presented as supporting evidence where it is reasonable to assume the dependent variables (i.e temperature, water table).

The temperature sensitivity (Q_{10}) of the carbon dioxide fluxes were assessed by first fitting an exponential function to the mean monthly data as by Fang *et al.* 2005 (Eq.1):

$$D = a\, e^{bT} \tag{1}$$

where D = decomposition pool or process, a and b are fitted parameters, and T is the soil temperature in °C. By definition, the value of Q_{10} is the factor by which the rate differs for a temperature interval of 10 °C (Eq.2):

$$Q_{10} = D_{T+10} / D_T \tag{2}$$

where R_T and R_{T+10} are respiration rates at temperature of T and T+10. Combining equation 1 and 2, Q_{10} was estimated by Eq.3:

$$Q_{10} = e^{10b} \tag{3}$$

3.3 Results

3.3.1 Effect of shading on vegetation composition and *Sphagnum* biomass

After 14 months, shading had significantly reduced total *Sphagnum* biomass per soil area within the gas chambers ($P < 0.01$; $F = 12.72$) to 60 % of the control (fig. 3-3). Shading caused a significant reduction in *S. capillifolium* biomass ($P < 0.05$). In the shaded treatment this species was identified by both red and green pigmentation (plate 3-3). Red pigmented *S. capillifolium* decreased ($P < 0.01$) from 76 to 50 % of total biomass (40 % of the control). The reduction in this species biomass was therefore associated with a reduction in red pigmentation (plate 3-4) and an increase in chlorophyll-rich green *S. capillifolium* ($P < 0.05$) from 2 to 24 % of total biomass (700 % of the control). No significant differences in *S. cuspidatum* or *S. papillosum* were found. Shading did not significantly affect the number of *Sphagnum* species.

Figure 3-3 Effect of shading on vegetation composition and *Sphagnum* biomass. Mean ± standard error (total biomass) (n=10).

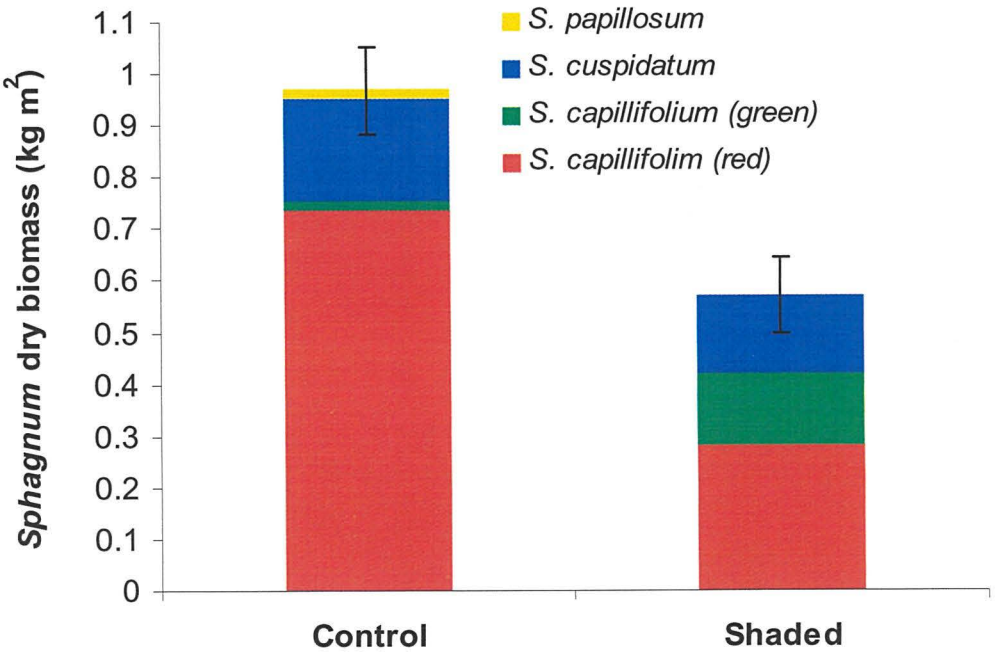
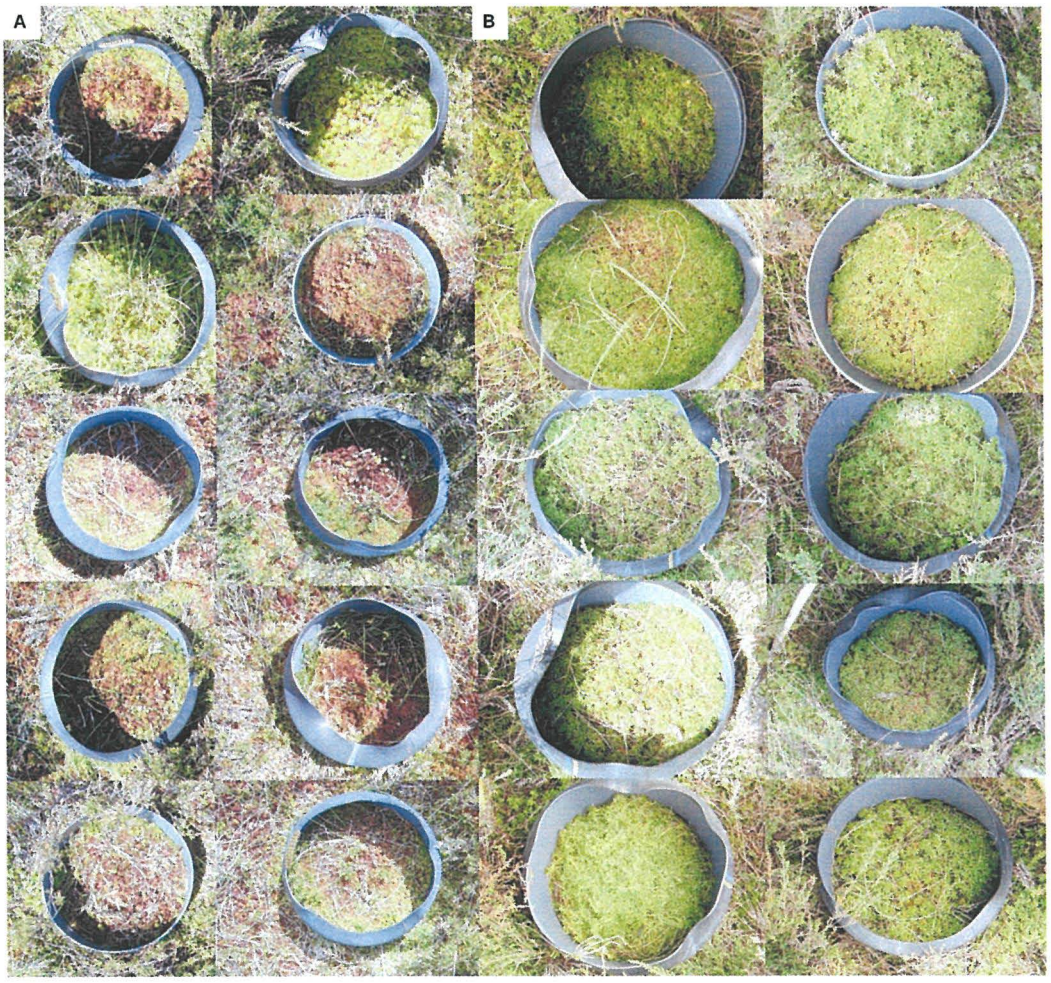


Plate 3-3 Reduction in red pigmentation of *S. capillifolium* by shading.

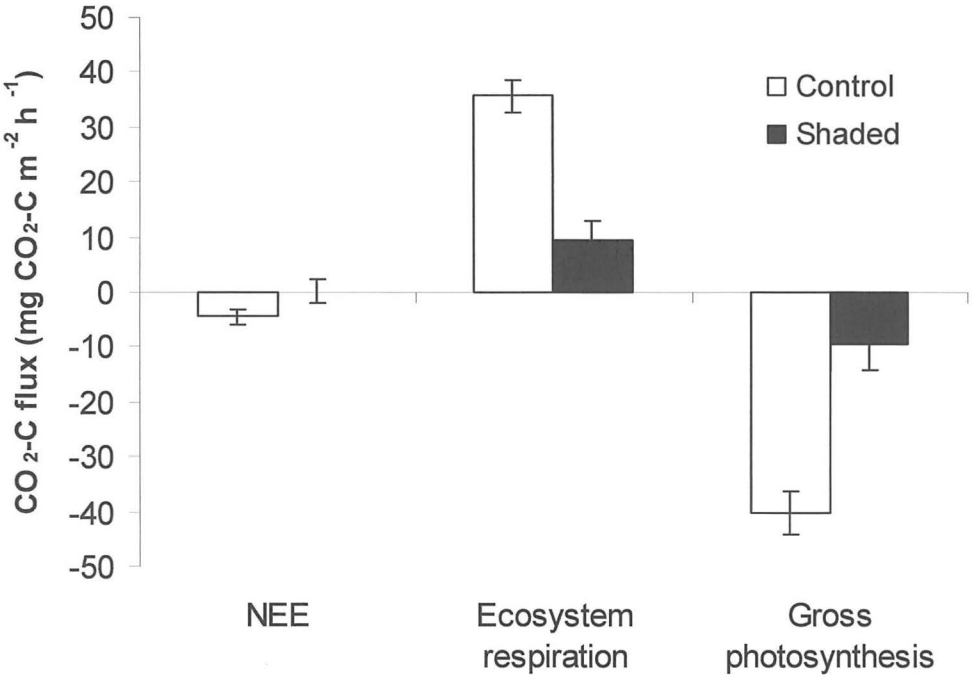
Plate 3-4 Species composition and reduction of red pigmentation of *S. capillifolium* by shading in the field plots where A = control plots and B = shaded plots.



3.3.2 Effect of shading on carbon dioxide fluxes

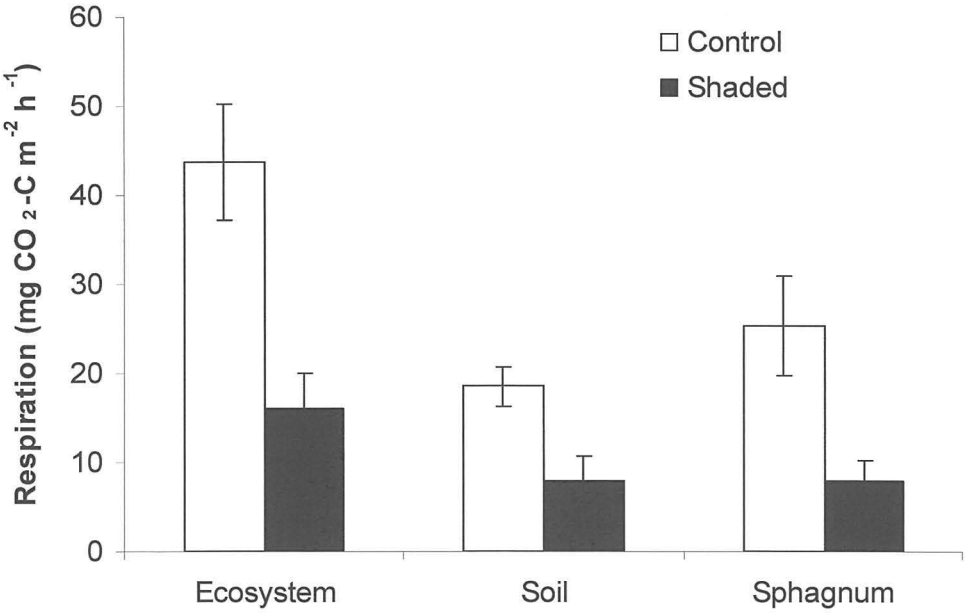
In order to determine the instantaneous effect of shading on gross photosynthesis, net ecosystem exchange (NEE) and ecosystem respiration per unit soil area, flux rates were determined with the shaded treatment remaining shaded throughout the gas incubation period after 14 months growth in the shade. Results show that shading did not have a significant effect on NEE (fig. 3-4). The reason for this was that shading significantly reduced both ecosystem respiration ($P < 0.001$; $F = 33.39$) and estimated gross photosynthesis ($P < 0.001$; $F = 24.11$) to 27 % and 23 % of the control respectively. Gross photosynthetic activity per unit of total *Sphagnum* dry biomass was also significantly lower in the shaded treatment (36.26 ± 4.23 μg CO_2-C g^{-1} h^{-1} control; 13.83 ± 4.18 μg CO_2-C g^{-1} h^{-1} shaded) ($P < 0.01$; $F = 14.21$).

Figure 3-4 Instantaneous effect of shading on net CO_2-C ecosystem exchange (NEE), ecosystem respiration and estimated gross photosynthesis after 14 months of growth in the shade (n=5).



In June 2004, it was found that shading significantly reduced ecosystem respiration ($P < 0.01$; $F = 13.14$), soil respiration ($P < 0.05$; $F = 8.85$) and *Sphagnum* respiration ($P < 0.05$; $F = 8.25$) (fig. 3-5). Ecosystem respiration in the shaded treatment was 36 % of the control, soil respiration was 43 % of the control, and *Sphagnum* respiration was 32 % of the control. However, in both treatments, soil and *Sphagnum* respiration made up 45 and 55 % of ecosystem respiration respectively.

Figure 3-5 Effect of shading on ecosystem, soil and *Sphagnum* respiration after 14 months growth in the shade (n=5).

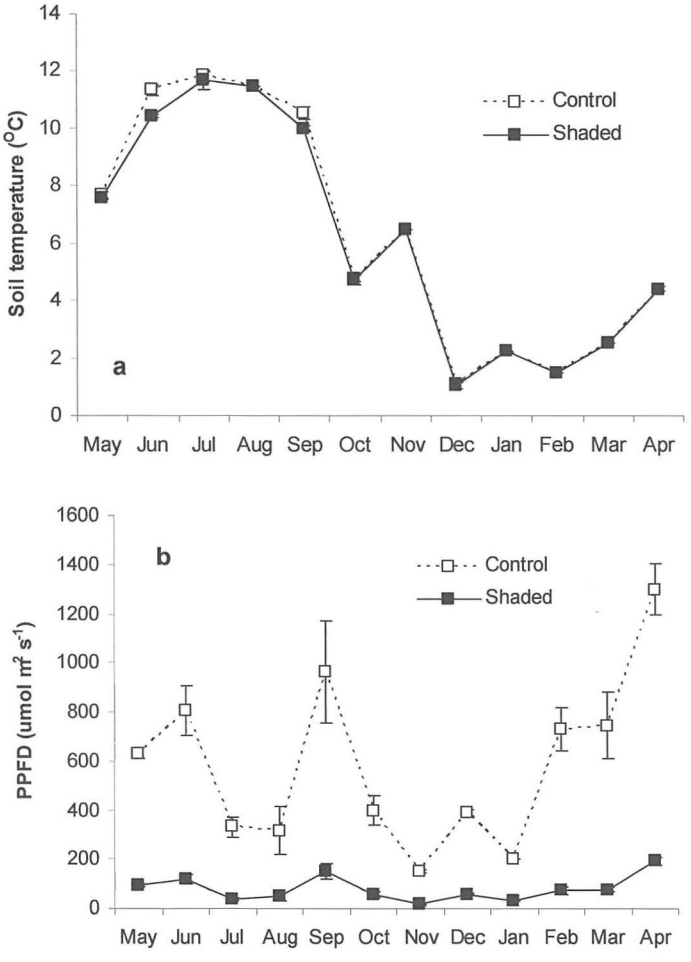


3.3.3 Seasonal variation of abiotic factors, carbon dioxide fluxes, organic C pools and soil enzyme activities

Abiotic factors

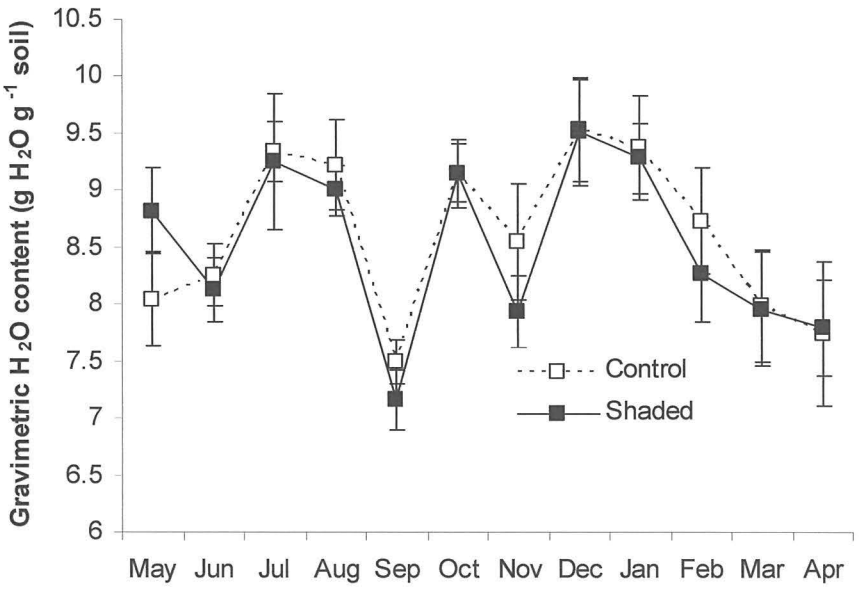
Soil temperature ranged from 0 to 12.5 °C (control) and 0.5 to 13 °C (shaded) (fig. 3-6a). Soil temperature was highest during summer and varied significantly throughout the year for both treatments ($P < 0.001$). Shading did not significantly affect soil temperature. Air temperature ranged between 0 and 22 °C. Photosynthetic photon flux density (PPFD) ranged between 100 - 1500 μmol m^{-2} s^{-1} (control) and 13.5 – 231 μmol m^{-2} s^{-1} (shaded) (fig. 3-6b). PPFD varied significantly throughout the year for both treatments ($P < 0.001$) and was significantly reduced in the shaded treatment ($P < 0.001$) to approximately 15 % ambient PPFD.

Figure 3-6 Seasonal variation of soil temperature (a) and photosynthetic photon flux density (under growth conditions) (b). Mean ± standard error (n=10).



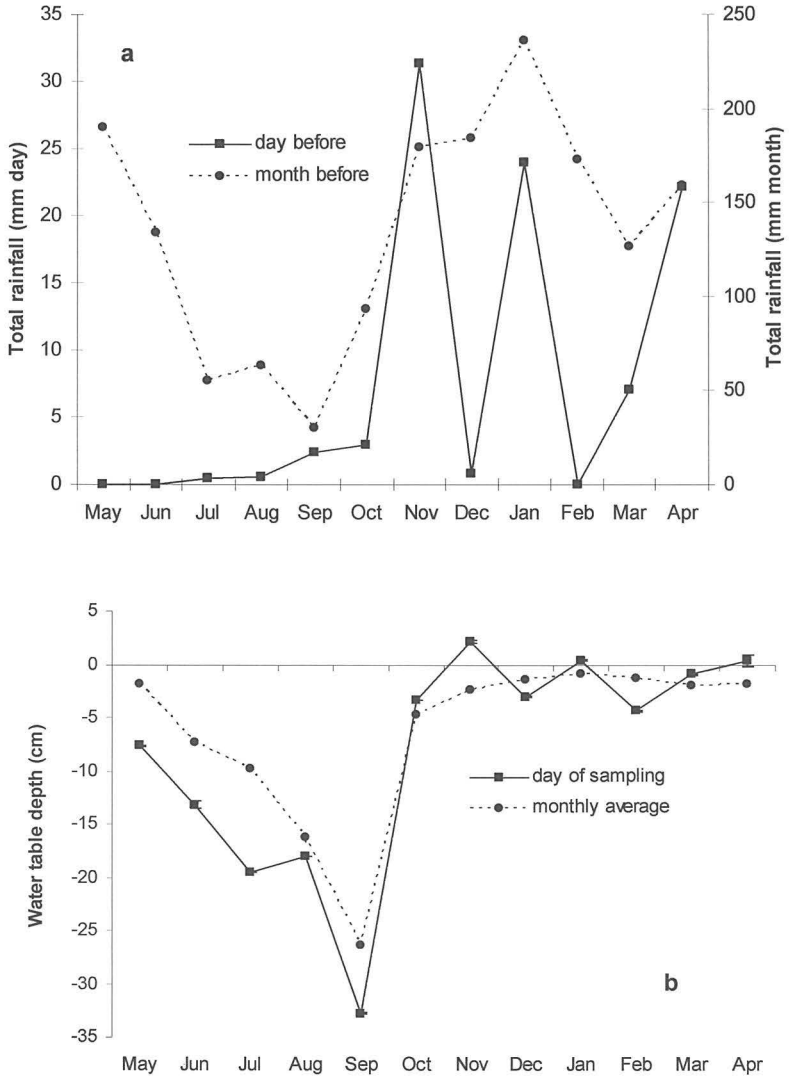
Gravimetric water content ranged from 5.1 to 12.4 g H_2O g^{-1} dry soil weight in the control and was significantly different between months ($P < 0.001$; $F = 2.79$) (fig. 3-7). In the shaded treatment it ranged from 5 to 12.5 g H_2O g^{-1} dry soil weight and was significantly different between months ($P < 0.001$; $F = 3.72$). Shading did not significantly affect the gravimetric water content. Total porosity averaged 95.7 % throughout the year. Water-filled porespace averaged 99.7 % throughout the year.

Figure 3-7 Seasonal variation of the gravimetric water content. Mean ± standard error (n=10).



Total rainfall and water table depth data were taken from ECN and thus differences between treatments were not determined. Total monthly rainfall ranged from 30 (Sept) to 236 (Jan) mm (fig. 3-8a) and was lowest in spring/summer. Average rainfall per hour was significantly different between months ($P < 0.001$). On the day before sampling, rainfall was highest in November, January and April. The depth of the water table ranged from 4 cm above the surface to 56 cm below the surface (fig. 3-8b). The depth of the water table was significantly different between months ($P < 0.001$) and was lowest in spring/summer.

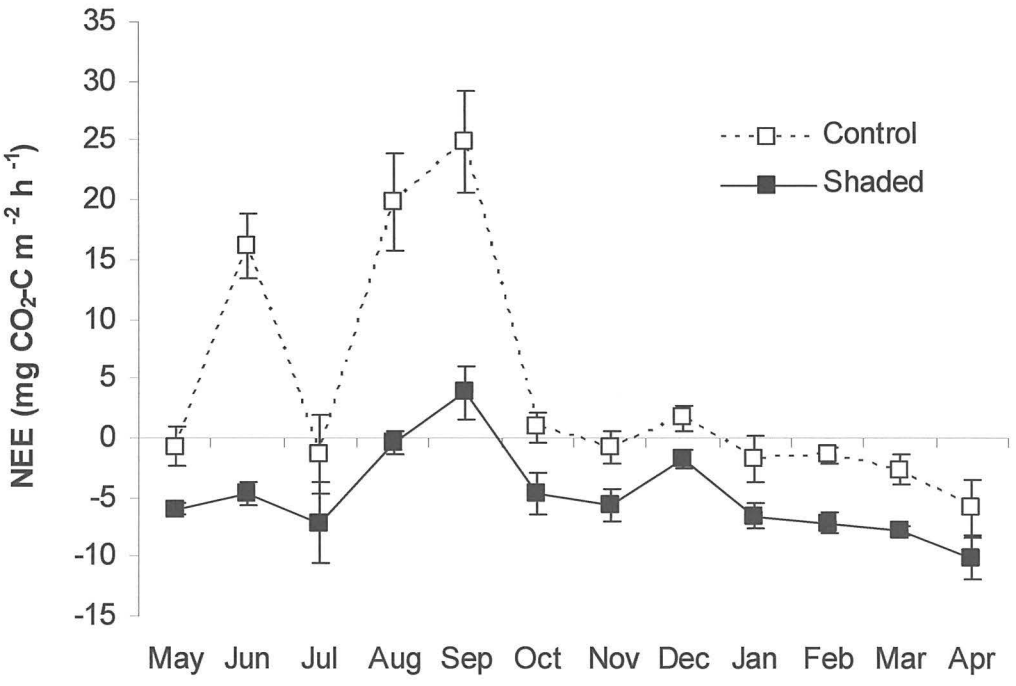
Figure 3-8 Seasonal variation of total rainfall the day before sampling and one month prior to sampling (a), and water table depth on the day of sampling and the average of four weeks prior to sampling (b). Mean ± standard error (n=10).



Carbon dioxide fluxes under natural PPFD conditions

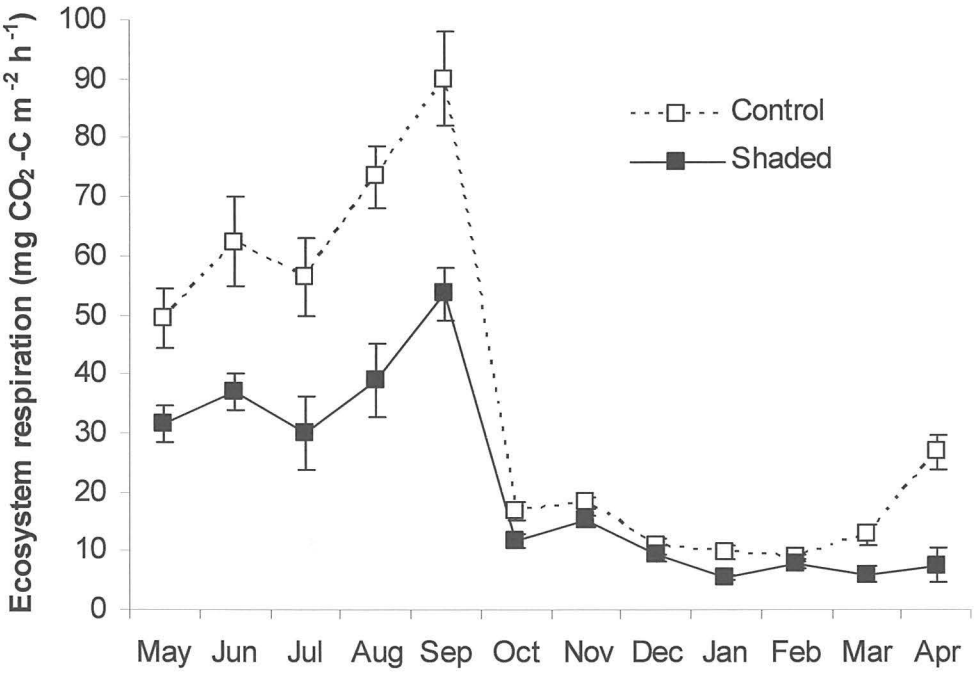
NEE of the control treatment ranged from -11.47 to 36.1 mg CO_2-C m^{-2} h^{-1} and varied significantly throughout the year ($P < 0.001$) (fig. 3-9). The control was a source of CO_2 between June – Sept and a sink between Oct – Apr. However, there was a source – sink switch in July. NEE of the shaded treatment measured under non-shaded conditions ranged from -18.8 to 8.85 mg CO_2-C m^{-2} h^{-1} and varied significantly throughout the year ($P < 0.001$). NEE was significantly different between treatments ($P < 0.001$). Removal of the canopy shading caused the shaded treatment to significantly sequester atmospheric CO_2 throughout the year except in September.

Figure 3-9 Seasonal variation of net CO_2-C ecosystem exchange under natural PPFD conditions. Mean ± standard error (n=10).



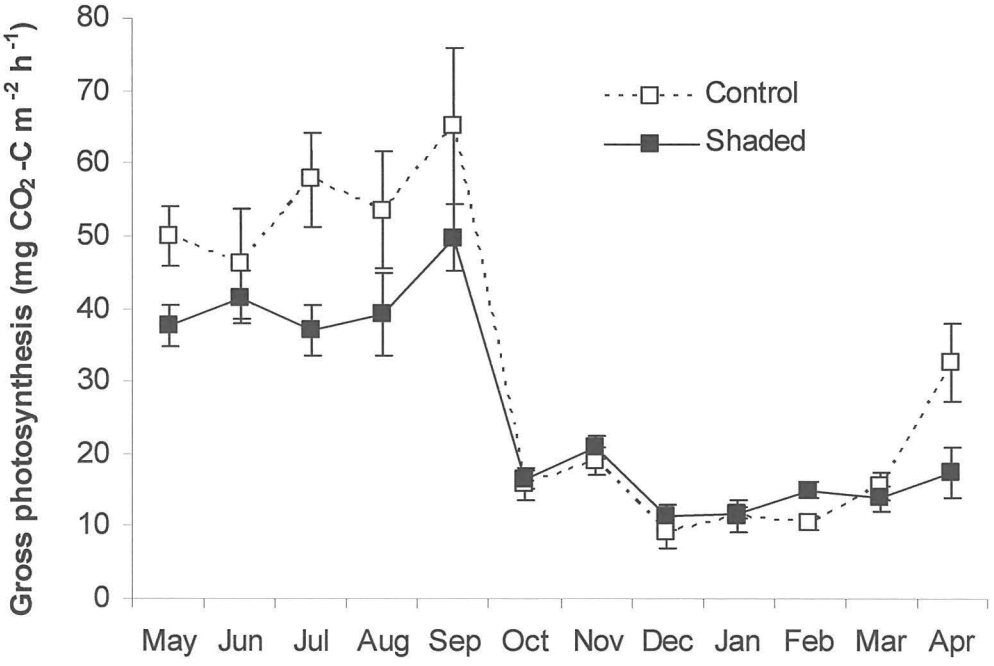
Ecosystem respiration of the control treatment ranged from 6.6 to 118.4 mg CO_2-C m^{-2} h^{-1} (fig. 3-10) and was significantly different between months ($P < 0.001$). Ecosystem respiration was significantly higher in spring / summer compared to autumn / winter ($P < 0.001$). Ecosystem respiration of the shaded treatment ranged from 1.3 to 64.5 mg CO_2-C m^{-2} h^{-1} (fig. 3-8) and was significantly different between months ($P < 0.001$). Shading significantly reduced ecosystem respiration ($P < 0.001$) and was 53 % of the control in spring / summer and 72 % of the control in autumn / winter.

Figure 3-10 Seasonal variation of ecosystem respiration. Mean ± standard error (n=10).



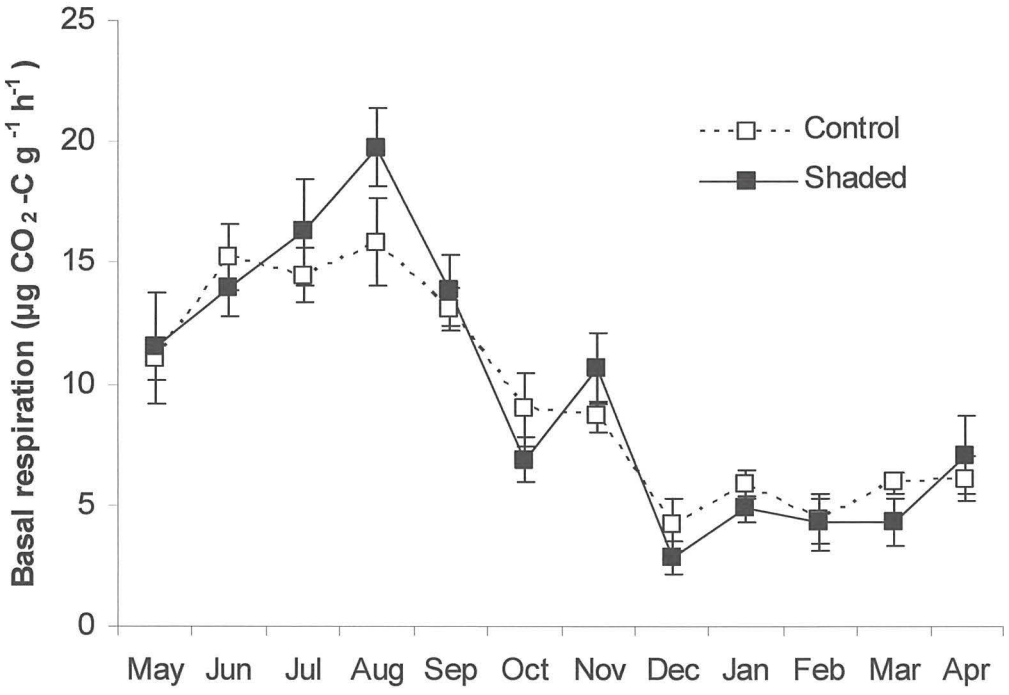
Estimated gross photosynthesis of the control ranged from 4.9 to 107 mg CO_2-C m^{-2} h^{-1} (fig. 3-11) and varied significantly throughout the year ($P < 0.001$). Estimated gross photosynthesis was highest in summer / spring and lowest in autumn / winter. Estimated gross photosynthesis of the shaded treatment ranged from 8 to 63 mg CO_2-C m^{-2} h^{-1} (fig. 3-9) and varied significantly throughout the year ($P < 0.001$). Gross photosynthesis was not significantly different between treatments throughout the year but was reduced between May and September ($P < 0.001$). Between May and September, gross photosynthesis of the shaded treatment under non-shaded conditions was reduced to 72 % of the control. Between October and March gross photosynthesis in the shaded treatment was 112 % of the control but was not significantly different.

Figure 3-11 Seasonal variation of estimated gross photosynthesis under natural PPFD conditions. Mean ± standard error (n=10).



Basal CO_2 respiration of the control ranged from 0.6 to 22.6 μg CO_2-C g^{-1} h^{-1} (fig. 3-12) and varied significantly throughout the year ($P < 0.001$). Basal CO_2 respiration was highest in spring / summer and lowest in autumn / winter. Basal CO_2 respiration of the shaded treatment ranged from 0.17 to 32 μg CO_2-C g^{-1} h^{-1} and varied significantly throughout the year ($P < 0.001$). Shading did not significantly affect basal CO_2 respiration.

Figure 3-12 Seasonal variation of basal CO_2 respiration. Mean ± standard error (n=10).

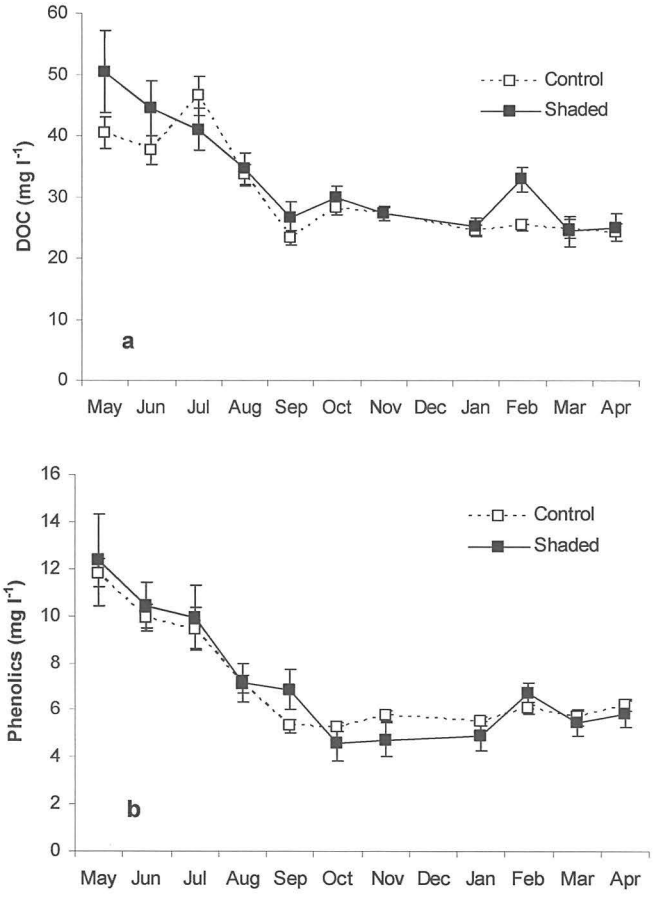


Soil organic carbon pools

DOC of the control ranged from 18.3 to 56 mg l^{-1} and varied significantly throughout the year ($P < 0.001$) (fig. 3-13a). DOC of the shaded treatment ranged from 14 to 90 mg l^{-1} and varied significantly throughout the year ($P < 0.001$). DOC was significantly different between treatments ($P < 0.05$) and was higher in soil beneath the shaded treatment in May and February. DOC of the control peaked in July but in the shaded treatment was highest in May.

Soil phenolics in the control ranged from 4 to 14 mg l^{-1} and varied significantly throughout the year ($P < 0.001$) (fi.g. 3-13b). Soil phenolics in the shaded treatment ranged from 1.3 to 21 mg l^{-1} and varied significantly throughout the year ($P < 0.001$). Soil phenolics were not significantly affected by shading.

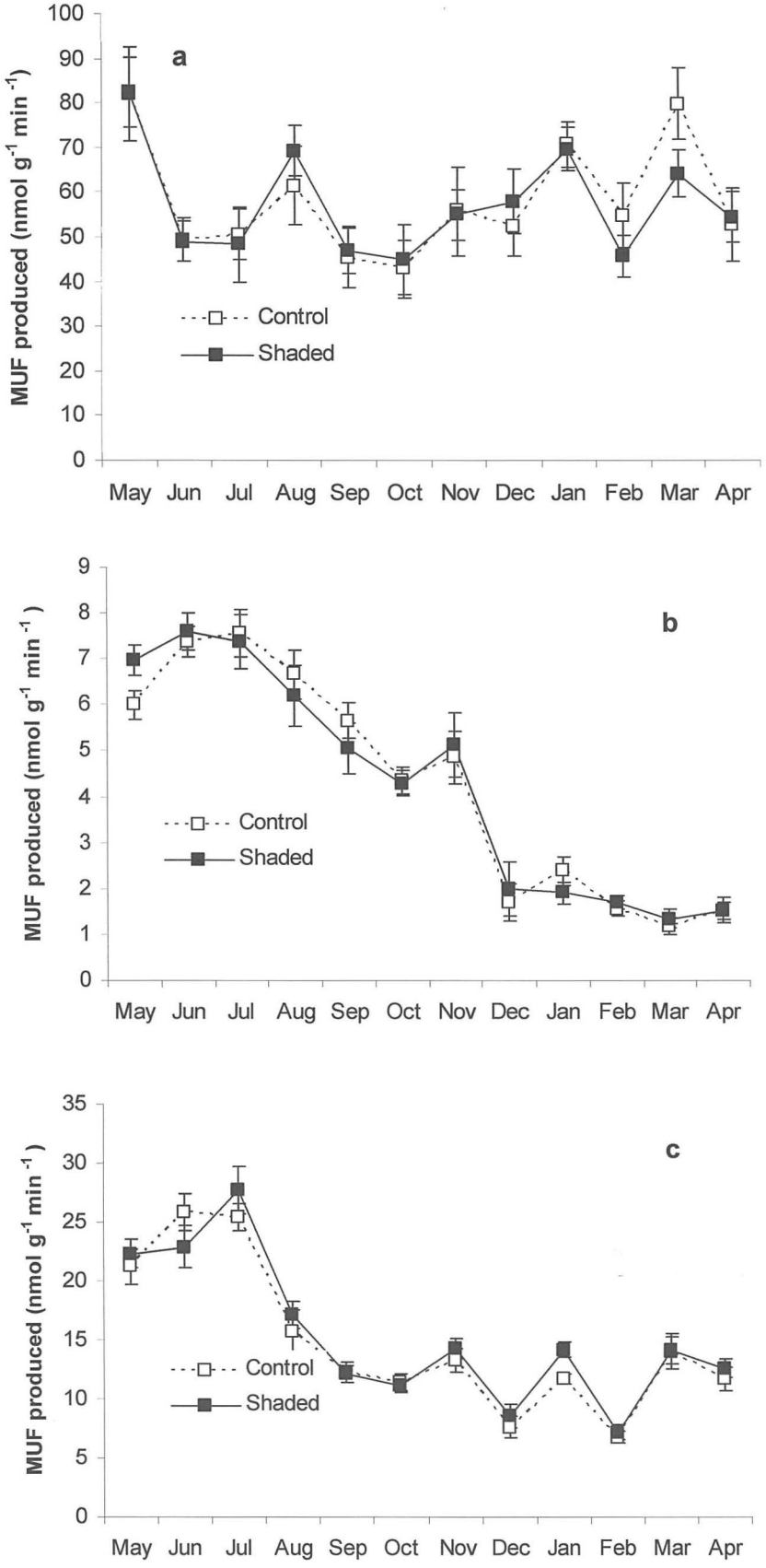
Figure 3-13 Seasonal variation of DOC (a) and soil phenolics (b). Mean ± standard error (n=10). Results for December are missing due to frozen water samplers.



Soil enzyme activities

Phosphatase activity of the control ranged from 19.7 to 142 nmol MUF g^{-1} min^{-1} (fig. 3-14a) and varied significantly throughout the year ($P < 0.01$). Phosphatase activity of the shaded treatment ranged from 17 to 111 nmol MUF g^{-1} min^{-1} and varied significantly throughout the year ($P < 0.001$). Shading did not significantly affect phosphatase activity. Sulphatase activity of the control ranged from 0.3 to 11 nmol MUF g^{-1} min^{-1} (fig. 3-14b) and varied significantly throughout the year ($P < 0.001$) Sulphatase activity of the shaded treatment ranged from 0.6 to 12 nmol MUF g^{-1} min^{-1} and varied significantly throughout the year ($P < 0.001$). Shading did not significantly affect sulphatase activity. β-glucosidase activity of the control ranged from 3.6 to 34 nmol MUF g^{-1} min^{-1} (fig. 3-14c) and varied significantly throughout the year ($P < 0.001$). β-glucosidase activity of the shaded treatment ranged from 4.2 to 38 nmol MUF g^{-1} min^{-1} and varied significantly throughout the year ($P < 0.001$). Shading did not significantly affect β-glucosidase activity.

Figure 3-14 Seasonal variation of phoshatase (a), sulphatase (b) and β-glucosidase (c) enzyme activities. Mean ± standard error (n=10).



Principal component analysis

Table 3-1 shows eigenanalysis of the correlation matrix. The first principal component (PC1) explained 56.3 % of the variation, PC2 explained 18.3 % (cumulative 74.6 %) and PC3 explained 13.9 % (cumulative 88.5 %). The first five principal components explained 96 % cumulatively although PC4 and PC5 each explained only 5 % and 2.5 % respectively. Figure 3-15 shows a plot of the first two principal components showing three groups. The minimum and maximum of each variable in each group were then further characterized by the coefficient of PC3 (table 3-1).

The results suggest that soil temperature, PPFD, ecosystem respiration and estimated gross photosynthesis were related by two PCs. However, figure 3-15 suggests that soil temperature may be more closely related to the CO_2 fluxes than PPFD that was separated by the third PC. Ecosystem respiration and gross photosynthesis were lowest in cold months and highest in warmer months with a significant peak in September (table 3-2).

Basal CO_2 respiration, the enzymes β-glucosidase and sulphatase, and the organic C pools were closely related by two PCs. This group may also be related to the first group particularly in respect to temperature and basal respiration. Table 3-2 shows that these variables were lowest in cold months and highest in warmer months as with the carbon dioxide fluxes. Sulphatase activity appears closely related to basal CO_2 respiration whilst β-glucosidase appears more closely related to the organic C pools (fig. 3-15).

The gravimetric water content, phosphatase activity, water table depth on the day of sampling and total monthly rainfall were related by two PCs. The negative values of the raw-input water table data suggests that this variable may also be related to the carbon dioxide fluxes. Indeed, multiplying the data by -1 placed water table depth with the carbon dioxide fluxes and is supported by comparison of figures 3-8b, 3-10 and 3-11. The water related variables were lowest in September (when carbon dioxide fluxes were highest) and highest in colder months whilst phosphatase activity appears negatively correlated.

Table 3-1 Eigenanalysis of the correlation matrix. Variables are grouped according to coefficients within the first two principal components (solid lines) and first three principal components (dashed lines).

Variable	PC1	PC2	PC3	PC4	PC5
PPFD	-0.023	0.449	0.458	0.374	0.290
Temperature	-0.361	0.016	-0.060	-0.013	-0.279
Ecosystem respiration	-0.340	0.192	-0.003	-0.222	-0.044
Gross photosynthesis	-0.349	0.126	-0.094	-0.136	0.093
Basal respiration	-0.357	-0.029	0.091	-0.078	-0.240
Sulphatase	-0.345	-0.144	0.121	0.038	-0.353
B-glucosidase	-0.297	-0.270	-0.195	0.207	-0.088
DOC	-0.276	-0.380	-0.056	0.241	0.354
Phenolics	-0.239	-0.335	-0.357	0.221	0.218
H_2O	0.067	-0.389	0.533	0.021	0.418
Phosphatase	0.069	-0.290	-0.459	-0.751	0.157
Rainfall	0.254	-0.302	-0.263	0.092	-0.390
Water table	0.299	-0.259	-0.148	0.250	-0.335
Eigenvalue	7.3245	2.3785	1.8016	0.6509	0.3255
Proportion	0.563	0.183	0.139	0.050	0.025
Cumulative	0.563	0.746	0.885	0.935	0.960

Figure 3-15 Plot of the first two PCA coefficients. Variables in circles were related by two principal components that explained 64 % of the variation. R_e = ecosystem respiration; P_g = gross photosynthesis; R_s = basal respiration; Sul = sulphatase; Glu = β-glucosidase and Pho = phosphatase.

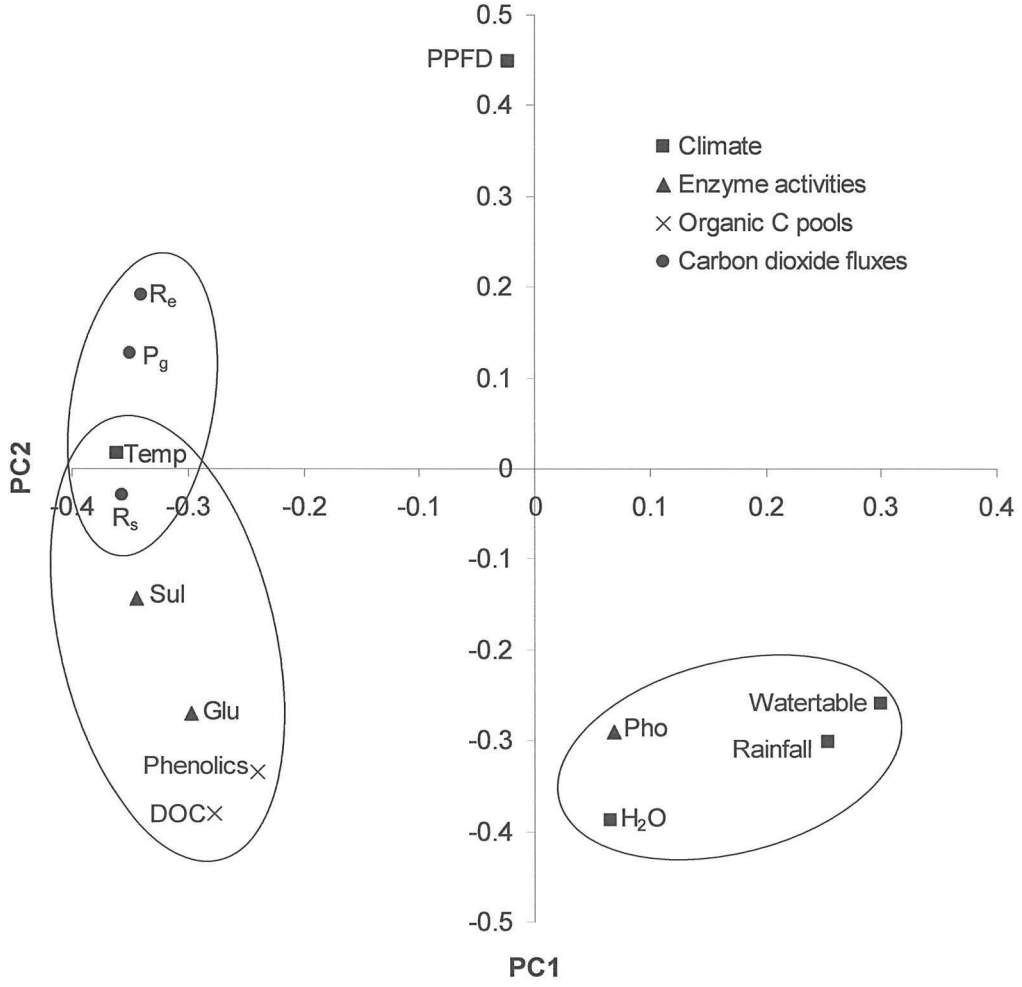


Table 3-2 Comparison of minimum and maximum monthly means for each variable (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Units are given in the methodology section. Variables are grouped according to PCA coefficients (see table 3-1) within the first two principal components (solid lines) and first three principal components (dashed lines).

Variable	**Min**	**Month**	**Max**	**Month**	***P*-value** ***(F value)***
PPFD	100	Nov	1500	Apr/Sep	*** (57.5)
Temperature	0	Dec	12.5	Jul	*** (1620)
Ecosystem respiration	6.6	Jan	118.4	Sep	*** (101.4)
Gross photosynthesis	4.9	Dec	107	Sep	*** (25.9)
Basal respiration	0.6	Dec	22.6	Aug	** (16.5)
Sulphatase	0.3	Dec	11.3	Jul	*** (77.7)
B-glucosidase	3.6	Dec	34	Jun	*** (93.8)
DOC	18.3	Sep-Apr	56	Jul	*** (71.6)
Phenolics	4	Sep-Jan	14	May	*** (92)
H_2O	5.1	Sep	12.4	Dec	*** (18.5)
Phosphatase	19.7	Feb	142	May	* (4.5)
Rainfall	30	Sep	236	Jan	-
Water table	-56	Sep	4	Nov	*** (1715)

Stepwise multiple regression analysis

Stepwise regression analysis was performed to determine the best abiotic predictors of seasonal ecosystem respiration. Factors added and removed from the model at $P < 0.15$ were air temperature, soil temperature, PPFD, water table depth and total rainfall. Linear regression analysis showed that soil temperature and water table depth individually explained 82 % of the variation ($P < 0.001$). Soil temperature and the depth of the water table together were the best predictors of ecosystem respiration (table 3-3; fig. 3-16). Anderson-Darling's test showed that the relationship was normally distributed and variation inflation factors (VIFs) were less than 2.5.

Table 3-3 Stepwise multiple regression analyses showing the best predictors of ecosystem respiration (R_e), gross photosynthesis (P_g), basal respiration (R_s), sulphatase activity (Sul) and β-glucosidase activity (Glu). (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) (n=12).

y	***Linear regression y = ax + b***	***P*-value**	$\mathbf{R^2}$
R_e	(-1.43 water table) + (3.45 temperature) + 2.448	***	94
P_g	(0.0145 PPFD) + (4.64 temperature) – 5.84	***	88
R_s	(1.025 temperature) + 2.99	***	96
Sul	(0.557 temperature) + 0.707	***	89
Glu	(0.694 DOC) – 5.79	***	77

Stepwise regression analysis was performed to determine the best abiotic predictors of seasonal gross photosynthesis. Factors added and removed from the model at $P < 0.15$ were air temperature, soil temperature, PPFD, water table and total rainfall. The first predictor added to the model was soil temperature and explained 82 % of the variation ($P < 0.001$). Soil temperature and PPFD were the best predictors of gross photosynthesis (table 3-3; fig. 3-17). Anderson-Darling's test showed that the relationship was normally distributed and variation inflation factors (VIFs) were less than 1.

Figure 3-16 Multiple regression analysis of soil temperature and water table depth as predictors of ecosystem respiration (n = 12). The legend indicates the growing season (May-September) and cold season (October-April).

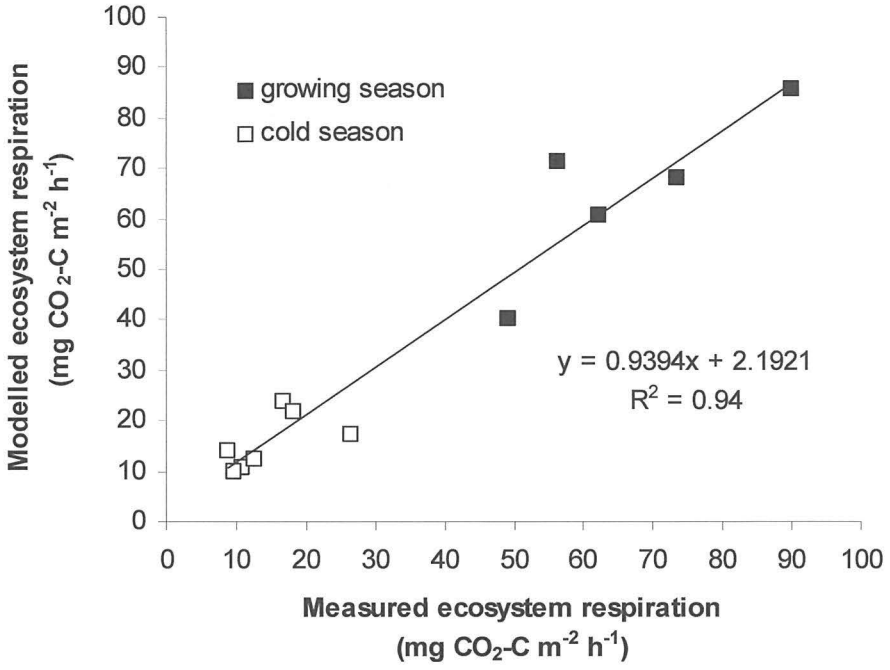
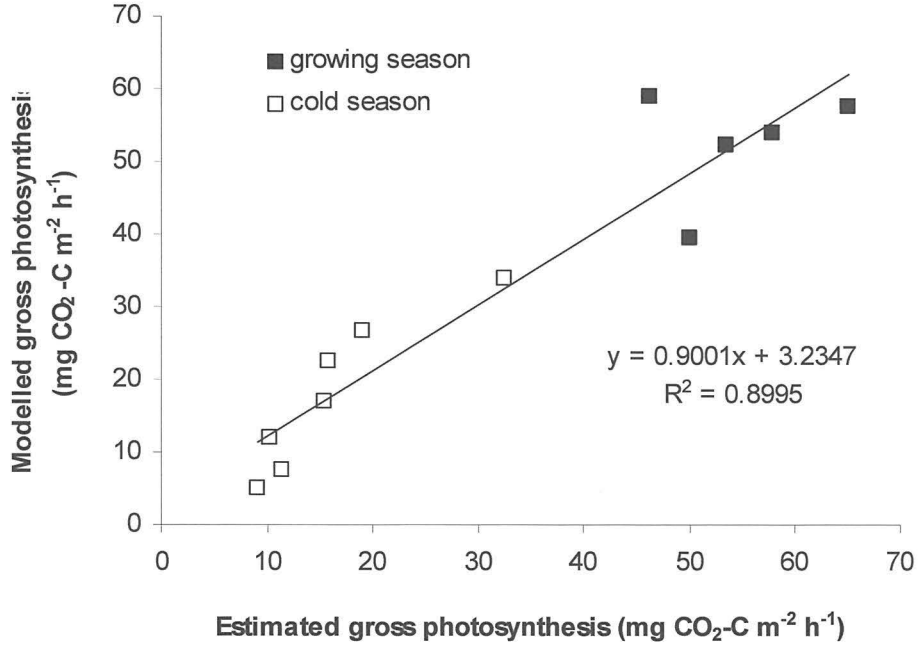


Figure 3-17 Multiple regression analysis of soil temperature and PPFD as predictors of estimated gross photosynthesis (n = 12). The legend indicates the growing season (May-September) and cold season (October-April).



A model of NEE was constructed using the multiple regression equations for ecosystem respiration and gross photosynthesis. For the model, 70 % of the NEE data was used and the remaining 30 % used for validation of the model. The model explained only 67 % of the seasonal variation of NEE.

Stepwise regression analysis was performed to determine the best predictors of seasonal basal CO_2 respiration. Factors added and removed from the model at $P < 0.15$ were air temperature, soil temperature, PPFD, water table, DOC and total rainfall. The first and only predictor added to the model was soil temperature (table 3-3). Anderson-Darling's test showed that the relationship was normally distributed.

Stepwise regression analysis was performed to determine the best predictors of enzyme activities. Factors added to the models were air temperature, soil temperature, PPFD, water table, DOC, soil water content and total rainfall. No factor determined phosphatase activity. For sulphatase, the first predictor added to the model was soil temperature and explained 89 % of the variation (table 3-3). Basal CO_2 respiration was also a significant predictor and explained 90 % of the variation. Anderson-Darling's test showed that the relationship was normally distributed. Multiple regression analysis of soil temperature and basal CO_2 respiration showed that these factors were multicolinear with VIFs of 29. For β-glucosidase, the best and only predictor added to the model was DOC (table 3-3). Anderson-Darling's test showed that the relationship was normally distributed. Since it is apparent that soil enzyme activities and the organic C pools interact, multiple regression analysis of factors affecting DOC and soil phenolics are not shown in table 3-3. However, stepwise regression analysis showed that as dependent factors, both pools were only affected by β-glucosidase activity.

Temperature sensitivity (Q_{10}s)

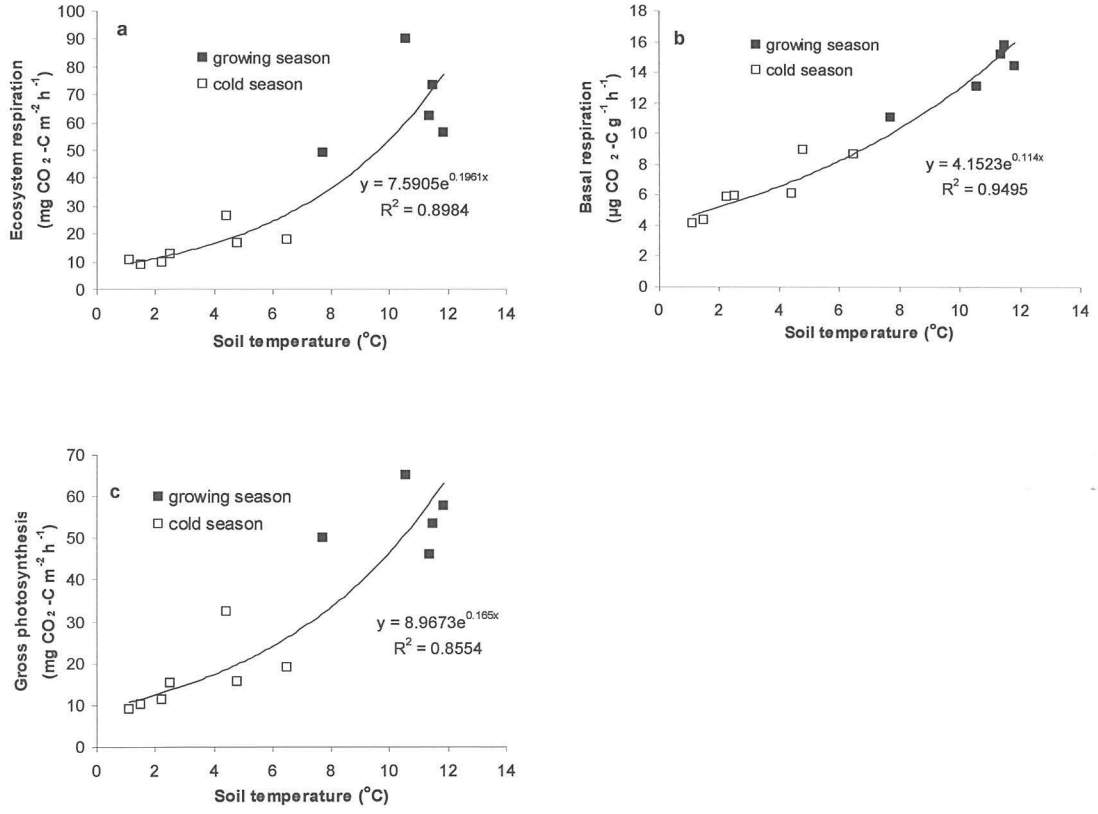
Regression analysis was performed on the mean data sets of the control treatment for ecosystem respiration, basal respiration and gross photosynthesis against soil temperature. Ecosystem respiration (fig. 3-18a), basal respiration (fig. 3-18b) and gross photosynthesis (fig. 3-18c) all showed significant exponential responses to soil temperature (table 3-4). Stepwise multiple regression analysis of the logarithms of ecosystem respiration and gross photosynthesis was repeated and

showed that soil temperature was the only significant predictor of ecosystem respiration and gross photosynthesis (Table 3-4).

Table 3-4 Exponential response of ecosystem respiration (R_e), basal respiration (R_s) and gross photosynthesis (P_g) to seasonal soil temperature variation. (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Variable	Equation	Q_{10}	P-value	R^2
R_e	$y = 7.5905e^{0.1961T}$	7.1	***	89
R_s	$y = 4.1523e^{0.114T}$	3.1	***	95
P_g	$Y = 8.9673e^{0.165T}$	5.2	***	85

Figure 3-18 Exponential response of ecosystem respiration (a), basal CO_2 respiration (b) and estimated gross photosynthesis (c) to seasonal soil temperature variation (n=12). Legend indicates the growing season (May-September) and cold season (October-April).



3.4 Discussion

3.4.1 Effect of shading on vegetation composition and *Sphagnum* biomass

Carbon dioxide absorbed by plants is reacted with a five-carbon substance ribulose bisphosphate (RuBP) by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) to form two molecules of a three-carbon substance glycerate-3-phosphate that is used to regenerate RuBP or form starch for growth (Taiz and Zeiger, 1998). Shading affects photosynthetic CO_2 fixation by reducing ribulose bisphosphate (RuBP) regeneration via reduced electron transport and ATP synthesis (Taiz and Zeiger, 1998). Plants have a number of mechanisms to maintain a positive C balance (see Fitter and Hay, 1987) which ultimately leads to reduced plant growth. In this study, shading (15 % of non-shaded conditions) led to a significant decrease in total *Sphagnum* biomass by 60 % after 14 months growth in the field. *Sphagnum capillifolium* is the dominant species at the site and accounted for approximately 75 % of total *Sphagnum* biomass in both control and shaded treatments. Thus it is not surprising that a reduction in *S. capillifolium* led to a reduction in total *Sphagnum* biomass whilst *S. cuspidatum* and *S. papillosum* biomass were not significantly affected by shading. Thus *S. capillifolium* may be particularly sensitive to light intensity as evident by the reduction in biomass and significant loss of red pigment in the capitula and upper stem (see Appendix 2).

Murray *et al.* (1993) showed that *Sphagnum* growth (*S. augustifolium, S. fuscum,* and *S. squarrosum*) was 2-3 times higher in shaded plots than in control plots, while significant reductions in growth were found in canopy removal plots. Flavonoids are the most widespread phenolics in bryophytes (Shaw and Goffinet, 2000). The red pigmentation of many plants is due a group of flavonoid pigments called anthocyanins. Shaw and Goffinet (2000) state that the typical red pigments reported from certain *Sphagnum* species may have a chemical relation to 3-deoxyanthocyanidins. Anthocyanins generally accumulate in peripheral tissues exposed to high irradiance or in obligatory shade plants due to an inbalance between light capture, CO_2 assimilation and carbohydrate utilization (Steyn *et al.*, 2002). They significantly modify the quantity and quality of light incident on chloroplasts (Krol *et al.*, 1995). Field *et al.* (2001) found that optical masking of

chlorophyll by anthocyanins reduces risk of photo-oxidative damage to leaf cells of Red-Osier dogwood as they senesce. Light attenuation by anthocyanins may help to re-establish a balance and so reduce the risk of photo-oxidative damage (Steyn *et al.*, 2002). It is hypothesized that *S. capillifolium* contains anthocyanins as a mechanism to reduce the incident light on chlorophyll and hence prevent photo-oxidative damage that Murray *et al.* (1993) showed can occur in other *Sphagnum* species. The response to shading may have caused a reduction in anthocyanins thus exposing chlorophyll in the upper light exposed regions of the plants enabling the species to maintain a positive photosynthetic C balance. This may also explain the insignificant effect of shading on *S. papillosum* and *S. cuspidatum* since they do not contain observable anthocyanins and could therefore potentially maintain higher net photosynthetic activity (see Appendix 2).

3.4.2 Effect of shading on carbon dioxide fluxes

The reduction in *S. capillifolium* biomass to 40 % of the control shows that shading affected metabolism directly through photosynthesis, and growth and development indirectly as a consequence of the immediate metabolic responses. Reduced biomass, gross photosynthetic activity per unit area of soil and per unit of total *Sphagnum* biomass shows that productivity was limited by PPFD in the shaded treatment (<200 μmol m^{-2} s^{-1}). This was unexpected since typically, photosynthesis of bryophytes saturate between 30 – 300 μmol m^{-2} s^{-1} (i.e. 5-10 % of full sunlight) (Shaw and Goffinet, 2000; Davey *et al.*, 1997). Thus the dominant species *S. capillifolium* may attain saturation at the high end of this range due to optical masking of chlorophyll by anthocyanins. At the start of the experiment, the reduction in gross photosynthesis by shading would have decreased *Sphagnum* respiration and hence productivity. *Sphagnum* respiration accounted for over 55 % of ecosystem respiration in both treatments at the start of the new growing season showing that *Sphagnum* productivity will affect the loss as well as gain of CO_2 in peatlands. However, the overall effect of shading on *Sphagnum* and soil CO_2 fluxes did not result in a significant change to NEE. Thus, reduced *Sphagnum* productivity due to light limitation may not impact on

the C balance of peatlands due to the concomitant decrease in ecosystem respiration. Also, light limits productivity directly through gross photosynthesis whilst respiration is affected indirectly due to the reduction in gross C fixation. Factors such as nitrogen may affect both processes directly that could have a significantly different effect on net productivity and hence NEE. Despite this, the significant reduction in gross C processes such as *in situ* soil respiration could have implications for long-term C storage. The reduction in soil respiration may have resulted from decreased input of labile DOC due to reduced *Sphagnum* C fixation although DOC concentrations (Section 3.3.3) suggest that this was not the case. An alternative hypothesis is that reduced productivity induced microclimatic differences in soil temperature and/or soil water content.

3.4.3 Seasonal variation of abiotic factors, carbon dioxide fluxes, organic C pools and decomposition processes

Abiotic factors

Climatic and physicochemical conditions varied significantly throughout the year with highest temperatures and lowest water table depth occurring between May and September. Soil temperature and soil water content did not differ significantly between treatments suggesting that these factors may not explain the reduction in soil respiration. Differences in the depth of the water table between treatments were not determined and cannot therefore be ruled out. However, it is unlikely that shading could have significantly affected the depth of the water table via reduced evapotranspiration.

Carbon dioxide fluxes

Net CO_2 ecosystem exchange of the shaded treatment was determined under natural PPFD conditions throughout the year. Thus the estimates of gross photosynthetic activity of the shaded treatment represent metabolic acclimation responses to shading or are due to the reduction in biomass as a consequence of metabolic responses. Plants acclimate to different light environments to optimize growth by reallocating resources and / or changing their morphology (Arp, 1991). Low light intensity primarily affects photosynthesis with morphogenetic

responses occurring secondarily (Fitter and Hay, 1987). The morphogenetic response was apparent in the reduction of anthocyanin pigments in the upper stems and capitula of *S. capillifolium* (see Appendix 2). Estimated gross photosynthetic activity of the shaded treatment under natural PPFD conditions was not significantly different throughout the year but was significantly lower in the growing season suggesting reduced productivity. In colder months, respiratory and morphological responses may have stabilized photosynthetic C gain, or more likely environmental conditions (i.e. temperature, PPFD) may have limited gross photosynthesis and growth. Shading significantly reduced ecosystem respiration to approximately 50 % of the control in the growing season that may represent the reduction in productivity or *Sphagnum* respiration as an acclimation response to shading that did not increase under natural PPFD conditions. However, the study of *Sphagnum* and soil respiration suggests that *in situ* soil respiration may also have been reduced. Overall, shading caused a source to sink switch in daytime NEE when incubated under natural PPFD conditions. This suggests that short-term exposure to natural PPFD conditions stimulated gross photosynthetic activity relative to respiration of the shaded treatment resulting in sequestration. However, it is hypothesized that long-term exposure to non-light limiting conditions may stabilize the balance between gross photosynthesis and respiration in the shaded treatment leading to an increase in productivity that would maintain the C balance. Basal CO_2 respiration was significantly higher in the growing season in both treatments but was not significantly affected by shading unlike *in situ* soil respiration. Since basal measurements were conducted under *ex situ* laboratory conditions, it is possible that conditions may have stabilized basal respiration of the shaded treatment.

Mean daytime NEE of the control treatment varied significantly throughout the year due to significant emission (June - Sept) followed by sequestration in colder months (Oct – May). These results agree with the known growing season for Moorhouse between May and October (Heal and Perkins, 1978). Ecosystem respiration and estimated gross photosynthesis were significantly higher in the growing season but gross photosynthesis was less than ecosystem respiration resulting in net CO_2 emission. However, the method used for estimating gross photosynthesis may have underestimated the flux due to a potential increase in *Sphagnum* respiration in the ecosystem respiration

chambers. The significant variation in ecosystem respiration and relatively higher flux rate compared to gross photosynthesis suggests that ecosystem respiration was the primary determinant of NEE. Bubier *et al.* (2003) found that soil respiration rates dominated interannual variability in summer NEE of a Canadian peatland whilst Valentini *et al.* (2000) found that respiration was more important than photosynthesis in explaining differences in NEE across a latitudinal gradient in European forests.

Principal component analysis (PCA) showed that the CO_2 fluxes were related positively with soil temperature and PPFD, and negatively with the depth of the water table. Linear regression analysis showed that soil temperature and water table depth best predicted ecosystem respiration both individually and interactively. Thus both direct kinetic effects of temperature and indirect effects of temperature and rainfall on water table depth resulting in soil aeration are critical determinants of ecosystem respiration. However, ecosystem respiration and basal CO_2 respiration were best explained by an exponential response to soil temperature. These results agree with Bridgham and Richardson (1992) who found that temperature was the best predictive environmental variable for the seasonal dynamics of *in situ* soil respiration of a North Carolina peatland, while water table depth was of lesser importance and soil moisture was insignificant. As with the differences between treatments, the insignificant effect of water table depth on basal respiration may have resulted from aeration by homogenization in the laboratory. Freeman *et al.* (1996) found that basal CO_2 respiration was not affected by a lowering of the water table and suggested that greater oxygenation does not always accompany a lowering of the water table.

Soil temperature and PPFD were the best predictors of gross photosynthetic activity. Thus the water table depth may be secondary to temperature and PPFD as a predictor of gross photosynthetic activity. Generally both high and low water contents can limit C uptake (Schipperges and Rydin, 1998; Titus *et al.*, 1983). Low water availability can inhibit photosynthetic enzyme activity, while slow CO_2 diffusion can be limiting at water saturation (Williams and Flanagan, 1996). Also, the effect of PPFD on *Sphagnum* gross photosynthesis is minimal compared to soil temperature variation as shown by the insignificant effect of PPFD as a single predictor. Bisbee *et al.* (2001) found that *Sphagnum* production was not correlated with PPFD. A hyperbolic function

was used to replace the linear PPFD part of the multiple regression equation to account for the known photosynthetic response of plants to light intensity (results not shown). Whilst the equation was significant, it did not improve the variation explained and did not improve prediction of estimated gross photosynthesis. The interactive effect of soil temperature, water table depth and the limited effect of PPFD due to photosynthetic saturation may explain this relationship. Experiments involving measurement of gross photosynthesis of *Sphagnum* mesocosms under different light intensities will resolve this issue. The PPFD underneath the shaded canopies ranged between 13 and 231 μmol m^2 s^{-1} and thus photosynthetic activity may be limited by PPFD values less than 300 μmol m^{-2} s^{-1}.

Ecosystem respiration, estimated gross photosynthesis and basal respiration of the control treatment all responded exponentially to seasonal variation in soil temperature with Q_{10}s of 7.1, 5.2 and 3.1 respectively. Since it is apparent from linear multiple regression analyses that both the depth of the water table and PPFD may have interactively affected the temperature sensitivity of ecosystem respiration and gross photosynthesis, it is not surprising that the Q_{10}s were higher than values that have been reported from controlled laboratory studies. NEE in this study agreed with Silvola (1986) that suggested that undisturbed Finnish peatlands tend to release CO_2 at the rate of 27 to 41 mg CO_2-C m^{-2} h^{-1} at summer temperatures of 10 to 15 °C. Lowering of the water table in one such Finnish peatland from 0-10 cm to 40-60 cm, increased the CO_2 output within a few weeks to 82 to 109 mg CO_2-C m^{-2} h^{-1}. Alm *et al.* (1999) showed that bogs can switch from sinks to sources under drought conditions, with enhanced rates of respiration and reduced plant production, particularly with *Sphagnum* mosses. However, these results do suggest that temperature may affect ecosystem respiration to a greater extent than photosynthesis as evident in the loss of CO_2 in the growing season (NEE) by both plant and soil respiration. Cox *et al.* (2000) and Houghton and Woodwell (1989) suggest that plant and soil respiration may increase at a faster rate than photosynthesis as ecosystems adjust to climate change. Oechel *et al.* (1993) showed that Alaskan tundra has become a source of carbon dioxide to the atmosphere that coincides with recent warming in the Arctic.

Long-term modelling studies indicate that both respiration and production in peatlands are highly sensitive to changes in the water balance (Hilbert *et al.*, 2000). Even small changes in NPP and decay rates may cause a permanent reduction in the current C sink capacity of peatlands (Wieder, 2001). Indeed, a significant switch from sink to source in June (or source to sink in July) did not correspond to any change in climatic or physicochemical conditions. This may have resulted in overestimation of gross photosynthesis. Despite this it is apparent that the site was a source of CO_2 during the daytime in the growing season due to the interactive effect of higher seasonal temperatures and a lower water table on ecosystem respiration. Seasonal temperature variation was the principal factor limiting *Sphagnum* growth rather than PPFD or the water table depth. This also supports the evidence that *Sphagnum* species are shade adapted (Shaw and Goffinet, 2000; Davey *et al.*, 1997) and therefore saturated at PPFD's above 300 μmol m^{-2} s^{-1}. Thus seasonal and long-term variation in temperature will be critical to the C balance of northern peatlands that will also be dependent on variation in water table depth.

Soil organic carbon pools

Shading caused a significant increase in DOC at the site in May and February. Fenner *et al.* (2003) showed that *Sphagnum* photosynthate rapidly contributes to the soil DOC pool. However, it is likely that the reduced productivity of *S. capillifolium* did not alter rhizodeposition of plant DOC since DOC was not significantly higher under shading in the growing season. Atmospheric CO_2 limitations on photosynthetic activity may be more important to *Sphagnum* rhizodeposition. Kang *et al.* (2001) found an increased DOC in pore-water under elevated CO_2 that was associated with an increase in the activities of phosphatase and N-acetylglucosaminidase but not β-glucosidase. Alternatively, under field conditions other factors affecting DOC such as microbial and heterotrophic production or consumption and enzyme activities may have hidden any change in *Sphagnum* DOC export. This hypothesis could be tested with the use of $^{13}CO_2$-C gas tracer applied to NEE gas chambers, fixed by living *Sphagnum* and measured over time in the DOC and microbial pools.

DOC in soil beneath *Sphagnum* varied significantly throughout the year and peaked in July. The DOC peak and concentrations may be a function of

Sphagnum dominated areas, topography or the depth of sampling due to the water table depth. PCA showed that DOC and soil phenolics were related to basal respiration and the enzymes β-glucosidase and sulphatase. A study by Worrall *et al.* (2004) showed that changes in soil temperature are responsible for only 12 % of a 78 % increase in DOC production at Moorhouse over a 30 year period. They suggested that temperature variation alone is insufficient to explain observed increases in DOC production and suggested that hydrological changes affect an enzymatic latch mechanism (Freeman *et al.* 2001a) that may account for increased decomposition. Indeed, multiple regression analysis suggests that β-glucosidase activity was related to DOC release explaining 77 % of the seasonal variation. Thus whilst temperature may directly be responsible for the loss of C as CO_2 from peatlands, the export of soil C as DOC may be determined by the response of soil enzyme activities and not climatically induced alterations in *Sphagnum* productivity.

Soil enzyme activities

The activities of phosphatase, sulphatase and β-glucosidase were not significantly reduced within the shaded treatment suggesting that reduction in *Sphagnum* productivity does not significantly affect soil enzyme activities in peatlands. This may be explained by immobilized enzymes bound to clay and humic colloids that can retain activity independently of microbial activity (Burns, 1982).

All enzymes varied significantly throughout the year and differed in response to abiotic factors. Phosphatase activity varied significantly throughout the year and was reduced in the growing season. PCA showed that phosphatase activity was related to the gravimetric water content, rainfall and water table depth. However, stepwise multiple regression analysis did not suggest a single, significant predictor to explain the seasonal variability. Factors not measured that may explain the variation in phosphatase activity include pH and ionic concentrations particularly PO_4^{3-} and cofactors such as K^+ (Kang and Freeman, 1999).

Sulphatase and β-glucosidase activities varied significantly throughout the year and were highest in the growing season that may be representative of plant and microbial growth. PCA showed that sulphatase and β-glucosidase

activities were related to basal respiration, the soil organic C pools and possibly soil temperature. Soil temperature affects enzyme activities indirectly through influencing microbial proliferation and also directly, by modifying enzyme kinetics (Freeman *et al.*, 1999). Linear regression analyses showed that soil temperature explained 89 % of sulphatase variation, and basal CO_2 respiration explained 90 % of the variation. Thus increased sulphatase activity may be explained by either kinetic effects of temperature or microbial proliferation. However, multiple regression analysis showed that soil temperature and basal CO_2 respiration were multicollinear possibly due to temperature effects on microbial activity. This suggests that *Sphagnum*, soil microbes and soil enzymes may not be linked but rather respond to seasonal temperature variation by similar magnitudes. This would not be surprising considering that all processes are principally enzymatic.

It has been reported that only a small amount (generally less than 10%) of DOC compounds are low molecular weight (LMW) compounds (e.g. free amino acid and carbohydrates), which are readily assimilated by microorganisms (Jones , 1998). The majority of DOC is composed of polymeric compounds, which need to be enzymatically depolymerised before utilization by heterotrophic microorganisms (Chróst, 1991). The relationship between sulphatase and β-glucosidase activities with DOC suggests that either enzyme production is induced by high concentration of high molecular weight (HMW) DOC or the enzyme reaction causes accumulation of LMW DOC (Kang, 1999). Indeed, site DOC was the best predictor of β-glucosidase activity explaining 77 % of the variation. Van Hees *et al.* (2005) showed that the flux of LMW compounds through the soil solution was rapid in forest soils and may contribute substantially to the total CO_2 efflux from the soil. Also, the production rates of these soluble transitory compounds could exceed HMW DOC production.

3.4.4 Conclusions

Shading significantly reduced *Sphagnum* productivity despite the fact that *Sphagnum* species are adapted to low PPFDs. Thus *S. capillifolium* may be adapted to higher light intensities due to the accumulation of anthocyanins that

reduce photo-oxidative damage. A reduction in *Sphagnum* productivity due to light limitation may not result in the expected loss of CO_2 from peatlands due to the concomitant decrease in *Sphagnum* respiration that was a significant fraction of ecosystem respiration. Upon short-term exposure to saturating light conditions, gross photosynthesis increased relative to respiration resulting in net CO_2 sequestration. However, it is hypothesized that long-term exposure of the shaded *Sphagnum* to saturating PPFDs would result in an increase in *Sphagnum* respiration and hence productivity thus stabilizing NEE. These results do not suggest that alteration to *Sphagnum* productivity by other factors (i.e. nitrogen) will not affect NEE since light intensity affects net photosynthetic fixation differently to other factors.

Ecosystem respiration was relatively more sensitive to soil temperature variation than gross photosynthesis and was thus the primary determinant of NEE that was predicted best by soil temperature and to a lesser extent the water table depth. Therefore, global warming may stimulate CO_2 release via effects on both *Sphagnum* and soil respiration relative to a potential increase in productivity. Management strategies that aim at reducing soil respiration and enzyme activities rather than increasing *Sphagnum* productivity may therefore be a more effective means of maintaining the C sink capacity of peatlands.

It is clear that under current climatic conditions, a reduction in *Sphagnum* productivity does not impact on soil organic C pools and soil enzyme activities. Thus, whilst temperature and water table depth may be responsible for the loss of C as CO_2 from peatlands, the export of soil C as DOC may be determined by the direct response of microbes and soil enzyme activities to climate change and not climatically induced alterations in *Sphagnum* productivity. Thus *Sphagnum*, soil microbes and soil enzymes may not interact directly under current climatic conditions but rather respond to seasonal temperature and water table variation by similar magnitudes. However, it is hypothesized that elevated CO_2 may increase soil enzyme activities indirectly via stimulation of *Sphagnum* productivity leading to an increased input of fixed C to the soil.

Chapter 4

Interactive effect of shading and increased N deposition on *Sphagnum* productivity and decomposition processes in peatland mesocosms

4.1 Introduction

As a rule, net primary productivity (NPP) in peatlands exceeds the rate of decomposition of organic matter despite low NPP in peatlands relative to faster carbon (C) cycling ecosystems such as grasslands and forests. Carbon sequestration in peatlands is partially dependent on the growth of *Sphagnum* (Berendse *et al.*, 2001). *Sphagnum* species promote conditions that strongly favour carbon sequestration such as waterlogged, nutrient-poor and acidic conditions (Van Breeman, 1995; Hoosbeek *et al.,* 2001), and yet they thrive because of a variety of adaptive physiological mechanisms, including efficient use of nutrients (Aldous, 2002a). Plants have evolved mechanisms for acclimatizing to different light, temperature and nitrogen environments, and may optimize survival, reproduction and growth under the prevailing environmental conditions by reallocating resources and/or changing their morphology (Arp, 1991). It remains unclear whether alteration to *Sphagnum* productivity impacts on soil decomposition processes via rhizodeposition of labile organic compounds that could lead to alteration of net CO_2 ecosystem exchange (NEE).

Bryophytes are generally considered to be shade plants, reaching photosynthetic light saturation at low irradiances (Shaw and Goffinet, 2000; Davey *et al.*, 1997). Despite this results from Chapter 3 showed that *S. capillifolium* productivity was reduced by PPFD less than 300 μmol m^{-2} s^{-1}. Reduction in productivity by shading decreased *in situ* soil respiration but did not significantly affect *ex situ* measurements of soil decomposition processes. This may have been the result of immobilized enzymes bound to clay and humic colloids that can retain activity independently of microbial activity (Burns, 1982). Nitrogen (N) is thought to limit plant growth in many terrestrial systems,

and thus *Sphagnum* productivity commonly increases following N additions (Turetsky, 2003; Aerts *et al.*, 1992). Limitations imposed on productivity by N allocation to photosynthetic tissues may result in increased rhizodeposition of fixed C to the soil. However, a number of studies have reported decreases in bryophyte growth with N addition (Gunnarsson *et al.*, 2004; Limpens and Berendse, 2003; Limpens *et al.*, 2003). *Sphagnum* species have very low nutrient demands due to low tissue nutrient concentrations, high nutrient use efficiency, and tight nutrient cycling, but also have a very low threshold for high atmospheric deposition (Bridgham, 2002). Bryophytes have poorly developed conduction systems, with water and solutes taken up over the entire plant surface. Thus bryophytes often serve as effective traps for water and nutrients. This also makes them more sensitive to atmospheric chemical deposition than vascular plants (Turetsky, 2003). Li and Vitt (1997) have shown that N is quickly sequestered by the moss layer and can be translocated from old to young tissues. Rydin and Clymo (1989) obtained evidence of movement, from old to young tissues of *Sphagnum recurvum*, of both carbon and phosphorus compounds, and demonstrated the presence of numerous plasmodesmata linking stem cells and thus affording a possible symplast pathway. Bridgham (2002) stated that efficient scavenging of atmospheric N and translocation of internal N can supply almost all of the nutrient demand of the plant. Strong linear relationships exist between nitrogen (N) and rubisco and chlorophyll with growth under low light intensity greatly increasing the partitioning of N into chlorophyll and thylakoids (Evans, 1989). Thus shading and increased N deposition are predicted to have an interactive effect on *Sphagnum* productivity.

Approximately 30-60% of net photosynthate is exported to the roots of vascular plants, and of this carbon approximately 50% is released as organic carbon into the rhizosphere (Lynch & Whipps, 1990; Liljeroth *et al.*, 1994). Soil microbes are dependent on this organic carbon, supplied as root exudates as a source of energy. Fenner *et al.* (2003) found that a very rapid significant ^{13}C enrichment of leachate DOC from labelled *Sphagnum* occurred within 4 h of labelling. Environmental factors such as light intensity and N deposition may affect the amount of dissolved organic carbon (DOC) released into the rhizosphere by *Sphagnum*, and therefore microbes may be affected indirectly by environmental factors via photosynthetic C fixation. This "priming" effect

(stimulation of soil organic matter decomposition caused by the addition of labile substrates) has the potential to stimulate CO_2 respiration (Cheng, 1999). An increase in DOC via *Sphagnum* rhizodeposition may invoke changes in soil enzyme activities due to an increase in substrate availability for hydrolytic reactions and / or increased microbial substrate for enzyme synthesis (Freeman *et al.*, 1997).

Chapter 3 showed that reduction in *Sphagnum* productivity by shading did not significantly affect *ex situ* measurements of soil decomposition processes but did reduce *in situ* soil respiration. The aim of this research was to determine whether the interactive effect of shading and increased N deposition on *Sphagnum* productivity would impact on soil decomposition processes in the short-term. Natural variations in PPFD and N deposition are unlikely to have a major impact on *Sphagnum* productivity to the extent that it could impact on decomposition in the short-term. Thus the aim was to utilize shading and N as a means to alter *Sphagnum* productivity to levels above natural conditions that may impact on soil decomposition. It was hypothesized that shading would reduce photosynthetic activity resulting in reduced productivity whilst increased N deposition would result in increased productivity. Of added interest was whether N deposition may ameriolate the limiting effect of shading on *Sphagnum* productivity. Productivity was then hypothesized to have a positive feedback effect on soil decomposition processes via rhizodeposition.

Alteration to *S. capillifolium* pigmentation by shading and increased N deposition was also studied to test the hypothesis that this species acclimatizes to shaded conditions by breaking down anthocyanin pigments and allocating N to photosynthetic pigments. Since this hypothesis was not an aim of this thesis, the results are presented in Appendix 2.

To answer these hypotheses, *Sphagnum* mesocosms were subjected to two levels of light intensity and N supply for 9 months. The experiment involved the determination of CO_2 fluxes, labelled ^{13}C cycling by *S. capillifolium* for determination of net C fixation and respiration, plant tissue analysis and analysis of soil decomposition processes following 9 months treatment.

4.2 Materials and methods

4.2.1 Experimental design

Forty peatland mesocosms were collected from Moorhouse, Upper Teesdale, Cumbria in November 2003. The site is described in Chapter 3. The mesocosms contained *Sphagnum capillifolium, Sphagnum papillosum* and *Sphagnum cuspidatum* and were made of PVC tubing measuring 400 mm depth x 110 mm diameter. Cores were collected by first cutting the peat around the base of the tube and then carefully pushing down on the tube into the peat. The cores were then removed from the peat by digging around the cores and removing the surrounding peat. The bases of the mesocosms were tightly sealed with polythene bags and tape to prevent soil aeration. Holes were then drilled 5 cm below the soil surface and 3 mm tubing inserted with 20 ml syringes for soil leachate collection. A glass wool filter was placed between the tubing and the syringe to reduce contamination from particulate organic matter.

The forty mesocosms were split into two groups of twenty replicates depending on the composition of *Sphagnum* species and the quality of plant material in a greenhouse. Twenty replicates (control) were placed under high light intensity bulbs with a timer set for a 12 hour photoperiod during day time (>300 μmol m^{-2} s^{-1}) in a lattice square design. The remaining twenty replicates were placed beneath a shading mesh within the greenhouse (<40 μmol m^{-2} s^{-1}). During the spring months (after April) when days were brighter and longer, supplementary lighting was not required.

All mesocosms were watered automatically with artificial rainwater using peristaltic pumps for 30 minutes each day from November 2003. The flow rate was approximately 1.4 ± 0.03 ml min^{-1} giving approximately 84 ± 2 ml day^{-1} to each mesocosm. The flow rate used was an approximation of the evapotranspiration rate (determined by measuring the change in weight of randomly selected cores over a couple of days). However, water tables were maintained by watering with distilled water every other day until saturated. Both treatments received 0.5 g N m^{-2} yr^{-1} between November and March 2003.

After four months incubation, ten replicates within each light level were subjected to increased N deposition at the start of April 2003, two weeks prior to

gas analysis in April. This was a factorial design for shading and N interaction. The increased N deposition mesocosms received 50 ml of a 0.511 g l^{-1} NH_4NO_3 solution in artificial rainwater once a week. This gave final concentrations of 0.5 g N m^{-2} yr^{-1} for the low N level and 20 g N m^{-2} yr^{-1} for the increased N deposition level.

4.2.2 Plant analysis

At the end of the experiment in August 2003, the cores were dismantled and separated into plant and soil fractions. Total living *Sphagnum* biomass (red and green) and decaying biomass (brown) were determined by drying at 70 °C for 3 days. Samples of *S. capillifolium* capitulum were ground with a ball mill and analyzed for total C and N on a CN analyzer (Vario EL Elemental Analyser System E GmbH, Hanau, Germany), ^{13}C by IR-GCMS (NERC Stable Isotope Facility), or pigmentation by spectrophotometry (Appendix 2).

4.2.3 *Sphagnum* $^{13}CO_2$-C labelling

In August 2003, the *Sphagnum* vegetation was labelled with 99.99 atom % $^{13}CO_2$. Within each treatment, eight cores were labelled with 40 ml of 99.99 atom % $^{13}CO_2$ in the headspace over an 8 hour period and two cores left for natural abundance. Natural abundance cores were separated from the labelled cores under similar light conditions. For each labelled core, a gas chamber was attached and 10 ml of gas extracted into a 12 ml exetainer and 10 ml of 99.99 atom % $^{13}CO_2$ injected into the headspace. The procedure was then repeated for each core every 2 hours for a total of 8 hours (4 injections of 10 ml $^{13}CO_2$ giving 40 ml of $^{13}CO_2$ in 8 hours). The shaded treatment remained shaded throughout labelling to purposefully reduce photosynthetic fixation. Ten ml of $^{13}CO_2$ is equivalent to a concentration of 20 ppt of $^{13}CO_2$ in air. This concentration can be found in the acrotelm and also allowed full saturation of the gas chambers (Smolders *et al.*, 2001). The mass of $^{13}CO_2$ label added to each core was 22.39 mg $^{13}CO_2$-C or 2714 mg $^{13}CO_2$-C m^{-2}. Following labelling, one individual *S.*

capillifolium capitula was removed from each core for determination of the amount of labelled ^{13}C fixed by the *S. capillifolium* per unit biomass and soil area. Five days post-labelling one individual *S. capillifolium* capitulum was removed from each core for determination of the amount of fixed ^{13}C-label retained.

Isotope equations

The $\delta^{13}C$ of *S.capillifolium* capitulum directly after labelling and 5 days post-labelling were converted to ^{13}C atom % (Eq. 4-1) (see Boutton, 1991):

$$^{13}C\ (\text{atom \%}) = (100 \times (((\delta^{13}C / 1000) + 1) \times 0.0112372)) / (1 + (((\delta^{13}C / 1000) + 1) \times 0.0112372)) \quad (4\text{-}1)$$

Estimates of ^{13}C fixation into *S. capillifolium* tissue immediately following labelling and the amount retained after 5 days are reported as atom % excess (sample atom % - natural abundance atom %). The amount of fixed ^{13}C-label lost via respiration and rhizodeposition over 5 days was calculated as the atom % excess fixed – atom % excess retained. The atom % excess of fixed, retained and lost ^{13}C was converted to mg ^{13}C g biomass by generating an 'F value' (Eq. 4-2 and 4-3) and converted to the total amount of ^{13}C per m^2 biomass (Eq. 4-4).

$$F = (\text{incubated atom \%} - \text{average NA atom \%}) / (99.99\ \text{\%} - \text{average NA atom \%}) \quad (4\text{-}2)$$

$$\text{mg } ^{13}C \text{ g biomass} = F \times 1000 \quad (4\text{-}3)$$

$$\text{mg } ^{13}C\ m^{-2} \text{ biomass} = \text{mg } ^{13}C \text{ g biomass} \times \text{g biomass } m^{-2} \quad (4\text{-}4)$$

The proportion of ^{13}C-label added that was fixed was determined by equation 4-5 where the amount of label added to each headspace was converted to the amount per m^{-2} (2714 mg ^{13}C m^{-2}).

$$\% \ ^{13}\text{C-label fixed} = (\text{mg } ^{13}\text{C m}^{-2} \text{ biomass fixed / mg } ^{13}\text{C label m}^{-2}) \times 100 \quad (4\text{-}5)$$

The proportion of fixed ^{13}C-label retained after 5 days was determined by equation 4-6. The proportion of fixed ^{13}C that was lost via respiration and rhizodeposition over 5 days was determined by equation 4-7.

$$\% \text{ fixed } ^{13}\text{C-label retained} = (\text{mg } ^{13}\text{C m}^{-2} \text{ biomass retained / mg } ^{13}\text{C m}^{-2} \text{ biomass fixed}) / 100 \quad (4\text{-}6)$$

$$\% \ ^{13}\text{C-fixed lost over 5 days} = ([\text{mg } ^{13}\text{C fixed} - \text{mg } ^{13}\text{C lost}] / \text{mg } ^{13}\text{C fixed}) \times 100 \quad (4\text{-}7)$$

4.2.4 Carbon dioxide fluxes

Static headspace gas sampling chambers were made of PVC tubing measuring 50 mm depth x 110 mm diameter with transparent acrylic lids and central septa sealed to the top with adhesive and silicon sealant. A time course experiment was carried out on three cores from each treatment to determine the optimal incubation period. Net CO_2 ecosystem exchange (NEE) was determined for all treatments under non-shaded conditions in order to determine productivity non-destructively. Ecosystem respiration was determined using black-out covers to inhibit photosynthesis. The flux of CO_2 was constant for 2 hours with no change between incubation periods (either half an hour or one hour). Within each treatment, cores were paired according to ecosystem respiration rates in order to measure NEE (light) and ecosystem respiration (dark) simultaneously. Gas measurements were made by sealing a gas chamber to the top of each core using electrical tape. Ten millilitres of gas were extracted into 5 ml gas exetainers and the headspace replaced with 10 ml of N_2 gas. After two hours incubation, 10 ml of gas was placed in a second exetainer. The gas samples were injected into a Perkin Elmer Gas Chromatograph equipped with a Porapak QS column and flame ionization detector. The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1}. Background gas concentrations were subtracted from incubated chamber concentrations to give an estimate of flux and converted to mg CO_2-C m^{-2} h^{-1}.

Gross photosynthesis was estimated as the difference between NEE and ecosystem respiration of paired replicates. Gas measurements were made after two months incubation in February and March. Following N addition, gas measurements were made in April and May.

4.2.5 Soil analysis

In August, soil sub-samples were either dried at 105 °C for 1 day, ground with a ball mill and analyzed for total C and N on a CN analyzer (Vario EL Elemental Analyser System E GmbH, Hanau, Germany), or immediately incubated for measurement of basal CO_2 respiration and extracellular enzyme activities. Basal CO_2 respiration was determined as in Chapter 3. The activity of three extracellular hydrolase enzymes (β-glucosidase, phosphatase, sulphatase and *N*-acetylglucosaminase activity) were determined using fluorogenic methylumbelliferyl (MUF)-substrates (Freeman *et al.,* 1995) as described in the method of Chapter 3. Soil leachate was collected from each mesocosm at a depth of 5 cm below the soil surface in June 2003. Samples collected were filtered through 0.45 μm filters. Dissolved organic carbon (DOC) was analyzed by the chemistry department at CEH Merlewood/Lancaster using the method described in Chapter 3. Soil phenolics were determined by the method of Box (1983) in Chapter 3.

4.2.6 Statistical analysis

All statistical analyses were performed with Minitab Release 13 (Minitab Inc.). Differences between treatments were determined using GLM and one-way ANOVA analysis and the residuals tested for normality using Anderson-Darlings normality test. Data that were non-normally distributed were ranked and tested using Kruskal-Wallis analysis. Spearman Rank correlation analysis of pigments was performed due to non-normal data.

4.3 Results

4.3.1 Plant analysis

Total *Sphagnum* biomass was significantly reduced by shading under low N deposition ($P < 0.001$; $F = 28.48$) and increased N deposition ($P < 0.01$; $F = 14.47$), and was significantly increased by N in the shaded treatment ($P < 0.05$; $F = 4.38$) (fig. 4-1a). There was no significant difference in dead or decaying biomass between treatments.

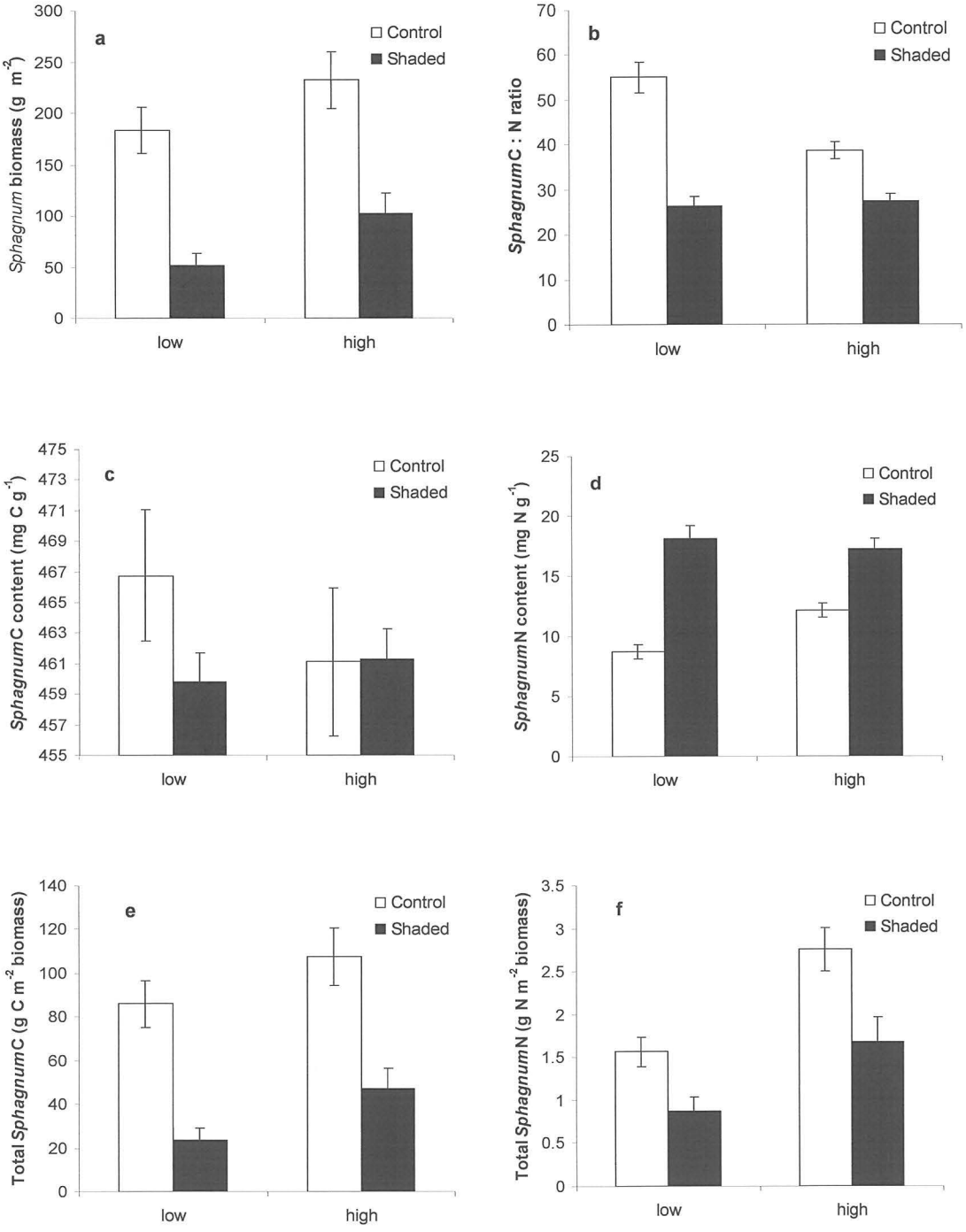
The C : N ratio of *S. capillifolium* capitulum was significantly reduced by shading under low N deposition ($P < 0.001$) and increased N deposition ($P < 0.001$; $F = 21.22$), and was significantly reduced by increased N deposition in the control treatment ($P < 0.001$; $F = 18.42$) (fig. 4-1b).

Capitulum C content was not significantly affected by shading or increased N deposition (fig. 4-1c). The reduction in *S. capillifolium* C : N ratio by shading was due to a significant increase in the capitulum N content under low N deposition ($P < 0.001$) and increased N deposition ($P < 0.001$; $F = 20.87$) (fig. 4-1d). Increased N deposition significantly increased capitulum N content in the control treatment ($P < 0.001$; $F = 16.46$). A significant interaction between shading and increased N deposition on capitulum N content was found ($P < 0.05$; $F = 6.48$).

Shading significantly reduced the total amount of biomass C per unit area under low N deposition ($P < 0.001$; $F = 26.4$) and increased N deposition ($P < 0.01$; $F = 14.05$) (fig. 4-1e). Increased N deposition increased the total amount of biomass C per unit area in the shaded treatment ($P < 0.05$; $F = 4.37$).

Shading significantly reduced the total amount of biomass N per unit area under low N deposition ($P < 0.01$; $F = 7.99$) and increased N deposition ($P < 0.01$; $F = 7.93$) (fig. 4-1f). Increased N deposition increased the total amount of biomass N per unit area in the shaded ($P < 0.05$; $F = 5.58$) and control treatments ($P < 0.01$; $F = 14.02$).

Figure 4-1 Effect of shading and increased N deposition on total *Sphagnum* biomass (a), *S. capillifolium* C:N ratio (b), *S. capillifolium* C content (c), *S. capillifolium* N content (d), *S. capillifolium* C content per soil area (e), and *S. capillifolium* N content per soil area (f). Mean ± standard error (n = 10).

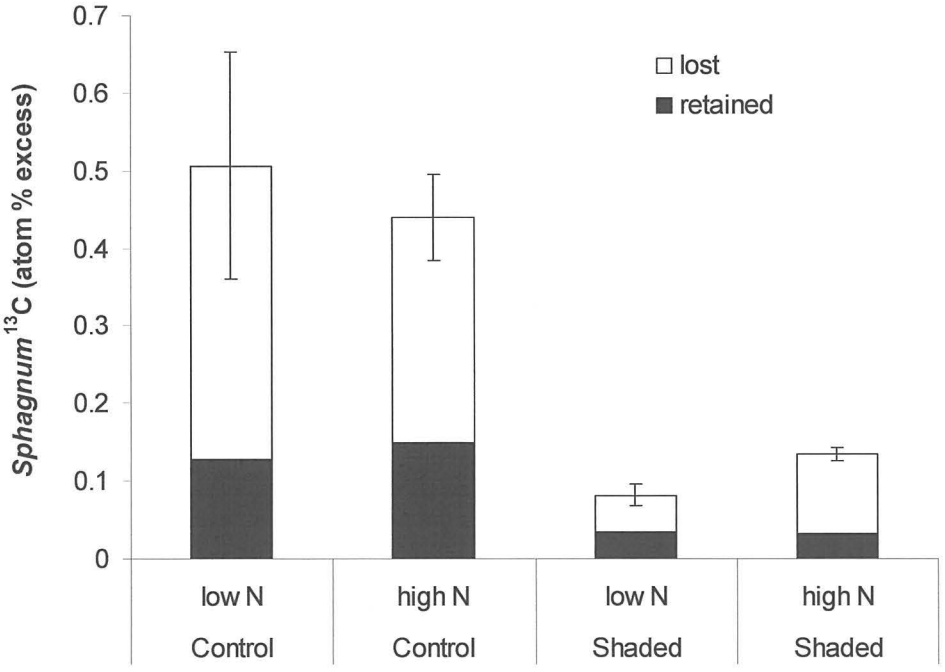


4.3.2 *Sphagnum* $^{13}CO_2$-C labelling

Sphagnum ^{13}C per unit biomass

The isotopic composition of natural abundance *S. capillifolium* capitulum averaged 1.0804 ± 0.0003 atom % (-28.027 ± 0.2559 $\delta^{13}C$). Shading significantly reduced the fixation of ^{13}C-label per unit biomass immediately after labelling in both the low ($P < 0.05$) and increased ($P < 0.01$; $F = 16.8$) N supply treatments (fig. 4-2). Increased N deposition significantly increased the fixation of ^{13}C-label per unit biomass in the shaded treatment only ($P < 0.05$; $F = 10.2$).

Figure 4-2 Effect of shading and increased N deposition on the isotopic composition above natural abundance of *S. capillifolium* capitulum after labelling, 5 days post-labelling (solid bars) and the atom % excess lost over 5 days (open bars).



Shading did not significantly reduce the retention of fixed ^{13}C-label per unit biomass after 5 days in both N supply treatments (fig. 4-2). Increased N deposition did not significantly affect the retention of fixed ^{13}C-label per unit biomass after 5 days under either controlled or shaded conditions. The proportion

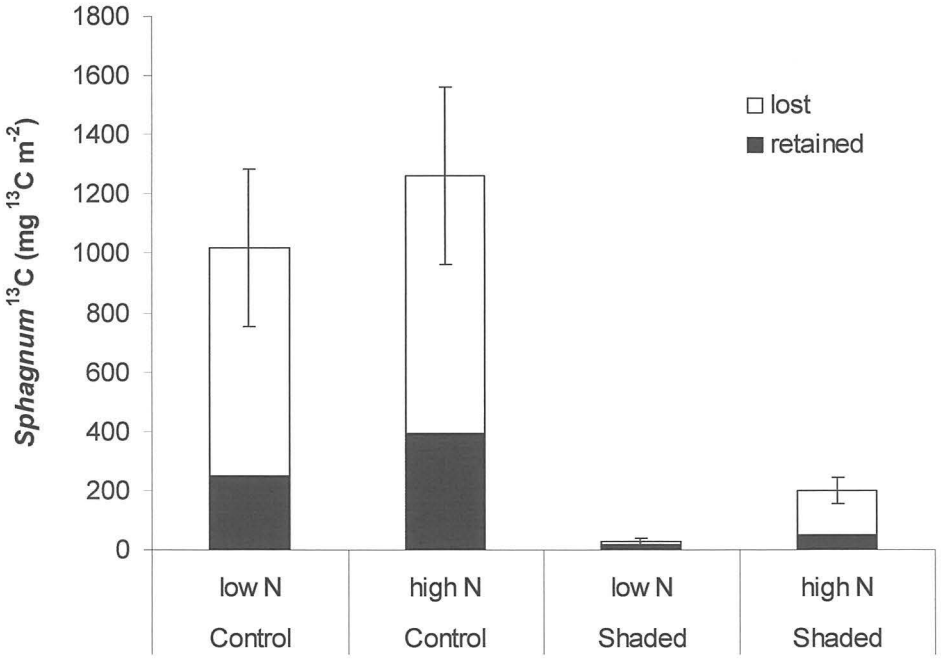
of fixed ^{13}C per unit biomass retained after 5 days was not significantly affected by either treatment and averaged 32 % across all treatments.

Shading significantly reduced the loss of fixed ^{13}C-label per unit biomass over 5 days in the low ($P < 0.05$) and increased ($P < 0.05$; $F = 6.62$) N deposition treatments (fig. 4-2). Increased N deposition significantly increased the loss of fixed ^{13}C-label per unit biomass over 5 days in the shaded treatment only ($P < 0.05$; $F = 18.16$). However, the proportion of fixed ^{13}C per unit biomass lost over 5 days was not significantly affected by either treatment and averaged 68 % across all treatments.

Sphagnum ^{13}C per unit area

Shading significantly reduced the fixation of ^{13}C-label per unit area in both the low ($P < 0.05$; $F = 6.43$) and increased ($P < 0.05$; $F = 7.11$) N deposition treatments (fig. 4-3). Increased N deposition increased the fixation of ^{13}C-label per unit area in the shaded treatment only ($P < 0.05$; $F = 14.33$).

Figure 4-3 Effect of shading and increased N deposition on *Sphagnum* ^{13}C per unit area after labelling, retained 5 days post-labelling (solid bars) and lost over 5 days (open bars).



Shading significantly reduced the retention of fixed ^{13}C-label per unit area after 5 days in both the low ($P < 0.05$; $F = 7.45$) and increased ($P < 0.05$; $F = 10.05$) N deposition treatments (fig. 4-3). Increased N deposition did not significantly affect the retention of fixed ^{13}C-label per unit area after 5 days under either controlled or shaded conditions. The proportion of fixed ^{13}C-label retained per unit area after 5 days was not significantly affected by either treatment and averaged 31 % across all treatments.

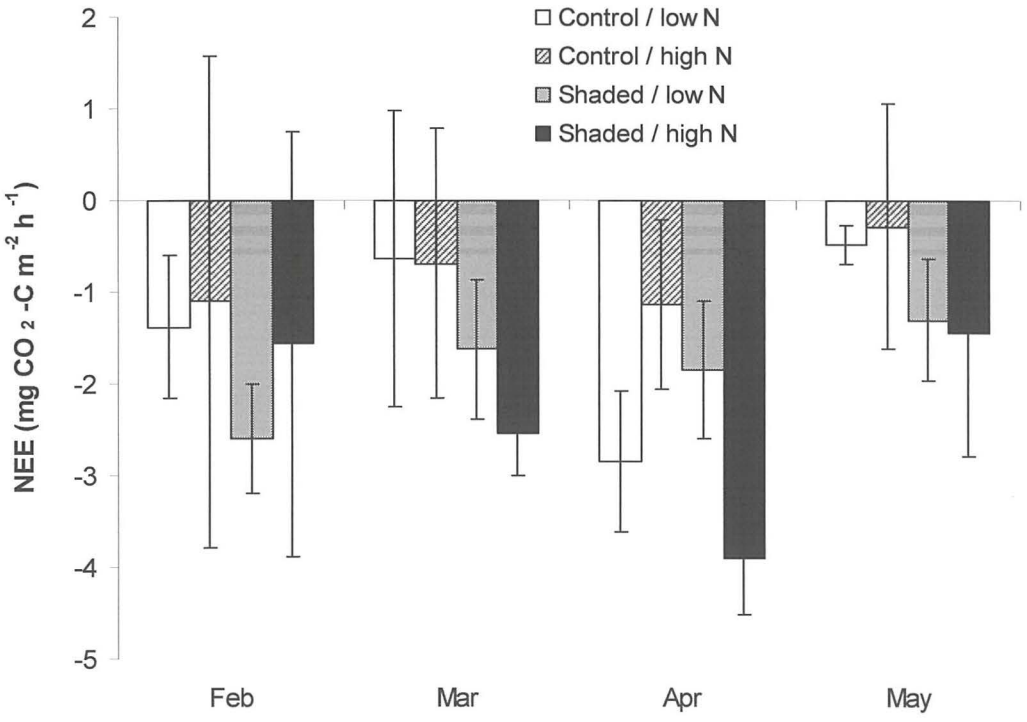
Shading significantly reduced the loss of fixed ^{13}C-label per unit area over 5 days in both the low ($P < 0.05$; $F = 5.43$) and increased ($P < 0.05$) N deposition treatments (fig. 4-3). Increased N deposition significantly increased the loss of fixed ^{13}C-label per unit area over 5 days in the shaded treatment only ($P < 0.01$; $F = 22.37$). The proportion of fixed ^{13}C-label lost over 5 days by was not significantly affected by either treatment and averaged 69 % across all treatments.

4.3.3 Carbon dioxide fluxes

Net CO_2 ecosystem exchange (NEE) under natural PPFD conditions

GLM ANOVA analysis showed that NEE was not significantly different between months. Shading and increased N deposition did not have significant effects on NEE over all months (fig. 4-4). However, one-way ANOVA analysis showed that shading significantly increased sequestration in April in the increased N deposition treatment ($P < 0.05$; $F = 6.25$). In April, C sequestration was significantly increased by increased N deposition in the shaded treatment ($P < 0.05$; $F = 4.5$).

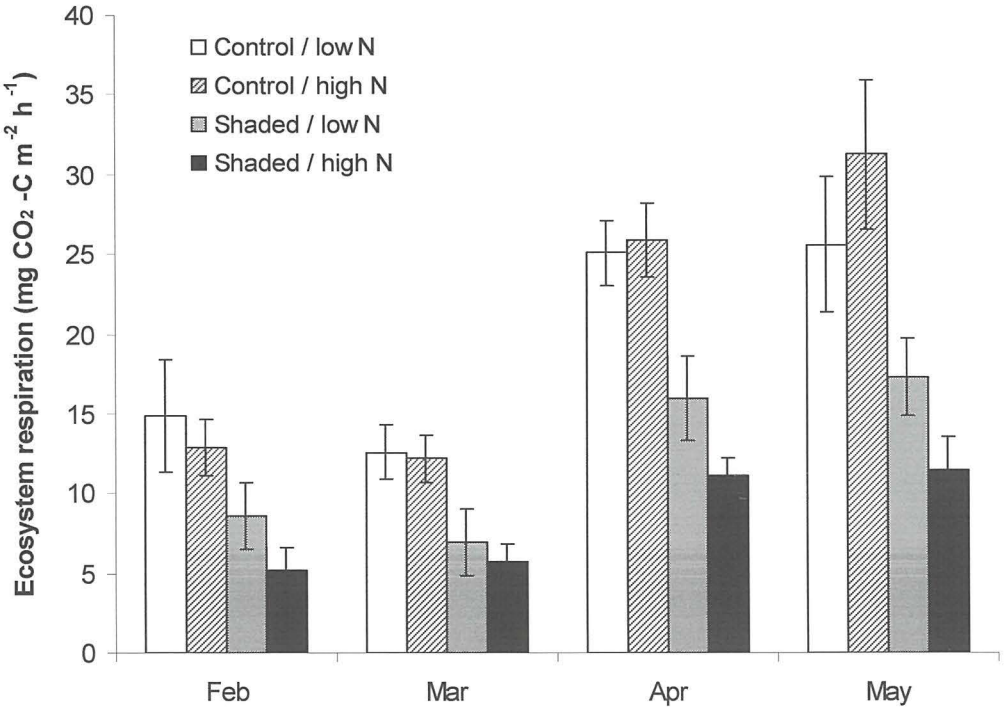
Figure 4-4 Effect of shading and increased N deposition on NEE over four months following three months prior incubation since November (n=5). Measurements of NEE were carried out under natural PPFD conditions. Nitrogen treatments began in April.



Ecosystem respiration

GLM ANOVA analysis showed that ecosystem respiration was significantly different between months ($P < 0.001$; $F = 30.62$) and that shading ($P < 0.001$; $F = 65.97$) significantly reduced ecosystem respiration over all months (fig. 4-5). Increased N deposition did not have a significant effect on ecosystem respiration after addition in April. However, Kruskal-Wallis analysis showed that ecosystem respiration was significantly reduced by increased N deposition in the shaded treatment after addition in April ($P < 0.05$). GLM ANOVA analysis showed that shading and increased N deposition interactively affected ecosystem respiration after N addition in April ($P < 0.05$; $F = 4.87$).

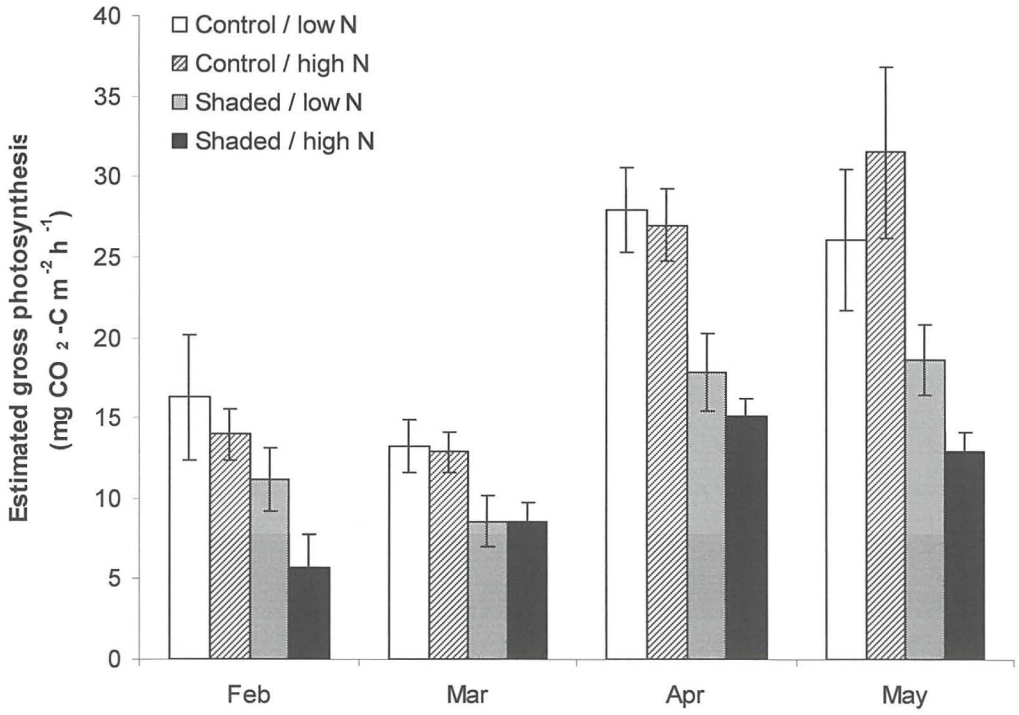
Figure 4-5 Effect of shading and increased N deposition on ecosystem respiration over four months following three months incubation since November (n=5). Nitrogen treatments began in April.



Estimated gross photosynthesis under natural PPFD conditions

GLM ANOVA analysis showed that gross photosynthesis was significantly different between months ($P < 0.001$; $F = 28.82$) and that shading ($P < 0.001$; $F = 48.03$) significantly reduced estimated gross photosynthesis over all months (fig. 4-6). However, month and shading interacted significantly ($P < 0.05$; $F = 2.74$) and Kruskal-Wallis analysis showed that shading did not significantly affect gross photosynthesis in February. Increased N deposition did not have a significant effect on estimated gross photosynthesis after addition in April. However, one-way ANOVA analysis showed that estimated gross photosynthesis was significantly reduced by increased N deposition in the shaded treatment in May ($P < 0.05$; $F = 5.31$).

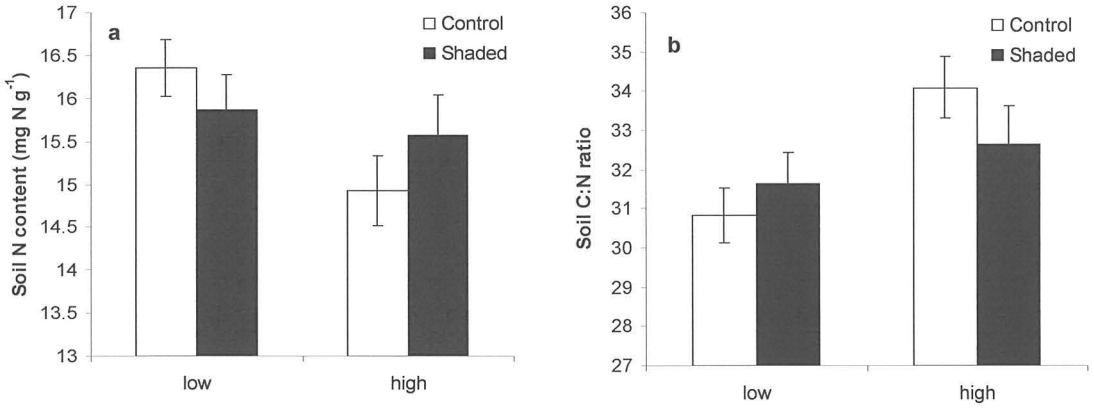
Figure 4-6 Effect of shading and increased N deposition on gross photosynthesis over four months following three months prior growth since November (n=5). Estimates were determined under natural PPFD conditions. Nitrogen treatments began in April.



4.3.4 Soil analysis

The soil N content was significantly reduced by increased N deposition in the control treatment ($P < 0.05$; $F = 7.48$) (fig. 4-7a). The soil C : N ratio was significantly increased by increased N deposition in the control treatment ($P < 0.01$; $F = 9.76$) (fig. 4-7b). Shading and increased N deposition did not significantly affect the soil C content, DOC, dissolved phenolics, extracellular enzyme activities or basal CO_2 respiration.

Figure 4-7 Effect of shading and increased N deposition on soil N content (a), and the soil C : N ratio (b). Mean ± standard error (n = 10).



4.4 Discussion

4.4.1 Plant analysis

Sphagnum productivity was significantly reduced over the nine months incubation under shaded conditions for both N deposition treatments. No significant difference was found in dead or decaying (brown) *Sphagnum* biomass below the canopy suggesting that shading slowed the production of new biomass rather than increasing death and decay of the original starting biomass. This supports the evidence of shade adaptation since despite reduction in productivity *Sphagnum* survived incubation under shaded conditions. Unfortunately, parameters of growth (i.e. the number of individual *Sphagna* and average stem heights per treatment) were not determined but visual observation showed that morphological changes to capitula and branches occurred that also accounted for the reduced biomass (plate 4-1). This included a significant reduction in anthocyanin pigments and a significant increase in photosynthetic pigmentation of the dominant species *S. capillifolium* (Appendix 2).

Plate 4-1. Observed reduction in growth and anthocyanin pigmentation and alteration to capitulum morphology of *S. capillifolium* by shading after four months. The control treatment is left and the shaded treatment is right.

Sphagnum productivity under natural PPFD conditions was not limited by N in the short-term or more likely N deposition was excessively high leading to a nutrient imbalance or toxicity. The increased N deposition (20 g N m^{-2} y^{-1}) was at the high end of published atmospheric deposition experiments examining *Sphagnum* productivity that range from 0.3 – 23 g N m^{-2} y^{-1} (Limpens *et al.*, 2003; Van der Heijden *et al.*, 2000; Aldous, 2002a and b). Annual atmospheric N deposition typically ranges from 0.1-0.5 g N m^{-2} y^{-1} (Bowden, 1986) reaching as high as 4 mg N m^{-2} y^{-1} (Killham, 2001). Gunnarsson and Rydin (2000) and Vitt *et al.* (2003) suggested that *Sphagnum fuscum* NPP is inhibited above critical N loading rates between 1.4 – 3.4 g N m^{-2} y^{-1}. Higher deposition rates can exceed plant tolerance levels, potentially with toxic effects (Aerts *et al.*, 1992; Gunnarsson and Rydin, 2000). Thus lower levels of N deposition (i.e. 0.5 – 4 g N m^{-2} y^{-1}) may have had a more significant positive effect on productivity in the short-term.

At N-limited sites, increased N deposition will result in increased growth, up to a certain capitulum concentration (Vitt *et al.*, 2003). Above this point, *Sphagnum* growth will be limited by other factors, such as phosphorus (Aerts *et al.*, 1992), and the high N concentration may result in a nutrient imbalance or even be toxic. *Sphagnum* growth has been found to decrease when the capitulum N concentration becomes too high, owing to moderate influxes (*c.* 1 g N m^{-2} y^{-1}) over long periods (Rochefort *et al.*, 1990; Gunnarsson and Rydin, 2000), or high influxes (> 15 g N m^{-2} y^{-1}) over a shorter period (Van der Heijden *et al.*, 2000). The capitulum N content of *S. capillifolium* grown under non-shaded conditions was significantly increased by increased N deposition from 8 to 12 mg N g^{-1} dry biomass since *Sphagnum* species have no known mechanism for regulating their uptake of N (Jauhiainen *et al.*, 1998). Van der Heijden *et al.* (2000) suggested that capitulum N contents in excess of 15 mg N g^{-1} dry biomass were detrimental to photosynthesis. The increased capitulum N content in this study led to a significant reduction in the capitulum C:N ratio but since productivity was not affected, increased N deposition was neither limiting or toxic. Aerts *et al.* (1992) found that at high N deposition sites, phosphorus supply caused an almost threefold increase in productivity, but increased N deposition did not result in any productivity increase. Thus, in an area with a high atmospheric N supply,

productivity is P-limited instead of N-limited. Thus, it is possible that *Sphagnum* at Moorhouse may be subject to high atmospheric N deposition.

Interestingly, shading resulted in a significant increase in capitulum N content and thus reduction in the capitulum C:N ratio under both N deposition treatments. Aldous (2002a, 2002b) found that *S. capillifolium* can translocate N from old to young tissues from mineralized N. Thus the increase in capitulum N content by shading must have been the result of translocation of N from old to new tissues leading to retention of N in photosynthetic tissues. Also, increased N deposition did not have a significant effect on capitulum N content in the shaded treatment suggesting that translocation is a more important N source for *S. capillifolium* grown under shaded conditions than retention of atmospheric N. Aldous (2002a, 2002b) used $^{15}NH_4{}^{15}NO_3$ tracer to determine whether N retention and translocation in *Sphagnum* would be greater in low N deposition sites compared to high N deposition sites. Aldous (2002b) demonstrated the importance of N translocation showing that 1 to 32 % of ^{15}N was translocated into newly growing *Sphagnum* tissue over 1 yr in the low deposition sites, and 64 to 83 % of ^{15}N was translocated in the high deposition sites. Aldous (2002a) suggested that N retention from atmospheric deposition satisfies only a small proportion of the annual N budget for moss production with the remainder supplied from internal N cycling particularly mineralized N. Aldous (2002b) estimated that between 0.5 and 11 % of the annual N requirement of *S. capillifolium* was met by translocation. Wells and Brown (1996) found that new growth of *Rhytidiadelphus squarrosus* was supported entirely by the nutrient content of the existing growth, with elements translocated from the latter. However, *Sphagnum* grown under shaded conditions were limited by N since productivity was increased by increased N deposition. Also, a significant interaction between shading and N deposition on tissue N content was found. Thus the translocation of N from decaying old tissues or soil and subsequent allocation to younger photosynthetic tissues under shaded conditions may have been affected by increased N deposition since bryophytes tend to become less efficient at retaining N under high deposition (Aerts *et al.*, 1992; Woodin and Lee, 1987). Aldous (2002b) concluded that a combination of internal N cycling via mineralization, N capture from atmospheric deposition and N translocation are the three key processes supplying N to growing mosses. Results from

Appendix 2 show that the translocated N was allocated to photosynthetic membranes and pigmentation to maintain positive growth under shaded conditions.

4.4.2 *Sphagnum* $^{13}CO_2$-C labelling

It is apparent that increased N deposition altered the effect of shading on the cycling of ^{13}C-label by *S. capillifolium*. First, results are discussed for the effect of shading independently of N deposition on ^{13}C turnover of *Sphagnum* grown for 9 months under shaded conditions. The isotopic composition above natural abundance of *S. capillifolium* capitulum immediately after labelling was significantly reduced by shading. Thus the PPFD of the shaded treatment was below the PPFD at which photosynthesis is saturated (P_{max}) whilst the PPFD of the control plants was probably above the photosynthetic capacity leading to potential photoinhibition and thus accounting for the higher concentration of anthocyanins (Appendix 2). The reduced fixation of ^{13}C per unit biomass suggests that physiological processes relating to C fixation were suppressed by shading e.g. RuBP regeneration, ATP synthesis. The isotopic composition immediately after labelling was approximately 50 % above natural abundance for *S. capillifolium* grown under non-shaded conditions and only 10 % above natural abundance for shade grown plants. Fenner *et al.* (2003) used $^{13}CO_2$-C label to determine the fate and turnover of C by *S. subnitens.* They found that labelled tissues 26 h post-labelling contained 61 % more ^{13}C than natural abundance. Fenner *et al.* (2003) stated that 'non-vascular species, such as *Sphagnum*, are likely to have greater access to respired sources of CO_2 (plant and soil) that would have been less ^{13}C enriched during the course of $^{13}CO_2$ pulse labeling.' The total amount of ^{13}C-label fixed per unit soil area was also significantly reduced by shading showing that reduced biomass in the shaded treatment affected the total amount of ^{13}C-label fixed. The proportion of ^{13}C-label fixed was significantly reduced by shading from 38 % to 1 % of ^{13}C-label added. Thus shading affected metabolism directly through photosynthesis, and growth and development indirectly as a consequence of the immediate metabolic responses.

Fenner *et al.* (2003) stated that 'photosynthate carbon release via respiration and exudation is probably occurring continuously, with dilution by carbon fixed from non-labelled $^{12}CO_2$ and refixation of respired $^{13}CO_2$ contributing to changes in *Sphagnum* tissue ^{13}C content.' The retention of fixed ^{13}C-label five days post-labelling per unit biomass was lower in the shaded treatment but was not significantly lower. It is likely that this was due to the sampling since the amount of ^{13}C fixed and retained per unit biomass were determined on different *Sphagnum* individuals. Indeed, the retention of fixed ^{13}C per unit area was significantly reduced by shading due to reduced productivity. Also, shading significantly reduced the loss of ^{13}C per unit biomass and per unit area showing that respiratory losses were reduced as well as photosynthetic fixation. The proportion of fixed ^{13}C-label retained or lost over five days was not significantly different with approximately 33 % of fixed ^{13}C-label retained or 67 % lost over five days in both light treatments. However, the respiration of fixed ^{13}C may have stabilized in both treatments five days post-labelling since Fenner *et al.* (2003) showed a rapid decline in ^{13}C content following maximum incorporation on day 2. These results suggest that respiration was reduced leading to reduced productivity but maintaining positive net photosynthetic C fixation under shaded conditions. The loss of ^{13}C-label over five days was probably the result of carbon loss as CO_2 via stem and shoot respiration of recent ^{13}C labelled photosynthates, such as monosaccharides and amino acids, and dilution by subsequent assimilation of natural abundance CO_2-C (Fenner *et al.*, 2003). Reassimilation of pulse derived ^{13}C, respired by the plant and soil heterotrophs as CO_2, also contributed to the stabilization of ^{13}C tissues. Refixed CO_2 is thought to be important in the growth of *Sphagnum* (Turetsky and Wieder, 1999; Smolders *et al.*, 2001). Bryophytes and vascular plants have similar $\delta^{13}C$ values averaging – 27 ‰. Substantially more negative $\delta^{13}C$ values are seen in aquatic bryophytes utilizing a substantial proportion of respired CO_2 (e.g. *S. cuspidatum* [Proctor *et al.*, 1992]). In this study, natural abundance $\delta^{13}C$ of *S. capillifolium* averaged -28.03 ± 0.26 ‰ that may reflect a relatively small refixation of respired $^{13}CO_2$-C.

Increased N deposition did not have a significant effect on the cycling of ^{13}C-label in the control treatment grown under natural PPFD conditions. This supports the evidence that productivity of *S. capillifolium* was not limited by N

supply. However, increased N deposition stimulated fixation of ^{13}C-label per unit biomass and per unit area for plants grown under shaded conditions suggesting that metabolic processes were N limited. Thus limitations on photosynthetic activity imposed by the shaded conditions may have been ameriolated by N supply that was allocated to chlorophyll and accessory pigments in order to maintain net C fixation (Appendix 2). Marshall and Proctor (2004) suggested that the low saturation values for *Sphagnum* photosynthesis probably reflect high diffusion resistance to CO_2 uptake – but this may be only a secondary limitation because low availability of N and P impose their own constraints on possible rates of production in most bog habitats. The loss of fixed ^{13}C per unit biomass and per unit area was significantly increased by increased N deposition in the shaded treatment suggesting that respiration was also increased by N deposition under shaded conditions. However, the proportion of fixed ^{13}C retained or lost over five days was not significantly affected by increased N deposition under shaded conditions or shading under increased N deposition showing that the balance between photosynthesis and respiration was maintained.

4.4.3 Carbon dioxide fluxes

Shading significantly reduced ecosystem respiration throughout February and May, and estimated gross photosynthesis between March and May. The one month lag between shading effects on ecosystem respiration and gross photosynthesis suggests that (1) gross photosynthetic activity of the control treatment may have been temperature limited in February, (2) *Sphagnum* biomass may not have been significantly reduced by shading in February, and (3) reduced plant respiration may have been an immediate short-term acclimation response to shading. Ambient temperature was significantly lower between February / March (8 - 9 °C) compared to April / May (13 - 14 °C) and this would explain the increase in ecosystem respiration and gross photosynthesis in April / May. Estimated gross photosynthetic activities of both light treatments were determined under natural PPFD conditions and thus represent changes in growth, physiology and morphology relating to shade acclimation. Reduction in growth and anthocyanin pigmentation, and alteration to capitulum morphology were

visually observable from March (Plate 4-1; Appendix 2) after 4 months incubation.

Long-term responses represent acclimation if net photosynthetic activity (or NPP) of shade grown *Sphagnum* is higher relative to natural PPFD grown *Sphagnum* measured under shaded conditions. Unfortunately the gross photosynthetic activities of the control and shaded treatments were not determined under both natural and shaded PPFD and thus the importance of these responses as acclimation strategies is not conclusive. However, it appears reasonable to hypothesize that photosynthetic activity of the shaded treatment per unit biomass would have been higher than the control treatment under shaded conditions.

The reduction in ecosystem respiration may be explained by a reduction in respiration of *Sphagnum* incubated under shaded conditions in order to maintain a positive net C balance by reducing the compensation point. However, results from the *in situ* field experiment in Chapter 3 showed that shading reduced both soil and *Sphagnum* respiration to 43 % and 32 % of ambient fluxes respectively. *Sphagnum* productivity was reduced by shading that would explain the reduction in ecosystem respiration and thus net CO_2 fixation must have decreased. By April / May, PPFD and temperature increase would have significantly increased productivity of the control treatment. The reduced biomass of *Sphagnum* in the shaded treatment would then account for the reduction in gross photosynthetic activity per unit soil area under natural PPFD. Also, the visually observable changes in pigmentation (Appendix 2) and morphology may have made *S. capillifolium* particularly vulnerable to photoinhibition that would also have reduced gross photosynthetic activity under ambient PPFD. Bryophytes are essentially shade-adapted plants and suffer photoinhibition and pigment bleaching at quite low irradiances (Critical Loads Advisory Group., 1996).

Increased N deposition did not have a significant effect on ecosystem respiration or estimated gross photosynthesis of natural PPFD grown mesocosms after addition in April. This is not surprising since the mesocosms had only been subjected to increased N deposition for two months. However, ecosystem respiration was significantly reduced by increased N deposition in the shaded treatment after April. These estimates were determined under natural PPFD and

thus the increased N allocation to photosynthetic pigments by shading may have resulted in an imbalance between C fixation and respiration. Under shaded conditions, the loss of fixed ^{13}C was increased by N suggesting that respiration and thus growth was stimulated by N under shaded conditions. Also, increased N deposition interacted significantly with shading suggesting that increased N deposition may have weakly stimulated respiration of the control treatment. Estimated gross photosynthesis was significantly reduced by increased N deposition in the shaded treatment in May. Thus as with shading there was an apparent one month lag in N treatment effect between ecosystem respiration and gross photosynthesis. The increased N deposition may then have had a toxic effect on the shaded treatment due to an imbalance between internal C and N cycling within the tissues under natural PPFD conditions.

All mesocosms were net sinks of CO_2-C between February and May due to relatively higher rates of gross photosynthesis relative to ecosystem respiration. In April, net C sequestration was significantly greater in the shaded treatment than the control. Since NEE of the shaded treatment was determined under natural PPFD this suggests that the reduction in *Sphagnum* productivity reduced ecosystem respiration to a greater extent than gross photosynthesis.

4.4.4 Soil analysis

Moss leaves are only one cell thick and are particularly susceptible to cytosolic leakage (Gerdol, 1991). Nutrient-binding organochemicals, phenolics, and uronic acid are excreted and leached by *Sphagnum* into the surrounding water (Rasmussen *et al.*, 1994). These lower the pH of the peatland and are inhibitory to some microfungi (Heil *et al.*, 2002), but are utilized by others (Thormann *et al.*, 2002). Shading and increased N deposition did not significantly affect DOC, dissolved phenolics, soil enzyme activities or basal CO_2 respiration. Thus the morphophysiological and biochemical response of *S.capillifolium* to shaded conditions did not induce changes to the soil rhizosphere that could have affected decomposition processes. This is understandable since under high PPFD, anthocyanins prevent photooxidative damage and hence cytosolic leakage, and under low PPFD photooxidation would not occur.

Extracellular enzyme activities and basal CO_2 respiration are sensitive to changes in labile DOC (Shackle *et al.*, 2000; Freeman *et al.*, 2004). Fenner *et al.* (2003) found that a very rapid significant ^{13}C enrichment of leachate DOC from *S. fuscum* occurred within 4 h of labelling. Up to 4 % of the total DOC in the peat leachate during labelling was derived from the $^{13}CO_2$ pulse. Thus recently fixed carbon from the *Sphagnum* community specifically makes an important contribution to the peatland DOC pool over a short time period (Fenner *et al.*, 2003). Whilst the effect of shading and increased N deposition on *Sphagnum* rhizodeposition is not conclusive, it is clear that shading and increased N deposition do not significantly alter the soil DOC pool via effects on *Sphagnum* productivity. Thus, light or N induced feedback interactions between *Sphagnum* and soil decomposition processes via rhizodeposition are probably not as important to C sequestration as the individual gross C processes.

Surprisingly, the soil N content was significantly reduced by increased N deposition in the natural PPFD treatment. Thus it is proposed that the *Sphagnum* was an efficient scavenger of atmospheric N deposition despite the increased N deposition. Aldous (2002a) found that N retention in *Sphagnum* moss followed an exponential decline from capitula to deeper segments, indicating that the capitula acts as 'apical nets' for capturing N as it is deposited from the atmosphere and before it reaches deeper zones. Shading did not significantly affect the soil N content and thus the reduction of *Sphagnum* biomass and altered morphology by shading may have permitted accumulation of N in the soil. The reduction in soil N content increased the soil C:N ratio that has been shown to have a negative impact on soil decomposition (Killham, 2001). The C:N ratio of a residue decomposing in soil is only an approximate indicator to net mineralization (Berg and Staff, 1981), largely because the elemental ratios take no account of the rates at which different forms of C and N in the residue become available to microorganisms (Gibbs and Barraclough, 1998). A C:N ratio of approximately 20 for arable soils is regarded as the approximate threshold between net mineralization and net immobilisation (Killham, 2001). The C:N ratio increased from 31 to 34 and thus microbial activities of both N levels were potentially limited by the supply of available N. Whilst the reduction in soil N content and hence increase in the soil C:N ratio did not impact on soil decomposition in the short-term, there is potential for increased *Sphagnum*

productivity to alter soil decomposition in the long-term via retention of atmospheric N deposition.

4.4.5 Conclusions

Sphagnum species are adapted to shaded conditions and typically reach photosynthetic saturation between PPFDs of 30 to 300 μmol m^{-2} s^{-1}. This study has shown that shading (<40 μmol m^{-2} sec^{-1}) can significantly reduce net photosynthetic C fixation of *S. capillifolium* as evident by a reduction in productivity. However, positive growth was maintained due to a concomitant reduction in respiration relative to photosynthesis (i.e. a lowering of the compensation point). Increased N deposition did not significantly affect *Sphagnum* biomass, gross photosynthesis, ecosystem respiration or NEE in the short-term suggesting that either productivity was not limited by N or more likely that the high N deposition rate was toxic. However, N increased productivity of the shaded treatment by translocation/allocation to photosynthetic pigments.

Alteration to *Sphagnum* productivity did not impact on *ex situ* measurements of soil decomposition in the short-term. However, lower N deposition rates may have a greater effect on productivity and hence a significant effect on rhizodeposition that could impact on soil decomposition processes. Despite this, increased *Sphagnum* productivity may reduce soil decomposition in the long-term via retention of atmospheric N deposition. Thus *Sphagnum* species may interact indirectly with soil decomposition processes by limiting the amount of atmospheric N available to soil microorganisms.

Chapter 5

Short-term interactive effect of atmospheric CO_2, temperature and waterlogging on net CO_2 ecosystem exchange and soil enzyme activities in peatland mesocosms

5.1 Introduction

Peatland ecosystems represent a significant global sink of atmospheric carbon (C) due to suppressed microbial decomposition of accumulated organic matter (Gorham, 1991; Van der Heijden *et al.*, 2000). Northern peatlands contain approximately one-third of the world's soil carbon stock (Gorham, 1991) and therefore stimulation of the processes regulating net C sequestration have the potential to feedback on predicted global climate change (Updegraff *et al.*, 1995; Zak *et al.*, 1993). The ability of peatlands to sequester atmospheric carbon dioxide (CO_2) under global change scenarios will be dependent in part on the response of plants and microorganisms (Ovenden, 1989; Van der Heijden *et al*, 2000) to change in the atmospheric CO_2 concentration, temperature and the depth of the water table. Approximately 60 Gt of CO_2 is cycled through plants and microorganisms via photosynthesis and decomposition that is greater than the annual net addition to the atmosphere (Goudriaan, 1992). Thus small changes to net primary productivity (NPP) or decomposition processes have the potential to significantly alter the flux of CO_2 between soil and atmosphere (Hoosbeek *et al.*, 2001). Because elevated CO_2 may strongly influence NPP, species abundance and community composition (Mooney *et al.*, 1999) and the chemical and physical composition of plant material, and therefore the decomposability of plant litter, strong feedbacks to the soil carbon pool are also expected (Hoosbeek *et al.*, 2001). Net C sequestration at a site is the combination of net exchange of CO_2, and the loss of carbon as CH_4 emissions and the loss or gain of carbon as DOC (Waddington and Roulet, 2000). While the latter two exchanges are significant in some circumstances, their contribution to the carbon budget is secondary to that of CO_2. Waddington and Roulet (2000) found that non-CO_2 fluxes accounted for less than 1 % of the carbon exchange of a boreal peatland.

Many vascular plants with high growth rates are known to acclimatise to elevated CO_2 in the long-term via down-regulation of photosynthetic capacity (Hsiao *et al.*, 1999). It remains unclear whether *Sphagnum* species will exhibit the same physiological response. Van der Heijden *et al.* (2000) found that elevated CO_2 increased *Sphagnum* dry biomass by 17 % after 6 months growth. At the start of their experiment, photosynthesis was stimulated by elevated CO_2, but it was down-regulated to control levels after three days of exposure. Jauhianinen *et al.* (1994) did not find any effect of elevated CO_2 on *Sphagnum fuscum*. Hoosbeek *et al.* (2001) found that after 3 years of exposure, increased CO_2 concentration (560 ppm) had no significant effect on *Sphagnum* biomass supposedly due to potassium limitation (Hoosbeek *et al.*, 2002). Smolders *et al.* (2001) showed that, at least in wet conditions, *Sphagnum* species recycle soil derived CO_2 from decomposition because concentrations in the acrotelm can reach 12 000 ppm. This may explain the limited response of *Sphagnum* species to elevated atmospheric CO_2 (500-700 ppm).

Elevated CO_2 has been found to increase rhizodeposition in vascular plants that can result in an increase in dissolved organic carbon (DOC) in the rhizosphere (Cheng, 1999; Cheng & Johnson, 1998). This may invoke a positive feedback in net C sequestration under atmospheric CO_2 conditions (Zak *et al.*, 1993) because increased exudation may exacerbate the so-called "priming" effect on soil nitrogen mineralisation, which may, as a consequence, increase plant growth due to the increased nitrogen availability (Cheng, 1999). For vascular plants, the increase in rhizodeposition has been due to the allocation of photosynthetically fixed C to roots. Since *Sphagnum* species do not possess 'true' roots, the effect of environmental factors on rhizodeposition of *Sphagnum* species is unclear. However, Fenner *et al.* (2003) showed that the *Sphagnum* community rapidly contributes recently synthesized carbon to the peatland DOC pool. An increase in DOC via *Sphagnum* rhizodeposition may invoke changes in soil enzyme activities due to an increase in substrate availability for hydrolytic reactions and / or increased microbial substrate for enzyme synthesis (Freeman *et al.*, 1997). Results from Chapter 3 and 4 showed that shading and nitrogen effects on *Sphagnum* productivity did not affect soil enzyme activities or basal CO_2 respiration. However, the amount of rhizodeposition will depend on the fixation of atmospheric CO_2 as CO_2 is the primary limitation on photosynthesis

in the short-term. Thus plant-soil interactions may be critical to the sequestration of atmospheric CO_2 in the future due to potentially higher rates of rhizodeposition that may feedback on decomposition via stimulation of soil enzyme activities. Plants may also respond to elevated CO_2 by increasing nutrient-acquiring activities such as phosphatase and sulphatase activities (Moorhead & Linkins, 1997).

Numerous results of field and laboratory experiments confirm that soil decomposition processes increase with temperature and aeration (Peterjohn *et al.*, 1994; Katterer *et al.*, 1998) raising the possibility of significant greenhouse gas emissions from peatland soils under warmer and drier climatic regimes (Neff & Hooper, 2002). Temperature is predicted to have significant kinetic effects on soil enzyme activities which would increase the decomposition of stored soil C to the atmosphere that may not be mitigated by an increase in photosynthetic activity. Results from Chapter 3 suggested that temperature is the primary control on NEE in peatlands. Thus in order to predict the response of peatland ecosystems to global climate change it is important to determine the effect of CO_2 and temperature when they are interacting.

Waterlogging of the soil may mitigate any increase in enzyme activities caused by CO_2 fertilisation or temperature due to anaerobic suppression of microbial enzyme synthesis, change in the redox status of the soil and / or an increase / decrease in enzyme inhibitors / activators (Pulford & Tabatabai, 1988; Freeman *et al.*, 1996). Since peatland ecosystems are defined by waterlogged conditions, increased rainfall is likely to have a significant effect on soil microbial processes in summer. Whilst some areas are predicted to experience drought in the future, many areas in northern Britain (Cooper & McGechan, 1996), northern Canada and Scandinavia (Wellburn, 1994) may experience increased precipitation and hence higher water tables. How elevated CO_2, elevated temperature and a saturated watertable interact to affect C cycling in peatland ecosystems in the short term is important for determining the potential for these systems to feedback on global climate change in the next century.

Sphagnum mesocosms were placed in solardomes under a factorial interactive design of CO_2, temperature and waterlogged treatments in May 2002. Net CO_2-C ecosystem exchange (NEE) was determined in May, August and October. Soil enzyme activities were determined in August.

CO_2, temperature and waterlogging were hypothesized to affect NEE in the following way:

(1) elevated CO_2 would increase sequestration, reduce emission or cause a source to sink switch under all levels of temperature and water table depth,
(2) elevated temperature would reduce sequestration, increase emission or cause a sink to source switch under all levels of CO_2 concentration and water table depth, and
(3) waterlogging would increase sequestration, reduce emission or cause a source to sink switch under all levels of CO_2 concentration and temperature.

CO_2, temperature and waterlogging were hypothesized to affect soil enzyme activities in the following way:

(1) elevated CO_2 would increase soil enzyme activities under all levels of temperature and water table depth,
(2) elevated temperature would increase soil enzyme activities under all levels of CO_2 concentration and water table depth, and
(3) waterlogging would reduce soil enzyme activities under all levels of CO_2 concentration and temperature.

5.2 Materials & Methods

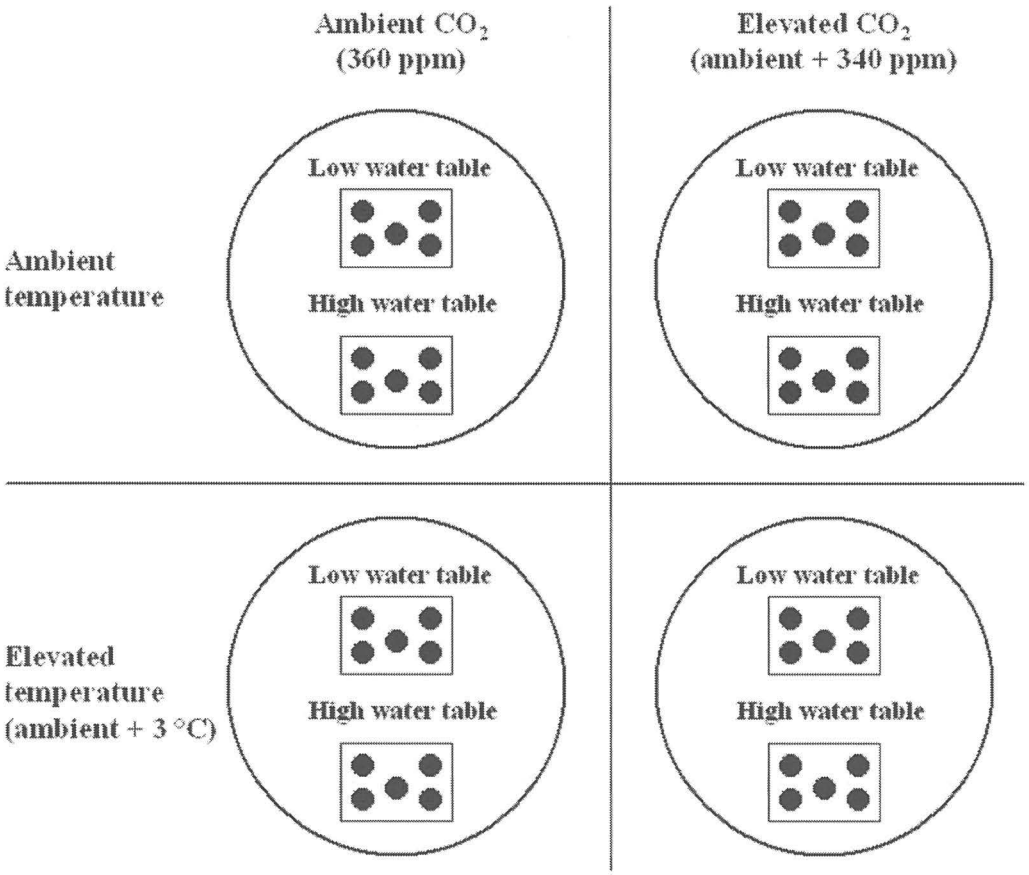
5.2.1 Experimental design

Forty peat cores were collected from Moorhouse National Nature Reserve, Upper Teesdale, UK (UK grid reference NY 755 336) in April 2002. The site is described in Chapter 3. The cores were collected in PVC drainpipes measuring 11cm diameter x 40cm depth and contained *Sphagnum* species only. Cores were collected by first cutting the soil around the base of the tube and then carefully pushing down on the tube into the soil to reduce soil disturbance. The cores were then removed from the peat by digging around the cores and removing the surrounding peat. Five replicate cores each were then placed at random in plastic crates (8 crates; 5 replicates per crate). The cores were incubated between May and October 2002 in solardomes, situated at the University of Wales farm at Abergwyngregyn, Bangor, Wales (plate 5-1). The solardomes provided computer-controlled maintenance of differential temperatures at ambient or 3 °C tracked above ambient; and computer-operated mass flow controllers to regulate carbon dioxide at either ambient (360 ppm) or elevated (ambient + 340 ppm) concentrations.

Plate 5-1 Solardome facility at the University of Wales farm at Abergwyngregyn, Bangor, Wales

Two plastic crates were placed in either of the following treatments: A= control; C = elevated CO_2; T = elevated temperature; CT = elevated CO_2 and elevated temperature (fig. 5-1). In each treatment one crate was maintained at a watertable 10 cm below the soil surface (low watertable) by drilling holes 10 cm below the soil surface, and the other was maintained at soil saturation (waterlogged). This factorial design allowed for all possible interactions between CO_2, temperature and watertable. Cores were watered in the solardomes daily by an automatic system that maintained the watertable depth. PPFD and temperature measurements were taken from automatic sensors at the site.

Figure 5-1 Schematic overview of the factorial experimental design. Circles represent each solardome with the two crates containing five replicate mesocosms each at either low or high watertables.



5.2.2 Net CO_2-C ecosystem exchange

Net CO_2-C ecosystem exchange (NEE) was determined by attaching polyethylene bags on top of the cores and collecting a 20 ml gas sample in a gas-tight exetainer using a gas-tight 20 ml syringe. After 2 hours a second sample was taken from each core. Gas samples were injected into an Ai Cambridge model 92 Gas Chromatograph equipped with a Porapak QS column and flame ionisation detector (Freeman *et al.*, 1994). The carrier gas was nitrogen at a flow rate of 13 cm^3 min^{-1} for CO_2. The flux rate was calculated from the difference between the first and second sample as mg CO_2-C m^{-2} h^{-1}. NEE was determined in May, August and October, and 2 weeks, 4 weeks and 12 weeks following switching of atmospheric CO_2 concentrations.

5.2.3 Soil enzyme activities

Extracellular enzyme activities were determined in August 2002 following 4 months incubation. The activities of extracellular hydrolase enzymes (β-glucosidase, phosphatase, sulphatase and *N*-acetylglucosaminidase) were determined using fluorogenic methylumbelliferyl (MUF) substrates by the method described in Chapter 3 (Freeman *et al.*, 1995). Phenol oxidase activity was determined by the method of Pind *et al.* (1994) as described in Chapter 2.

5.2.4 Statistical Analysis

Statistical analysis was performed using General Linear Model ANOVA for interactions and one-way ANOVA analysis in Minitab 13.1 (Minitab Inc). For NEE, first 4-way analysis was performed with time, CO_2, temperature and waterlogging as the factors. Then, 3-way ANOVA analysis was performed for each month with CO_2, temperature and waterlogging as the factors. For the soil enzyme activities, first 3-way ANOVA analysis was performed for each month with CO_2, temperature and waterlogging as the factors. Differences between

specific treatments were then tested using one-way ANOVA analysis. Temperature and PPFD between months were compared using one-way ANOVA analysis. The residuals from ANOVA analysis were used to test for normality using Anderson-Darling normality test. For data that were non-normally distributed, the data were ranked.

5.3 Results

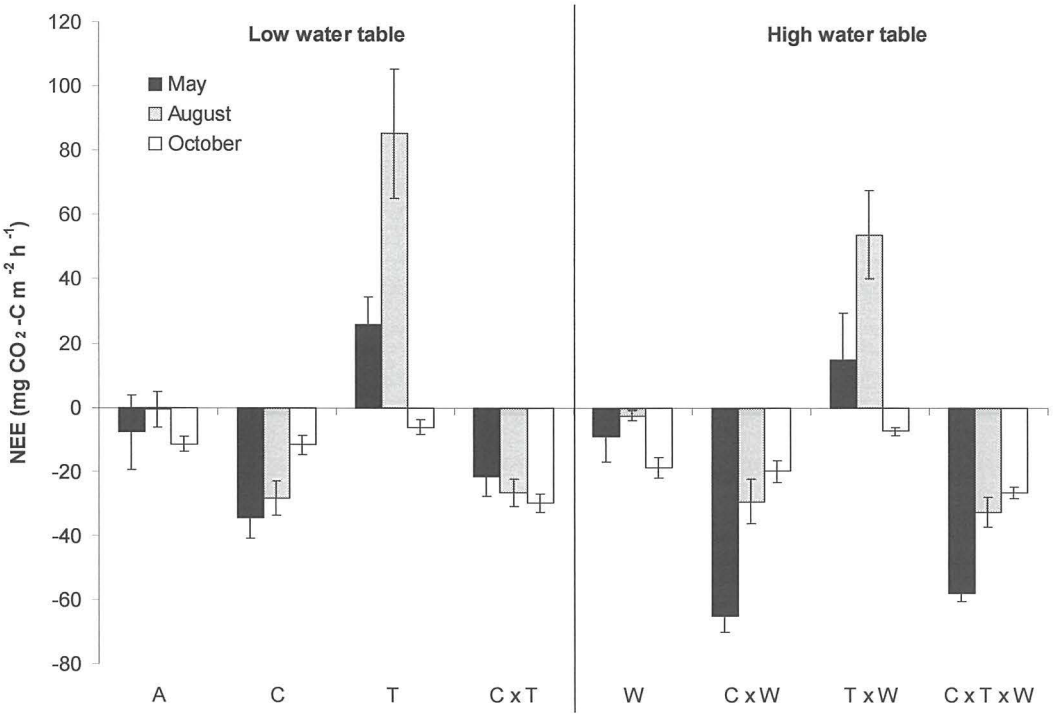
5.3.1 Climatic conditions

Light intensity decreased significantly between May and August ($P < 0.05$), and May and October ($P < 0.001$). The average daytime photon flux densities (mean ± standard deviation) were 797 ± 192 μmol m^{-2} s^{-1} in May, 599 ± 283 μmol m^{-2} s^{-1} in August and 238 ± 135 μmol m^{-2} s^{-1} in October. Air temperature increased significantly between May and August ($P < 0.001$). The maximum air temperature was 25 °C in May, 27.5 °C in August and 20°C in October.

5.3.2 Net CO_2-C ecosystem exchange

General linear model (GLM) analysis with the three factors (CO_2, temperature and waterlogging) and time as a fourth factor with interactions was performed. Results from the 4-way ANOVA analysis are shown in table 5-1 and show that NEE differed significantly over time. Figure 5-2 suggests that between May and August ecosystem respiration increased relative to a smaller increase in gross photosynthetic activity due to an increase in seasonal temperature. This caused an increase in net CO_2 emission in the elevated temperature treatment and a reduction in net CO_2 sequestration in the other treatments. By October, ecosystem respiration and gross photosynthesis were probably reduced due to seasonal reduction in temperature and PPFD that resulted in net CO_2 sequestration in all treatments.

Figure 5-2 Effect of CO_2, temperature and waterlogging on net CO_2-C ecosystem exchange of peatland mesocosms over 6 months. Carbon dioxide and temperature treatments are separated into water table treatments. A = control, C = elevated CO_2, T = elevated temperature and W = waterlogged conditions. Mean ± standard error (n=5).



Elevated CO_2 significantly affected NEE as seen by an increase in net CO_2 uptake (fig. 5-2; table 5-1). The effect of elevated CO_2 interacted significantly with time. Elevated temperature significantly affected NEE causing a sink to source switch or reduction in net CO_2 sequestration. The effect of elevated temperature interacted significantly with time. Elevated CO_2 and temperature interacted significantly resulting in net CO_2 sequestration. Waterlogging significantly affected NEE by increasing net CO_2 sequestration or reducing net CO_2 emissions.

Table 5-1 Four-way GLM ANOVA analysis for the main and interactive effects of time, CO_2, temperature and waterlogging on NEE (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *df*= degrees of freedom).

Factor	*df*	*F*-value	*P*-value
Time	2	6.01	**
CO_2	1	234.95	***
Temp	1	8.57	**
Water	1	10.28	**
Time x CO_2	2	18.12	***
Time x Temp	2	4.40	*
Time x Water	2	2.93	ns
CO_2 x Temp	1	19.41	***
CO_2 x Water	1	1.95	ns
Temp x Water	1	0.04	ns
Time x CO_2 x Temp	2	0.97	ns
Time x CO_2 x Water	2	1.56	ns
Time x Temp x Water	2	1.96	ns
CO_2 x Temp x Water	1	0.04	ns
Time x CO_2 x Temp x Water	2	0.20	ns

Three-way ANOVA results for the main and interactive effects of CO_2, temperature and waterlogging on NEE in May, August and October are shown in table 5-2. In May, atmospheric CO_2 increased net CO_2 sequestration, temperature forced a sink to source switch in CO_2 and waterlogging significantly increased sequestration but only under elevated CO_2 since CO_2 and waterlogging interacted significantly. In August, CO_2 and temperature significantly affected NEE as main effects. However, CO_2 and temperature interacted significantly resulting in net CO_2 sequestration. In October, atmospheric CO_2 significantly altered NEE as a main effect. However, CO_2 and temperature interacted significantly as did temperature and the level of the water table.

Table 5-2 Three-way GLM ANOVA analysis for the main and interactive effects of CO_2, temperature and waterlogging on NEE in May, August and October (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, numbers in brackets = F value, degrees of freedom = 1).

Factor	May	August	October
CO_2	*** (66.99)	*** (85.42)	*** (35.77)
Temp	** (9.81)	*** (26.49)	ns
Water	** (10.29)	ns	ns
CO_2 x Temp	ns	*** (27.56)	*** (31.87)
CO_2 x Water	* (4.87)	ns	ns
Temp x Water	ns	ns	* (5.64)
CO_2 x Temp x Water	ns	ns	ns

Hypothetical model of gross photosynthetic activity and ecosystem respiration

A simple model was used to show how photosynthetic and respiratory responses to elevated CO_2 and temperature could explain the changes in net CO_2 ecosystem exchange in August when fluxes were greatest (fig 5-3). The model used the empirical NEE data for August to show the lowest probable fluxes of gross photosynthesis and ecosystem respiration. Since NEE is determined by gross photosynthesis and ecosystem respiration, the maximal photosynthetic activity, known from a year-long field survey of the peatland site (approximately 110 mg CO_2-C m^{-2} h^{-1} in summer time (see Chapter 3)), was used to model the ecosystem respiration rate of the control treatment (R_e [A]) using the empirical NEE data of the control treatment (NEE [A]) (Eq. 1):

$$R_e[A] = NEE[A] + 110 \quad (1)$$

It was then assumed that the modelled ecosystem respiration rate of the control (R_e [A]) would equal ecosystem respiration of the elevated CO_2 treatment (R_e [C]). *Sphagnum* respiration may have increased due to an increase in gross photosynthetic activity so the rate represents a minimal possible rate. Therefore

the minimal rate of gross photosynthesis under elevated CO_2 (P_g [C]) was calculated by equation 2 using the empirical NEE data (NEE [C]):

$$P_g [C] = NEE [C] - R_e [A] \quad (2)$$

This is the same as adding the difference between NEE of the control and CO_2 treatments to the gross photosynthetic rate of the control treatment.

In the elevated temperature treatment both gross photosynthesis and *Sphagnum* / soil respiration may have increased. However, photosynthesis is limited by the atmospheric CO_2 concentration and therefore the response to temperature in terms of CO_2 fixed may be considerably less than that lost by respiration i.e. the photosynthetic enzyme rubisco is limited by substrate and not thermodynamic activity. Therefore ecosystem respiration in the elevated temperature treatment (R_e [T]) was assumed to be equal to ecosystem respiration of the control treatment plus the difference between empirical NEE of the control and temperature treatments (Eq. 3):

$$R_e [T] = R_e [A] + (NEE [T] - NEE [A]) \quad (3)$$

Gross photosynthesis in the elevated temperature treatment (P_g [T]) was then calculated using equation 4:

$$P_g [T] = NEE [T] - R_e [T] \quad (4)$$

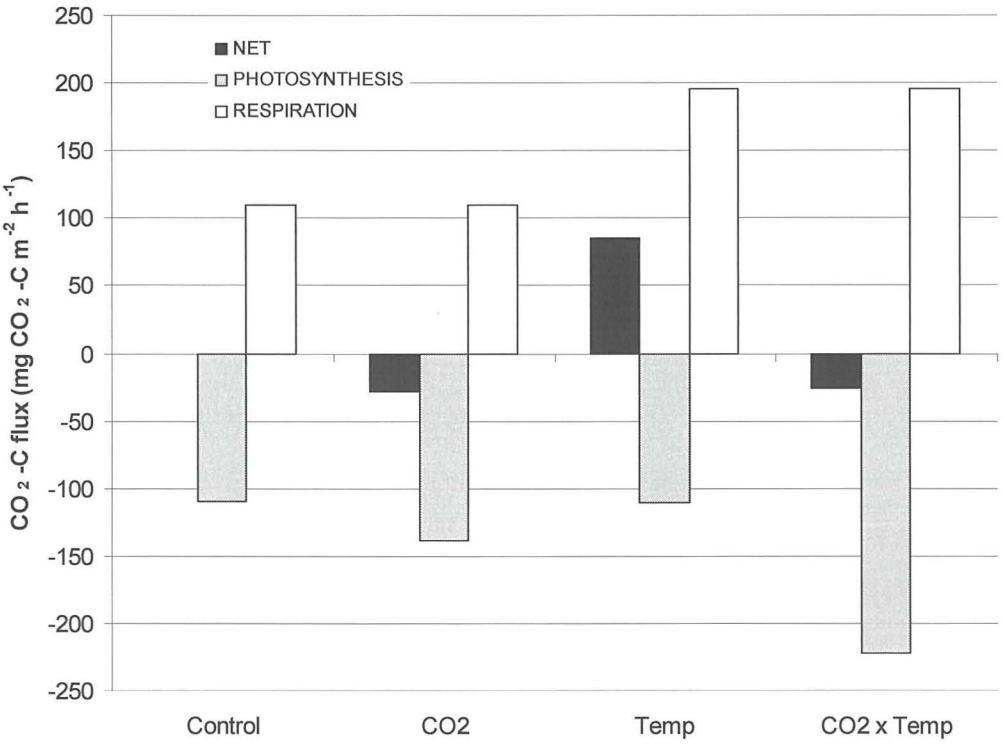
Finally it was assumed that ecosystem respiration was equal between the temperature and the CO_2 x temperature treatments [C x T]. Again *Sphagnum* respiration may have increased and thus the flux represents the minimal rate. Therefore photosynthesis in the CO_2 x temperature treatment was calculated using equation 5:

$$P_g [C \times T] = NEE [C \times T] - R_e [T] \quad (5)$$

The model (fig. 5-3) shows that elevated CO_2 primarily increased gross photosynthesis. An increase in ecosystem respiration by elevated CO_2 would

have resulted in a greater gross photosynthetic flux under elevated CO_2 than that shown. Elevated temperature primarily increased ecosystem respiration. An increase in gross photosynthesis by elevated temperature would have resulted in a greater respiratory response to temperature but would have remained less than ecosystem respiration. Of most importance, the model shows that elevated CO_2 and temperature had an additive effect on gross photosynthesis resulting in net C sequestration. If ecosystem respiration was stimulated by both elevated CO_2 and elevated temperature, then gross photosynthesis would still be greater under elevated CO_2 and temperature than under elevated CO_2 alone. Thus it is likely that elevated temperature may have also stimulated gross photosynthesis as a main effect.

Figure 5-3 Hypothetical model of the minimal effects of CO_2 and temperature on gross photosynthetic activity and ecosystem respiration calculated from measured NEE data from August.



5.3.3 Soil enzyme activities

Results from three-way ANOVA analysis for the effects of CO_2, temperature and waterlogging on soil enzyme activities in August are shown in Table 5-3.

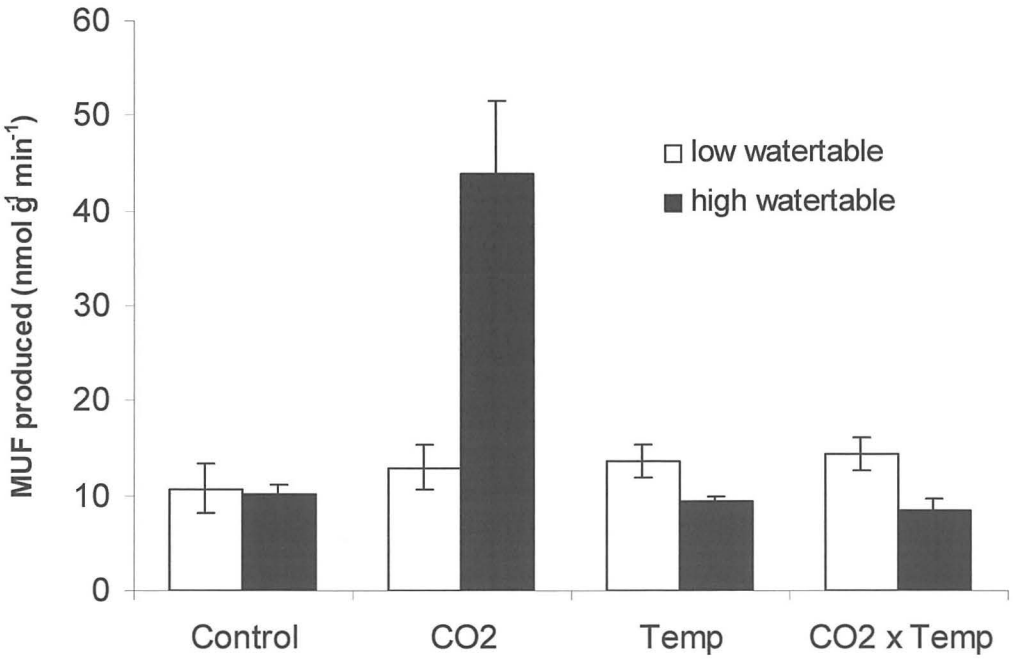
Table 5-3 Three-way GLM ANOVA analysis for the main and interactive effects of CO_2, temperature and waterlogging on β-glucosidase (Glu), *N-acetylglucosaminidase* (NAG), phosphatase (Pho) and sulphatase (Sul) activity in August (ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, degrees of freedom = 1).

Factor	**Glu**	**NAG**	**Pho**	**Sul**
CO_2	* (5.15)	*** (39.57)	*** (21.81)	*** (15.45)
Temp	ns	ns	ns	ns
Water	ns	ns	ns	* (5.80)
CO_2 x Temp	* (5.30)	ns	ns	ns
CO_2 x Water	ns	ns	ns	ns
Temp x Water	* (12.88)	ns	ns	ns
CO_2 x Temp x Water	ns	ns	ns	ns

β-glucosidase activity

Three-way ANOVA analysis showed that CO_2 significantly affected β-glucosidase activity (fig. 5-4; table 5-3). However, there were significant interactions between CO_2 and temperature, and temperature and water table depth. One-way ANOVA analysis showed that elevated CO_2 increased activity only under waterlogged conditions ($P < 0.01$). Waterlogging significantly increased activity under elevated CO_2 ($P < 0.01$; $F = 14$) and significantly reduced activity under elevated temperature ($P < 0.05$; $F = 5.21$). Elevated CO_2 and waterlogging increased activity under ambient temperature ($P < 0.01$; $F = 15.87$) and decreased activity under elevated temperature ($P < 0.05$; $F = 5.48$).

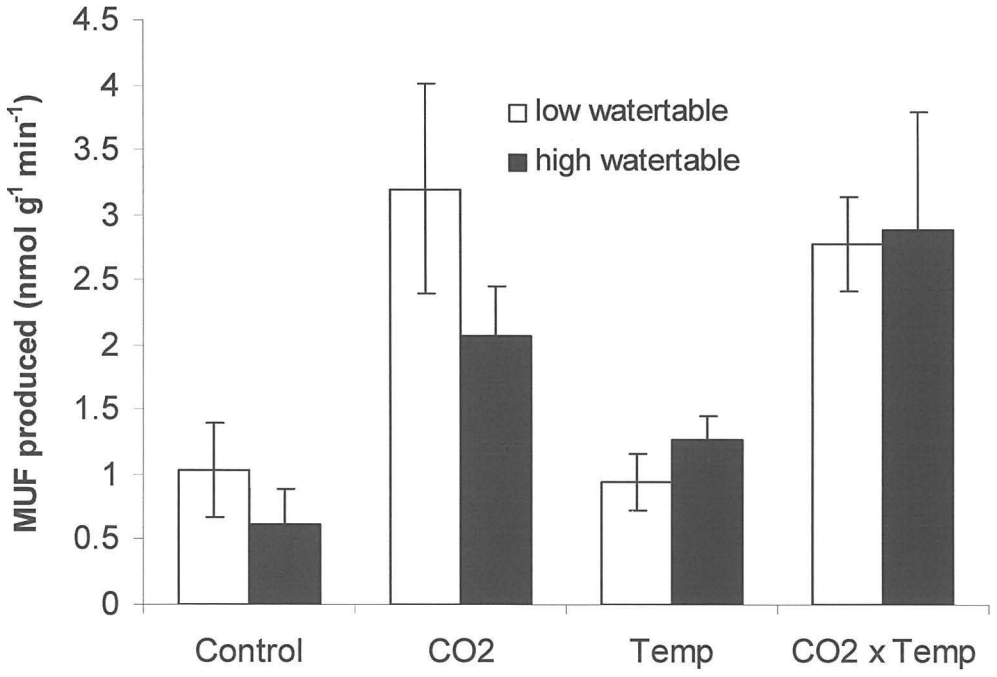
Figure 5-4 Effect of CO_2, temperature and waterlogging on β-glucosidase activity. Mean ± standard error (n=5).



N-acetylglucosaminidase activity

GLM analysis showed that atmospheric CO_2 significantly increased *N*-acetylglucosaminidase activity with no significant interactions (fig. 5-5; table 5-3). One-way ANOVA analysis showed that elevated CO_2 increased activity under ambient temperature ($P < 0.05$; $F = 6.04$) and elevated temperature ($P < 0.01$; $F = 18.74$). Elevated CO_2 increased activity under waterlogged conditions ($P < 0.01$; $F = 9.66$). Activity was significantly increased by elevated CO_2 and temperature under low water table ($P < 0.01$; $F = 11.55$) and waterlogged conditions ($P < 0.05$). Elevated CO_2 and waterlogging increased activity under elevated temperature ($P < 0.05$).

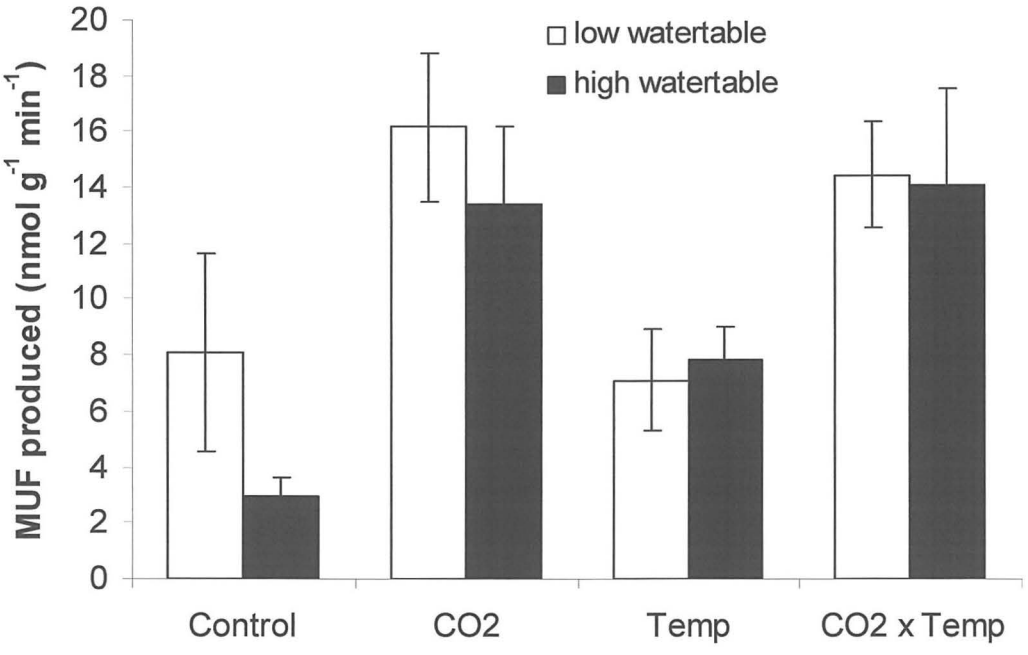
Figure 5-5 Effect of CO_2, temperature and waterlogging on *N*-acetylglucosaminidase activity. Mean ± standard error (n=5).



Phosphatase activity

Three-way ANOVA analysis showed that atmospheric CO_2 significantly increased phosphatase activity with no significant interactions (fig 5-6; table 5-3). One-way ANOVA analysis suggested that elevated CO_2 increased activity under elevated temperature ($P < 0.05$; $F = 7.75$) or waterlogged conditions ($P < 0.01$; $F = 13.56$). Under waterlogged conditions, elevated temperature increased activity ($P < 0.01$; $F = 13.8$), and elevated CO_2 and temperature together increased activity ($P < 0.01$; $F = 10.37$).

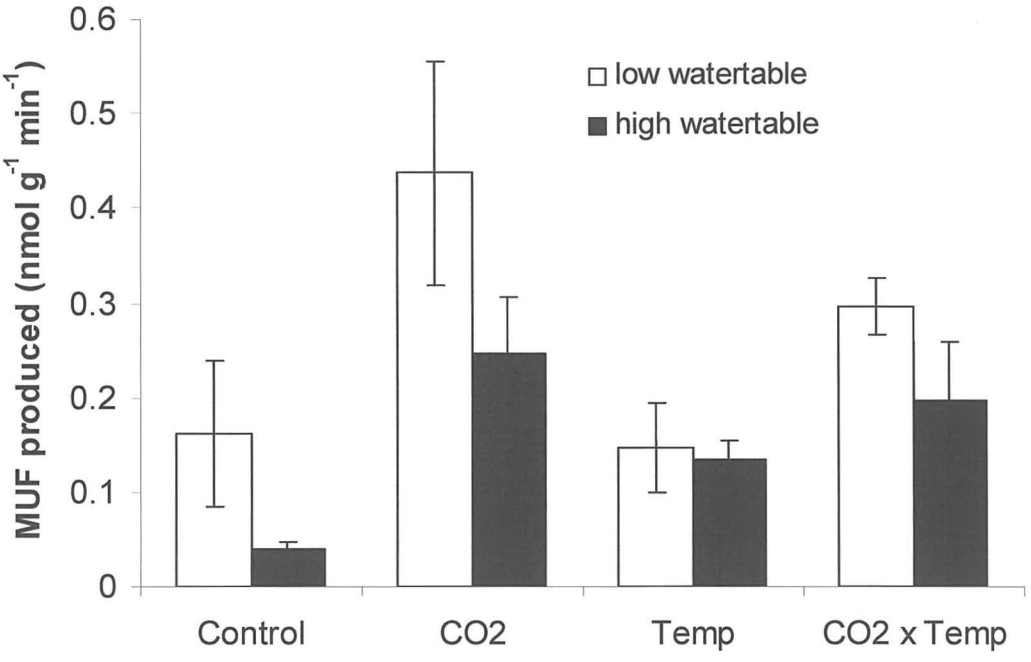
Figure 5-6 Effect of CO_2, temperature and waterlogging on phosphatase activity. Mean ± standard error (n=5).



Sulphatase activity

GLM ANOVA analysis showed that atmospheric CO_2 and significantly increased sulphatase activity with no significant interactions whilst waterlogging decreased activity (fig 5-7; table 5-3). One-way ANOVA analysis suggested that elevated CO_2 increased activity under elevated temperature ($P < 0.05$; $F = 7.2$) or waterlogged conditions ($P < 0.01$; $F = 11.7$). Under waterlogged conditions, elevated temperature increased activity ($P < 0.01$; $F = 18.93$), and elevated CO_2 and temperature together increased activity ($P < 0.05$; $F = 5.88$).

Figure 5-7 Effect of CO_2, temperature and watertable on sulphatase activity. Mean ± standard error (n=5).



Phenol oxidase activity

Phenol oxidase activity was not significantly affected by CO_2, temperature or water table depth.

5.4 Discussion

5.4.1 Climatic conditions

The transplanting of the mesocosms from Moorhouse to Aber farm inevitably resulted in the mesocosms being subjected to higher air temperatures. The maximum air temperature in May was 25 °C, 27.5 °C in August and 20 °C in October. Air temperatures at Moorhouse can reach as high as 27 °C in August and thus whilst the transplanting may have increased gross processes, the temperatures were within the natural range of Moorhouse. Light intensity was significantly higher in May than in August but remained at PPFD values that would fully saturate *Sphagnum* photosynthetic activity. By October, PPFD was significantly reduced to levels that may have reduced *Sphagnum* photosynthetic activity.

5.4.2 Net CO_2-C ecosystem exchange

Elevated CO_2, elevated temperature and waterlogging affected NEE and significant variation occurred within both the elevated CO_2 and temperature treatments over time. This suggests that seasonal variation in temperature and light intensity and potentially down-regulation of photosynthetic capacity (i.e. limited nutrient availability) could have affected the response of ecosystem respiration and gross photosynthesis.

Elevated CO_2 forced net CO_2 sequestration via stimulation of gross photosynthesis and productivity in May, August and October. However, in August and October, CO_2 and temperature were interacting significantly and together forced net CO_2 sequestration. Thus, the effect of elevated CO_2 was significantly different at each level of temperature (under ambient temperature, elevated CO_2 increased net CO_2 sequestration; under elevated temperature, elevated CO_2 forced a source to sink switch). Thus gross photosynthesis was enhanced by CO_2 and temperature to a greater extent than ecosystem respiration. By October, all treatment mesocosms were sequestering atmospheric CO_2 and there was no main effect of temperature suggesting seasonal temperature

limitation on ecosystem respiration. NEE of the elevated CO_2 mesocosms were at the control level flux rate and thus other factors such as nutrient availability, temperature and light intensity may have limited the photosynthetic response to elevated CO_2. The elevated CO_2 x temperature treatment was still sequestering atmospheric CO_2 at a rate higher than the control. This suggests that the interactive effect of CO_2 and temperature can support greater rates of photosynthetic activity at the end of the growing season since temperature may limit the effect of elevated CO_2 on photosynthetic C fixation. Since gas measurements were per unit soil area and not per unit biomass, it is likely that these responses were due to differences in productivity due to higher rates of net C fixation at the start of the experiment. Van der Heijden *et al.* (2000) found that photosynthesis of *Sphagnum recurvum* photosynthesis was stimulated by elevated CO_2 but was down-regulated to control levels after three days of exposure but increased *Sphagnum recurvum* biomass by 17 % after 6 months incubation. Our current knowledge suggests that an increase in globally averaged temperature by 2-5 °C will lead to the release of C by an increase in soil microbial decomposition in northern latitudes (Peterjohn *et al.*, 1994; Katterer *et al.*, 1998). These results suggest that in the short-term, loss of soil C from peatlands by soil microbial processes may be 'offset' by an increase in *Sphagnum* productivity from the predicted rise in atmospheric CO_2 that will accompany global warming (IPCC, 2001). However, long-term studies (> 3 years) have shown that elevated atmospheric CO_2 has a limited effect on *Sphagnum* biomass (Hoosbeck *et al.*, 2001; Berendse *et al.* 2001) and thus the response of NEE to elevated CO_2 in the long-term will be dependent on secondary limiting factors such as temperature, and nutrients (Hoosbeck *et al.*, 2002). Also, atmospheric CO_2 concentrations will increase slowly over the next century and thus the growth response of plants will be slow and not immediate.

In May, temperature had a main effect on NEE and did not interact with CO_2 or waterlogging that resulted in a switch in NEE from a sink to a source suggesting increased ecosystem respiration relative to gross photosynthesis. Elevated temperature continued to stimulate ecosystem respiration in August as evident as an increase in net CO_2 emission. The effect of temperature was significantly different at each level of atmospheric CO_2 (under ambient CO_2, elevated temperature forced net CO_2 emission; under elevated CO_2, elevated

temperature had no apparent effect on NEE). This suggests that under ambient atmospheric CO_2, temperature primarily increased ecosystem respiration over gross photosynthesis resulting in the net emission of CO_2. This is further supported by the seasonal effect of temperature on NEE that resulted in a switch from sink to source between May and August. Cox *et al.* (2000) and Houghton and Woodwell (1989) suggest that plant and soil respiration may increase at a faster rate than photosynthesis as ecosystems adjust to climate change. Oechel *et al.* (1993) found that tundra of Alaska has recently turned from a sink of CO_2 to a source due to recent warming. Thus it is apparent that temperature may affect ecosystem respiration to a greater extent than photosynthesis due to its effect on both plant and soil respiration. These results also agree with the seasonal effect of temperature on NEE at Moorhouse (Chapter 3). Net photosynthesis may also have been increased by temperature meaning that the soil respiration flux may have been considerably greater than the modelled flux shown in fig 5-3. However, under elevated CO_2 and temperature, photosynthesis 'offset' the increase in ecosystem respiration by elevated temperature resulting in sequestration (fig. 5-2). Thus CO_2 and temperature had an additive effect on gross photosynthesis or the photosynthetic response to elevated CO_2 was limited by temperature. Under elevated CO_2, *Sphagnum* photosynthesis *'wins out'* over respiration regardless of the temperature, resulting in the net sequestration of atmospheric CO_2 in the short-term.

Waterlogging generally reduced net CO_2 emission or increased net CO_2 sequestration under elevated CO_2 or temperature. This is not unexpected since it is known that waterlogging reduces soil respiration due to the anerobic conditions (Mitsch and Gosselink, 2000) and increases *Sphagnum* photosynthetic activity. Rochefort *et al.* (2002) showed that development of *Sphagnum* species can be enhanced by limited periods of shallow flooding. Generally both high and low water contents can limit C uptake (Schipperges and Rydin, 1998; Titus *et al.*, 1983). Low water availability can inhibit photosynthetic enzyme activity, while slow CO_2 diffusion can be limiting at water saturation (Williams and Flanagan, 1996). Water contents below 500 % were associated with declining photosynthetic rates of most *Sphagnum* species (Titus *et al.*, 1983). Waterlogging of the soil increased the sink strength of the elevated CO_2 treatment in May. Elevated CO_2 and waterlogging interacted suggesting that waterlogging

enhanced sequestration under elevated CO_2 but not under ambient CO_2. Thus photosynthetic fixation by *Sphagnum* was probably limited by the depth of the water table. In August, waterlogging did not significantly affect NEE suggesting that seasonal variation in light intensity and temperature may have been more limiting than the depth of the water table. In October, waterlogging interacted with temperature that suggests that waterlogging increased net CO_2 sequestration under ambient temperature but the effect of waterlogging was mitigated by elevated temperature. Oechel *et al.* (2000) showed that Arctic ecosystems returned to summer sink activity during the warmest and driest periods observed over four decades indicating a capacity for ecosystems to metabolically adjust to long-term changes in climate.

5.4.3 Soil enzyme activities

GLM ANOVA analysis suggested that elevated CO_2 increased all hydrolase enzyme activities. However, one-way ANOVA analysis suggested that the positive effect of elevated CO_2 on hydrolase enzyme activities was dependent on the temperature and waterlogged conditions and on the type of enzyme. The only enzyme that responded to elevated CO_2 as a main effect was *N*-acetylglucosaminidase. Chitin is a polymer of β-(1,4)-*N*-acetyl-D-glucosamine (NAG) units, and the second most abundant polysaccharide in nature after cellulose (Chen *et al.*, 1994). Chitin is recognized as a significant fraction of humus-bound nitrogen in soil (Aber and Mellilo, 1991). Thus *Sphagnum* and / or microbial growth under elevated CO_2 may have been limited by nitrogen. Kang *et al.* (2005b) also found that elevated CO_2 only increased the activity of *N*-acetylglucosaminidase of a bog and suggested that enzymes involved in N or P mineralization only increase under elevated CO_2 when nutrient limitation is strongly exerted. It is hypothesized that the increase in *N*-acetylglucosaminidase activity was principally due to an increase in microbial activity via labile C rhizodeposition rather than *Sphagnum* enzyme exudation since a number of authors have shown that N supply has a negative or no effect on *Sphagnum* biomass exposed to elevated CO_2 (Berendse *et al.*, 2001; Hoosbeck *et al.*, 2002). The activities of *N*-acetylglucosaminidase, phosphatase and sulphatase were

significantly altered by elevated CO_2 under either elevated temperature or water logged conditions. It appears that elevated temperature reduced the variability in activities whilst the waterlogged conditions reduced activity of the control. These effects were not significant but suggest that the effect of elevated CO_2 on soil enzyme activities is dependent on temperature and the depth of the watertable.

The significant increase in hydrolase enzyme activities associated with the N, P and S cycles by elevated CO_2 under elevated temperature or waterlogged conditions could be explained by an increase in enzyme production by *Sphagnum* due to low soil nutrient availability (i.e. elevated CO_2 promoted plant growth resulting in higher rates of nutrient immobilisation that induced enzyme synthesis). Bryophytes acquire the majority of nutrients from atmospheric deposition but there is evidence that the substratum as well as the atmosphere may be an important source of minerals for bryophytes (Bates, 1992). Studies indicate that nitrate uptake and early metabolism in *Sphagnum* (Press and Lee, 1982; Woodin *et al.*, 1985), and phosphate uptake in several mosses (Wells and Richardson, 1985; Chapin *et al.*, 1987) are conventional. Press and Lee (1983) found that intracellular acid phosphatase activity in 11 species of *Sphagnum* was negatively correlated with the total phosphorus concentration and, in experiments, increased under conditions of phosphate starvation. Exudation of intracellular enzymes to the rhizosphere would increase the availability of limiting nutrients. Moorhead and Linkins (1997) found greater phosphatase activities of roots, mycorrhizae and soil organic matter in root regions of arctic tussocks exposed to elevated CO_2. They stated that 'increased carbon availability at elevated CO_2 may stimulate efforts by roots, root symbionts and soil microorganisms to produce other nutrients.'

Sphagnum rhizodeposition of labile organic compounds may also have enhanced microbial mineralization activities leading to an increase in the nutrient pool (a positive feedback) (Zak *et al.*, 1993). Díaz *et al.* (1993) suggested that the increased microbial biomass would cause higher microbial nutrient uptake, and decrease available nutrients to plants (a negative feedback). An increase in substrate abundance for microbial metabolism supports synthesis of new enzymes (Küster, 1993). Kang *et al.* (2001) showed that elevated CO_2 increased primary productivity of fen vegetation that stimulated net CO_2 emission as a consequence of an increased DOC supply from the vegetation to the soil

microbes. However, they did not observe any significant differences in soil enzyme activities in the bulk soil but suggested that this may have been due to a greater availability of limiting nutrients in fens compared to ombrotrophic bogs. A number of other authors have reported higher soil enzyme activities and microbial activities under elevated CO_2 (Körner and Arnone, 1992; O'Neill *et al.*, 1987; Rogers *et al.*, 1992; Runion *et al.*, 1994).

Elevated temperature significantly increased the activities of phosphatase and sulpatase under waterlogged conditions but not *N*-acetylglucosaminidase activity. Kang *et al.* (2005a) found that a rise in soil temperature from 2 to 5 °C did not result in a significant increase in *N*-acetylglucosaminidase activity in 39 northern latitude wetlands. The significant effect of elevated temperature under waterlogged conditions appears to be due to the reduced activity of the control. Waterlogging of soils results in: a) changes to microbial populations; b) a decrease in soil Eh; and c) electrochemical and chemical changes. In the absence of O_2, anaerobic microorganisms utilise oxidized soil components such as NO_3^-, MnO_3, $Fe(OH)_3$, SO_4^{2-} and dissimilation products of organic matter as electron acceptors in their respiration. Decrease of redox potential indirectly influences enzyme activities by changes in the microbial population (i.e. shift to anaerobes), inhibition of *de novo* enzyme synthesis, or mobilising of reduced metal ions which have inhibitory effects on the enzymes (Pulford and Tabatabai, 1988). Therefore raising of the watertable could reduce enzyme activities by a) decrease in microbial activity and *de novo* enzyme synthesis, or b) by an increase in enzyme inhibitors such as Fe, Mn or phenolics (Freeman et al., 1996). However, enzyme activities may have been limited by waterlogging under both water table depths that would have affected the response to elevated CO_2 and temperature.

Enzyme activities associated with C cycling such as β-glucosidase, cellulase and phenol oxidase should be lower under elevated CO_2 due to a greater availability of simple carbohydrates because production of C enzymes declines when simpler carbohydrates are available (Chróst, 1993). The results from GLM ANOVA analysis suggested that CO_2 and temperature, and temperature and waterlogging interactively affected β-glucosidase activity. The significant increase in β-glucosidase activity under elevated CO_2 occurred under waterlogged conditions but did not occur under elevated temperature suggesting that the increase may not be due to elevated CO_2. The results also suggest that

waterlogging increased activity under elevated CO_2. Phenol oxidase was not significantly affected by any treatment. Kang *et al.* (2004) did not find any effect of elevated CO_2 on β-glucosidase activity suggesting little direct impact on carbon mineralization. Moorhead and Linkins (1997) found lower levels of cellulase activities but higher levels of cellulase activities associated with ectomycorrhizal rhizomorphs that is similar to reports of higher levels of xylanase activity in soils at elevated CO_2 (Körner and Arnone, 1992). Moorhead and Linkins (1997) suggested that greater availabilities of simple carbohydrates could have a priming effect on mycoorhizae-mediated cellulose decay or the overall degradation of cellulose enhances access to organically-bound nutrients in litter. Despite the inconclusive effect of elevated CO_2 on β-glucosidase activity, waterlogging reduced activity under elevated temperature. This supports the overall negative effect of waterlogging on hydrolase enzyme activities and reduced variability of activities under elevated temperature.

5.4.4 Conclusions

In the short-term, elevated CO_2 increased the sink capacity of the cores for atmospheric CO_2 since photosynthetic activity was limited by C substrate. However, it appears that the fertilization effect of elevated CO_2 may be dependent on climatic and physicochemical limiting factors. Elevated temperature increased ecosystem respiration relative to gross photosynthesis forcing a sink to source switch whilst waterlogging either increased net CO_2 sequestration or reduced net CO_2 emission of the elevated CO_2 and temperature treatments. However, under elevated CO_2 and temperature, photosynthesis was amplified relative to ecosystem respiration resulting in sequestration. Thus, in the short-term, the increase in ecosystem respiration by temperature may be 'offset' by an increase in *Sphagnum* photosynthetic activity from the predicted rise in atmospheric CO_2 that will accompany global warming. This interactive effect of elevated CO_2 and temperature may also lead to an increase in the length of the growing season resulting in higher annual rates of net CO_2 sequestration. However, published data on the long-term limited effect of elevated CO_2 on *Sphagnum* productivity due to nutrient availability suggests that increased net

CO_2 sequestration may be short-lived. This agrees with our current knowledge that suggests that an increase in global average temperature will lead to release of CO_2 from peatland ecosystems by an increase in soil microbial decomposition.

Elevated CO_2 had an indirect impact on soil mineralization by stimulating soil enzyme activities for the release of inorganic nutrients for plant / microbial growth. This may lead to increased nutrient availability that may support increased *Sphagnum* productivity under elevated CO_2 and temperature. Thus the magnitude and duration of this CO_2 induced stimulation of soil enzyme activities may be the key mechanism determining the C balance of *Sphagnum* dominated peatlands. Also, the effect of waterlogging on NEE and soil enzyme activities appears to be greater under elevated CO_2 and temperature that may enhance net CO_2 sequestration. Thus multiple, interacting climatic factors and positive nutrient feedback from increased soil enzyme activities will determine the C balance of peatland ecosystems in the future.

Chapter 6

Discussion

6.1 Preamble

Models of global environmental change are dependent on accurate predictions of the processes regulating carbon (C) and nutrient cycling in terrestrial ecosystems. Currently much research is focused on the biogeochemical cycles of peatlands due to their involvement in the terrestrial sequestration of atmospheric carbon dioxide (CO_2). Northern peatlands contain approximately one-third of the world's soil carbon stock (Gorham, 1991) and therefore alteration to the processes regulating C and nutrient cycling have the potential to feedback on predicted global climate change via CO_2 loss to the atmosphere (Updegraff *et al.*, 1995; Zak *et al.*, 1993). This study examined the climatic and physicochemical factors that regulate biogeochemical cycling in *Sphagnum* dominated peatlands. Also the effect of *Sphagnum* productivity on soil decomposition processes under current and future climate change scenarios was examined since this interaction may feedback on CO_2 processes by increasing the availability of limiting nutrients.

Field surveys of a riparian peatland and a blanket bog identified a number of climatic and physicochemical factors that regulate carbon dioxide fluxes and soil enzyme activities. Peatland mesocosm experiments identified the effect of *Sphagnum* productivity on net CO_2 ecosystem exchange and soil enzyme activities under current and future climate change scenarios. The implications of these findings to peatland biogeochemistry are discussed below.

6.2 Climatic and physicochemical factors regulating carbon dioxide fluxes

Waddington and Roulet (2000) found that non-CO_2 fluxes accounted for less than 1 % of the carbon exchange of a boreal peatland. Thus the C balance of northern peatlands is primarily dependent on the climatic and physicochemical factors that regulate gross photosynthesis, plant respiration and soil respiration.

The net result of these processes is the net CO_2 ecosystem exchange and is an approximation of the C balance of a peatland.

Net CO_2 ecosystem exchange (NEE) showed that the riparian peatland was a sink for atmospheric CO_2 between early spring – late summer and a source between autumn – winter (Chapter 2). The seasonal variability of NEE of the *Sphagnum* dominated blanket bog was the reverse of the riparian peatland (Chapter 3) showing net CO_2 emission in spring – summer and net CO_2 sequestration in autumn – winter. The difference in NEE between these peatland types is likely due to greater vascular and non-vascular plant productivity at the riparian site due to nutrient replenishment in summer and soil saturation throughout most of the year (Mitsch and Gosselink, 2000). In the blanket bog, *Sphagnum* gross photosynthesis was also highest in spring – summer but was less than ecosystem respiration per unit area resulting in net CO_2 emission due to the temperature sensitivity (Q_{10}) of ecosystem respiration relative to gross photosynthesis. NEE of the riparian peatland ranged between -32.4 to 81.1 mg CO_2-C m^2 h^{-1} whilst NEE of the blanket bog ranged between -11.47 to 36.1 mg CO_2-C m^2 h^{-1}. Thus it is apparent that the riparian peatland exhibited greater seasonal variation in NEE that may be explained by nutrient replenishment and a fluctuating water table (Mitsch and Gosselink, 2000). This has implications for predicting the future C balance of peatland types since the CO_2 fertilization effect on vegetation is limited by nutrient availability (Chapin *et al.*, 1980; Oechel & Billings, 1992). Thus NPP and hence NEP may increase in riparian systems under climatic change whilst blanket bogs may release stored soil C due to nutrient limitations and the greater sensitivity of ecosystem respiration to temperature. Basal CO_2 respiration ranged from 18.1 to 185.9 μg CO_2-C g^{-1} h^{-1} in the riparian peatland and ranged between 0.6 to 22.6 μg CO_2-C g^{-1} h^{-1} in the blanket bog that further suggests that soil respiration is limited in blanket bogs relative to riparian peatlands due to nutrient availability (i.e. N, P and K).

Results from Chapter 2 suggested that nitrogen and phosphorus were limiting in the riparian peatland due to low soil organic matter decomposition. Increased N deposition (Chapter 4) did not have a significant effect on gross photosynthesis or ecosystem respiration of the blanket bog in the short-term suggesting that *Sphagnum* productivity was not limited by N. Multiple regression analysis suggested that basal CO_2 respiration of the riparian peatland was limited

by the availability of PO_4^{3-} and Mg^{2+}. Basal CO_2 respiration was also related positively with both pH and soil temperature (Chapter 2). In particular, the results suggested that labile DOC from extracellular hydrolysis may limit soil CO_2 respiration under higher summer temperature. For the blanket bog, temperature and water table depth were the best predictors of NEE and ecosystem respiration whilst temperature and PPFD were the best predictors of *Sphagnum* gross photosynthesis. Unfortunately, dissolved ionic concentrations were not determined at the blanket bog and cannot therefore be ruled out as factors affecting CO_2 fluxes. For the blanket bog, soil temperature was the best and only predictor of basal CO_2 respiration though again nutrient availability cannot be ruled out as a limiting factor.

It is apparent that seasonal temperature variation is currently the major factor controlling CO_2 fluxes in both types of peatlands and that nutrient and labile C availability, plant types, and water table depth may account for the differences in NEE between peatland types. Long-term modelling studies indicate that both respiration and production in peatlands are also highly sensitive to changes in the water balance (Hilbert *et al.*, 2000). Ecosystem respiration had a greater Q_{10} response to temperature than *Sphagnum* gross photosynthesis in the blanket bog (Chapter 3) that suggests that global warming may lead to significant release of C from *Sphagnum* dominated peatlands. A peatland mesocosm experiment supported this conclusion in that elevated temperature resulted in a switch from CO_2 sink to source in the short-term (Chapter 5) both seasonally and due to elevated temperature. Also, *Sphagnum* respiration accounted for approximately 50 % of ecosystem respiration and thus the response of both *Sphagnum* productivity and soil respiration to climatic change will affect the loss of CO_2 from peatland ecosystems.

The effect of global warming in the future on NEE will be dependent on nutrient availability, the water table depth and the effect of elevated CO_2 on plant productivity. In the short-term, elevated CO_2 increased the sink capacity of *Sphagnum* mesocosms for atmospheric CO_2 since photosynthetic activity was limited by C substrate (Chapter 5). However, it appears that the fertilization effect of elevated CO_2 may be dependent on the climatic and physicochemical limiting factors mentioned above (Hoosbeck *et al.*, 2002). Under elevated CO_2 and temperature, photosynthesis was amplified relative to the temperature effect

alone on ecosystem respiration resulting in net CO_2 sequestration. Thus, in the short-term, the increase in respiration by temperature may be 'offset' by an increase in photosynthetic activity from the predicted rise in atmospheric CO_2 that will accompany global warming. This interactive effect of elevated CO_2 and temperature may also lead to an increase in the length of the growing season resulting in higher annual rates of net C sequestration. The effect of waterlogging on NEE appears to be greater under elevated CO_2 and temperature resulting in either increased net CO_2 sequestration or reduced net CO_2 emission. The effect of temperature on ecosystem respiration relative to gross photosynthesis, and the short-term effect of elevated CO_2 on *Sphagnum* productivity suggest that in the long-term *Sphagnum* dominated peatlands may release stored C to the atmosphere as CO_2. This response will clearly be dependent on the availability of limiting nutrients for both plant and microbial growth and thus peatland types are likely to respond differently to climatic change. Published long-term studies support this finding in that the positive effect of elevated CO_2 on *Sphagnum* productivity is short lived due to nutrient limitation and thus elevated temperature may eventually force C emission from northern latitude peatlands (Van der Heijden *et al.*, 2000; Hoosbeck *et al.*, 2001; Berendse *et al.*, 2001; Smolders *et al.*, 2001). Thus the C balance of peatland ecosystems in the future may be dependent on the response of soil enzyme activities to changes in plant productivity and climatic/physicochemical conditions that regulate the supply of limiting nutrients to plants and soil microorganisms.

6.3 Climatic and physicochemical factors regulating soil enzyme activities

In the decomposition of organic matter and nutrient cycling in peatlands, extracellular hydrolysis has been reported as a critical step for three reasons. First, other sources of nutrients (i.e. weathering, atmospheric deposition) are generally insignificant for the nutrient demand of plants and microbes. Second, almost all microorganisms and plants in peatlands are not permeable to high molecular weight (HMW) compounds (Paul and Clark, 1989). Therefore, extracellular hydrolysis of organic matter is a crucial step prior to nutrient uptake (Chróst, 1991). Finally, the extracellular hydrolysis rate is much lower than the

rate of uptake of low molecular weight (LMW) substrates, which implies the importance of extracellular hydrolysis as a rate limiting step in nutrient cycles (Hoppe *et al.*, 1988). Plant and microbial processes are limited by nutrient availability in peatland ecosystems and thus CO_2 fluxes are indirectly dependent on the climatic and physicochemical factors that regulate soil enzyme activities. Thus the response of soil enzyme activities to future climate change and plant productivity may limit the effect of climatic change on net CO_2 sequestration.

In the riparian peatland, it was not clear which climatic and physicochemical factors regulate soil enzyme activities (Chapter 2). Principal component and multiple regression analyses suggested that DOC, nitrogen, phosphorus and soil water content as well as magnesium and potassium may be important. The limited response of soil enzyme activities to soil temperature may limit microbial activity since microbes are dependent on the production of labile LMW DOC. Also, limitations imposed on soil enzyme activities may allow leaching of particulate HMW DOC to rivers draining peatlands. Thus DOC export may be more important in terms of the C balance than the loss of CO_2 from riparian peatlands. Factors such as low pH, low ion concentrations, low oxygen content and low microbial proliferation (Brock and Bregman, 1989) may have limited the enzyme activities (Kang and Freeman, 1999; Kang, 1999).

B-glucosidase activity was higher in the riparian peatland (28 - 76 nmol MUF g^{-1} min^{-1}) relative to the blanket bog (3.6 - 34 nmol MUF g^{-1} min^{-1}). At the riparian site, principal component analysis suggested that β-glucosidase activity was not related to DOC. However, multiple regression analysis suggested that DOC had a positive relationship with β-glucosidase, whilst Mg^{2+} and K^{+} had negative relationships. Results from the blanket bog showed that DOC and β-glucosidase activity were positively associated. This suggests that either enzyme production is induced by high concentration of HMW DOC or the enzyme reaction causes accumulation of LMW DOC. However, in the blanket bog, soil temperature may have affected the seasonal variation in activity. DOC was lower in the riparian peatland (4.3 - 34.6 mg l^{-1}) compared to the blanket bog (18.3 – 56 mg l^{-1}) although DOC in the blanket bog was collected from a lower soil depth where enzymic hydrolysis may be lower. A study by Worrall *et al.* (2004) showed that changes in soil temperature are responsible for only 12 % of a 78 % increase in DOC production at Moorhouse over a 30 year period. They suggested

that temperature variation alone is insufficient to explain observed increases in DOC production and suggested that hydrological changes affect an enzymatic latch mechanism (Freeman *et al.* 2001a) that may account for increased decomposition. Indeed, multiple regression analysis suggests that β-glucosidase activity in the blanket bog was related to DOC release explaining 77 % of the seasonal variation. Overall it is apparent that there may be differences in the factors controlling β-glucosidase activity and DOC production between riparian and blanket bog peatlands due to the variability in abiotic conditions.

Sulphatase activity was also higher in the riparian peatland (4.8 - 17.5 nmol MUF g^{-1} min^{-1}) relative to the blanket bog (0.3 - 11 nmol MUF g^{-1} min^{-1}). The controls on sulphatase activity in the riparian peatland are unclear since the best predictors were O_2 and Mg^{2+}. However, in the blanket bog, sulphatase activity was related to the same climatic factors that affected β-glucosidase activity. Basal CO_2 respiration was also related to sulphatase activity and thus the low microbial proliferation in the blanket bog may partially explain the relatively lower activity. Surprisingly, both sulphatase and β-glucosidase appeared more responsive to seasonal temperature variation in the blanket bog than the riparian peatland despite lower activities.

Phosphatase activity was lower in the riparian peatland (25 – 64 nmol MUF g^{-1} min^{-1}) relative to the blanket bog (19.7 - 142 nmol MUF g^{-1} min^{-1}). A possible explanation for this may be phosphate limitation in the blanket bog since it is one of the most limiting nutrients after nitrogen. For the blanket bog, phosphatase activity did not correlate with any measured variable but since dissolved ionic concentrations were not measured they cannot be ruled out. The negative effect of soil phenolics on phosphatase activity found in the riparian peatland (Chapter 2) may have interacted with other factors to affect the seasonal variability at the blanket bog since soil phenolics were lower in the riparian peatland (0.26 to 3.97 mg l^{-1}) relative to the blanket bog (4 to 14 mg l^{-1}).

Elevated CO_2 increased *N*-acetylglucosiminidase activity, and increased phosphatase and sulphatase activity under elevated temperature and/or waterlogged conditions. Thus the response of soil enzyme activities to global warming may be less than the short-term indirect effect of elevated atmospheric CO_2 that potentially increased DOC availability. This has important implications

for the cycling of N, P and S under future climate change since the C balance of peatlands may be dependent on the availability of limiting nutrients.

6.4 Plant-soil interactions in peatland ecosystems

Plant productivity and soil decomposition are the primary controls on the C balance of peatland ecosystems. The results from Chapters 2, 3 and 4 of this thesis suggest that feedback between plant and soil processes (i.e. labile C rhizodeposition leading to increased mineralization) does not play an important role in C sequestration under current environmental conditions. Seasonal variability in light intensity, temperature, and atmospheric N deposition did not significantly alter *Sphagnum* productivity to an extent that would affect soil enzyme activities and lead to significant nutrient feedback between plant and soil processes. Thus the C balance of peatlands is presently controlled by the direct effect of climate and physicochemistry on plant and soil processes with little feedback occurring between biological processes. The rapid transfer of photosynthate C inputs to the soil microbial community are well known in grassland and forest ecosystems (Freeman *et al.*, 1998b; Ostle *et al.*, 2003; Johnson *et al.,* 2002; Reynolds *et al.*, 2003). Limitations imposed by the waterlogged conditions, acidic conditions and low temperature variability on NPP may account for the insignificant rhizodeposition by *Sphagnum* and hence the insignificant effect on soil decomposition processes.

The results from Chapter 5, however, suggest that elevated CO_2 may lead to an increased complexity to our current understanding of plant-soil interactions in peatland ecosystems. Elevated CO_2 significantly altered C sequestration in the short-term by increasing *Sphagnum* photosynthetic activity (or NPP) that increased soil enzyme activities possibly by increased rhizodeposition of labile C. This suggests that elevated CO_2 may lead to significant feedback between plant and soil process by increasing plant rhizodeposition and nutrient availability. This has important implications for modelling the C balance of peatlands since nutrient feedback between plant and soil processes may significantly alter the direct effect of elevated CO_2, temperature and the water table depth on C related processes.

6.5 Implications for peatland biogeochemistry

Climatic change is expected to be most pronounced in boreal and arctic ecosystems, with a predicted surface temperature increase of 2-4 °C in the summer and as much as 6-15 °C in the winter and spring (Gates *et al.*, 1992; Meehl *et al.*, 1993; IPCC, 2001). Given the great uncertainty in global climate models, the ultimate effect of climate change on C cycling in *Sphagnum* dominated ecosystems is difficult to quantify. Interactions between temperature, atmospheric CO_2, precipitation, soil moisture, evapotranspiration, vegetation, gas exchange, nutrient availability and decomposition processes are complex and only partially understood (O'Neill, 2000).

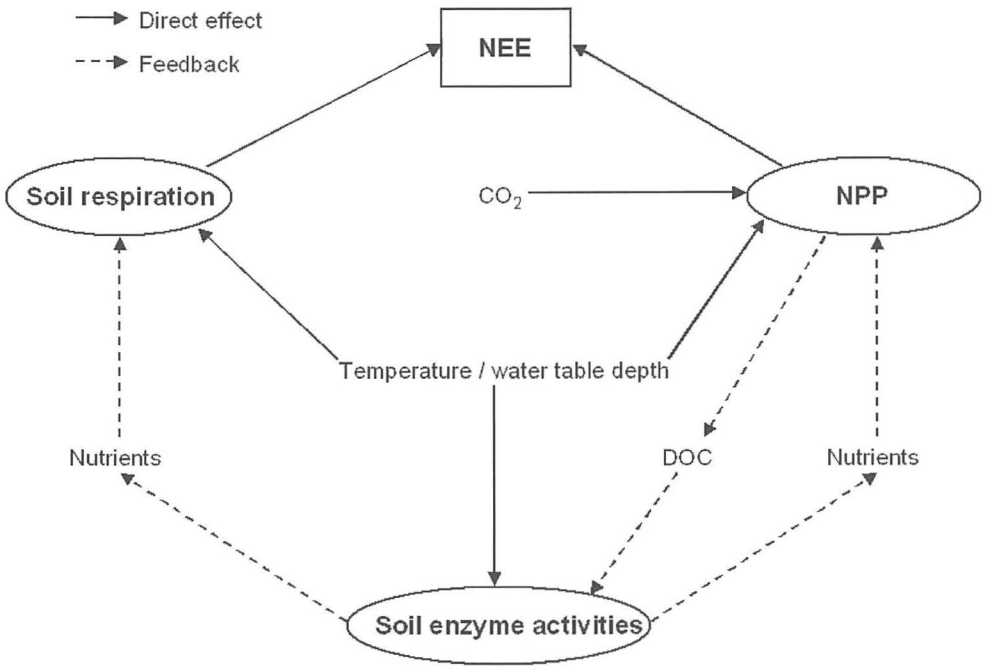
Over short-time scales, exposure to elevated CO_2 significantly increases rates of photosynthesis and C accumulation in many arctic species. Results from this study support these findings in that *Sphagnum* productivity maybe increased by elevated CO_2 and temperature in the short-term resulting in net CO_2 sequestration and possibly extending the length of the growing season. However, both chamber and *in situ* studies of northern species show that this response may be short-lived (Billings *et al.*, 1984; Oechel *et al.*, 1994). The long-term response to elevated CO_2 and temperature may by limited by nutrient availability which may eventually reduce the increased capacity of peatland ecosystems to sequester atmospheric CO_2. Sustaining elevated rates of photosynthesis over long periods of time requires either availability of sufficient nutrients (Chapin *et al.*, 1980) or improved nutrient use efficiency (Oechel & Billings, 1992). Because most northern ecosystems are nutrient-limited, the direct effect of CO_2 enhancements may be minimal (Oechel *et al.*, 1994, Oechel & Vourlitis, 1997). However, in this study (Chapter 5), it is apparent that CO_2 indirectly increased soil enzyme activities that may lead to an increase in nutrient availability for plant and microbial growth which may extend the CO_2 fertilization effect on NPP. Also, Nadelhoffer *et al.* (1991) suggested that warmer and drier soil conditions may relieve nutrient limitations by increasing decomposition and release of stored nutrients, thus stimulating ecosystem productivity and C accumulation (Shaver *et al.*, 1992). Without the CO_2 fertilization effect, Mack *et al.* (2004) showed that over a 20 year period, nutrient availability in Alaskan tundra can actually stimulate net C loss. NPP doubled during the experiment but losses of C were

substantial and more than offset the C storage in plant biomass. It was suggested that projected release of soil nutrients associated with high-latitude warming may further amplify C release from soils, causing a net loss of ecosystem C and a positive feedback to climate warming.

Ultimately, future carbon source-sink potential of *Sphagnum* dominated peatlands will be determined by the temperature response of NPP compared to that of organic matter decomposition (Kirschbaum, 1995). In this study, ecosystem respiration had a relatively higher Q_{10} response to temperature than gross photosynthesis. Over short-time scales, soil decomposition is expected to be more responsive to changes in climate (Pastor & Post, 1993; Oechel & Vourlitis, 1997). However, over longer time-scales changes in plant species composition (Smith & Shugart, 1993), NPP and increased nutrient availability via decomposition (Rastetter *et al.*, 1992) could improve ecosystem productivity and increase the amount of C stored in plant biomass. This increased C storage could balance or potentially exceed the short-term loss of C by enhanced soil respiration (O'Neill, 2000).

Overall, the results from this thesis suggest that multiple, interacting factors will determine the effect of global warming on the C balance of peatlands in the future. Positive feedback mechanisms such as the CO_2-plant-enzyme-nutrient interaction (fig. 6-1) may have important implications for future C storage in these systems. The magnitude and duration of this indirect enzymic response to elevated CO_2 may be the key to the C balance of peatland ecosystems in the future by extending the CO_2 fertilization effect on NPP. Without this mechanism, global warming will probably lead to the release of stored soil C to the atmosphere as CO_2 from northern peatlands.

Figure 6-1 A model for the effect of climatic factors on NEE. These climatic factors will interactively affect NEE. Of key importance is the effect of CO_2 on NPP that may indirectly stimulate soil enzyme activities that will feedback on NPP and soil respiration by increasing nutrient availability.



Future work

It is clear from this thesis that *Sphagnum* productivity reduced *in situ* CO_2 respiration but did not affect soil enzyme activities. This may be explained by immobilized enzymes bound to clay and humic colloids that can retain activity independently of microbial activity (Burns, 1982). Laboratory experiments assessing the effect of *Sphagnum* productivity on rhizodeposition are required to determine the quantity and quality of exudates released by *Sphagnum* under varying degrees of productivity and other factors that may account for the reduction in *in situ* soil respiration.

It is not clear whether the increase in *Sphagnum* productivity by elevated CO_2 will continue due to seasonal limiting factors such as temperature, PPFD and nutrient availability. Long-term studies (over 10 years) addressing the effect of CO_2 and temperature on peatland NEE, soil enzyme activities and plant and soil nutrient status/availability under both *in situ* and climatically controlled conditions are required.

It is apparent that vascular and non-vascular productivity may impact differently on soil decomposition and NEE. Modelling of both vascular and non-vascular dominated areas in peatlands to account for differences in plant-soil interactions may be import for accurately determining the impact of future climate scenarios on the C balance of peatland ecosystems. Also, the extent of feedback between plant and soil processes under elevated CO_2 due to increased enzyme activities requires assessment since it may affect the response of C related processes to global warming. This is of importance since models that do not account for feedback between plant and soil processes under future climate change scenarios may result in inaccurate prediction of the future C balance of northern peatlands.

Appendix

Appendix 1 Gas and enzyme methodology development

Figure A1-1 Gas timecourses for ecosystem CO_2 respiration. Mean ± standard deviation (n=5).

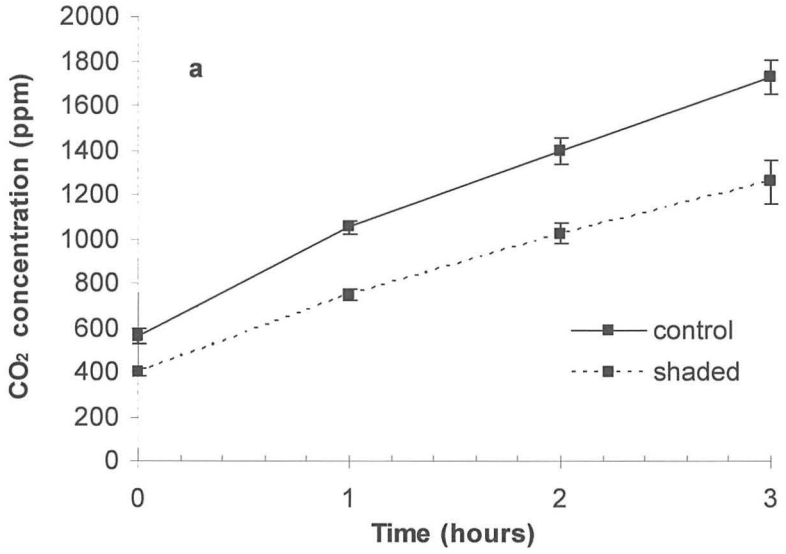


Figure A1-2 Linear regression analysis of methylumbelliferyl enzyme standard concentration and standard in peat for quenching correction.

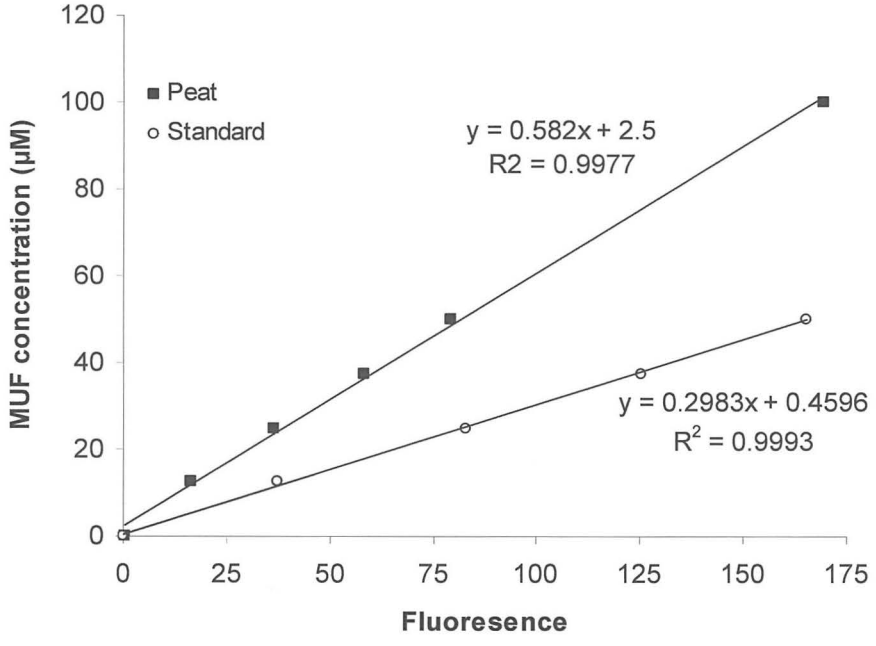


Figure A1-3 Effect of MUF-substrate concentration on enzyme activities. Mean ± standard deviation (n=3). MUF-substrate concentration is slurry diluted i.e. double the concentration was added to soil slurry.

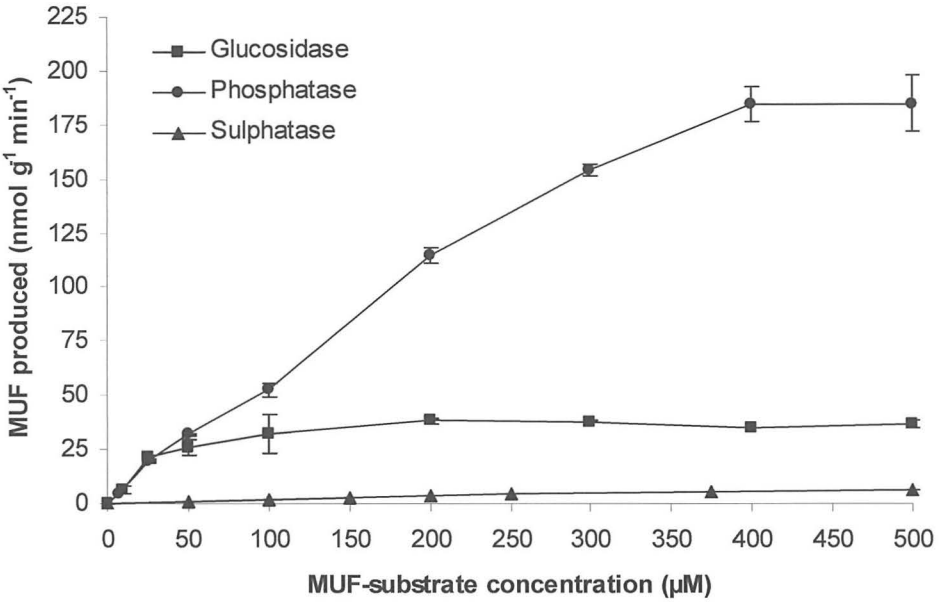
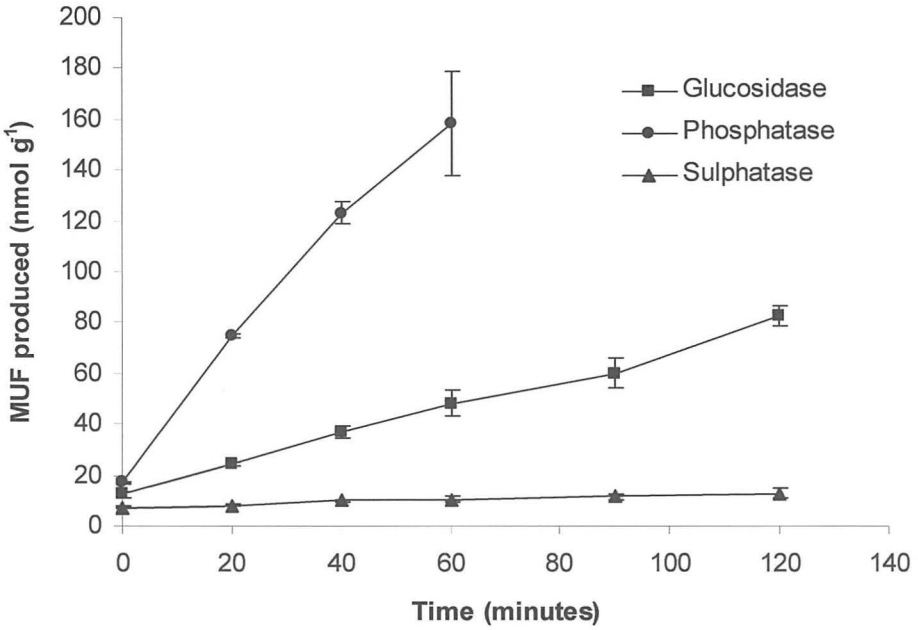


Figure A1-4 Effect of incubation time on enzyme activities. Mean ± standard deviation (n=3).



Appendix 2 Effect of shading and increased N deposition on *Sphagnum capillifolium* pigmentation

A2.1 Introduction

Bryophytes are generally considered to be shade adapted plants, reaching photosynthetic light saturation at low irradiances (Shaw and Goffinet, 2000; Davey *et al.*, 1997). Typically, photosynthesis of bryophytes saturate between 30 – 300 μmol m^{-2} s^{-1} (i.e. 5-10 % of full sunlight) (Shaw and Goffinet, 2000; Davey *et al.*, 1997). Shade adapted plants have thinner leaves, larger chloroplasts, are richer in chlorophyll and contain a higher proportion of chlorophyll *b* relative to chlorophyll *a* (Boardman, 1977). Carotenoids, chlorophyll *b* and chlorophyll *a* are usually associated intimately with both antenna and reaction center pigment proteins and are integral constituents of the thylakoid membrane (Taiz and Zeiger, 1998). The energy of light absorbed by carotenoids is rapidly transferred to chlorophylls, so carotenoids are termed accessory pigments. Carotenoids called xanthophylls play an essential role as photoprotective agents by rapidly quenching the excited state of chlorophyll (Taiz and Zeiger, 1998). Strong linear relationships exist between nitrogen (N) and rubisco and chlorophyll. Growth under low light intensity greatly increases the partitioning of N into chlorophyll and thylakoids (Evans, 1989).

Flavonoids are the most widespread phenolics in bryophytes (Shaw and Goffinet, 2000). The red pigmentation of many plants is due a group of flavonoid pigments called anthocyanins. Shaw and Goffinet (2000) state that the typical red pigments reported from certain *Sphagnum* species may have a chemical relation to 3-deoxyanthocyanidins. Anthocyanins generally accumulate in peripheral tissues exposed to high irradiance or in obligatory shade plants due to an inbalance between light capture, CO_2 assimilation and carbohydrate utilization (Steyn *et al.*, 2002). They significantly modify the quantity and quality of light incident on chloroplasts (Krol *et al.*, 1995). Field *et al.* (2001) found that optical masking of chlorophyll by anthocyanins reduces risk of photo-oxidative damage to leaf cells of Red-Osier dogwood as they senesce. Light attenuation by

anthocyanins may help to re-establish a balance and so reduce the risk of photo-oxidative damage (Steyn *et al.*, 2002).

The aim of this research was to determine the interactive effect of shading and increased N deposition on *Sphagnum capillifolium* pigmentation. Results from Chapter 3 suggested that this species may acclimate to shaded conditions by breaking down anthocyanin pigments. It was hypothesized that *S. capillifolium* contains anthocyanins as a mechanism to reduce the incident light on chlorophyll and hence prevent photo-oxidative damage that Murray *et al.* (1993) showed can occur in other *Sphagnum* species. Shading was hypothesized to increase the allocation of N to photosynthetic pigmentation. Thus N was hypothesized to increase photosynthetic pigmentation under shaded conditions. This research was carried out as part of the experiment described in Chapter 4.

A2.2 Materials and methods

The experimental design is described in Chapter 4.

Anthocyanins in *S. capillifolium* capitulum were determined by the method of Pietrini *et al.* (2002). 0.02 g of dry, ground *S. capillifolium* capitulum were dissolved in 2 ml of 1% HCL in methanol in a centrifugation vial for 4 hrs at 4 °C to avoid degradation of chlorophylls followed by centrifugation at 13000 rpm for 10 minutes. The absorbance of the supernatant was measured using a spectrophotometer at 534 and 661 nm, and $A_{534} - 0.25 \times A_{661}$ was used to account for interference from chlorophylls (Mancinelli, 1984). Anthocyanin content was calculated as cyanide-3-glucoside ($\mu g\ g^{-1}$) using 29600 as extinction coefficient and 445 MW (Wrolstad, 1976).

Chlorophyll *a*, *b* and total carotenoids were determined by the methods of Pietrini *et al.* (2002) and Lovelock and Robinson (2002). 0.02 g of dry, ground *S. capillifolium* capitulum were dissolved in 2 ml acetone-water (80% v/v) in centrifugation vials for 24 hrs at 4 °C followed by centrifugation at 13000 rpm for 10 minutes. The absorbance of the supernatant was measured with a spectrophotometer at 470, 643 and 661 nm. Chlorophyll *a*, chlorophyll *b* and total carotenoids were determined in units of $\mu mol\ ml^{-1}$ using the equations of Lichtenthaler (1987) below and converted to $\mu g\ g^{-1}$ using the following

molecular weights: Chl *a* = 893.5 g mol^{-1}, Chl *b* = 907.5 g mol^{-1}, and carotenoids = 550 g mol^{-1} where:

$$\text{Chlorophyll } a = 0.01261*A_{661} - 0.001023*A_{534} - 0.00022*A_{643}$$

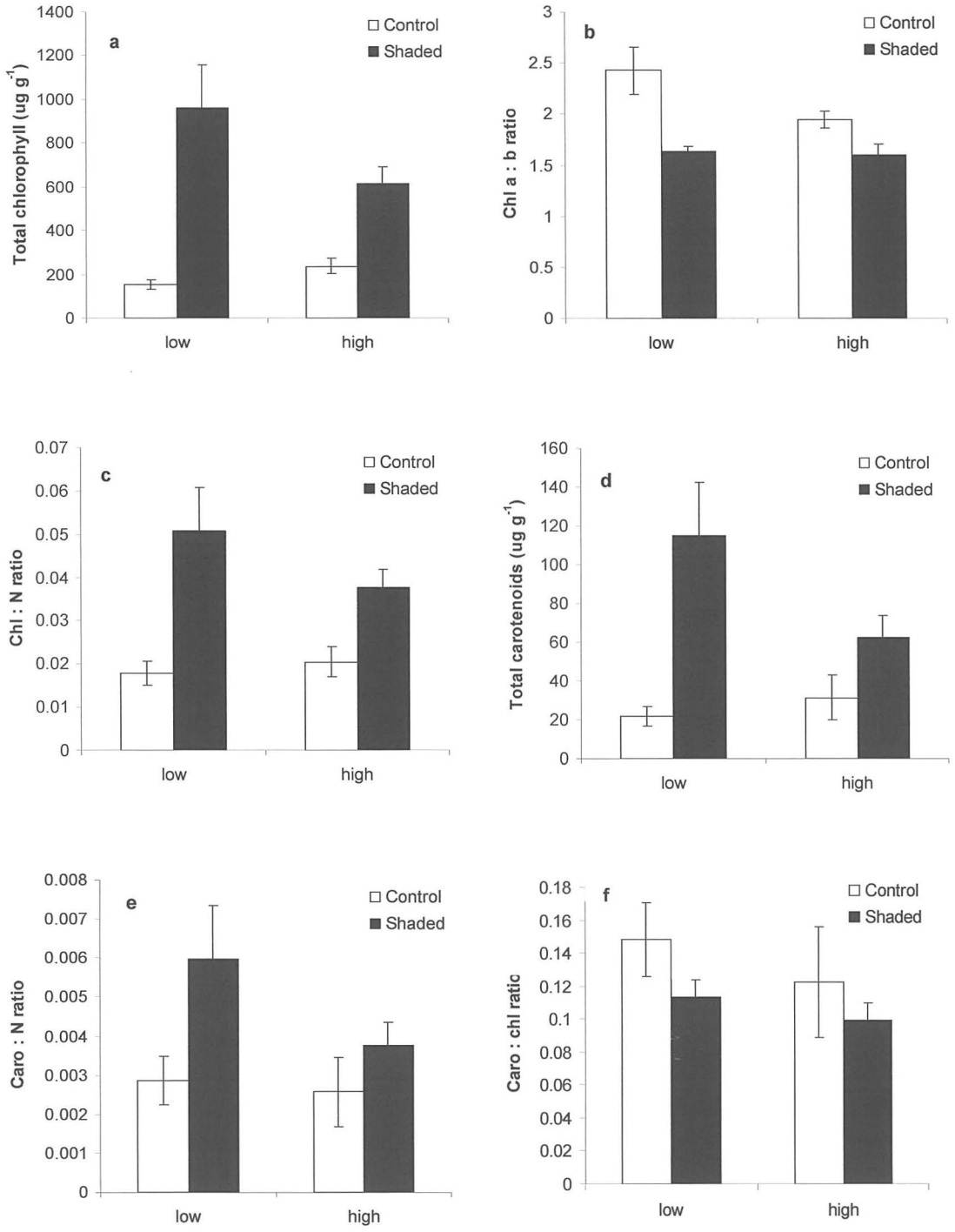
$$\text{Chlorophyll } b = 0.02255*A_{643} - 0.00439*A_{534} - 0.004488*A_{661}$$

$$\text{Caretenoids} = (A_{470} - 17.1*(\text{Chl } a + \text{Chl } b) - 9.479*\text{Antho}) / 119.26$$

A2.3 Results

Total chlorophyll of *S. capillifolium* capitulum was significantly increased by shading under low N deposition ($P < 0.001$) and increased N deposition ($P < 0.001$; $F = 19.27$) (fig. A2-1a). Total chlorophyll was significantly increased by increased N deposition in the control treatment ($P < 0.05$; $F = 4.64$). Chlorophyll *a* of *S. capillifolium* capitulum was significantly increased by shading under low N deposition ($P < 0.001$) and increased N deposition ($P < 0.001$; $F = 19.52$). Chlorophyll *a* was significantly increased by increased N deposition in the control treatment ($P < 0.05$; $F = 4.18$). Chlorophyll *b* of *S. capillifolium* capitulum was significantly increased by shading under low N deposition ($P < 0.001$) and increased N deposition ($P < 0.001$; $F = 17.42$). Chlorophyll *b* was significantly increased by increased N deposition in the control treatment ($P < 0.05$; $F = 5.06$). The chlorophyll *a* : *b* ratio was significantly reduced by shading under low N deposition ($P < 0.01$) and increased N deposition ($P < 0.05$; $F = 6.41$) (fig. A2-1b). The chlorophyll *a* : *b* ratio was significantly reduced by increased N deposition in the control treatment ($P < 0.05$). The chlorophyll : N ratio was significantly increased by shading under low N deposition ($P < 0.01$) and increased N deposition ($P < 0.01$; $F = 9.76$) (fig. A2-1c). Total carotenoids were significantly increased by shading under low N deposition ($P < 0.001$; $F = 17.82$) and weakly increased by shading under increased N deposition ($P < 0.064$) (fig. A2-1d). The total carotenoid : N ratio was significantly increased by shading under low N deposition ($P < 0.05$; $F = 4.76$) (fig. A2-1e). The total carotenoid : total chlorophyll ratio was not significantly affected by either shading or increased N deposition (fig. A2-1f).

Figure A2-1 Effect of shading and increased N deposition on *S. capillifolium* photosynthetic pigmentation: (a) total chlorophyll, (b) chlorophyll *a* : *b* ratio, (c) total chlorophyll : N ratio, (d) total carotenoids, (e) total carotenoids : N ratio and (f) total carotenoids : total chlorophyll ratio. Mean ± standard error (n = 10).



Total anthocyanins were significantly reduced by shading under low N deposition ($P < 0.05$) and increased N deposition ($P < 0.05$) (fig. A2-2) but were not significantly affected by increased N deposition. Total chlorophyll, chlorophyll *a*, chlorophyll *b* and total carotenoids were all strongly correlated with capitulum N content and with each other (Table A2-1). Total anthocyanins did not correlate with capitulum N content or photosynthetic pigmentation.

Figure A2-2 Effect of shading and increased N deposition on the total concentration of anthocyanins in the capitulum of *S. capillifolium*. Mean ± standard error (n=10).

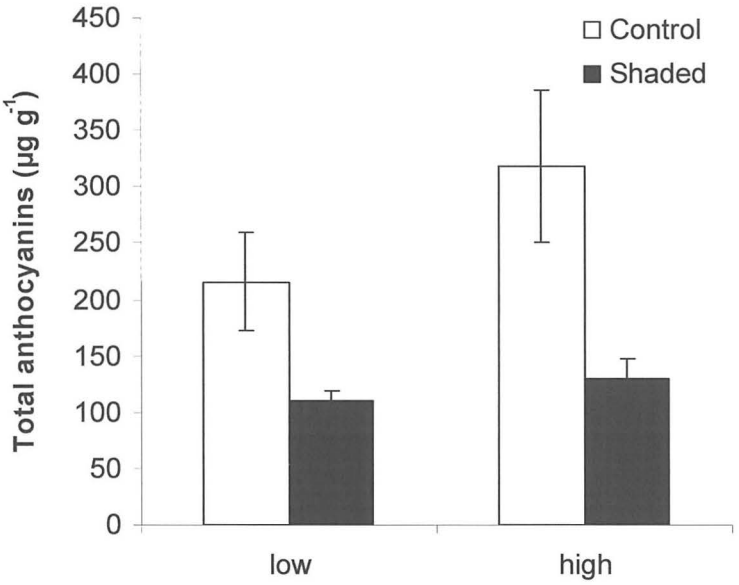


Table A2-1 Spearman rank correlation coefficients between capitulum N content and pigments. (n = 40) (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

	N	Chl	Chl *a*	Chl *b*	Caro
Chl	0.839***				
Chl *a*	0.839***	1.000***			
Chl *b*	0.830***	0.995***	0.995***		
Caro	0.712***	0.859***	0.859***	0.849***	
Antho	-0.347	-0.258	-0.258	-0.243	-0.211

A2.4 Discussion

The increase in capitulum N content (Chapter 4) under shaded conditions via translocation was probably associated with an increased requirement for compounds associated with photosynthetic capacity. Photosynthetic capacity in vascular plants is related to the N content primarily because the proteins of the Calvin cycle and thylakoids represent the majority of leaf N (Evans, 1989). Within vascular species there are strong linear relationships between N and both rubisco and chlorophyll (Evans, 1989). Total chlorophyll including both chlorophyll *a* and *b* and total carotenoids were significantly increased by shading under both N deposition treatments. In many species, growth under shading greatly increases the partitioning of N into chlorophyll and thylakoids, while the electron transport capacity per unit of chlorophyll declines (Evans, 1989). Also, total chlorophyll, chlorophyll *a* and *b* and total carotenoids correlated significantly with the capitulum N content. Thus the translocated N was allocated to photosynthetic pigmentation to increase the absorption of photons. The difference between the capitulum N content of ambient and shaded treatments, and total chlorophyll content of ambient and shaded treatments suggested that approximately 8 % of translocated N was allocated to chlorophyll for shaded *Sphagnum* under both N supply treatments. Also, the total chlorophyll : N ratio was significantly increased by shading under both N supply treatments. Thus the remaining proportion of translocated N may have been allocated to soluble protein (i.e. rubisco) and pigment-protein/reaction centre complexes that contain the majority of thylakoid N (60-85 %) (Evans, 1989).

The chlorophyll *a* : *b* ratio was significantly reduced by shading under both N supply treatments from 2.4 to 1.6. Bryophytes typically have low chlorophyll *a* : *b* ratios that would generally be regarded as characteristic of shade plants (Valanne, 1984) with reported values mostly lying within the range from 1.5 to 3.0 (Martin and Churchill, 1982; Kershaw and Webber, 1986; Marschall and Proctor, 2004). This implies that the light-harvesting chlorophyll *a* : *b* protein complex makes up a large proportion of the total chlorophyll present (Marschall and Proctor, 2004). Martin and Churchill (1982) found generally higher chlorophyll *a* : *b* ratios for mosses on an exposed sandstone outcrop than in an oak-hickory forest in Kansas. The forest mosses also showed a striking

increase in total chlorophyll concentration. Increased N deposition significantly increased total chlorophyll, chlorophyll *a* and chlorophyll *b* under ambient PPFD suggesting that photosynthetic capacity may have been limited by N. This also resulted in a decrease in the capitulum chlorophyll *a* : *b* ratio.

Total carotenoids were significantly increased by shading under low N deposition suggesting that carotenoids not associated with photoprotection or non-photochemical quenching were increased for absorption of light energy. Total carotenoids correlated positively with total chlorophyll and the chlorophyll : carotenoid ratio was not significantly different between light or N deposition treatments. Johnson *et al.* (1993) found a very close correlation between total chlorophylls and total carotenoids in their sample of vascular plants, hence rather little variation in the chlorophyll : carotenoid ratio. However, Marshall and Proctor (2004) presented data from 39 species of mosses and 16 liverworts for ratios of chlorophylls and carotenoids and found that high chlorophyll : carotenoid ratios were associated with bryophytes growing in more or less deep shade. Rosevear *et al.* (2001) showed that the main determinant of leaf pigment content and composition is the environmental factors experienced during development, rather than any genetic predisposition to a given pigment complement. Johnson *et al.* (1993) found highly significant correlations of the proportions of lutein (positive) and the xanthophyll-cycle pigments (negative) with shade tolerance. Marshall and Proctor (2004) stated that it is reasonable to surmise that at least a part of the very wide range of chlorophyll : carotenoid ratio seen in bryophytes is due to large amounts of xanthophyll-cycle pigments in the species of sunny sites, particularly in view of the extreme NPQ levels shown by many of these plants at high irradiance (Demmig-Adams, 1998). Rosevear *et al.* (2001) thus stated that 'deep-shade plants may reflect a particular importance of these two pigments in light harvesting, increasing the efficiency of light harvesting at very low irradiances. Therefore it is hypothesized that *S. capillifolium* has characteristics of intermediately shaded species and may increase carotenoids not associated with non-photochemical quenching (i.e. lutein and neoxanthin) under shaded conditions to increase the efficiency of light capture. Rosevear *et al.* (2001) concluded that at any level of shade tolerance a similar range of carotenoid concentrations occur in all species, indicating that the ability to produce carotenoids in general does not play a major role in

determining tolerance of high light or shade. Carotenoids were not significantly affected by increased N deposition in either light treatment unlike chlorophyll. Total carotenoids correlated positively with capitulum N content. The conversion of xanthophylls-cycle pigments can be slow when the plant is nutrionally deprived of N or stressed by high-light conditions (Taiz and Zeiger, 1998).

Anthocyanins were significantly decreased by shading under both N deposition treatments thus leading to a significant decrease in the anthocyanin : N ratio. Plate A2-1 shows that the loss of anthocyanins occurred in the capitulum and upper light-exposed stem where the photosynthetic apparatus is located.

Plate A2-1 Reduction in anthocyanin pigmentation due to shading in the capitulum and upper stem.

It is proposed that for *S. capillifolium,* anthocyanins play an important role in photoprotection rather than carotenoids involved in photoprotection and NPQ. Field *et al.* (2001) found evidence that anthocyanins in senescing leaves of red-osier dogwood form a pigment screen that shields the photosynthetic apparatus from excess light energy during nutrient retrieval. If PPFDs are sufficiently high and prolonged, then anthocyanin accumulation is induced and red-osier dogwood appears to show a facultative anthocyanin production (Field *et al.*, 2001).

Anthocyanins absorb light in blue wavelengths that could be captured by free chlorophyll, and because anthocyanins are localized in the cell vacuole, they may be poised to scavenge oxygenated radicals leaking from chloroplasts as well as mitochondria and peroxisomes (Yamasaki, 1997; Grace and Logon, 2000). Attenuation by anthocyanins may help to re-establish a balance and so reduce the risk of photo-oxidative damage (Steyn *et al.,* 2002).

Responses of plants to changes in light intensity are mediated by three main receptor systems: chlorophyll for photosynthesis, phytochrome for many photomorphogenetic responses, and flavins for tropisms and high-energy photomorphogenesis (Fitter and Hay, 1987). The control of anthocyanin accumulation or breakdown appears unrelated to the controls on photosynthetic pigmentation due to the lack of correlations between anthocyanins, photosynthetic pigments and capitulum N content. It is intuitively reasonable that poikilohydric photosynthetic organisms should be adapted to function at relatively low light levels (Marschall and Proctor, 2004). During periods of bright, dry sunny weather bryophytes will generally be dry and metabolically inactive. Most of their photosynthesis takes place in rainy or cloudy weather, when irradiance may often be < 20 % of full sunlight. The major physiological need during transient exposures to bright sunshine as the plant dries out is likely to be for photoprotection rather than energy capture. Marshall and Proctor (2004) showed that *Sphagnum* species saturate at relatively low PPFDs, but have high non-photochemical quenching indicating a high level of photoprotection consistent with their unshaded habitat. Thus anthocyanins appear to be the critical mechanism of photoprotection in *S. capillifolium* rather than carotenoids that may principally serve as accessory pigments.

Conclusions

S. capillifolium responded to shading via morphophysiological and biochemical alterations to the photosynthetic tissues such as (1) break down of anthocyanins involved in photoprotection of chloroplasts, (2) translocation of N from mineralized N or old tissues (Chapter 4), and (3) allocation of translocated N to photosynthetic pigments. Increased N deposition potentially ameriolated the

negative effect of shading by allocation to chlorophyll though productivity was primarily limited by light intensity. Overall these results suggest that *S. capillifolium* can tolerate both low and high light intensities via morphophysiological and biochemical responses but does not acclimate to deep-shade since productivity was reduced. These species grow in non-shaded habitats and are thus exposed to a wide range of light intensities. Anthocyanins rather than carotenoids appear to play an essential role in photoprotection with translocation serving as an important source of N. Whether these mechanisms are dynamic (i.e. reversible) requires assessment but they may provide *S. capillifolium* with a selective advantage over other *Sphagnum* species that could explain the dominance of these species in northern peatlands.

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