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The effects of climate change on dissolved organic carbon release from peatlands

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THE EFFECTS OF CLIMATE CHANGE ON DISSOLVED ORGANIC CARBON RELEASE FROM PEATLANDS

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SUMMARY

Dissolved organic carbon (DOC) concentrations in UK rivers have been rising significantly, with the largest increases occurring in peat dominated catchments. These catchments are major sources of potable water in the UK and DOC compounds have adverse effects on many aspects of water quality, from serving as precursors for the formation of disinfection by-products and causing low residual chlorine (limiting its protection against biological contamination), to reducing aesthetic value. The potential effects of some major climate change predictions for northern regions on the quantity and quality of DOC produced in peatland catchments have therefore been examined. Such predictions include elevated atmospheric CO_2 concentrations (eCO_2), warmer temperatures (eTemp), an increased frequency of summer droughts and increased rainfall. Both eCO_2 and eTemp increased leachate DOC concentrations with selective enrichment of recalcitrant phenolic compounds. Under eCO_2, such increases were associated with the stimulation of plant inputs (biomass and exudation), reduced extracellular enzyme activities and suppressed DOC decomposition. Warmer conditions provided evidence for enzymic mobilization of DOC and phenolic compounds from the peat matrix. These treatments in combination (eCO_2/eTemp) apparently interact to produce the highest concentrations of DOC and phenolic enrichment. Stable isotope (^{13}C) labelling studies revealed that increased exudation of recently synthesized DOC potentially has a crucial role in this response. Successive droughts seemingly induced an increasing trend for DOC concentrations, due to an increased diversity of aerobic, aromatic degrading bacteria and enhanced enzyme activities stimulating mobilization of the peat matrix. Conversely, increased rainfall simulations showed reduced bacterial diversity and enzymic inhibition, conducive to the accumulation of high molecular weight DOC. Potential effects of climatic changes on biofilm communities in the recipient waters were also studied. Elevated CO_2 and eTemp in combination were found to increase DOC and phenolic compound production when photoautotrophs were present, but also to compromise the removal of these increased inputs by the biofilm heterotrophs. It is therefore proposed that increased DOC production (in the peatland system and by the biofilm) coupled with reduced biodegradation of this material could account for the rising DOC concentrations in UK rivers.

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CHAPTER 1: INTRODUCTION

1.01 DEFINITION OF WETLANDS

Wetlands are possibly the most difficult of all ecosystems to define. They can be considered as transitional zones between terrestrial and aquatic ecosystems and are highly diverse according to their genesis, location, hydrological regime, chemistry, dominant plant species and soil characteristics (Vymazal, 1995). Many different types of wetland can exist in close proximity and substantial variation can occur within a single wetland area. Despite this heterogeneity, wetlands can be assigned three distinguishing characteristics; the presence of water, a unique soil type, and vegetation adapted to waterlogging (Mitsch & Gosselink, 2000). One of the most widely accepted definitions of wetlands considers them as:

> 'lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface.....Wetlands must have one or more of the following attributes 1) the area is saturated with water at some time during the growing season, 2) the substrate is predominantly undrained hydric soil, and/or 3) hydrophytes are the dominant vegetation' (modified the from the US Fish and Wildlife Service definition, Cowardin *et al.*, 1979; from Mitsch & Gosselink, 2000).

Wetlands are frequently found at the margins of deep water and terrestrial systems leading to wetlands being considered as ecotones or transition zones of 'tension' between two or more communities (Clark, 1954), such as lakes and uplands. Both the terrestrial and aquatic systems could therefore be assumed to influence the wetland. This can however, be disputed (e.g., Tiner, 1993) since many wetlands occur effectively as distinct systems, rather than boundaries between terrestrial and aquatic systems. Furthermore, the wetland soil and plant communities are often distinct, bearing no resemblance to the surrounding communities. In some cases the input of materials, such as nutrients, to a wetland can be large as can the outputs (e.g., riparian systems). Conversely, the influence of communities surrounding the wetland may be small (e.g., ombrotrophic bogs) with inputs and outputs being little other than rainfall and atmospheric gases (Mitsch & Gosselink, 2000). The ability of wetland soils to greatly modify nutrient inputs from surrounding areas means that even wetlands with substantial inputs may be little influenced by them, excepting the outer margins.

Those definitions that refer to wetlands as 'transitional communities' may also be misleading as this suggests that wetlands are temporary habitats which will evolve

either into uplands or deep water. However, unless wetland hydrology is substantially changed as a result of major factors (such as climate change), leading to significantly different plant and soil communities, this may not be the case (Tiner, 1993). On the other hand, wetland stability may be less affected by some changes in climate because the development of certain wetlands is governed by a number of internal and external factors, in addition to climate (Frenzel, 1983), such as the decay inhibiting properties of certain types of litter (Verhoeven & Toth, 1995).

Wetlands therefore, are unique systems, often bearing little resemblance to adjacent ecosystems irrespective of whether or not there is an interaction with such systems. And, they posses a stability that may be resistant to the influence of external factors. Many diverse ecosystems can however still be classed as wetlands, for example northern peatlands in the UK and Canada, tidal salt marshes in coastal areas across many parts of the world, and mangrove swamps in the tropics. Wetlands can occur in a range of locations from high mountain regions to low-lying coastal regions and although wetlands, by definition, contain vegetation which is adapted to waterlogging, such vegetation is also highly diverse, including bryophytes (within which are the *Sphagnaceae*), sedges such as *Juncus*, along with the *Cypraceae* and *Quercus* in the hardwood forests of the US. This study focuses on northern peatlands (those found at high latitude over boreal and sub Arctic areas), the most common wetland type in Britain.

1.02 PEATLANDS

Peat can be defined as an accumulation of non-consolidated, partially decomposed plant material in bogs (acid peats) and fens (alkaline peats) (Lincoln *et al.*, 1998) and is homogenous in neither space nor time. The biological, chemical and physical properties of peat are discussed by Clymo (1983). The terms *peatland* and *mire* are often used interchangeably. Peatlands are however, by definition, areas containing peat soils whereas mires are systems which are currently actively forming peat (Immirzi *et al.*, 1992). All mires are therefore classed as peatlands, but the term *peatland* can also include areas such as drained agricultural land provided they contain peat (Immirzi *et al.*, 1992). Peatlands contain a high percentage of organic carbon but the content of soils classified as peat varies considerably. Most peats contain less than 20% inorganic matter, while *Sphagnum* peats often contain less than 1% inorganic material and, by commercial standards, soils containing as much as 55% inorganic matter can be termed 'peat' (Clymo, 1983).

Peatland vegetation often exhibits a significant degree of adaptation to the stresses that are associated with the habitat. Both structural and physiological adaptations can be found, enabling toleration or regulation strategies to be employed. These stresses may include: i) a lack of oxygen, ii) a lack of available mineral nutrients and iii) an accumulation of toxic compounds formed *via* anaerobic decay. In order to cope with the absence of oxygen in the soil, peatland plants often: i) possess large intercellular spaces to improve oxygen supply and allow its diffusion from aerial parts of the plant to the roots, ii) have a reduced oxygen consumption, and iii) allow oxygen to escape from the roots generating an aerobic zone around the roots (Mitsch & Gosselink, 2000). *Sphagnum* (perceived as important agent of palludification in northern peatlands) is adapted to nutrient poor conditions, obtaining its nutrients from precipitation. Many peatland plants are also adapted to conserve nutrients. An example is *Eriophorum*, which translocates nutrients back into perennating organs prior to litterfall. Adaptations also exist that allow further sources of nutrients to be obtained, for instance by trapping insects (e.g., *Saracenia*). Whether the plant secretes enzymes to digest its captive or simply allows the material to decay is debatable (Freeman pers comm.).

The balance between the rate of primary production and the rate of decomposition determines the amount of organic matter (OM) that builds up in a given ecosystem (e.g., Swift *et al.*, 1979). Carbon sequestration occurs in peatlands because the rate of above ground primary production is greater than the rate of decomposition below ground (e.g. Kuhry & Vitt, 1996) and the latter has been the focus of attention, being attributed to many factors. These include low soil temperatures, low soil pH, a lack of mineral nutrients, the presence of microbial and plant toxins, and the chemical characteristics of the litter (e.g., Dickinson, 1983; Updegraff *et al.*, 1995). Generally though, decomposition rates are considered to be limited primarily by the waterlogged nature of the peat. With the onset of waterlogging, the pore spaces in a soil are filled with water which limits oxygen diffusion by approximately 10 000 times in comparison to an aerobic soil (Greenwood, 1961). The rate of oxygen diffusion becomes insufficient for the requirements of aerobic microorganisms and the available oxygen is rapidly consumed therefore rendering the soil anaerobic. The productivity of aerobic microorganisms is limited by the subsequent low oxygen concentrations and anaerobic assemblages proliferate. Although the bulk of the peat tends to be anaerobic, oxygen may still be present in the shallow surface layers as a result of diffusion from the air, algal production, and surface mixing by wind and convection currents (Gambrell & Patrick, 1978). This aerobic zone may be disproportionately important in, for example, allowing nutrient cycling in peats by transforming nutrients which can then diffuse into

the lower anaerobic zones (Mitsch & Gosselink, 2000). Such nutrients might otherwise be unavailable if anaerobic conditions prevented their production.

In certain types of peat (e.g., those supporting *Sphagnum* vegetation) factors such as the presence of plant toxins may be important in governing rates of decomposition. It is thought that the preservation of 'bog bodies' is due to the properties of certain degradation products of *Sphagnum*, which allow tissue preservation and suppress microbial activities (Børsheim *et al.*, 2000; Painter, 1983; 1991). *Sphagnum* homogenates, when added to peat, have been found to retard decomposition, an effect which has been attributed in part to the resistance of the plant cell walls to decay and to the cells containing antimicrobial substances (Børsheim *et al.*, 2000; Verhoeven & Toth, 1995). However, the ubiquitous phenolic compounds may also be important in relation to microbial inhibition, since decomposition in the absence of *Sphagnum* can also be impaired (Freeman *et al.*, 2001a).

Peatland formation

The development of peat is strongly dependent on climate, occurring where precipitation exceeds evapotranspiration and typically where annual precipitation is over 500 mm (e.g., Gignac & Vitt, 1994). Peat formation requires the inflow of water at a given site to exceed the outflow, i.e., an impediment of natural drainage resulting in the retention of water for at least part of the year (Immirzi *et al.*, 1992). In areas essentially closed to streamwater, the water input from precipitation need only exceed evapotranspiration. Where there is an inflow and outflow of streamwater, for example along river margins, inflows *via* precipitation and streamflow must exceed outflows *via* evapotranspiration and streamwater. The necessary reduced drainage can occur as a result of the relief of the peatland or its characteristics and this forms a basis for peatland classification. Primary peats form in depressions, the depression acting as a water reservoir. Peat forming beyond a depression, allowed by the water storage capacity of the peat itself, it is referred to as secondary peat. Tertiary peat develops beyond contact with groundwater when the peat itself impedes drainage and raises the water table (Immirzi *et al.*, 1992).

A peatland's primary water source and nutrient supply to such waters can also be used to classify peatlands. Peatlands which receive all of their water as rain or snow are called ombrotrophic bogs and are consequently nutrient poor because precipitation has a relatively low nutrient content. Rheotrophic fens receive the bulk of their water from ground and surface sources, the result being soils with a relatively high nutrient content

(in comparison to the bogs) due to the influence of the underlying rock. Oligotrophic, mesotrophic and eutrophic can further be used to describe peatlands, referring to low, medium and high peat water nutrient content respectively (see Gore, 1983; Immirzi *et al.*, 1992). Plant species can be used in peatland classification (e.g., Rodwell, 1991; Tansley, 1939), although care is required because of interacting factors which determine vegetation type and peat development (Gore, 1983).

Peatland distribution

Peatlands are mainly distributed in the Northern Hemisphere; northern boreal and sub Arctic wetlands are mainly peatlands, although collectively smaller areas also occur in the Southern Hemisphere (see figure 1.01). There are still major discrepancies between estimates of the area covered by peatlands and even the definition of different wetland types in different countries is not always consistent. Approximately 4-6% of the earth's land is covered by wetlands (Mitsch & Gosselink, 2000), approximately half of this is composed of peatlands (Immirzi *et al.*, 1992) and the peatlands of Britain account for around 0.4% of this (Immirzi *et al.*, 1992). There are only six countries that have larger total areas of peatland than Britain and these include Canada and the former USSR (Taylor, 1983). The high proportion of peatlands in Britain is attributed to a range of climatological and geological conditions and Taylor (1983) discusses this further.

Peatland biogeochemistry

Wetlands, including peatlands, therefore cover a significant but not substantial proportion of the land area of the earth. In biogeochemical terms however, wetlands can be considered important ecosystems and this is illustrated by the fact that they contribute an estimated 20% of the total dissolved organic carbon (DOC) exported from the continent to the oceans (Lugo *et al.*, 1989).

Biogeochemistry can be traced back to the Greeks between 450 and 270 B.C., who used the concept of the "balance of nature" because the earth can be considered as a system that is effectively closed with respect to matter, resulting in total internal cycling of materials (Gorham 1991a). Wetlands (and other systems on a smaller scale) are however, unlikely to be closed to matter. There may be considerable inputs of energy and materials into a wetland in the form of atmospheric gases and nutrients *via* rain, ground or surface water etc. If the earth is assumed to be a closed system, the implication is that the composite smaller systems must also have outputs. There are three possible scenarios (Begon *et al.*, 1990):

1. Inputs = outputs

2. Inputs > outputs = net storage
3. Outputs > inputs = net loss

The process of carbon accretion in peatlands is described by situation two, with the rate of OM input to the soil as plant remains exceeding that of the decomposition of this material, resulting in net storage of carbon. This property makes peatlands important on a global scale in that historically they have been net carbon sinks (Gorham, 1991b). There is uncertainty as to the precise amount of carbon sequestered in peat. Northern peatlands (boreal and sub Arctic) contain *circa* one third, or 455 Pg (1 Pg = 10^{15} g), of the global store of organic carbon (Gorham, 1991b), with accumulation rates of 20-40 g C m^{-2} yr^{-1} during the last 5000 to 10 000 years (Tolonen & Turunen, 1996). Immirzi *et al.* (1992) estimate that temperate peatlands contain 390 Pg of carbon and that tropical peatlands could add a further 70 Pg to give a total of 460 Pg (but realistically this may be in the region of 329-528 Pg). A change in either the rate of production or the rate of decomposition could produce no net change of carbon accretion or a net loss of carbon, climate change being an example of a factor which could result in peatlands losing their ability to act as a net carbon sink. This is to be the concern of this thesis with particular reference to DOC and water quality.

Wetlands in general exhibit unique biogeochemistry compared to upland or aquatic ecosystems and for this reason they have been one of the main subjects for biogeochemical studies. It is necessary to briefly summarize the important biogeochemical processes in wetlands before the forms of carbon (and the reactions in which it is involved) are discussed in more detail.

First, on the depletion of oxygen and decrease in redox potential, various electron acceptors other than oxygen are sequentially employed by micoorganisms. These reduction/oxidation processes include denitrification/nitrification, manganese or iron reduction/oxidation, sulphate reduction/sulphide oxidation, and methanogenesis /methane oxidation. Radiatively active gases (e.g., CO_2, N_2O and CH_4) are among the products or by-products of these reactions and are of concern in relation to climate change (Nykänen *et al.*, 1995). The above reactions are found in upland or aquatic ecosystems but the unique property with respect to wetlands is that they occur together in close proximity or rapidly change from one to another.

Secondly, inextricably linked to the above, are decomposition and mineralization processes, which in wetlands are quite different from those of the upland grassland

systems. In northern peatlands, carbon (sequestered by photosynthesis) accretes due to exceptionally low decomposition rates (Clymo, 1983). As mentioned above, this has been attributed to waterlogging (and the consequent decrease in redox potential), low temperature, low pH, and/or low nutrient availability (Dickenson, 1983; Updegraff *et al.*, 1995 etc.). Nutrients such as nitrogen, phosphorus and calcium are 'locked up' within the peat matrix and the mineralization rate is typically retarded in comparison to upland soils. From a global perspective, northern peatlands therefore represent a substantial carbon sink. However, in systems such as temperate riparian wetlands where accumulation of OM is minimal (therefore, mineral soils predominate) and productivity/decomposition is higher than upland forest (Archibold, 1995), this may not be applicable.

Thirdly, at the landscape scale, wetlands represent important transformers in nutrient cycling. Many types of wetlands (e.g., riparian, marshes, estuarine wetlands, etc.) are hydrologically open and various chemical transformations are carried out in the wetlands allowing the transport of chemicals from/to adjacent ecosystems. The hydrochemistry of passing water can be altered substantially through wetlands and hence the biogeochemistry of adjacent streams, rivers or even the oceans can be influenced (Chen, 1999; Lugo *et al.*, 1989). Generally, wetlands represent a source for organic carbon and a sink for mineral nutrients (N, P, Ca, etc.) (Mitsch & Gosselink, 2000) however, this can depend on wetland types and even on seasons within a single wetland.

1.03 FORMS OF CARBON

In general, wetlands and stream water contain three principal forms of carbon; DOC, particulate organic carbon (POC) and dissolved carbonates (the former being the focus for attention here because this is the mode *via* which nearly all carbon is exported from wetlands (Hope *et al.*, 1994)). Wetland and riverine organic carbon can be divided into three categories depending on origin (modified from Hope *et al.*, 1994):

1. allochthonous sources derived from terrestrial OM,
2. an autochthonous pool from *in situ* biological production , and
3. an anthropogenic pool derived from agricultural, domestic and industrial activities (Degens, 1982).

Traditionally dissolved organic matter (DOM) was determined by chemical oxidation, however many recent studies have used automated carbon analysis by water sample combustion. In the first instance DOM is reported, in the latter case DOC. The two can

be interchangeable provided DOM contains 45-50% organic carbon by mass. DOC is an operational definition and is separated from POC by passing the sample through a 0.45-0.5 μm filter, leaving the latter fraction behind (Thurman, 1985). DOM and particulate organic matter (POM) are differentiated on a similar basis. Filtration is also commonly used to subdivide the POM into size classes. Usually these are:

1. Course (CPOM) > 1 mm,
2. Fine (FPOM): 1 mm-53 μm, and
3. Very Fine (VPOM): 53 μm-0.45-0.50 μm.

Plant litter, algal debris, invertebrates, eroded soil, OM and detritus are all components of POM. In practice though, it is probable that the dissolved fraction also contains some colloidal OM, viruses and smaller bacteria. Allen (1995) suggests that colloidal OM has a size range of 0.01-0.5 μm. Such matter usually comprises 20% of the dissolved fraction, rising to 50% in some Canadian rivers (Lock *et al.*, 1977). Similarly, Allen (1995) found 29-53% of total DOM was made up of this fraction in a range of Canadian waters, although according to Thurman (1985) the colloidal fraction accounts for less than 10% in most cases.

Natural DOC consists of a range of molecules, from a variety of sources, in a complex composite with a continuous range of sizes (figure 1.02). Humic substances (which constitute around 50% of the total DOC pool (Alarcon-Herrera *et al.*, 1994)) arise from the associations of plant and animal debris transformation products (Tombácz, 1999) during humification. There is a huge variety of multifunctional source structures and so humic substances can be thought of as consisting mainly of cross-linked components giving rise to fractal-like entities (Tombácz, 1999). Chemical characteristics can be used to further classify such matter (Thurman, 1985). Fulvic and humic acids make up the bulk of the dissolved fraction (50-70%), accounting for 5-10% of the total anion load in streams and rivers (Thurman, 1985). Humic compounds, particularly humic acids, are the cause of much of the colour present in many rivers (Visser, 1984), bogs and wetlands (Thurman, 1985).

The classical fractionation technique for the separation of fulvic and humic acids from soil organic matter (SOM) involves dispersion by sodium hydroxide (NaOH) or sodium pyrophosphate ($Na_4P_2O_7$). The fraction not dispersible by the peptizing action of the sodium, the chelating action of the pyrophosphate, and the hydrogen bond-breaking activity of very alkaline pH values is referred to as humin (Paul & Clark, 1989). The

term 'humic acid' is given to the dispersible materials precipitated at acidic pH values and those which remain in solution are known as fulvic acids (Schnitzer & Khan, 1978). Due to the randomness of formation, a uniform behaviour is not expected and only trends can be predicted for the properties of humic substances (Tombácz, 1999). There is still extensive debate as to whether humic substances, especially humic and fulvic acids, are large macromolecules or associations, aggregates or micelles of relatively small molecules (Senesi, 1999). The molecular weight values recorded for humic substances range from a few hundred Daltons (Da) for certain fulvic acids to over a million Da for some humic acid fractions (Table 1.01).

Table 1.01. Molecular weights reported for humic substances*

Method	Type of material		
	Humic acid	Fulvic acid	Unspecified
Ultracentrifugation			
Sedimentation-diffusion	53 000-100 000†		
Sedimentation-viscosity	22 000-28 000		
Equilibrium sedimentation	24 000-28 000		
Viscosimetric properties	36 000		
Freezing-point depression	25 000	640-1000	670-1680
Osmotic pressure		951§	47 000-53 800
Light scattering	65 000-66 000		
Small-angle X-ray scattering	200 000-1 000 000		

* Measured in Daltons (Da). From Senesi (1999) adapted from Stevenson (1994).
† Range of 2000-1 360 000 Da for highly fractionated samples.
§ Range of 275-2110 Da following fractionation by gel filtration.

Fulvic acids

Fulvic acids are generally described as being low molecular weight materials (1000-30 000 Da) that remain soluble at pH 2 when extracted by NaOH. They are composed of highly oxidized aromatic rings with numerous side chains. Phenolic acids and benzene carboxylic acids are fundamental units held together primarily by hydrogen bonding or van der Waals' forces and ionic bonding (Paul & Clark, 1989). X-Ray analysis, electron microscopy and viscosity measurements suggest that fulvic acids have a relatively open, flexible structure perforated with voids of various dimensions. Organic and inorganic compounds that fit into the voids can become trapped provided the charges are complementary. The fulvic acid fraction tends to contain a large amount of polysaccharide in addition to low molecular weight fatty acids and cytoplasmic constituents of microorganisms. Fulvic compounds are said to be linear, flexible colloids at low concentrations, and spherical colloids at high solution concentration and

low pH. Their associations with each other and with soil inorganic constituents governs their shape in nature.

Humic acids and humin

Humic acids are composed of higher molecular weight materials (10 000-100 000 Da) extractable from soil by dilute alkali but precipitable at pH 2. These materials contain aromatic rings and nitrogen in cyclic and peptide chain form. As such materials are formed by the polycondensation of similar but not identical constituents, rather than enzymes (as is the case for cellulose and protein), no two molecules will be identical (Paul & Clark, 1989). There is some evidence from ^{13}C-Nuclear Magnetic Resonance (^{13}C-NMR) spectroscopy (Aiken *et al.*, 1985) that SOM is not as aromatic in nature as previously was proposed (e.g., Stevenson, 1994). However, structures tend to be depicted with polyaromatic and non polyaromatic units held together by ether linkages, cyclic nitrogen and hydrogen bonding. Purified humic acids contain little carbohydrate, while carbon is said to make up around 57% of humic acids and there may be up to 4% nitrogen (Paul & Clark, 1989). The primary functional groups are COOH, phenolic OH, alcoholic OH groups and a small amount of ketonic oxygen (Paul & Clark, 1989). The absorbance of humic acids over the wavelength range of 250-350nm has been found to decrease with increasing fraction size in humic acids derived from peat (Tombácz, 1999). As the nominal size fractions increased, the ratio of absorbance at 465 and 665nm (the E4/E6 ratio) was also found to decrease significantly from 6.6 to 14.7. This perhaps indicates a systematic change in chemical composition, since this parameter is related inversely to the degree of condensation of aromatic groups or the molecular weight (Rao & Choppin, 1995). Tombácz (1999) also looked at fluorescence emission spectra at several excitation wavelengths and found significant differences in the emissions of the humic acid fractions. The smaller the size fraction the greater the fluorescence emission. Cross Polarization Magic Angle Spinning (CPMAS) ^{13}C-NMR was used to study the differences in chemical composition of the humic acid fractions. Aliphatic character was found to predominate in the unfractionated peat humic acid and became more pronounced in the largest sized fractions. Aromaticity was seen to increase with decreasing fraction size. Similarly, the abundance of functional groups (O-containing groups) increased as molecular sizes decreased.

Humates are adsorbed to clay minerals by polyvalent cations (e.g., Ca^{2+} & Fe^{3+}) and by association with hydrous oxides, either *via* coordination (ligand exchange) or by anion exchange through positive sites which exist on iron and aluminium oxides at pH values below 8 (Paul & Clark, 1989).

The non-NaOH-dispersible fraction of SOM is referred to as humin. Upon fractionation, the majority of soils produce relatively equal amounts of fulvic acids, humic acids and humin. Removal of those materials that NaOH failed to extract, along with further extraction, suggests that humin is composed of fulvic acids plus humic acids, in addition to insoluble plant and microbial components such as undecomposed cellulose, ligniferous matter, microbial cell walls and some charcoal (Paul & Clark, 1989).

1.04 WETLAND CARBON SOURCES

Atmospheric inputs of organic carbon to the catchment are generally low in relation to internal fluxes (Hope *et al.*, 1994). The organic carbon in precipitation tends to be in dissolved form, in concentrations between 1 and 10 mg L^{-1} (Likens *et al.*, 1982; Thurman, 1985) and of low molecular weight compared to soil and stream water (Cole *et al.*, 1984; Lock & Ford, 1986). The main inputs of carbon to wetlands are photosynthesis by *in situ* plants, algae and bacteria, although allochthonous carbon input could be substantial depending on the wetland type (and season within a single type).

The term photosynthesis generally refers to any chemical combination aided by light but if used without qualification is taken to mean the reaction by which carbohydrates are synthesized from CO_2 and water, using sunlight as the energy source and chlorophyll as the catalyst (Krauskopf & Bird, 1995). The process can be summarized as:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

In reality it is much more complex. Photosynthesis ultimately provides virtually all of the OM in plants and animals including carbohydrates, fats and proteins (Krauskopf & Bird, 1995).

Plants consist of 15-60% cellulose, 10-30% hemicellulose, 5-30% lignin and 2-15% protein but it is difficult to separate plant residue and SOM decomposition (Paul & Clark, 1989). Plant residue decomposition and SOM formation have been reviewed by Flaig *et al.* (1975), Stevenson (1994) and Tate (1987). Cellulose (the earth's most abundant plant residue) is frequently associated with hemicellulose and lignin (Paul & Clark, 1989). It is described as being composed of glucose units with ß(1-4) linkages, occurring in a semi crystalline state and with a molecular weight of 10^6. Unbranched chains of glucose residues are cross-linked by hydrogen bonds. Lignin is a complex

aromatic polymer comprising *circa* 25% of the land-based biomass on earth (Harwood & Parales, 1996) and can form 30% of the total dry mass of peat in the earlier stages of humification, rising to 50-60% in the later stages (Clymo, 1983). The monomeric phenylpropane units in lignin are linked by several different carbon-carbon and ether linkages (rather than a single intermonomeric linkage), most of which are not readily hydrolysable. Thus, lignin is more persistent in the environment than cellulose (Krauskopf & Bird, 1995).

While much of the nutrients and labile organic compounds are efficiently metabolized, retained and recycled by the microbiota, a large proportion of the plant OM is exported as relatively recalcitrant DOM (Wetzel, 1979, 1990). Despite the fact that algae can synthesize relatively recalcitrant organic compounds, these are produced in much greater quantities in the structural tissues of higher plants (Wetzel, 1992). Hence, virtually all of the humic loading to fresh waters (including the wetland) is dissolved organic compounds derived from partial decomposition of plant tissues (Wetzel, 1992). These plant derived humic and phenolic compounds form a major component of the dissolved organic acids of fresh waters, which frequently constitute up to 80% of the total DOM (Wetzel, 1983, 1984). Concentrations of 4-8 mg L^{-1} of organic acids or more are common (Perdue & Gessing, 1990) and such materials are considered later in relation to enzymatic inhibition, with particular reference to phenolic compounds. Phenolic substances can occur free, as glycosides in vacuoles or as esters of plant cell wall structures (Wetzel, 1993). Phenols, polyphenols and polymeric phenols act as intermediates in humic compound formation (Wetzel, 1993). Condensation and polymerization transformations are microbially mediated leading to the formation of these more stable fulvic and humic acids (Wetzel, 1993). Although actively degraded, the more recalcitrant organic compounds of plant origin have slow turnover rates, persisting in the dissolved phase for a considerable amount of time. Average decomposition rates of 0.1 to 1% per day have been reported and phenolic acids usually dominate this pool (Wetzel, 1984, 1991).

1.05 DECOMPOSITION AND MINERALIZATION OF ORGANIC MATTER

Microorganisms convert organic materials to CO_2, completing the biological carbon cycle that was initiated with photosynthesis (Paul & Clark, 1989). In the wetland ecosystem key biogeochemical processes are facilitated *via* decomposition. Decomposers are responsible for the majority of the nutrient replenishment for example, by mineralization. The continuous microbial quest for the energy tied up in the C-H bond is the driving force behind virtually all the nutrient cycling reactions

involving organic compounds in soils and sediments (Paul & Clark, 1989). When OM is introduced to wetlands, decomposition by various microorganisms and fauna occurs. During this biodegradation process, carbon compounds are either released as organic acids (e.g., acetate & lactate), CO_2, CH_4, or incorporated into biomass. Transformation of inorganic materials (i.e., N, P, S, Ca, K, Mg and other micronutrients) among several forms takes place and eventually these are taken up by plants and microorganisms, or lost from the system either as a solute or a gas. Decomposition serves two major functions: the mineralization of essential elements and the formation of SOM (Swift *et al.*, 1979). Decomposers, physico-chemical environment (i.e., climate or edaphic conditions) and resource quality (e.g., composition of soluble carbohydrate, cellulose, hemicellulose, and lignin) are the three primary factors said to regulate decomposition rates (Coûteaux *et al.*, 1995).

In aerobic conditions (where there is ample free oxygen), organic carbon is used as an energy source and released as CO_2. This reaction is essentially the reverse of photosynthesis, with CO_2 and water as the end products (Krauskopf & Bird, 1995). Under anaerobic conditions (in the absence or near absence of oxygen), fermentative metabolism is dominant resulting in the production of various low molecular weight organic acids, alcohols, and CO_2 (Ponnamperuma, 1972). These, in turn, can be utilized by methanogenic bacteria, which produce CH_4 as an end product (Atlas & Bartha, 1987). Anaerobic decay is complex and can take many directions depending on temperature, the nature of the original material and the degree of exclusion of oxygen (Krauskopf & Bird, 1995). The bacterial assemblage is also important which may act not only on the OM itself but also other substances, for example, in the reduction of sulphur from SO_4^- to form pyrite (Krauskopf & Bird, 1995).

The original input of organic carbon is mainly of high molecular weight (e.g., cellulose, hemicellulose, lignin etc.) in either of the two decomposition pathways and thus the degradation of these materials into utilizable monomers is often the rate-limiting step in carbon cycling (Chróst, 1991). Extracellular hydrolysis (see below) has been reported as a critical step in the decomposition and nutrient cycling in wetlands for two reasons. First, there are generally insufficient nutrients from other sources (i.e., weathering of parent rocks and atmospheric deposition) for the requirements of the vegetation and microorganisms. Secondly, microorganisms and plant roots in wetland systems tend to be impermeable to high molecular weight OM (Geller, 1985) with which most of the essential nutrients are combined (e.g., cellulose, protein, urea, or phospholipid) (Paul & Clark, 1989). Therefore, the extracellular hydrolysis rate is substantially slower than

the rate of uptake of low molecular weight substrates, implying the importance of the former as a rate-limiting step in nutrient cycling (Hoppe *et al.*, 1988).

1.06 ENZYMES

Definition and origin

Soil enzymes are biological catalysts that function in soil ecosystems (Dick & Tabatabai, 1993) and are largely of microbial origin but also include contributions from plants and animals (Ladd, 1978). The term 'soil enzymes' traditionally referred solely to 'abiontic' enzymes i.e., those not affiliated with living cells (Skujiňs, 1976). Since later studies have shown the importance of microorganisms as the main producers, the soil enzyme concept was extended to include both abiontic and microbial enzymes (Ladd, 1978). Abiontic enzymes can be temporally or spatially displaced from the microorganisms that synthesized them, representing a functional legacy to the community (Nannipieri *et al.*, 1983). Burns (1982) separated soil enzymes into categories according to their location in the soil, and proposed a possible role for abiontic enzymes. The proposed categories were enzymes affiliated with 1) living cells (cytoplasmic, periplasmic, or cell-attached enzymes), 2) dead cells (leaked or cell-attached enzymes), 3) non-proliferating cells, 4) dissolved enzymes (free or substrate-associated enzymes), and 5) immobilized enzymes (clay-adsorbed or humus-bound enzymes). He further postulated that immobilized enzymes play a role in substrate catalysis as a microbial signal for the initiation or repression of extracellular enzyme production. An analogous situation to that of the soil can be found in aquatic biofilm (Lock *et al.*, 1984), where extracellular enzymes are mainly associated with bacteria and fungi but also algae & protozoa. Chróst (1991) defined extracellular enzymes in aquatic ecosystems associated with viable cells as 'ectoenzymes'. These are extracellular enzymes bound to the cell surface of microorganisms or found in the periplasmic space in Gram negative bacteria (usually predominant in aquatic environments) that act outside the cell (Chróst, 1990). In this study, the term 'extracellular enzyme' refers to the cell-bound enzymes defined by Chróst (1990) but also includes the free enzymes that may be released into the environment (within the soil or biofilm polysaccharide matrix (PSM) (see later) and/or bound to detritic particles). This is because extracellular enzymes may remain active (80% of their initial activity) for at least three days after being detached from the cell (Decho & Herndl, 1995) and some studies suggest much longer periods, for example, Kiss *et al.* (1975) suggest a year or more.

Various techniques can be employed to determine the source of these enzymes, such as sterilizing treatments, for example toluene (Frankenberger and Johanson, 1982), azide (Tena *et al.*, 1986), antibiotics (Nannipieri *et al.*, 1983), fungicide (Nakasa *et al.*, 1987) or γ-radiation (Lensi *et al.*, 1991). These treatments can eliminate certain microbial populations responsible for the production of enzymes (e.g., applying fungicide would remove fungal inputs provided the treatment was efficient), thus revealing the contributions from different sources.

Importance of extracellular enzymes

Extracellular enzymes are the primary mechanisms for polymeric & macromolecular OM degradation, generating low molecular weight molecules that can cross the bacterial cell membrane (Rogers, 1961). The rate of OM decomposition is dependent on this enzymatic activity (e.g., Lock, 1990), which therefore plays a key role in carbon cycling within the peatland (Freeman *et al.*, 2001a; Freeman *et al.*, 2001b) and aquatic environments (Chróst, 1994; Turley, 1994). Such activity prevents the excessive accumulation of detrital OM in the environment, supplies photosynthetic organisms with nutrients and is an indication of the growth and development of bacterial communities (Gajewski & Chróst, 1995). Bacterial uptake of dissolved organic molecules is governed by molecular size and composition (Kaplan & Bott, 1983). Microorganisms generally are unable to assimilate organic molecules over 1000 Da (e.g., Confer & Logan, 1998) and preferentially utilize low molecular weight molecules (Meyer *et al.*, 1987). Peatlands are perceived as an accumulation of high molecular weight, refractory, aged organic carbon (c.f. Clymo, 1983; Freeman *et al.*, 2001 a, b) and similarly less than 10% of the DOM in rivers is composed of low molecular weight, labile compounds (Thurman, 1985). However, extracellular hydrolysis of autochthonous, low molecular weight algal exudates may also be necessary prior to cellular uptake (Lancelot, 1984) as may be true in the case of plant exudates in the peat system.

Extracellular enzyme activities studied in this thesis

The carbon cycling enzymes phenol oxidase and ß-glucosidase (or cellobiase) are the focus of attention in this study due to the large amounts of their substrates (lignin and cellulose respectively) in the wetland environment.

Cellulose is hydrolysed to glucose by the enzyme complex cellulase. A variety of bacteria (e.g., *Pseudomonas*, *Chromobacterium*, *Bacillus*, *Clostridium*, *Streptomyces* and *Cryptophaga*) and fungi (including *Trichoderma*, *Chaetomium* and *Penicillium*)

can degrade cellulose using cellulases (Paul & Clark, 1989), although less may be able to degrade the original crystalline form (Shewale, 1982). It is widely accepted that at least three types of cellulase enzyme exist: exo-ß-1,4-glucanase or ß-1,4-glucan cellobiohydrolase (EC 3.2.1.91), endo-ß-1,4-glucanase or ß-1,4-glucan glucanohydrolase (EC 3.2.1.4) and ß-glucosidase or cellobiase (EC 3.2.1.21), and that all are required to degrade crystalline cellulose. The exo-enzyme exo-ß-1,4-glucanase hydrolyses the cellulose chain by removing cellobiose from the non reducing ends (e.g., Eriksson & Pettersson, 1975a, b; Selby, 1968). The cellulose chain is cleaved at random internal points by endo-ß-1,4-glucanase, increasing the number of chain ends available for exo-ß-1,4-glucanase activity (Eriksson & Pettersson, 1975a; Wood, 1975). Prolonged endo-glucanase activity produces cellobiose and higher cellodextrins, with ß-glucosidase catalyzing the hydrolysis of cellobiose and cellodextrins to glucose (Shewale, 1982). Small oligomers containing ß-D-glucose linkages (such as those from algal excretion) are degraded by the cleavage of the terminal ß-D-glucose residue (Desphande & Eriksson 1988). Such activity might be especially relevant since its end product, glucose, is energetically important for the microbial community (Chróst, 1990). Exo- and endo-glucanases are inhibited by cellobiose (e.g., Halliwell & Griffin, 1973; Wood, 1975) and therefore ß-glucosidase plays a critical part in large scale sacchrification by removing it (Shewale, 1982). ß-glucosidase activity is an indicator of cellulose decomposition (Sinsabaugh *et al.*, 1991) and of carbon mineralization rates (McLatchey & Reddy, 1998; Sinsabaugh *et al.*, 1991) thus, assays to determine activity provide a useful way of determining changes in carbon cycling.

A large proportion of peat is composed of lignin (up to 60%, Clymo, 1983) and the general class of enzymes referred to as phenol oxidases have the ability to attack the relatively recalcitrant aromatic molecules (McLatchey & Reddy, 1998) that make up this three dimensional polymer. The white-rot fungi are the most active degraders of lignin (producing CO_2 and water) but only in the presence of adequate oxygen (Paul & Clark, 1989). *Coriolus versicolor* decompose the aromatic ring, methoxyl, or longer side-chain components and *Pleurotus ostreatus* along with *Phanerochaete chrysosporium* also cause complete degradation (Paul & Clark, 1989). Brown-rot fungi (e.g., *Poria* and *Gloeophyllum*) decompose the polysaccharides associated with lignin, removing the CH_3 sub-groups and R-O-CH_3 side chains, but leave phenol behind (Paul & Clark, 1989). In wet situations, the soft-rot fungi are important (*Chaetomium* and *Preussia* being representative organisms (Paul & Clark, 1989)). Lignin is also decomposed by actinomycetes such as *Streptomyces* and *Nocardia* as well as aerobic, Gram negative bacteria such as *Pseudomonas* and *Azotobacter* (Paul & Clark, 1989).

Phenol oxidase was recently recognized as a major regulator of carbon storage in organic rich northern soils (Freeman *et al.*, 2001a). Since then (as a result of work associated with this study) it has also been shown that the activity of phenol oxidase and ß-glucosidase may generate dissolved phenolic compounds and DOC respectively from the peat matrix and that warming as a result of climate change can stimulate activities, thus increasing DOC production in peatlands (Freeman *et al.*, 2001b).

Also included in this thesis are sulphatase and phosphatase because they provide an indication of the mineralization of organic sulphur and phosphate compounds respectively (Sinsabaugh *et al.*, 1991). Enzyme activities directed towards the acquisition of carbon, nitrogen and phosphorus may shift under a changing climate. Any increase in nutrient cycling (c.f. Zak *et al.*, 1993) may stimulate plant growth, leading to increased carbon inputs to the wetland system and providing positive feedback to rising DOC concentrations. Conversely, reduced nutrient cycling may cause a negative feedback to plant growth (Diaz *et al.*, 1993; Zangerl & Bazzaz, 1984) and therefore DOC production. Furthermore, in wetland soils, OM decomposition can proceed through a pathway that involves both carbon and sulphur cycles, the fermentation: sulphate reduction pathway (Howes *et al.*, 1984). In order to convert sulphate to sulphide, sulphur-reducing bacteria require an organic substrate, usually of low molecular weight, as an energy source and fermentation can conveniently supply such compounds (e.g., lactate) (Mitsch & Gosselink, 2000). Generally, oxidation of organic carbon by CH_4 production is dominant in freshwater wetlands, whereas in saltwater wetlands it is sulphate reduction that dominates (Capone & Kiene, 1988).

Controlling variables

Numerous regulatory factors control soil enzyme activities, interacting over a wide range of spatio-temporal scales. Microbial activity or species composition regulates enzyme production at the ecosystem level, in the long term, and this in turn is determined by temperature, water content, nutrient availability and/or vegetation type (e.g., Insam, 1990). Conversely, enzyme activities are functions of edaphic conditions such as temperature, inhibitors (phenolic compounds and iron, for example), activators (e.g., magnesium and calcium), and redox potential, at a higher resolution (such as the microenvironment), in the short term. Phenolic compounds are of particular importance in the peatland system in comparison to other ecosystems due to the relatively high concentrations.

Hydrolytic enzymes catalyze the cleavage of covalent bonds such as C-O (esters and glycosides), C-N (proteins and peptides) and O-P (phosphates) and often these enzymes can also affect synthesis by condensation reactions. The cleavage of C-C bonds however, is relatively rare and hydrolytic enzymes do not react with aromatic nuclei of phenolic compounds, though they react with phenolic hydroxyl and other substituted functional groups that often occur in phenolic compounds (Wetzel, 1991). As mentioned above, polyphenolic compounds form a major component of the dissolved organic acids of freshwaters and these substances can induce precipitation of proteins by binding to one or more sites on the protein surface to give a monolayer that is less hydrophilic than the protein itself (Hazlam, 1988; Hazlam & Lilley, 1988; Spencer *et al.*, 1988). Aggregation and precipitation follow, and enzyme activities are substantially inhibited (e.g., Ladd, 1985). More aromatic and condensed molecules can distort bound enzymes to a greater extent than the simpler compounds (e.g., fulvic acid) because of a more rigid structure (Ladd & Butler, 1975). Any increase in phenolic compound concentrations as a result of climatic changes therefore has the potential to reduce DOC decomposition, either in the wetland or the recipient aquatic system, allowing its accumulation.

Plant polyphenols complex protein molecules very effectively due to their polydentate ligands (Wetzel, 1991). Many phenolic groups and aryl rings on the periphery of the molecule mean such ligands possess numerous potential hydrogen binding sites (Beart *et al.*, 1985). Polyphenolic compounds have the molecular size and structure to form stable cross-linked structures with several different protein molecules (Wetzel, 1991). Enzyme inhibition is of the classical non-competitive type in which the polyphenol inhibitor and substrate bind simultaneously to the enzyme (Wetzel, 1991). Partial or entire inactivation of the enzyme can ensue as a result of the formation of polyphenolic-enzyme complexes. The reaction is often reversible by means such as UV photolytic degradation of the phenolic substances or digestion by other enzymes. The formation of enzyme complexes as a result of polyphenolic and related DOM loading to an aquatic system can represent a dominant regulatory mechanism of phosphorus and hence photosynthetic activity and respiratory metabolism (Wetzel, 1991).

Those phenolics that include an array of plant substances which posses an aromatic ring with one or more hydroxyl substituents are said to be especially important for complex formation. In this case, these are predominantly phenolics of structural tissue origin (phenols, phenolic acids, phenylpropanoids and polymers) because of the large quantity of these materials received from wetland and littoral sources. Other phenolic

compounds (e.g., flavanoid and other pigments, flavanols, flavones) are less significant in quantitative terms but may have important regulatory functions in some instances (Wetzel, 1991).

Different phenolic compounds differ in their reactivity with proteins. Phenylpropanoids (e.g., ρ-hydroxycinnamic, ρ-coumaric, caffeic, and ferrulic acids), for example, are more reactive with proteins than some simple phenols and phenolic acids (e.g., ρ-hydroxybenzoic acid) (Wetzel, 1991). Hydrolysable tannin polymers (gallo- and ellagitannins) esters of gallic and diphenic acids with glucose strongly complex proteins. Protonated amino groups and other positively charged groups can bind electrostatically with the negatively charged, ionized organic acids. Organic acid adsorption increases as their charge decreases and their molecular weight and hydrophobic properties increase (Perdue & Gessing, 1990). A large percentage of the carboxylic acid groups will be ionized at the pH of most natural waters (pH 5-8) (Wetzel, 1991). Complex formation with enzymes, and therefore inactivation, can thus be reduced by the reaction between organic acids and the major cations (e.g., Ca^{2+}). Humic acids in both soils and freshwaters are known to reversibly bind with enzymes by cation exchange mechanisms (Ladd, 1972; Ladd & Butler, 1970; Tipping *et al.*, 1988). Enzymes can be displaced from complexes by high concentrations of inorganic (particularly divalent) cations by the latter binding to the humic acid carboxyl group where the enzyme would bind.

Determination of enzyme activities

Two approaches can be used to determine soil enzyme activities. One considers the soil as a whole system and introduces a substrate into soils to determine its fate, or that of the product. Valuable information can be gained on process rates by this method and it has the operational advantage that it is simple to conduct for large numbers of samples. Since ecological studies require analysis of multiple samples and information on the system as a whole, this approach is often employed (e.g., litter decomposition assessment). However, the clear elucidation of enzyme activities in soils is confounded by the heterogeneity of soils and the various statuses of enzymes. For example, adsorption of substrates or products, the presence of inhibitors, low substrate diffusion rates, non-specific reactions of different enzymes with a single substrate, or contributions from several isoenzymes, can all affect the results. Such problems can be avoided by the alternative, involving extraction and/or purification of enzymes from a soil matrix by various extractants or biochemical procedures (e.g., Nannipieri *et al.*,

1974). This approach is generally used by those interested in the specific characteristics of a particular enzyme and can provide detailed information about the enzymes in soils (e.g., determination of isoenzymes, or fractionation of free and humus-bound enzymes). However, this method also has its limitations because soil enzymes bind to soil humus to varying extents and through various mechanisms, hence it is difficult to obtain large quantities of purified enzymes. Furthermore, natural soil structure can be destroyed in the extraction or purification step. Thus, enzyme activities determined in this way rarely reflect the natural process rates in the field.

In this study, the former approach (using fluorometric and colorimetric techniques) has been used for the determination of enzyme activities in peatlands, coupled with incubation conditions that are as close to those found in the environment as is practicable. Given the oligotrophic nature of the systems studied here and the cool temperatures along with other features that constrain enzyme activities, this approach was favoured over that of reporting potential enzyme activities (using optimum conditions for incubation periods).

1.07 TRANSFORMATION AND DEGRADATION OF ORGANIC CARBON IN THE RIVER SYSTEM

Several studies identify wetlands as being the major contributor of organic carbon to river water within a catchment where they exist (e.g., Hope *et al.*, 1997; Wetzel, 1992). Wetlands dominate the export of DOC to the receiving waters, releasing more DOC per unit area than any other major biogeographic type in the world, estimated at 20% of the total flux to the oceans (Lugo *et al.*, 1989). Riverine DOC is thought to contain significant amounts organic carbon consistent with the age of terrestrial inputs (Palmer *et al.*, 2001). Due to the predominance of small, shallow freshwater bodies, most DOC of lacustrine and riverine ecosystems is derived from the photosynthesis of plants and microflora associated with detritus (including sediments) and is only augmented by photosynthesis of phytoplankton (Wetzel, 1992). Partial utilization of dissolved organic compounds generated in the wetland and littoral interface regions, as they move toward the open water, effects a selective increase in organic recalcitrance (Wetzel, 1992). The transformation/degradation processes of carbon in the wetland are also applicable to the stream ecosystem (Wetzel, 1992) and are considered below. Indeed, in many cases it may be difficult to determine where a wetland ends and a stream begins with the former perhaps being an extreme of the latter (or *vice versa*). Biological, physical, and

chemical processes are all capable of modifying both the flux and the composition of organic carbon.

Biological processes

Breakdown of particulate organic matter

Particulate organic matter in streams is decomposed by mechanical comminution and stream biota, the latter being widely thought to be especially important. Hope *et al.* (1994) describe an ordered decomposition process, beginning with the rapid leaching of fresh plant matter. Microorganisms, particularly fungi, then colonize the particles. Course particles are broken down by crane fly, caddis fly and other such shredders, which excrete up to 60% as faeces following ingestion (Moss, 1988; Swank, 1986). Fine particles are consumed by filter feeders and collectors while algae are removed from exposed surfaces by scrapers. This activity causes comminution of POM and nutrient mineralization, with the potential for significant contributions of DOC to stream water (Meyer and Tate, 1983).

Oxidation and respiration

Carbohydrates, amino acids and phenolic compounds of a reactive nature are produced as a result of the continuous biotic oxidation of both POM and DOM by microorganisms and invertebrates within the stream (Degens, 1982). Carbon can therefore be exported to the atmosphere directly, *via* oxidation and respiration of CO_2, and this was recently recognized as an important flux to the atmosphere in the global carbon cycle (Richey *et al.*, 2002). Microbial oxidation can account for DOC levels of 4-20 mg C L^{-1} in the interstitial water of aerobic sediments, whereas when anaerobic conditions prevail (e.g., in bogs) such concentrations can be 10-30 mg C L^{-1} (Thurman, 1985).

Immobilization

Stream biota may be involved in the transitory storage of carbon within the stream by the conversion of DOC to POC and back again. The biofilm (or epilithon) is important in lotic OM processing because of the high wetted surface area to water volume ratio (Sinsabaugh & Linkins, 1988). Numerically, epilithic biofilm are dominant by two or three orders of magnitude (Geesey *et al.*, 1977; 1978) and metabolically they are an order of magnitude more active (Ladd *et al.*, 1979). It has been shown that as much as 75% of DOC additions to a river can be removed within a 30m stretch (Hynes *et al.*, 1974). These assemblages of algae, bacteria, fungi and protozoa form the primary site for the processing of removed carbon (Mickleburgh *et al.*, 1984) and occurs on all

wetted surfaces (Lock, 1993). It is bound by a gelatinous PSM, which is said to attract charged molecules (anions and cations) and function as an ion exchange system. Concentration and retention of compounds (especially those of high molecular weight) tends to occur due to a reduced diffusion rate within the biofilm (Lock, 1981). The close proximity of autotrophic and heterotrophic components allows efficient cycling of photosynthetically fixed carbon, with extracellular degradative capacity potentially being conserved within the PSM (Lock, 1981; Lock *et al.*, 1984). It is assumed that material from the biofilm is transferred to the stream ecosystem *via* scrapers, grazing and progressive sloughing to give fine POM. However, the 'microbial loop' (the trophic system found to be separate from the typical photosynthetic organisms-grazers food chain) could be responsible for the processing of a considerable amount of OM (Azam *et al.*, 1983; Ducklow, 1994).

Increasing the low molecular weight component of DOC tends to enhance heterotrophic metabolism (e.g., Kaplan & Bott, 1983). Conversely, recalcitrant, high molecular weight compounds (Freeman *et al.*, 1990; Freeman & Lock, 1992) & humic compounds (Wetzel, 1993) in these waters may inhibit the metabolic activity of the biofilm heterotrophs. 'Brown water' rivers (such as those that drain peatlands) are usually rich in such materials and are especially likely to exhibit such a phenomenon (Freeman & Lock, 1992). Some researchers, however, have demonstrated decomposition of some humic materials, despite their high molecular weight. And, in addition to the immobilization of low molecular weight material, Fiebig and Lock (1991) found that of the DOM removed from solution 10-26% was of high molecular weight. Furthermore, Amon and Benner (1996) demonstrated that a large proportion of high molecular weight DOC compounds are more fresh and bioreactive than those of low molecular weight in oceanic environments, therefore composition could be more important than molecular weight. Changes in the nature of organic loading to the stream, as a result of climate change, are important in terms of DOC concentrations because altered biofilm activity could modify carbon uptake and processing within the aquatic system.

Production in-situ

DOC can originate from biogenesis within the stream (known as an autochthonous source). The principal in-stream sources are the epilithon, aquatic plants, suspended bacteria, phytoplankton, periphyton, bryophytes, invertebrate excretions and microflora associated with detritus (e.g., Wetzel, 1992). Three categories of autochthonous material are identified:

1. Secretions of living organisms, such as those from algae (e.g., organic acids, polysaccharides, polypeptides and vitamins) (Brock, 1966).
2. Soluble OM released from dead organisms. Depinto and Verhoff (1977) found that 20-50% of the organic substances in cells may be released immediately.
3. Bacteria, viral particles and other 'viable biogenic material' of colloidal nature (Mill, 1976).

Carbon transfer from primary to secondary producers is facilitated by algal exudates (e.g., Chróst, 1984; Coveney & Wetzel, 1989; Kaplan & Bott, 1982) or by algal cell lysis (Haack & McFeters, 1982a; Lovell & Konopka, 1985). A proportion of carbon assimilated by algae during photosynthesis is released (Bjoernsen, 1988; Sell, 1994) and this is related to the availability of inorganic nutrients (Margalef, 1997). Such algal exudates produce a nutrient-rich zone around themselves that is likely to enhance attached bacterial growth (Murray *et al.*, 1986). Haack and McFeters (1982b) reported that a heterotrophic population may only utilize OM from photosynthesizing cells as a carbon source, and algal exudates were found to support 45% of epilimnetic bacterial production in an oligotrophic lake (Larsson & Hagstrom, 1982). In eutrophic waters such exudates are less important (Brock & Clyne, 1984).

Algal exudates contain low molecular weight compounds (Bjoernsen, 1988) including carbohydrates, lipids, peptides, organic phosphates, volatile substances, vitamins, toxins and antibiotics (Fogg, 1966). Glycollic acid is the major component in phytoplankton excretion (Fogg, 1977) and is also excreted by cyanobacteria (Fründ & Cohen, 1992). These exudates are regarded as labile carbon sources (Fogg, 1966; Hellebust, 1974; Kaplan & Bott, 1985; Miller, 1987) but may still require extracellular processing prior to uptake (Lancelot, 1984), as mentioned earlier. Furthermore, certain components of these exudates can be refractory and/or inhibitory (Nishizawa *et al.*, 1985; Wetzel, 1992). However, decaying algae and/or heterotrophic organisms may also represent a source of autochthonous material, along with the release of organic compounds by the heterotrophs (such as vitamins to algae) (Lock, 1993).

Chemical processes

Thurman (1985) identified four major riverine chemical processes that involve dissolved organic compounds: adsorption, precipitation, oxidation/reduction and complexation.

Adsorption

DOC can be removed from the water column in appreciable amounts as a result of adsorption by iron and aluminium oxides present in the stream sediment (Dahm, 1981). Up to 60% of natural DOC can be removed from solution by adsorption onto alumina (Davis, 1982). In streams supplemented with iron (III) and aluminium chloride DOC removal from the water column *via* the sediments occurred (Hall *et al.*, 1985). Such a process is said to resemble that which occurs in the B horizon of the soil, apparently dampening fluctuations in DOC (McDowell, 1985). Despite this, microbial removal over several days was reported to account for the largest proportion of DOC. Close associations frequently occur between iron and DOC in upland streams (Reid *et al.*, 1980). Where the pH decreases, salinity increases or there is an increase in the concentration of polyvalent cations (Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}) precipitation of DOC can occur, particularly where upland streams meet lowland rivers (Thurman, 1985). Dahm (1981) has studied abiotic uptake by clays and chemical complexing with oxides of iron and aluminium, finding that up to a third of the DOC removal in the water column could be attributed to abiotic processes and that this could occur rapidly.

Oxidation

Oxidation can be induced photochemically by ultra violet radiation (UV) (Gjessing, 1970) or chemically (Thurman, 1985). Several studies suggest that photodegradation is an important mechanism in the transfer of stream DOC to the sediment particulate carbon pool and to the atmosphere (e.g., Granéli *et al.*, 1996; Molot & Dillon, 1997; Vahatalo *et al.*, 1999). Zuo and Jones (1997) studied the production of carbon monoxide (CO) from lake and wetland waters and found that when globally averaged effective sunlight flux was considered, photoformation of CO leads to a turnover of 2-10 years for DOC in sunlit waters. Photodecay may account for all of the DOC losses *in situ* to the atmosphere and sediments in lakes with low DOC concentrations (<4 mg L^{-1}), but cannot account for all of that lost at high DOC concentrations (>4 mg L^{-1}) (Molot & Dillon, 1997). Vahatalo *et al.* (1999) demonstrated that 17-21% of the ^{14}C labelled carbon in synthetic lignin and 18-23% of the indigenous carbon was mineralized within 7 days to produce CO_2 and soluble organic photoproducts. Rates of photodegradation apparently depend on the nature of the humic substances involved, as

do the products formed (c.f. Lindell *et al.*, 2000; Moran *et al.*, 2000; Vodacek *et al.*, 1997), leading to a wide range of figures being reported for the loss of DOC and the change in optical properties. Furthermore, Tranvik and Kokalj (1998) found that dissolved humic matter became less available to microbes following UV radiation in the presence of a ^{14}C labelled algal extract, suggesting a pathway for recalcitrant DOC production. However, biological degradation of photobleached DOC can be more rapid than that of unbleached material in the estuarine environment (Moran *et al.*, 2000).

Complexation

According to Stumm and Morgan (1981) and Thurman (1985), the complexation of metal ions by OM can occur in both dissolved and suspended phases as well as bottom sediments. Dissolved OM is an efficient complexing agent for iron, copper, aluminium, zinc, mercury and other metals, influencing their solubility, transport and toxicity (e.g., Buffle, 1984; Schnitzer & Khan, 1972).

Flocculation

Since bubbles in the marine environment can have DOC adsorbed onto their surfaces, resulting in particle formation on the collapse of the bubble, Lush and Hynes (1973) looked at whether a similar phenomenon occurred in stream water. They found that it probably does especially with the entrainment of air bubbles in fast flowing waters to give foam. Particles were described as up to 60µm in size with structure-less aggregations and plate-like sheets. Microbial colonization followed giving further alteration and finally degradation. Factors such as pH, turbulence and the ionic properties of the water are said to influence particle formation.

Longitudinal trends in river systems

The export of terrestrial carbon to streams, rivers and eventually the oceans is an important component of the global carbon cycle. Estimates put total global transport of organic carbon at between 0.4 and 0.9 x 10^{15} g yr^{-1} (Degens, 1982; Degens *et al.*, 1991; Meybeck, 1982; Schlesinger & Melack, 1981). Peatlands in particular are a major source of DOC and streams draining temperate peatland catchments commonly contain between 10 and 45 mg L^{-1} (Urban *et al.*, 1989). DOC release from a catchment represents the resultant of the capacity of the upland and wetland/aquatic components to produce and consume such matter.

Once carbon has entered the river, the biological, chemical and physical storage/retention of carbon and the extent to which it is exported through downstream

water flow, will determine its flux. This concept is referred to as 'carbon spiralling' and is summarized by Newbold *et al.* (1982). A further influence on such spiralling is the form of carbon involved, relatively labile material being turned over rapidly while that of a more refractory nature will turnover at a slower rate (Lush, 1981). The average travel distance in the stream between organic fixation of a carbon atom and its loss through respiration or degassing is a definition of turnover length (Newbold *et al.*, 1982). Such a measure is also described as a practical method for determining carbon spiralling. Temperate systems have been examined by Minshall *et al.* (1983), who found a tendency for increased turnover lengths with distance downstream. Lengths were found to vary between 0.9 and 10.8km in headwaters, between 1.0 and 129km in mid sections and ranged from 31 to 246km in downstream reaches. Similarly, Naiman *et al.* (1987) proposed that turnover lengths increase exponentially downstream in boreal forest watersheds from 8 to 436km.

1.08 CLIMATE, GEOLOGY, SOIL TYPE AND HYDROLOGY

River basins and watershed systems are said to have characteristics that are largely governed by climate and geology (Gregory & Walling, 1973). Both the geomorphology and rates of weathering (physical and chemical) are greatly influenced by geology (Lotspeich, 1980). Interactions between climate and vegetation ultimately affect sediment OM exports through impacts on runoff, erosion, bedform and stability.

Hope *et al.* (1994) describe a link between climate and the export of organic carbon in rivers because primary production and decomposition rates govern the quantities of organic carbon that are potentially available. Arctic, alpine and arid environments are said to present low DOC productivity and export. Moderate exports are recorded in temperate and tropical regions where, despite high productivity, there is also rapid decomposition. Meybeck (1981) and Thurman (1985) identify high exports from wetlands spanning a range of biomes as a result of moderate production levels but slow decomposition rates.

The amount and distribution of organic-rich *versus* mineral soils is of great importance in determining organic carbon concentrations in recipient waters (Freeman *et al.*, 2001b; Hope *et al.*, 1994). Consistently higher DOC concentrations were found in Scottish catchments where hill peats dominated (Creasy, 1984; Cresser & Edwards, 1987). A positive relationship between mean annual DOC concentrations and the coverage of hill peat was also reported in the same area. The variation in the discharge:concentration relationship for DOC has been found to relate to the coverage

of mineral *versus* peat dominated soils in six upland catchments in Scotland (Grieve, 1991). Flow paths were also thought to account for complex DOC:discharge relationships in several New Zealand catchments (Moore & Jackson, 1989).

1.09 CLIMATE CHANGE

Wetlands and climate change

Wetland ecosystems perhaps are particularly vulnerable to climate change, being shallow water features, and the predictions for future climate have the potential to cause considerable impacts. This may be particularly true for northern peatlands specifically because aspects of climate i.e., high rainfall and low temperatures, contribute to peat formation and biogeochemical processes within the peat. Northern ecosystems may be especially vulnerable to climate change because they are located in regions where warming is expected to be greatest (IPCC, 2001a), and due to the large current temperature constraints on biological activity (Kattenburg *et al.*, 1996; Körner & Larcher, 1988). Furthermore, due to the large amounts of carbon involved, even a small change in exports may have a considerable impact on the global carbon cycle. In northern latitudes, where plant species diversity is relatively low, climatically mediated changes in species composition or abundance are likely to have large effects on the ecosystem (Pastor *et al.*, 1996). Some of the major factors (relating to climate change) with the potential to influence carbon cycling in northern peatland systems are identified below:

1. Increased concentrations of atmospheric CO_2. Carbon dioxide levels have been increasing in the atmosphere since pre-industrial times and models project that by 2100 atmospheric CO_2 concentrations will be between 540 and 970ppm (90 to 250% above the concentration of 280ppm in 1750) (IPCC, 2001a). Atmospheric CO_2 is an important control on plant activity being a primary resource for photosynthesis. Elevated CO_2 may, for example, increase the labile carbon input to the peat system as a result of increased photoautotrophic exudation, thus accelerating decomposition and increasing DOC concentrations. Changes in the amount (Ball, 1997) or quality (Cotrufo *et al.*, 1994; Lambers, 1993; O'Neill & Norby, 1996; Strain & Bazzaz, 1983) of litter inputs from the plant community and changes in the species composition (Agren *et al.*, 1991) have also been suggested, which could lead to modified decomposition rates. An alteration in the balance between production and decomposition may also affect carbon exports from the ecosystem.

2. Increasing soil temperatures. Global average surface temperatures are also rising (perceived to be due to increased concentrations of radiatively active gases) and are set to reach between 1.47°C and 5.87°C by 2100 (IPCC, 2001a). The rate of warming is projected to be especially rapid in northern latitudes (IPCC 2001a) where much of the world's peat reserves are located. Temperature has a key role in controlling the rates of biogeochemical processes in soils, including peat soils, with effects upon DOC generation (Freeman *et al.*, 2001b), CO_2 (e.g., Moore & Dalva, 1993), CH_4 (e.g., Wilson *et al.*, 1989) and N_2O emissions (Bailey & Beauchamp, 1973; Lensi & Chalamet, 1982), as well as the availability of nutrients (Koerselman *et al.*, 1993; Ross, 1985). Temperature is also inextricably linked to hydrology (see below) because higher temperatures cause more evapotranspiration leading to decreased soil moisture.

3. Altered hydrological regime. Both an increase and decrease in precipitation has been projected by the IPCC (2001a) depending on the region in question, and so both of these scenarios are considered below.

a) Increased frequency, duration or magnitude of summer drought, either as a result of increased evapotranspiration at higher temperatures or due to changing rainfall patterns (Hulme *et al.*, 2002; IPCC, 2001a). Drier conditions may alter plant community structure and therefore the potential for carbon accumulation. Such conditions have the potential to stimulate carbon cycling enzyme activities and therefore mobilization of the peat matrix, leading to intensified DOC release (Freeman *et al.*, 1996).

b) Increased precipitation has largely been ignored in the literature perhaps due to the perceived importance of water to wetland existence. Radiative effects of anthropogenic changes in atmospheric composition are expected to cause an intensification of the global water cycle (Cubasch, 2001). Many areas are predicted to receive increased rainfall (IPCC, 2001a) and those areas include northern Canada, Scandinavia (Wellburn, 1994) and the north of Britain (Cooper & McGechan, 1996; Hulme *et al.*, 2002; IPCC, 2001a; Mansell, 1997), where some of the largest peat reserves are located. More intense rainfall events (Palmer & Räisänen, 2002) and flooding events (Milly *et al.*, 2002) are said to have occurred in recent times and are predicted for the future. DOC movements from upland soils to aquatic ecosystems are driven by precipitation events (Wetzel, 1992) and increased rainfall has been correlated with increased humic acid concentrations in Swedish lakes (Forsberg, 1992). Since anaerobic, waterlogged conditions tend to favour a greater proportion of DOC products (Ponnamperuma, 1972), increasing areas of anaerobiosis within wetlands (or rewetting

of relatively well drained soils) may increase DOC exports from such areas. Of course, it is possible that some areas may experience both increased precipitation in winter and drought in the summer giving greater extremes of hydrological regime, or that the timing of events is changed.

Wetland investigations are justified with regard to climate change because not only are they affected by climatic conditions, but they also have the potential to exert an influence over climate (representing significant sources and sinks of radiatively active gases). There is concern that changes in climate may cause a degradation of the southern boundaries of wetlands faster than any northward expansion potential, leading to an imbalance between biomass loss and increase respectively. Implications for the carbon cycle can be foreseen whereby wetlands could undergo a reversal from sinks to sources of atmospheric carbon, providing a positive feedback to climate change. Furthermore, climatic change may induce modifications in the adjacent aquatic or terrestrial systems to which they also are linked, due to changes in the quantity and/or quality of exported materials, DOC being a prime example which has repercussions on the quality of the receiving waters. The latter is the focus of this study.

Water quality

Water supplies are affected by climatic factors and this in turn is linked to the issue of water quality. Generally, demand for water is increasing due to population growth and economic development, although it is falling in certain countries as a result of more efficient usage (IPCC, 2001b). According to the Intergovernmental Panel on Climate Change (2001b), the demand for freshwater will be enhanced by warmer average or extreme temperatures and more frequent droughts, particularly for direct human consumption and agriculture. Water quality would be degraded by the higher temperatures projected as a result of decreased volumes of water from a given catchment and therefore increased nutrient loading (DOC, nitrogen, phosphorus etc.) per unit volume. An associated increase in eutrophication is expected with high production of aquatic biomass, decreasing species diversity, deterioration of oxygen levels, and adverse effects on water quality. Even where wetland and lake levels remain constant, water quality may be affected due to reduced throughflow. In regions where most water comes from surface sources, decreasing lake volumes and lower water quality may cause serious problems for human use. During warmer, possibly drier, summers water supplies would need to be maintained through transfer from wetter regions (of water surplus) or larger storage (IPCC, 2001b). The possibility of increased

precipitation at certain times of year and in certain regions may mitigate to a certain extent some degradations in water quality by increasing dilution.

Upland peatlands and water quality

Wetlands export more DOC to the oceans than any other major biogeographic area of the world (Lugo *et al.*, 1989) and, as previously mentioned, peatland streams commonly contain between 10 and 45 mg L^{-1} DOC (Urban *et al.*, 1989). This is significant because when DOC concentrations are high there is a tendency for levels of colour, turbidity, chlorine demand and total trihalomethanes concentrations (TTHM) in drinking water to also become undesirably high (Alarcon-Herrera *et al.*, 1994). Trihalomethanes are formed when chlorine reacts with the natural organic compounds in raw waters and include chloroform, dibromochloromethane, bromodichloromethane and bromoform, all of which are regulated as 'total trihalomethanes' (Betts, 1998). The Environmental Protection Agency (EPA) has listed chloroform, bromodichloromethane and bromoform as probable human carcinogens and TTHM are commonly used as a surrogate for a range of disinfection by-products (DBPs) because they are easily measured (Betts, 1998). Chlorine dioxide and ozone (popular disinfection alternatives) also generate a variety of DBPs including some associated with health risks and chloramines tend to result in the same DBPs as chlorine, although in lower concentrations (Betts, 1998). There have been a number of recent studies suggesting an association between TTHM and reproductive problems including miscarriages and neural tube defects (see Betts, 1998 and references therein).

Humic substances not only serve as precursors for the formation of chlorinated compounds but also possess ion exchange and complexing properties that include association with toxic elements and micro-pollutants (Alarcon-Herrera *et al.*, 1994; Pardue *et al.*, 1993). Furthermore, they cause low residual chlorine (limiting its protection against biological contamination) (Worrall *et al.*, in press), compete with pollutant compounds for adsorption sites in activated carbon adsorption and precipitate in the distribution system (Alarcon-Herrera *et al.*, 1994). Many workers have observed that humic acids chelate metals such as iron (e.g., Chin *et al.*, 1998; Fenner *et al.*, 2001; Goodman *et al.*, 1991; Heikkinen 1990) which further contributes to raw water colour (e.g., Heikkinen, 1990), the pale brown/yellow waters being aesthetically displeasing to the consumer. The nature and concentration of humic substances vary with season and geographical location, constituting a major influence on the performance of conventional water treatment processes (Alarcon-Herrera *et al.*, 1994).

Organic carbon also affects numerous other processes in stream water such as the transport of organic pollutants (Carter & Suffet, 1982), particle surface and colloid chemistry (Tipping, 1986), photochemistry (Zafiriou *et al.*, 1984) and nutrient availability (Stewart & Wetzel, 1981). Such matter can contribute significantly to the acidity of surface waters and has a role in pH buffering (Thurman, 1985). It can also influence the distribution of ions between water and sediment phases (Baas-Becking & Moore, 1959). Finally, both DOM and POM form an important energy source for stream biota (Fisher & Likens 1973; Wetzel, 1992).

In north and mid Wales, over a 12-15 year period, upland sites draining forest and moorland catchments have presented a statistically significant and consistent trend of increasing concentrations of DOC (Reynolds *et al.*, 1997; Robson & Neal, 1996). Increased stream water colour (related to DOC) has also been reported in Wales (Kay *et al.*, 1989) and other British studies (e.g., in the Pennines (McDonald *et al.*, 1989)). More recently, these rising trends have been found over a 30 year period (Worrall *et al.*, in press) and across a wide range of deposition, soil types, topography, land use and geographical location (Freeman *et al.*, 2001b), suggesting that DOC increases in upland waters may extend well beyond the UK and could be due to some aspect of climate change. This study focuses on the aforementioned upland peatland catchments because freshwater DOC concentrations are linked to the storage of carbon in catchment soils (Aitkenhead *et al.*, 1999; Hope *et al.*, 1997) and increases are greatest at sites with the largest stores of soil carbon, i.e., peatlands (Freeman *et al.*, 2001b). Furthermore, upland peat dominated catchments are major sources of potable water in the UK (Worrall *et al.*, in press) and with the abstraction/depletion of lowland water supplies the cheaper upland water resources are likely to increase in importance. This, along with the increased transportation of waters from such areas (of relative surplus) to those of deficit (IPCC, 2001b), will potentially expose a greater proportion of the population to water of reduced quality.

1.10 OBJECTIVES OF THE THESIS

The primary objective of this thesis is to determine whether climate change is likely to increase concentrations and/or alter the quality of DOC exports from upland peatland catchments, therefore potentially reducing water quality. The following chapters aim to examine the effects of:

1. Elevated concentrations of atmospheric CO_2
2. Warmer soil temperatures
3. An increased frequency of summer droughts

4. Increased rainfall events

The main study site was located in the Plynlimon catchment in Powys, mid Wales, which is a peat dominated catchment typical of the Welsh uplands (Hughes *et al.*, 1996). This site was chosen due to the availability of long term DOC and hydrochemical data collected since 1992 and held at the University of Wales, Bangor and Centre for Ecology and Hydrology (CEH), Bangor.

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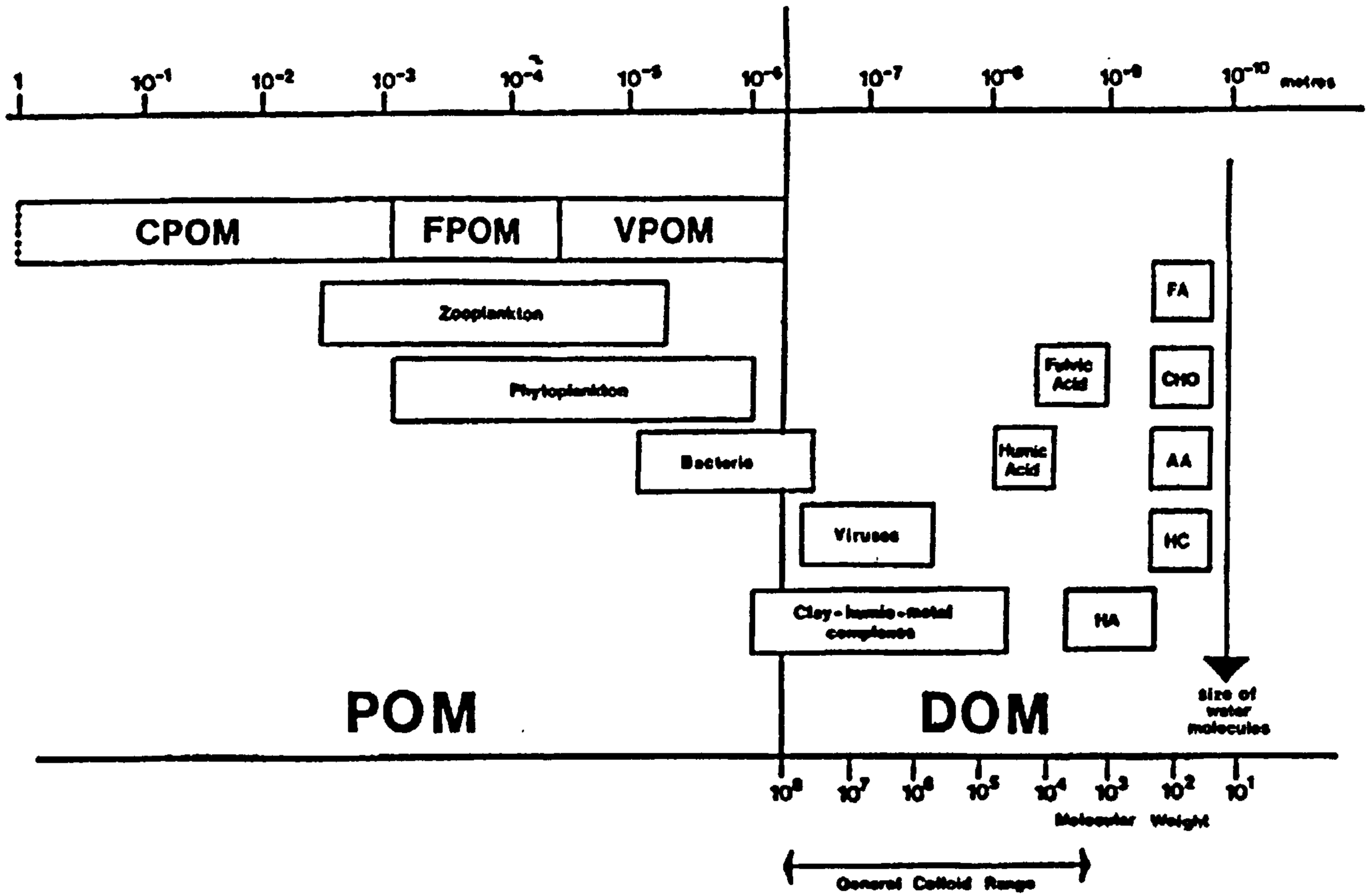

Figure 1.02. Size range of particulate organic matter (POM), dissolved organic matter (DOM) & carbon compounds in natural waters. AA denotes amino acids; CHO, carbohydrates; CPOM, course particulate organic matter; FA, fatty acids; FPOM, fine particulate organic matter; HA, hydrophilic acids; HC, hydrocarbons & VPOM, very fine particulate organic matter (adapted by Hope *et al.*, (1994) from Thurman (1985)).

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CHAPTER 2: THE EFFECTS OF ELEVATED ATMOSPHERIC CO_2 AND ELEVATED TEMPERATURE ON DOC RELEASE

2.01 INTRODUCTION

Concentrations of CO_2 in the atmosphere are continuing to increase as a result of fossil fuel burning and changes in land use (mainly deforestation) (IPCC, 2001). Much (ca. 50%) of that extra CO_2 is absorbed in 'sinks' on land and in the oceans (Schimel, 1996). However, with increasing atmospheric CO_2 a decreasing fraction of anthropogenic CO_2 emissions will be taken up by the land and the ocean (IPCC, 2001). Models indicate that the net effect of land and ocean climate feedbacks is to further increase atmospheric CO_2 concentrations by reducing uptake from both the land and ocean. Carbon cycle models project that by 2100 atmospheric CO_2 concentrations will be between 540 and 970 ppm (90 to 250% above the concentration of 280 ppm in 1750) (IPCC, 2001), and the most recent UK report suggests a similar range (see Hulme *et al.*, 2002). Global average surface temperatures have also increased (perceived to be a consequence of the increased CO_2 concentrations) and are likely to continue to do so, being set to rise between 1.47°C and 5.87°C by 2100 (IPCC, 2001). The rate of warming is projected to be especially rapid in northern latitudes (IPCC, 2001) where much of the world's peat reserves are located.

Numerous studies have addressed the possible impacts of elevated CO_2 (eCO_2) on the terrestrial ecosystem (Körner & Arnone, 1992). The 'fertilizer effect' whereby eCO_2 stimulates plant primary productivity has, at least in the short term, been widely noted (Hunt *et al.*, 1991). Enhanced root exudation rates in various species have also been inferred from several radiolabelling experiments (e.g., Norby *et al.*, 1987; Paterson *et al.*, 1996; Rattray *et al.*, 1995). A 12-14% increase in root derived carbon from *T. aestivum* under 700 ppm CO_2 was observed in a long term labelling experiment (Billes *et al.*, 1993). This increase however, could be accounted for by the larger root systems that had developed (Billes *et al.*, 1993). In contrast, no effect of atmospheric CO_2 concentration was reported on the distribution of ^{14}C between shoots, roots and soil in *Zea mays* (Whipps, 1985), and responses are likely to be species specific. Jones *et al.* (1998) reported increased dissolved organic carbon (DOC) concentrations in the leachate water from model grasslands and attributed this to increased carbon allocation below ground to the root and soil. Thus, the increased input of recently synthesized carbon (as a result of increased carbon assimilation under eCO_2) may contribute to the rising DOC concentrations reported across a wide variety of catchments types

(Freeman *et al.*, 2001a), since this would also be a common factor in addition to the increased temperatures the authors discuss (chapter 3).

In contrast to the terrestrial system, less has been done to elucidate the potential impacts of eCO_2 on wetland ecosystems (Drake, 1992; Drake *et al.*, 1996). Curtis *et al.* (1990) have reported substantial increases in the root biomass of different estuarine marsh communities under eCO_2, while effects on *Sphagnum* growth have been found to be species dependent (e.g., Van de Heijden *et al.*, 2000). *Juncus bulbosus* responded positively up to 500 ppm CO_2, with the root system being especially stimulated (Svedang, 1992). *Festuca ovina* leaf and litter dry weight was also stimulated under eCO_2 (Leadley & Stocklin, 1996). Though none of these studies were concerned with DOC concentrations, they illustrate a similar potential for increased carbon input to the system (*via* exudation), and therefore increased DOC concentrations as in the truly terrestrial systems. Indeed, Kang *et al.* (2001) found increased DOC concentrations in a study on the biogeochemistry of fen peat and also attributed this to an increase in carbon allocation to the roots and soil.

In addition to exudation, there are at least four other factors that could affect DOC concentrations relating to changes in organic matter (OM) decomposition (one of the rate-limiting factors in ecosystem processes (Norby *et al.*, 1994)) under eCO_2. The first is a direct effect on microbial activity. It is feasible that increased CO_2 concentrations could inhibit activity directly (because CO_2 represents an unwanted waste product of metabolism (Freeman *et al.*, 1998)) and therefore reduce degradation of OM dissolved in the pore waters, allowing the accumulation of DOC. However, it is generally thought that such direct inhibition is unlikely since CO_2 concentrations are naturally 10-50 times higher in soils than in the atmosphere (Lamborg *et al.*, 1983). Having said this, Koizumi *et al.* (1991) found that microbial respiratory activity was reduced by around 50% when the CO_2 concentration of ventilated air was increased from 0 to 1000 ppm (Koizumi *et al.*, 1991; Nakadai *et al.*, 1993). Nevertheless, it remains probable that the dominant effects of eCO_2 on soil processes would be indirect given that the concentration of soil CO_2 is measured in terms of 10^4 ppm (van Veen *et al.*, 1991).

Secondly, eCO_2 may affect soil decomposition by changing the plant species composition of an ecosystem (Agren *et al.*, 1991) and this is known to have a significant effect on litter decomposition rates (Kelly & Beauchamp, 1987). There is though, only limited information regarding long term effects of eCO_2 on the species composition of ecosystems (Ball, 1997). *Sphagnum* has been found to be particularly

resistant to decay under ambient conditions (Painter, 1983, 1991). And *Sphagnum* homogenates have been found to retard decomposition when added to peat, which has been attributed in part to the resistance of the cell walls to decay and to antimicrobial substances within the cells (Børsheim *et al.*, 2000; Painter, 1991; Verhoeven & Toth, 1995). Thus, any change in species composition may mean enhanced degradation, releasing DOC to the system that would otherwise be sequestered as *Sphagnum* structural tissue for extended periods.

Thirdly, eCO_2 has the potential to increase the amount of carbon available for export due to increased plant biomass production (above and/or below ground) because vegetation has responded *in situ* by significant increases, not only in carbon uptake and plant growth, but also litter production (Ball, 1997). Increased OM input would increase the carbon accumulation in the soil carbon reservoir (Smith & Shugart, 1993) and since there is a strong relationship between the soil carbon content of a catchment and DOC released (Aitkenhead *et al.*, 1999; Hope *et al.*, 1994), an increase in the release of carbon to rivers could be foreseen. As mentioned previously, substantial increases in the above ground biomass of *J. bulbosus* (Svedang, 1992) and *F. ovina* (Leadley and Stocklin, 1996) have been reported, along with increases in root biomass of different estuarine marsh communities (Curtis *et al.*, 1990) and *J. bulbosus* (Svedang, 1992), under eCO_2.

Finally, eCO_2 may change the chemical composition of plant litter and such changes are likely to have considerable effects on soil decomposition (e.g., O'Neill & Norby, 1996; Strain & Bazzaz, 1983). An increase in the carbon content of the non-structural material in green leaf tissue was observed by Körner and Arnone (1992). Decreases in leaf litter nitrogen concentration have also been reported (Curtis *et al.*, 1989; Körner & Arnone, 1992) as have changes in secondary metabolite concentrations such as tannins (Johnson *et al.*, 1994; Lindroth & Kinney, 1993). Should the litter reflect these changes in green leaf tissue composition, it is proposed that decomposition rates could be decreased (Melillo, 1983). However, many perennial plants re-translocate nutrients from leaves to storage tissue prior to litterfall, thus the nutrient content of the litter can differ considerably from green leaf concentrations. Increased translocation under eCO_2 has also been proposed, which would further reduce litter quality (Johnson *et al.*, 1994). There is at present little information regarding the decomposition of stem and root material, both of which contribute to ecosystem decomposition. It has been postulated that the higher C:N ratio of leaf litter grown under eCO_2 may retard microbial decomposition rates (Cotrufo *et al.*, 1994), and Melillo (1983) found that

leaves grown at high CO_2 contained higher levels of soluble phenolics. Similarly, Gill *et al.* (2002) reported that the phenolic content of the roots of C3/C4 grassland species increased exponentially with increasing atmospheric CO_2 concentrations. Although, Van der Heijden *et al.* (2000) found that a doubling of CO_2 did not promote secondary metabolite production in either *S. balticum* or *S. papillosum*, except in the latter species under increased nitrogen deposition. Of course, species specific responses are likely. Any increase in exports of phenolic compounds as a result of eCO_2 would adversely effect water quality because such materials are both persistent and impair the degradation of DOC in the receiving waters (Freeman *et al.*, 1990). A further consideration is that the relative input of leaf and root materials may change, therefore altering litter composition and DOC leaching.

Elevated atmospheric CO_2 concentrations could affect soil enzyme activities, and therefore decomposition of organic carbon, in several ways. First, an increase in root exudation may lead to a general activation of microbes, which are often carbon limited, and an increase in enzyme production/activity (e.g., Dhillion *et al.*, 1996). Secondly, carbon related enzyme production (e.g., cellulase) may be inhibited by an increased supply of easily-utilizable carbon (e.g., monosaccharides), whilst other enzyme activities (e.g., phosphatase) may be increased to relieve nutrient limitation (e.g., Barrett *et al.*, 1998; Moorhead & Linkins, 1997). Thirdly, a general decrease in microbial activity may be induced if actively growing vegetation competes against soil microbes for inorganic nutrients, giving reduced enzyme production and/or activities (Freeman *et al.*, 1998). Other possibilities include inhibition as a result of increased phenolic compound production or other chemical changes in plant derived OM, as mentioned above. Few studies have considered the effects of eCO_2 on peatland enzyme activities and production, although Moorhead and Linkins (1997) found reduced cellulase activities in tussock tundra that were said to be consistent with an increase in carbon exudation from plant roots.

Soil warming experiments have also demonstrated the potential for increased DOC release from organic upland soils (Ineson *et al.*, 1995; Tipping *et al.*, 1999) and from the study site in this experiment (Freeman *et al.*, 2001a). The latter attributed the enhanced DOC concentrations in peat pore waters to enzymic generation from the peat matrix (see chapter 3). However, the literature on the combined effect of eCO_2 and elevated temperature (eTemp) is sparse, despite the potential for amplified DOC release with the attendant reduction in water quality as a result of an interaction between these factors.

Wetlands contribute only ca. 4-6% of the world's land surface (Immirzi *et al.*, 1992; Mitsch & Gosselink, 2000) yet they play an important role in global and local biogeochemistry. It has been demonstrated that wetlands represent a significant source of DOC for freshwater streams (Schiff *et al.*, 1998) and can affect the functional processes of the receiving waters substantially by modifying the amount and nature of DOC present (Wetzel, 1992). Lugo *et al.* (1989) estimate that globally, wetlands contribute 20% of DOC exports from the continent to the oceans. Northern ecosystems may be especially vulnerable to climate change because they are located in regions where warming is expected to be greatest (IPCC, 2001) and due to the large current temperature constraints on biological activity (Kattenburg *et al.*, 1996; Körner & Larcher, 1988). Such peatlands store approximately ⅓ (455 Pg) of the world's soil carbon (Gorham 1991) and so even a small change in exports may have a considerable impact on water quality and global carbon cycling. Furthermore, in northern latitudes, where plant species diversity is relatively low, climatically mediated changes in species composition or abundance are likely to have large effects on the ecosystem (Pastor *et al.*, 1996).

Upland peat dominated catchments are major sources of potable water in the UK (Worrall *et al.*, in press). Given the potential for increased DOC release as a consequence of eCO_2 and eTemp separately (shown by previous studies in the literature), the combined effect of eCO_2 and eTemp may be especially pronounced with serious implications for the production of disinfection by-products (DBPs) upon chlorination of raw waters. The primary objective of this investigation was therefore to determine whether eCO_2 and/or eTemp had the potential to alter the quantity and/or quality of DOC available for export from northern peatlands. It was hypothesized that:

1. DOC concentrations in the peat leachate waters would increase under eCO_2 due to increased plant inputs (biomass and exudation).
2. Elevated temperatures would stimulate extracellular enzyme activities, increasing the generation of DOC *via* decomposition of the peat matrix.
3. The combination of the above mechanisms would produce amplified DOC concentrations under eCO_2/eTemp.

Intact soil cores were therefore incubated under the following conditions at the CEH solar dome facility at Abergwyngregyn.

1. Ambient CO_2/ambient temperature (control).

2. Elevated (235 ppm above ambient, ca. 605 ppm) CO_2/ambient temperature (eCO_2).
3. Ambient CO_2/elevated (3°C above ambient) temperature (eTemp).
4. Elevated CO_2 (235 ppm above ambient)/elevated (3°C above ambient) temperature (eCO_2/eTemp).

In addition to DOC and phenolics concentrations, a suite of biogeochemical properties were measured, with the focus being on extracellular enzyme activities in the soils, to determine whether carbon cycling is likely to change under a future climate. Concentrations of Poly-ß-hydroxyalkanoate (PHA), an indicator of microbial nutritional stress (e.g., Freeman *et al.*, 1993a; Macrae & Wilkinson, 1958; Malmcrona-Friberg *et al.*, 1986), were also analyzed at the end of the experiment. Trace gases were measured to determine the likelihood of feedback to climate change because wetlands can be substantial sources of greenhouse gases such as CH_4 (e.g., Greenup *et al.*, 2000; Whiting & Chanton, 1993), CO_2 and N_2O (e.g., Freeman *et al.*, 1993b).

2.02 MATERIALS AND METHODS

Site description

The site chosen for study, a pristine wetland in the Upper Wye catchment on Plynlimon (UK NGR SN 820 866), is a small gully typical of many in the uplands of Wales (Hughes *et al.*, 1996). The mire is characterized by *Sphagnum* and *Juncus* communities and has a pore water pH, at a depth of 10 cm, in the range of 3.9-4.8.

Peat collection

Twenty cores (0.11m diameter x 0.25m deep) were carefully excavated using a sharp knife and the perfusion systems (used for housing the peat monoliths, based on the design described by Freeman, *et al.*, 1993c, d) as templates. Each core could then be collected with minimal disturbance by easing the perfusion cylinder around the peat in small stages. Five replicate cores were collected for each of the four treatments at the solar dome facility. As *Sphagnum cuspidatum* is the main vegetation type of the gully mire, cores were taken from homogenous *S. cuspidatum* stands as far as possible but some *S .subunitens* was also included. This also enabled the effects of treatments on the vegetation composition to be studied. Since *Sphagnum* gains much of its nutrients from rain water absorbed above ground, synthetic rain water (table 2.01a & b) was applied to provide the same volume per day as that calculated from the average annual rainfall (over the last 10 years) recorded at the sample site. The peat cores were allowed to equilibrate for a month prior to treatment in an attempt to minimize disturbance effects.

Table 2.01a. Composition of synthesized rain water

Formula	Chemical	g
NH_4NO_3	ammonium nitrate	13.77
NH_4Cl	ammonium chloride	2.35
KCl	potassium chloride	2.01
$MgSO_4.7H_2O$	magnesium sulphate	32.66
$CaCl_2.6H_2O$	calcium chloride hexahydrate	13.80
NaCl	sodium chloride	61.77
Na_2SO_4	sodium sulphate anhydrous	3.20

The amounts shown above were dissolved individually in 250 mL of ultra pure (MilliQ™) water and subsequently mixed. Sulphuric acid (0.7 mL) was then added gradually until the pH reached 4.56 (conductivity 223 µS). This stock solution was then diluted (2 mL in 10 L ultra pure water) to give the following concentrations.

Table 2.01b. Final concentrations of synthesized rainwater components

Compound	Final concentration (mg L^{-1})
Na	2.53
K	0.11
Ca	0.25
Mg	0.32
Cl	4.45
SO_4	2.79
NH_4	0.39
NO_3	1.07

Modified from Cocksedge (1988).

Routine sampling and chemical analysis of peat pore water

Pore water samples were extracted at approximately monthly intervals from the top 3 cm of the peat profile using lateral sampling ports in the perfusion systems. The samplers were constructed according to the design described by Freeman *et al.* (1993c, d) aimed at minimizing the extraction volume and any unnecessary dead volume. This ensured that excessive disturbance of the core was avoided and that the integrity of the extracted sample (in particular the redox potential) was unaffected. Plastipak^R syringes (2.5 cm^3) were cut off at 1.5 cm from the Luer tip. The Luer end was retained, packed with glass wool and connected to Tygon^R auto analyzer transmission tubing, through which suction could be applied using a 5 cm^3 Plastipak^R syringe (into which the samples were collected). The opposite end of the modified syringe tip was connected to the peat housing *via* drilled holes. Following collection, all samples were filter

sterilized (0.2 µm diameter membranes, Whatman, Kent, UK) and refrigerated (4°C) immediately to minimize bacterial degradation and remove POC.

DOC concentrations

Dissolved organic carbon (DOC) was determined by the difference between total carbon (TC) and inorganic carbon (IC) in the samples. Total Carbon and IC were measured with a Total Organic Carbon analyzer (Shimadzu 5000) using a 33 µL injection volume. Standard curves were prepared using 0-100 mg L^{-1} of potassium hydrogen phthalate solution (for TC) or 0-100 mg L^{-1} of Na_2HCO_3/Na_2CO_3 solution (for IC).

Phenolic compound concentrations

Phenolic contents were assayed according to the method of Box (1983) with the following ratios of sample, Na_2CO_3 and Folin-Ciocalteau reagent. To 1 mL of sample, 150 µL of Na_2CO_3 solution (200 mg L^{-1}) was added and subsequently 50 µL of Folin-Ciocalteau reagent (Sigma-Aldrich Co., Ltd., Dorset). The mixture was incubated 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 of the reactants was measured spectrophotometrically at 750 nm. When the samples were out of range of the phenol standard curve, the samples were diluted with ultra pure water and the procedure was repeated. Generally, samples required 0-10 fold dilution depending on season.

Anion concentrations

Anion concentrations (fluoride, chloride, nitrate, phosphate and sulphate) were measured using a Dionex 200i ion chromatograph (Dionex UK Ltd., Leeds) and an AS4A anion column, with Na_2HCO_3/Na_2CO_3 eluent solution and H_2SO_4 regenerant solution.

Trace gas fluxes

Whole core trace gas fluxes (CO_2, CH_4 and N_2O) were measured by sealing the perfusion systems with an Osma double socket and inspection cover (Freeman *et al.*, 1993d). Evolved gases were allowed to accumulate over 2 hours. A 10 cm^3 gas syringe fitted with a needle was then used to remove samples through a Subaseal (septum) in the cap. The 5 replicates from each treatment were collected at midday and an Ai Cambridge model 92 Gas Chromatograph (GC) (with a twin Porapak QS column at

35°C and N_2 carrier gas flow rate of 30 cm min^{-1}) was used for sample analysis. The GC included a flame ionization detector incorporating a CO_2 to CH_4 catalytic converter and an electron capture detector. The increase in trace gas concentration (above the initial background concentration) was used to estimate gaseous fluxes from the cores.

Plant community structure

The percentage cover of each plant species present was estimated for each core and the average recorded.

Stable isotope (^{13}C) labelling experiment

Before destructive sampling began, the eTemp and eCO_2/eTemp cores were used in a ^{13}C pulse labelling experiment (see chapter 4B).

Destructive laboratory analysis

Cores were transported back to the laboratory to allow above ground and below ground plant biomass, bulk density, porosity, saturation, enzyme activities and PHA concentrations to be measured.

Above ground plant biomass was obtained by cutting the vegetation at the soil surface while below ground plant biomass (i.e., root material) was separated from the peat using a 2 mm sieve and flowing water. Brown Sphagnum material was considered to be 'below ground'. Both above and below ground material was then dried to constant weight at 106°C. Bulk density was determined throughout the peat profile in 2 cm increments along with porosity and saturation, using standard soil techniques. Phenol oxidase activities were determined using a random sub-sample of peat from the surface layer of each core as were β-glucosidase and phosphatase activities.

Extracellular phenol oxidase activities

Phenol oxidase activities were determined using 10 mM L-DOPA (dihydroxy phenylalanine) (Sigma-Aldrich) solution as a substrate according to Pind *et al.* (1994). A homogenate of 1 cm^3 of peat per 2 mL of ultra pure water was prepared using a stomacher (Seward Colworth model 400) in order to minimize cell disruption. The homogenate was diluted 1:1 with ultra pure water and 1 mL aliquots were transferred to 2.5 mL centrifuge tubes. A 10 mM solution of L-DOPA (Sigma-Aldrich) was used throughout due to poor solubility at higher concentrations. One mL of L-DOPA solution or ultra pure water (control) (following incubation at the appropriate

temperature, i.e., the treatment temperature) was added to each tube. The tubes were shaken and incubated at the appropriate temperature for 1 or 3mins and immediate centrifugation at 72000 g (10000 rpm, Whatman micro-centrifuge) for 5mins terminated the reaction (Pind *et al.*, 1994). The supernatant was filtered (Whatman GF/C) and the absorbance measured at 460 nm. Original samples were dried to constant weight to determine the dry weight used in each assay. Activity was expressed in terms of nmol 2,3-dihydroindole-5,6-quinone-2-carboxylate (here referred to as diqc) min^{-1} g^{-1} peat (dry weight). The difference in absorbance, as a result of the two incubation periods above, was used to determine the rate of product formation by using Beers Law and the molar absorbancy coefficient for dicq of $3.7x10^4$ (Mason, 1948).

Extracellular ß-glucosidase activities

Two mL of Methylcellosolve: 2-Ethoxyethanol (Ethylene Glycol Monoethyl Ether) (Sigma-Aldrich) was used to pre-dissolve all substrates (Hoppe, 1983), which were freshly prepared for each assay. Methylcellosolve does not affect enzyme activities but increases substrate solubility (Hoppe, 1983). Substrates and replicate 1 cm^3 peat cubes from each core, in each treatment, were allowed to equilibrate in the incubator at the treatment temperature before each assay was carried out.

Each cube of peat was gently homogenized for 30 sec with 1 mL of ultra pure water. One mL of this slurry was pipetted into 20 mL vials and the Methylumbelliferyl (MUF)-substrates (Sigma-Aldrich) added (either 3.5 mL of 500 µM MUF-ß-D-glucosidase, 1000 µM MUF-phosphate or 1000 µM MUF-sulphate solution). The final incubation concentrations of the different substrates were 150 µM, 600 µM and 700 µM respectively. These concentrations were above the concentration at which substrate availability limits activity but below the concentration at which substrate inhibition occurs (Freeman *et al.*, 1995). The samples were mixed and allowed to incubate for 1 hour at the appropriate temperature. One and a half mL of the slurry was then transferred to an eppendorf tube and centrifuged at 72000 g for 5 min. Half a mL of the supernatant was transferred to a cuvette containing 2.5 mL of ultra pure water and 0.5 mL of pH 6.6 buffer solution (0.05 M glycine/0.2 M NaOH-buffer). The latter to convert the MUF into the more fluorescent anionic form (Chróst & Krambeck, 1986). The immediate centrifugation and addition of buffer to the samples following incubation prevents further enzyme activity (c.f. King, 1986).

Since the intensity of MUF fluorescence is pH dependent, this necessitated using a MUF standard range measured at the same pH as the samples (an alternative would be

applying a correction formula (Chróst & Krambeck, 1986)). In order to correct for quench, a range of standard concentrations of MUF free acid (Sigma-Aldrich) within the range of the sample activities was made up in the sample matrix (i.e., peat slurry incubated under identical conditions as those described above, but without the MUF substrates). Saturation concentrations for the assay were established by varying concentrations of MUF-substrate (0-2 mM) added to peat samples and incubated under identical conditions to those described above.

A Perkin-Elmer LS50 Luminescence Spectrometer was used to determine fluorescence, at 450 nm emission and 330 nm excitation (slit setting 1). This was done immediately after centrifugation and buffer addition to avoid any decreases in fluorescence over time.

Poly-ß-hydroxyalkanoate (PHA) concentrations

A modification of the ion exclusion High Performance Liquid Chromatograph (HPLC) method of Karr *et al.* (1983) was used to determine the PHA content of the peatland microorganisms (Freeman *et al.*, 1993a). Peat samples were dried, digested in sulphuric acid at 90°C (to convert PHA to crotonic acid) and the resulting acid quantified using a HPLC.

Samples were digested in batches using a heating block and then allowed to cool for 30mins. Before analysis by HPLC, the digested samples were diluted 20 fold with ultra pure water. The HPLC system used included a CECIL instruments CE1100 series HPLC with a CE1200 variable wavelength monitor (UV detection set at 214 nm) fitted with an Aminex HPX organic acid column, using 0.028 M H_2SO_4 as the eluent (Freeman *et al.*, 1993a) at a flow rate of 0.7 mL min^{-1}. Crotonic acid eluted at 26.5 min. A calibration curve produced using crotonic acid (Sigma-Aldrich) was linear up to at least 50 mg L^{-1}.

2.03 STATISTICAL CONSIDERATIONS

Data was tested for normality using the Kolmogorov-Smirnov test. Five replicates (one from each core) per treatment were used for each sampling point to compare against the control using ANOVA and Dunnetts simultaneous tests (Minitab version 13.32, Minitab Inc.). Due to concerns regarding normality, a nonparametric test (Kruskall-Wallis, Minitab version 13.32, Minitab Inc.) was used to confirm the results for the percentage cover data. Significance values will refer to the Dunnetts simultaneous tests unless stated otherwise.

2.04 RESULTS

DOC concentrations

DOC concentrations are shown in figure 2.01a with statistical significance for each point in comparison to the control. Pre-readings taken before treatments began showed that DOC concentrations in all treatments were not significantly different ($P>0.05$) in relation to the control, being between approximately 45 and 60 mg L^{-1}. Generally, concentrations remained relatively constant through time though the tendency was for increased levels during spring/early summer 2001 in all treatments. Elevated CO_2 and eTemp cores showed significant increases in DOC concentration relative to the control at certain times of year (particularly in the growing season), while DOC levels under eCO_2/eTemp were consistently and significantly increased.

Mean DOC concentrations were significantly higher than the control for the first time following 86 days of treatment, on 07/02/01, ($P<0.05$) in the eCO_2 cores, after which concentrations were on average 51.11% higher and significantly different with respect to the control on 5 out of the 8 remaining sampling occasions.

Under eTemp conditions, mean DOC concentrations were of a similar magnitude to those of the eCO_2 treatment and were significantly increased by 143 days of treatment, on 05/04/01, ($P<0.01$), after which point concentrations were 35.11% higher than the control on average and significantly different on 2 sampling occasions of the remaining 5.

The eCO_2/eTemp treatment rapidly and dramatically increased mean DOC concentrations found in the peat pore water in relation to the ambient situation, over and above that observed in the separate treatments. The increased DOC concentrations were significant with respect to the control dome following only 18 days of treatment, on 01/12/00, ($P<0.001$), when a peak in concentrations occurred immediately after the start of treatment (an increase of 104.05%). Despite the fact that this concentration was not maintained, relative levels apparently stabilized at between approximately 98.20 and 118.75% above the control (since control concentrations declined somewhat) and significance at the $P<0.05$ level or less was maintained throughout the remainder of the experiment. On average concentrations were 105.65% higher than the control following 01/12/00.

Phenolic compound concentrations

Figure 2.01b shows the mean phenolic compound concentrations under each climate change treatment and significance in relation to the control. Pre-readings for phenolic compound concentrations showed no significant differences between treated samples and the control ($P>0.05$), ranging between approximately 9 and 12 mg L^{-1}. Mean concentrations strongly reflected those of DOC but with an accentuated response to season producing maxima in all treatments in December and May. Increased phenolics concentrations tended to occur in the growing season with respect to the control in all treatments.

Elevated CO_2 produced mean phenolic concentrations that became significantly different to the control following 176 days of treatment, on 08/05/01, ($P<0.05$), after which concentrations were 125.77% higher than the control on average and significantly different on 3 out of the 4 remaining sampling occasions.

Elevated temperature exhibited concentrations that became significantly different to the control earlier than those of the eCO_2 treatment, 143 days after the treatments began, on 05/04/01, ($P<0.01$). From then on average concentrations were 108.24% higher than the control and significantly different on 1 out of the 5 remaining sampling occasions.

Phenolics concentrations became significantly increased with respect to the control ($P<0.001$) following 18 days of treatment, on 01/12/00, under eCO_2/eTemp and remained so throughout the experiment ($P<0.001$), with average concentrations being 271.46% higher than the control.

Community structure

Tables 2.02a-c show the mean percentage cover of the dominant northern peatland species present under each of the climate change scenarios (numbers in parentheses represent standard error of the mean). Percentages changes in relation to the mean of the control cores are shown (Δ%) and P values where ns denotes non significant, ns (10%) significant at the $P<0.1$ level only, * significance at the $P<0.05$ level, ** at the $P<0.01$ level and *** at the $P<0.001$ level. These conventions will apply throughout unless stated otherwise. Figure 2.02 illustrates the mean percentage cover of the species found under each of the climate change simulations.

Table 2.02a. Mean percentage *Sphagnum cuspidatum* cover of peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	*S. cuspidatum* (% cover)	%Δ	P
control	89 (7.5)		
eCO_2	88 (5.6)	-1.1	ns
eTemp	48 (15.4)	-46.0	*
eCO_2/eTemp	33 (13.0)	-62.9	**

Mean *S. cuspidatum* cover remained similar under eCO_2 in comparison to the ambient control, but was significantly and dramatically reduced under conditions of eTemp (-46.0%, $P < 0.05$) and eCO_2/eTemp (-62.9%, $P<0.01$).

Table 2.02b. Mean percentage *Juncus effusus* cover of peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	*J. effusus* (% cover)	Δ%	P
control	8 (8.0)		
eCO_2	0 (0.0)	-100.0	ns
eTemp	0 (0.0)	-100.0	ns
eCO_2/eTemp	30 (11.0)	275.0	ns

Mean *J. effusus* cover was not significantly different in any treatment though eCO_2/eTemp cores exhibited an increase of 275% in relation to the control.

Table 2.02c. Mean percentage *Festuca ovina* cover of peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	*F. ovina* (% cover)	Δ%	P
control	1 (1.0)		
eCO_2	11 (5.1)	1000	ns
eTemp	50 (13.9)	4900	**
eCO_2/eTemp	29 (11.9)	2800	ns

The only significant increase in *F. ovina* cover occurred in the eTemp treatment (4900%, $P<0.01$), however large non significant increases were also found in the eCO_2 (1000%) and eCO_2/eTemp (2800%) treatments.

Mean percentage *P. commune* cover was similar in all treatments ranging between 0% cover in the control and 8% cover in the eCO_2/eTemp cores.

Plant Biomass

Table 2.03. Mean above ground, below ground and total plant biomass for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	Above (g)	Δ%	P	Below (g)	Δ %	P	Total (g)	Δ %	P
control	2.33(0.46)			1.28(0.23)			3.61(0.57)		
eCO_2	4.51(0.79)	93.64	ns	2.25(0.23)	75.66	ns	0.99(0.99)	87.26	ns
eTemp	3.91(0.72)	67.87	ns	3.25(0.51)	153.35	**	0.92(0.92)	98.23	ns (10%)
eCO_2/eTemp	8.39(0.91)	260.31	***	6.38(0.51)	397.66	***	14.77(1.39)	309.09	***

The above ground biomass increased appreciably under all treatments but only the increase under eCO_2/eTemp was significant (260.31%, $P<0.001$) (see figure 2.03). Below ground biomass also increased in all treatments and this was significant in the eTemp and eCO_2/eTemp treatments (153.35%, $P<0.01$ and 397.66% $P<0.001$ respectively). Total biomass therefore also increased under all treatment conditions but only the 309.09% increase in the eCO_2/eTemp conditions was significant ($P<0.001$), the increase under eTemp being significant only at the $P<0.1$ level only.

Peat Saturation

Table 2.04. Percentage changes in mean percentage saturation for the uppermost peat layers in relation to the control for elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp cores

Treatment	2-3 cm		4-5 cm		6-7 cm	
	Δ%	P	Δ %	P	Δ %	P
eCO_2	0.44	ns	0.31	ns	2.98	ns
eTemp	-17.69	**	-6.59	ns	-8.66	ns
eCO_2/eTemp	-30.22	***	-16.35	*	-2.73	ns

Under eCO_2 mean percentage peat saturation was very similar to the control at all depth increments, while eTemp induced a significant reduction at the 2-3 cm depth (-17.69%, $P<0.01$) as did eCO_2/eTemp at the 2-3 cm (-30.22%, $P<0.001$) and the 4-5 cm depth (-16.35%, $P<0.05$). A profile of the mean saturation values for each treatment is shown in figure 2.04.

Enzyme activities

Table 2.05a. Mean phenol oxidase enzyme activities for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	Phenol oxidase (nmol g^{-1} min^{-1})	Δ%	P
control	11.40 (1.69)		
eCO_2	8.55 (0.92)	-25.01	ns
eTemp	14.25 (1.92)	24.98	ns
eCO_2/eTemp	4.75 (0.89)	-58.34	*

Phenol oxidase activities were suppressed in both the eCO_2 and eCO_2/eTemp treatments but only in the latter case was this significant (-58.34%, $P<0.05$). The tendency was for increased phenol oxidase activities under eTemp but again this was not significant. Phenol oxidase activities are shown in figure 2.05a.

Table 2.05b. Mean hydrolase enzyme activities for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	ß-glucosidase (nmol mg^{-1} h^{-1})	Δ%	P	Phosphatase (nmol mg^{-1} h^{-1})	Δ %	P
control	2.12 (0.17			12.86 (0.50)		
eCO_2	1.74 (0.19)	-17.67	ns	17.16 (1.01)	33.43	*
eTemp	2.40 (0.20)	13.46	ns	10.98 (1.14)	-14.63	ns
eCO_2/eTemp	1.55 (0.07)	-26.82	ns (10%)	15.99 (1.30)	24.36	ns

ß-glucosidase activities (figure 2.05b) followed the same direction as the phenol oxidase activities but these were not significant at the $P<0.05$ level. The reduction in activity under eCO_2/eTemp (-26.82%) was though, significant at the $P<0.1$ level.

Phosphatase activities showed the opposite response to that of the carbon cycling enzyme activities (see figure 2.05c), increasing in the eCO_2 and the eCO_2/eTemp treatments but only the latter reached significance (33.43%, $P<0.05$). The tendency was for suppressed phosphatase activities under eTemp but again this was non significant.

Poly-ß-hydroxyalkanoate (PHA) concentrations

Table 2.06. Mean Poly-ß-hydroxyalkanoate (PHA) concentrations for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	Mean PHA concentration (mg crotonic acid g^{-1})	Δ%	P
control	95.04 (5.77)		
eCO_2	123.38 (3.91)	29.82	**
eTemp	113.03 (4.88)	18.93	ns (10%)
eCO_2/eTemp	143.02 (5.87)	50.48	***

Poly-ß-hydroxyalkanoate concentrations (illustrated in figure 2.06) significantly increased under both the eCO_2 (29.82%, $P<0.01$) and the eCO_2/eTemp (50.48%, $P<0.001$) treatments, indicating increased microbial nutritional stress. The increase in the eTemp treatment was only significant at the $P<0.1$ level.

Trace gas fluxes

Table 2.07a. Mean CO_2 flux for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	CO_2 (ng cm^{-2} d^{-1})	Δ %	P
control	4320.09 (1136.71)		
eCO_2	5241.23 (1279.26)	21.32	ns
eTemp	5347.39 (1204.11)	23.78	ns
eCO_2/eTemp	7729.18 (1656.63)	78.91	ns

Mean CO_2 flux from all treatments (figure 2.07a) increased in relation to the control although such increases were not significant. Similar increases were observed under eCO_2 and eTemp conditions (21.32% and 23.78% respectively), with eCO_2/eTemp producing a much greater increase in CO_2 emissions (78.91%).

Table 2.07b. Mean CH_4 flux for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	CH_4 (ng cm^{-2} d^{-1})	Δ%	P
control	6.06 (4.25)		
eCO_2	22.12 (10.87)	265.26	ns
eTemp	10.26 (2.60)	69.44	ns
eCO_2/eTemp	7.16 (3.86)	18.26	ns

Changes in mean CH_4 flux as a result of the climate change simulations were again non significant although all increased emissions relative to the control (figure 2.07b), the eCO_2 treatment inducing a dramatic increase of 265.26%, while eTemp substantially stimulated release (69.44%). The eCO_2/eTemp treatment showed a less pronounced increase compared to the other treatments (18.26% with respect to the control).

Table 2.07c. Mean N_2O flux for peat cores maintained under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp

Treatment	N_2O (ng cm^{-2} d^{-1})	Δ%	P
control	0.12 (0.03)		
eCO_2	0.31 (0.02)	149.51	***
eTemp	0.40 (0.01)	227.61	***
eCO_2/eTemp	0.20 (0.04)	65.16	ns

Nitrous oxide fluxes (figure 2.07c) were less variable than those of CO_2 or CH_4 and the large increases in relation to the control found in the eCO_2 and the eTemp treatments (149.51% and 227.61% respectively) were significant ($P<0.001$), while the 65.16% increase under eCO_2/eTemp was not.

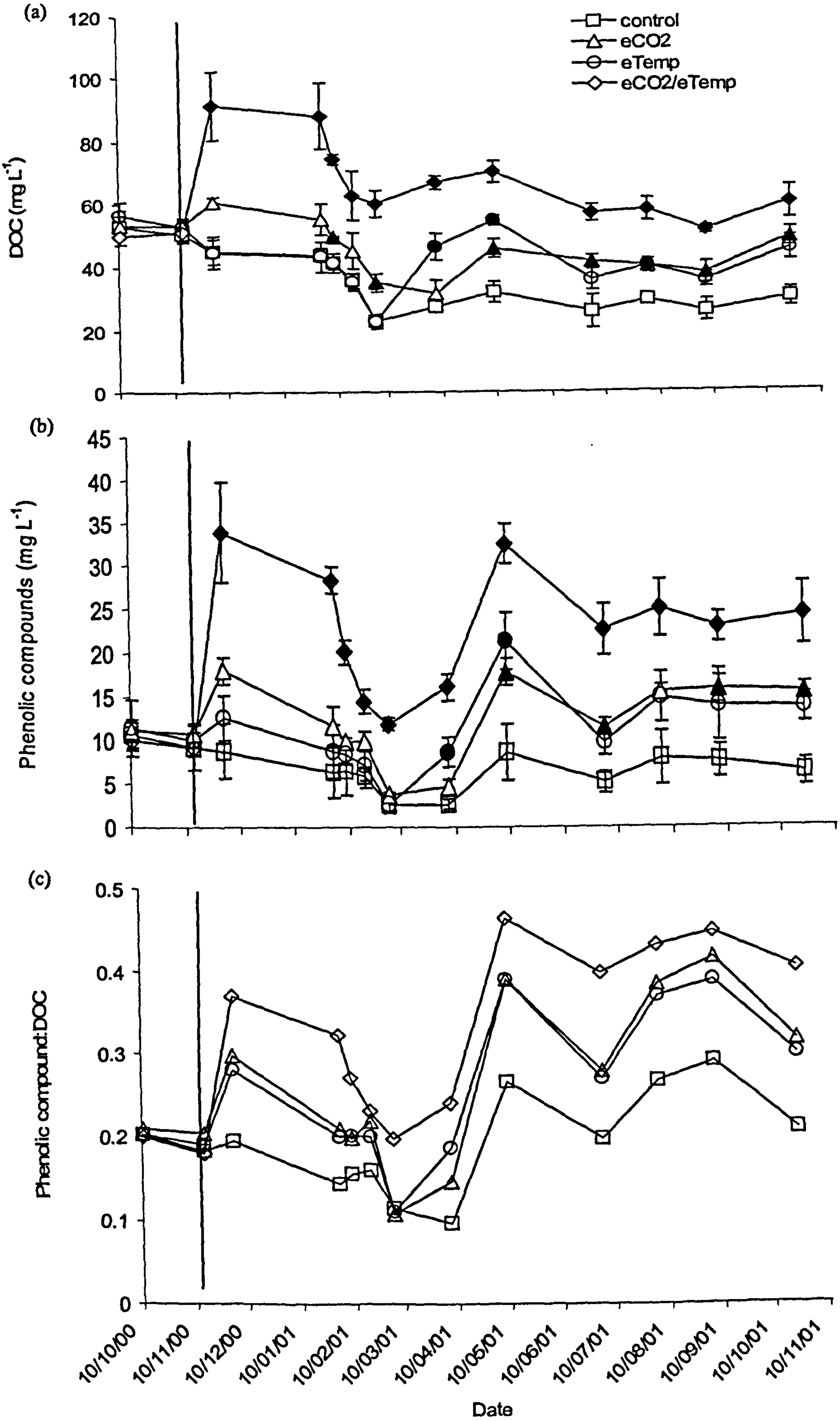

Figure 2.01. Pore water (a) DOC concentrations (b) phenolic compound concentrations & (c) phenolic compound:DOC ratios in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores. Filled symbols denote statistical significance in relation to control cores at $P<0.05$ or below. Vertical line denotes the start of treatment, error bars represent standard error of the mean, n=5.

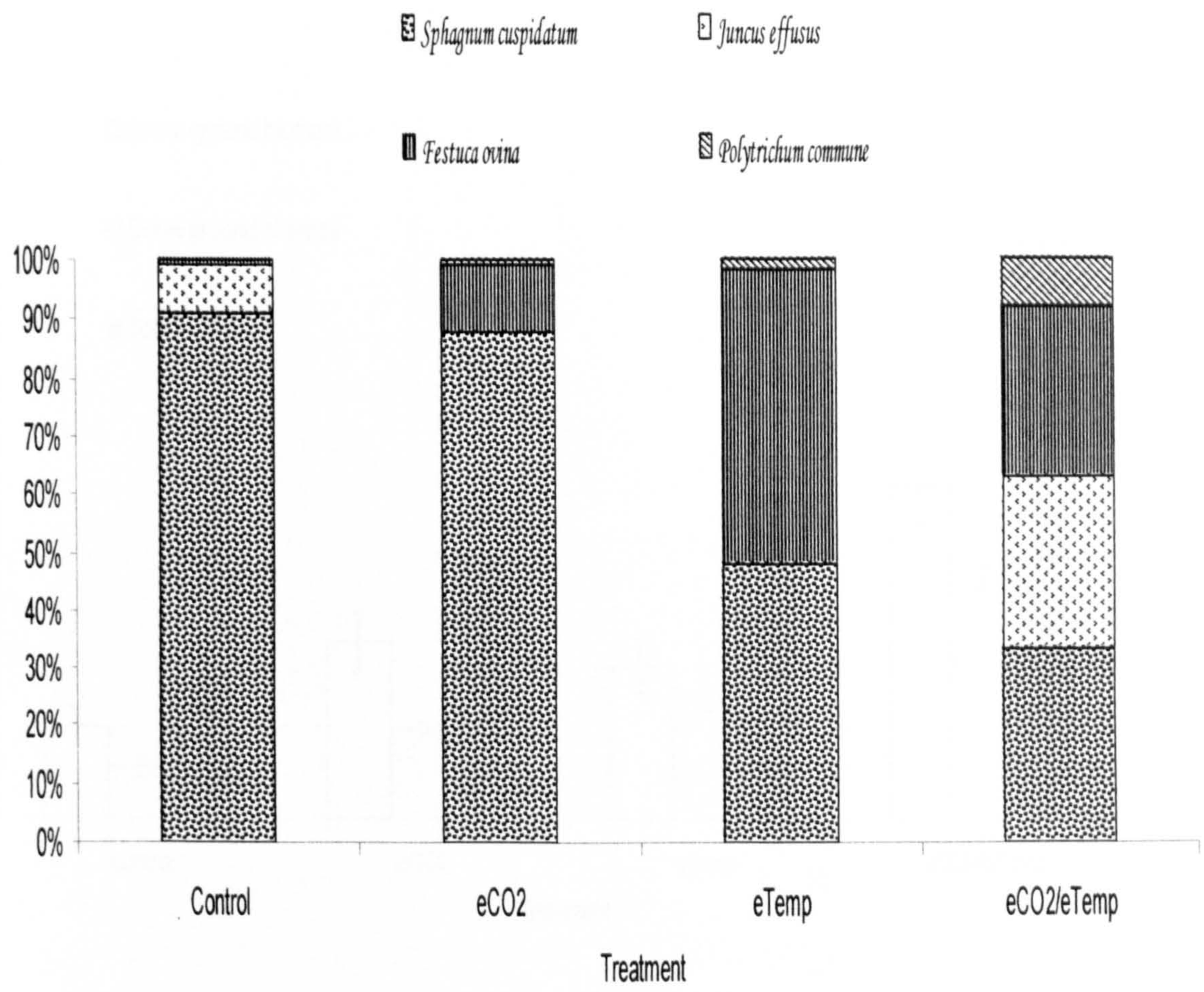

Figure 2.02. Plant species composition (percentage cover) in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores following ca. 1 year of treatment.

2.03

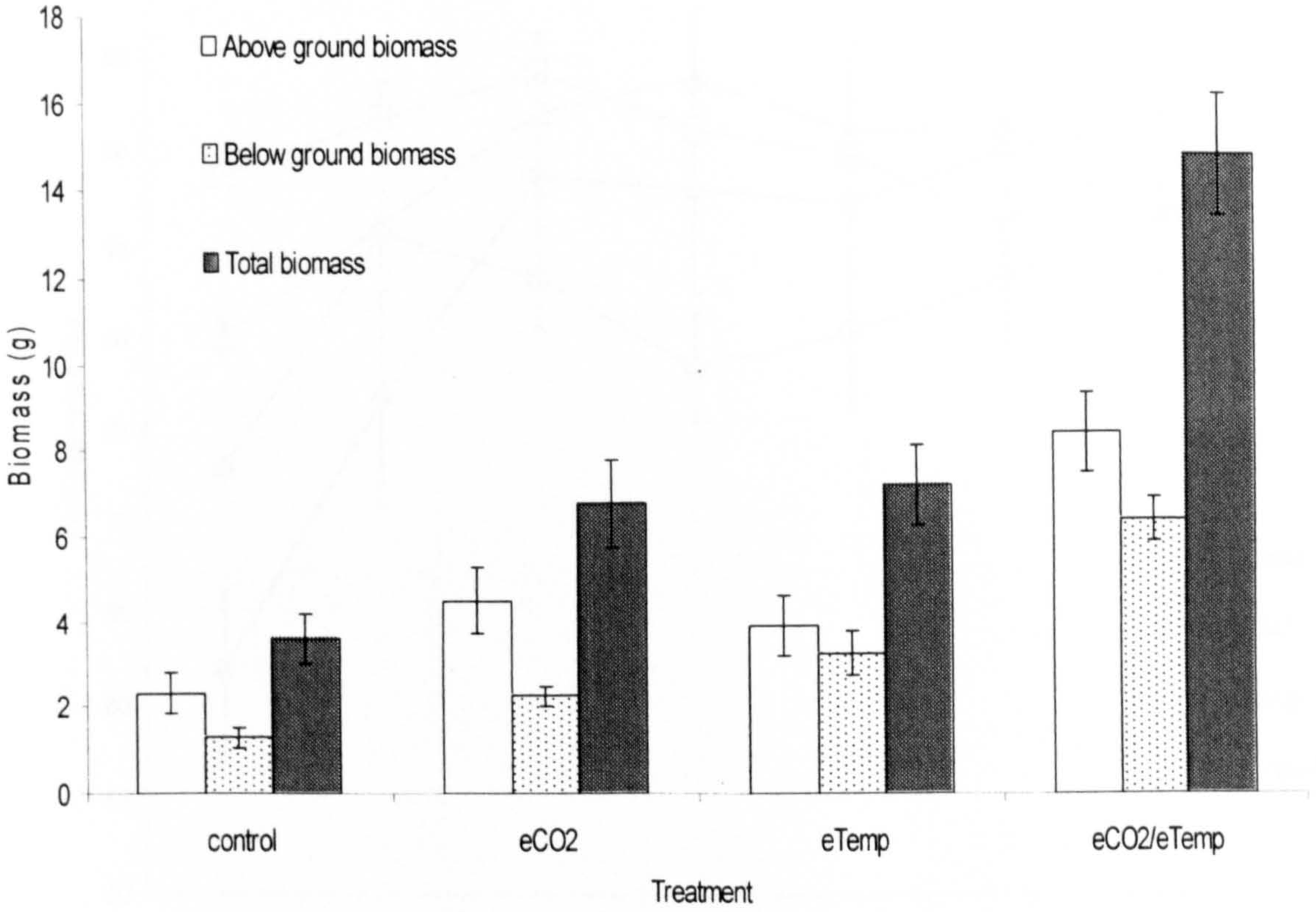

Figure 2.03. Plant biomass in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores following ca. 1 year of treatment. Error bars represent standard error of the mean, n=5.

2.04

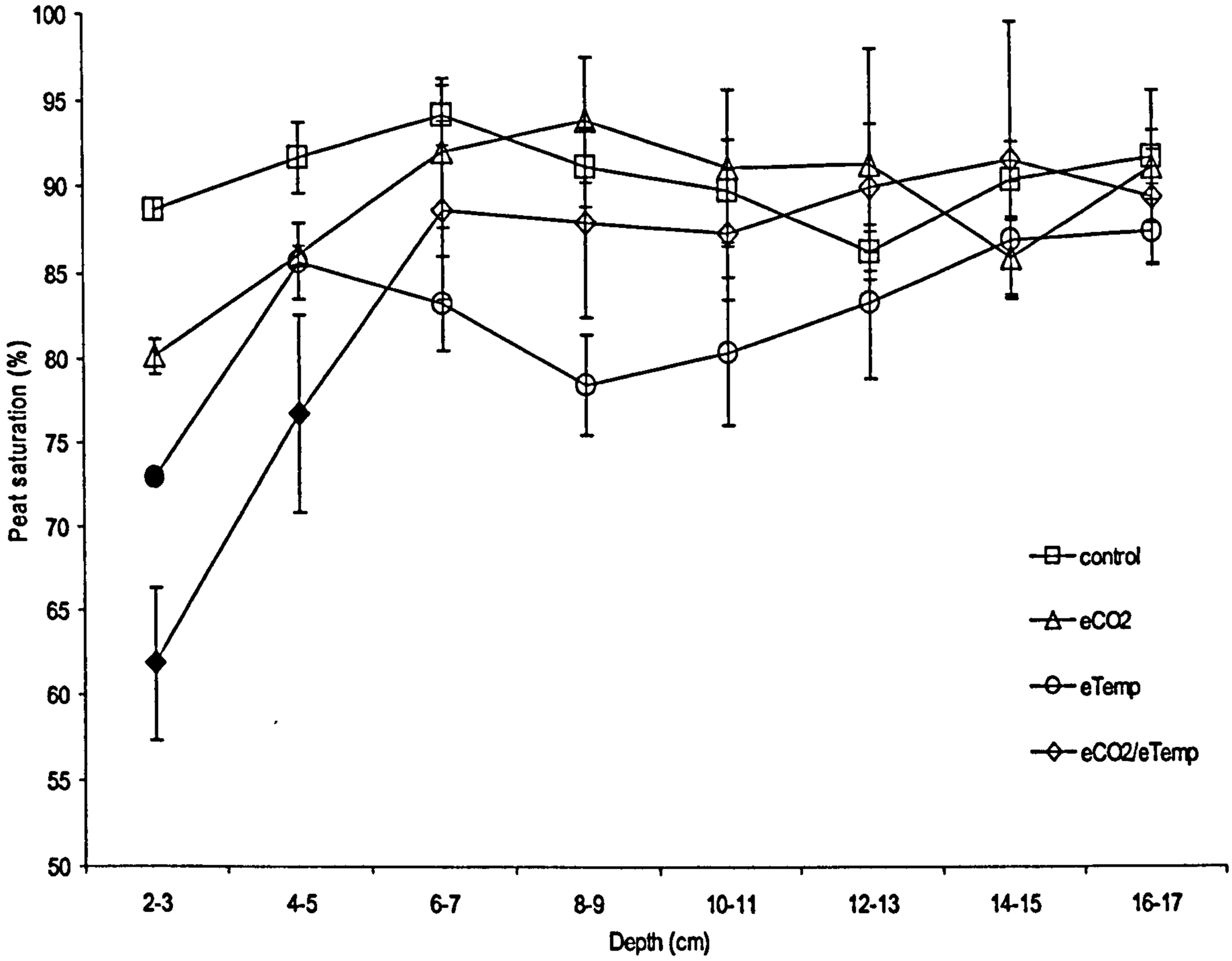

Figure 2.04. Peat saturation in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores. Filled symbols denote statistical significance in relation to control cores at $P<0.05$ or below. Error bars represent standard error of the mean, n=5.

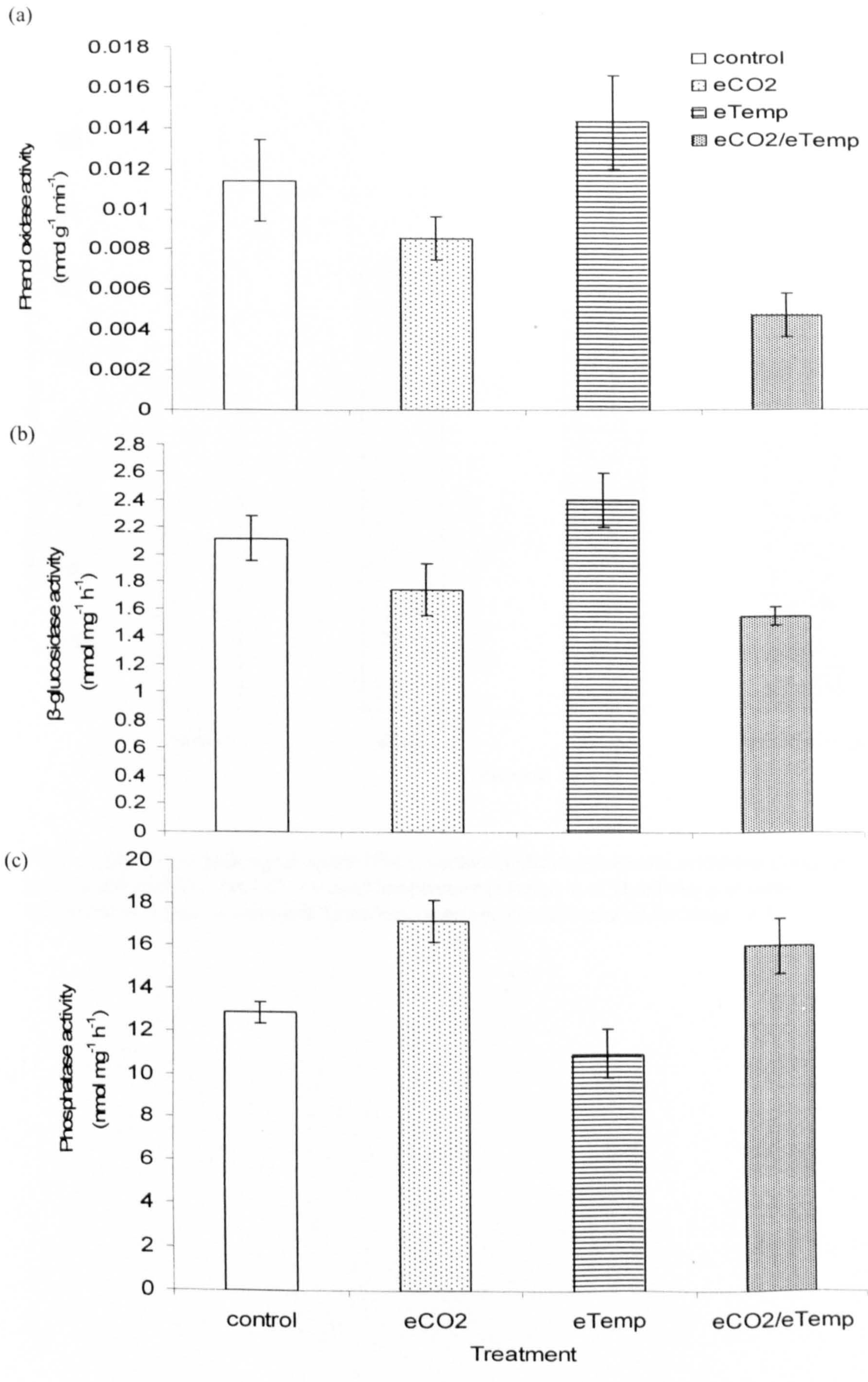

Figure 2.05 (a) phenol oxidase, (b) β-glucosidase & (c) phosphatase activities in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores following ca. 1 year of treatment. Error bars represent standard error of the mean, n=5.

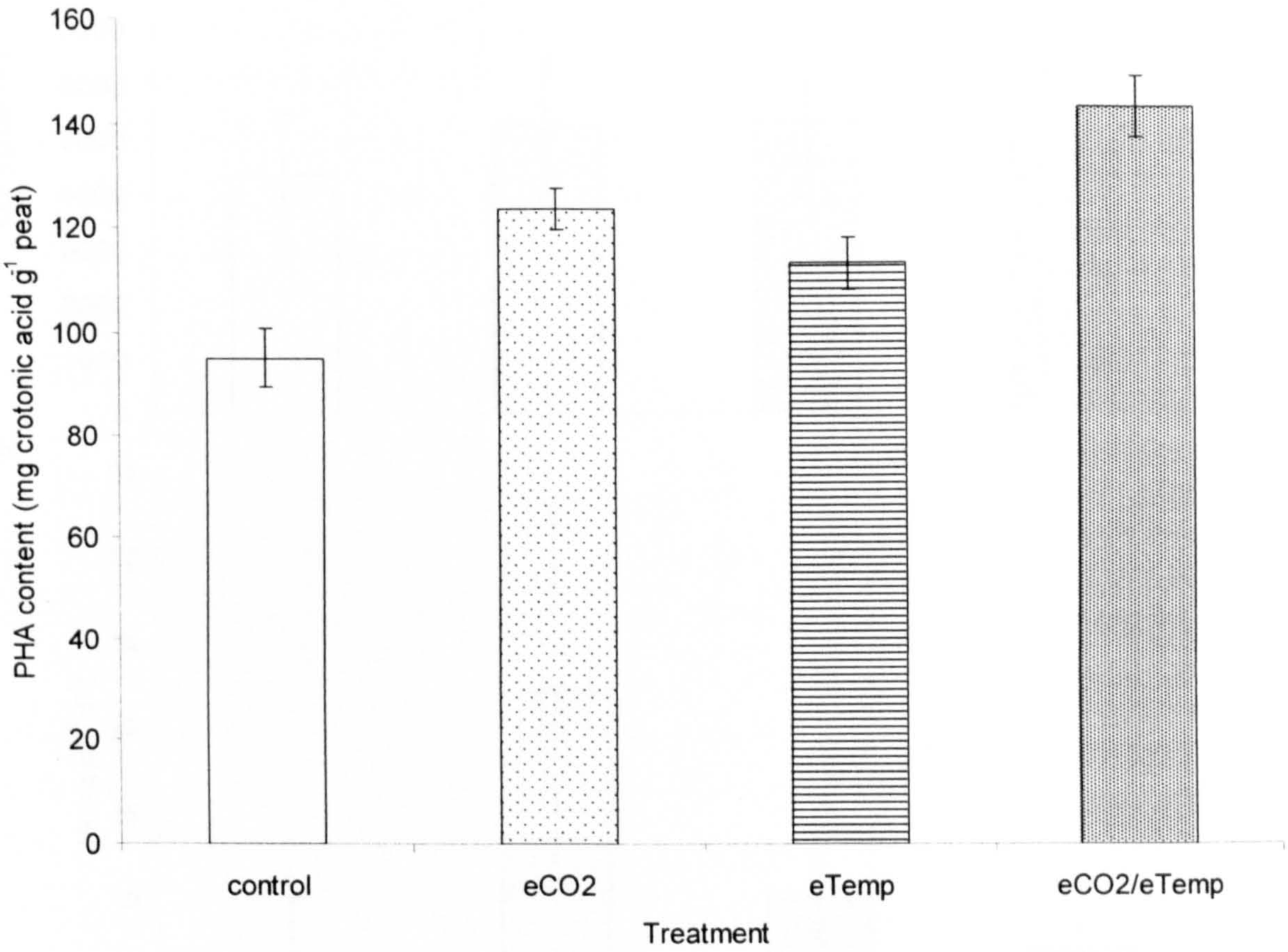

Figure 2.06. Poly-ß-hydroxyalkanoate (PHA) content (indicating bacterial nutritional stress) in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores following ca. 1 year of treatment. Error bars represent standard error of the mean, n=5.

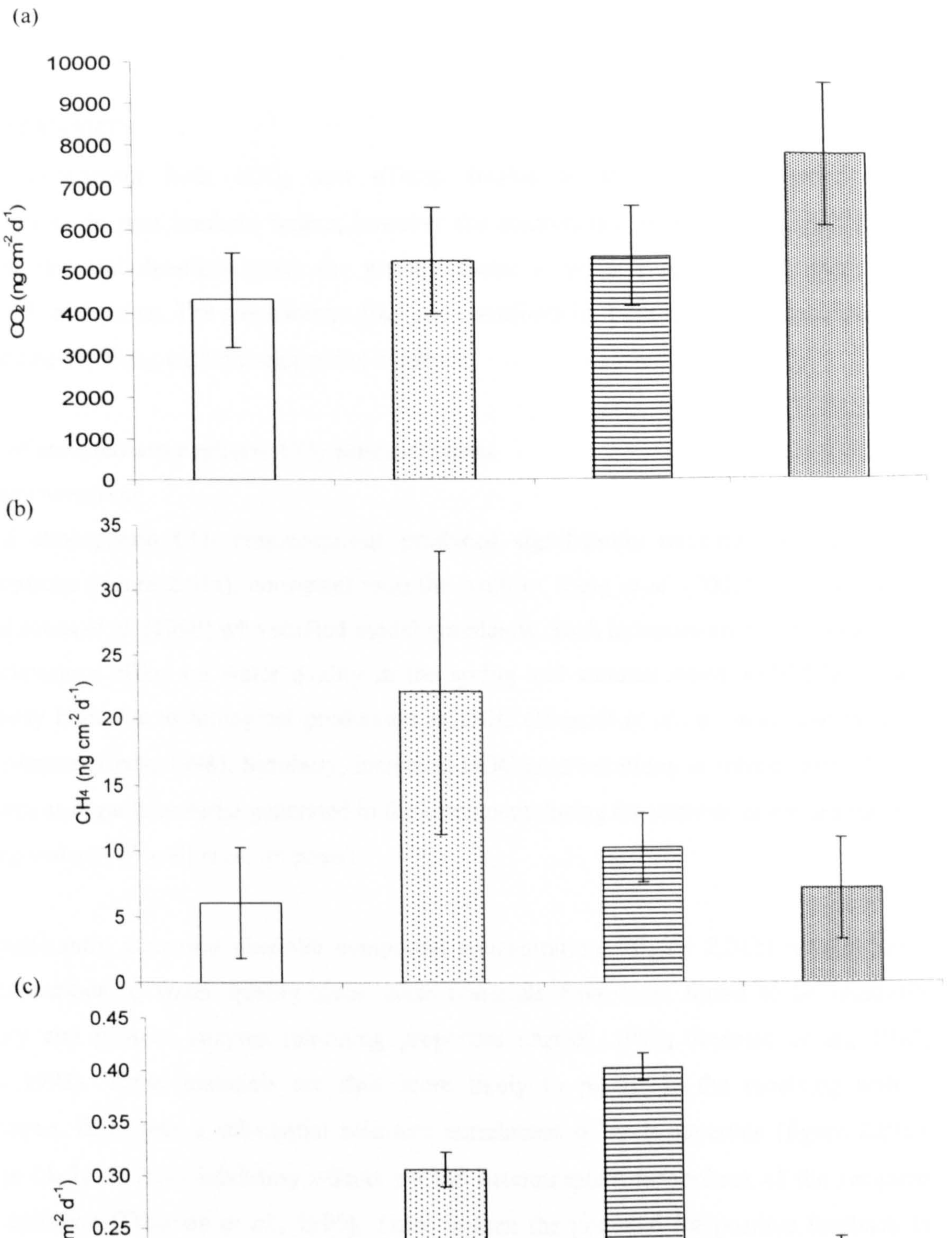

Figure 2.07 (a) CO_2 (b) CH_4 & (c) N_2O flux in control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp peat cores following ca. 1 year of treatment. Error bars represent standard error of the mean, n=5.

2.05 DISCUSSION

In this experiment both eCO_2 and eTemp treatments significantly increased DOC concentrations in peat leachate waters, however the combination of these treatments further increased this and therefore poses the greatest threat to water supplies from upland peat dominated catchments. The mechanisms likely to contribute to these findings in each case are discussed below along with the supporting evidence.

Effects of elevated atmospheric CO_2 concentrations

DOC concentrations

Elevated atmospheric CO_2 concentrations produced significantly increased leachate DOC concentrations (figure 2.01a), consistent with the work of Kang *et al.* (2001) working on fen peat and Jones *et al.* (1998) who studied model grasslands. Such increases are likely to have the most deleterious effect on water quality in the spring and summer when TTHM levels are particularly high due to biological production of DOC (Worrall *et al.*, in press) and reduced water volumes (Betts, 1998). Similarly, increased DOC concentrations in autumn may also be of concern as organic material generated in the catchment during the summer is washed into the receiving waters (Worrall *et al.*, in press).

The significantly increased phenolic compound concentrations (figure 2.01b) may be even more deleterious to water quality since these materials have been found to be relatively refractory and possess enzyme inhibiting properties (Appel, 1993; Freeman *et al.*, 1990; Wetzel, 1992). These materials are thus more likely to persist in the receiving waters. Furthermore, there was a substantial selective enrichment of such materials (figure 2.01c), which is likely to have inhibitory effects on the heterotrophic metabolism of the recipient stream epilithon (Freeman *et al.*, 1990). There is then the potential for positive feedback to increasing DOC concentrations and reduced water quality, with even the decomposition of the most labile DOC being impaired.

Increased DOC concentrations may be due to an increased input of recently assimilated carbon derived from *Sphagnum* exudates. However, given that this species has rhizoids rather than vascular roots, it is more likely that the contribution from the monocotyledonous (monocot) species (*F. ovina*) would be more significant. Moreover, a modest increase in the cover of *F. ovina* was observed (figure 2.02) suggesting more vigorous growth (conducive to the release of greater amounts of photosynthetically fixed carbon), while *Sphagnum* cover was reduced. It was anticipated that *Sphagnum* cover would not be directly affected by eCO_2 because its growth strategy (in close proximity to the peat surface) means it is likely to be naturally exposed to much higher concentrations of CO_2 than have been imposed here (as a result of soil

respiration), in contrast to the taller members of the community. Such results agree with those of the FACE (Free Air CO_2 Enrichment) experiments that took place on predominantly ombrotrophic peatbog-lawns in Finland, Sweden, The Netherlands and Switzerland, where no significant effect on *Sphagnum* or vascular plants was found after 3 years of treatment (560 ppm CO_2 concentration) (Hoosbeek *et al.*, 2001). Jauhiainen *et al.* (1997) also reported no distinct trends in *S. fuscum* grown at different CO_2 concentrations. Other studies have shown increased *Sphagnum* growth or no effect depending on the species studied. Van de Heijden *et al.* (2000), for example, found that the oligotrophic species *S. balticum* did not respond to eCO_2, supporting the results found here, while the structural biomass of the oligo-mesotrophic *S. papillosum* was stimulated significantly. Conversely, the monocots are more likely to be limited by lower concentrations of CO_2 and therefore respond when the eCO_2 treatments are imposed. Indeed, positive effects on *F. ovina* have been reported (Leadley & Stocklin, 1996).

Changes in plant species composition are inextricably linked to both the above ground and below ground biomass, which in turn will affect the exudation potential of the system. Above ground and below ground biomass increased substantially but not significantly (figure 2.03). The latter at least is likely to be due to the changes in the percentage cover and/or growth strategy of *F. ovina* given that *Sphagnum* lacks vascular roots. As mentioned previously, Leadley and Stocklin (1996) also reported a positive effect of eCO_2 on *F. ovina* growth as have other studies on grassland communities as a whole including this species (e.g., Warwick *et al.*, 1998). The increased root biomass is likely to represent an increased potential for exudation of recently assimilated carbon and such a theory is supported by the work of Cotrufo and Gorissen (1997), who found eCO_2 significantly enhanced below ground ^{14}C carbon allocation to the root and soil of *F. ovina* in grassland phytotron experiments. Similarly, Gorissen and Cotrufo (2000) again reported increased ^{14}C labelled carbon allocation to the roots in *F. ovina*, which may mean increased carbon flow from the root to the soil.

The increased phenolic compound concentrations observed are not inconsistent with increased exudation, since the organic materials found on, in or near roots reveal a wide range of aromatic and aliphatic acids, amides, sugars and amino sugars, along with the insoluble substances that occur in the root such as lignin and cellulose (Paul & Clark, 1989). There is apparently, little information on the effects of eCO_2 on the composition of root exudates though. Given that increased CO_2 levels can produce increased soluble phenolics concentrations (Gill *et al.*, 2002; Melillo, 1983) and often induce higher C:N ratios in root tissue (e.g., Curtis *et al.*, 1990; Gorissen *et al.*, 1995; Torbert *et al.*, 1995), it may be reasonable to speculate that this could also occur in the exudates.

Of course plants are only the primary receptors of increased atmospheric CO_2 levels (Sadowsky & Schortemeyer, 1997) within a plant-soil-microbe system and there are many ways in which eCO_2 may facilitate increased DOC concentrations. An increase in the input of what is often said to be easily utilizable (i.e., low molecular weight) DOC to the soil (in the form of exudates) may affect many biogeochemical processes mediated by soil microbes, at least in close proximity to the root where the effects of climate change are likely to first manifest themselves. The peatland system, given its retarded decomposition and nutrient cycling rates (e.g., Dickinson, 1983; Updegraff *et al.*, 1995), may be especially sensitive to increased labile DOC. Such inputs may fuel anaerobic respiration, which is said to favour the production of DOC products (Ponnamperuma, 1972), because eCO_2 did not induce significant changes in peat saturation (figure 2.04).

The general increase in plant biomass (above and/or below ground) is likely to mean increased carbon accumulation in the soil carbon reservoir (Smith & Shugart, 1993), which in turn may have produced the increased DOC concentrations observed in the leachate waters (c.f. Aitkenhead *et al.*, 1999; Hope *et al.*, 1994). This may also explain the increased phenolics concentrations (as much of the plant biomass would be composed of lignin), but the selective enrichment of phenolics suggests that there was a relative increase in phenolics production, for example, within the tissues of the vegetation (Gill *et al.*, 2002; Melillo, 1983). A further factor of relevance is the modest change in species composition to vegetation that is probably less refractory, in view of the fact that *Sphagnum* has been found to be particularly resistant to decay and may possess antimicrobial properties (Børsheim *et al.*, 2000; Painter, 1983, 1991; Verhoeven & Toth, 1995). Dissolved organic carbon and phenolic compounds may therefore be more readily released from the tissues of *F. ovina* and the latter at a relatively accelerated rate.

Despite the potential for increased substrates for microbial growth (such as increased exudates, root tissues and less recalcitrant plant litter), extracellular phenol oxidase and β-glucosidase activities decreased and the latter significantly (figures 2.05a & b, tables 2.05a & b respectively) suggesting an increased accumulation of pore water DOC is likely. However, this study provided no means of differentiating between changes in enzyme activity and production. End product inhibition due to increased amounts of labile exudates may account for such a finding, but many labile exudates in the aquatic system still require extracellular cleavage (Lancelot, 1984) and an analogous situation may be true in the peatland system. Cellulase activity and production is said to decline in the presence of the simpler carbohydrates (Chróst, 1993), thus an increased availability of labile materials in the root region may suppress the decomposition of more complex polymers (Moorhead & Linkins, 1997). If the same principle

is applied to the degradation of phenolic compounds by phenol oxidase, this may account for the selective enrichment of phenolic materials observed. Alternatively, inhibitory mechanism(s), such as increased phenolic compound concentrations, altered tissue composition or lack of nutrients, may be exerting an influence on the activities of the microbial population. These reduced carbon cycling enzyme activities may mean less decomposition of pore water DOC (e.g., root exudates). However, such reduced activities could eventually impair nutrient cycling; a negative feedback to plant growth (Baxter *et al.*, 1994; Zangerl & Bazzaz, 1984) and therefore decrease DOC production. This is significant in terms of positive feedback to climate change because less sequestration of CO_2 *via* the plant community would allow atmospheric CO_2 concentrations to rise more rapidly as a result of anthropogenic activities.

Given the versatility of microbial catabolism (e.g., Arcangeli & Arvin, 1995), it seems unlikely that all the species producing aromatic degrading enzymes would be inhibited under the relatively modest climate changes imposed. However, degradation of lignin and cellulose is a community scale process because few organisms posses the entire suite of enzymes needed for complete degradation of such materials (Sinsabaugh *et al.*, 1994), and cellulytic and lignolytic fungi may be particularly sensitive to climate change (Schimel & Gulledge, 1998). Thus, it may be that adverse effects upon only a single species within the community may be sufficient to have a substantial impact on carbon processing, favouring the DOC mode of carbon export rather than rapid mineralization to CO_2.

Elevated CO_2 stimulated phosphatase activities considerably (figure 2.05c, table 2.05b), in contrast to the carbon cycling enzymes, and this is consistent with the increased levels of DOC being of a plant derived nature as enhanced plant growth would require increased phosphate uptake. Microbial phosphatase activities may have increased to relieve phosphate limitation, in line with the findings of Moorhead and Linkins (1997) and Barrett *et al.*, (1998). Similarly, in their model MARCIE (Microbial Allocation of Resources Among Community Indicator Enzymes), which predicts mass loss as a function of extracellular enzyme activity, Sinsabaugh and Moorhead (1994, 1997) suggest that the production of carbon acquiring enzymes is constrained by the need to enzymatically acquire nitrogen and phosphorus. The presence of increased carbon substrates may therefore allow a shift in enzyme production (i.e., energy expenditure) to phosphate acquisition. The altered plant species composition may also have had an effect, for example, *F. ovina* may require more phosphate for growth than *S. cuspidatum*.

However, care is required as the potential impacts of climate change on enzyme activities are likely to be complex and mechanisms may differ spatially and temporally. For instance, in the

short term cellulase production may be inhibited by increased exudation and rhizodeposition, but in the longer term enhanced root production may provide more organic carbon (e.g., dead root material) which is a substrate for cellulase producing organisms. Furthermore, impacts could differ between the rhizospheric area and the bulk soil. Moorhead and Linkins (1997) found increased phosphatase activities under eCO_2, but only on the root surface or in soil within close proximity to the root. The results in this study were obtained from the bulk soil and as enzyme activities were measured after a year it is believed that the systems would be well equilibrated. Thus, the enzyme activities would be expected to be representative of activities in the *bulk soil* of the northern peatland system. Perhaps in hindsight though, a measure of rhizosphere enzyme activities would have proved informative, since eCO_2 may intensify carbon processing in the rhizosphere.

A further explanation for the suppressed phenol oxidase and β-glucosidase but stimulated phosphatase activities is that the vegetation is out competing the microbes for inorganic nutrients (Freeman *et al.*, 1998), thus reducing microbial carbon degradation and causing increased phosphatase production to relieve inorganic nutrient stress. Freeman *et al.* (1998) found little evidence to suggest that an increase in microbial competitiveness would occur (looking at the growth of *Poa alpina* under 700 ppm CO_2), but rather that the plant was at an advantage. It must be noted however, that propagules were grown in sand, rather than natural soil, to ensure that the soil microflora was dependent on plant exudates as the primary source of carbon (unlike the situation in natural soil, where other organic materials would be present). They used ^{14}C labelled CO_2 to trace carbon from the atmosphere into the plant and soil microflora in order to determine whether the microbes would benefit from an increased flux of organic carbon under eCO_2. Labelled phosphate (^{32}P) was used to determine whether the soil microflora were sequestering organic nutrients more effectively than the plants. Though 114% ($P<0.05$) more ^{14}C was incorporated into the plants grown at 700 ppm in relation to those at 350 ppm CO_2, 31% less label was incorporated into the microbial lipids ($P<0.05$). Apparently, microbial metabolism was limited by factors other than the availability of organic substrates (c.f. van Veen, 1991). Similarly, at the high CO_2 levels plants incorporated 44% ($P<0.05$) more phosphate, while the microbes under the same conditions showed 26% ($P<0.05$) less ^{32}P incorporation (Freeman *et al.*, 1998). Such findings imply that under eCO_2, it is the plants that are favoured rather than the microbes in the competition for inorganic nutrients.

Significantly increased PHA concentrations were found in this study (29.82%, $P<0.01$, figure 2.06) suggesting an increase in microbial nutritional stress due to eCO_2, consistent with the hypothesis of Freeman *et al.* (1998). Reduced carbon acquiring enzyme activities and a stimulation of phosphate acquiring enzyme activities may thus produce such unbalanced

microbial growth. Acidic mires tend to be poor in terms of available nutrients, which may exacerbate any competition for inorganic nutrients between the plant and the microbial community, with root growth becoming more extensive as the vegetation attempts to capture such resources.

Both the results in this chapter and those of Freeman *et al.* (1998) conflict with the theory that an increased microbial biomass would enhance nutrient uptake and therefore reduce the nutrients available to plants (Diaz *et al.*, 1993). However, from first principles, eCO_2 would be considered beneficial for plants as it represents an increase in the abundance of a primary resource for photosynthesis, but an unwanted burden of metabolic waste product for microbes (Freeman *et al.*, 1998). Since microorganisms mediate nutrient cycling, this would mean reduced rates of inorganic nutrient release from the decomposition of previous generations of plants. This presumably would eventually represent a negative feedback to increased plant derived DOC concentrations.

Clearly, further investigation is required in order to elucidate the cause of the reduced microbial activity at high CO_2. Increased PHA concentrations are consistent with the competition theory proposed by Freeman *et al.* (1998). However, the increased phenolic compound concentrations suggest a more complex situation with enzyme inhibition perhaps contributing to the increased DOC concentrations under eCO_2. The source of the phenolic substances is not clear but is likely to be derived from plant exudates and/or ligniferous structural material. In this study, the balance between plant production and microbial decomposition of organic materials would be important in determining DOC concentrations available for export in leachate waters. Elevated CO_2 seemingly shifts this balance in favour of increased plant production without inducing a similar increase in the consumption of that material by the microbial population.

Trace gas fluxes

Trace gas fluxes were variable but tended towards increasing CO_2 emissions (figure 2.07a). Altered plant community structure is known to effect trace gas emissions from wetlands because many vascular plants growing in flooded soils produce aerenchyma (a tissue that contains extensive gas spaces) (Sculthorpe, 1985). Aerenchyma transports oxygen to the root tips in saturated soil and also CO_2 and CH_4 to the atmosphere (Holzapfel-Pschorn *et al.*, 1986) without it being fixed by photosynthetic algae or microorganisms at the soil surface. Such plants serve as direct conduits between reducing and oxidizing environments (Chanton *et al.*, 1992) and can be responsible for up to 90% of total CH_4 flux (Shannon *et al.*, 1996). Increased CO_2 flux may therefore be a simple physical effect whereby greater amounts of vascular plant biomass provides more channels for the escape of gases (produced as a result of microbial

activity). Since trace gases were measured at the end of a year period, there may also have been changes to the production rate rather than simply an increased transport of these gases from an unchanged reservoir beneath the peat surface. Altered litter composition and carbon inputs (as a consequence of the presence of vascular plants and their increased contribution of root and shoot biomass, for example) may increase microbial respiration, thus increasing CO_2 concentrations within the peat. Enhanced root exudation would be expected to stimulate microbial respiration due to the increase in labile carbon availability (c.f. Körner & Arnone, 1992), although in this case the microbial population is seemingly inhibited. Given that root derived respiration can comprise 35-45% of total soil respiration in organic soils (Silvola *et al.*, 1996), it is feasible that root respiration accounts for the increase but it is impossible to confirm this with the present study. Such results suggest that northern peatlands may represent a positive feedback to climate change, releasing CO_2 as the product of the decomposition of a larger pool of decaying biomass, for example, rather than sequestering CO_2 as biomass. The recent study by Cox *et al.* (2000) (which examines the ability of land and ocean ecosystems to absorb CO_2) highlights the importance of understanding climate change feedback mechanisms. They present results from a fully coupled, three-dimensional, carbon-climate model indicating that climate change could accelerate significantly over the 21st century due to carbon cycle feedback. The authors calculate a global mean warming of 8°C over land, compared to 5.5°C without the carbon-cycle feedback, by the year 2100.

Peatlands are a major source of atmospheric CH_4, contributing an estimated 25-30% of the total released to the atmosphere each year (Cicerone & Oremland, 1988). It is estimated that CH_4 has approximately 21 times the atmospheric warming potential of CO_2 over a 100 year time period (Schimel *et al.*, 1996). A substantial but insignificant increase in CH_4 flux was found under conditions of eCO_2 (figure 2.07b). *Sphagnum* is generally considered to have no role in the conduction of CH_4 because of its lack of roots or ventilation system and because significantly lower CH_4 fluxes have been reported from areas of pure *Sphagnum* (Thomas *et al.*, 1996). In tundra systems, high rates of CH_4 oxidation have been reported in the moss layer (Vercherskaya *et al.*, 1993) and the soil surface (Whalen & Reeburgh, 1990; Whalen *et al.*, 1996). This lead to the hypothesis that *Sphagnum* may limit CH_4 release (Greenup *et al.*, 2000). However, these authors reported no significant changes in CH_4 emissions when the *Sphagnum* was removed in the field, suggesting no limitation of diffusion or production and that *Sphagnum* did not act as a site for CH_4 oxidation. Methane was found to be present at depths below the *Sphagnum* but escaping at a retarded rate by molecular diffusion or ebullition (bubbling) from the peat to the atmosphere. A shift toward a monocot dominated system and increased biomass, leading to enhanced gas transport, may therefore explain the large increase in CH_4 fluxes from the eCO_2 cores. However, plants can also affect CH_4 dynamics through the translocation of photosynthate

to their roots and, subsequently it passage into the rhizosphere (van Veen *et al.*, 1989), increasing substrate availability to the methanogens. Thus, the increased CH_4 flux again does not conflict with the theory of an increased labile substrate supply, in the form of DOC, to the methanogenic population *via* photosynthesis. Enhanced CH_4 emissions from CO_2 enriched systems have been observed by several workers (e.g., Dacey *et al.*, 1994; Hutchin *et al.*, 1995; Megonigal & Schlesinger, 1997) and this was also attributed to increased plant productivity stimulating methanogenic bacteria by supplying more root exudates and/or increasing CH_4 transport through the plant.

Nitrous oxide is an even more potent greenhouse gas with an atmospheric warming potential of ca. 200 times that of CO_2 (Rodhe, 1990) and atmospheric concentrations are increasing at the rate of 0.2-0.3% per year (IPCC, 1996). Elevated CO_2 can modify soil carbon and nitrate availability (Körner & Arnone, 1992), which are important controls on N_2O production by denitrification (Bowden *et al.*, 1992; Seitzinger, 1994). The flux of N_2O seemingly increased considerably under eCO_2 (149.51%, $P<0.001$; figure 2.07c), consistent with enhanced carbon availability to microorganisms in the soil, activating the denitrification/nitrification process (Davidson, 1991). Increased rhizosphere denitrifier activity under eCO_2 has been reported in mineral soil planted with wheat (Smart *et al.*, 1997) and to increase N_2O flux from a calcareous grassland (Arnone & Bohlen, 1998). No attempt was made in this study to determine whether increased N_2O emission was produced by enhanced denitrification or nitrification. This could be done using an inhibition soil incubation method (e.g., an acetylene blocking method).

These results suggests implications for the management of peatlands as sources or sinks of trace gases, as any change leading to a increase in vascular plant coverage could lead to a concomitant increase in emissions, in line with the findings of Greenup *et al.* (2000) regarding CH_4.

Effects of elevated temperature

DOC concentrations

Elevated temperatures (eTemp) also appeared to significantly increase pore water DOC concentrations, particularly during the summer months (figure 2.01a) when water quality is already likely to be relatively low. Several studies demonstrate increased DOC concentrations under warmer conditions (Ineson *et al.*, 1995; Tipping *et al.*, 1999; see chapter 3) including Freeman *et al.* (2001a) working on the same study site (see chapter 3). Warming apparently allowed slightly earlier 'spring' DOC peaks, perhaps as a result of stimulated microbial activity earlier in the year than for ambient temperature treatments. Since it is widely found that DOC concentrations tend to increase due to biological activity in the summer months (Hughes *et al.*,

1990; Worrall *et al* in press), eTemp may increase DOC export due to a lengthened period in the year that is conducive to this activity. Such a phenomenon may contribute to the rising DOC concentrations in UK rivers, as there is evidence to suggest that spring is getting earlier (Gian-Reto *et al.*, 2002) and the growing season longer (Gian-Reto *et al.*, 2002; Hulme *et al.*, 2002; Menzel & Fabian, 1999; Roetzer & Chmielewski, 2000).

Elevated temperature also induced significantly higher phenolic compound concentrations (figure 2.01b) (as is reported in chapter 3) with a selective enrichment of the DOC pool (figure 2.01c), suggesting the potential for positive feedback to reduced water quality. Such findings conflict with those of Tipping *et al.* (1999) who found no changes to the hydrophilic/hydrophobic ratio and optical absorbance of DOC due to higher temperatures in their moorland study. This may indicate site specific responses should be expected.

The observed increases in DOC and phenolics concentrations may again be due to increased root exudation and/or biomass inputs because there was a dramatic effect of eTemp on the plant community structure (figure 2.02, tables 2.02a-c), perhaps due to *S. cuspidatum* experiencing direct climate associated stresses under warmer conditions but also as a result of enhanced evapotranspiration (leading to drier conditions in the surface layer of the peat). This might both reduce *S. cuspidatum* cover and favour the monocot invasion directly, while the latter would accelerate the former due to increased peat aeration as a consequence of more extensive rooting and evapotranspiration. Saturation measurements confirmed significant drying in the surface layer 2-3 cm (-17.69%, $P<0.01$) and reductions at depth, with a consistently lower percentage saturation being observed than in the ambient cores until at least 10-11 cm (figure 2.04). Such changes could account for the increased DOC concentrations by causing increased chemical oxidation of the peat (Worrall *et al.*, in press), even without taking enzymatic generation into consideration (see below). Transient increases in DOC release have also been demonstrated by Tipping *et al.* (1999). However, it was concluded that increased temperature *per se* would not lead to sustained increases in DOC export but that a combination of warming and drying was necessary. This is supported by the work of Worrall *et al* (in press) who found that two out of three catchments studied in the Pennines showed increasing DOC and colour trends over ca. thirty years. While all three catchments, it was assumed, had been exposed to the same increasing summer temperatures, the two exhibiting increasing DOC had been subject to drainage. It may be that the effective warming and drying that occurs under the eTemp conditions is responsible for the increased DOC concentrations in this situation.

Increased drying within the surface layer of the peat may induce the formation of a hydrophobic layer that would insulate against rehydration, setting up a positive feedback to

reduced saturation and increased peat oxidation. Such conditions may also be conducive to cracking and pipe formation in the environment (Gilman & Newson, 1980). Changes to the drainage of the peat (e.g., increased pipe and overland flow) may allow DOC-rich water to bypass absorption sites *en route* to the stream channel. Thus, reduced peat saturation and the potential for positive feedback suggests that warmer soils could have severe consequences for the stability of northern peatlands, accelerating aeration and therefore, it is assumed, decomposition even at depth. The reduction or loss of these peatlands has implications for water quality, flooding, habitat/species loss, global carbon budgets and feedback to climate change.

Enhanced microbial decomposition both of the peat matrix and dissolved materials in the pore waters was anticipated, hence increasing DOC concentrations from the former source but reducing it from the latter, either as a result of the higher temperatures or more aerobic conditions in a greater proportion of the peat. Indeed, increased phenol oxidase and ß-glucosidase activities (figures 2.05a & b) were recorded, in contrast to the eCO_2 treatment and consistent with recent work suggesting that these enzymes may generate soluble products (Freeman *et al.*, 2001a; see chapter 3). Phenol oxidase may, for example, generate dissolved phenolic compounds faster than they can be consumed by the microbes in the system. Similarly, it has been reported that increased enzyme activities will accompany higher temperatures and more frequent drought events predicted by climate change models (Freeman *et al.*, 1996). Enzymic generation is likely to be most pronounced in the summer months, at a time when water quality is already compromised, further amplifying the DOC production *via* chemical oxidation. DOC concentrations in the leachate will thus represent the net effect of numerous interacting processes, and enhanced matrix decomposition (as a result of stimulated enzyme activities) may play a major role in inducing increased DOC export. Increased labile plant inputs have the potential to effectively 'prime' previously energy limited heterotrophs, allowing enhanced degradation of the more recalcitrant materials of the peat matrix and enhancing mobilization of aged DOC. Indeed, it is thought that some lignolytic microorganisms (e.g., fungi) degrade lignin only in the presence of some other labile substrate as the primary energy source (Paul & Clark, 1989). Similarly, the phenomenon of co-metabolism is well documented in the field of biodegradation. Another possible factor of relevance in producing enhanced DOC release is increases in enchytraeid activity (Briones *et al.*, 1998) and this may be an intermediate in the enzyme response observed (see chapter 9), aerating the soil and promoting nutrient cycling. Increased nutrient availability would further promote plant growth and enhance DOC concentrations both directly and as a result of priming effects.

Surprisingly, warming did not stimulate phosphatase activities (figure 2.05c) and this may mean one of several things. First, factors other than low temperatures may be limiting phosphatase activities in the peat system, such as the presence of inhibitory phenolic compounds. Secondly, warmer temperatures may mean that there is relatively less competition from the vegetation for inorganic nutrients as a result of increased microbial enzyme activities. Thirdly, there may have been a change in the relationship between plant and microbial components of the system (e.g., in the nature of root exudates) altering phosphate acquisition requirements. However, while bulk soil enzyme activities decreased, rhizosphere activities may have increased associated with the supply of labile carbon from the roots. Seemingly, microbial enzyme production in the bulk soil has been shifted in favour of carbon acquisition, perhaps due to increased microbial growth rates facilitating nutrient cycling and rapid utilization of labile DOC. As mentioned previously though, it may be that extracellular enzyme activities are generating soluble DOC products faster than they can be incorporated into microbial biomass, especially if these are refractory in nature.

Elevated temperatures did not significantly increase microbial nutritional stress as indicated by PHA concentration (figure 2.06). This is in line with the increased carbon cycling enzyme activities without an increase in phosphatase activities, indicating balanced growth and reinforcing the notion that it is the microbes that are stimulated under eTemp. Thus, there are likely to be different mechanisms contributing to the enhanced DOC concentrations in comparison to the eCO_2 treatment.

Any differences in soil water content (induced either directly by eTemp or indirectly *via* the change in plant community) could potentially modify the growth of plants, microbes and the interactions between them. Little is known about the effect of saturation on the deposition of plant exudates in the rhizosphere (Drew, 1990) and the subsequent growth of rhizosphere microorganisms. Sadowsky and Schortemeyer (1997) postulated that decreased soil water would affect microorganisms directly, by decreasing water activity below certain critical growth tolerance limits, and indirectly, by altering the types and quantities of root exudates released into the rhizosphere as well as by altering root growth. However, in wetlands enzyme activities have been found to increase upon drainage (Freeman *et al.*, 1997; McLatchey & Reddy, 1998). Bacteria have narrow tolerance limits to low water potential, so microbial activity in dry soils will be dominated by fungi and actinomycetes (Brown, 1990). A change in the microbial community may alter degradatory processes including DOC production and consumption. For example, it is widely accepted that fungi are major producers of phenol oxidases which may accelerate mobilization of the peat matrix under a future, warmer climate, increasing phenolic compound release into the pore waters.

Trace gas fluxes

Under eTemp there was a tendency was for increasing CO_2 fluxes (figure 2.07a) but this showed relatively large natural variation and was not statistically significant. Again, it is possible that this is due to the altered community structure acting as a more efficient conduit. Some studies have suggested that a lowered water table can release CH_4 trapped in deep peat (Romanowicz *et al.*, 1995) and the reduced saturation under warmer conditions may induce a similar effect in CO_2 flux. Others find no such seasonal change in deep peat CH_4 concentrations from sites with seasonal fluctuations in the water table (Clymo & Pearce, 1995). Increased CO_2 fluxes may represent an increase in microbial respiration due to the eTemp and/or increased labile carbon substrates. Altered litter composition and carbon inputs (as a consequence of the presence of vascular plants and their increased contribution of root and shoot biomass) are equally likely to increase CO_2 fluxes. Again it is feasible that root respiration was also enhanced. A positive linear relationship between CO_2 emissions and temperature has been reported for a range of soil types in laboratory experiments (e.g., Howard & Howard, 1993; Wiant, 1967) and increased CO_2 emissions under warmer temperatures were also found in the thermal gradient study (discussed in chapter 3).

Despite the substantial rise in DOC concentrations, which would provide a substrate for methanogenesis, the eTemp treatment did not significantly increase CH_4 fluxes (although the tendency was for increased emissions) (2.07b). However, the dual function of aerenchymous tissues in transporting oxygen to the roots (Roura-Carol & Freeman, 1999) may negate the positive effects of a conduit system and photosynthesis on CH_4 emissions. Strictly anaerobic methanogenic bacteria being inhibited (Watson *et al.*, 1997) and methanotrophic bacteria (the activity of which is constrained by oxygen transport from the roots to the otherwise anoxic rhizosphere (Rosalev & King, 1994)) stimulated by the increased oxygen. Net CH_4 emissions are therefore a balance between these processes. Another possibility is that the considerable algal presence on the surface of the cores (as observed in fen studies, Kang, 1999) had a significant influence. Methane emissions were determined during the day when photosynthesis and hence oxygen production by the algae would be expected to be high. This may diffuse into the soil thus inhibiting the methanogenic bacteria and/or activating CH_4 oxidizing bacteria. Higher temperatures may either favour algal oxygen production, inhibit the methanogens and/or activate methane oxidizers.

Elevated temperature seemingly induced the largest increase in N_2O flux (227.61% $P<0.001$, figure 2.07c). The enhanced carbon availability for microorganisms in the soil may again be important, activating the denitrification/nitrification process (Davidson, 1991), but also N_2O

producing microbes may be especially temperature limited in cool peatlands and therefore benefit from the warmer conditions directly or indirectly (e.g., *via* increased nutrient availability or reduced waterlogging).

Effects of elevated CO_2 and elevated temperature

DOC concentrations

The dramatically increased DOC, phenolics concentrations and selective enrichment for the latter (figures 2.01a-c) suggests that the greatest threat to water quality is posed by eCO_2/eTemp in combination, with an interaction occurring between the two factors. The response was remarkably rapid, becoming significantly different to the control within 18 days of treatment and producing a peak in concentrations over ca. one month, which perhaps suggests that exudation is important in producing the amplified DOC concentrations. Changes in the amount of biomass and chemical changes in litter composition would be expected to occur over a longer time period. Physical disturbance of the cores may have caused the initial peak in concentrations but as this did not occur in the control cores and the DOC concentrations remained significantly higher throughout the year, this seems improbable. Perhaps then, the initial peak was induced by the change in environmental conditions and a period of acclimation was necessary, after which DOC concentrations stabilized.

Elevating both temperature and CO_2 levels induced the most dramatic reduction in mean *S. cuspidatum* cover (-62.88%, $P<0.01$) and mean *J. effusus* cover was substantially but not significantly increased (figure 2.02, table 2.02b). *Juncus effusus* may represent the greatest exudation potential because it has a far more substantial, deep rooting system in relation to the other species present. Carbon dioxide has been found to stimulate the growth of another *Juncus* species, *J. bulbosus*, up to a concentration of 500 ppm however, higher CO_2 pressure restrained growth (Svedang, 1992). In this study, both eCO_2 and eTemp were required for *J. effusus* to increase its coverage. *Juncus* may be more efficient in terms of water use under the warmer conditions than the other species allowing it to gain an advantage from the eCO_2 and dominate. In contrast, *Sphagnum* is said to be poikilohydric and relies on physiological mechanisms to avoid death when desiccation occurs, *S. cuspidatum* being confined to wet habitats (Smith, 1978). Since eCO_2 results in decreased soil water stress along with increased carbon input to the soil (Owensby *et al.*, 1993), the increased water use efficiency (WUE) is likely to allow more aggressive growth when warmer temperatures are also imposed than under the separate treatments and so even more exudation and/or biomass inputs.

Seemingly, eCO_2 and eTemp interact to exacerbate changes in community structure, perhaps because both effectively cause drying and warming, the former indirectly and the latter directly

and indirectly through changing the plant species composition. The even greater reduction in saturation in the surface layer of the peat (30.22%, $P<0.01$) than the eTemp treatment and consistent reduction down to at least 6-7 cm (figure 2.04) supports such a theory. It follows that chemical oxidation of the peat is likely to be most intense under such conditions, providing DOC from the degradation of the matrix for export upon rehydration. These conditions may also facilitate nutrient cycling, allowing species other than *Sphagnum* (which is adapted to nutrient poor environments, gaining nutrients ombrotrophically rather than *via* uptake from the soil) to invade, providing positive feedback to the alteration of peat properties. The nature of litter produced from different plant species is likely to promote DOC release as previously mentioned, although the quality of the monocot litter eventually may change as a result of eCO_2 with implications for DOC release.

Significant increases in above ground biomass (260.31%, $P<0.001$, figure 2.03) may be due to a fertilizing effect of eCO_2 on the plant community already present but also to the dramatic shift towards a monocot dominated system. An enhanced supply of photoassimilates may lead to the production of greater root biomass (397.66%, $P<0.001$, figure 2.03), representing an increased DOC input to the soil both as biomass and exudates. Warmer conditions may further promote growth and evapotranspiration, necessitating more extensive rooting to capture water and other nutrients. Increased aeration of the peat will be further reinforced by increased oxygen release from a more substantial root system, which may further exacerbate mobilization of the peat matrix *via* chemical oxidation.

Both amplified plant inputs (exudation and structural material) and chemical oxidation of the peat are likely to produce increased DOC concentrations along with the change in nature of the litter produced. However, despite the huge potential for increased substrates for microbial growth and warmer temperatures conducive to microbial proliferation, extracellular phenol oxidase and β-glucosidase activities decreased significantly, as was the case in the eCO_2 treatment (see figures 2.05a & b, tables 2.05a & b respectively). This conflicts with the original hypothesis that enzymic generation of DOC from the peat matrix would occur. Again this may be as a result of those mechanisms already described such as end product inhibition, but PHA concentrations suggest nutritional stress; eCO_2/eTemp seemingly inducing the greatest stress (50.48%, $P<0.001$, figure 2.06). This is consistent with the suppressed carbon cycling enzyme activities and the stimulated phosphatase activities observed (figure 2.05a-c respectively). Thus, these results imply vigorous plant growth, contributing increased organic carbon to the system, while forcing increased microbial enzyme allocation to acquire inorganic nutrients and unbalanced growth. It seems then, that the enhanced DOC concentrations in relation to the other treatments is due to further increased plant inputs and chemical oxidation of the peat

matrix, coupled with reduced microbial degradation of such materials, allowing their accumulation in the pore waters.

Trace gas fluxes

The largest increase in CO_2 flux (though still non significant) was found when both the atmospheric CO_2 concentration and the temperature was elevated (figure 2.07a). The mechanisms at work under the separate eCO_2 and eTemp treatments seemingly combine to amplify CO_2 emissions, indicating that positive feedback to a changing climate is likely (in view of the fact that CH_4 and N_2O emissions were not suppressed).

Methane emissions were not amplified in comparison to the separate treatments (figure 2.07b) but tended to increase nonetheless. Since the eCO_2/eTemp cores contained more root biomass than the other treatments, more oxygen would perhaps be expected to reach the deeper peat. This would be transported more extensively by the increased root system, strongly inhibiting methanogenic bacteria (Jespersen *et al.*, 1998). The slight increase in CH_4 emissions in relation to the control may reflect the net effect of a greatly enlarged labile substrate pool for methanogenic bacteria and/or an increased conduit effect that is virtually negated by considerable oxidation due to increased oxygen availability and drying.

Nitrous oxide emissions tended to increase but not significantly so (figure 2.07c), unlike the other treatments, suggesting that there is some factor constraining microbial N_2O production, perhaps mediated by the much increased plant growth (e.g., nutritional limitation or increased aeration).

Increased trace gas emissions, though less pronounced than under the separate treatments, again emphasize the potential for positive feedback to climate change and the need for accurate modelling of such systems.

2.06 CONCLUSIONS

From the results observed it is clear that there is the potential for increased DOC concentrations as a result of eCO_2, eTemp and a combination of eCO_2 and eTemp. The latter is likely to be the most deleterious to water quality since this treatment produced not only the greatest enhancement of DOC concentrations but also the greatest selective enrichment of phenolic compounds. Such selective enrichment was pronounced in all treatments, highlighting the extensive potential for positive feedback to increased DOC concentrations and reduced water quality as a result of impaired degradation of even the most labile DOC in the recipient waters.

Considerable shifts in the plant community structure and therefore exudation potential and biomass inputs were observed, especially under eCO_2/eTemp, mediating marked changes in the biogeochemistry of the peat. The original hypothesis that eCO_2 would increase DOC concentrations as a result of increased plant inputs (exudates and/or biomass) seemingly holds true, but suppressed enzyme activities suggest reduced degradation of such materials allowing an accumulation in the leachate waters. Stimulated carbon cycling enzyme activities under eTemp implied that enzymic mobilization of the peat matrix generated increased DOC concentrations, while increased labile plant inputs potentially primed the degradation of refractory peat materials, amplifying the DOC release due to enhanced chemical oxidation under drier conditions. Under the combined eCO_2/eTemp treatment, DOC concentrations were further enhanced but this was attributed to even more vigorous plant growth increasing organic carbon inputs, exacerbating chemical oxidation and further reducing DOC consumption by the microbial component (rather than increased enzymic mobilization of the peat matrix).

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CHAPTER 3: RISING TEMPERATURE AS A DRIVING MECHANISM FOR DOC RELEASE

3.01 INTRODUCTION

Global average surface temperatures are set to rise by between 1.47°C and 5.87°C by 2100 (IPCC, 2001). The rate of warming is projected to be especially rapid in northern latitudes where much of the world's peat reserves are located (IPCC, 2001). Temperature has a key role in controlling the rates of biogeochemical processes in soils, including peat soils, with effects upon dissolved organic carbon (DOC) release (e.g., Briones *et al.*, 1998; Freeman *et al.*, 2001a; Ineson *et al.*, 1995; Tipping *et al.*, 1999) carbon dioxide (CO_2) (e.g., Moore & Dalva, 1993), methane (CH_4) (e.g., Wilson *et al.*, 1989) and nitrous oxide (N_2O) emissions (e.g., Bailey and Beauchamp, 1973; Lensi & Chalamet, 1982), as well as the availability of nutrients (e.g., Koerselman *et al.*, 1993; Ross, 1985).

Strong trends for rising DOC concentrations have been found for over a decade in receiving waters in north and mid Wales (including those draining the study site) (Freeman *et al.*, 2001a; Reynolds *et al.*, 1997; Robson & Neal, 1996) at a time of rising global temperature. Recently it has been suggested that riverine DOC has been pre-aged prior to reaching the ocean, such that older, more refractory compounds of terrestrial origin have come to dominate the recipient ecosystem (Raymond & Bauer, 2001). Northern peat accumulating wetlands have exceptionally low rates of decomposition creating a vast global store (Gorham, 1991) of highly aged (Clymo, 1983) organic matter (OM). Such material is of an age comparable with the average age of marine organic carbon (Smith & Hollibaugh, 1993), according to radio carbon dating (Williams & Druffel, 1987). Monitoring of DOC within UK rivers has revealed a strong trend for rising concentrations. DOC has risen significantly ($P<0.05$) at 20 out of 22 UK fresh water Acid Monitoring Network sites and at some sites annual mean DOC concentrations have more than doubled since 1988 (Freeman *et al.*, 2001a). Annual increases, on average 5.4%, were said to be proportionate to mean DOC concentration ($R^2 = 0.81$, $P<0.001$). Since freshwater DOC concentrations are linked to storage of carbon within the catchment soil (Aitkenhead *et al.*, 1999; Hope *et al.*, 1997), this indicated that regionally consistent processes within this carbon store are driving increases and that they are greatest at sites with large stores of soil carbon, such as peatlands. Although an inverse relationship has been proposed between mineral acidity and the generation of DOC (Krug & Frink, 1983), similar proportional

increases in DOC were reported at remote, unacidified sites as well as those recovering from anthropogenic acidification (Freeman *et al.*, 2001a) and the observed changes could not be accounted for by changes in rainfall, river discharge or land use. These rising trends have occurred across a wide range of deposition, soil types, topography, land-use and geographical location, suggesting that DOC increases in upland waters over the last two decades may extend beyond the UK.

Approximately ⅓ of the world pool of soil organic carbon (or 455 Pg) is stored in northern boreal and sub Arctic peatlands (Gorham, 1991). This vast accumulation of peat is allowed by the exceptionally low rates of decomposition (e.g., Freeman *et al.* 2001b; Gorham, 1991) which are generally attributed to low oxygen availability in waterlogged soils. Phenol oxidase was recently recognized as a regulator of carbon storage in organic-rich northern soils (Freeman *et al.*, 2001b). Under conditions of low oxygen the activity of this enzyme is low, which is thought to allow phenolic compounds to accumulate. These materials inhibit the action of other hydrolase enzymes in the peatland system (which are not oxygen limited) leading to retarded rates of decay (Freeman *et al.*, 2001b). Temperature is a major regulator of microbial metabolism, which in turn is responsible for the production of many of these enzymes (Kang & Freeman, 1999). However, little is known about the effects of warming on them despite the fact that they may be critical in determining carbon exports from this globally important ecosystem. The effects of temperature on the carbon cycling enzymes phenol oxidase and ß-glucosidase activities have therefore been studied in order to estimate the likely impact of the warming observed in recent years in relation to DOC concentrations.

A temperature gradient bar (figure 3.01) has been used to apply a thermal gradient of 2-20°C to northern peatland soil. In addition to measuring total DOC concentrations in the peat pore water, phenolic compound concentrations (within this DOC pool) have also been determined due to their perceived importance in the inhibition of hydrolase enzyme activities and recalcitrant nature (Appel, 1993; Freeman *et al.*, 1990; Wetzel, 1992). The DOC apparent molecular weight (AMW) spectra was examined in order to ascertain whether increasing temperature would induce spectral shifts, indicating altered DOC processing. Phenol oxidase activity was measured due to its control on carbon storage (Freeman *et al.* 2001b), ability to attack recalcitrant phenolic materials and its participation in lignin degradation (McLatchey & Reddy, 1998). ß-glucosidase activity (which

specifically hydrolyzes cellobiose as is present in cellulose (Killham, 1996)) was also determined because it is an indicator of cellulose decomposition (Sinsabaugh *et al.*, 1991) and of carbon mineralization rates (McLatchey & Reddy 1998; Sinsabaugh *et al.*, 1991). Carbon dioxide flux from the peat surface was measured to provide an indication of microbial respiration.

3.02 MATERIALS AND METHODS

Operation

A thermal gradient (2-20°C) was maintained along an aluminium temperature bar (figure 3.01). The apparatus was operated in a constant temperature environment in order to ensure close temperature control, given the absence of a thermostatic temperature control. The temperature gradient which occurs down through the soil profile was minimized by operating the bar in an environment with a temperature in the range of the thermal gradient.

Soil collection

Peat was collected in winter (January), spring (April), summer (August) and autumn (November) from an acid gully mire (described in chapter 2) to a depth of 10 cm. The surface layer of vegetation was subsequently removed.

Preparation and incubation of samples

Peat was homogenized by hand for 10 min in order to reduce spatial heterogeneity. A 2 cm layer of this soil was placed on the temperature gradient bar within two layers of clingfilm to prevent water loss without inhibiting gas exchange (Gordon *et al.*, 1987). Soil water loss has been found to be negligible (between 0.4 and 0.9% of the total water content of the peat soil) using a single layer of clingfilm but a second layer is suggested to ensure complete water retention (Dowrick, 1998). Significant water losses can occur if incubation temperatures of 30°C and above are used (Bremner & Douglas, 1971). Peat was incubated along a thermal gradient between 2°C and 20°C for two weeks.

Gas and hydrochemical analysis

Following two weeks of incubation, phenolic substances and DOC concentrations were measured in peat waters obtained by centrifugation (10 000 g for 2 hours) from 2°C intervals across the thermal gradient. The former using the method of Box (1983) and the latter a Shimadzu 5000 Total Organic Carbon analyzer (described in chapter 2).

DOC Apparent Molecular Weight (AMW) Spectra

A CECIL 1100 series High Performance Liquid Chromatograph (HPLC) with a gel filtration column (PL-GFC 8 µm, 300 Å, 300 mm × 7.5 mm inner diameter, Polymer Laboratories Ltd., Shropshire) was used to determine the AMW distribution of DOC in the samples, with a CECIL 1200 variable wavelength monitor detecting at 254 nm. The flow rate was 3 mL min^{-1} and the loop size was 120 µL. Several proteins were used to calibrate the column: albumin (molecular mass, MM = 67 000), ovalbumin (MM = 43000), chymotripsinogen A (MM = 25 000), ribonuclease A (MM = 13 700), cytochrome C (MM = 12400), and vitamin B12 (MM = 3500) (Alarcon-Herrera *et al.*, 1994). In addition, polystyrene standards ranging between MM 5000 and 200 000 (Sigma-Aldrich Co. Ltd., Dorset) were used for calibration (Chin *et al.*, 1998) and to cross check both the performance range of the column and the effect of the molecular weight on retention time. Tris (Tris hydroxymethane aminomethane) hydrochloric acid (0.01M, pH 7.5) was used as the eluent (according to the recipe of Dawson *et al.*, 1989), after being degassed and filtered using a 0.45 µm membrane filter (Whatman, Kent, UK). Samples were diluted with this eluent solution to avoid the production of artefacts.

Extracellular phenol oxidase activities

Phenol oxidase activities were determined using 10 mM L-DOPA (dihydroxy phenylalanine) solution as a substrate according to Pind *et al.* (1994). Four 1 cm^3 peat cubes from 2°C intervals in the thermal gradient bar were taken and allowed to equilibrate in an incubator at the appropriate temperature (that at which it was incubated within the thermal bar), before each assay was carried out according to the protocol described in chapter 2.

Extracellular ß-glucosidase activities

Substrates were prepared and activities measured from 2°C temperature intervals as described in chapter 2.

Microbial respiration (CO_2 emissions)

Carbon dioxide emissions along the temperature gradient bar were determined by measuring concentration increases above background after 1 hour, using 90 mm by 13 mm petri-dish bases as headspaces placed upside down on the soil surface. The dishes were

pressed 1-2 mm into the peat at 2°C temperature intervals. Tubing (PVC, 10 cm long, 3 mm diameter, 1 mm bore), connected to a gas syringe and running under the peat into the dishes, was used to collect gas samples. Each dish was removed following every sampling replicate run (of which 4 were carried out) and flushed with ambient air before emissions were again determined. Five mL of gas was taken from each chamber for analysis using an Ai Cambridge model 92 gas chromatograph.

3.03 STATISTICAL CONSIDERATIONS

In order to provide some measure of standard error, two thermal gradient bars were run simultaneously for each season. Error bars shown represent two pseudo-replicates from each bar taken from the appropriate temperatures and pooled. Data was tested for normality using the Kolmogorov-Smirnov test and the response of a given determinand to increasing temperature ($°C^{-1}$) was estimated using regression analysis (Minitab version 13.32, Minitab Inc.). Significant correlations (Pearson correlation coefficient) between determinands within a given season are also presented.

3.04 RESULTS

Thermal optima

In a given season, broad maxima (referred to here as peaks) were present in all the determinands at a temperature (referred to as the thermal optimum) approximately predicted by the ambient temperature recorded at the time of peat collection. This feature was 'superimposed' onto an underlying 'baseline' trend produced with increasing temperature across the gradient bar. To avoid confusion AMW spectra will be described in terms of fractions.

Mean ambient temperatures recorded at the time of peat collection were 2.53 ± 2.03°C, 11.03 ± 2.20°C, 16.8 ± 3.53°C and 5.23 ± 1.76°C in the winter (January), spring (April), summer (August) and autumn (November) of 2000 respectively. However, it should be noted that these values represent an average temperature obtained during 2 days of field work only.

Tables 3.01-3.04 show the temperature ('thermal optima') in the thermal gradient bar (2-20°C) at which the peak solute concentrations, enzyme activities and CO_2 emissions were

found along with ranges of concentrations/activities from peat collected during winter, spring, summer and autumn respectively.

Table 3.01. Solute concentrations, enzyme activities and CO_2 emissions for peat collected during winter

Determinand	Minimum	Maximum	Thermal optimum (°C)
DOC (mg L^{-1})	38.85 (6.55)	80.65 (3.57)	2
Phenolic compounds (mg L^{-1})	1.88 (0.84)	5.6 (0.52)	2
Phenol oxidase (nmol g^{-1} min^{-1})	0.72 (0.35)	1.14 (0.27)	2
β-glucosidase (nmol mg^{-1} h^{-1})	0.07 (0.023)	0.178 (0.017)	2
CO_2 (mg m^{-2} h^{-1})	6.6 (1.1)	15.7 (2.8)	2

Numbers in parentheses denote standard error of the mean and this convention applies throughout, except where stated otherwise.

The winter peat generally showed the lowest concentrations/activities in all determinands in comparison to the other seasons. Peaks in all determinands were observed at 2°C in the thermal gradient bar (figures 3.02a-c), which coincided with the approximate temperature recorded in the field at the time of peat collection (2.53 ± 2.03°C). Leachate collected from the winter peat exhibited a DOC spectra with two distinct AMW fractions (>5000 to <90000 Da, referred to as the primary fraction, and >200000 Da) along with a certain amount of lower molecular weight material (<5000 Da) in two smaller fractions (figure 3.02d). The primary fraction was most prominent at 2°C in comparison to the higher temperatures and 0°C, while the other AMW fractions were relatively similar at the temperatures studied. All AMW fractions showed a tendency to decrease with increasing temperature.

Table 3.02. Solute concentrations and enzyme activities for peat collected during spring

Determinand	Minimum	Maximum	Thermal optimum (°C)
DOC (mg L^{-1})	51.81 (3.16)	229.7 (5.96)	12
Phenolic compounds (mg L^{-1})	0.87 (0.99)	6.2 (0.59)	12
Phenol oxidase (nmol g^{-1} min^{-1})	0.4 (0.15)	1.42 (0.11)	12
β-glucosidase (nmol mg^{-1} h^{-1})	0.11(0.045)	0.5 (0.037)	12

Generally, the spring peat showed mid concentrations of determinands. Peak concentrations and activities were found at 12°C (figure 3.03a & b) and this coincided approximately with the ambient temperature recorded at the time of peat collection (11.03 ± 2.20 °C).

Table 3.03. Solute concentrations, enzyme activities and CO_2 emissions for peat collected during summer

Determinand	Minimum	Maximum	Thermal optimum (°C)
DOC (mg L^{-1})	88.91 (1.43)	152.44 (3.59)	20
Phenolic compounds (mg L^{-1})	4.46 (0.48)	10.99 (0.47)	20
Phenol oxidase (nmol g^{-1} min^{-1})	1.001 (0.17)	1.52 (0.15)	20
β-glucosidase (nmol mg^{-1} h^{-1})	0.17 (0.046)	0.31 (0.04)	20
CO_2 (mg m^{-2} h^{-1})	17.1 (5.99)	49.8 (2.57)	20

The summer peat produced leachate with a mid range of solute concentrations and enzyme activities, similar in magnitude to those in the spring peat. Peaks in all determinands were found to occur at approximately 20°C (figure 3.04a-c) and the temperature recorded at the time of peat collection was somewhat lower (16.8 ± 3.53°C). The summer DOC spectra (figure 3.04d) showed the same primary AMW fraction as that of the winter peat, but the >200000 Da AMW fraction was not detected. The highest molecular weight fraction instead corresponded to an AMW of >90000 to <200000 Da and it was this fraction that was the most sensitive to warming, increasing with increasing temperature, while the primary fraction remained similar but tended to decrease with increasing temperature. The low AMW material was barely detectable in the summer DOC spectra.

Table 3.04. Solute concentrations and enzyme activities for peat collected during autumn

Determinand	Minimum	Maximum	Thermal optimum (°C)
DOC (mg L^{-1})	96.09 (3.55)	313.52 (9.79)	6
Phenolic compounds (mg L^{-1})	7.6 (0.99)	24.13 (1.7)	6
Phenol oxidase (nmol g^{-1} min^{-1})	1.3 (0.15)	1.3 (0.19)	6
β-glucosidase (nmol mg^{-1} h^{-1})	0.22 (0.055)	0.63 (0.062)	6

Autumn showed the greatest DOC concentrations and enzyme activities. Peaks in all determinands were found at approximately 6°C (figure 3.05a & b) and ambient temperatures were 5.23 ± 1.76°C. A secondary peak (lesser in magnitude) was also evident at between approximately 14 and 20°C.

For comparative purposes DOC concentrations in peat collected from all four seasons are shown in figure 3.06. The thermal optima apparently shifted as the ambient field temperature at the time of peat collection changed with season.

Tables 3.05-3.08 show only the significant correlations found between the determinands in peat collected from each season. P values are summarized where * denotes significant at the $P<0.05$ level, ** at the $P<0.01$ and *** at the $P<0.001$ level.

Table 3.05. Significant correlations between determinands for peat collected during the winter

	DOC	ß-glucosidase	Phenolic compounds	Phenol oxidase
DOC		0.910 **	0.9111***	0.9417 ***
ß-glucosidase	0.910 **		0.9061***	0.8990 ***
Phenolic compounds	0.9111 ***	0.9061***		0.962 **
Phenol oxidase	0.9417 ***	0.8990 ***	0.962 **	
CO_2	0.9012 ***	0.9221***	0.9469 ***	0.9354 ***

Table 3.06. Significant correlations between determinands for peat collected during the spring

	DOC	ß-glucosidase	Phenolic compounds	Phenol oxidase
DOC		0.8464 **	0.9680 ***	0.8470 **
ß-glucosidase	0.8464 **		0.9047 ***	0.8307 **
Phenolic compounds	0.9680 ***	0.9047 ***		0.9218 ***
Phenol oxidase	0.8470 **	0.8307 **	0.9218 ***	

Table 3.07. Significant correlations between determinands for peat collected during the summer

	DOC	ß-glucosidase	Phenolic compounds	Phenol oxidase
DOC		0.9205 ***	0.9471 ***	0.8683 **
ß-glucosidase	0.9205 ***		0.8832 ***	0.7833 **
Phenolic compounds	0.9471 ***	0.8832 ***		0.9380 ***
Phenol oxidase	0.8683 **	0.7833 **	0.9380 ***	
CO_2	0.9583 ***	0.9381 **	0.9724 ***	0.8609 **

Table 3.08. Significant correlations between determinands for peat collected during the autumn

	DOC	ß-glucosidase	Phenolic compounds	Phenol oxidase
DOC		0.7166 *	0.9502 ***	0.8078 **
ß-glucosidase	0.7166 *		0.7654 **	0.9454 ***
Phenolic compounds	0.9502 ***	0.7654 **		0.8779 ***
Phenol oxidase	0.8078 **	0.9454 ***	0.8779 ***	

Thermally induced trends in peat response

In an attempt to clarify the underlying or 'baseline' response of the peat to increasing temperature, values that were observed at the ambient field temperature (during peat collection) and the value flanking this were removed. This enabled regression analysis to be conducted and increases in solute concentrations/enzyme activities ($°C^{-1}$) to be calculated, since the baseline response was assumed to be linear.

Tables 3.09-3.13 show changes (Δ) in a given determinand ($°C^{-1}$) and the significance in terms of P values (statistical conventions apply and ns denotes non significant ($P>0.05$)) for DOC concentrations, phenolic compound concentrations, phenol oxidase activities, ß-

glucosidase activities and microbial respiration (CO_2 emissions) respectively, along with Q10 values and the corresponding percentage increases where appropriate.

Table 3.09. Thermally induced change in leachate DOC concentrations of peat collected from all seasons

Season	R^2	ΔDOC (mg $L^{-1}°C^{-1}$)	P
Winter	0.35	0.69	ns
Spring	0.58	3.21	*
Summer	0.90	3.30	**
Autumn	0.69	7.95	*

Peat from all seasons showed significantly increased DOC concentrations with increasing temperature, excepting the winter peat which was non significant (figure 3.07). Both the spring and the summer showed increases of over 3 mg $L^{-1}°C^{-1}$ ($P<0.05$ and 0.01 respectively), while the autumn peat exhibited the most pronounced response to warming with DOC release increasing by 7.95 mg $L^{-1}°C^{-1}$.

Table 3.10. Thermally induced change in leachate phenolic compound concentrations in peat collected from all seasons

Season	R^2	ΔPhenolic compounds (mg $L^{-1}°C^{-1}$)	P
Winter	0.11	-0.023	ns
Spring	0.93	0.130	***
Summer	0.81	0.332	**
Autumn	0.67	0.439	*

Spring, summer and autumn showed a significant positive response in terms of phenolic compound concentrations, and winter a non significant, slight negative response (figure 3.08). Phenolic compound release increased from spring (0.13 mg $L^{-1}°C^{-1}$, $P<0.001$) to autumn (0.44 mg $L^{-1}°C^{-1}$, $P<0.05$), summer values being between these (0.32 mg $L^{-1}°C^{-1}$, $P<0.01$).

Table 3.11. Thermally induced change in phenol oxidase activity in peat collected from all seasons

Season	R^2	ΔPhenol oxidase activity (nmol dicq $min^{-1}g^{-1}°C^{-1}$)	P	Q10	Δ%
Winter	0.06	-0.001	ns	0.99	-0.88
Spring	0.48	0.018	ns (10%)	1.29	28.57
Summer	0.66	0.026	*	1.36	36.36
Autumn	0.84	0.062	**	1.46	46.15

Phenol oxidase activities followed a similar pattern to that shown by phenolic compound concentrations, with the winter peat showing a non significant, slight negative response to increasing temperature (figure 3.09). The increase in activity was more pronounced in autumn (0.062 nmol $g^{-1}min^{-1}°C^{-1}$, $P<0.01$) than summer (0.026 nmol $g^{-1}min^{-1}°C^{-1}$, $P<0.05$), and more pronounced in summer than spring (0.018 nmol $g^{-1}min^{-1}°C^{-1}$, significant at the $P<0.1$ level only). Accordingly, the highest Q10 value (1.46) was found in the autumn peat (corresponding to a 46.15% increase) compared to values of 1.36 in the summer and 1.29 in the spring peat (corresponding to percentage increases of 36.36 and 28.57% respectively).

Table 3.12. Thermally induced change in ß-glucosidase activity in peat collected from all seasons

	R^2	Δß-glucosidase activity (nmol $mg^{-1}h^{-1}°C^{-1}$)	P	Q10	Δ%
Winter	0.36	0.001	ns	1.09	8.59
Spring	0.98	0.018	***	2.20	119.57
Summer	0.73	0.0067	*	1.35	35.45
Autumn	0.77	0.016	**	1.68	67.60

As was the case for DOC concentrations, ß-glucosidase activities (figure 3.10) increased with increasing temperature in all seasons and the winter response was not statistically significant. The largest increase was found in the spring peat though (0.018 nmol $mg^{-1}h^{-1}$ $°C^{-1}$, $P<0.001$, corresponding to a Q10 value of 2.20). The summer peat showed a lesser increase in activity (0.0067 nmol mg^{-1} $h^{-1}°C^{-1}$, $P<0.05$, corresponding to a Q10 value of

1.35), while the autumn peat showed a similar increase to the spring peat (0.016 nmol mg^{-1} $h^{-1}°C^{-1}$, $P<0.01$, corresponding to a Q10 of 1.68).

Table 3.13. Thermally induced change in microbial respiration (indicated by CO_2 emissions) for peat collected in the winter and summer

	R^2	ΔCO_2 (mg $m^{-2}h^{-1}°C^{-1}$)	P	Q10	Δ%
Winter	0.0007	0.007	ns	1.01	0.83
Summer	0.8239	1.75	**	1.99	99.04

Microbial respiration (figure 3.11) showed a non significant response in the winter, while the summer peat produced a dramatic and significant increase of 1.75 mg $m^{-2}h^{-1}°C^{-1}$ ($P<0.01$), which corresponded to a Q10 of 1.99 (i.e., a percentage increase of 99.04%).

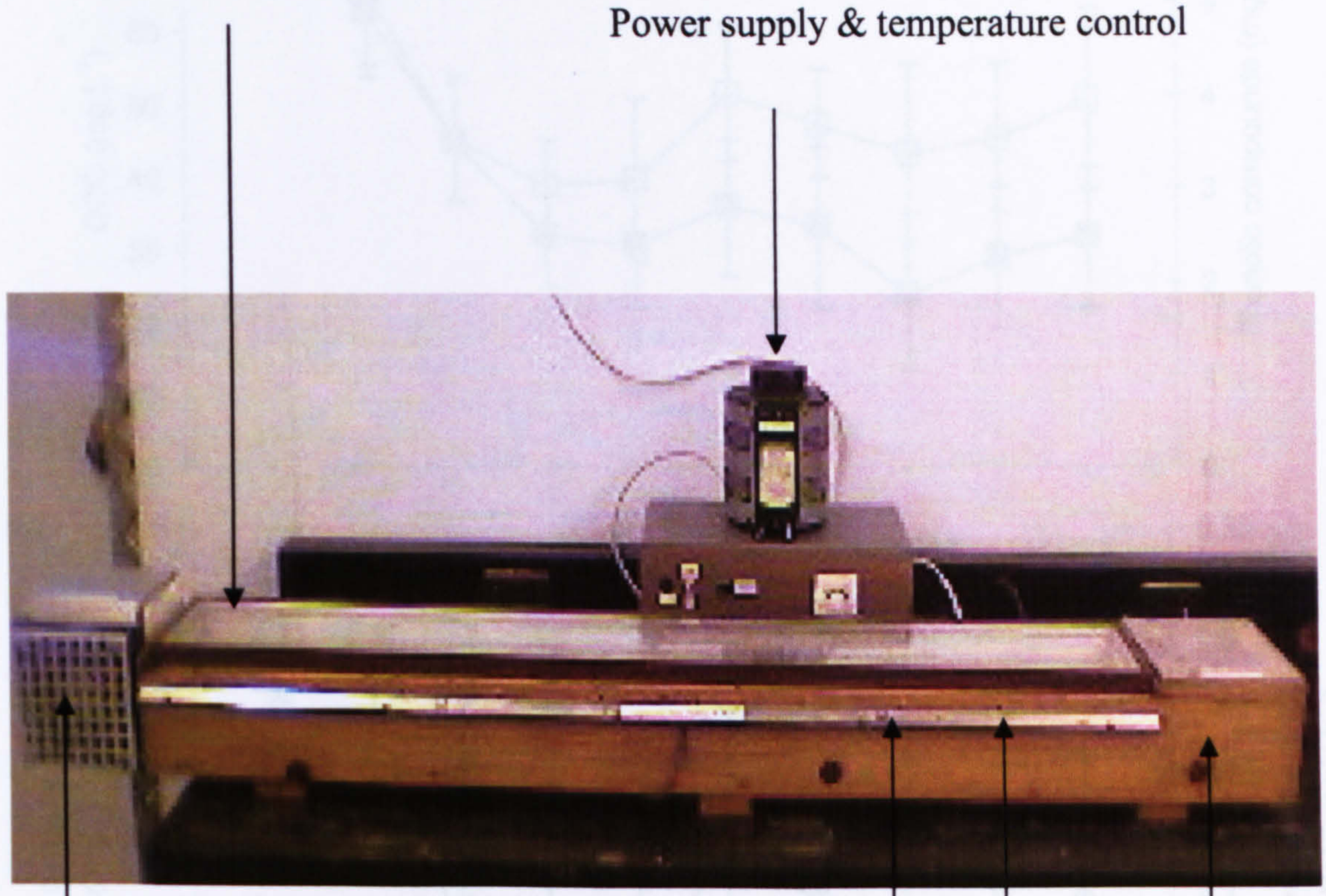


Figure 3.01. Thermal gradient bar used to incubate peat over a range of 2-20°C.

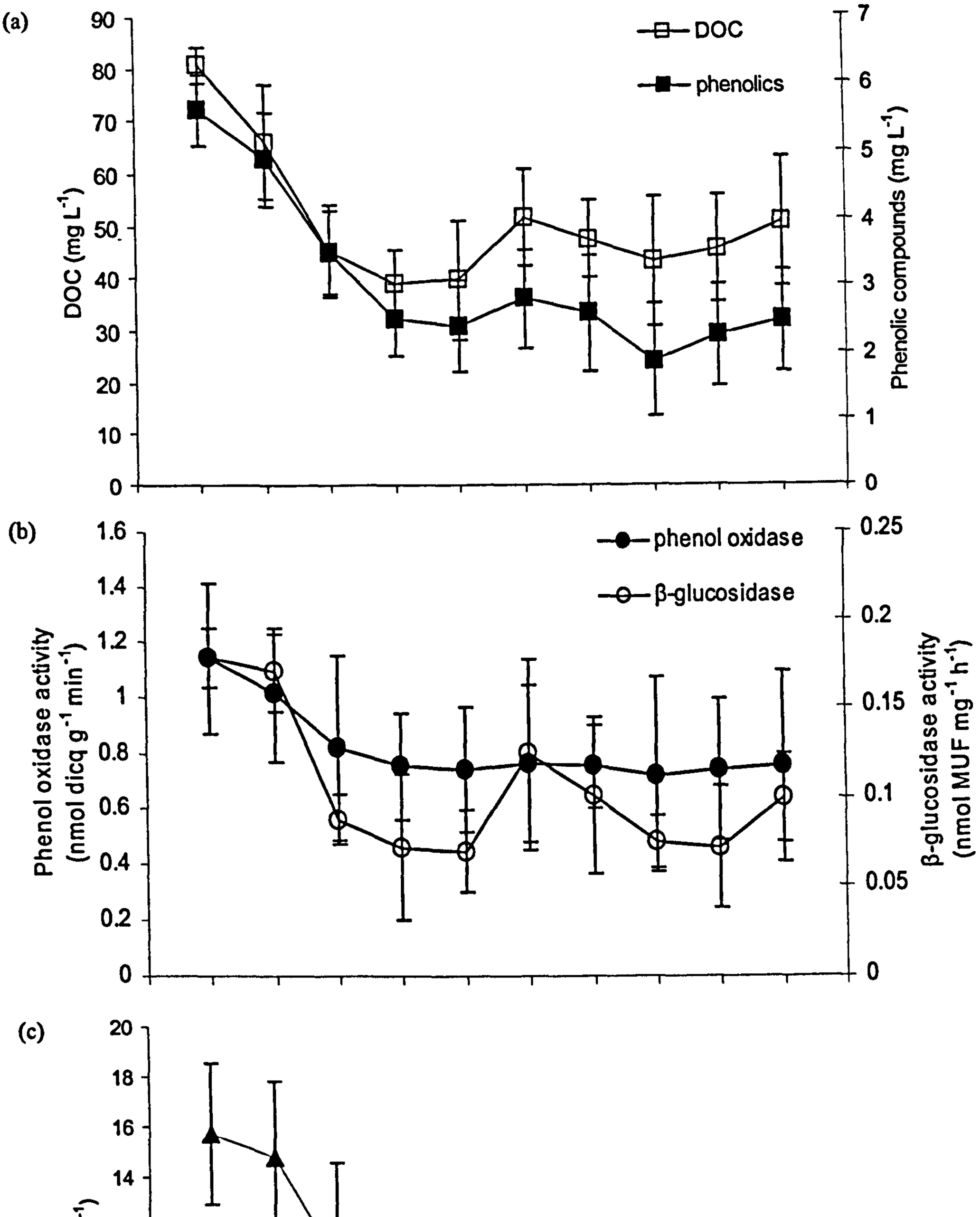


Figure 3.02 (a) pore water DOC & phenolics concentrations, (b) phenol oxidase & β-glucosidase activities, & (c) microbial respiration (indicated by CO_2 emissions) across a thermal gradient for peat collected during winter. Error bars represent standard error of the mean, n=4.

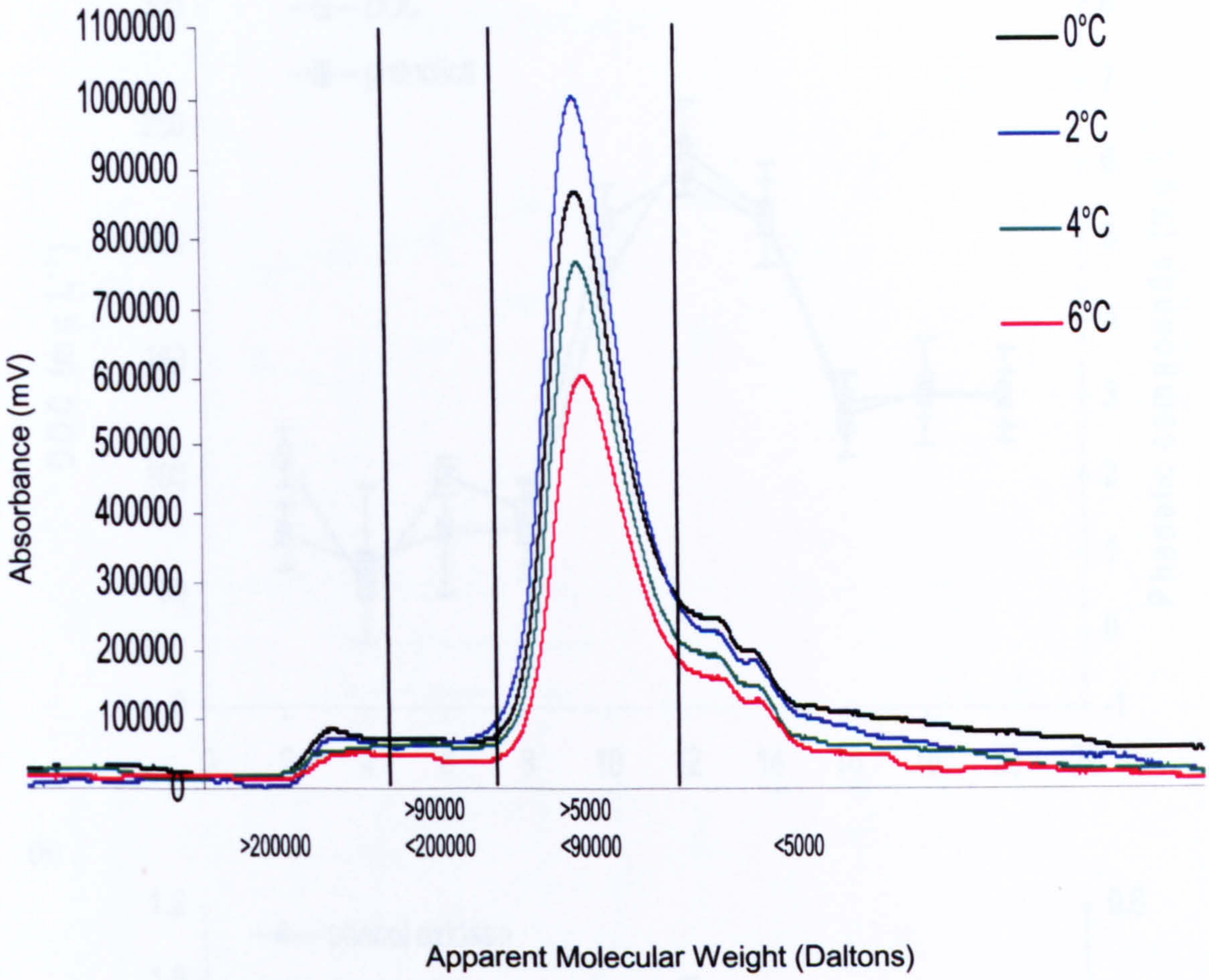


Figure 3.02 (d) pore water DOC apparent molecular weight spectra across a thermal gradient for peat collected in winter.

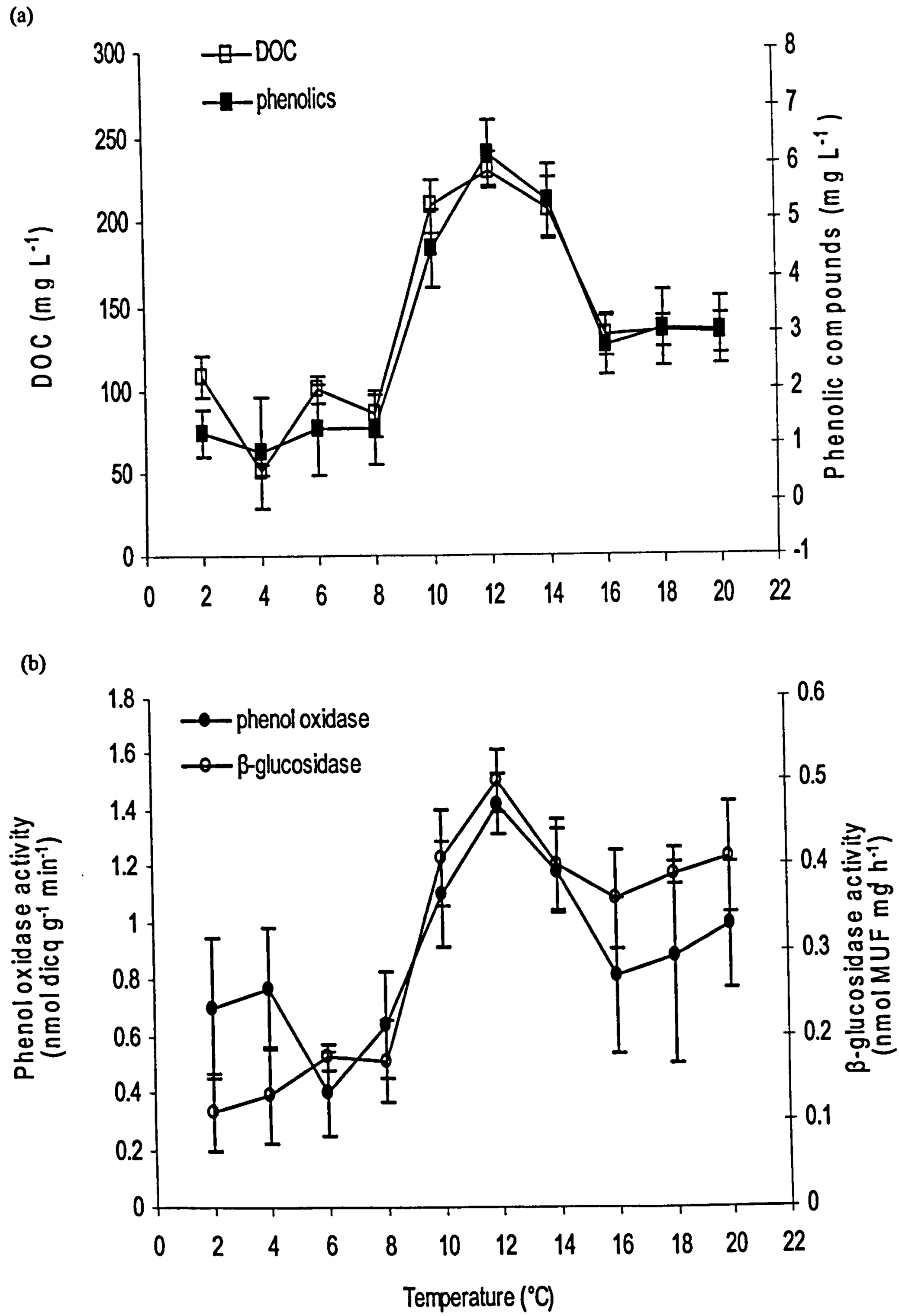


Figure 3.03 (a) pore water DOC & phenolics concentrations, & (b) phenol oxidase & β-glucosidase activities across a thermal gradient for peat collected during spring. Error bars represent standard error of the mean, n=4

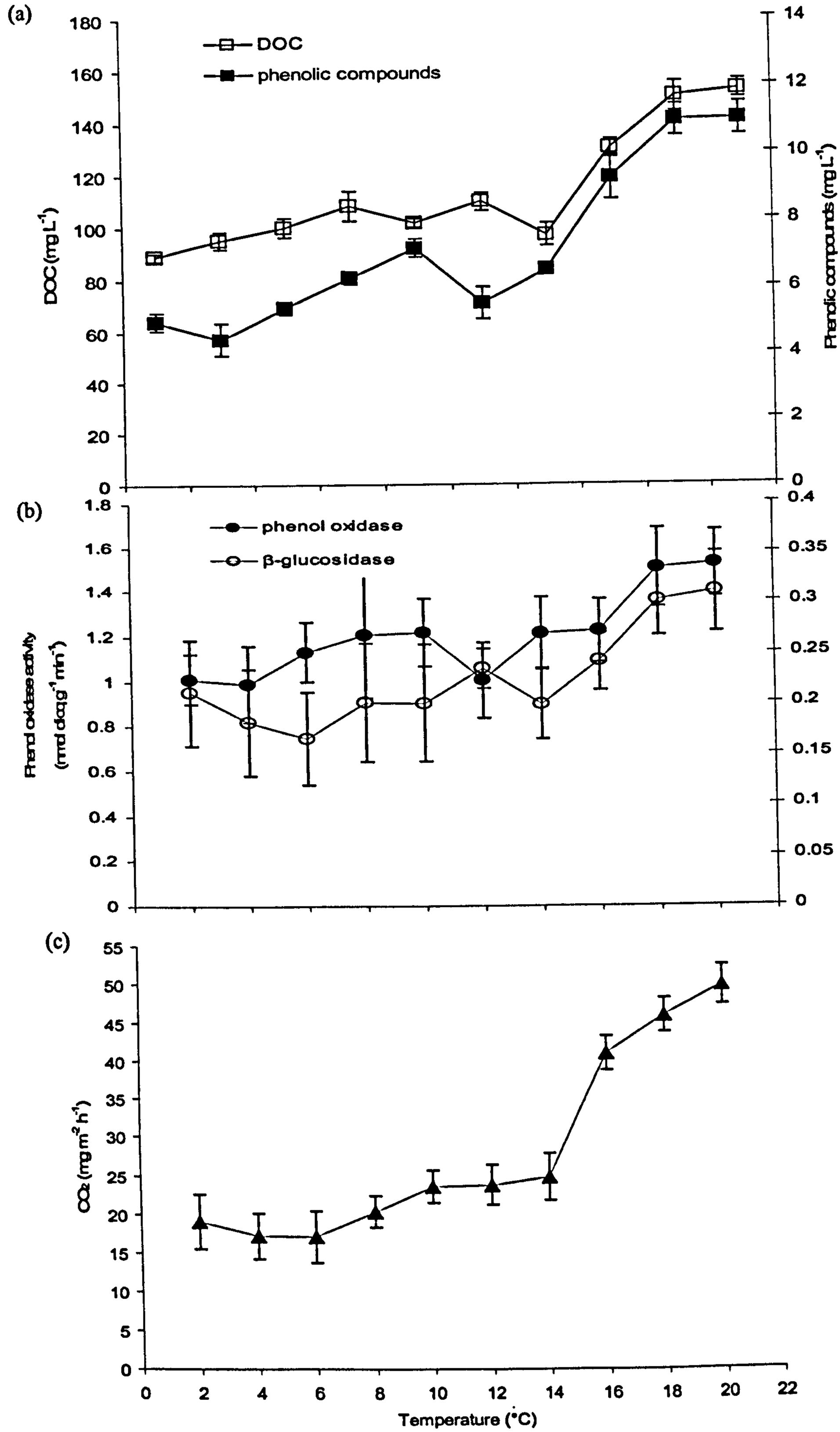


Figure 3.04 (a) pore water DOC & phenolics concentrations, (b) phenol oxidase & β-glucosidase activities, & (c) microbial respiration (indicated by CO_2 emissions) across a thermal gradient for peat collected during summer. Error bars represent standard error of the mean, n=4

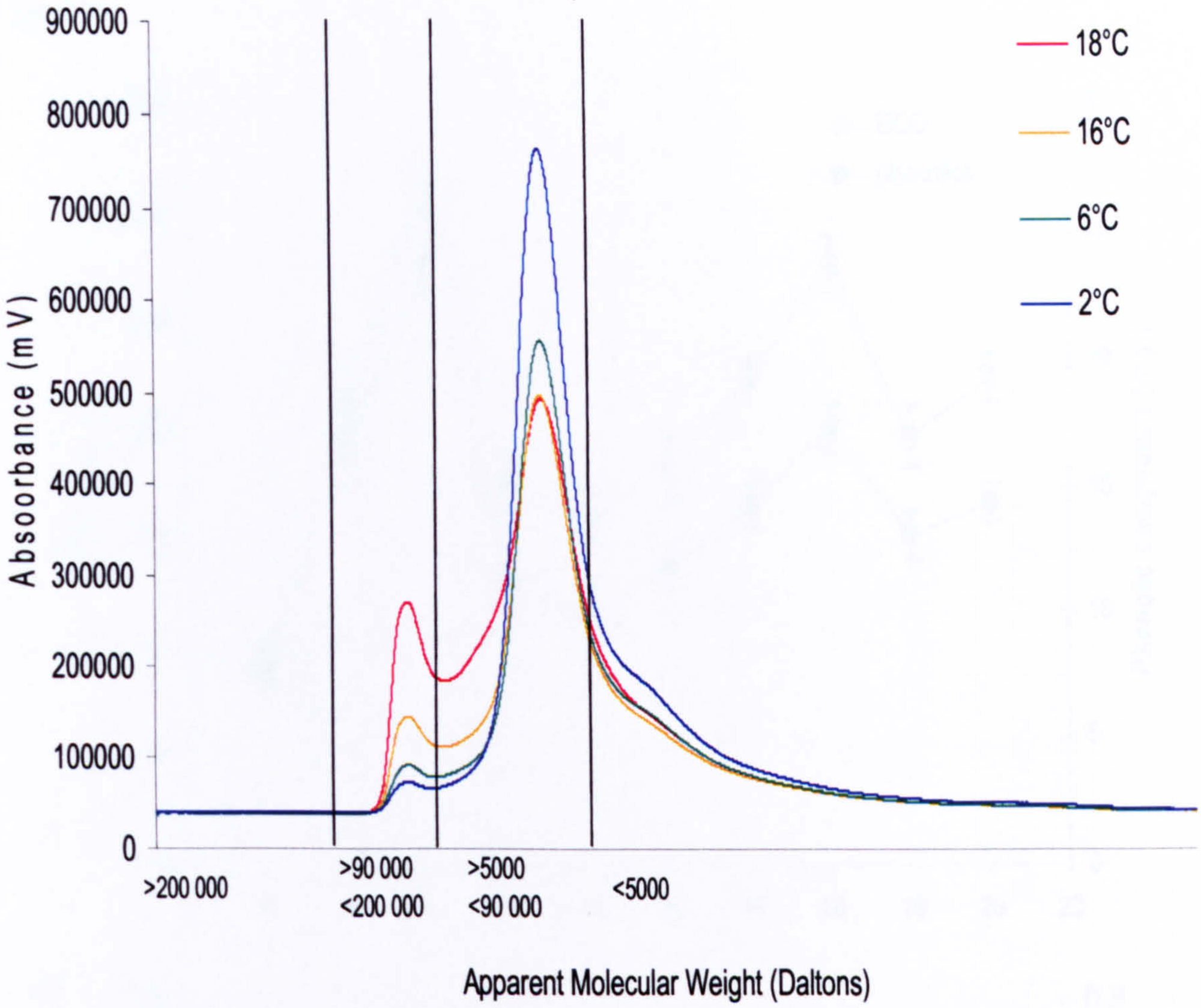


Figure 3.04 (d) pore water DOC apparent molecular weight spectra across a thermal gradient for peat collected during summer.

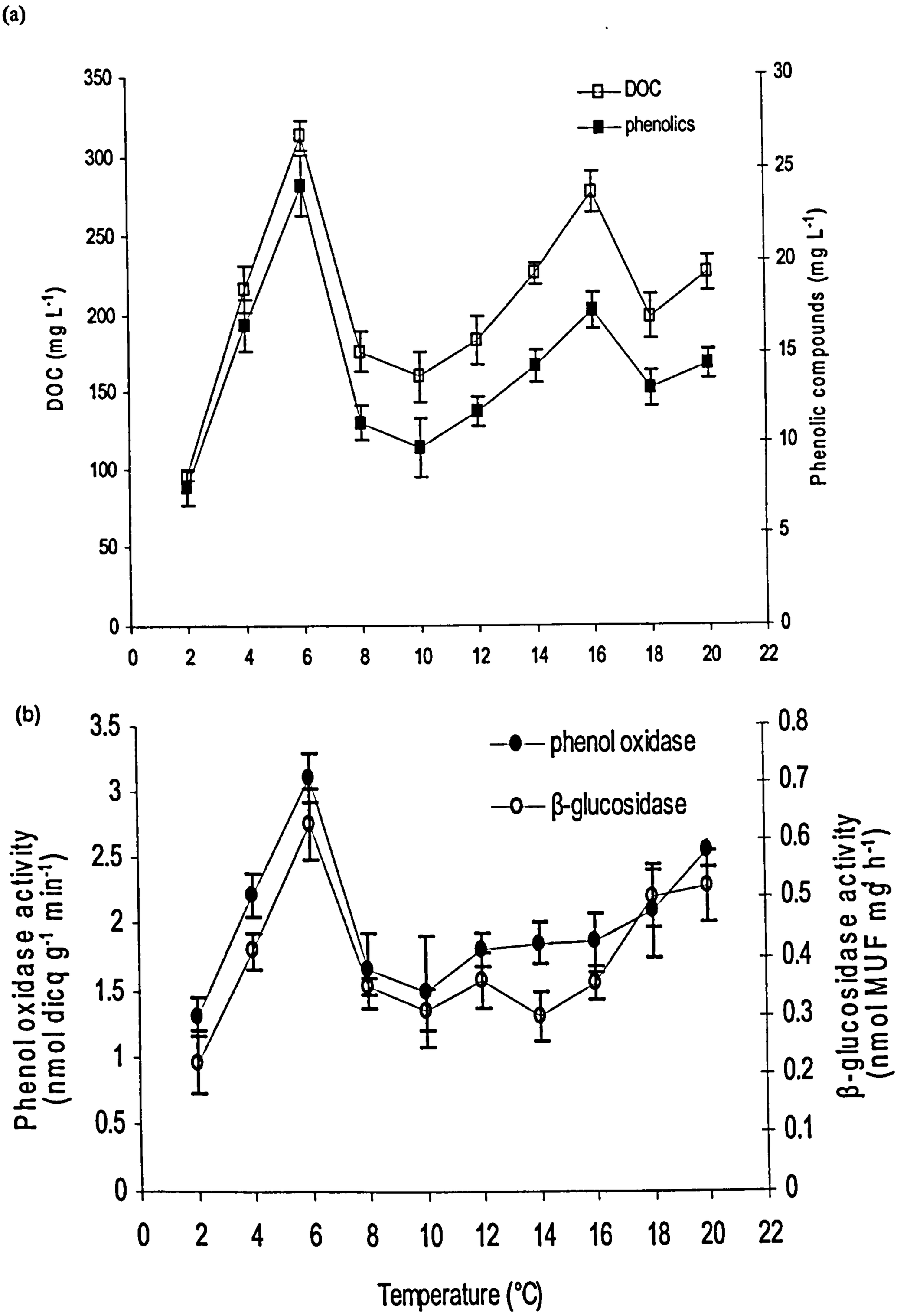


Figure 3.05 (a) pore water DOC & phenolics concentrations, & (b) phenol oxidase & β-glucosidase activities across a thermal gradient for peat collected during autumn. Error bars represent standard error of the mean, n=4

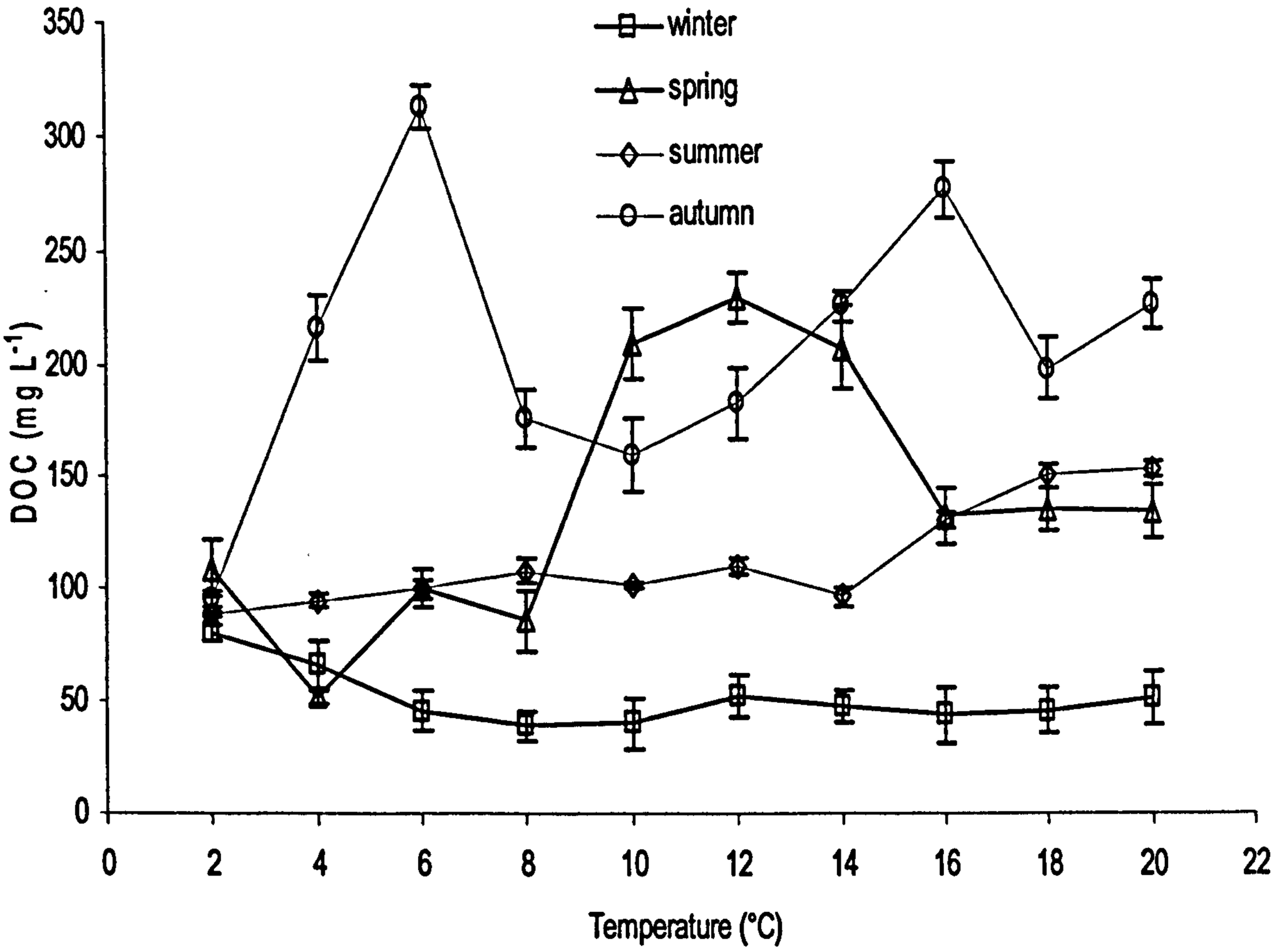
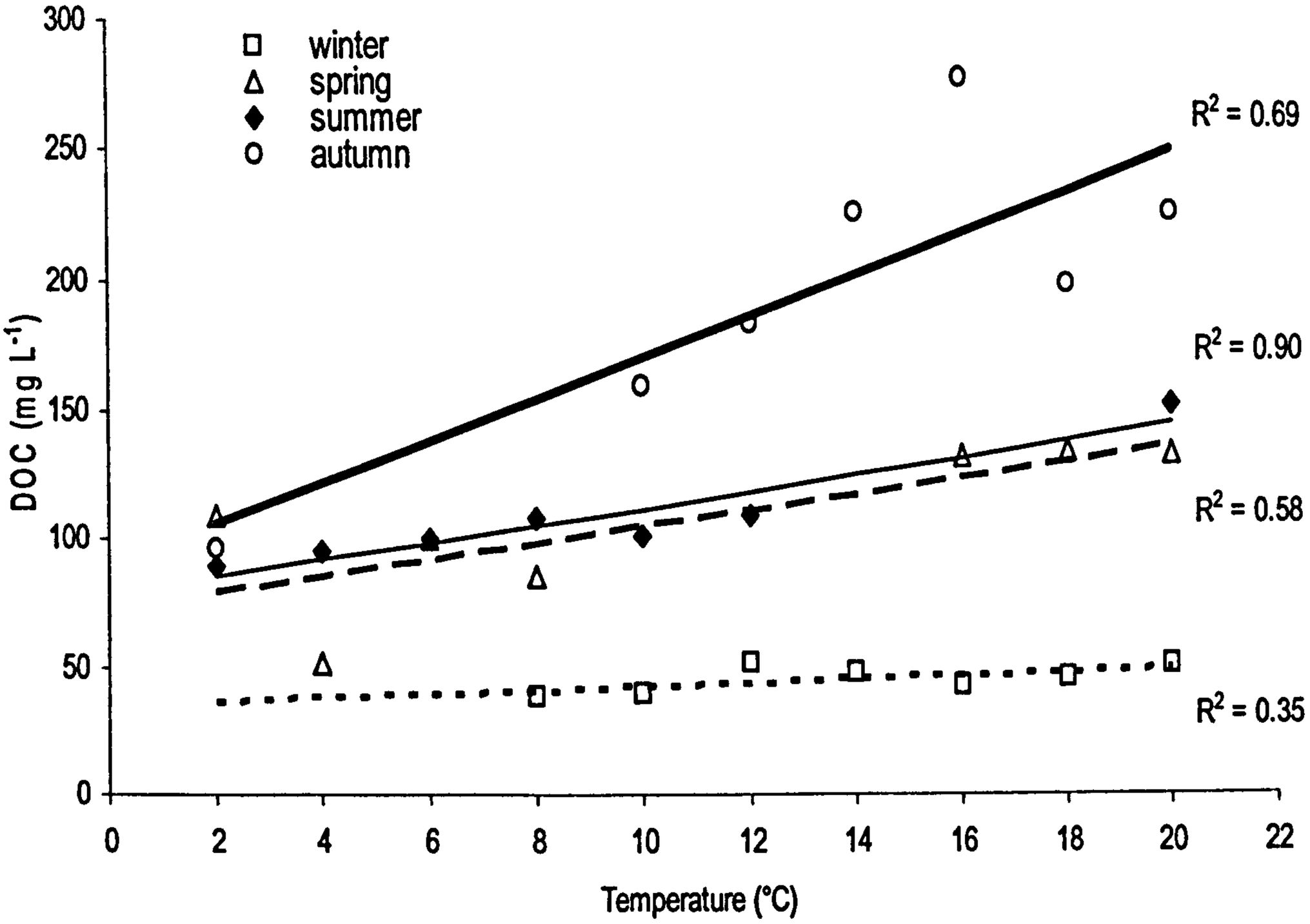
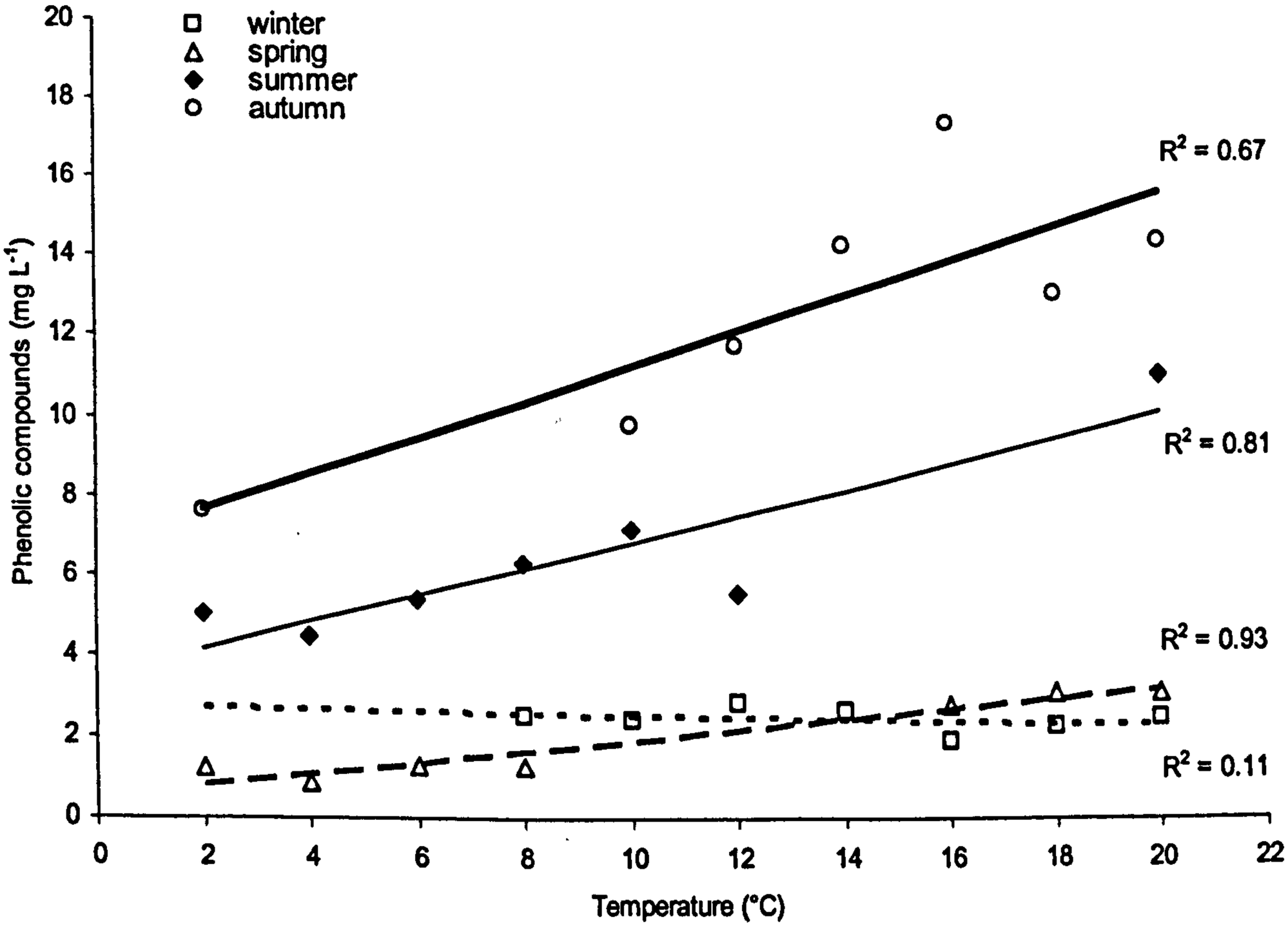


Figure 3.06. Pore water DOC concentrations across a thermal gradient in peat collected during all seasons. Error bars represent standard error of the mean, n=4

3.07

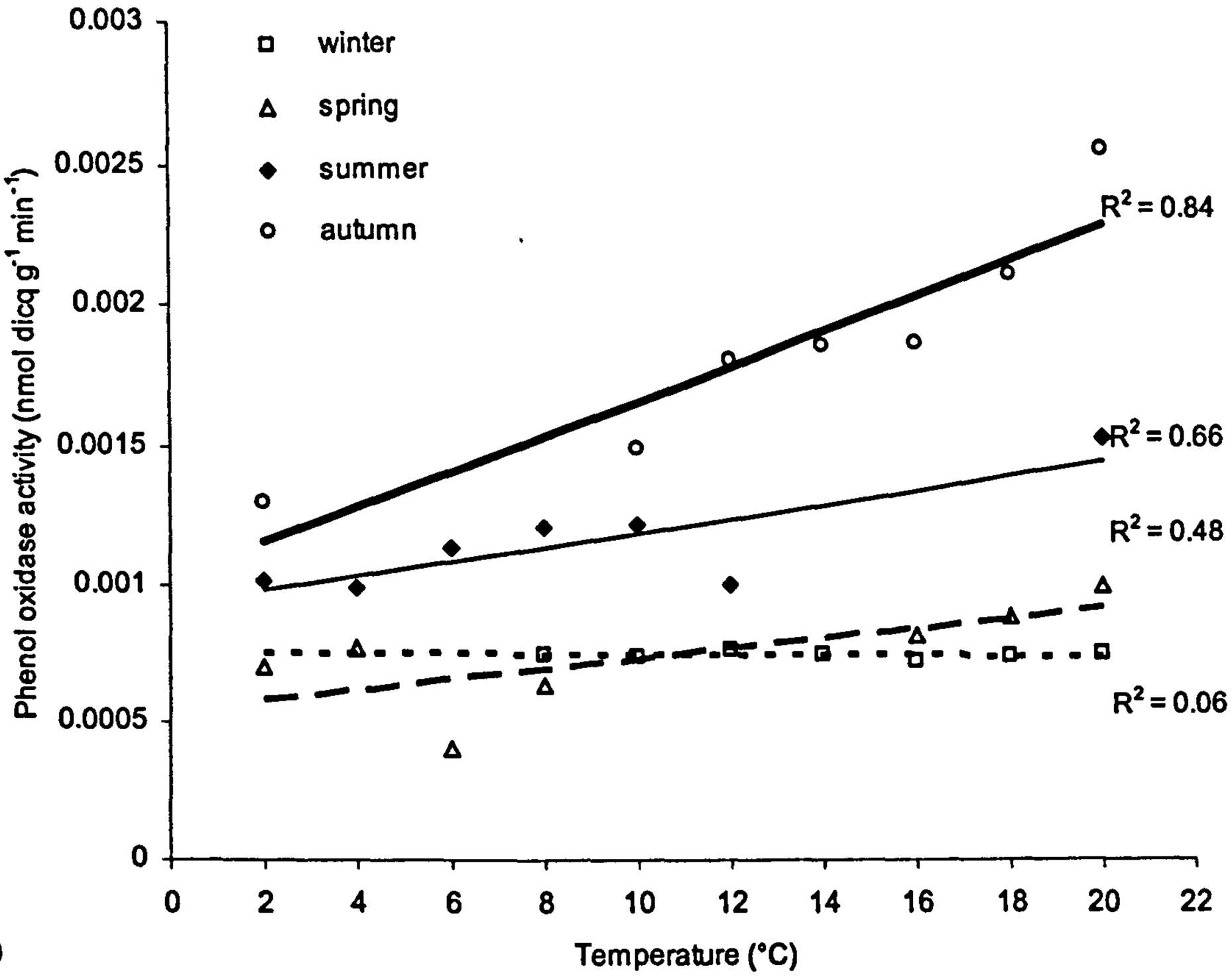


3.08

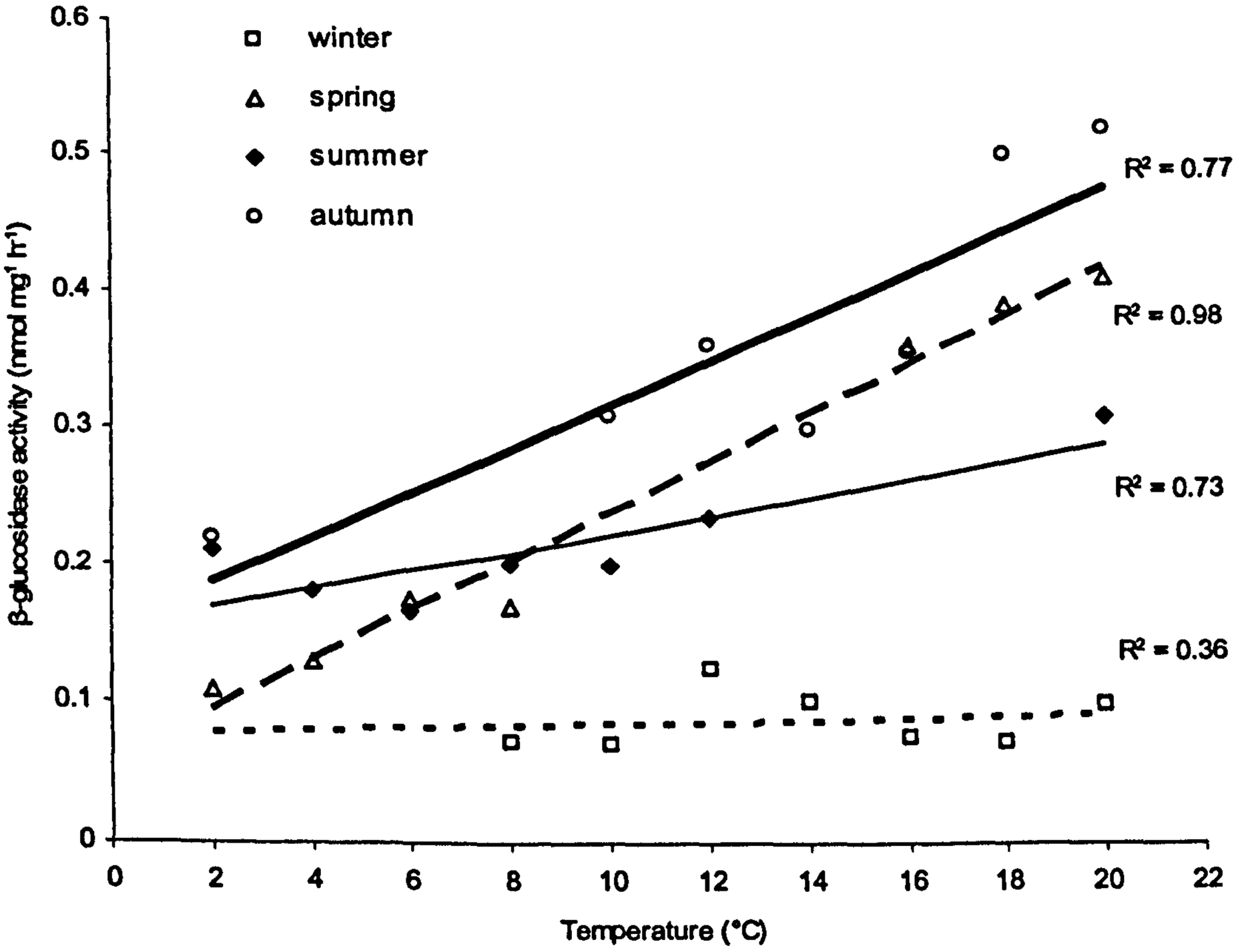


Trends in pore water DOC (figure 3.07) & phenolic compound (figure 3.08) concentrations across a thermal gradient for peat collected during all seasons. Regression lines are shown where dots denote winter, dashes spring, thin lines summer & bold lines autumn.

3.09



3.10



Trends in phenol oxidase (figure 3.09) & β-glucosidase (figure 3.10) activities across a thermal gradient for peat collected during all seasons. Regression lines are shown where dots denote winter, dashes spring, thin lines summer & bold lines autumn.

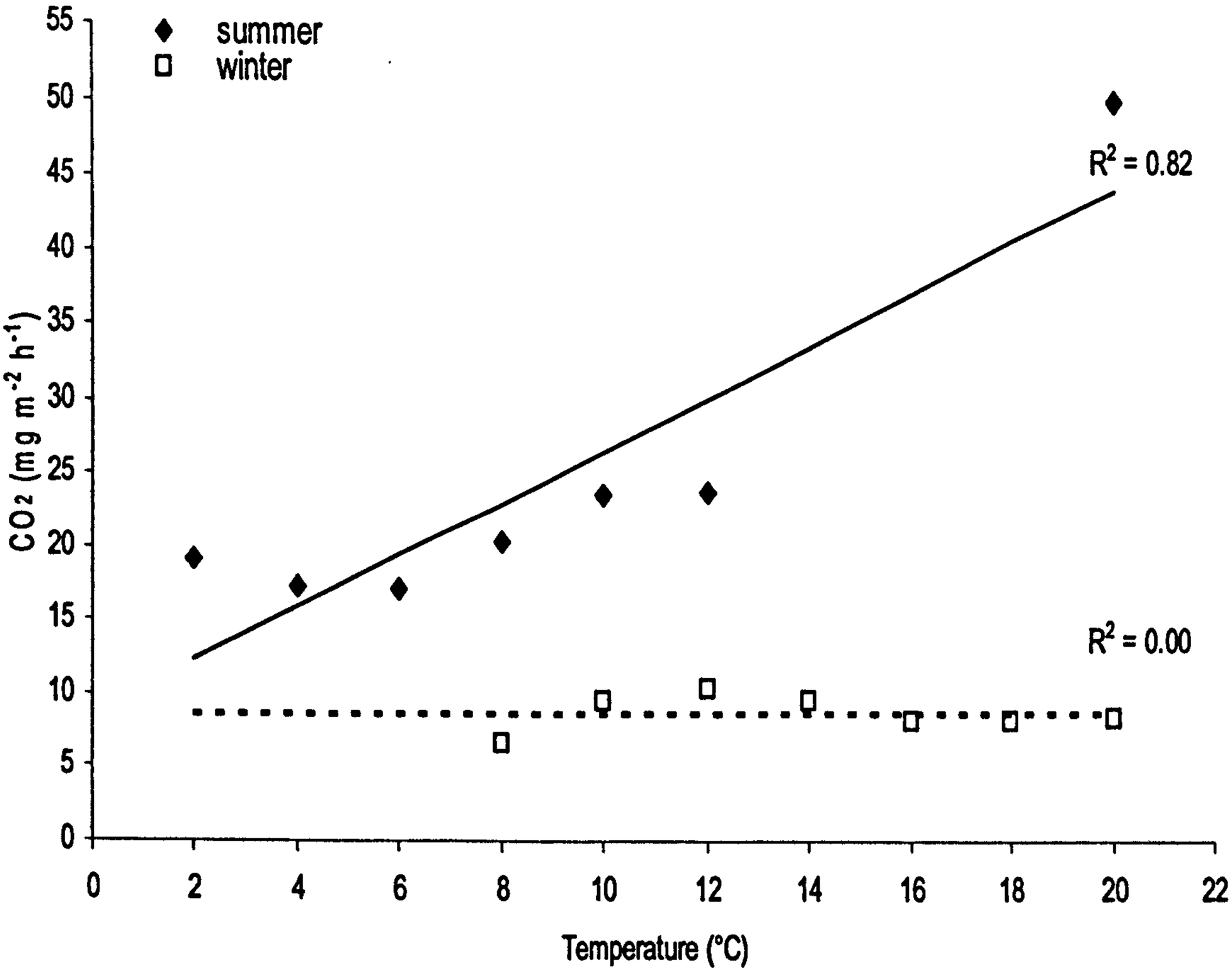


Figure 3.11. Microbial respiration (indicated by CO_2 emissions) across a thermal gradient for peat collected during winter & summer. Regression lines are shown where dots denote winter & the thin line summer.

3.05 DISCUSSION

Thermal optima

The most striking feature of these data was that temperature ranges (referred to as thermal optima) were found within which raised concentrations (referred to here as peaks) of DOC and phenolic compound concentrations, along with increased enzyme activities and CO_2 emission all occurred (figures 3.02a-3.05b). These peaks in a given season coincide with approximate ambient temperatures in the field at the time of peat collection. This implies that microbial respiration along with phenolic compound and more labile polysaccharide degradation (indicated by phenol oxidase and ß-glucosidase activities respectively) is adapted to the field temperature. Few microbial species posses the entire suite of enzymes required to completely degrade cellulose or lignin to CO_2 (Sinsabaugh *et al.*, 1994). ß-glucosidase indicates cellulase activity (Sinsabaugh *et al.*, 1991) and phenol oxidase is involved in lignin degradation (McLatchey & Reddy, 1998). The thermal optima of these enzymes occurs at the same temperature in a given season and corresponds to that of microbial respiration, implying that community degradation is occurring and seemingly the generation of dissolved phenolic compounds and DOC from the peat matrix as a result.

When the seasonal data are collated (figure 3.06), the thermal optima are reminiscent of temperature growth ranges and optima of the major classes of microorganism; pyschrophile, mesophile and thermophile. The organisms in this system are likely to be psychrophiles (optimal growth temperatures of under 20°C) and mesophiles (optimal growth temperatures between 20 and 50°C) and thus, the observed pattern may represent the proliferation of organisms and their enzymes within these classes as environmental conditions change with season. A microorganism can proliferate only when the environmental temperatures are restricted to the thermal growth range of that organism, and the ability of a microorganism to compete for survival in a given system is increased when temperatures are close to its optimal growth temperature (Atlas, 1988). The differences in optimal growth temperatures and temperature growth ranges result in spatial separation of these organisms in nature and since temperatures are changing through time, temporal separation also. Some microbes can survive in a dormant state (through production of endospores) but do not grow at temperatures outside their thermal growth range (Atlas, 1988), and this may be the mechanism which allows a secondary peak to occur in the autumn peat at 14°C and above (figure 3.05a & b). Certain species of the microbial population present in the summer peat may survive as dormant endospores with the decline in temperatures towards autumn and in this case can proliferate again when warmed in the thermal gradient bar. The peat may therefore possess a 'microbial memory', allowing optimal activities should the prevailing conditions become favourable to growth.

The microbial community present in the environment at any particular time is likely to represent a complex interaction between stenothermal microbes (that only grow at temperatures near their optimal range) and eurythermal microbes (that grow over a wider range), even without taking into consideration numerous growth substrates that are available. It thus becomes apparent that even a slight change in temperature as a result of climate change is likely to alter the intricate balance of the community structure and possibly function, with implications for DOC processing. Given that it is generally accepted that the maximal growth rates of thermophiles are greater than those of mesophiles, which in turn are greater than those of psychrophiles, increasing summer temperatures are likely to increase DOC concentrations. The differential response with season suggests that the timing of soil warming could play a key role in determining the response of the peat system to climatic change.

The AMW spectra of DOC in the winter peat (figure 3.02d) showed a primary peak of mid AMW material (>5000 to <90 000 Da) that was also present in the greatest amount at the approximate ambient temperature recorded during peat collection. This fraction may indicate the degradation potential of the community in the external environment, representing a balance of enzymic generation of high AMW material (>200 000 Da) from the matrix (*via* the action of phenol oxidase for example) and community degradation of such material to the mid (primary fraction) and lower AMW fractions (<5000 Da) due to the activities of enzymes such as ß-glucosidase. Eventually these potentially more labile materials may be mineralized to produce CO_2. In the spectra produced by the summer peat (figure 3.04d), a somewhat different situation occurred in that it was the intermediate AMW fraction (>90 000 to <200 000 Da), absent in the winter peat, that appeared to be temperature sensitive and increased with increasing temperature. The reason for the differing DOC spectra may relate to the different microbial communities present and thus catabolism of OM *via* different pathways. The lack of the highest AMW fraction (>200 000 Da) and relatively high enzyme activities in the summer peat might imply that efficient decomposition prevents its accumulation, once generated from the matrix. Alternatively, this fraction may not be generated at all, with the >90 000 <200 000 AMW fraction perhaps being generated from the matrix in its place. A further possibility is that the latter fraction is synthesized by the polymerization of lower AMW material and that the microbes responsible for this are more active in the summer peat. This may be due to increased ambient temperatures and/or increased plant contributions, in the form of labile exudates or nutrients for example. Low molecular weight fractions may be difficult to detect given their labile nature in a system where refractory, high AMW, plant/humic material is quantitatively dominant (Wetzel, 1992) and this is especially true for the summer peat when decomposition (and polymerization) rates are probably higher. The increased CO_2 release in the summer

compared to the winter peat (figures 3.04c & 3.02c respectively) supports this, indicating increased mineralization.

Temperature-dependent solubilities could account for the observed peaks in determinands, but this seems unlikely because all the different determinands peak at the same temperature in a given season, including microbial respiration (e.g., figures 3.02a-d). From this we could infer that the dominant microbial population is adapted to the external conditions in the field and that their contributions to the biogeochemistry of the peat are considerable. Their sensitivity to ambient seasonal conditions implies that soil warming could have a profound effect on the quantity and/or quality of carbon exports, both to the aquatic system and the atmosphere. Furthermore, the seasonal differences in the biogeochemical properties of the peat demands consideration in future studies as very different results can be produced simply by collecting sample material at different times of year. The response of peat to environmental stimuli cannot therefore be predicted by a single sample point in time, complicating the modelling of such systems. Seemingly, the response of peat not only differs between sites in its response to temperature changes (Updegraff *et al.*, 1998) but also to warming within the same site depending on season. Thus, without knowing the thermal history of the peat we may be unable to predict its response to climate change. Similarly, Updegraff *et al.* (1998) suggest that our ability to predict the effects of climate change on trace gas fluxes may be constrained by hysteresis in the temperature response of CO_2 and CH_4 production in peat soils.

Conventionally, phenol oxidase activity is perceived to be inversely correlated with phenolic compound concentration, i.e., the higher the phenol oxidase activity the more phenolic materials are removed form the system (Freeman *et al.*, 2001b; Pind *et al.*, 1994). However, this experiment suggests that the action of phenol oxidase can generate phenolic substances by mobilizing the peat matrix, rather than simply degrading those already dissolved in the pore waters, since phenolic compound concentrations correlated strongly and positively with phenol oxidase activities in all seasons (winter 0.962, $P<0.01$; spring 0.9218, $P<0.001$; summer 0.9380, $P<0.001$; autumn 0.8779, $P<0.001$). It could be argued that the increased phenolic compound concentrations were already present and induced the increased phenol oxidase activities (substrate induction) observed at the thermal optima in each season. This is thought to be improbable because optimum phenol oxidase activities occur at ambient temperatures and if the enzyme were only capable of degrading dissolved material in the pore waters then a negative correlation would be expected at these temperatures. However, further work would be required in order to prove either theory (for example, enzyme addition experiments). A positive correlation suggests that phenolic substances are being liberated from the vast peat resource faster than they can be consumed/transformed by the microbial community, i.e. net production

over consumption is occurring. The same appears to be true in the case of β-glucosidase, in that activities correlated strongly and positively with DOC concentrations (winter 0.910, $P<0.01$; spring 0.8464, $P<0.01$; summer 0.9205, $P<0.001$; autumn 0.7166, $P<0.05$). Care is therefore required because in experiments involving purely pore waters but lacking any soil matrix there would be a fixed amount of dissolved material available for removal by the enzyme and a negative correlation would be apparent. In contrast, the presence of a matrix could allow an increased potential for the release of materials due to enzymic mobilization, leading to a positive correlation being produced. This illustrates a further complexity that must be understood if we are to accurately model carbon cycling within the peatland system.

Phenolic compounds have enzyme inhibiting properties (Appel, 1993; Wetzel, 1992) and the concentration relative to that of DOC is thought to be important in determining enzyme activities in fresh water aquatic systems (Freeman *et al.*, 1990). The relative activities of phenol oxidase and β-glucosidase in this experiment appear to be crucial in determining the concentrations of dissolved phenolic compounds and DOC available for export. Thus, these enzymes not only seem to play a major role in carbon cycling within the peatland itself, but also may influence the concentrations of phenolic substances and DOC persisting in the aquatic systems that drain such areas.

Thermally induced trends in peat response

The second notable feature of the data, the underlying trend or 'baseline' response with increasing temperature, may represent the activity of abiontic enzymes (since these can persist for long periods when bound to OM (e.g., over a year, Kiss *et al.*, 1975)) but also contributions of microorganisms that were not adapted to prolific growth under the prevailing environmental conditions at the time of peat collection. Similarly, physico-chemical effects of warming may also contribute to the trends discussed below.

Generally, DOC concentrations were found to increase from winter to autumn (figure 3.07) in line with other work (e.g., Hughes *et al.*, 1998; Fenner *et al.*, 2001; Tipping *et al.*, 1999), and all seasons excepting winter showed a significant positive response to increasing temperature, i.e., increasing DOC concentrations (table 3.09). Many workers have also reported that increasing temperatures can increase the release of DOC (e.g., Briones *et al.*, 1998; Ineson *et al.*, 1995; Tipping *et al.*, 1999), although the seasonal response tends not to be accounted for. Similarly, phenolic compound concentrations increased with increasing temperature in all seasons excepting winter (figure 3.08, table 3.10). Hence, there is the potential for increased DOC levels in the waters draining such peatlands for a large proportion of the year, indicating

that water quality would be reduced. Furthermore, from these results, it seems likely that concentrations of phenolic compounds will also increase for much of the year should soil temperatures increase. This may be especially important because phenolic substances are relatively recalcitrant and therefore will be more persistent in the recipient stream. Moreover, their enzyme inhibiting properties (Appel, 1993; Freeman *et al.*, 1990; Wetzel 1992) may lead to positive feedback to impaired degradation of even the most labile DOC and further reduce water quality.

In the winter peat, surprisingly phenol oxidase and β-glucosidase activities were not significantly increased with increasing temperatures (figures 3.09 & 3.10, tables 3.11 & 3.12 respectively). Thus, there was no significant additional release of DOC or phenolic compounds and the latter even showed a slight negative response, perhaps indicating net consumption. The similar AMW spectra (figure 3.02d) produced with increasing temperatures support this showing slight reductions in the two highest AMW fractions (>5000 to <90 000 Da and >200 000 Da) at 4 and 6°C. Such results suggest that temperature is not the limiting factor governing DOC production at this time of year. It may be that it is the production of the labile carbon pools that is sensitive to warming (c.f. Davidson *et al.*, 2000) and that in winter there is a lack of such material due to, for example, reduced root exudation. However, the more obvious relatively low AMW fractions (<5000 Da) remaining in the winter DOC spectra (compare with summer spectra, figure 3.04d) suggest an inhibitory mechanism in relation to decomposition may be at work. A possibility is that the life cycle of microorganisms responsible for the production of enzymes and degradation of the lower AMW material in winter is determined by factors other than temperature (e.g., substrate quality or day length), and therefore such microbes remain inactive even when warmed. The lack of a significant effect on DOC and phenolic compound concentrations (tables 3.09 & 3.10 respectively) suggests that even if winters get warmer there may be little change in water quality due to increased DOC concentrations generated from peatlands, although this takes no account for changes in hydrology. Mean winter temperatures in Central England are thought to have shown little trend over the 20th century and Central England temperatures correlate ($R^2 = 0.78$) with those of the entire Northern Hemisphere (DETR, 1999), but unfortunately no statistical significance values were provided. Similarly, the latest climate change report for the UK predicts that warming is likely to be greatest in the summer and autumn (Hulme *et al.*, 2002). There is, as ever though, contradictory evidence because there has been a long term warming trend in the north Pennine uplands over the past seven decades and most of the warming is concentrated during the winter months (Holden & Adamson, 2001). However, the annual warming at Moor House meteorological station has been greater than that experienced at the nearby Durham station (450m lower), perhaps indicating site specific climatic changes and therefore responses are

probable, complicating matters further. Despite the potential for winter warming, the results from this study suggest that winter DOC exports may remain similar in the near future. Rates of biological DOC production and enzymic generation from the peat matrix are likely to remain low as a result of conditions that are still relatively cool. Having said this, there is the question of whether DOC exports in future, warmer, winters would resemble those of the spring or autumn period (see below).

Microbial respiration also did not increase significantly with increasing temperatures in the winter (table 3.13), which is to be expected if DOC failed to increase since organic carbon can represent a source of energy for the production of trace gases (c.f. van Veen *et al.*, 1989). Data from the decomposition rates of organic carbon in mineral soils suggests that temperature does not limit microbial activity and that increased temperature alone will not stimulate decomposition (Giardina & Ryan, 2000a). However, these results were in the context that decomposition rates in these mineral soils were remarkably constant across a global-scale gradient in mean temperature. Of course, such a system might differ greatly from peatlands, but it serves to illustrate that a suite of suitable conditions may be required before microbial growth is stimulated. There is however, criticism of the work of Giardina and Ryan (2000a) because estimates were based on the assumption that soil organic matter (SOM) can be represented as a single homogenous pool. The widely varying age of SOM (from months to millennia) can have a large effect on calculated turnover times when soil carbon fractions that cycle at different rates are averaged together (Trumbore, 2000). Davidson *et al.* (2000) discuss the inappropriate nature of Giardina and Ryan's approach and the authors defend their work (see Giardina & Ryan, 2000b). The winter fluxes of both CO_2 and CH_4 in some natural boreal peatlands in eastern Finland were found to be connected to some extent with the dynamics of peat temperature, but not exclusively and not throughout the winter (Alm *et al.*, 1999), consistent with the results presented here.

Increasing temperatures stimulated phenol oxidase activities in the spring peat by 0.018 nmol $g^{-1}min^{-1}°C^{-1}$ ($P<0.1$ only, corresponding to a Q10 of 1.29) and β-glucosidase activities by 0.018 nmol $mg^{-1}h^{-1}°C^{-1}$ ($P<0.001$, corresponding to a Q10 of 2.20) (figure 3.09 & 3.10 respectively), enhancing DOC and phenolic compound concentrations by 3.21 ($P<0.05$) and 0.13 mg $L^{-1}°C^{-1}$ ($P<0.001$) respectively (figure 3.07 & 3.08 respectively). This may be due to a stimulation of *in situ* abiontic enzyme activities and/or an increase in enzyme production by microbes that are able to rapidly respond to increased temperatures. Enhanced temperature sensitivity in relation to the winter peat may be facilitated by increased labile DOC inputs, provided by root exudates or microbial degradation products, when conditions become more favourable to growth at the start of the growing season. Indeed, ß-glucosidase showed unusually high activities compared

to those of the other seasons, which may be induced by the availability of such labile materials. Increased phenolic compound release from the peat represents a potential source of inhibition for DOC consumption in the receiving waters, with the possibility of positive feedback to reduced water quality. These results may be of concern because total trihalomethane (TTHM) levels increase dramatically from the spring to autumn period associated with increased DOC concentrations (Alarcon-Herrera *et al.*, 1994; Betts, 1998; Worrall *et al.*, in press). Furthermore, Alarcon-Herrera *et al.*, (1994) identify high TTHM concentrations in April and May, attributing this to the spring turnover in water bodies, an effect which may be exacerbated in peat dominated catchments due to thermally induced DOC release. There is also evidence to suggest that currently spring is beginning earlier (Gian-Reto *et al.*, 2002; Menzel & Fabian, 1999). This lengthens the time over which the enhanced spring DOC release is likely to occur, rather than the preceding winter exports. However, care is required since bias can be introduced into reports of climate related trends in the phenology of spring events by giving the calendar date of such occurrences each year, as opposed to their timing relative to the vernal equinox (e.g., see Sagarin, 2001).

From the summer peat incubation, increasing summer temperatures pose a greater threat to water quality than increasing temperatures in the spring. While DOC concentrations increased by a similar amount (3.3 mg $L^{-1}°C^{-1}$, $P<0.01$), increases in phenolic compound concentrations were more than double those of the spring peat (0.33 mg $L^{-1}°C^{-1}$, $P<0.01$, figure 3.08) associated with stimulated phenol oxidase activities (0.026 nmol g^{-1} $min^{-1}°C^{-1}$, $P<0.05$). Each 10°C rise produced a 36.36% increase in such activities i.e., a Q10 of 1.36 (figure 3.09) despite the fact that waterlogged peat soils generally exhibit highly constrained phenol oxidase activities (Freeman *et al.*, 2001a). We might thus infer that the enzymes present in the summer peat are adapted to warmer temperatures (originally coming from a microbial community that is adapted to summer conditions), therefore allowing increased activities with increasing temperatures when such conditions arise. Or, that other factors allow this response in the summer peat; as with the spring results, the effect of plant inputs such as labile exudates may be important.

The DOC AMW spectra (figure 3.04d) highlights the potential for substantial increases in high molecular weight material (>90 000 to <200 000 Da) with increasing summer temperatures, in line with the increasing phenolic compound concentrations discussed above. These results suggest that in the summer months refractory materials may also be sensitive to temperature, as well as the low AMW fractions (c.f. Davidson *et al.*, 2000), perhaps for the reasons mentioned. The barely detectable low AMW fraction supports the theory that decomposition is more

efficient, allowing mineralization to CO_2 (figure 3.11), in comparison to the situation in the winter where decomposition seemingly is inhibited.

Mean summer temperatures have risen to over twice the long term average and the Central England Temperature Record shows that mean temperatures were 0.66°C higher in the 1990s than in the three preceding decades (DETR, 1999). The Intergovernmental Panel on Climate Change (IPCC) has proposed that we could experience up to a 5.9°C additional warming this century (IPCC, 2001), thus we could see up to a 19.47 mg L^{-1} (or 20.45%) and 2.0 mg L^{-1} (or 38.21%) increase in the export of aged DOC and recalcitrant phenolic compounds respectively (c.f. tables 3.09 & 3.10 respectively). Elevated DOC concentrations would also impact on the observation that much of the young DOC can be selectively degraded over the residence times of river and coastal waters, leaving an even older and more refractory component for oceanic export. Increasing the abundance of phenolic compounds in receiving waters impairs the metabolism of the remaining DOC (Freeman *et al.*, 1990). More phenolic compounds would therefore slow degradation of exported materials irrespective of origin, amplifying the total flux to the ocean. The rising DOC exports that have been reported are therefore likely to increase further as global warming intensifies.

In contrast to the situation in winter, microbial respiration increased dramatically (1.75 mg $m^{-2}h^{-1}°C^{-1}$, $P<0.01$) with increasing temperature in the summer peat (figure 3.11), consistent with the results from the AMW spectra, indicating that at this time of year temperature is an important control on biological activity. This is supported by the work of Silvola *et al.* (1996) who found that the temperature of the surface layer of peat governed summer CO_2 release. A positive linear relationship between CO_2 emissions and temperature has also been reported for a range of soil types in laboratory experiments (e.g., Howard & Howard, 1993; Wiant, 1967). Summer temperatures are likely to become warmer and so CO_2 emissions from peatlands are also likely to increase, enhancing the potential for global warming (i.e., a positive feedback). The recent study by Cox *et al.* (2000), which examines the ability of land and ocean ecosystems to absorb CO_2, highlights the importance of understanding climate change feedback mechanisms. They present results from a fully coupled, three-dimensional, carbon-climate model indicating that climate change could accelerate significantly over the 21st century due to carbon cycle feedback. The authors calculate a mean global warming of 8°C over land, compared to 5.5°C without the carbon-cycle feedback, by the year 2100. Based on the results described, this may produce a 52% (or 2.66 mg L^{-1}) increase in the concentrations of phenolic compounds available for export (c.f. table 3.10).

Freeman *et al.* (2001b), using data from a three month field survey, found that every doubling in phenol oxidase activity was accompanied by an approximate doubling in CO_2 production. In this study, phenol oxidase activities correlated strongly with CO_2 emissions in winter (0.9354, $P<0.001$) and in summer (0.8609, $P<0.01$). Since there seems to be a strong link between phenol oxidase and DOC concentration in all seasons (tables 3.05-3.08), it could be hypothesized that DOC is indicative of CO_2 release. Indeed, DOC and CO_2 correlate in both winter and summer (0.9012, $P<0.001$ and 0.9583, $P<0.001$ respectively). Hence, increased DOC concentrations in waters draining peatlands could represent a signal that climate change is occurring and is affecting both aquatic carbon transport and loss to the atmosphere.

Autumn peat was the most sensitive to warming, showing a similar increase in ß-glucosidase activities to that of the summer peat (0.016 µmol $g^{-1}h^{-1}°C^{-1}$, $P<0.01$ and a Q10 value of 1.68), but an even more pronounced increase in phenol oxidase activities (0.062 nmol $g^{-1}min^{-1}°C^{-1}$, $P<0.01$ and a Q10 value of 1.46, figure 3.09). This stimulation of activities was associated with the most dramatic rise in DOC and phenolic compound concentrations (7.95 ($P<0.05$) and 0.44 mg $L^{-1}°C^{-1}$ ($P<0.05$) respectively, figure 3.07 & 3.08 respectively) of all the seasons studied. Such sensitivity may relate to the substantial inputs of DOC as a consequence of plant senescence. In particular, high molecular weight, lignified materials are likely to be a substrate for phenol oxidase, which is seemingly limited by temperature in the autumn. Warming significantly stimulated this enzyme and apparently caused a significant increase in the generation of dissolved phenolic materials. Since Hulme *et al.* (2002) predict warming in the summer and autumn period as a result of climate change in the UK, increased DOC release poses a serious threat to the quality of drinking water. Moreover, this would occur at a time when the potential for the production of disinfection by-products (DBPs) is already high (Alarcon-Herrera *et al.*, 1994; Worrall *et al.*, in press) as a result of DOC generated over the summer period being washed into the recipient waters (Worrall *et al.*, in press). In addition, the growing season in the UK is longer now than at any other time since records began in 1772 (Hulme *et al.*, 2002). A lengthened growing season has also been reported by Menzel and Fabian (1999) who analyzed data from observations in Europe over more than 30 years. They found spring events had advanced by 6 days, whereas autumn events have been delayed by 4.8 days. The average annual growing season has therefore increased by 10.8 days since the early 1960s and these shifts can be attributed to changes in air temperature (Menzel & Fabian, 1999). Gian-Reto *et al.* (2002) also found evidence for a link between climate change and longer growing seasons in different ecosystems and review this area of research. Dissolved organic carbon release as a result of longer periods conducive to plant production, microbial degradation of this material and enzymic generation from the peat matrix may continue later

into the year, when traditionally concentrations decreased with the onset of cooler winter temperatures.

3.06 CONCLUSIONS

Thermal optima were observed in the peat where maximal DOC concentrations, enzyme activities and microbial respiration in a given season were produced. This coincided with the approximate ambient temperature recorded during peat collection, suggesting that the microbial community is adapted to the external conditions and is dynamic, shifting with changing environmental stimuli. Thus, changes in DOC processing are likely under a changing climate. Such seasonality in the response of the peatland system needs to be taken into account when modelling carbon flux in and from these systems, otherwise grossly different responses to climate change may be predicted from those that occur in reality.

Significantly increased DOC and phenolic compound generation for most of the year (spring, summer and autumn) as a result of higher soil temperatures could indicate that a major terrestrial carbon store is being relocated to the oceans. The exodus of recalcitrant phenolic compounds is of particular concern, potentially representing a positive feedback to declining water quality, with even the biodegradation of the most labile material being potentially impaired. This mechanism may account for the rising trend in DOC concentrations reported in UK rivers across a variety of catchment types (Freeman *et al.*, 2001a; Worrall *et al.*, in press etc). DOC export is likely to increase as global warming intensifies, further reducing the quality of drinking water. Moreover, increased use of relatively inexpensive upland waters and transportation to areas of deficit with increasing temperatures is likely to expose more of the population to water of reduced quality. Further research into the fate of that extra organic material in the recipient ecosystem is required.

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CHAPTER 4A: STABLE ISOTOPE METHOD DEVELOPMENT

4.01 INTRODUCTION

Our current knowledge of carbon (C) dynamics relies upon inventories of soil, plant and atmospheric C pools (Howard *et al.*, 1995; Parton *et* al., 1993), although measurements of changes in the size of C pools do not adequately describe the sources and magnitude of C turnover *in situ* (Thornley, 1998). Radio isotope (^{14}C) tracer techniques have proved invaluable in determining the fate of plant assimilated C *in vitro* and have been used to measure plant photosynthesis under various conditions (Vose, 1980), C transfer to the soil (Martin & Kemp, 1986), and root turnover (e.g. Milchunas & Lauenroth, 1992). Unfortunately their application has been limited to field studies in experimental agronomic systems due to high costs and safety considerations associated with the use and analysis of C radio isotope tracers (Swinnen *et* al., 1995a, b). Medium to long term soil C dynamics have also been quantified using natural abundance ^{14}C values of plant organic matter (Harkness & Harrison, 1989). This approach utilizes the assimilation and processing of atmospheric $^{14}CO_2$ originating from atmospheric nuclear weapons testing in the late 1950s and early 1960s. The technique enables a crude measurement of soil C turnover but the resolution of the ^{14}C input pulse cannot always be used to quantify short term (hours-days) and medium term (weeks-months) C turnover.

Stable isotope technologies now allow the tracing and quantification of C in studies of terrestrial nutrient dynamics *in situ* (Boutton *et al.*, 1999). Moreover, ^{13}C (1.11% natural abundance) is considered a more faithful tracer of ^{12}C (98.89% natural abundance) than ^{14}C because of the smaller mass difference that exists between ^{13}C and ^{12}C in comparison to that between ^{12}C and ^{14}C (Schimel, 1993). A number of investigations have recently applied ^{13}C tracer techniques to grassland systems. An *in vitro* $^{13}CO_2$ pulse labelling system was developed by Hungate *et al.* (1997) to investigate the effects of ambient and elevated CO_2 (eCO_2) concentrations on C allocation and turnover in prairie soil/plant systems. They demonstrated that plant C assimilation and partitioning was altered to favour photosynthate C turnover through rapidly metabolized pools under eCO_2. Stewart and Metherell (1999) studied the effect of plant community composition and soil type on the C turnover in grazed New Zealand grasslands. Their experiment involved the acidification of ^{13}C labelled inorganic C sources (Na_2CO_3) to produce a pulse of CO_2 at elevated concentrations (>1000 ppm). They established that a stable isotope pulse could be detected in plant

matter for at least 21 days. However, neither of these studies were concerned with the assimilation and turnover of pulse derived CO_2-C at ambient atmospheric concentrations (i.e., 350-360 ppm CO_2) in the field.

The flow of C through peatlands is poorly understood despite their importance in the global carbon cycle (see chapter 1). In particular, little is known about the roles that different species, especially bryophytes, play in carbon flux and storage. *Sphagnum* is a characteristic taxon of peat bogs being the dominant component of bogs and poor fen peat (Gajewski *et al.*, 2001). On a global basis, no other group of mosses is as ecologically dominant (Andrus, 1986). Over half of the world's peat originated from *Sphagnum*, which represents 10-15% of the total carbon stock, and there is more carbon sequestered in dead and living *Sphagnum* than is fixed in one year by all terrestrial vegetation (Clymo & Hayward, 1982).

In spite of the importance of *Sphagnum* dominated wetlands in carbon accumulation and *Sphagnum's* unusual physiological features (such as being poikilohydric, the presence of a preservative oxopolysaccharide (Painter, 1983) and phenolic compounds (Rasmussen *et al.*, 1995) etc.), such plants have received little attention regarding exudation. This is probably due to the fact that they possess rhizoids rather than the root systems associated with vascular plants. These structures anchor the bryophyte to the surface layer, while nutrients and water are taken up ombrotrophically.

The aim of this pilot study was to determine whether the ^{13}C pulse labelling technique was sufficiently sensitive to study the effect of eCO_2 on exudation from peatland plant communities, i.e., whether stable isotope could be detected in the leachate water DOC (that passing through a 0.45 μm filter) and if so over what time scale. The pilot also allowed us to investigate whether *Sphagnum cuspidatum* could significantly contribute to the short term total DOC production of the peat system, as a result of exudation, using a controlled pulse of ^{13}C labelled CO_2 at ambient concentrations (~350 ppm). Since *Sphagnum* is perceived to have an important role in C accumulation, we hypothesized that *Sphagnum* would a) allocate photosynthates into structural fractions and therefore b) exude little C. To test this, a stable isotope tracer approach was used that involved the measurement of $^{13}C/^{12}C$ ratios of leachate organic C, respired CO_2 and plant matter sampled from intact peat monoliths following 5 hours (h) of $^{13}CO_2$ pulse labelling.

4.02 MATERIALS AND METHODS

Peat cores

Eight intact monoliths (11 cm diameter x 25 cm deep) of *S. cuspidatum* dominated peat were collected in perfusion systems (Freeman *et al.*, 1993a, b). Some *S. subunitens* was also included. The study site was a pristine wetland in the Plynlimon catchment, mid Wales, UK (NGR 820 866) described in chapter 2.

As *Sphagnum* gains much of its nutrients from rain water absorbed above ground, synthetic rain water (as described in chapter 2) was applied to provide the same volume per day as that calculated from the average annual rainfall (over the last 10 years) recorded at the sample site. The peat cores were allowed to equilibrate for 1 month prior to labelling in an attempt to minimize disturbance effects.

Stable isotope delivery

A mobile stable isotope delivery (SID) laboratory (Ostle *et al.*, 2000) was used to label vegetation with ^{13}C enriched CO_2 at ambient concentrations. The SID system had the capacity to simultaneously remove atmospheric CO_2, introduce $^{13}CO_2$ and deliver a controlled flow of gas to multiple vegetation cover chambers whilst monitoring headspace CO_2 and H_2O concentrations. The headspace isotope chambers used were those described by Ostle *et al.* (2000) and are shown in chapter 4B (figure 4.11a).

Experimental $^{13}CO_2$ pulse, sampling and $^{13}C/^{12}C$ determinations

A ^{13}C labelled (99.99 atom % ^{13}C) CO_2 pulse was supplied to 4 of the 8 perfusion systems at ambient concentrations (350-360 ppm) over a period of 5 h on the 21/06/00. Flow rates were maintained at 5 L min^{-1} to ensure that photosynthesis was not constrained by reduced CO_2 concentrations. The 4 remaining perfusion systems were maintained and sampled during the same time period to provide natural abundance controls (NAB). Chambers were opened to the atmosphere at the end of the $^{13}CO_2$ labelling period. Complete *S. cuspidatum* plants were carefully removed from the replicate chambers and immediately frozen at -20°C. Gas samples for ^{13}C analysis were also taken and stored in pre-evacuated 12 mL glass vials (Labco Ltd., UK). Ten mL of leachate water were collected with a 20 mL palstipak® syringe *via* a surface lateral sampling port (3 cm below the core surface) and a deep port (at 20 cm depth) on the perfusion system. The leachate was immediately frozen at -20°C in 20 mL glass vials. Vegetation, gas and leachate samples from each perfusion system were taken every 2 h before, during and immediately after pulse labelling. Sampling was then gradually

reduced after the first 24 h in regular increments during the 23 days post $^{13}CO_2$ labelling.

Processing

Sphagnum tissue samples were subsequently freeze-dried for 16 h (Beta 1-8, Christ, Germany) and sectioned at 1 cm intervals in order to trace the pulse of ^{13}C from the photosynthetically active capitulum through the *Sphagnum* plant and into the rhizoids. A method was developed to allow the conversion of organic C within the individual samples to CO_2 in sealed, evacuated vials. This is achieved by oxidation of the sample with potassium dichromate and a 2:1 mixture of sulphuric and orthophosphoric acids (Ostle pers comm.). Assuming that organic C has an average valence or oxidation state of zero, the reaction can be described as:

$$2KCr_2O_7^{2-} + 3C^0 + 16H^+ \rightarrow 4Cr^{3+}\ 3CO_2 + 8H_2O$$

The reagents used were as follows:

1. Potassium dichromate ($2KCr_2O_7$) solution (0.06667M)
2. Sulphuric and phosphoric acid. Concentrated sulphuric acid (667 mL) was added to a 1 L glass measuring cylinder and 333 mL of 85% orthophosphoric acid was added slowly. The mixture was then transferred to a 1 L reagent bottle.

Organic C in the samples could then be oxidized using the following procedure:

1. One mL of leachate was placed in a pre-evacuated 12 mL glass 'exetainer' vial (Labco Ltd.) and 2-3 anti-bumping granules added.
2. 0.25 mL of potassium dichromate solution was then added to each exetainer along with 2 mL of mixed acid in rapid succession.
3. Exetainers were capped and immediately evacuated using a syringe fitted with a standard hypodermic needle and carefully swirled to mix their contents, ensuring none of the digestion mixture made contact with the cap.
4. Exetainers were placed in a boiling water bath (in a fume cupboard) for at least 90 minutes. The rubber septa in the exetainer caps were kept dry.
5. Exetainers were then allowed to cool completely.

Evaluation of the technique

In addition to the samples, the following organic standard solutions were prepared at dilutions of 1, 5, 10, 20, 25, 50, 100 and 200 μg C 100 mL^{-1} from a concentrated stock:

1. Asparagine

2. Glutamine
3. Serine
4. Glucose
5. Mannose
6. Sucrose

Similarly, solutions of phenolic compounds were also used to investigate recovery of more biologically recalcitrant material and mixtures of standard compounds were also used to determine whether their interaction would alter C recovery. Standard solutions were oxidized in the same way as the samples and the CO_2 concentrations in the headspace of the exetainers were then measured using a GC (Autosystem XL, Perkin Elmer, UK). Using 25 µg C 100 mL^{-1} as an example, the calculations are as follows:

Each exetainer contains 3.25 mL of solution and has an 8.75 mL headspace into which will pass 25 µg of C or 91.6 µg of CO_2.

One mole or 44.0098 g of CO_2 occupies approximately 24 L at room temperature.

Therefore, 91.6 µg CO_2 will occupy ((24 × 91.6) / 1000) × 44.0098 = 0.04995 mL.

Thus, the concentration of CO_2 in the remaining headspace will be 0.04995 in 8.75 mL:

$= (0.04995 \times 10^6) / 8.75$

= 5708.57 ppm CO_2 in the headspace

This assumes that the headspace is at atmospheric pressure.

In order to ensure that the headspace was at atmospheric pressure, a polythene bag filled with N_2 was used to equilibrate the exetainer. A double-ended needle (Vacutainer, Rutherford, New Jersey) allowed the N_2 into the exetainer; one end of the needle was placed completely within the bag and the other end was then brought down through the rubber septum of the exetainer. Gas from the equilibrated exetainers could then be analyzed as described below. The percentage recovery was then calculated from the actual concentration of CO_2 measured and the expected concentration (calculated as shown above). Control digests were performed in the same way to account for reagent and ultra pure water (MilliQ™) CO_2 production.

Analysis

All leachate samples and standard solutions were analyzed for DOC using a Shimadzu 5000 Total Organic Carbon analyzer (see chapter 2).

Analytical determinations of $^{13}C/^{12}C$ ratios were made at the NERC Stable Isotope Facility (SIF-Merlewood). Plant matter was analyzed using a Roboprep Elemental Analyzer coupled with a Tracermass Isotope Ratio Mass Spectrometer (IRMS), with an analytical precision of ± 0.1$\delta^{13}C‰$ (PDZ, Europa, Crewe, UK). Ratios of $^{13}C/^{12}C$ in atmospheric and headspace CO_2 samples were determined using a trace gas pre-concentration unit coupled to a Micromass Isoprime IRMS (Manchester, UK). Headspace gas obtained from the oxidation of leachate and standard solution sub-samples was also injected into a GC (Autosystem XL, Perkin Elmer) immediately after ^{13}C analysis in order to measure CO_2 concentrations and quantify recovery.

4.03 EQUATIONS AND STATISTICAL CONSIDERATIONS

Atom % values of the sample (leachate, plant tissue and gas) were calculated as follows:

$$^{13}C \text{ atom } \% = [R_{sample} - (R_{sample} + 1)] \times 100$$

Where R_{sample} is the $^{13}C/^{12}C$ ratio determined by IRMS on analyte CO_2 gas from the sample.

Estimates of $^{13}CO_2$ derived C incorporation into whole plant tissues (mg C g plant tissue C^{-1}) and leachate DOC (μg mg^{-1} DOC) were calculated by first generating an 'F' value using the equation;

$$F = (\text{sample atom } \% - \text{average NAB atom } \%)/(99.99 - \text{average NAB atom } \%)$$

Where F is the fraction of sample derived from 99.99 atom % $^{13}CO_2$ and NAB is the natural abundance control sample.

So that:

$$F * 1000 = {}^{13}C \text{ mg g plant tissue}^{-1} \text{or } {}^{13}C \text{ in } \mu g \text{ mg}^{-1} \text{ DOC}$$

To calculate the proportion of leachate DOC that is derived from pulse ^{13}C;

$$^{13}C \text{ (}\mu g \text{ mg}^{-1} \text{ DOC)}/(\text{DOC(mg L}^{-1})/100)$$

Statistical differences in ^{13}C content between labelled and NAB control cores over time were calculated using one way analysis of variance (ANOVA) (Minitab version 13.32, Minitab Inc.), and are reported in terms of P (Steel & Torrie, 1980).

Atom % values were used for statistical analysis (rather than, for example, the pulse derived ^{13}C as a percentage of DOC) because in this experiment there was no treatment as such, only labelled and NAB cores.

4.04 RESULTS

Atmospheric CO_2 concentrations (taken from ca. 1.5 m above the core surface) were taken to ensure that there was no ^{13}C enrichment in the atmosphere due to influences from fossil fuel burning. Signatures were found to be in the range expected (ca. -7δ ^{13}C‰). Following the removal of the chambers at the end of the pulse labelling, CO_2 concentrations monitored at ground level within the *Sphagnum* canopy were higher at night (500-700 ppm) than in daylight hours (200-400 ppm). Such nocturnal augmentation in CO_2 is a consequence of reduced photosynthesis by the vegetation cover and an accumulation of soil/plant respired CO_2 within the canopy. As morning light levels and photosynthetic activity increased the CO_2 concentrations in the vegetation decreased.

Recovery of C from all the standard solutions following the digestion procedure ranged between 70.6 and 100% over the range of 0-100 mg L^{-1}, with a mean recovery of 82.9% which increased to 94.2% over the range of the leachate DOC concentrations. Leachate DOC concentrations were relatively consistent over time in both the NAB and labelled cores (figure 4.01a), being in the range 4.66 ± 4.66 to 18.27 ± 0.11 mg L^{-1}. Maximum concentrations were observed in daylight and minimum concentrations during darkness. Labelled and NAB leachate DOC concentrations were not significantly different ($P>0.05$). Figure 4.01b shows the rapid ^{13}C enrichment of leachate DOC in the labelled cores (within 4 h of labelling commencing at 16.00 (21/06/00), $F = 71.00$, $P<0.01$) and a gradual decline over the experimental period towards the NAB levels. Similarly, the proportion of pulse derived ^{13}C within the total DOC pool is shown for comparative purposes (figure 4.01c).

The isotopic composition of unlabelled control vegetation remained within the typical range of C3 plants (1.083 ± 0.0357 to 1.084 ± 0.0004 atom %), while $^{13}CO_2$ pulse labelled tissue atom % values increased markedly to 1.748 ± 0.1246. Results of $^{13}C/^{12}C$ analysis of *S. cuspidatum* capitula (figure 4.02) showed a significant incorporation of pulse derived ^{13}C after 4 h of pulse labelling ($F = 52.59$, $P<0.001$). Enrichment continued to increase and reached a maximum of 1.75 ± 0.13 atom % at ca. 26 h after labelling ceased (19.30 on 22/06/00), i.e., a 61.39% increase in ^{13}C content compared to the unlabelled NAB controls (1.08 ± 0.0003 atom %) ($F = 28.49$, $P<0.01$). A rapid

decline in ^{13}C content was then observed over the next 48 h, followed by a more gradual decline over the next 4 weeks. ^{13}C labelled capitula remained significantly enriched until 14/07/00, ca. 23 days after ^{13}C pulse labelling, relative to the NAB controls ($F = 182.11$, $P<0.001$).

The pulse derived ^{13}C incorporation into the various 1 cm depth increments of single *S. cuspidatum* plants, below the capitulum, over time, is illustrated in figures 4.03a-d and tracks the changes at the apex. Initial incorporation values were maximal at 1 cm depth, immediately after labelling ended (1.81 and 1.08 atom % in the labelled and NAB *Sphagnum* tissue respectively). Maximum ^{13}C enrichment could be seen in progressively deeper sections of the plant. This ^{13}C enrichment was attenuated with time and no enrichment was found at a depth of 8 cm or more (i.e., the final 1 cm of the *Sphagnum* plant) throughout the study period.

Figure 4.04 shows the pulse derived ^{13}C in the gross respired CO_2-C. A rapid increase in ^{13}C enrichment occurred within 24 h of labelling, with maximum enrichment measured on the 22/06/00 when ^{13}C labelled and NAB cores produced values of 1.104 ± 0.0021 and 1.099 ± 0.0002 atom % respectively ($F = 7.76$, $P<0.05$). Following this there was a relatively rapid decline in ^{13}C enrichment, although the ^{13}C labelled respired CO_2-C remained significantly different to that of the NAB cores for 3 days after the pulse labelling event (on 24/06/00 $F = 8.42$, $P<0.05$).

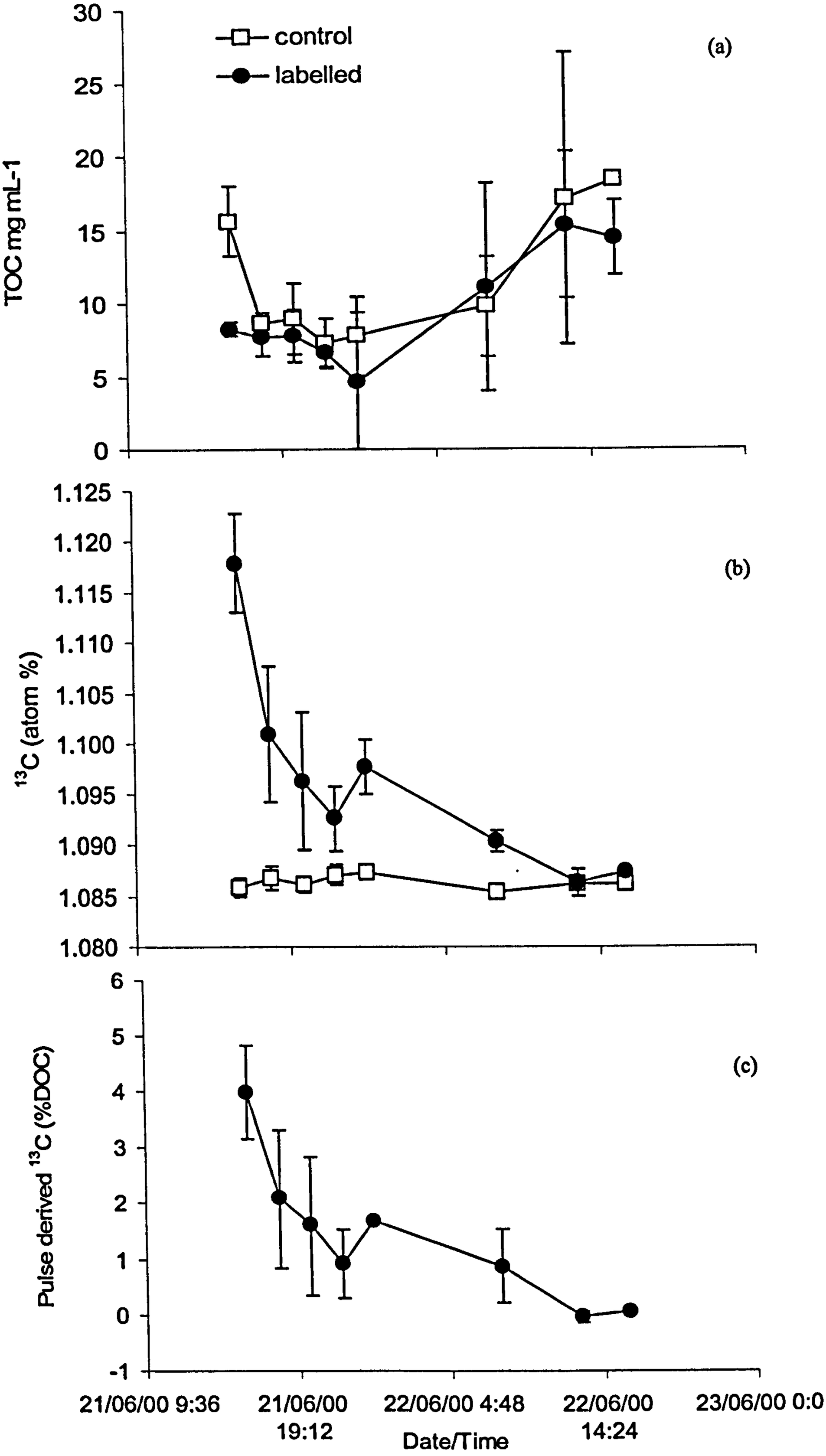

Figure 4.01 (a) leachate DOC concentrations from natural abundance control & $^{13}CO_2$ pulse labelled *Sphagnum* peat cores (b) isotopic composition of leachate DOC from control & labelled cores, & (c) pulse derived ^{13}C as a percentage of the total leachate DOC pool. Error bars represent standard error of the mean, n=3.

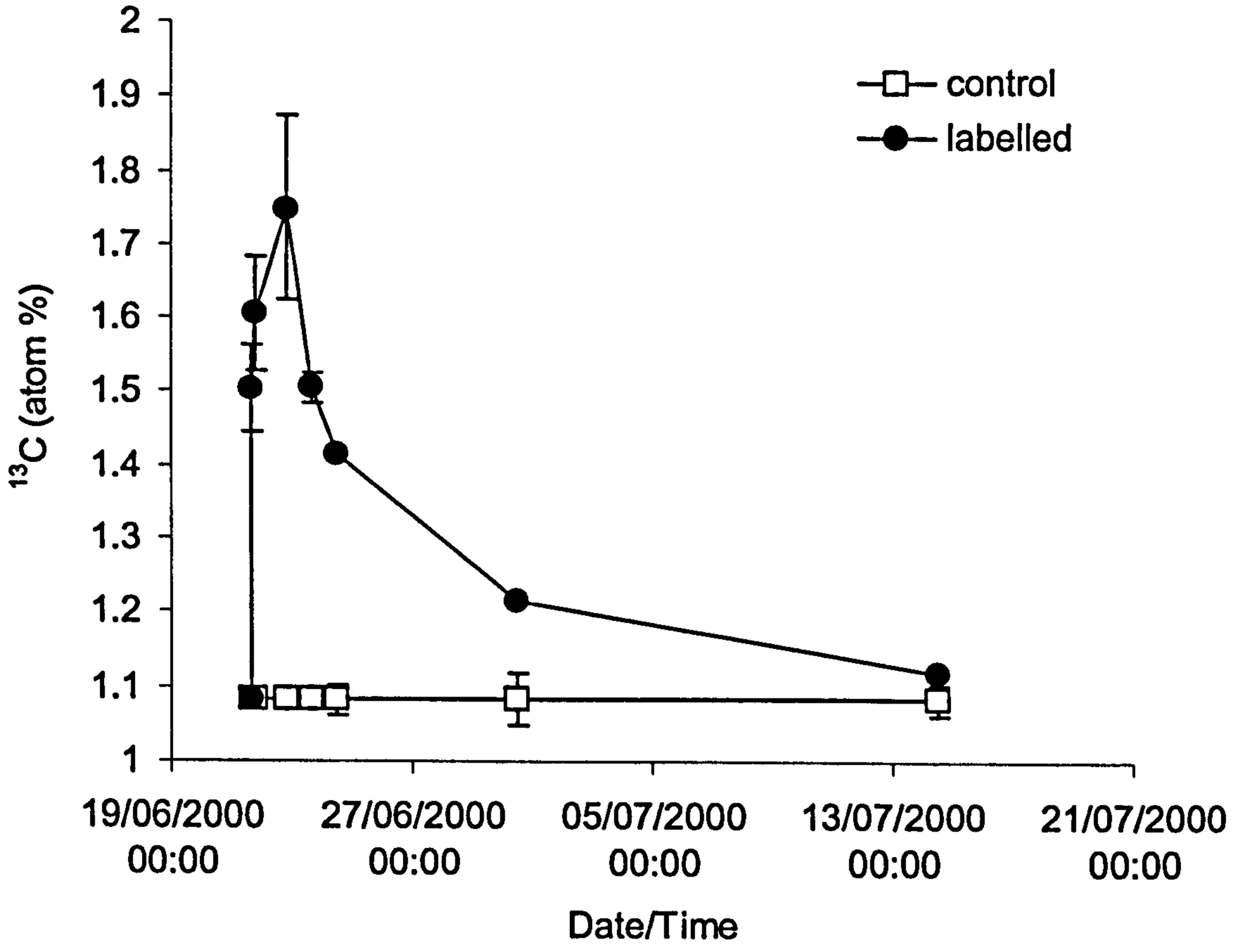

Figure 4.02. Isotopic composition of natural abundance control & $^{13}CO_2$ pulse labelled *Sphagnum cuspidatum* capitula. Error bars represent standard error of the mean, n=4.

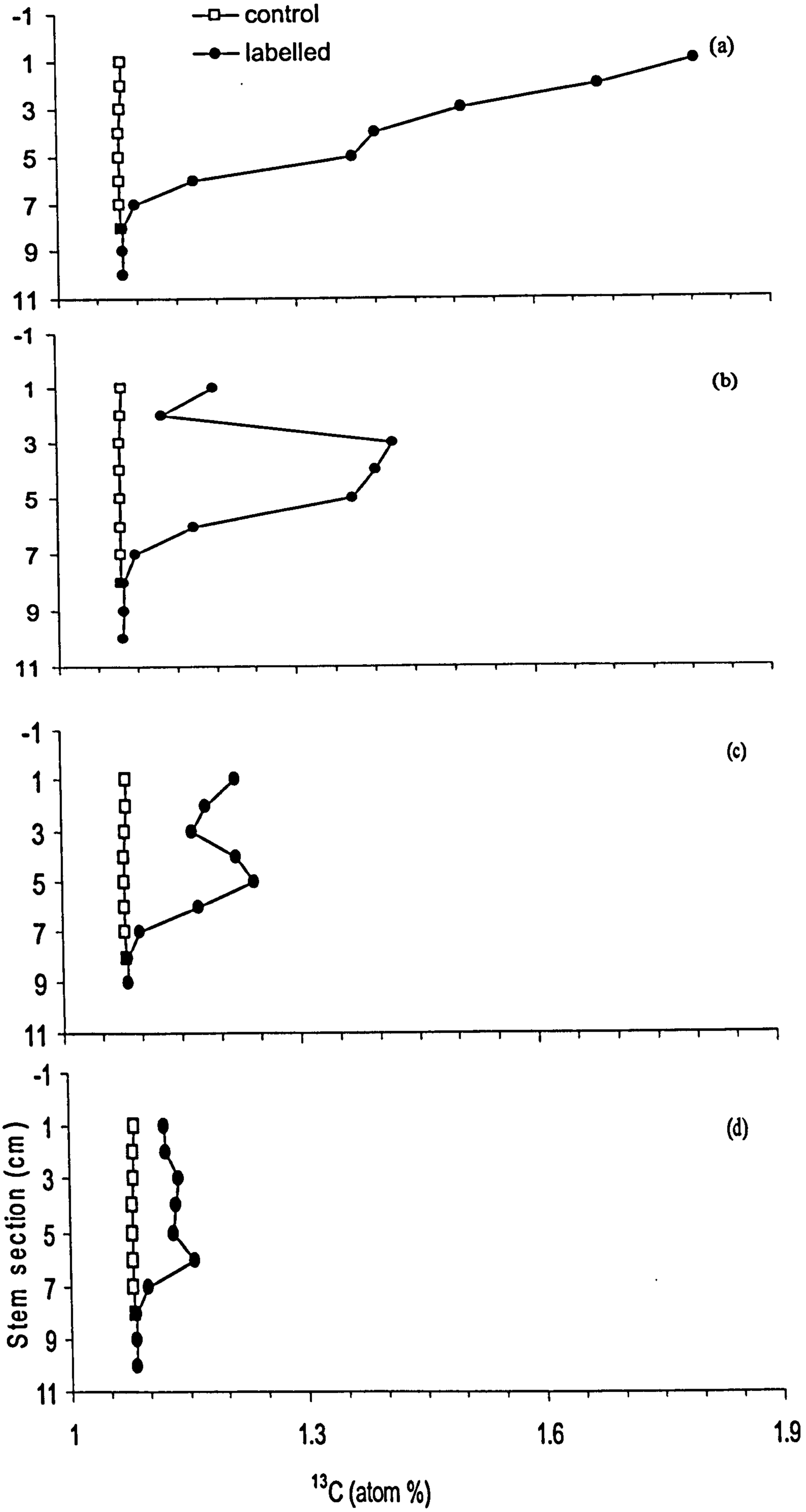

Figure 4.03 showing the isotopic composition through a *Sphagnum cuspidatum* plant during ((a) 21/06/00) & after ((b) 30/06/00 (c) 14/07/00 (d) 18/10/00) $^{13}CO_2$ pulse labelling.

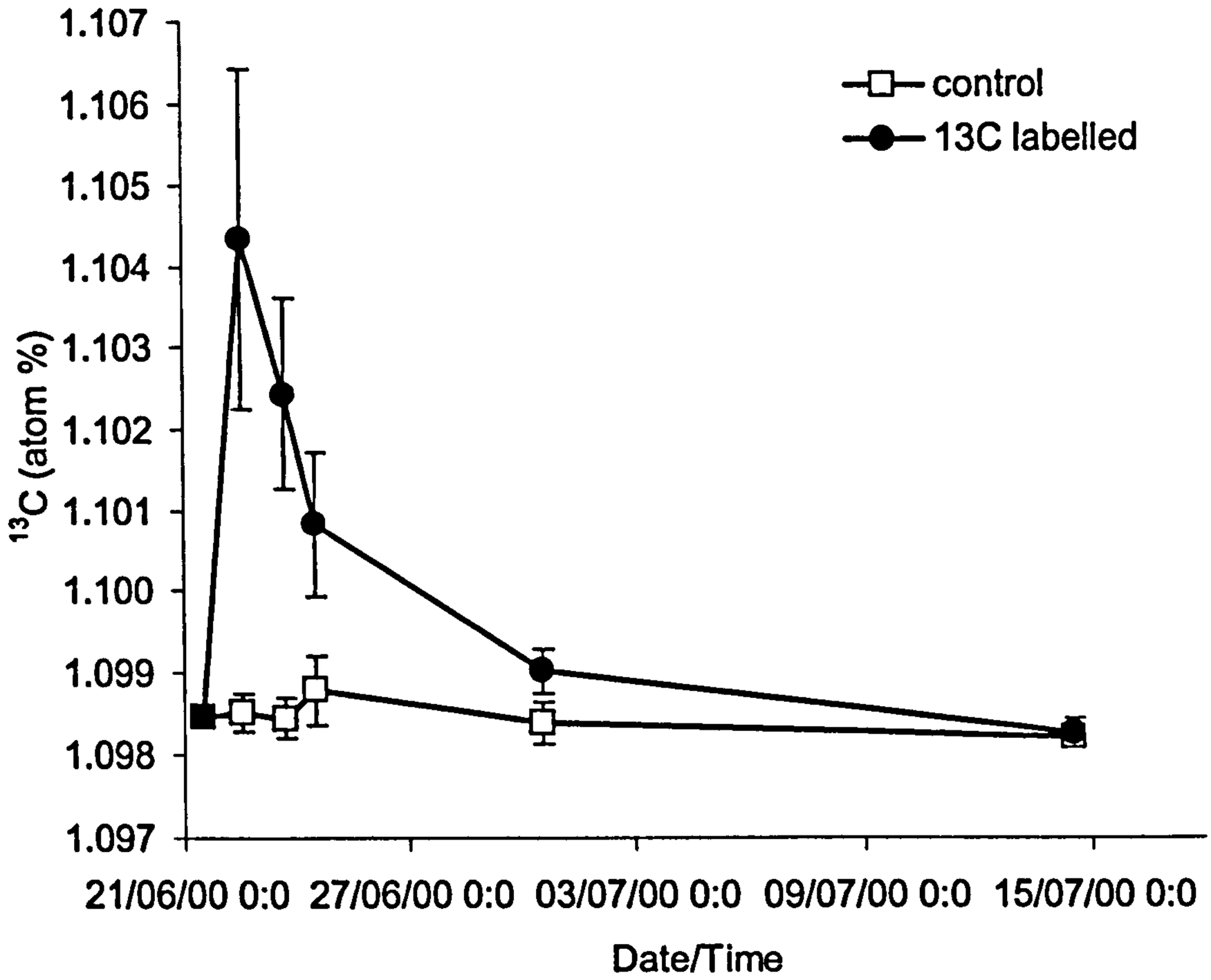

Figure 4.04. Pulse derived ^{13}C in gross respired CO_2-C from natural abundance control & $^{13}CO_2$ pulse labelled *Sphagnum* peat cores. Error bars represent standard error of the mean, n=4.

4.05 DISCUSSION

The chemical digestion technique was developed and applied because the amounts of C in leachate samples are frequently too small for combustion-IRMS, which requires 50 μg of solid C, whereas the instruments used for trace gas-IRMS require only 100 ng of CO_2-C. The mean recovery of C from all the standard solutions ranged between 70.6 and 100% (mean 82.9%) at concentrations of 0 to 100 mg L^{-1} but above this percentage recoveries declined. Higher percentage recoveries (mean 94.2%) were found for standards over the range of 0 to 20 mg L^{-1}, i.e., in the range of DOC concentrations observed in the leachate samples (4.66 ± 4.66 to 18.27 ± 0.11 mg L^{-1}).

As might be expected, leachate DOC concentrations were similar over time in both the NAB and labelled cores (figure 4.01a) and there was some evidence for a diurnal pattern, with maximum concentrations being observed in daylight and minimum concentrations during darkness, consistent with increased exudation as a result of photosynthesis under light conditions.

Despite the rapid and significant ^{13}C enrichment of the labelled *Sphagnum* capitula (e.g., 26 h after labelling ceased where the maximum 61.39% increase ($F = 28.49$, $P<0.01$) occurred, figure 4.02), there is seemingly much less initial direct incorporation of $^{13}CO_2$ in comparison to the above ground tissues of other vascular grassland species. For example, relatively high $^{13}CO_2$ pulse label assimilation rates have been reported for *Festuca ovina* (Ostle pers. comm.) and for an *Agrostis capillaris* sward (Ostle *et al.*, 2000). This could be due to a) higher rates of photosynthesis or, more likely, b) the greater accessibility of the $^{13}CO_2$ to vascular plants with active leaves that 'reach' into the $^{13}CO_2$ pulse stream. Non-vascular species of bryophyte such as *Sphagnum*, however, will have greater access to respired sources of CO_2 (soil and plant) that would have been less ^{13}C enriched during the course of $^{13}CO_2$ pulse labelling.

Dynamic measurements made after $^{13}CO_2$ pulse labelling showed a rapid decline in ^{13}C content following maximum incorporation. This initial rapid decline in pulse derived ^{13}C concentrations in the photosynthetically active capitula was probably the product of C loss as CO_2 *via* rhizoid and shoot respiration, dilution by subsequent assimilation of non ^{13}C labelled CO_2-C, and loss as exudate. In contrast, over the next 4 weeks there was a more gradual decline in ^{13}C content. This two-phase C release is possibly the result of catabolic respiration of more recent ^{13}C labelled photosynthates, such as monosaccharides and amino acids to produce CO_2, with only a relatively small component of pulse derived C being incorporated into more biologically stable structural molecules including lignin, cellulose and hemicellulose (Ostle *et al.*, 2000). Reassimilation of pulse derived ^{13}C that has been respired by the plant and soil heterotrophs as

CO_2, could account for the stabilization of ^{13}C concentrations (see chapter 4b). Indeed, this refixed CO_2 is thought to be important in the growth of *Sphagnum* (Turetsky & Wieder, 1991). In the current tracer study, ^{13}C from a single 5 h period of photosynthesis was detected over 21 days after $^{13}CO_2$ labelling ceased with the labelled capitula remaining significantly ^{13}C enriched (on 14/07/00 $F = 182.11$, $P<0.001$). The concentrations of pulse derived ^{13}C found in plant tissues tended to be higher in *Sphagnum* than in other vascular species (Ostle *et al.*, 2000) and the upland grass species dominated by *F. ovina* (Ostle pers. comm.). This suggests that despite lower growth rates, *Sphagnum* may have a higher potential to accumulate and retain CO_2 derived C than other upland species, which confirms the importance of *Sphagnum* in peatland C sequestration.

Estimates of $^{13}CO_2$ derived C incorporation into plant matter can be made from measurements of tissue ^{13}C and ^{12}C composition. It is accepted that this does not constitute a quantification of total photosynthesis and this was not the aim of the experiment (but rather to determine the potential for recently fixed C to enter the leachate DOC). It is though, a 'tracer' of the assimilation of the $^{13}CO_2$ pulse derived component of total photosynthetically fixed CO_2 and as such, its fate and turnover rate should reflect that of the unlabelled fraction of C assimilated during the same period (Ostle *et al.*, 2000). In order to eliminate unlabelled sources of CO_2 (e.g., soil respiration), *Sphagnum* could have been grown in sterilized sand or other appropriate supporting medium but it is doubtful that this would be representative of natural conditions.

Figures 4.03a-d show the pulse derived ^{13}C incorporated into the various 1 cm depth increments of the *Sphagnum* plant below the photosynthetically active capitulum. It is likely that there are complex mechanisms governing the changes in ^{13}C enrichment down the length of the plant and over time. Photosynthate C release *via* respiration and exudation (see below) is probably occurring continuously, with dilution by C fixed from non labelled $^{12}CO_2$ and refixation of respired $^{13}CO_2$ contributing to changes in *Sphagnum* tissue ^{13}C content. Results from the current study showed that fixed ^{13}C was initially found in the photosynthetically active capitulum from where it was translocated through the plant into the structural components towards the base of the plant. Evidence of ^{13}C from 5 h pulse labelling was found up to ca. a month later, longer than has been reported for the vascular species *A. capillaris* (Ostle *et al.*, 2000) and *F. ovina* dominated systems (Ostle pers comm.). This suggests that *Sphagnum* has a greater metabolic capacity to 'retain' photosynthetic carbon. Seemingly, there is no ^{13}C enrichment at 8 cm depth or beyond and this might be due to the presence of hyaline cells at the base of *Sphagnum* plants, where water collects.

Carbon dioxide concentrations measured in the *Sphagnum* canopy represent a measure of gross CO_2 flux from the system that includes both soil and rhizoid respiration. Although contamination of gas samples from the redistribution of $^{13}CO_2$ pulse gas can and does occur for between 2 and 3h after labelling (Ostle pers. comm.), by 4 h it was possible to begin to determine the rapidity of the pulse ^{13}C return to the atmosphere as respired CO_2. Figure 4.04 shows the pulse derived ^{13}C in gross respired CO_2-C. As with the plant tissue results, there was a rapid initial increase in ^{13}C enrichment within 24 h of sampling ($F = 7.76$, $P<0.05$) due to plant respiration of ^{13}C assimilated *via* photosynthesis. There was, despite the relatively rapid loss of most assimilated ^{13}C from the plant, evidence of 5 h of photosynthesis ca. 3 days after the $^{13}CO_2$ pulse labelling event (on 24/06/00 $F = 8.42$, $P<0.05$). This later ^{13}C enrichment of respired CO_2-C was probably due to delayed soil heterotrophic and/or rhizoid respiration. Grassland vegetation dominated by *A. capillaris* showed the same trend but respiratory release of ^{13}C was more rapid with relatively less retention of ^{13}C within the plant tissues (Ostle pers comm.). This suggests that although initial ^{13}C assimilation was lower in *Sphagnum*, the potential for C accretion may be greater than for other upland species.

Most of the ^{13}C was released *via* respiration however, this is not the only route by which photosynthetically derived C can leave the plant. Indeed, the transfer of C into the soil *via* plant exudates is widely accepted, particularly in grassland plant species. However, this route is poorly studied in wetland systems and no information could be found regarding the amount of recent photosynthates contributing to DOC in pore waters, or the speed at which these photosynthate-derived exudates could enter the soil. This is surprising given that a) several studies identify wetlands as being the major contributor of DOC to river water within the catchment where they exist (e.g., Hope *et al.* 1994; 1997; Wetzel, 1992), b) river DOC is thought to contain significant amounts relatively recent (post-AD 1955) organic C (Palmer *et al.*, 2001), and c) *Sphagnum* peatlands are assumed to be dominated by highly aged (Clymo, 1983) and relatively recalcitrant organic material (Evans *et al.*, 2002; Freeman *et al.*, 2001a; Freeman *et al.*, 2001b).

Statistical analysis of atom % values showed a significant ($F = 71.00$, $P<0.01$) and very rapid ^{13}C enrichment of the leachate DOC within 4 h of the start of labelling (21/06/00 at 16.00) (figure 4.01b), with the mean ^{13}C content after labelling being significantly higher than that of the NAB cores over the entire experimental period ($F = 35.17$, $P<0.001$). Although the size of plant C and leachate DOC pools remained constant, the pulse derived ^{13}C as a percentage of the total DOC pool was also calculated (figure 4.01c) to ensure that slight fluctuations in NAB ^{13}C and pool sizes were accounted for. Surprisingly, up to 4.8% (on average 4.0%) of the total DOC in the peat leachate was derived from only 5 h of $^{13}CO_2$ pulse labelling at ambient

concentrations. This demonstrates that recently fixed C from the *Sphagnum* community makes an important contribution to the peatland DOC pool over a very short time period. In reality, there would be contributions of recently fixed C from that morning and from the day before that were not ^{13}C labelled, making the calculated 4.0% contribution to DOC an underestimate. Thus, much of the DOC measured in peat pore waters could be composed of recent photosynthate-C, rather than ancient (Clymo, 1983, Evans *et al.*, 2002; Ohlson & Okland, 1998; Turunen *et al.*, 2001), relatively refractory (Evans *et al.*, 2002; Freeman *et al.*, 2001a; Freeman *et al.*, 2001b) material as might be presumed from the bulk of the literature. We could speculate that after a given number of days all the leachate DOC would be recently fixed, however it is beyond the scope of this study and further work would be required. Together these results again are consistent with the notion that *Sphagnum* is crucial in the process of C accumulation in peatlands.

There is the possibility that *Sphagnum* is not entirely responsible for the observed results because of small amounts of other plant species included within the *Sphagnum* stands. Algae may also take up and exude pulse derived ^{13}C. In order to prove that only *Sphagnum* produced this effect further research would be necessary, for example, using *Sphagnum* plants supported in sterile containers (as mentioned previously). Photosynthates could then be collected and the presence of isotope would indicate that *Sphagnum* alone was capable of exuding photosynthetically derived C. Nevertheless, it is assumed that the results presented from this experiment are representative of contributions of recently fixed C to peat leachate DOC in the natural system. Furthermore, as described above, ^{13}C incorporation can be traced through the *Sphagnum* plant to the soil interface, although it is not clear whether C is exuded from the hyaline cells, the rhizoid structures or the photosynthetic cells further towards the capitulum of the plant.

4.06 CONCLUSIONS

This study demonstrated that the $^{13}CO_2$ pulse labelling technique is sufficiently sensitive to allow the fate of ^{13}C to be traced through a *Sphagnum* plant and into the leachate DOC pool, despite the importance of such species in C accumulation. Thus, any increases in organic C exudation as a result of eCO_2 could also potentially be detected. The approximate time scale over which sampling would be necessary in order to perform this follow up experiment was also determined.

The *Sphagnum* community showed less initial incorporation of ^{13}C into the capitulum tissue compared to upland grassland species labelled simultaneously (Ostle pers comm.), possibly indicating slower growth or a different source of CO_2. There was however, evidence of greater

^{13}C retention for a longer period of time after labelling ceased and less respiratory loss, consistent with the original hypotheses and the perception that *Sphagnum* plays a major role in peatland C accumulation. Nevertheless, the *Sphagnum* community apparently rapidly contributed a significant amount of recently synthesized DOC to the leachate pool. There is therefore the potential for much of the DOC we measure in peat leachate waters to be recently assimilated rather than ancient material, as could be assumed from the bulk of the literature.

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CHAPTER 4B: EFFECTS OF ELEVATED CO_2 & TEMPERATURE ON THE CONTRIBUTIONS OF RECENTLY ASSIMILATED CARBON TO PEATLAND DOC

4.11 INTRODUCTION

Peatlands represent an important reservoir of terrestrial carbon (C) storing ⅓ (455 Pg) of the world's soil organic carbon (SOC) (Gorham, 1991); annual accumulation rates have been estimated at 20-40 g C m^{-2} over the last 5000 to 10 000 years (Tolonen & Turunen, 1996). The potential for peatland soils to release this C as a result of climate change is of considerable importance in relation to rising DOC concentrations in receiving waters. Furthermore, sequestration from, and release to the atmosphere of peatland C has implications for feedback to future climate change.

Sphagnum peatlands are important accumulators of C due to their slow decomposition rates (e.g., Kuhry & Vitt, 1996). Nonetheless, wetlands export more DOC than any other major biogeographic area in the world, accounting for 20% of the total terrestrial DOC exported to the oceans (Lugo *et al.*, 1989). Peatlands in particular are a major source of DOC and streams draining temperate peatland catchments commonly contain between 10 and 45 mg L^{-1} (Urban *et al.*, 1989). Consistent and statistically significant increasing trends for DOC concentrations have been reported at upland sites in Wales over more than a decade (Robson & Neal, 1996), including the sampling site for material used in this study (Reynolds *et al.*, 1997). A 65% increase in DOC concentration in the freshwaters draining from upland catchments in the UK has also recently been reported by Freeman *et al.* (2001). This increase has been attributed to temperature increases as a result of climate change, since these sites span a range of acid deposition levels, soil types, topographies, land uses and geographical locations. In addition to elevated temperatures (eTemp), elevated atmospheric CO_2 concentrations (eCO_2) are also likely to be a common feature and can effect soil leachate DOC concentrations (Jones *et al.*, 1998; Kang *et al.*, 2001). Such findings were attributed to increases in photosynthetically fixed C allocated below ground to the roots and soil.

The response of intact *Sphagnum* peat monoliths to various climate change simulations have been monitored over a year (see chapter 2) using the solar dome facilities (Centre for Ecology and Hydrology (CEH) Bangor). Substantially increased DOC concentrations were reported in the leachate waters under eTemp with respect to control cores however, this was further increased in the eCO_2/eTemp treatment (chapter 2, figure 2.01a). Increased extracellular enzyme activities were prominent in the former

but not the latter treatment and there was a significant increase in root biomass (with respect to the eTemp treatment) in the eCO_2/eTemp cores. Thus, increased DOC concentrations were attributed to enhanced enzymic generation under eTemp and increased exudation and/or biomass DOC inputs in the eCO_2/eTemp simulation. If this is the case then the magnitude and source of DOC (old *versus* more recent C pools) could shift in response to projected climate change and increases in CO_2 concentrations.

From preliminary $^{13}CO_2$ pulse labelling measurements made in 2000 (chapter 4A), the *Sphagnum* community was found to rapidly contribute a significant amount of recently synthesized C to the leachate waters (a minimum 4.0% of the total DOC pool). It was hypothesized that eCO_2 concentrations would increase the contribution of recently assimilated photosynthate C (through increased rates of photosynthate release into the soil solution as exudate), and that this is the mechanism for the observed increase in DOC concentrations under eCO_2/eTemp. In contrast, enzymic mobilization of dissolved materials from the peat matrix would be expected to liberate increased amounts of aged C and therefore show no such enrichment of recently synthesized DOC. In order to test this hypothesis, intact peat cores that had been exposed to eCO_2/eTemp and those that had been maintained at eTemp only for a year were labelled with $^{13}CO_2$, using the Stable Isotope Delivery (SID) system described by Ostle *et al.* (2000). The stable isotope approach adopted in this study allowed the differentiation between recent ^{13}C pulse derived photosynthate C and older plant and SOC in leachate DOC.

4.12 MATERIALS AND METHODS

Over a period of a year, intact peat monoliths (11 cm diameter x 25 cm deep) contained in perfusion systems (Freeman *et al.* 1993a, b) have been exposed to eTemp (3°C above ambient) and to eCO_2 (ambient + 235 ppm)/eTemp in the CEH Bangor solar dome facility (as described in chapter 2). Both treatments were replicated 5 times and had lateral leachate collection ports located at 3 and 20 cm depths below the peat surface. For each treatment level 2 natural abundance cores (NAB) and 3 labelled ($^{13}CO_2$) cores were used.

The perfusion systems were pulse labelled with $^{13}CO_2$ (99 atom % ^{13}C) (figure 4.11a & b) at ambient concentrations (350 ppm) for 5 hours (h) to enable ^{13}C enrichment of the peatland plant material by natural photosynthetic assimilation of CO_2-C. Leachate samples were taken at regular intervals before, during, and after $^{13}CO_2$ labelling.

Similarly, vegetation samples (*Sphagnum cuspidatum*, *Juncus effusus*, *Festuca ovina* and *Polytrichum commune*) were taken and analyzed for $^{13}C/^{12}C$ ratios as described in chapter 4A. Above ground and below ground biomass was determined as described in chapter 2.

Isotopic data was used to determine the passage of pulse derived C through the plant community and into leachate DOC. DOC samples were first chemically oxidized in sealed exetainers to form CO_2, using the method developed in chapter 4A, before $^{13}C/^{12}C$ analysis by Trace Gas-Isotope Ratio Mass Spectrometry (TG-IRMS). The vegetation samples were analyzed for $^{13}C/^{12}C$ by Elemental Analysis-IRMS (EA-IRMS). Cores were removed from the perfusion systems at the end of the experiment, sectioned into 2 cm increments and frozen with the intention of conducting bulk $^{13}C/^{12}C$ isotopic analysis at a later date. These data could then be used to calculate dynamic changes in the tracer ^{13}C contributions to DOC.

4.13 EQUATIONS AND STATISTICAL CONSIDERATIONS

Equations and statistical considerations were as described in chapter 4A, except that the ^{13}C content (mg g plant tissue C^{-1}) and the percentages of DOC that were pulse derived could be used for statistical analysis (ANOVA/ANCOVA), since this experiment included two levels of CO_2 treatment (as well as NAB controls). Differences in ^{13}C incorporation between different species were determined using ANOVA and differences in ^{13}C retention over time using regression and testing for equality of slopes (Minitab version 13.32, Minitab Inc.).

4.14 RESULTS

Table 4.11. Range of DOC concentrations under elevated temperature (eTemp) and elevated CO_2/elevated temperature (eCO_2/eTemp) in peat leachate water collected from 3 cm and 20 cm depths.

	eTemp		eCO_2/eTemp	
Depth (cm)	Minimum ($mg\ L^{-1}$)	Maximum ($mg\ L^{-1}$)	Minimum ($mg\ L^{-1}$)	Maximum ($mg\ L^{-1}$)
3	26.51 (3.96)	44.91 (14.04)	13.04 (2.68)	26.89 (10.86)
20	38.64 (5.34)	46.88 (13.55)	17.81 (9.17)	33.46 (8.84)

Standard error of the mean is given in parentheses and this convention applies throughout unless stated otherwise. Leachate DOC concentrations for samples taken from 3 cm and 20 cm below the core surface were not significantly different between treatments ($P>0.05$).

Figure 4.12a shows the pulse derived ^{13}C as a percentage of the total DOC pool in the peat leachate from the labelled eTemp and eCO_2/eTemp treatments at 3 cm depth. The proportion of leachate DOC derived from pulse $^{13}CO_2$ was considerably greater in eCO_2/eTemp cores in comparison to eTemp cores. Overall, mean values after labelling were ca. 6 fold higher than those of the eTemp treatment ($F = 51.9$, $P<0.05$), the eCO_2/eTemp leachate showing significant enrichment in relation to the eTemp treatment 4 h and 24 h after labelling ceased (11/10/01 at 20.00 $F = 39.64$, $P<0.01$ and 12/10/01 at 16.00 $F = 33.67$, $P<0.05$ respectively). The maximum difference was observed 24 h after labelling ceased (12/10/01 at 16.00) when the eCO_2/eTemp leachate contained $2.45 \pm 0.45\%$ DOC that was ^{13}C pulse derived, while that of the eTemp cores contained $0.24 \pm 0.14\%$ pulse derived DOC, i.e., a difference of over 10 fold.

Evidence of enrichment under eCO_2/eTemp could be found at the 20 cm sampling depth (figure 4.12b) with the mean pulse C as a percentage of the DOC ($1.64 \pm 1.03\%$) being over 20 fold higher than that of the eTemp cores (0.064 ± 0.043 %), though samples were unfortunately limited and this was not statistically significant.

Table 4.12. Mean ^{13}C natural abundance values (atom %) for each plant species present in the intact peat cores under elevated temperature (eTemp) and elevated CO_2/elevated temperature (eCO_2/eTemp) simulations.

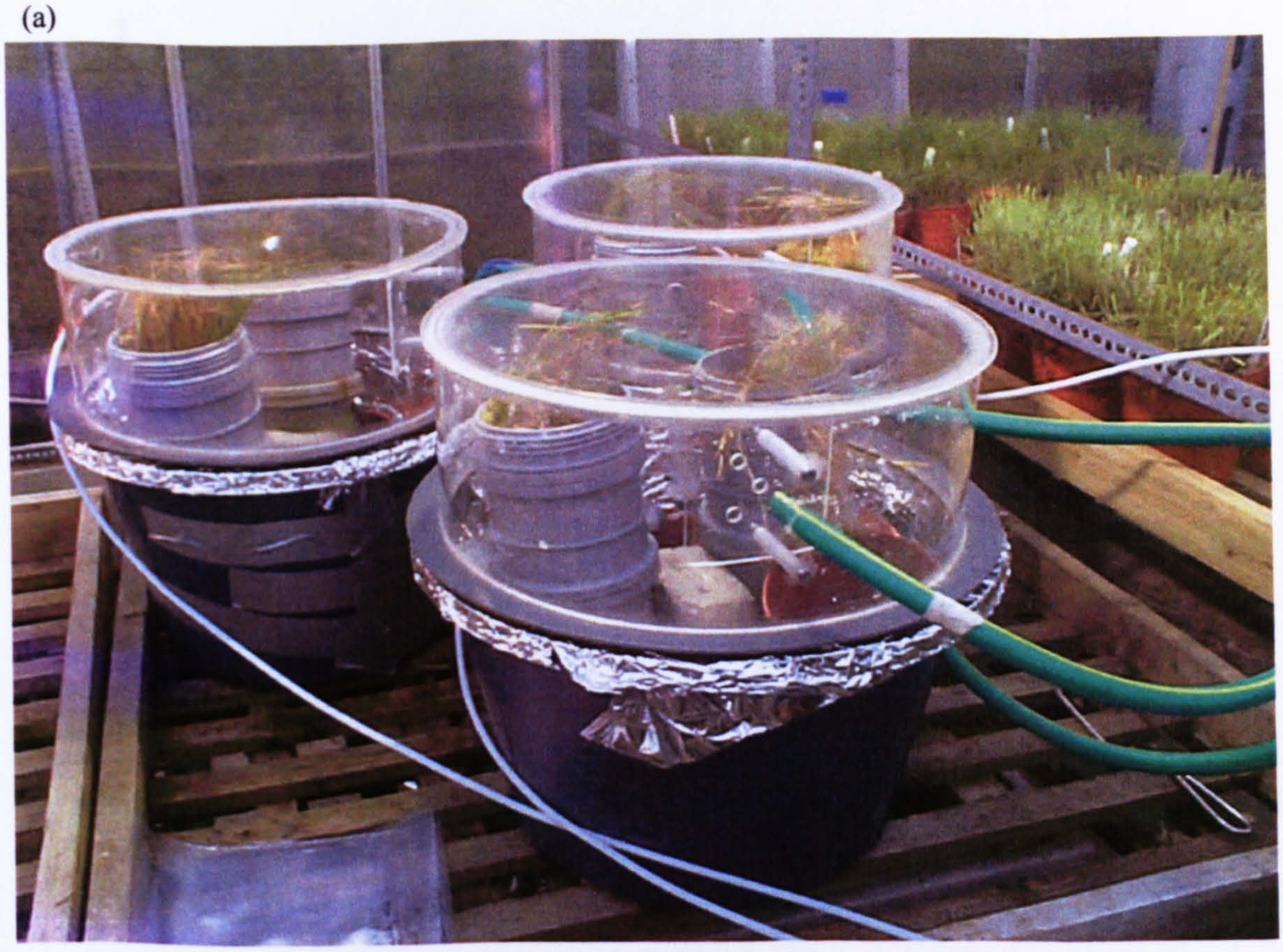
Species	eTemp (^{13}C atom %)	eCO_2/eTemp (^{13}C atom %)
Sphagnum cuspidatum	1.08 (0.0006)	1.07 (0.0006)
Juncus effusus	NA	1.07 (0.0004)
Festuca ovina	1.08 (0.0003)	1.07 (0.0006)
Polytrichum commune	1.08 (0.0013)	1.07 (0.0009)

NA denotes no available data (due to the absence/limited abundance of the species under a given treatment).

NAB ^{13}C atom % values for all species did not significantly change over labelling in either the eCO_2/eTemp or the eTemp treatments. The difference between NAB values for the two treatments, irrespective of labelling, was due to the tank CO_2 (used to elevate atmospheric concentrations in the solar dome facility) being relatively ^{13}C depleted. This difference was accounted for in the tracer mixing equation described in chapter 4A.

For a given species, no significant difference was found in ^{13}C incorporation into the plant tissue between the treatments. However, differences between species were observed. *Sphagnum* showed significantly less initial incorporation of ^{13}C per unit plant C mass in comparison to the other wetland species studied ($F = 5.62$, $P<0.01$). Immediately after ^{13}C labelling ceased *Sphagnum* tissue contained 5.86 ± 0.61 and 4.27 ± 0.93 mg ^{13}C g plant C^{-1} in the eTemp and eCO_2/eTemp cores respectively. This remained remarkably constant ($F = 4.94$, $P<0.05$) with values of 7.28 ± 2.76 and 4.10 ± 3.18 mg ^{13}C g plant C^{-1} respectively, even 48 h after labelling had ceased. Figure 4.13a depicts this response over time under both the eTemp and eCO_2/eTemp conditions. In contrast, despite higher initial incorporation values, a rapid decline in ^{13}C enrichment in the tissues of the other species studied occurred over time ($F = 28.17$, $P<0.001$), and this is illustrated in figures 4.13b-d.

(a)



(b)

Figure 4.11 showing perfusion systems for maintaining intact peat cores (a) inside $^{13}CO_2$ pulse labelling isotope chambers & (b) with lateral sampling ports for leachate collection.

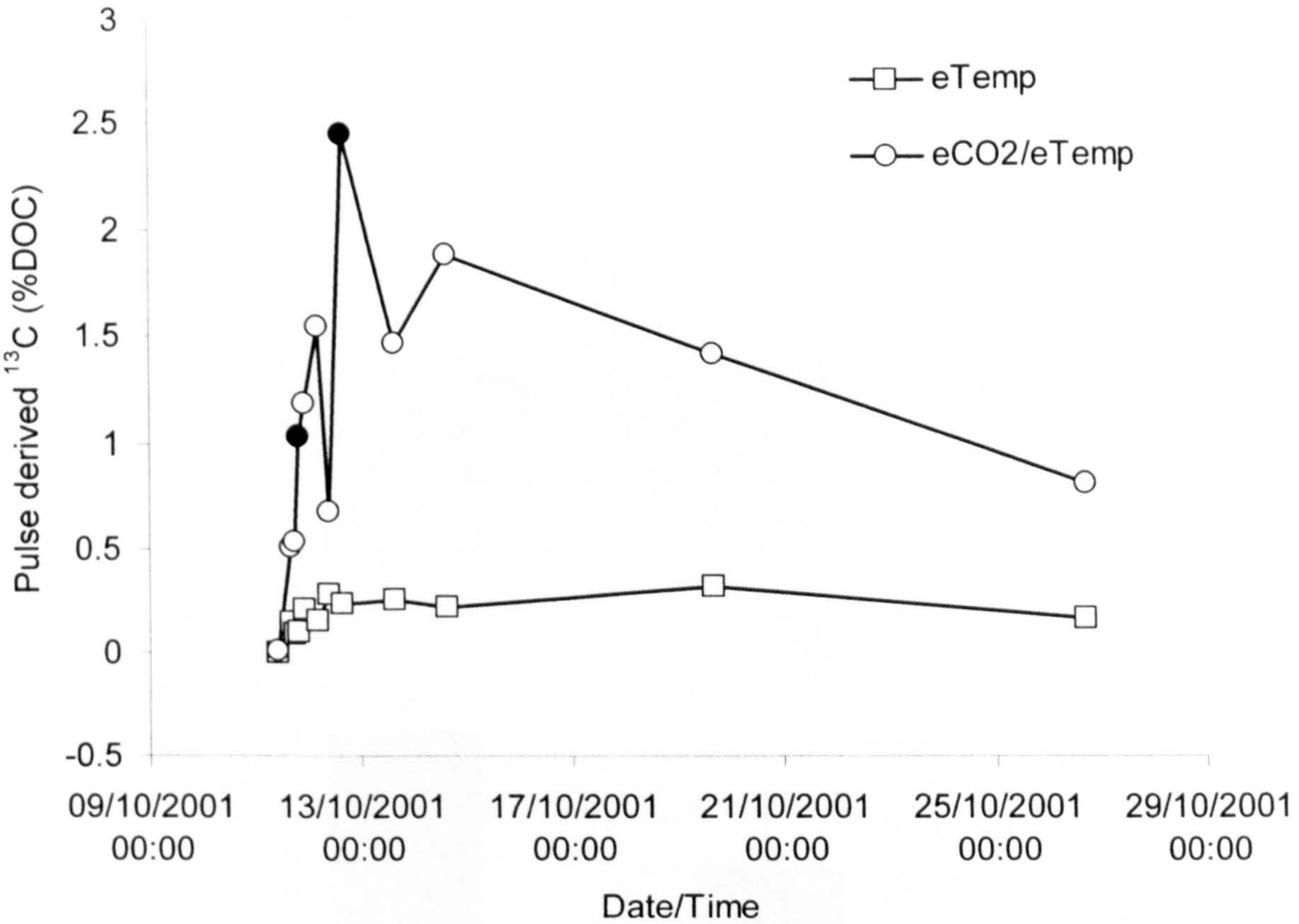


Figure 4.12 (a). Pulse derived ^{13}C as a percentage of the total leachate DOC pool collected at 3cm depth from elevated temperature (eTemp) & elevated CO_2/eTemp (eCO_2/eTemp) peat cores during & after $13CO_2$ pulse labelling. Filled symbols denote a significant difference between treatments at $P<0.05$ or below. Standard errors omitted for clarity (typically ± 0.08 under eTemp & 0.56 under eCO_2/eTemp conditions), n=3.

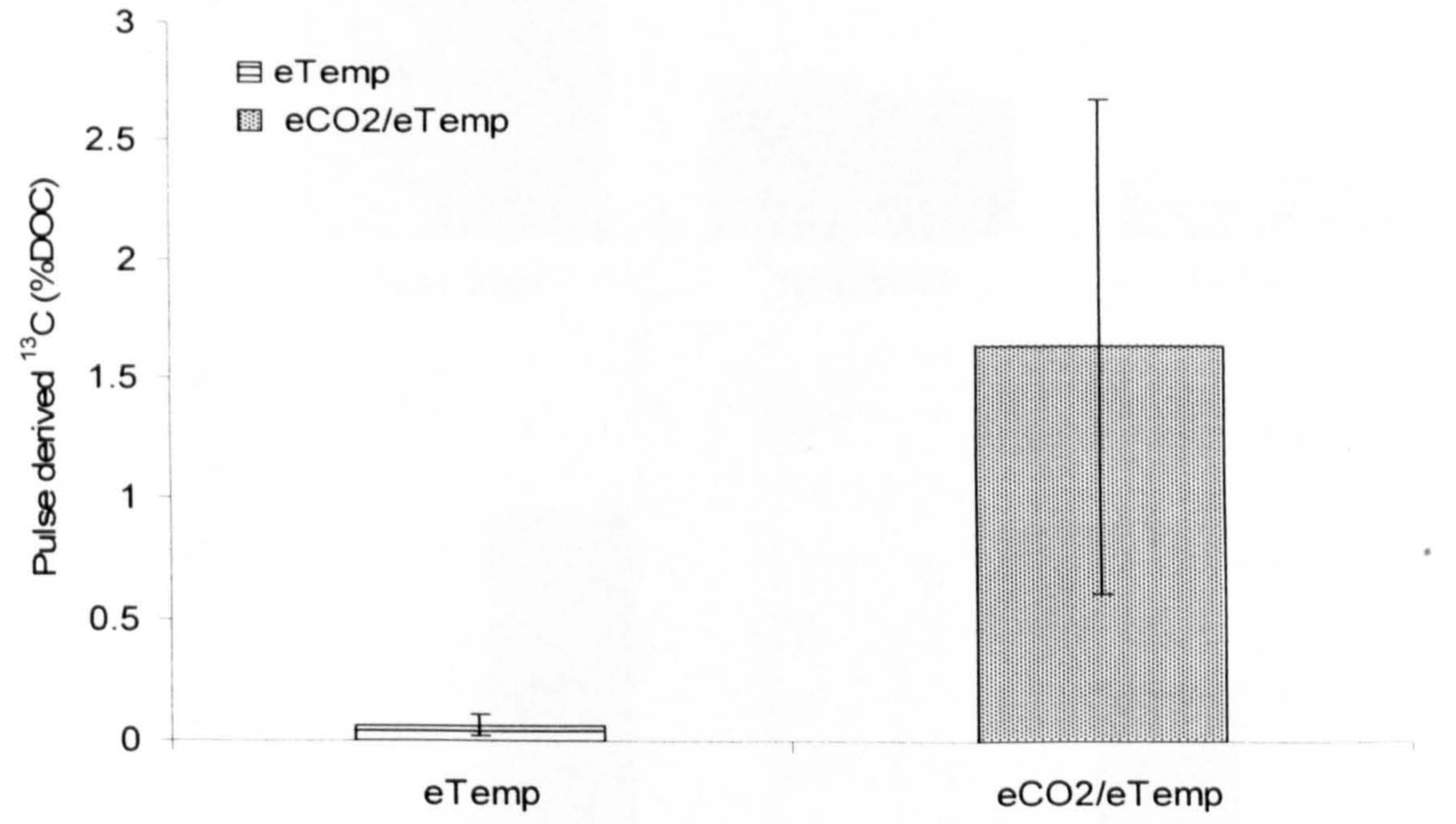


Figure 4.12 (b). Pulse derived ^{13}C as a percentage of the total leachate DOC pool collected at 20cm depth from eTemp & eCO_2/eTemp peat cores ca. 2 weeks after $^{13}CO_2$ pulse labelling (26/10/01). Error bars represent standard error of the mean, n=3.

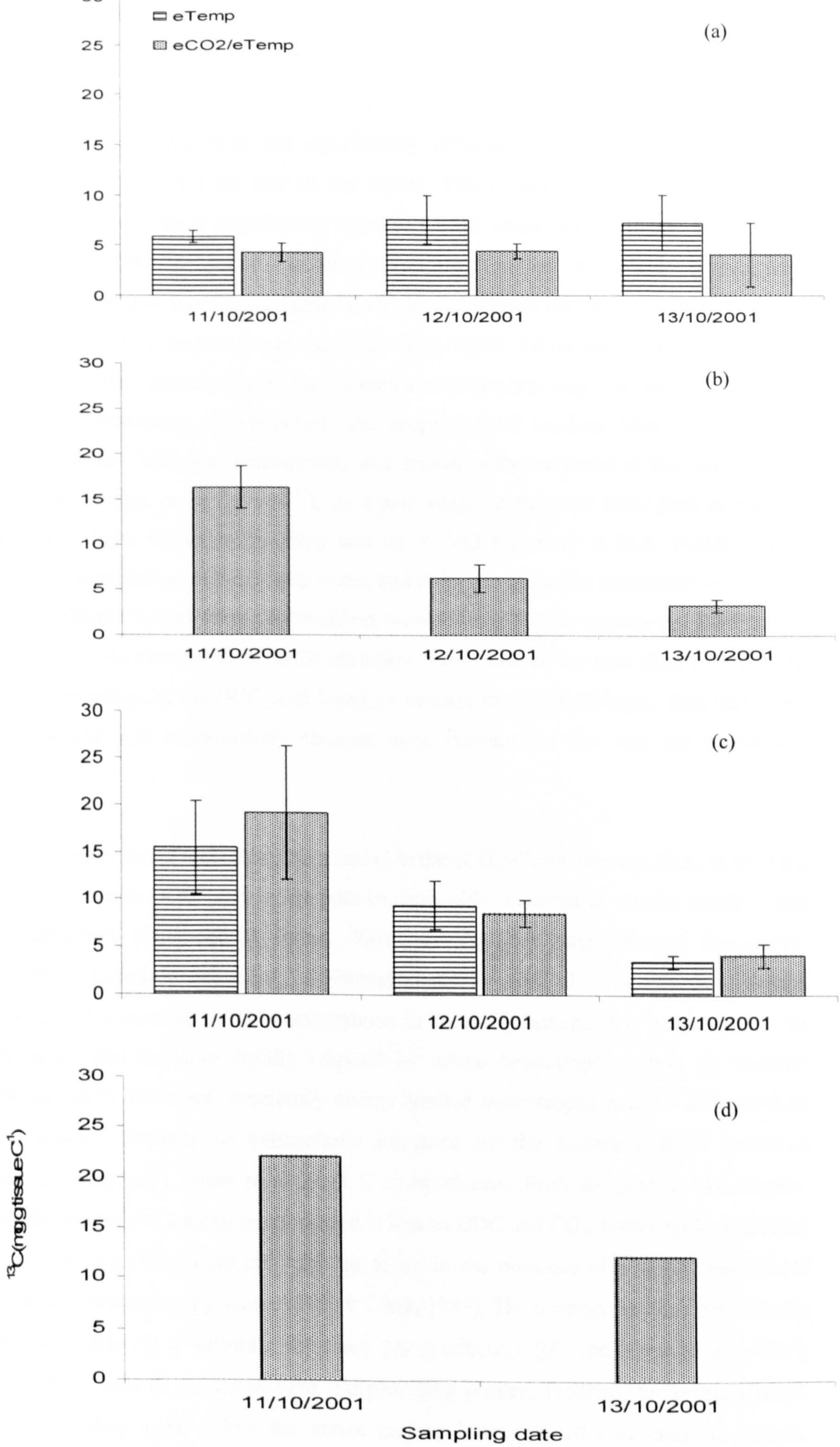


Figure 4.13. Pulse derived ^{13}C retention in (a) *Sphagnum cuspidatum*, (b) *Juncus effusus*, (c) *Festuca ovina* & (d) *Polytrichum commune* tissues following $^{13}CO_2$ pulse labelling. Error bars represent standard error of the mean, n=3 except *P. commune* where n=1.

4.15 DISCUSSION

Mean DOC concentrations were not significantly different between the eTemp and the eCO_2/eTemp cores at both 3 cm and 20 cm depths. This contradicts the monitoring results (chapter 2) somewhat, since significantly increased DOC concentrations were found under eCO_2/eTemp. However, the greatest differences during treatment in the solar domes were observed in the summer months, whereas labelling was carried out in October when levels tended to converge to a certain extent (see chapter 2, figure 2.01a) and measurements were made over a short time period only. Some disturbance of the cores may also have occurred due to pre-labelling procedures. Nevertheless, the proportion of leachate DOC derived from recently assimilated $^{13}CO_2$ was considerably and significantly increased in the eCO_2/eTemp peat cores. On average, pulse derived ^{13}C as a percentage of the total DOC pool in leachate from the 3 cm depth following labelling was ca. 6 fold higher ($F = 51.9$, $P<0.05$) under eCO_2/eTemp in comparison to the eTemp cores, and at its maximum the difference was over 10 fold ($P<0.01$) (figure 4.12a) when contributions were 2.5 and 0.2% respectively. Evidence of ^{13}C enrichment could even be found at 20 cm below the surface of the peat (figure 4.12b), the pulse C as a percentage of the DOC pool being on average over 20 fold higher than that of the eTemp cores, although unfortunately samples were limited and this was not statistically significant.

An increased proportion of recently synthesized C in the eCO_2/eTemp cores is likely to alter the quality of the leachate DOC pool in the peat because older material is usually complex and relatively refractory (Raymond & Bauer, 2001), having undergone selective degradation (Wetzel, 1992). Increased exudation of photosynthetically derived C is therefore perhaps unlikely to directly increase DOC concentrations in receiving waters, if it is assumed to be labile in nature and therefore rapidly respired by active heterotrophs within the wetland (although see later). However, previously energy limited heterotrophs may be stimulated to produce increased amounts of extracellular enzymes by the increased labile exudated substrates, allowing older, more recalcitrant C to be cleaved from the peat matrix, thereby 'priming' the system and leading to intensified C loss as DOC and CO_2. Some lignin degrading microorganisms (e.g., fungi) are only able to do so in the presence of a readily degradable substrate as the primary energy source (Paul & Clark, 1989). The increased availability of labile DOC may also provide a substrate for trace gas production (cf. van Veen *et al.*, 1989), reinforcing this C loss to the atmosphere and providing positive feedback to climate change. Even relatively deep peat, below the active surface layer (ca. 10 cm), may be primed, increasing its decomposition. This process has the potential to reduce the current C storage in peatlands, accelerating the DOC exodus (Freeman *et al.*, 2001) and reducing water quality.

Furthermore, longer growing seasons (e.g., Gian-Reto *et al.*, 2002) could potentially further increase exudation, amplifying this effect.

The organic C supply in small streams, especially in upland and wetland areas, is typically dominated by allochthonous, terrestrial DOC (Hope *et al.*, 1994; Hope *et al.*, 1997; Wetzel, 1992). Evidence from a combination of stable isotope and ^{14}C dating studies has been used to identify the main sources and processes controlling streamwater DOC and total inorganic carbon in a Scottish, temperate, non-forested watershed (Palmer *et al.*, 2001). Dissolved organic carbon $\delta^{13}C‰$ values for soil solution and stream water were consistent with those of the surrounding soil and terrestrial vegetation, indicating that the terrestrial ecosystem is the dominant source of aquatic DOC. Increases in the proportion of recently synthesized C as a result of eCO_2/eTemp may thus be quantitatively significant in a future climate. And this would be particularly true if decomposition of such material was impaired as a result of competition between the vegetation and microorganisms for inorganic nutrients (Freeman *et al.*, 1998), as could be inferred from the results of chapter 2. There is evidence from ^{14}C dating that although peat had been accumulating in the Scottish watershed for at least 2700 yrs, C of relatively recent origin (post-AD 1955) was predominant in the soil pore water and streamwater DOC (Palmer *et al.*, 2001). The results from our experiment confirm that in the wetland, recently formed C could be a major source of C for stream water (even under ambient CO_2 conditions in October; 0.3% contribution from 5h pulse labelling). Furthermore, preliminary work carried out under ambient conditions using only *Sphagnum* in June showed a greater contribution of pulse derived DOC in the leachate water (4.0% of the DOC), probably as a result of the increased light intensity, longer day length and warmer summer conditions allowing increased C release *via* exudation (see later).

As discussed in chapter 2, changes in plant species composition may provide a clue as to the mechanism(s) responsible for this increase in recently fixed C in the leachate water of eCO_2/eTemp cores. Elevating atmospheric CO_2 concentrations and temperatures in the current experiment apparently produced a shift from a *Sphagnum* and *Festuca* co-dominated community to one where other monocotyledons and vascular species became dominant (especially *J. effusus*) (chapter 2, figure 2.02). Monocotyledons, as a result of their growing strategy/architecture, are likely to be CO_2 limited because they are usually surrounded by relatively low CO_2 concentrations. *Sphagnum* on the other hand, would be subject to much higher CO_2 concentrations in the environment due to the influence of soil respiration from the peat surface (CO_2 concentrations are naturally 10-50 times higher in soils than in the atmosphere (Lamborg *et al.*, 1983)).

As expected, there was no significant difference in the incorporation of ^{13}C per gram of plant C between *Sphagnum* tissue from the eTemp and eCO_2/eTemp cores (figure 4.13a) because incorporation of ^{13}C is facilitated by photosynthesis, which in turn depends on the number of chloroplasts and the light intensity per unit area, factors likely to be similar within a given species. And, the same was true for the other species studied (*J. effusus*, *F. ovina* and *P. commune*, figures 4.13b-d respectively). However, analysis of the different species present showed differential processing of ^{13}C derived from photosynthesis.

Despite less initial incorporation of ^{13}C in comparison to the other wetland species studied ($F = 5.62$, $P<0.01$), ^{13}C concentrations remained remarkably constant in the *Sphagnum* tissue over the sampling period. In marked contrast, the other species studied (*J. effusus*, *F. ovina* and *P. commune*) showed a similar and strong decline in enrichment over the same period (overall $F = 28.17$, $P<0.001$), indicating rapid C processing (significantly different to that of *Sphagnum*: $F = 4.94$, $P<0.05$). This is consistent with the perceived role of *Sphagnum* in C sequestration and the following mechanisms are proposed:

1. Retention of ^{13}C within the structural components and/or,
2. Refixation (or 'reflux') of $^{13}CO_2$ from root and/or soil heterotrophic respiration.

Sphagnum may receive more recycled $^{13}CO_2$ than the other species of the peatland community due to its closer proximity to the peat surface. This recycling of plant and soil derived CO_2 is also thought to play an important role in short term C cycling in dense vegetation such as grassland (Furness & Grime, 1982). Refixation, i.e., the photosynthetic fixation of soil produced or respired CO_2, has largely been ignored in research on peatland C cycling. Turestsky and Wieder (1999) are among the few who have quantified it. They transplanted groups of nonradioactive *S. fuscum* into plots that had been radioactively labelled by exposure to $^{14}CO_2$. *Sphagnum* transplanted into plots that received both nitrogen and sulphur was found to have greater refixed activities (Bq g^{-1}) than control transplants. When extrapolated on a m^2 basis, transplanted *Sphagnum* was found to refix 3.5-5.4% of the total ^{14}C initially incorporated into the living vegetation and top 10 cm of peat during labelling. Thus, refixation may be an important pathway for C cycling within peatlands, potentially capturing significant proportions of peat-produced or respired CO_2 before it escapes to the atmosphere.

The initial incorporation of significantly more ^{13}C into the tissues of *J. effusus* and *F. ovina*, and much faster release than *Sphagnum* is consistent with independent preliminary work on *F. ovina* (Ostle pers comm.) and published studies (Ostle *et al.*, 2000). From the literature, respiration is presumed to be the major route through which ^{13}C is lost from the plant, but exudation could also be important. Given the differential processing of C between the species

studied and the depth at which evidence for ^{13}C enrichment could be found (20 cm), it is postulated that increased exudation of recently synthesized material under eCO_2/eTemp was a response of the monocotyledons (vascular plant species) rather than the *Sphagnum*. The former have a greater potential for exudation due to their extensive root system in comparison to non-vascular *Sphagnum* rhizoids. *Juncus effusus* roots were observed to be particularly thick, deep and extensive. Indeed, root biomass was significantly and dramatically increased in the eCO_2/eTemp cores as was total plant biomass (see chapter 2, figure 2.03). Such changes mean deeper roots, occupying a greater area, that would allow increased exudation from species that initially assimilate more ^{13}C per unit plant tissue C than *Sphagnum*, but which then lose this more rapidly through exudation and respiration. Positive feedback to community change as a result of increased root oxygen release, evapotranspiration and aeration is probable, accelerating soil drying due to enhanced warming. The potential for C accumulation in peatlands is therefore likely to decline as the *Sphagnum* is out-competed. Increased drying is also widely believed to stimulate decomposition and hence CO_2 flux from peatlands (e.g. Aerts & Ludwig, 1997; Moore & Knowles, 1989; Scanlon & Moore, 2000), underlining the potential for positive feedback to a changing climate.

When the results from the pilot study (chapter 4A) and the follow up experiment were compared, the initial ^{13}C incorporation into the capitulum tissue under ambient CO_2 concentrations was very similar (4.25 ± 0.59 and 5.86 ± 0.61 mg g plant C^{-1} respectively). Release of incorporated ^{13}C was more rapid (through respiration and exudation) in the pilot study and this was probably because sampling occurred in June whereas the latter experiment was carried out in October. Increased light intensity, day length and warmer temperatures etc. are likely to have caused the more rapid response in the former study, as mentioned previously. Such seasonally different growth rates and C allocation is perhaps to be expected and probably influences the biogeochemistry of the peat profoundly (e.g., see chapter 3), which may be an important consideration when modelling such systems.

4.16 CONCLUSIONS

There are likely to be both local and global implications of climate change on peatland C processing. A significant increase in the proportion of recently synthesized C under eCO_2/eTemp was observed which will potentially increase peatland DOC exports directly but also alter the quality of the leachate DOC pool, since older material is more refractory (Raymond & Bauer, 2001). Previously energy limited heterotrophs (even in relatively deep peat) may be stimulated by an increase in labile exudates, allowing older, more recalcitrant C to be degraded, thereby 'priming' the system and leading to intensified C loss to the aquatic

system. Such material may provide increased substrates for trace gas production (c.f. van Veen *et al.*, 1989) providing positive feedback to climate change. Increased biodegradation could reduce the C storage capacity of peatlands and thus accelerate the increasing concentrations of CO_2 in the atmosphere. Moreover, the reduction in *Sphagnum* cover is likely to exacerbate this and the invasion of vascular species promote aerobic degradation of the peatland, with positive feedback to altered plant and microbial community structure. Increased drying stimulates CO_2 flux from peatlands (e.g., Aerts & Ludwig 1997; Moore & Knowles 1989; Scanlon & Moore, 2000), underlining the potential for positive feedback to a changing climate. The fate of recently assimilated DOC within wetlands and the implications for C processing in the recipient aquatic ecosystems demands further research.

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CHAPTER 5: THE EFFECTS OF SUMMER DROUGHT ON DOC MOBILIZATION

5.01 INTRODUCTION

Water is the single most important regulator of wetland biogeochemistry (Ponnamperuma, 1972), thus biogeochemical cycling will probably be profoundly affected should there be any changes in water availability. Gorham (1991) has estimated that the cool, wet, climate of northern latitudes supports 346 million hectares of peat accumulating wetland. However, as a result of climate change the rainfall patterns that have allowed such development may change. Recent global circulation models predict an increased frequency of drought (e.g., IPCC, 2001) and the most recent UK Climate Impacts Programme report suggests that summers across the UK have already become drier, and will continue to do so with a decrease in precipitation of up to 50% by the 2080s under the high emissions scenario (Hulme *et al.*, 2002). In the past, attention has been focused on the effects of drought due to the perceived dependence of wetlands on waterlogging for their persistence and stability (Freeman *et al.*, 1998). Examples include studies on vegetation (Laine *et al.*, 1995), trace gas emissions (Freeman *et al.*, 1993a; Moore & Knowles, 1989; Moore & Dalva, 1993), hydrochemistry (Freeman *et al.*, 1993b, c; VanHaesbroeck *et al.*, 1997) and enzymic decomposition processes (Freeman *et al.*, 1996).

Over the last 12-15 years stream chemistry data has been collected from upland sites draining moorland and forest catchments in north and mid Wales, including the study site for this investigation. A consistent and statistically significant trend of increasing concentrations of dissolved organic carbon (DOC) (Freeman *et al.*, 2001a; Reynolds *et al.*, 1997) has been reported at a time when the frequency of warm dry summers has been relatively high (Robson & Neal, 1996). Recently the increasing DOC trend has been detected in 20 out of 22 UK Acid Waters Monitoring Network sites, and this was attributed to rising temperatures (Freeman *et al.*, 2001a). However, specific climatic events, such as drought, have been associated with increases in colour and DOC (e.g., Hughes *et al.*, 1998; Worrall *et al.*, in press) therefore, drought events may have serious implications for the treatment of upland waters. Wetlands, both natural and constructed, are important nutrient sinks and have the potential to be used in the filtration of a variety of pollutants (e.g., nitrate runoff from agricultural land). The efficiency of this sink can be reduced by a drop in water level due to the aeration of the soil. Moreover, the peat matrix may release its

nutrients as a consequence of increased decomposition (e.g., Freeman *et al.*, 1993c; Heathwaite, 1990).

Research into the effects of drought on wetlands has already been undertaken at the study site for this investigation and the Centre for Ecology and Hydrology (CEH) final report for the year 2000 summarizes all the findings at the site since 1992 (Hughes *et al.*, 2000). This involved subjecting the wetland to 3 successive years of simulated summer drought (1992-1994) and monitoring its recovery until 1998 (Hughes *et al.*, 1998), with a final simulated drought in 1999 which formed part of this study. The natural summer maxima of DOC were found to be significantly reduced as a result of drought, whilst the natural autumn/winter sulphate peaks in the peat water were increased. In view of their findings, the authors suggest that droughts may reduce the negative impacts of peatland derived DOC on water quality in the short term. In the long term however, it is proposed that an increased frequency of summer droughts may initiate a trend for rising DOC exports because this was identified in the pore waters of the experimental wetland (Hughes *et al.*, 1998). While this supported the trends identified in stream water from the site, the results were described as 'unexpected', since drier conditions are thought to promote aerobic decomposition which favours CO_2 as an end product of metabolism (Freeman *et al.*, 1993c; Ponnamperuma, 1972). Waterlogged anaerobic conditions on the other hand, tend to favour a greater proportion of DOC end products such as aldehydes and ketones (Ponnamperuma, 1972).

The primary aim of the current investigation was to examine the extracellular enzymic response of the wetland during a simulated summer drought over a period of a year, because a suggested cause of the long term DOC response is an increase in the potential for mobilization of the peat matrix due to enzymic hydrolysis under such conditions (Freeman *et al.*, 1996). The activities of the carbon cycling enzymes phenol oxidase and ß-glucosidase were therefore measured along with sulphatase and phosphatase activities.

Secondly, it was hypothesized that there would be increased decomposition of relatively labile DOC under more aerobic conditions leaving the more recalcitrant material. Thus, a gradual shift under drought conditions towards higher concentrations of phenolic compounds would be anticipated, these tending to be relatively refractory and inhibitory to the metabolism of DOC in recipient waters (Appel, 1993; Freeman *et al.*, 1990; Wetzel,

1992). This would mean an increase in the amount of persistent DOC in the system (that is more likely to arrive at a water treatment plant rather than being decomposed/consumed *via* in-stream processes), and an increased potential for an accumulation of readily utilizable material. The latter due to the inhibitory action of phenolic substances on the activity of hydrolase enzymes (Freeman *et al.*, 2001b).

Trace gases were monitored with the aim of detecting feedbacks to climate change as a result of simulated drought.

5.02 MATERIALS AND METHODS

Site description and hydrological manipulation

The study site, Cerrig-yr-Wyn in the Upper Wye catchment on Plynlimon (UK NGR SN 820 866), is a small gully typical of many in the uplands of Wales (Hughes *et al.*, 1996) where flush wetlands have formed as discontinuous serial cascade systems. *Sphagnum* and *Juncus* communities characterize the gully mire, which has peat with a pH range of 4-4.8. Freeman *et al.* (1993b) describe the original flow manipulation system, hydrology and geology of the site. For the current project, the experimental wetland was subjected to a simulated drought from the first week in June 1999 to the end of September 1999 and this provided the final experimental period for the CEH long term monitoring work. Subsequent rewetting was carried out using pipes to distribute surface recharge (stream flow) across the head of the wetland. A further simulated drought was imposed from mid May 2000 to allow enzyme activities and phenolic compound concentrations to be studied, and this ended with a natural flood toward the end of September 2000. The areas of the control and experimental wetlands were, 348m^2 and 336m^2 respectively (Hughes *et al.*, 1997).

Sampling procedure

Temperature and pH were measured in the field using standard electrometric techniques. Water table levels were measured using two variants of the dip-well technique 1) a standard 5 cm radius unlined dip-well (Ingram, 1983) located in the centre of the transect through each wetland, and 2) a wide, shallow (60 cm depth, 15 cm diameter) well, also sited near the centre of each wetland (Hughes *et al.*, 1997).

Initially, samples of peat water were extracted prior to the experimental drought in 1999 to determine whether the two wetlands (control and experimental) were behaving similarly. This was done, usually at monthly intervals, using soil solution samplers (Freeman *et al.*, 1993b). Samplers were placed at a depth of 10 cm in the peat profile, 2.5 m apart, along a transect of the two wetlands (each wetland having 5 replicate samplers installed). The extraction and dead volumes were kept to a minimum to ensure that excessive disturbance of the wetland was avoided and that the integrity of the sample (in particular redox potential) was unaffected. Sampling from both the experimental and control wetland continued throughout the period of simulated summer drought and recovery. Five replicate peat samples from the surface layer (0-10 cm depth) were also taken (one from each sample point in the transect) and transported back to the laboratory in airtight containers for enzyme analysis.

Measurements of CO_2, CH_4 and N_2O fluxes from the wetlands were made at monthly intervals at least, using a closed chamber technique. Five 4.5 L capacity, opaque, polyethylene wide neck bottles, which had been sawn off at the base, were placed along a transect (at 2.5 m spacing) in each wetland. A small hole was drilled in the lid of each bottle and a short piece of Tygon® Auto analyzer transmission tubing was inserted into the hole to give an airtight fit. Gas samples were collected (within 30 minutes of midday) following a 2 hour accumulation period, using 10 cm^3 capacity glass Pressure-Lok® gas sample syringes. Each chamber was left open between sampling dates to ensure that growth of wetland vegetation within them was unimpeded.

Sample preparation

On returning to the laboratory, samples were filtered using a 0.2 µm diameter membrane syringe filter (Whatman, Kent, UK) and maintained at 4°C for reasons already mentioned (chapter 2).

Sample analysis

Concentrations of DOC were determined using a Shimadzu 5000 Total Organic Carbon Analyzer, phenolic compound concentrations using Folin-Ciocalteau reagent (Box, 1983), enzyme activities using fluorogenic substrates, and trace gas concentrations using an Ai Cambridge model 92 gas chromatograph (GC), as described in chapter 2.

5.03 STATISTICAL CONSIDERATIONS

All statistical analyses were performed using Minitab version 13.32 (Minitab Inc.). Data were tested for normality using the Kolmogorov-Smirnov test. Means for each of the five sampling points within the control and experimental wetlands were calculated for distinct time periods to enable comparisons with long term CEH data. The control and experimental wetlands were compared using a two-sample unpaired t-test. No assumption of equal variances was made. Although a paired t-test may have been appropriate before the successive treatments began at the site, an unpaired test was favoured because there is some evidence to suggest that differences in behaviour between the two wetlands have occurred over time (perhaps as a result of successive droughts). Where there was a significant difference between the control and experimental wetlands prior to the drought simulation, analysis of covariance (ANCOVA) was used to determine whether the experimental drought induced differences in response having taken into account the pre drought levels.

Prior to looking for an underlying trend in DOC concentrations, it was necessary to eliminate seasonal effects that were clearly visible (figure 5.01a & b) and to overcome the problems of data being recorded at varying frequencies throughout the study period (9 years). The fact that data was available for only part of the year 2000 (due to flooding and foot and mouth restrictions) also had to be accounted for to avoid bias in the trend. Individual determinations of DOC were coded by both their year of measurement and to 10 equal divisions within the year. An analysis of variance (ANOVA) with the year and division as factors was conducted to produce least square means for each year. These adjusted means were then subject to regression to determine if there were trends over time.

5.04 RESULTS

During 1999 and 2000, the simulated droughts decreased the water table by 5-10 cm compared to the control. Temperature was increased by 3.77% $P<0.001$ and 3.92% $P<0.001$ respectively in the experimental wetland under these simulation conditions, while pH remained similar. A difference in temperature (4.84%, $P<0.01$) remained in the autumn-spring period of 1999/2000 with pH values being the same for both wetlands.

Table 5.01. Mean solute concentrations and enzyme activities for distinct time periods with significance of the t-test for the control and experimentally droughted wetlands

Determinand	1999 simulated summer drought			autumn-spring 1999/2000			2000 simulated summer drought		
	C	E	Δ%	C	E	Δ%	C	E	Δ%
DOC (mg L^{-1})	8.3	5.4	-34.94	2.64 (0.23)	2.76 (0.26)	4.55	12.16 (2.62)	7.22 (0.36)	-40.63
Phenolic compounds (mg L^{-1})	1.06 (0.43)	0.60 (0.20)	-43.18	0.37 (0.02)	0.39 (0.04)	5.41	1.72 (0.37)	0.90 (0.06)	-47.67
Phenol oxidase (nmol dicq g^{-1} min^{-1})				1.48 (0.57)	1.47 (0.34)	-0.68	0.78 (0.23)	1.43 (0.35)	83.33
ß-glucosidse (nmol MUF mg^{-1} h^{-1})				0.39 (0.06)	0.57 (0.12)	46.15	0.33 (0.06)	0.85 (0.10)	157.58 **
Sulphatase (nmol MUF mg^{-1} h^{-1})				0.17 (0.05)	0.23 (0.04)	35.29	0.19 (0.07)	0.39 (0.07)	105.26
Phosphatase (nmol MUF mg^{-1} h^{-1})				3.10 (0.04)	4.72 (0.30)	52.26 *	2.81 (0.55)	5.2 (0.57)	85.05 *

C and E denote values from control and experimental wetlands respectively, 1999 summer drought DOC values provided by S. Hughes (CEH Bangor). Numbers in parentheses indicate standard error of the mean. Percentage changes relative to the control are presented (Δ%) and P values where * denotes significance at the $P<0.05$ level, ** at the $P<0.01$ level and *** at the $P<0.001$ level. These conventions will apply throughout unless stated otherwise.

Generally, DOC concentrations in both the control and experimental wetland showed a seasonal response, being higher in the summer months (figure 5.01a). During 1999, the experimental wetland showed a non significant (ns) 34.94% decrease in DOC concentration under simulated summer drought conditions in relation to the control. The control and experimental wetlands behaved similarly in the autumn-spring of 1999/2000 with means of 2.64 and 2.76 mg L^{-1} (ns) respectively. DOC concentrations during the drought simulation in 2000 decreased by 40.63% (ns) compared to the control, consistent with the 1999 manipulation. Phenolic compound concentrations (figure 5.01b) were also higher in the summer months, correlating strongly and positively with those of DOC in the control (Pearson product moment 0.9745, $P<0.001$) and experimental (0.8475 $P<0.01$) wetlands. Mean phenolics concentrations were reduced by 43.18% (ns) during the 1999 drought simulation. Prior to the 2000 treatment, phenolics concentrations were not significantly different (means of 0.37 and 0.39 mg L^{-1} in the control and experimental site respectively) and with the onset of simulated drought phenolics concentrations in the experimental wetland decreased by 47.67% (ns), consistent with the previous simulation.

Phenol oxidase activities are shown in figure 5.02a and were similar in the control and experimental wetlands prior to the 2000 treatment (1.48 and 1.47 nmol $g^{-1}min^{-1}$ respectively, ns). The latter site showed a substantial but non significant increase of 83.33% under treatment conditions relative to the control. Similarly, ß-glucosidase activities (figure 5.02b) in the pre-treatment period were similar in the control and experimental sites (means of 0.39 and 0.57 nmol $mg^{-1}h^{-1}$ respectively (ns), while under treatment conditions the experimental wetland exhibited a substantial increase of 157.58% ($P<0.01$). Sulphatase activities (figure 5.02c) were also similar before treatment in the control and experimental sites (means of 0.17 and 0.23 nmol $g^{-1}h^{-1}$ respectively (ns)) and the treatment period showed a 105.26% increase ($P<0.1$, considered not quite significant) with respect to the control. During the treatment period the experimental site showed an 85.05% increase ($P<0.05$) in phosphatase activities in relation to the control (figure 5.02d). However, pre-treatment activities were also found to be higher (52.26%, $P<0.05$) than the control. Analysis of covariance revealed that the difference in activities under drought conditions could be accounted for by the pre-treatment levels.

Generally, due to large natural variation (especially in the experimental wetland), differences between trace gas fluxes in the two sites were not significantly different, thus peak emissions for a given year are presented below.

Table 5.02a. Peak trace gas emissions in the control and experimentally droughted wetlands during 1999 (n=5)

Trace gas	C (mg $m^{-2}d^{-1}$)	Date	E (mg $m^{-2}d^{-1}$)	Date
CO_2	1402.89 (615.71)	13/09/01	11506.30 (9791.99)	27/09/99
CH_4	35.77 (14.84)	25/10/99	62.62 (25.19)	11/10/99
N_2O	0.59 (0.19)	13/09/99	0.36 (0.19)	27/09/99

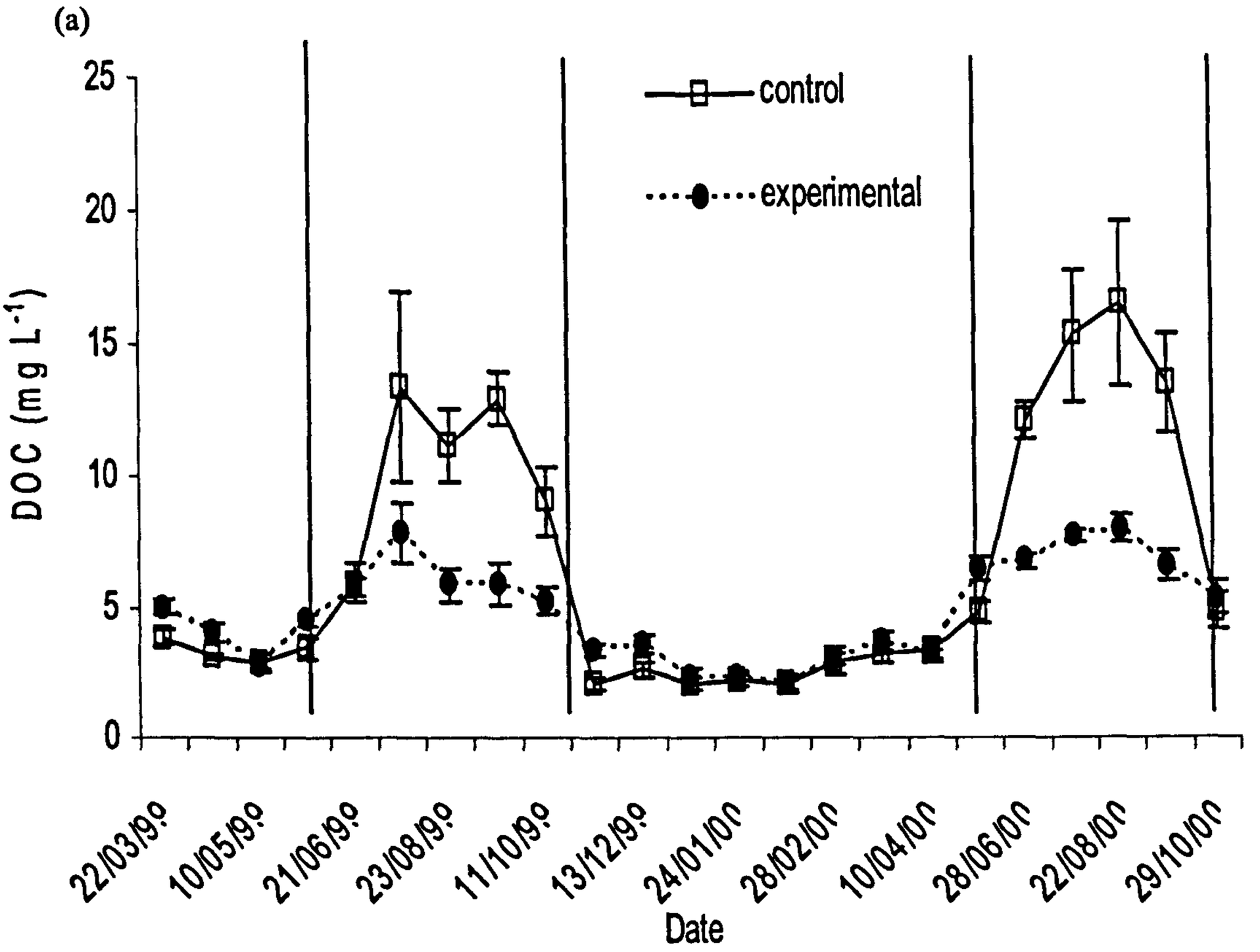
Table 5.02b. Peak trace gas emissions in the control and experimentally droughted wetlands during 2000 (n=5)

Trace gas	C (mg $m^{-2}d^{-1}$)	Date	E (mg $m^{-2}d^{-1}$)	Date
CO_2	1213.51 (373.77)	25/09/00	8321.78 (7538.58)	25/09/00
CH_4	141.31 (1.93)	25/09/00	154.07 (6.769)	25/09/00
N_2O	0.55 (0.14)	25/07/00	0.2 (0.11)	22/08/00

Carbon dioxide fluxes (figure 5.03a) measured for the current study (1999-2000) generally showed seasonal late summer maxima in the control and experimental wetlands. Peak CO_2 emissions in the control site were 1402.89 ± 615.71 and 1213.51 ± 373.77 mg $m^{-2}d^{-1}$ (on 13/09/99 and 25/09/00 respectively) and in the experimental site reached much higher levels (11506.30 ± 9791.99 and 8321.78 ± 7538.58 mg $m^{-2}d^{-1}$, on 27/09/99 and 25/09/00 respectively). Long term cumulative fluxes are presented in figure 5.03b, which shows both wetlands acting as a source of CO_2 with increased emissions from the experimental wetland.

Methane emissions generally showed seasonal increases with maxima in late summer/autumn (figure 5.04). The final drought manipulations in the summer of 1999 and 2000 did not significantly affect CH_4 emissions relative to the control (peak emissions being 62.62 ± 25.19 (11/10/99) and 154.07 ± 6.77 mg $m^{-2}d^{-1}$ (25/09/00) for the experimental site, while in the control wetland emissions reached 35.77 ± 14.84 (25/10/99) and 141.31 ± 1.93 mg $m^{-2}d^{-1}$ (25/09/00) respectively).

Nitrous oxide fluxes (figure 5.05) from the control and experimental wetlands also showed seasonal summer maxima. The control site exhibited maxima of 0.59 ± 0.19 (13/09/99) and 0.55 ± 0.14 mg $m^{-2}d^{-1}$ (25/07/00), while the experimental wetland showed reduced maxima of 0.36 ± 0.19 (27/09/99) and 0.2 ± 0.11 mg $m^{-2}d^{-1}$ (22/08/00).



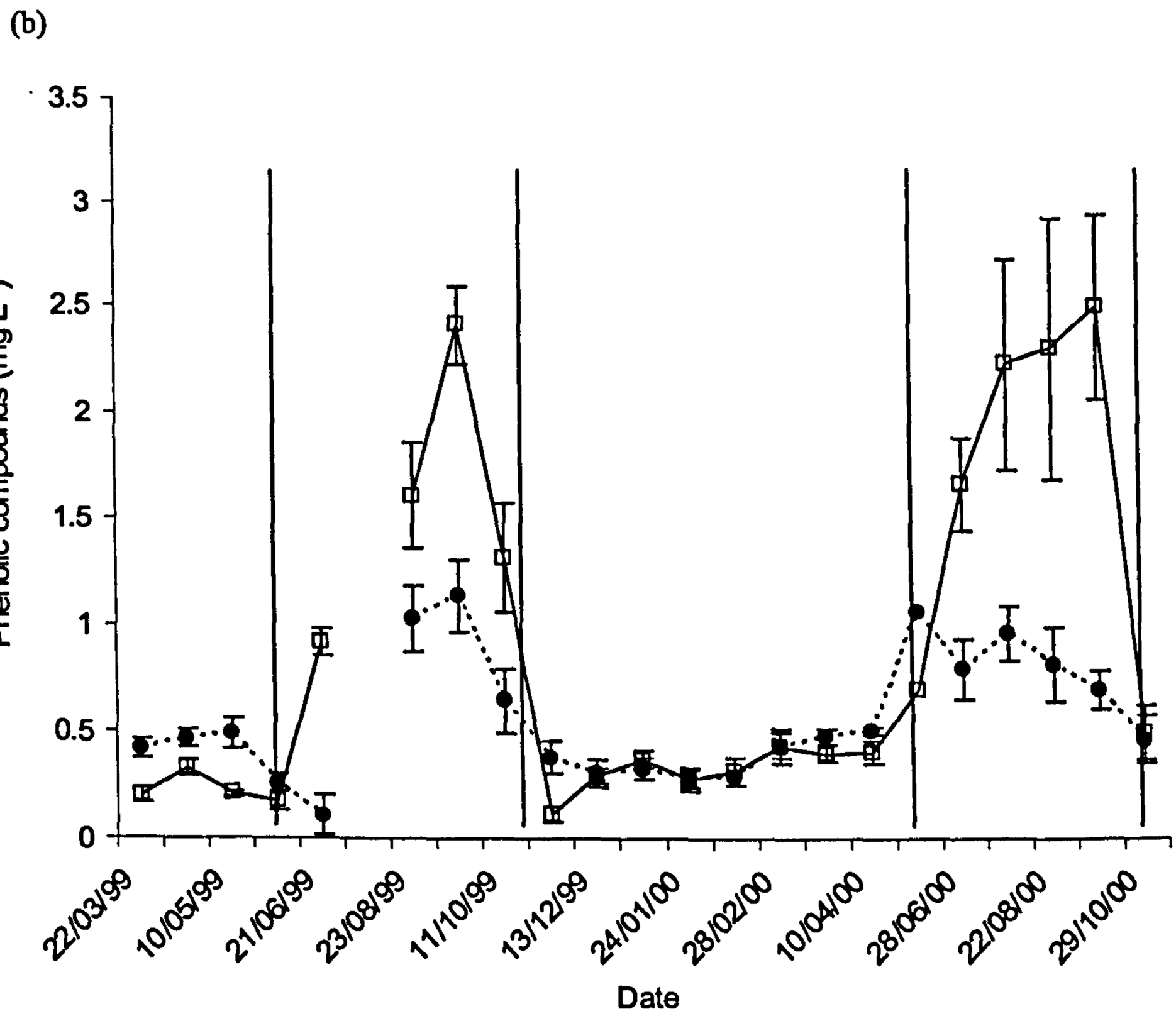
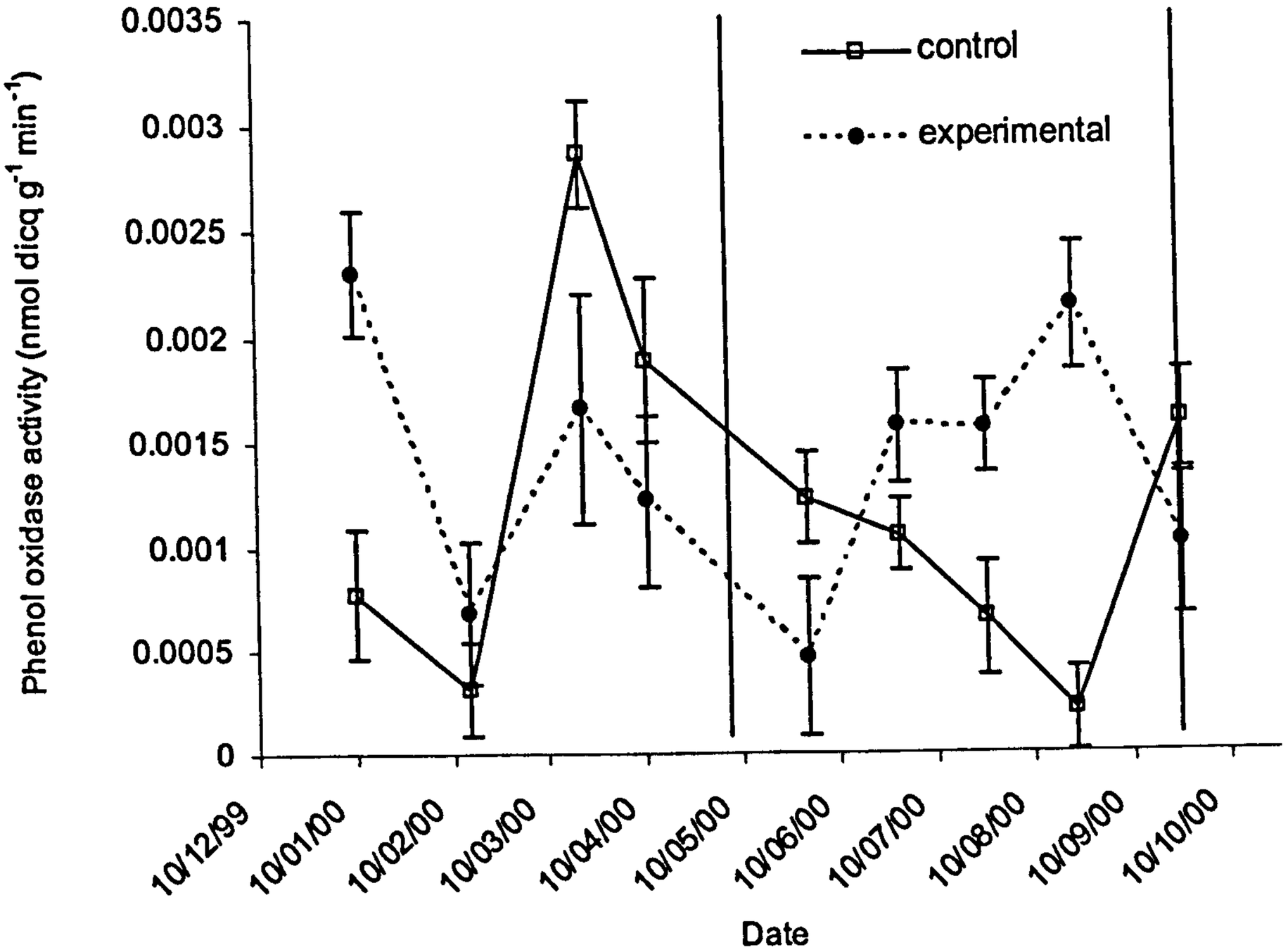


Figure 5.01. Leachate (a) DOC, & (b) phenolic compound concentrations in control & experimentally droughted wetlands. Vertical lines denote summer drought periods. Error bars represent standard error of the mean, n=5.

(a)



(b)

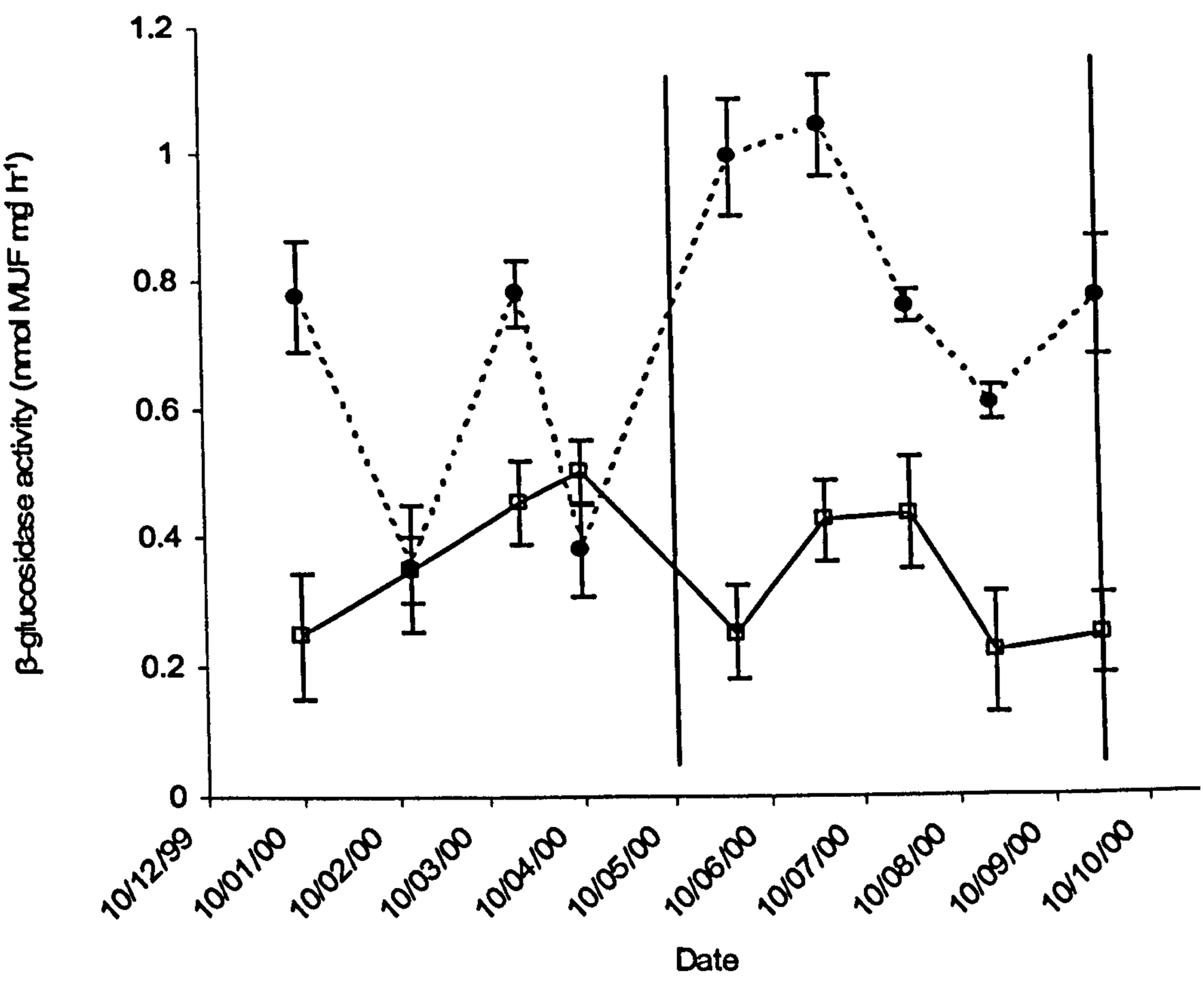
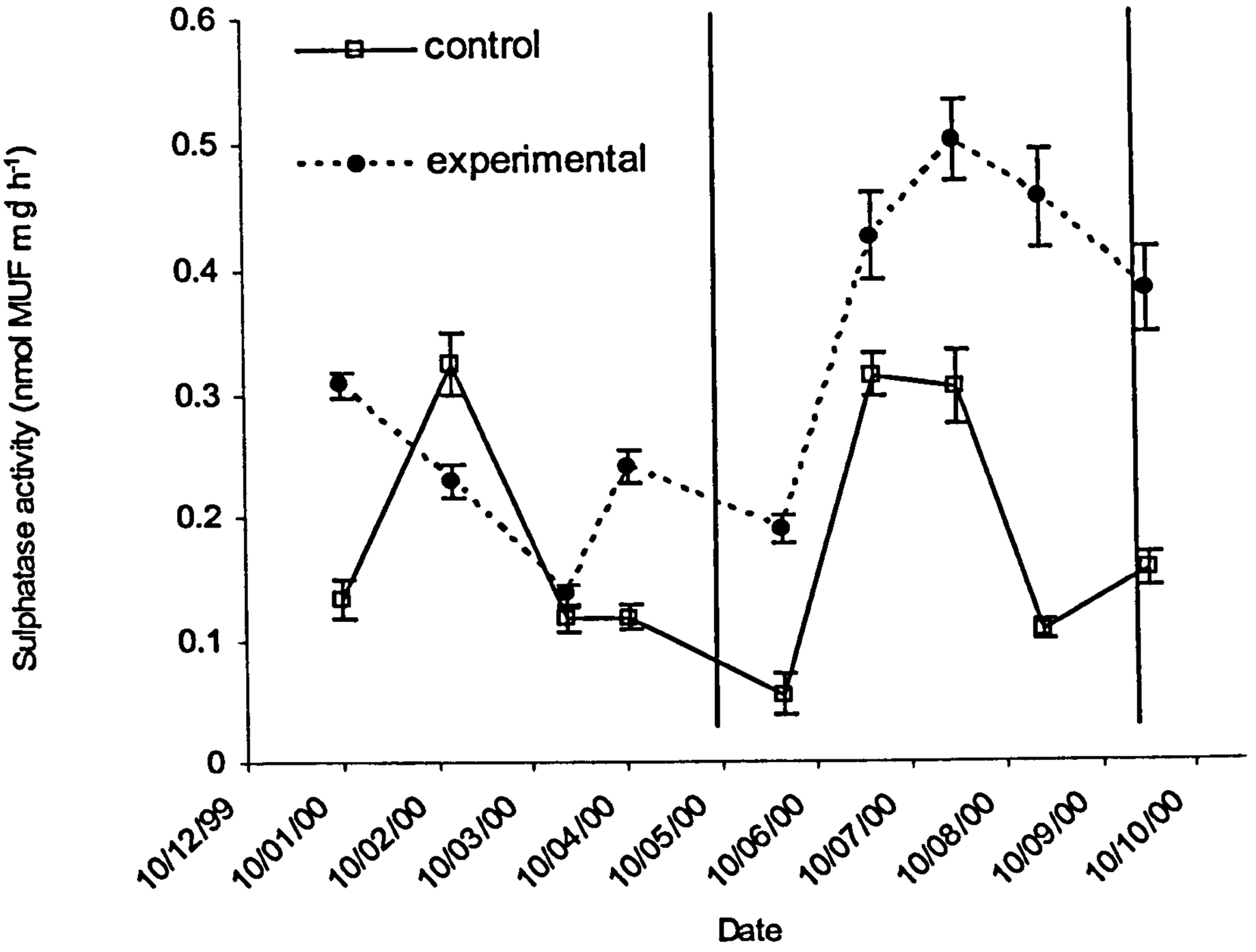


Figure 5.02. Peat (a) phenol oxidase, & (b) β-glucosidase activities in control & experimentally droughted wetlands. Vertical lines denote the summer drought period. Error bars represent standard error of the mean, n=5.

(c)



(d)

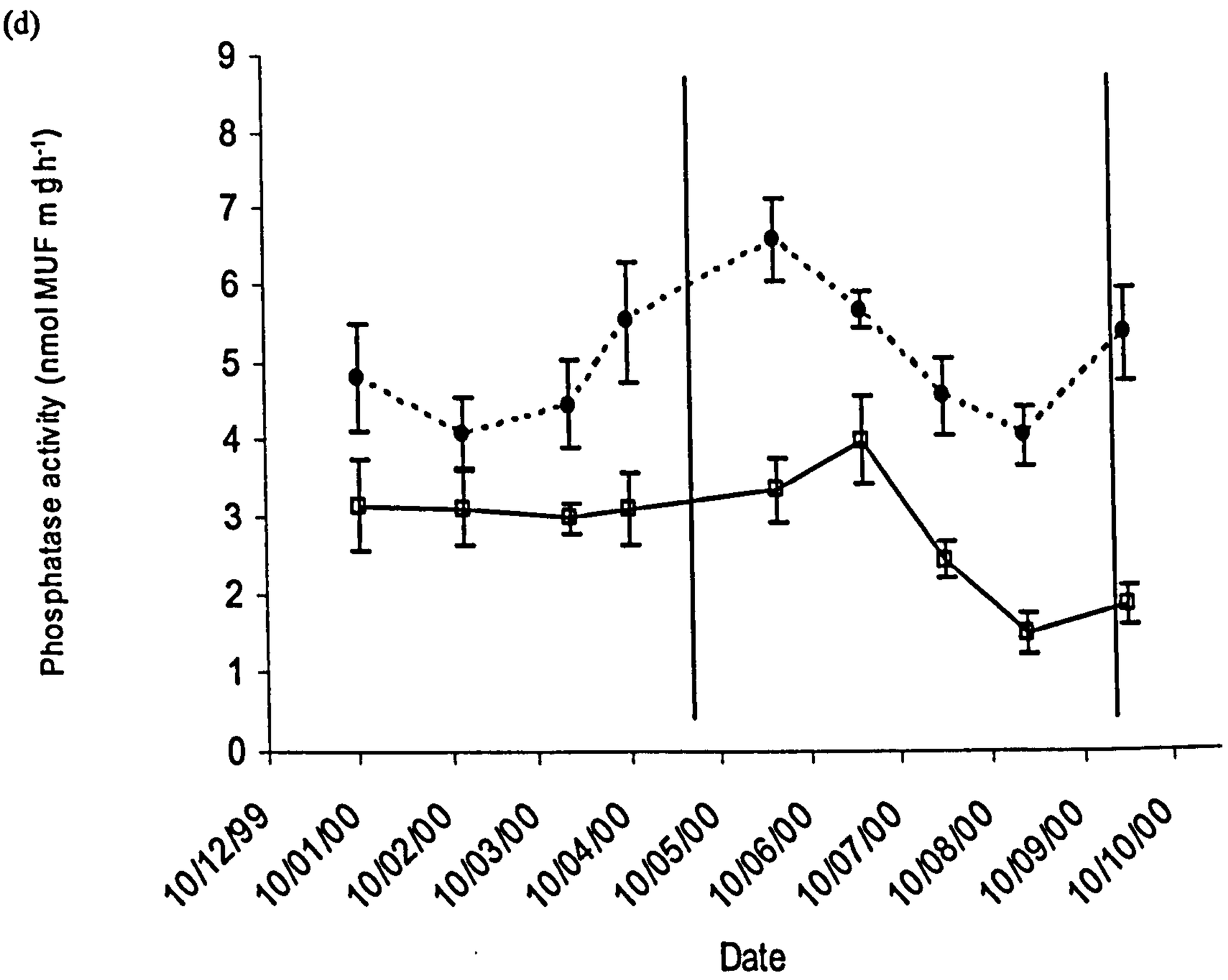
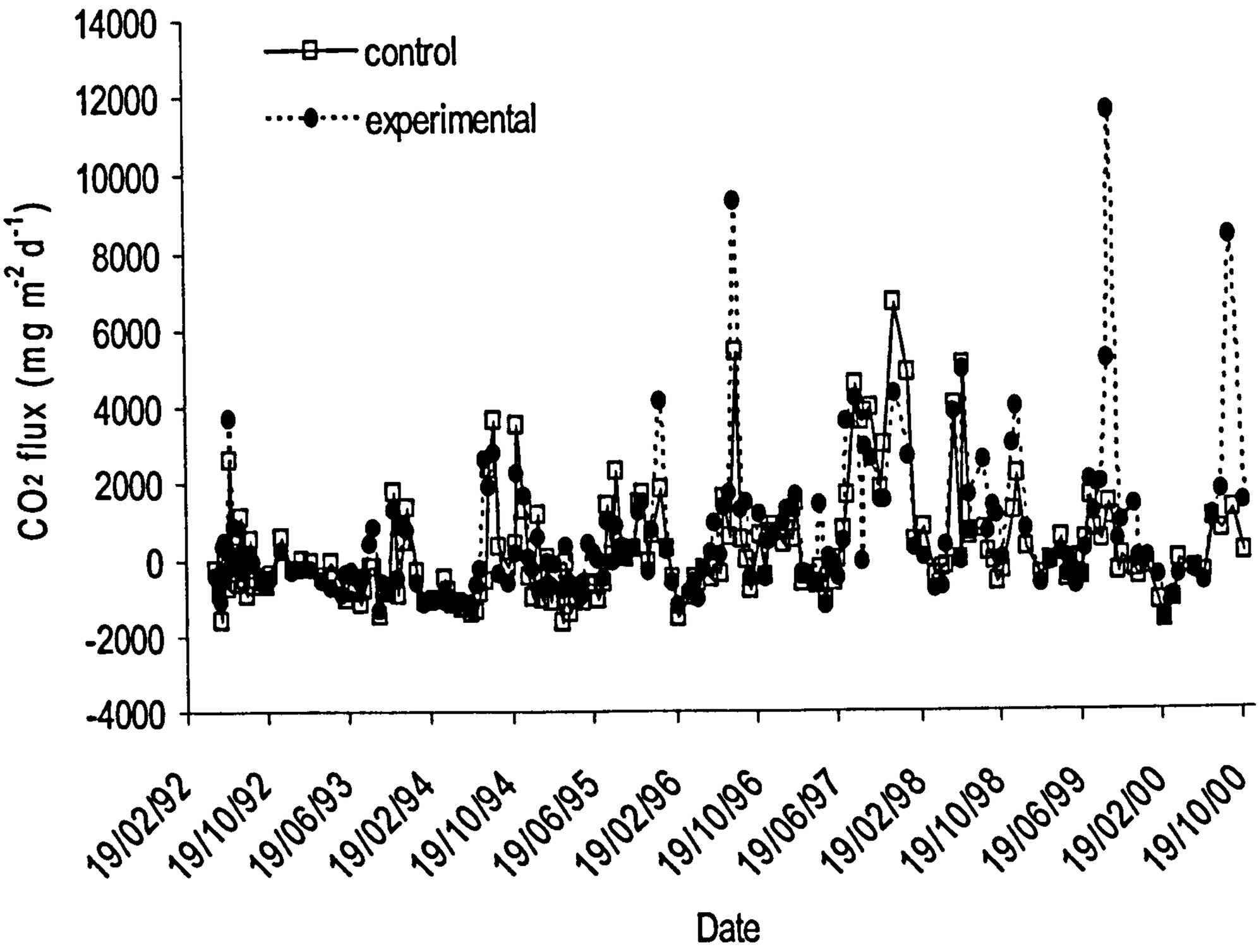


Figure 5.02. Peat (c) sulphatase, & (d) phosphatase activities in control & experimentally droughted wetlands. Vertical lines denote the summer drought period. Error bars represent standard error of the mean, n=5.

(a)



(b)

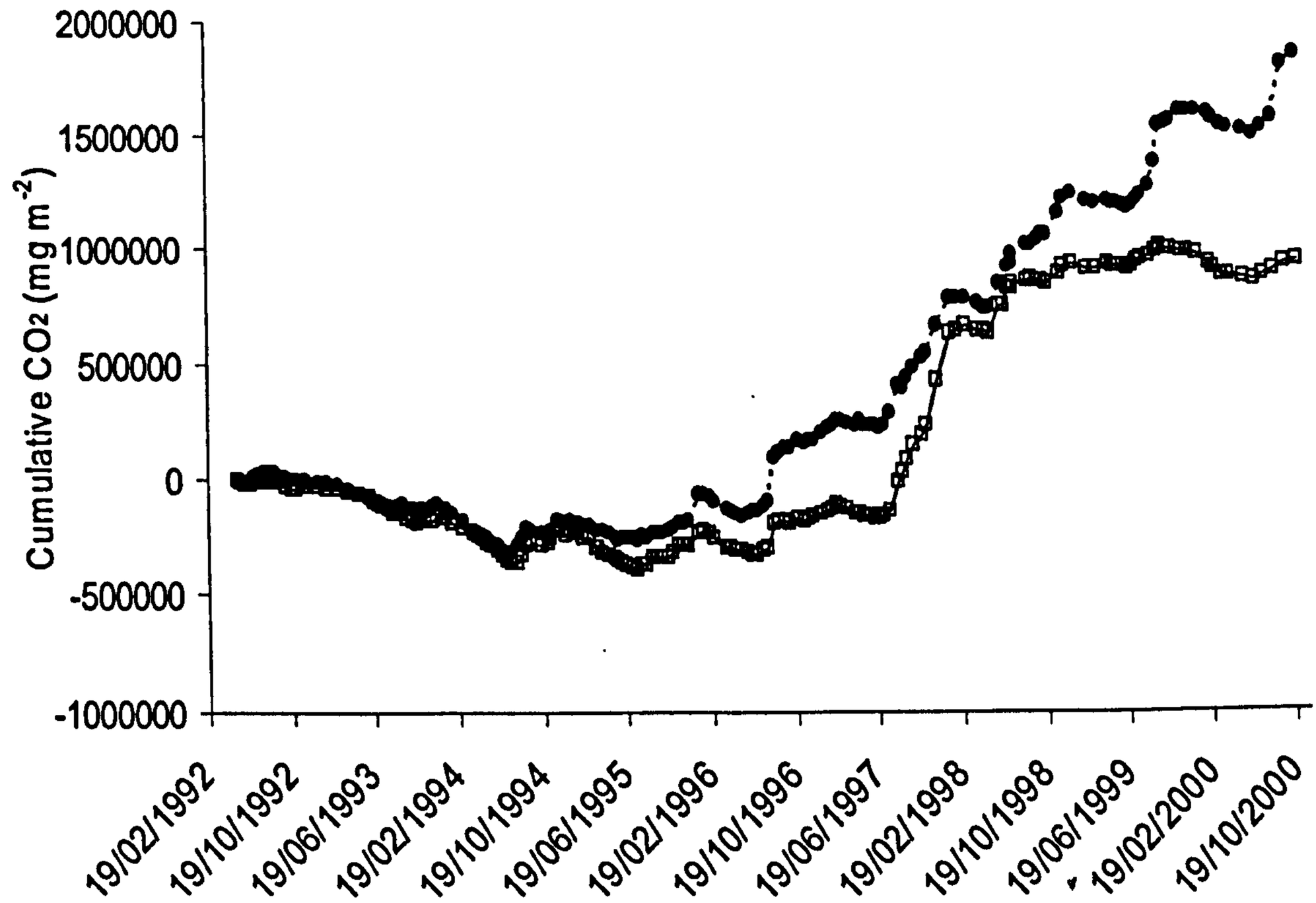


Figure 5.03 (a) CO_2 flux, & (b) cumulative CO_2 in control & experimentally droughted wetlands. Experimental droughts were induced from the end of May to the end of September except for a recovery period (1997 & 1998) & a natural summer drought (1995). Standard error of the mean omitted for clarity (on average control ± 316.31 & experimental ± 456.885mg m^{-2} d^{-1}), n=5.

Figure 5.04

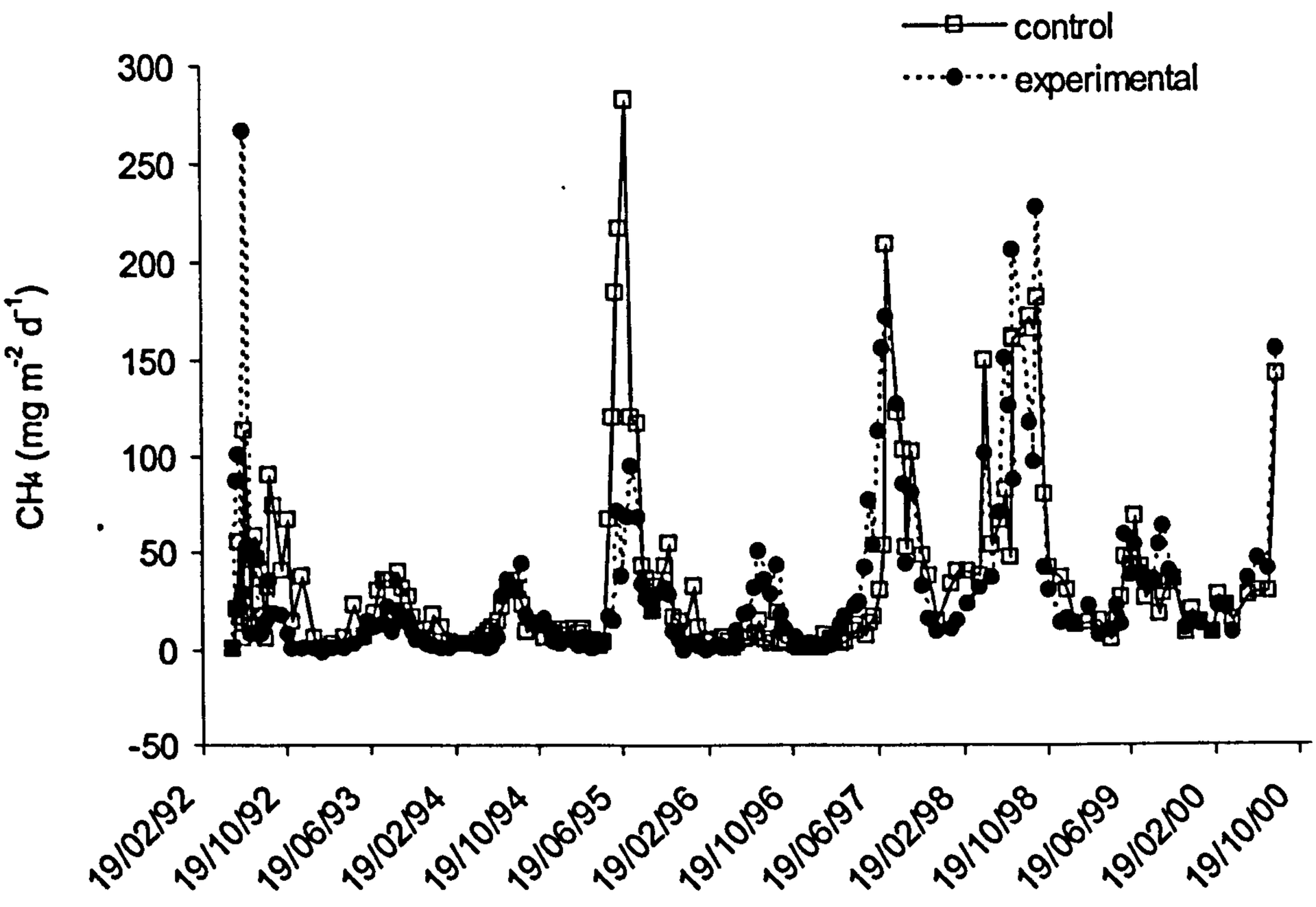
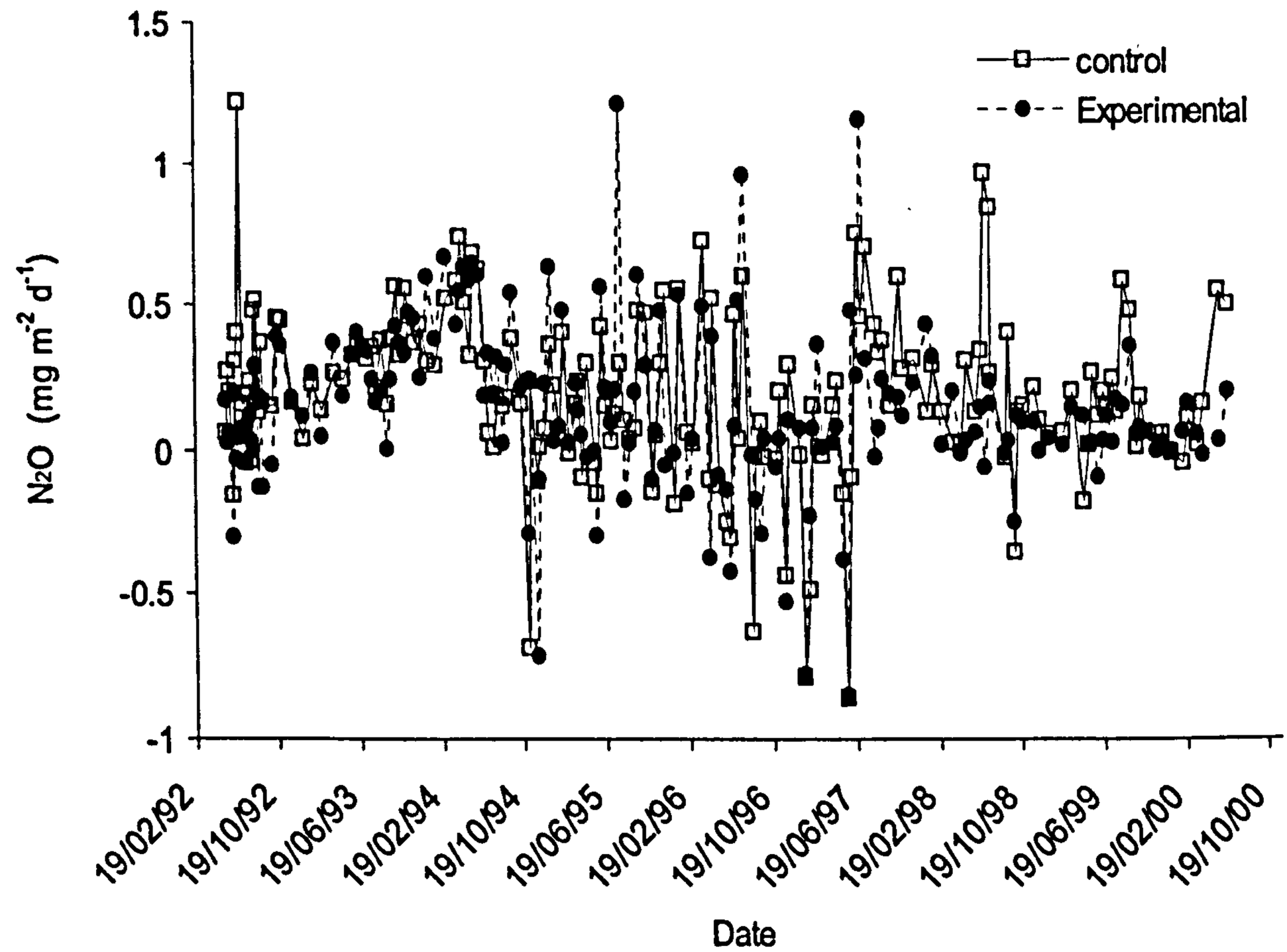


Figure 5.05



Methane (figure 5.04), & N_2O (figure 5.05) flux in control & experimentally droughted wetlands. Experimental droughts were induced from the end of May to the end of September except for a recovery period (1997 & 1998) & a natural summer drought (1995). Standard error of the mean omitted for clarity (on average control ± 12.6 & experimental ± 9.47mg m^{-2} d^{-1} for CH_4, control ± 0.12 & experimental ± 0.09mg m^{-2} d^{-1} for N_2O), n=5.

5.05 DISCUSSION

During 1999 and 2000, simulated droughts decreased the water table by a modest 5-10 cm and this is consistent with previous manipulations and the effects of a natural drought in 1995 (Hughes *et al.*, 1997; Hughes *et al.*, 2000), suggesting that the summer drought simulations were not unrealistic.

Effects of drought on DOC and phenolic compound concentrations

DOC concentrations in the experimental wetland were much lower in relation to the control during the 1999 and 2000 simulated summer droughts (figure 5.01a), although natural variability was relatively high. Such results are consistent with most of the earlier simulations (Hughes *et al.*, 1997; Hughes *et al.*, 1998), where averaged DOC concentrations over the summer simulations of 1992, 1993 and 1994 were significantly lower than those of the control for the same period, as was true for 1996, 1997 and 1998 ($P<0.01$). The natural summer drought of 1995 also reduced concentrations in relation to the control ($P<0.05$). Similarly, decreased DOC concentrations have also been reported in laboratory drought studies involving peat (Freeman *et al.*, 1993c; Freeman *et al.*, 1994). The reduced DOC levels can be explained by established theories of redox/organic sequestration processes leading to the co-precipitation of iron (Fe) (III) and DOC on the peat surfaces. As a result of a change to higher redox potential conditions in the experimental wetland (in response to drying), conversion of Fe (II) to Fe (III) is facilitated, which results in the much less soluble Fe (III) organic complexes precipitating from solution (c.f. Freeman *et al.*, 1993c). Moreover, the more humic-rich fractions of DOC tend to adsorb readily onto these precipitates, thus enhancing the process (Freeman *et al.*, 1993c; Tipping & Woof, 1990). Precipitation of large molecular mass humic acids is likely to be promoted by any increase in acidity in the experimental wetland induced by drought simulation (Hughes *et al.*, 1995; Hughes *et al.*, 2000), although in this case the pH remained similar. Microbial processes are also likely to contribute to the differences in DOC concentrations in the two wetlands. In the more anaerobic (low redox potential) conditions normally found in wetlands, a greater proportion of end products of metabolism include low molecular weight organic solutes (such as organic acids, alcohols, aldehydes and ketones) (Ponnanperuma, 1972), whereas in more aerobic conditions microbial metabolism favours the rapid production of oxidative, inorganic carbon (CO_2) end products (Freeman *et al.*, 1993c). Furthermore, aerobic respiration is often said to allow more efficient degradation and is energetically more feasible (Stainer *et al.*, 1986). This also partially explains the increased fluxes of CO_2 from the experimental wetland (figure 5.03a & b), since higher rates of mineralization by soil microbes may be expected to make a significant contribution to the net flux of CO_2 through the bog (see later).

Iron and DOC concentrations were strongly correlated in the wetlands ($R^2 = 0.788$, $P<0.001$ control; $R^2 = 0.739$, $P<0.001$ experimental) (Hughes *et al.*, 1997). This, together with the highly seasonal trends in DOC and Fe hydrochemistry in the mire, supports the view that the solubility of Fe in organic-rich waters is in part dependent on the relative abundance of natural organic ligands (in addition to pH and redox potential), and that much of the Fe is present in solution as dissolved/colloidal Fe-organo compounds (Heikkinen, 1994; Vaughan *et al.*, 1993). Concentrations of Fe did not decrease in the control wetland following the natural summer drought in 1995 (Hughes *et al.*, 1997). Moreover, Fe concentrations in the experimental wetland were significantly lower than those found in the control wetland during summer, even in years without drought (1997 & 1998). This may suggest that the differences in Fe concentrations between the two wetlands are mainly the result of inherent differences in the availability of Fe and in carbon cycling processes, rather than any effect of fluctuations in water table affecting Fe solubility (Hughes *et al.*, 2000). The need for an adequate pre-treatment period of data before experimental manipulations commence is therefore clearly demonstrated. However, such results may also suggest that the timing of a drought may be critical in determining the overall effects on this solute (Hughes *et al.*, 1997). Drought simulations were initiated early in the summer, before the natural cyclic peak of DOC production. Redox effects may therefore be more important in governing the solubility of Fe-organo compounds in the control wetland following the natural drought (Hughes *et al.*, 1997). In comparison to free Fe^{2+}, Fe (II) complexed to organic matter (OM) is stabilized and removed slowly from solution by oxidation and precipitation (Theis & Singer, 1975). This may occur in the control wetland following a natural drought, explaining the lack of a decline in Fe and DOC concentrations in comparison to the experimental site following a drought simulation.

The very seasonal patterns of DOC and Fe concentrations in both wetlands suggests that Fe exists in soil solution predominantly as organic complexes and that these solutes are undergoing strong biological control (Emmett *et al.*, 1994; Hughes *et al.*, 1990). Long term concentrations of DOC and Fe show seasonal peaks during the summer, both solutes strongly and positively correlating with peat temperature (DOC $R^2 = 0.84$ $P<0.001$; Fe $R^2 = 0.74$, $P<0.001$ control wetland; DOC $R^2 = 0.82$, $P< 0.001$; Fe $R^2 = 0.77$, $P<0.001$ experimental wetland) (Hughes *et al.*, 2000), with lower concentrations in the experimental wetland. This lends support to the theory that increasing soil temperatures as a result of global warming may well contribute to the rising trend in DOC concentrations (see chapter 3).

Despite the short term reductions of DOC concentrations in the wetland pore water under summer drought conditions, an increasing trend was detected of 0.16 mg $L^{-1}yr^{-1}$ ($P<0.01$) over the 9 years of experimental data. The trend identified by Hughes *et al.* (1998) thus continues to

rise and the increase reported here is similar in magnitude to that reported by Worrall *et al.*, (in press) for catchments in the Yorkshire Pennines (0.1 mg $L^{-1}yr^{-1}$). Dissolved organic carbon concentrations in the control wetland also tended to increase, though this was not significant (0.12 mg $L^{-1}yr^{-1}$). However, a second control wetland (referred to as 'lower', see chapter 6) in the wetland chain also revealed a significant increasing trend (0.87 mg $L^{-1}yr^{-1}$, $P<0.01$). Furthermore, this wetland has a naturally lower water table than the upper control, suggesting that soil moisture levels may be critical in governing a system's response to a changing climate.

Phenolic compound concentrations tracked total DOC concentrations in both wetlands (Pearson product moment 0.9745, $P<0.001$ control, 0.8475 $P<0.01$ experimental) with seasonal peaks being seen during the summer months (figure 5.01b). Phenolic concentrations may drive total DOC concentrations given that they have been described as inhibitory in relation to microbial metabolism (Appel, 1993; Freeman *et al.*, 1990; Wetzel, 1992) and therefore to the decomposition of DOC. Indeed, these results seem to support the proposed importance of phenolic materials in the regulation of biotic components of aquatic ecosystems (Wetzel, 1992). Lower phenolic compound concentrations were observed in the experimental wetland during the 1999 and 2000 simulated summer drought, consistent with Freeman *et al.* (1994). Unfortunately, there is no long term data on phenolic compounds at the site and further monitoring would be required to determine whether there was an increasing trend with successive droughts. The phenolics:DOC ratio is thought to be especially important in relation to degradation of DOC by biofilm in the receiving aquatic system (Freeman *et al.*, 1990). The relative proportion of phenolic compounds declined during the drought, contradicting the hypothesis that selective utilization of labile DOC would increase the amount of dissolved phenolic compounds remaining. This may be due to high molecular weight humic substances (including phenolic compounds) being particularly prone to precipitation (c.f. Hughes *et al.*, 1995; Hughes *et al.*, 2000; Tipping & Woof, 1990), and increased degradation *via* the action of phenol oxidase under more aerobic conditions (Freeman *et al.*, 2001b; McLatchey & Reddy, 1998) allowing these relatively refractory dissolved materials to be mineralized.

Effects of drought on enzyme activities

The 2000 simulated summer drought induced a dramatic increase in phenol oxidase activities in the experimental wetland in comparison to the control (figure 5.02a). Since much of the peat matrix is composed of lignin (up to 60%, Clymo, 1983), this suggests that the potential for enzymic hydrolysis of this matrix is much increased. However, phenolic compounds are also likely to be removed from the pore waters (Freeman *et al.*, 2001b; McLatchey & Reddy, 1998) and the pronounced fall in phenolic compound concentrations (figure 5.01b) under the simulated drought is consistent with this observation. During drought the more aerobic

conditions may therefore allow efficient consumption of dissolved organic matter (DOM) within the pore waters but also lignin degradation (*via* the stimulated phenol oxidase activity) is likely to release large amounts of dissolved and/or particulate OM from the peat matrix. The latter material would be available for wash out when the wetland becomes rehydrated. Such enzymic decay may also expose many more sites for other enzymes to attack the lignin polymer, such as those that cleave internal bonds only (e.g., endo lignases). Already one of the features that define northern peatlands (their low enzyme activities and capacity to sequester carbon) seemingly has been altered, with the drought apparently causing the wetland to exhibit properties more akin to a truly terrestrial soil.

The increased phenol oxidase activities reported here may be due to a recovery of the activity of enzymes already present in the peat matrix (e.g., immobilized in combination with OM), because enzymes can retain their activity and stability for up to a year or more (Kiss *et al.*, 1975), and/or due to *de novo* synthesis by microorganisms within the peat (aerobes or facultative aerobes). Increased CO_2 flux from the bog (see later) suggests microbial activity has increased and thus that enzyme production is likely, but further work would be required to determine the dominant processes involved. A change to drier conditions may well allow a different microbial community to proliferate (see chapter 7) that produces greater amounts of phenol oxidase.

There are clearly profound implications of increased phenol oxidase activity, in the context of climate change, as a feedback to the process of intensified carbon loss. Enhanced peat aeration as a result of droughts has the potential to eliminate a 'latch' mechanism restricting the re-release of 455 Pg of carbon to the atmosphere (Freeman *et al.* 2001b). Seemingly, there is also the potential for DOC release to the recipient aquatic system as a result of an increase in the activity of this key peatland enzyme. The 30 year trends for increasing DOC in two out of the three catchments in the Pennines, studied by Worrall *et al.* (in press), could be explained by this mechanism. The authors state that drainage of those two catchments showing increasing DOC trends had occurred, while increasing summer temperatures were assumed to have affected all sites. However, the authors believe that drying in itself would be sufficient to increase peat oxidation, and that this coupled with increased runoff could account for the upward trend in DOC. Furthermore, they would expect step changes in their long term records in response to summer droughts if a latch mechanism were operating.

Warming and drying are, however, inextricably linked. Warming promotes increased evaporation and, from the results above, inducing drought significantly increases temperatures in the surface peat. Increased temperatures would lead to enhanced microbial activity

(increasing enzymic decomposition of the matrix and therefore DOC release) and evaporation. The more the peat dries the higher the potential for chemical oxidation of OM and the greater the accumulation of organic carbon over the summer to produce increased fluxes in the autumn (Worrall *et al.*, in press). Similarly, increased enzymic mobilization of the peat matrix would amplify this effect. Since Tipping *et al.* (1999) found that temperature *per se* did not increase DOC release, other than for a transient period, it may be that a combination of warming and drying cycles leads to the initiation of increasing DOC concentrations. Worrall and co-workers therefore concluded that the 30 year trends may be due to changes in summer temperatures accentuated by drainage. Even if the intensity of these summer droughts were to remain constant, an increase in frequency may set up positive feedback to chemical and enzymic DOC mobilization, as well as aeration of the peat deposits, because the structure of peat can be changed by drought. Hydrophobic properties induced in the surface layer as a result of drought (Gilman & Newson, 1980; Hughes *et al.*, 1995; Worrall *et al.*, in press) insulate the peat below from recharge by rainfall, thus effectively intensifying the drought and exacerbating DOC release. Such a phenomenon might be especially detrimental in ombrotrophic bogs which receive water solely from rainfall.

Phenol oxidase activities in both the control and experimental wetland were arrested by the natural flooding in the autumn of 2000. Rewetting can kill up to 50% of the microbes present (Kieft *et al.*, 1987), presumably the more rapid the flooding event the higher the mortality. The complete cessation of activity is in accordance with the work of McLatchey and Reddy (1998) who reported that phenol oxidase activity was only detectable under aerobic conditions. Such a phenomenon may allow more of the phenolic compounds released during the previous drought to enter the receiving waters without being subject to biodegradation.

ß-glucosidase activities in the experimental wetland were considerably higher in the simulated drought period of 2000 than those of the control wetland (157.58% $P<0.01$, figure 5.02b). The reason for the enhanced ß-glucosidase activities, and therefore inferred cellulase activities (Sinsabaugh & Linkins, 1988; Sinsabaugh *et al.*, 1991), may be related to the increased phenol oxidase activities and reduced phenolic inhibition (Freeman *et al.*, 2001b). Indeed, there was a significant positive correlation between ß-glucosidase and phenol oxidase for the experimental wetland only (0.6848, $P<0.05$). Since stimulated ß-glucosidase activities indicate enhanced carbon mineralization (McLatchey & Reddy, 1998; Sinsabaugh *et al.*, 1991), drought seemingly has the potential to mobilize carbon from the peat, increasing the CO_2 flux to the atmosphere. Summer drought may effect a similar response in the entire suite of cellulase enzymes, increasing cellulose decomposition and therefore releasing DOC products from the peat matrix for washout upon rehydration.

As a result of successive droughts the experimental wetland may have come to respond differently to the control site since, for example, there is a peak in carbon cycling enzyme activities under non-drought conditions on the 21/3/00 that is absent in the control. This may suggest that the experimental site may have been 'sensitized' or 'primed' to certain environmental changes (e.g., a natural fall in water table or associated effects) allowing enzyme activities to increase more rapidly than in the control wetland, even if the change is relatively slight (although this is purely speculation). It seems feasible that the previous simulated and natural droughts have altered the properties (physical structure, plant species composition, microbial community etc.) of the experimental wetland, changing its enzymic response to environmental stimuli in relation to the control. Alternatively, it may be that the experimental wetland had not recovered from the previous drought simulation. Either situation suggests that an increased frequency of mild drought could have a prolonged impact on the carbon cycling of the mire.

The rapid convergence of ß-glucosidase activities in the experimental and control wetlands following the 2000 simulated drought may be due to the effects of the autumn flooding causing both wetlands to become fully saturated. The lack of phenol oxidase activity would be expected to allow the accumulation of phenolic materials and thus inhibit hydrolase enzyme activities (Freeman *et al.*, 2001b). Phenolic compound concentrations were higher than those of the control in the recovery period on average (table 5.01), but a more intensive sampling regime would be needed to determine whether a pulse of such material occurred upon rewetting. There may also be other overriding factors such as the declining temperature with the onset of autumn and associated with the increased water supply restored to the experimental wetland. Alternatively, it may be that the aerobic organisms that have been producing ß-glucosidase cannot proliferate/survive under flooded conditions.

Sulphatase activities were stimulated over the period of simulated drought in relation to the control (figure 5.02c). Many studies (including those from the field site used here) note increased sulphate concentrations following water table drawdown (Freeman *et al.*, 1993c; Hughes *et al.*, 1997; Ogden 1982). Generally, this response has been attributed to the re-oxidation of sulphides. Peatlands, however, usually contain large amounts of organically bound sulphate (>20% of the total sulphur pool (Wieder *et al.*, 1987)) that would provide substrates from which sulphatase could release sulphate (Freeman *et al.*, 1997a). Thus, Freeman and co-workers postulated that sulphatase could contribute to the increased sulphate abundances that are observed following droughts. Again the increased sulphatase activity under a drought regime may be related to the activity of phenol oxidase and the removal of phenolic inhibition.

The effect may though, be due to the lower levels of inhibitory metal ions (e.g., Fe^{2+}) and DOC levels. The latter may bind to enzymes, immobilizing them and lowering activity (Wetzel, 1992 etc.). Freeman *et al.* (1996) also observed increased sulphatase activities in response to water table drawdown and attributed this to a reduction in the concentration of inhibitors such as Fe and phenolics. However, as with the other enzymic responses, it may be that a change in the microbial community structure is responsible for the observed effects. Given the link with OM decomposition (Howes *et al.*, 1984), a stimulation of sulphatase activities could further intensify loss of carbon from peatlands, in addition to the increased threat of surface water acidification due to sulphate release. This increased inorganic nutrient cycling and associated decomposition (Freeman *et al.*, 1996) is likely to accelerate as is DOC release and export following rehydration.

Phosphatase activities were higher in the experimental wetland in relation to the control even during the winter-spring of 1999/2000 (figure 5.02d, table 5.01), i.e., under non-drought conditions. This increase in activities may be due to changes that have occurred in the wetland as a result of previous droughts (and to which phosphatase may be particularly sensitive). Such changes may include shifts in plant or microbial communities, flow paths and peat properties. From field observations the latter seemingly have become more fibrous as a result of successive droughts. Previous droughts might have allowed colonization by a different community of microorganisms that are able to reproduce quickly and contribute to biogeochemical processes when favourable conditions return. Since the water table fell more rapidly following the first simulated drought in subsequent simulations (Hughes *et al.*, 1995), drainage may play an important part in this altered response. Increased phosphorus cycling again indicates increased mineralization with associated carbon loss, consistent with the original hypothesis and the results from both the oxidase and hydrolases discussed above. However, it is possible that the difference in phosphatase activities between the two wetlands simply reflects inherent differences in phosphate availability that are not related to the hydrological manipulation, as no pre-treatment in data was available from the site. Phosphate concentrations were often below limits of detection and this may account for the high phosphatase activities in both wetlands regardless of induced drought.

Waterlogging is generally believed to increase the availability of phosphate because as ferric (Fe^{3+}) iron is reduced to more soluble ferrous (Fe^{2+}) compounds, phosphorus that is in a specific ferric phosphate form (analytically known as reductant-soluble phosphorus (Faulkner & Richardson, 1989; Gambrell & Patrick, 1978)) is released into solution. Other reactions that may also be important in releasing phosphorus are the hydrolysis of ferric and aluminium phosphates, and the release of that sorbed to OM, clay and hydrous oxides by anion exchange

(Ponnamperuma, 1972). Phosphorus release from insoluble salts can also occur when the pH is changed, either by the production of organic acids or by the production of nitric and sulphuric acids by chemosynthetic bacteria (Mitsch & Gosselink, 2000). Thus, the initiation of drought conditions would be expected to decrease the amount of available phosphorus, necessitating the production of enzymes involved in phosphate acquisition and perhaps explaining the increased difference between control and experimental wetlands under drought simulation. Freeman *et al.* (1996) also reported increased phosphatase activities following water table drawdown, but attributed this to a reduction in the concentration of inhibitors such as Fe and phenolics. The latter mechanism may be more likely in this case given the similar pH in the two sites.

Temperatures in the experimental wetland showed a small but significant increase in comparison to the control, consistent with previous data from the site (Hughes *et al.*, 1997). Soil temperature affects enzyme activities both directly, through modified enzyme kinetics, and indirectly, by influencing microbial proliferation (therefore enzyme production). Thus the increases in both the oxidase and the hydrolase enzymes studied could be solely due to these warmer temperatures However, this is unlikely given the observed increases in enzyme activities per °C rise in temperature found in chapter 2. Kang & Freeman (1999) speculate that water table drawdown and elevated temperature, both of which are predicted by climate change models (and which are in effect imposed on the experimental wetland here), would increase enzyme activities and consequently the regeneration of inorganic nutrients. Indeed, all of the enzymes studied here were only found to correlate significantly with their appropriate, anticipated substrates or products in the drought impacted wetland.

Effects of drought on trace gas flux

In addition to laboratory manipulations to demonstrate the suppression of hydrolase activities by phenolic compounds, Freeman *et al.* (2001b) state that for every doubling of phenol oxidase activity there was an approximate doubling of CO_2 production. ß-glucosidase activities in this study closely correlated with those of phenol oxidase in the experimental wetland (0.6848, $P<0.05$), and the former is said to indicate carbon mineralization rates (McLatchey and Reddy, 1998; Sinsabaugh *et al.*, 1991). On the basis that both phenol oxidase and ß-glucosidase activities increased under drought conditions, it would again seem sensible to predict increased CO_2 flux from the experimental wetland. There is however an alternative theory that *Sphagnum* peat does not degrade even under aerobic conditions due to the presence of residues of D-lyxo-5hexosulouronic acid (5-keto-D-mannuronic acid, 5KMA) in the hyaline cell walls (Painter, 1983). The results presented here seemingly conflict with the latter theory, as do the results previously reported at the site, because peak CO_2 fluxes through the experimental wetland were much higher than the control (figure 5.03a) suggesting increased aerobic decomposition. This is

consistent with the majority of studies in the literature (e.g., Aerts & Ludwig 1997; Scanlon & Moore, 2000) including those relating to *Sphagnum* peat (Prevost *et al.*, 1997). However, differences in mean fluxes were usually not statistically significant due to relatively large natural variation, especially in the experimental wetland. Should drought events become more frequent as a result of climate change there is the potential for increased emissions but also the transformation of such wetlands from a sink to a source of atmospheric CO_2 (figure 5.03b), i.e., a positive feedback to climate change. In the short term, the increased carbon mineralization may leave less DOC available for export from the surface layers to the recipient waters, but may indicate increased decomposition of the peat with the resultant rising DOC trends in the long term. While no attempt was made to separate soil and root respiration, the increased CO_2 emissions support the hypothesis that microbial activity has been stimulated as opposed to only abiontic enzyme activities.

Wetlands are a major contributor to the global CH_4 budget (Cicerone & Oremland, 1988) and drier summers may alter the CH_4 flux from northern wetlands with potential feedbacks to climate change (Bridgham *et al.*, 1995). Based on numerous reports from short term laboratory and field studies (e.g., Bridgham *et al.*, 1995; Christensen, 1993; Moore & Knowles, 1989; Moore & Roulet, 1993; Sebacher *et al.*, 1986; Vourlitis *et al.*, 1993), a consensus identifies water table drawdown as limiting CH_4 emissions from wetlands but it is important to test that assumption over the longer term.

Despite a lack of pre-manipulation data on CH_4 flux from the control and experimental sites, CH_4 emissions are considered to show some degree of acclimation (c.f. Hughes *et al.*, 1999) to consecutive, low intensity summer drought over the past 9 years (figure 5.04). The third summer simulation saw a return to control CH_4 emissions during the period of water table drawdown. Moreover, the experimental wetland was affected less by the natural drought than the control site, after having been subjected to summer water table drawdowns previously. Similarly, the final drought manipulations in the summer of 1999 and 2000 did not significantly affect CH_4 emissions relative to the control. Although it is widely accepted that reduced waterlogging increases the volume of peat in which oxidation can occur, increased diffusivity through the drier peat can counteract this effect (Hughes *et al.*, 1999). An increase in monocotyledonous (monocot) species may also allow an increased conduit effect, by-passing the zone of oxidation (e.g., Chanton *et al.*, 1992; Whiting & Chanton, 1993). The work of Rosalev and King (1996) suggests that oxidation is of secondary importance in the hydrological regulation of CH_4 emissions, as increased oxidation but also production occurred with short term decreases in water table levels. Several field studies also note rising emissions in the early stages of drought (Hughes *et al.*, 1999) and the mechanisms proposed to account for this

included increased diffusivity, removal of over-burden pressure and reduced rates of oxidation in the surface layers (Windsor *et al.*, 1992). Freeman *et al.*, (2002) also found no evidence for increased oxidation under drought conditions from the same study site as was used here. Furthermore, production has been found to respond exponentially to temperature, whereas oxidation was thought to be approximately linear (or at least with a much smaller exponent than production if the response was exponential) (Pearce & Clymo, 2001). Thus, warming as a result of water table draw down would favour CH_4 production, offsetting any reductions in efflux due to a deeper unsaturated zone and higher temperatures increasing oxidation.

The acclimation phenomenon might indicate that the wetland has a microbial 'memory', (as has been suggested in relation to temperature in chapter 3), i.e., retains the potential to adjust to periods of relatively slow and modest water table fluctuations, even though a two year recovery period had elapsed between the 1996 summer drought simulation and the 1999 simulation. In addition to the mineralization of DOC to produce CO_2, concentrations may be limited during the drought *via* consumption by the methanogens as peak CH_4 production in the peat profile is generally attributed to a localized introduction of labile DOC in the form of root exudates or detritus from previous plant growth (Roura-Carol & Freeman, 1999). An increased monocot presence is likely to represent an increased exudation potential (see chapter 4B) and an increase in the availability of labile DOC, favouring methanogenesis. However, given that DOC concentrations declined under drought conditions in the surface peat, it may be that methanogenic bacteria have migrated down the profile to the more anaerobic zones where DOC concentrations (and moisture levels) would remain unaltered. The former providing a pool of low molecular weight electron acceptors for the production of CH_4 (Mitsch & Gosselink, 2000).

Such observations of an acclimation phenomenon does not necessarily contradict the consensus view that water table drawdown restricts CH_4 emissions from wetlands. It may however, be prudent to exercise caution when examining CH_4 results from short term laboratory or field drought manipulations, and their possible use in mathematical CH_4 flux models that have yet to consider such acclimation (Cao *et al.*, 1996). The results found here may help to explain why it is difficult to discern relationships between water table position and CH_4 emissions within sites, whereas such relationships have been demonstrated between sites (Moore & Roulet, 1993; Whalen & Reeburgh, 1992). When considering the potential for acclimation, it should be noted that the simulated drought was not severe in comparison with many studies (Moore & Knowles, 1990). From the Cerrig-yr-Wyn results it seems that CH_4 emission-water table relations may be dynamic and dependent on patterns and frequencies of drought events. The complexity of interacting factors affecting wetland CH_4 emissions (Bridgham *et al.*, 1995; Bubier & Moore, 1994) makes long term field studies essential.

The first simulated drought produced one of the most pronounced of all effects observed in that the flux of N_2O was all but arrested (figure 5.05, Hughes *et al.*, 1995). At the time, this was suggested to be due to an elimination of the flow of nitrate (the substrate for denitrification/N_2O production) through the experimental wetland (Hughes *et al.*, 1995). However, the second and third simulated droughts produced far less dramatic effects with only a ca. 23% reduction in emissions being detected in the second year (as opposed to ca. 83% in the first year). And, a return to similar levels of emission to those of the control occurred in the third year and from then onwards to 2000.

The rate of release of N_2O from wetlands is governed by complex mechanisms that are beyond the scope of this study. Briefly, N_2O is produced during both denitrification and nitrification; it is an intermediate in the former and a by-product of the latter. The following conditions are said to be favourable for N_2O production (Bandibas *et al.*, 1994): 1) medium-high soil water content, limiting the oxygen diffusion rate, 2) high mineral-nitrogen (NO_3^- or NH_4^+) availability (which in turn are controlled by rates of mineralization and immobilization) and 3) high organic carbon availability. The latter is widely thought to enhance the loss of NO_3^- or N_2O^- through denitrification (Payne, 1981; Rolston, 1981), although other studies have shown that high carbon supply also decreases the ratio of N_2O:N_2 evolved (Smith & Tiedje, 1979), which may offset to some extent the positive effects on the process rate (i.e., denitrification). Studies using carbon supplied *via* an application of organic residues have reported not only increased N_2O production in soils, but also an increased rate of N_2O reduction to N_2 (Letey *et al.*, 1980).

Nitrous oxide production at the experimental site may be affected by any one or a combination of the above factors. It is feasible that the slower rate of water table drawdown during the first year may have had an adverse effect on N_2O production compared to the more rapid water table falls in the second and third years. In the first year, it is possible that there was a cessation of the normal mechanism of N_2O production (i.e., denitrification) in the drought impacted wetland, whilst the switch to the alternate aerobic process of N_2O production was very slow. In the proceeding years of drought simulation, the more rapid drainage may have enabled the switch in mechanisms to occur more effectively, without significantly affecting net N_2O production. Peat water hydrochemistry data for the control and experimental wetland suggests that the drought/rewetting cycle did not have a pronounced effect on the supply of substrates (NH_4^+ and NO_3^-) for N_2O production in these wetlands (Hughes *et al.*, 1995). Nitrous oxide emissions may therefore have been a by-product of the nitrification of ammonium released during mineralization (Freeman *et al.*, 1997b), particularly since DOC concentrations declined in the surface layers and carbon supply is an important control on denitrification (Bowden *et al.*

1992; Seitzinger, 1994). A further possibility that has been proposed in the literature is the denitrification of nitrate products that have diffused into the anaerobic zone (Freeman *et* al., 1993a, c; Seitzinger, 1994). However, Freeman *et al.* (1997b), who also noted a reduction in N_2O emissions under drought conditions from the same site, found increased nitrate concentrations in the waters draining the wetland and inferred that denitrification had been severely impaired. This perhaps suggests that it is unlikely that denitrifying bacteria are responsible for the acclimation of N_2O emissions.

5.06 CONCLUSIONS

An increased frequency of summer drought has the potential to eliminate the 'latch' mechanism represented by phenol oxidase that restricts the re-release of 455 Pg of carbon not only back to the atmosphere (Freeman *et al.* 2001b) but also to the adjacent aquatic systems as DOC, therefore threatening drinking water supplies. Stimulated hydrolase enzyme activities further substantiate this, indicating the potential for increased enzymic mobilization of the peat matrix. This mechanism may contribute to the significant rising trend in DOC concentrations detected in the pore waters of the drought-impacted wetland (0.16 mg $L^{-1}yr^{-1}$), amplifying the release due to chemical oxidation of the peat. Such changes, along with the increased CO_2 flux from, and inorganic nutrient cycling in the experimental wetland indicate a shift towards more aerobic decomposition processes and the potential for reduced carbon sequestration in northern peatlands. In addition to phenol oxidase activity, drought will also affect many factors traditionally thought to allow peat accumulation, namely by increasing temperatures, aeration, oxidation and nutrient cycling, along with changes to the botanical composition of the peat (e.g., by reducing *Sphagnum* content) and microbial community structure. There is the potential for certain processes (CH_4 and N_2O emissions) to acclimate to successive low intensity droughts. Thus, decreases in CH_4 and N_2O emissions reported following one drought in isolation should not be relied on to offset increased CO_2 release in terms of feedback to climate change, nor used for modelling purposes. The results presented may have management implications for the prevention of adverse effects on water quality and a change in the function of wetlands (from a source to a sink of nutrients and trace gases), with relevance to restoration practices and constructed wetlands.

5.07 REFERENCES

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CHAPTER 6: THE EFFECTS OF SIMULATED INCREASED RAINFALL ON DOC MOBILIZATION

6.01 INTRODUCTION

Throughout the peat covered catchments of the UK management problems exist due to the discolouration of raw waters by humic substances (Kay *et al.*, 1989). A seasonal pattern can be seen, with raw water colour levels peaking in late summer to autumn (Kay *et al.*, 1989; Worrall *et al.*, in press), and the annual variance of this cyclic pattern has increased in many upland catchments in recent years producing higher peak colour (Kay *et al.*, 1989). A consistent and statistically significant rising trend in dissolved organic carbon (DOC) has also been reported in waters draining a peat dominated catchment in Wales (Robson & Neal, 1996) and similarly in streams draining the site in question (Reynolds *et al.*, 1997). More recently this trend has been reported in 20 out of the 22 Acid Waters Monitoring Network UK sites (Freeman *et al.*, 2001a) and for two of the three catchments in the Yorkshire Pennines studied over a 30 year period by Worrall *et al.* (in press). Both iron (Fe) and colour are related to DOC, and when concentrations of humic substances (which account for approximately 50% of DOC in most natural waters) are high so too is turbidity and total carcinogenic trihalomethanes concentrations (TTHM) produced during treatment of raw waters for drinking purposes (Alarcon-Herrera *et al.*, 1994; Shin & Lim, 1996). Coloured waters are aesthetically displeasing to the consumer and low residual chlorine limits protection against biological contamination (Worrall *et al.*, in press). Humic substances not only serve as precursors for the formation of chlorinated compounds but also possess ion exchange and complexing properties that include association with toxic elements and micro-pollutants (Alarcon-Herrera *et al.*, 1994; Pardue *et al.*, 1993). Furthermore, they are precursors for organic compounds formed by oxidation during the ozonation process, they can compete with pollutant compounds for adsorption sites in activated carbon adsorption and they precipitate in the distribution system (Alarcon-Herrera *et al.*, 1994). Many workers have observed that humic acids chelate metals (e.g., Buffle, 1980; Chin *et al.*, 1998). Kerven *et al.* (1984) reported that copper was bound to a weakly acidic site in peat derived humic acids, although there is much uncertainty and apparent contradictory evidence regarding the mechanism of complexation. The literature on the behaviour of other metals is equally complex. Zinc, for example, was found to be chelated by both humic and fulvic acids (Himes & Barber, 1957).

In the future, relatively cheap upland water resources are likely to increase in importance because not only is demand *per capita* rising (Thomsen, 1990), but it has been suggested that the frequency of droughts may increase due to climate change in certain regions (Arnell, 1992;

Hulme *et al.*, 2002; IPCC, 2001) adding a further strain on water resources. Moreover, increased use and transportation from areas of water surplus would mean greater proportions of the population would be subject to water of a potentially reduced quality.

Radiative effects of anthropogenic changes in atmospheric composition are expected to cause an intensification of the global water cycle (Cubasch, 2001). Although some areas may become drier (Arnell, 1992; Hulme *et al.*, 2002; IPCC, 2001), many are predicted to receive increased rainfall at certain times of year and those areas include northern Canada, Scandinavia (Wellburn, 1994) and the north of Britain (Cooper & McGechan, 1996; Mansell, 1997) where some of the largest peat reserves are located. More intense rainfall events (Palmer & Räisänen, 2002) and flooding events (Milly *et al.*, 2002) are also anticipated and DOC movements from upland soils to aquatic ecosystems are driven by precipitation events (Wetzel, 1992). The impact of increased water input on a relatively well drained peatland system in the Plynlimon catchment, mid Wales (described by Hughes *et al.*, 1996), has been studied to determine whether water quality is likely to be reduced as a result of increased DOC release from such systems. The growing interest in the restoration of drained peatlands in Britain (Wheeler & Shaw, 1995) also has possible consequences for water quality and it is important to determine the effects of these changes in land management practices on biogeochemical processes in the peat. Similarly, afforestation has been found to increase DOC and Fe concentrations in the upper horizon of podzol soils in mid Wales and clearfelling enhanced this effect (Hughes *et al.*, 1990). Changes in soil moisture content could therefore play a key role in determining the levels of DOC and Fe produced within upland peatland catchments. Indeed, additional water input can significantly increase the export of dissolved organic matter (DOM) from upland moorland soils (brown earth and micropodzol) in the UK (Tipping *et al.*, 1999).

'Dramatic increases' in DOC and Fe in the peat pore water have previously been reported at the study site over a continuous, three year increased rainfall simulation in the gully mire (Hughes *et al.*, 1998). Following four years of experimental wetting, seasonal peak concentrations of DOC and Fe have since remained at these higher levels (Fenner *et al.*, 2001, figure 6.01a), in contrast to the response by bromide (Hughes *et al.*, 1998). The former may therefore be the result of a change in carbon cycling within the mire, whereas the latter has been attributed to a flush effect (Hughes *et al.*, 1998).

Recently, phenol oxidase has been recognized as having a key role in carbon storage in organic-rich northern soils (Freeman *et al.*, 2001b). This enzyme along with another carbon cycling enzyme, ß-glucosidase, has therefore been studied to determine whether carbon cycling in the

mire has altered as a result of increased water inputs, or whether the increased DOC concentrations are simply a flush effect with a long recovery period (beyond that of bromide). Since phenol oxidase activities are stimulated by bimolecular oxygen (Freeman *et al.*, 2001b) and cannot be detected under waterlogged conditions (McLatchey & Reddy, 1998), it was hypothesized that the increased rainfall simulation would suppress the activity of this enzyme. Reduced hydrolase enzyme (such as ß-glucosidase) activities would therefore be expected as a result of increased inhibitory phenolic compound concentrations (Freeman *et al.*, 2001b), inducing the further accumulation of DOC. In addition to total DOC and phenolic compound concentrations, sulphatase and phosphatase activities were also measured to provide an indication of the mineralization of organic sulphur and phosphate compounds respectively (Sinsabaugh *et al.*, 1991). The former may also be important because in wetland soils, organic matter (OM) decomposition can proceed through a pathway that involves both carbon and sulphur cycles, the fermentation: sulphate reduction pathway (Howes *et al.*, 1984).

In order to gain further insight into the nature of the association between DOC and Fe at the site in question and in numerous other studies (e.g., Chin *et al.*, 1998; Heikkinen 1990), the apparent molecular weight spectrum (AMW) of the DOC, and the Fe concentration in the various fractions was examined. Any differences in the AMW spectra between the experimental and control sites would also indicate whether carbon processing is altered as a result of enhanced water input. Many studies in the literature suggest an association between fulvic material (or at least the mid molecular weight fraction (1000 to 10 000 Daltons) of DOC) and Fe (e.g., Ghosh & Schnitzer, 1981; Goodman *et al.*, 1991). It was therefore hypothesized that the increased rainfall simulation site would yield a spectrum of DOC with selective enrichment of material corresponding to the mid AMW material, with which the majority of Fe would be accounted for.

Trace gas emissions from the control and experimental sites were also measured with the aim of detecting any feedback to climate change.

6.02 MATERIALS AND METHODS

Site description

The catchment chosen for study, Cerrig-yr-Wyn in the Upper Wye catchment on Plynlimon (UK NGR SN 820 866), is a small gully typical of many in the uplands of Wales (Hughes *et al.*, 1996) in which flush wetlands have developed as serial cascade systems. The control and experimental wetlands are located adjacent to those used for the droughting experiments (see chapter 5) and are characterized by *Sphagnum* and *Juncus* communities, with pore waters in the

range of pH 3.9-4.8 at a depth of 10 cm. Part of the gully mire has been naturally drained over time by the formation of, and changes to, natural soil pipes deep within the peat. This changed the normal hydraulic recharge of the mire from recharge by surface streamflow, to recharge from sources deeper within the peat profile (Hughes *et al.*, 1996).

The continuous increased rainfall simulation in the experimental wetland commenced in June 1995 and ended in October 1999, marking the end of the Centre for Ecology and Hydrology (CEH) work at this site. A further simulation was conducted in 2000 (May to September) to enable enzyme activities to be measured and this was effectively ended by the natural flooding towards the end of September 2000. The increased rainfall simulation involved diverting streamflow from an adjacent gully using PVC pipes and drip-feeding the streamwater onto the surface of the wetland. An adjacent naturally drained wetland was used for monitoring as a control. The original construction is described by Hughes *et al.* (1996). Water table depths ranged from 49 cm to 9 cm below the surface of the peat in the control wetland, and between 16 cm and 1 cm in the experimental wetland. Moisture contents in the control site ranged between 69.6% and 92.4%, while in the rewetted site, values ranged between 74.4% and 96.5% (Freeman *et al.* 1998).

Sampling and chemical analysis of peat and pore water

Peat samples were taken monthly from 5 replicate positions within each wetland (control and experimental) in a transect corresponding to the pore water sampling positions. Pore water samples were extracted at least monthly from the top 10 cm of the peat profile using solution samplers placed at 2.5 m spacing along a transect within the two wetlands. Five samplers installed in each of the two wetlands gave 5 replicates, "sample A" and "sample B" are used to denote 2 individual replicates of these containing high DOC concentrations. The samplers were constructed as described in chapter 2 and inserted into the peat. All aqueous samples were filtered through 0.2 μm diameter membranes (Whatman, Kent, UK), and analyzed for DOC and Fe. The former using a Shimadzu 5000 Total Organic Carbon Analyzer (see chapter 2) and the latter by flame atomic absorption spectrometry (AAS) at 248.3 nm. Phenolic compound concentrations within the total DOC pool were measured as described in chapter 2. The concentrations of these solutes were also analyzed in samples of stream water used for the rainfall simulation.

A CECIL 1100 series High Performance Liquid Chromatograph (HPLC) was used to determine the AMW distribution of DOC in the samples, as described in chapter 3. The various DOC fractions eluted from the HPLC were collected and AAS allowed the Fe concentration to be

measured. Ferric Chloride and Ferrous Sulphate solution (100 mg L^{-1} Fe content) were used to determine the point at which inorganic Fe would elute off the column, and Ferric citrate solution (100 mg L^{-1} Fe content) to ensure that there was no separation of Fe from the organic material as it passed through the column.

The absorbance spectrum between 200 and 900 nm was determined for all samples using a UVICON 943 spectrophotometer (BIO-TEK, Contron instruments, Contron Ltd., West Sussex).

6.03 STATISTICAL CONSIDERATIONS

All statistical analyses were performed using Minitab version 13.32 (Minitab Inc.) as described in chapter 5, however, it must be noted that only 5 years of data was available for the trend analysis in this case.

6.04 RESULTS

Effects of increased rainfall on DOC concentrations

Generally, DOC release in both the control and experimental wetland in 1999 and 2000 (figure 6.01a) showed a seasonal pattern (as did Fe, recorded simultaneously) with the largest concentrations observed in the summer/autumn months. Phenolic concentrations showed a very similar response (figure 6.01b), correlating strongly and positively with DOC concentrations in the control and experimental wetlands (Pearson product moment 0.8843, $P<0.01$ and 0.949, $P<0.01$ respectively).

Table 6.01 shows the mean solute concentrations and enzyme activities for distinct time periods along with the significance of the t-test for the control and experimental wetlands.

Table 6.01. Mean solute concentrations and enzyme activities for distinct time periods with significance of the t-test for the control and experimental (increased simulated rainfall) wetlands

Determinand	Continuous simulation period 1999			Recovery period winter 1999/2000			Summer simulation period 2000		
	C	E	Δ%	C	E	Δ%	C	E	Δ%
DOC (mg L^{-1})	6.89	55.02	698.43	4.33	22.79	426.62	8.26	24.9	201.31
	(0.78)	(3.68)	***	(0.35)	(3.95)	**	(2.12)	(2.29)	***
Phenolics (mg L^{-1})	0.58	7.00	1116.41	0.49	2.64	436.96	0.62	3.24	424.77
	(0.13)	(0.84)	**	(0.007)	(0.47)	**	(0.19)	(0.88)	ns (10%)
Phenol oxidase (nmols dicq g^{-1} dry peat min^{-1})				1.30	0.48	-63.08	2.10	-0.07	-103.33
				(0.09)	(0.02)	**	(0.05)	(0.05)	***
ß-glucosidse (nmols MUF mg^{-1} dry peat h^{-1})				0.17	0.22	32.65	0.26	0.38	45.41
				(0.04)	(0.07)		(0.02)	(0.10)	
Sulphatase (nmols MUF mg^{-1} dry peat h^{-1})				0.23	0.27	16.31	0.31	0.29	-4.10
				(0.02)	(0.02)		(0.02)	(0.08)	
Phosphatase (nmols MUF mg^{-1} dry peat h^{-1})				4.77	2.25	-52.78	4.41	2.47	-43.91
				(0.70)	(0.03)	*	(0.74)	(0.24)	ns (10%)

C and E denote values from control and experimental wetlands respectively. Numbers in parentheses indicate standard error of the mean, n=5. Percentage changes relative to the control are presented (Δ%) and P values where ns (10%) denotes significance at the P<0.1 level only, * significance at the P<0.05 level, ** at the P<0.01 level and *** at the P<0.001 level. These conventions will apply throughout unless stated otherwise.

Mean DOC concentrations were substantially higher (698.43%, P<0.001) in the experimental wetland than in the control during the continuous wetting phase in 1999 (figure 6.01a). Wetting ceased after 27/10/99 and DOC concentrations declined gradually until the second simulation imposed on 10/05/00. However, the DOC concentration in the experimental wetland remained higher than that of the control even without treatment (426.62%, P<0.01). The increase in DOC concentrations relative to the control under the increased rainfall simulation of 2000 (following the recovery period) was less than that produced under continual wetting. Nevertheless, a 201.31% increase (P<0.001) in concentrations was observed. Due to large natural variation and the fact that the experimental wetland apparently required further time to recover from the previous treatment, analysis of covariance (ANCOVA) revealed no significant difference between the two wetlands once the pre-treatment levels had been taken into account. However, a substantial increase in concentrations can be seen in the 2 months after the 2000 simulation began and on the 30/05/00 concentrations were 249.14% higher than the control at the P<0.1 level only.

Similar patterns were induced in phenolic compound concentrations (figure 6.01b); phenolics concentrations under the continual simulation were considerably enhanced with respect to the control (1116.41%, P<0.001), as was the ratio of phenolic compounds:DOC as a result. During the recovery period, phenolics concentrations remained higher than the control (436.96%, P<0.01) and the 2000 simulation produced an increase of 424.77% but this was only significant at the P<0.1 level. A substantial increase occurred on 28/6/00 during the latter experimental period where phenolic compound concentrations were increased by 555.64% (P<0.01) in relation to the control.

Effects of increased rainfall on enzyme activities

Mean phenol oxidase activities (figure 6.02) generally increased during the summer months in the control site but dramatically declined with simulated increased rainfall in the experimental wetland. During the 1999 recovery period, activities were significantly reduced in the experimental wetland (-63.08%, P<0.01) relative to the control and this activity was further suppressed during the 2000 simulation (-103.33%, P<0.001). ß-glucosidase activities (figure 6.03) were somewhat erratic but tended to increase during the summer and autumn in the control wetland. There was no significant difference between the mean activities for the control and experimental wetlands during the recovery period or the 2000 simulation. Sulphatase activities tended to increase during the summer months in both wetlands (figure 6.04), but showed no significant difference between wetlands over either the recovery or simulated increased rainfall period. Phosphatase activities (figure 6.05) were consistently lower in the

experimental in comparison to the control wetland, with suppressed activities even during the recovery period (-52.78%, $P<0.05$) before the 2000 simulation. The experimental wetland exhibited activities that were suppressed with respect to the control during the 2000 simulation (-43.91%, $P<0.1$ only).

Effects of increased rainfall on the apparent molecular weight (AMW) spectra of DOC

Table 6.02. Relative iron (Fe) concentrations of eluted DOC fractions from the experimental wetland.

AMW[a] (Daltons)	Relative Fe concentration[b]	
	Sample A	Sample B
>200000	0.00	0.00
<200000 >90000	0.07	0.02
<90000 >5000	0.24	0.07
<5000	0.00	0.00

[a]Apparent Molecular Weight
[b]Absorbance units
Samples were collected on 10/05/99 and the figures above refer to a five fold dilution.

Under the continuous treatment, the experimental wetland showed DOC AMW spectra with 3 substantial fractions (>200 000, <200 000 to >90 000 and <90 000 to >5000 Da) along with a considerable amount of material in the <5000 AMW range (figures 6.06a & b). The control wetland showed a much less dramatic spectra, with a small amount of material barely forming a distinct peak spanning the range of <90 000 to <5000 Da (figure 6.06c), i.e., corresponding to the lowest AMW fractions observed in the experimental wetland. The AMW fraction of DOC associated with the greatest Fe concentration in the experimental wetland samples was that of the >5000 to <90000 AMW range (table 6.02), and this was true for samples taken throughout the year. When the inorganic Ferric Chloride and Ferrous Sulphate solutions were passed through the column, the fraction giving the highest Fe concentration did not elute at the same time as that of the >5000 to <90000 AMW fraction of the sample, and therefore the Fe association with this material was not likely to be due to a simple artefact. Ferric citrate solution produced an AMW peak of DOC which corresponded to the eluted fraction that yielded the most Fe, demonstrating that the column used did not disrupt the OM-Fe association.

Effects of increased rainfall on trace gas flux

Due to large natural variation in trace gas fluxes at the field sites, the effects of the increased rainfall simulation were not statistically significant; means and peak values are thus provided below.

Table 6.03. Peak trace gas fluxes from control and simulated increased rainfall sites during 1999

Trace gas	C (mg $m^{-2} d^{-1}$)	Date	E (mg $m^{-2}d^{-1}$)	Date
CO_2	4045.6 (458.00)	19/07/99	4452.4 (1561.6)	19/07/99
CH_4	10.37 (0.18)	23/08/99	297.26 (297.63)	27/09/99
N_2O	0.2 (0)	10/05/99	0.8 (0.5)	21/06/99

Table 6.04. Peak trace gas fluxes from control and simulated increased rainfall sites during 2000

Trace gas	C (mg $m^{-2}d^{-1}$)	Date	E (mg $m^{-2}d^{-1}$)	Date
CO_2	2948.65 (772.8)	25/07/00	3059.55 (1156.4)	25/07/00
CH_4	33.33 (29.28)	22/08/00	206.63 (20.93)	25/09/00
N_2O	0.2 (0.1)	25/07/00	0.6 (0.2)	25/07/00

Carbon dioxide fluxes along with CEH long term data are shown in figure 6.07a. During the 1999 and 2000 simulations, fluxes were similar in the control and experimental sites ranging between ca. –2000 (i.e., the wetlands acting as a sink) and 15000 mg $m^{-2}d^{-1}$ (i.e., the sites were a source of CO_2). Cumulative values from the long term CEH data (figure 6.07b) show a slight decrease in CO_2 emissions from the experimental wetland in relation to the control, but generally both wetlands behaved as CO_2 sources after the first six months (when the sites had a net flux of virtually zero).

Methane emissions were relatively small from the two wetlands over the first two years of the experiment (figure 6.08a). However, with time a substantial increase in CH_4 release from the experimental site occurred, reaching a peak value of 297.26 ± 297.63 mg $m^{-2}d^{-1}$ during the 1999 simulation compared to 10.37 ± 0.18 mg $m^{-2}d^{-1}$ in the control. Cumulative CH_4 values (figure 6.08b) show a pronounced increase in CH_4 emission over that of the control, following a lag phase (where both wetlands were only slight sources of CH_4) that ended during July 1997 with both wetlands acting as a source of CH_4. Cessation of waterlogging during the autumn of 1999 resulted in a sharp fall in the water table and concomitant attenuation of CH_4 emissions from the experimental site. During the 2000 simulation, mean CH_4 emissions were substantially larger than the control, producing a peak value of 206.63 ± 20.93 mg $m^{-2}d^{-1}$ compared to the control wetland where 33.33 ± 29.98 mg $m^{-2}d^{-1}$ was the maximum observed.

Nitrous oxide emissions also eventually increased, reaching peak values of 2.0 ± 0.7 mg $m^{-2}d^{-1}$ in the experimental wetland and 0.9 ± 0.0 mg $m^{-2}d^{-1}$ in the control (figure 6.09), after a shorter lag phase than the CH_4 emissions. Nitrous oxide emissions then remained higher in the experimental wetland during both the 1999 and the 2000 simulation (peak values 0.8 ± 0.5 and 0.6 ± 0.2 mg $m^{-2}d^{-1}$ respectively, compared to control values of 0.2 ± 0.0 and 0.2 ± 0.1 mg $m^{-2}d^{-1}$ respectively)

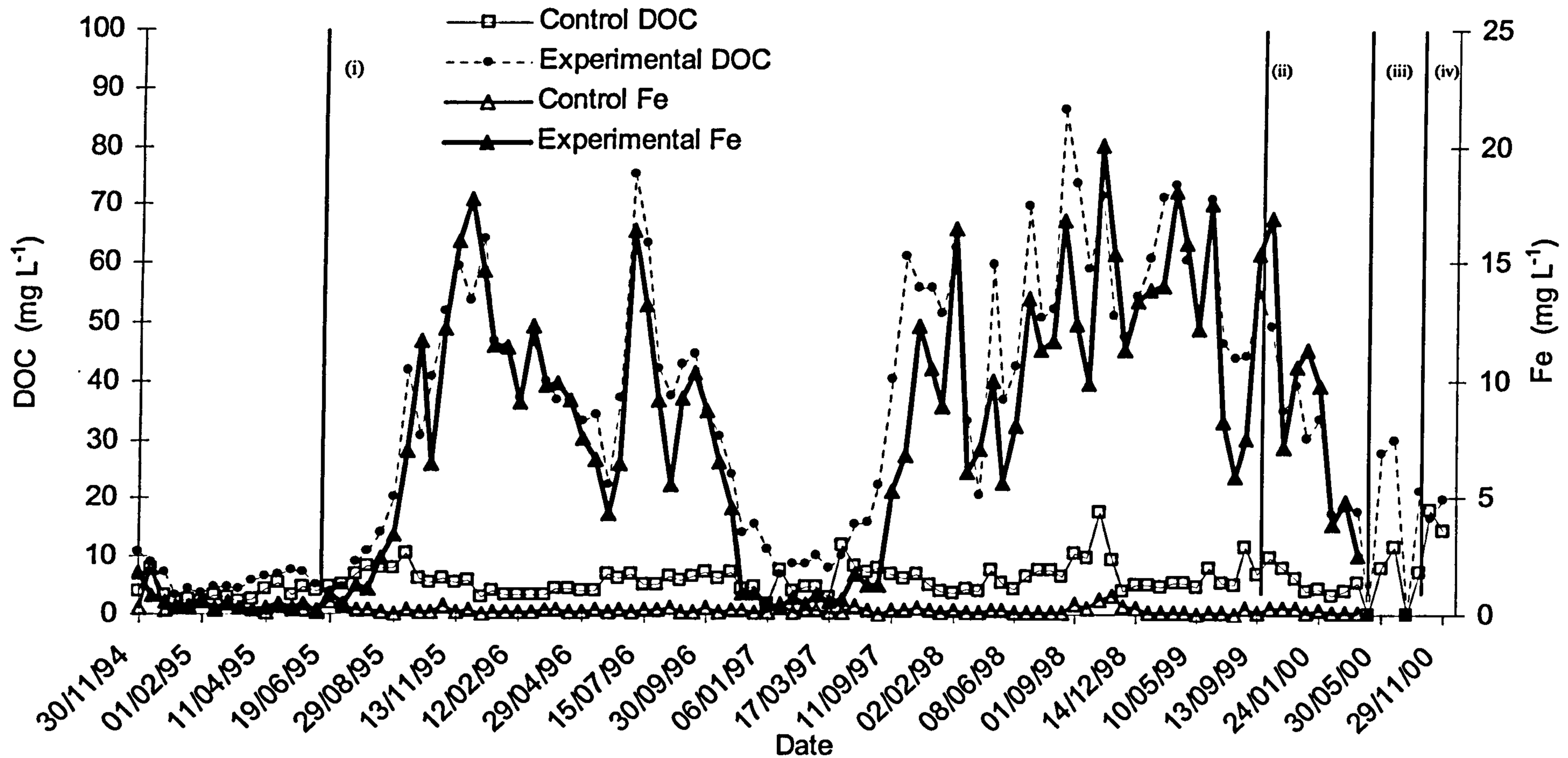


Figure 6.01(a). Mean DOC & Fe concentrations in peat pore waters from control & experimental wetlands.Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), (ii) & (iii) a recovery period, & (iii) & (iv) a shorter simulation. On average standard error of the mean for the control wetland DOC & Fe was 0.39mg L^{-1} & 0.04mg L^{-1} respectively, & for the experimental wetland 26.32mg L^{-1} & 3.51mg L^{-1} respectively, n=5.

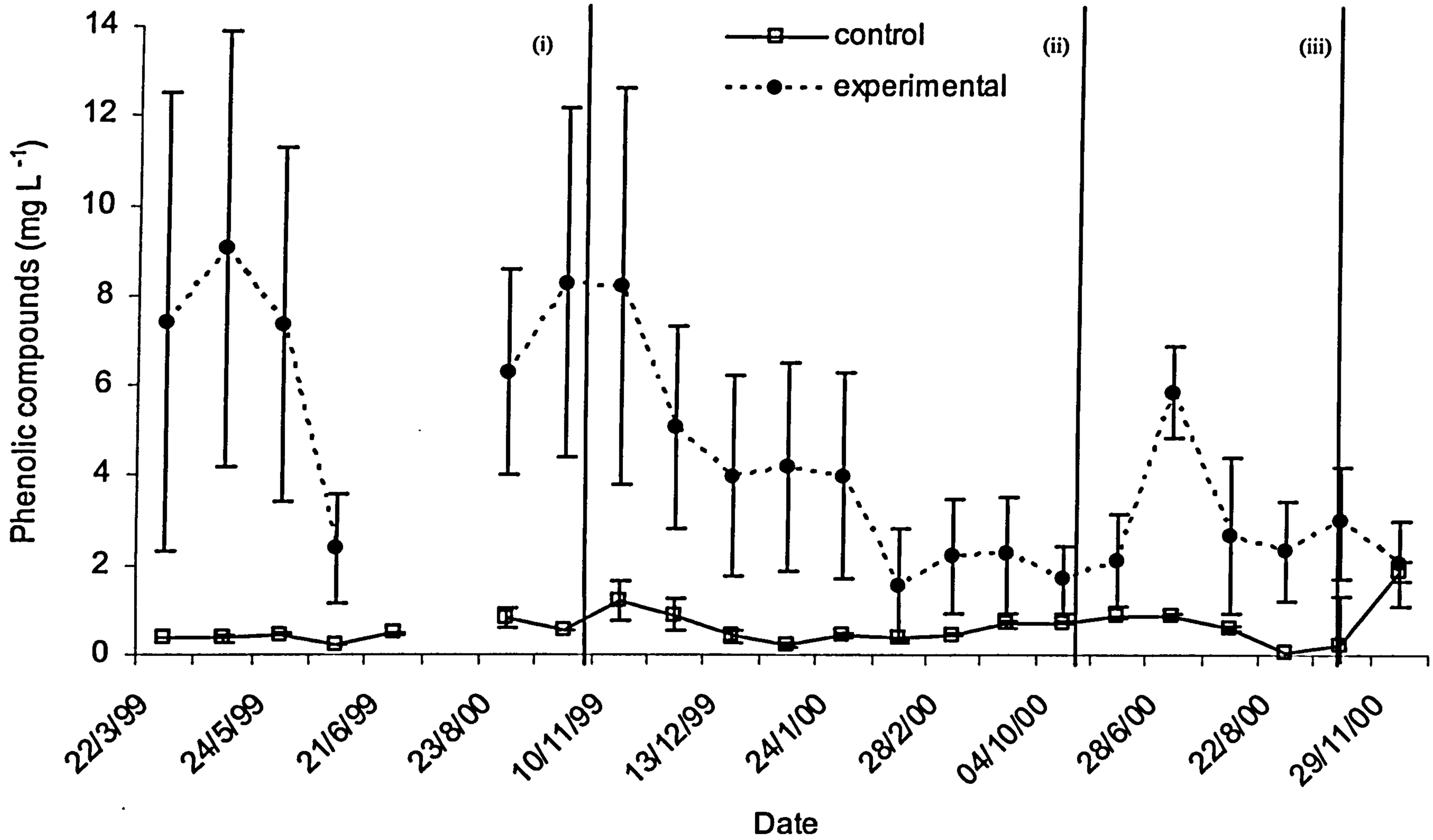
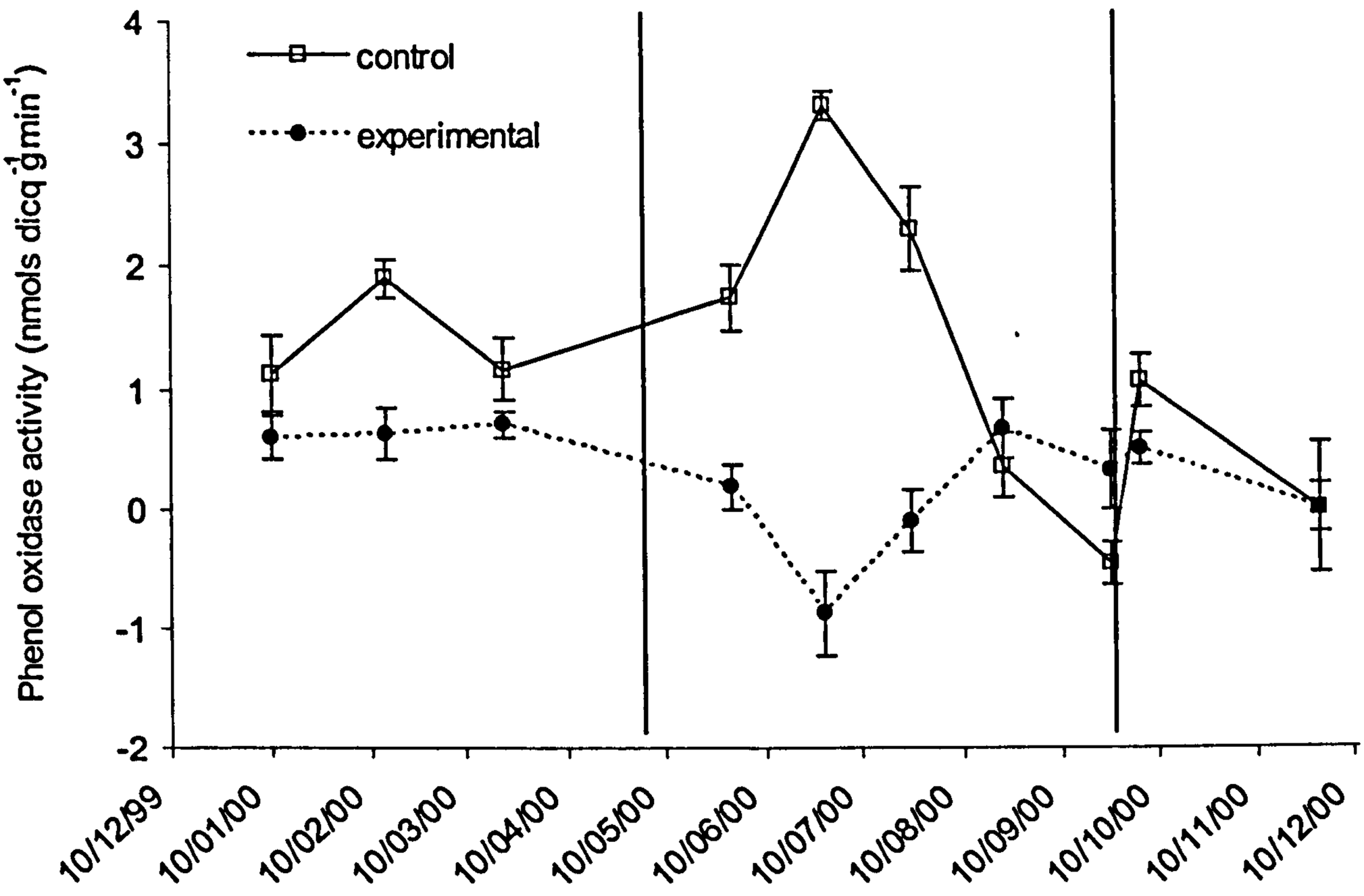


Figure 6.01(b). Phenolic compound concentrations in the pore waters of the control & experimental wetland. Vertical line (i) denotes the end of the continuous increased rainfall simulation, between (i) & (ii) a recovery period in 1999, & between (ii) & (iii) a second shorter simulation. Error bars represent standard error of the mean, n=5.

6.02



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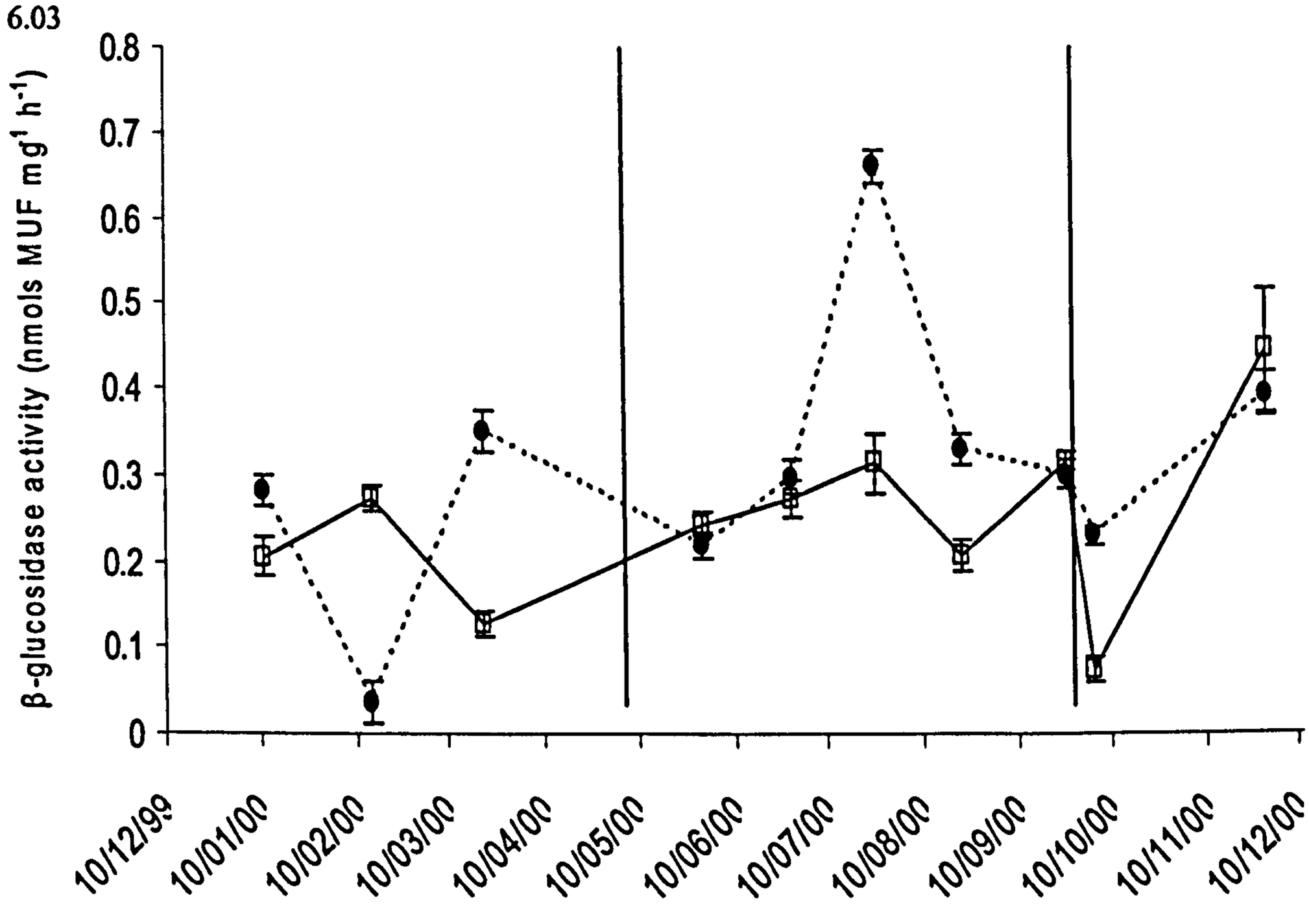
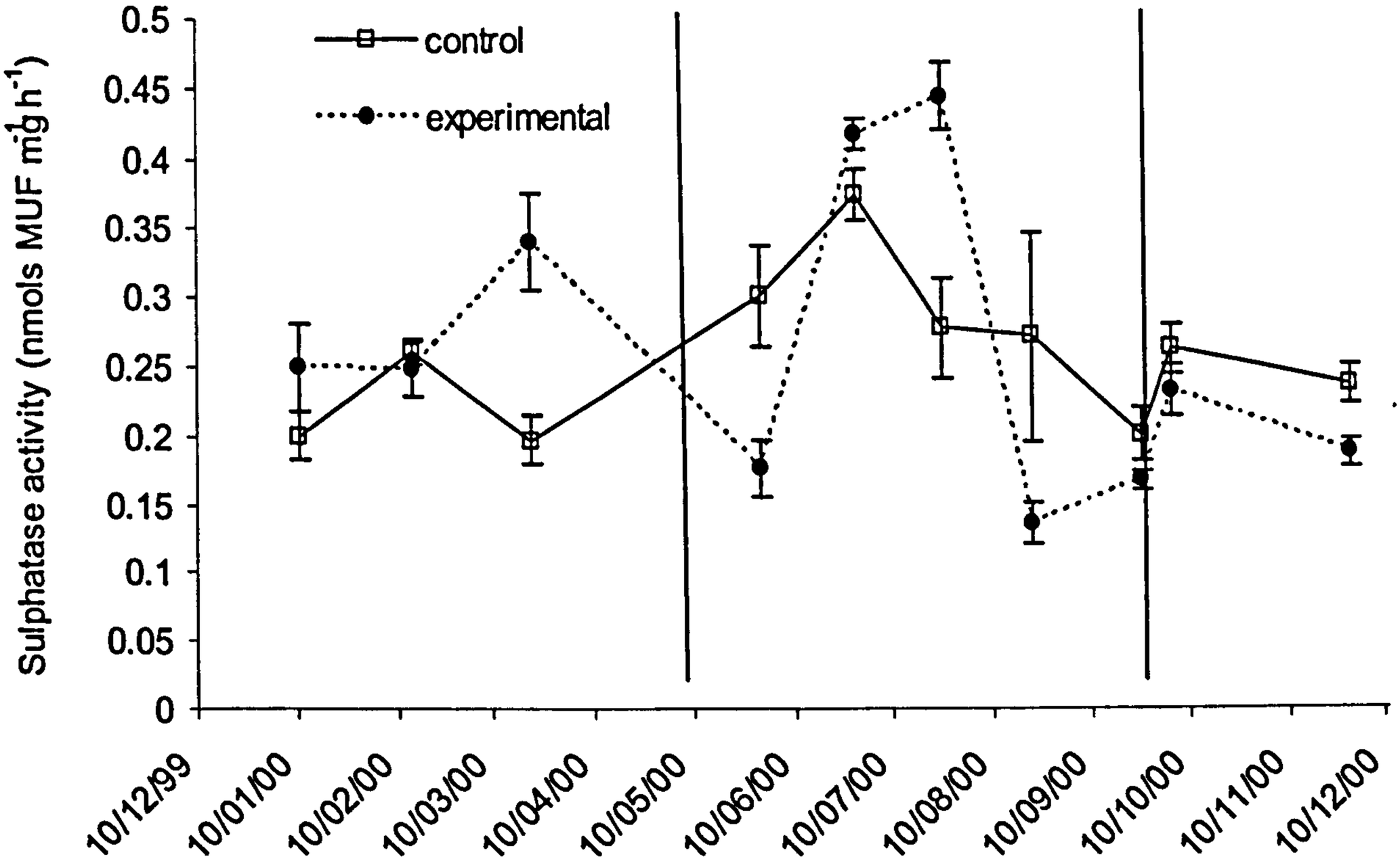


Phenol oxidase (figure 6.02), & β-glucosidase (figure 6.03) activities in control & experimental wetlands. Vertical lines denote the increased rainfall simulation period. Error bars represent standard error of the mean, n=5.

6.04



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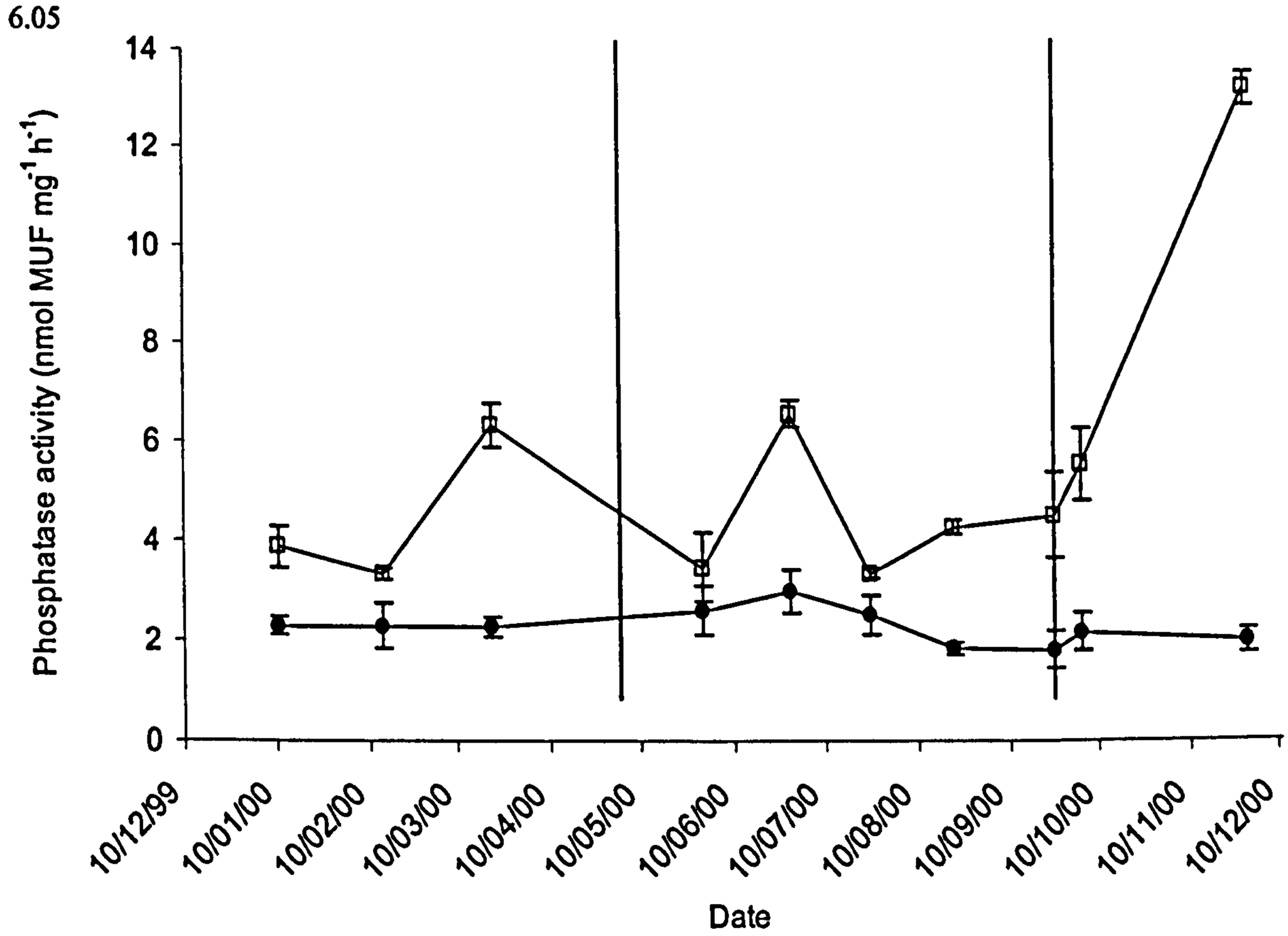

Sulphatase (figure 6.04), & phosphatase (figure 6.05) activities in control & experimental wetlands. Vertical lines denote the increased rainfall simulation period. Error bars represent standard error of the mean, n=5.

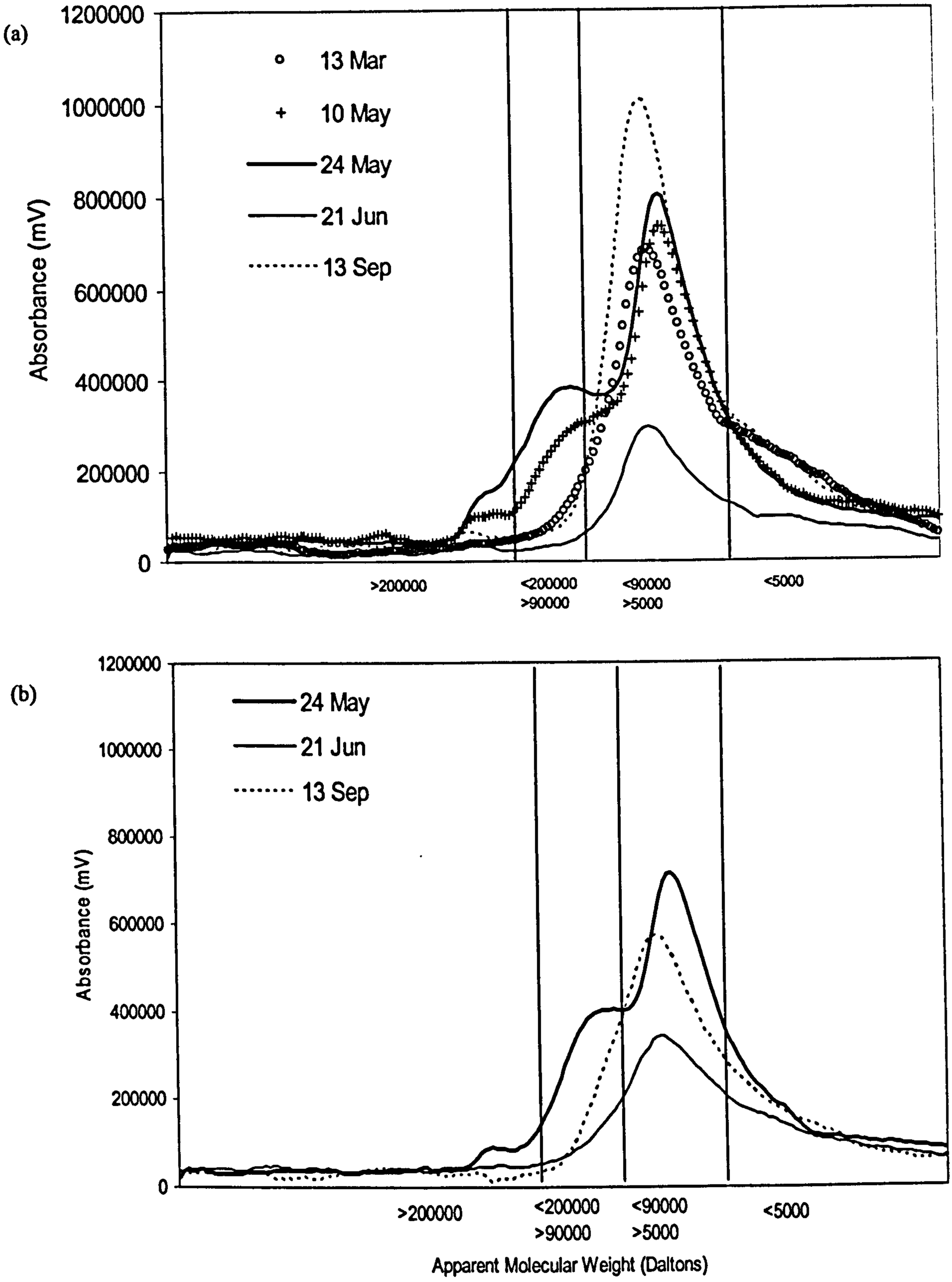

Figure 6.06 (a) seasonal changes in the AMW spectrum of pore water DOC collected from the experimental wetland in 1999 for (a) replicate sample A, where a 40x dilution was used in all cases except for samples taken on the 23/03/99 where a 50x dilution was necessary & (b) replicate sample B, where 20x dilution was used in all cases except for samples taken on the 23/03/99 where a 30x dilution was necessary.

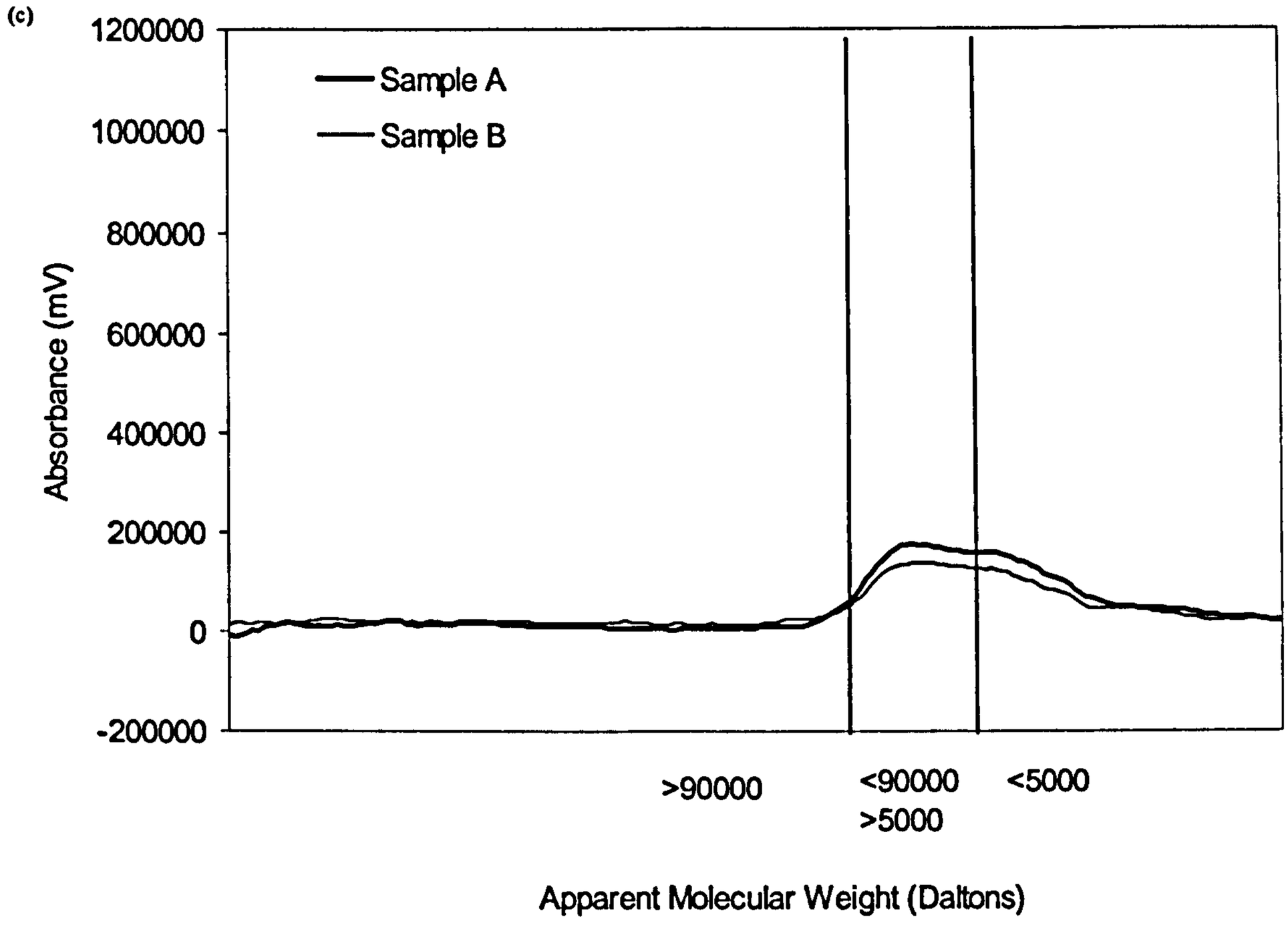

Figure 6.06 (c). A typical AMW spectrum of DOC for samples A & B collected from the control wetland. Samples were collected on 13/09/99 & a 2x dilution was used in all cases.

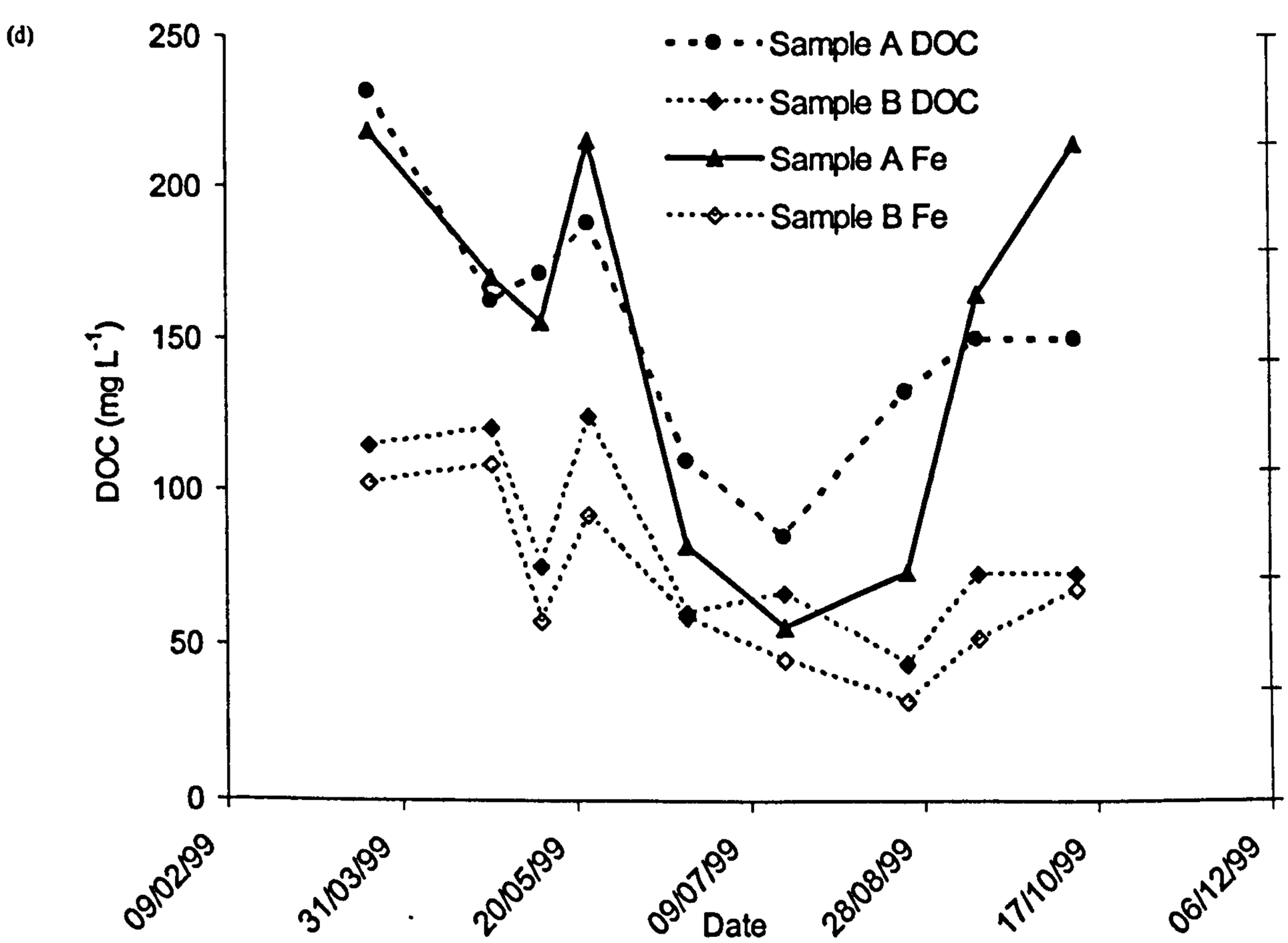

Figure 6.06 (d). Contribution of sample replicates A & B to the seasonal concentrations of DOC & Fe in the experimental wetland.

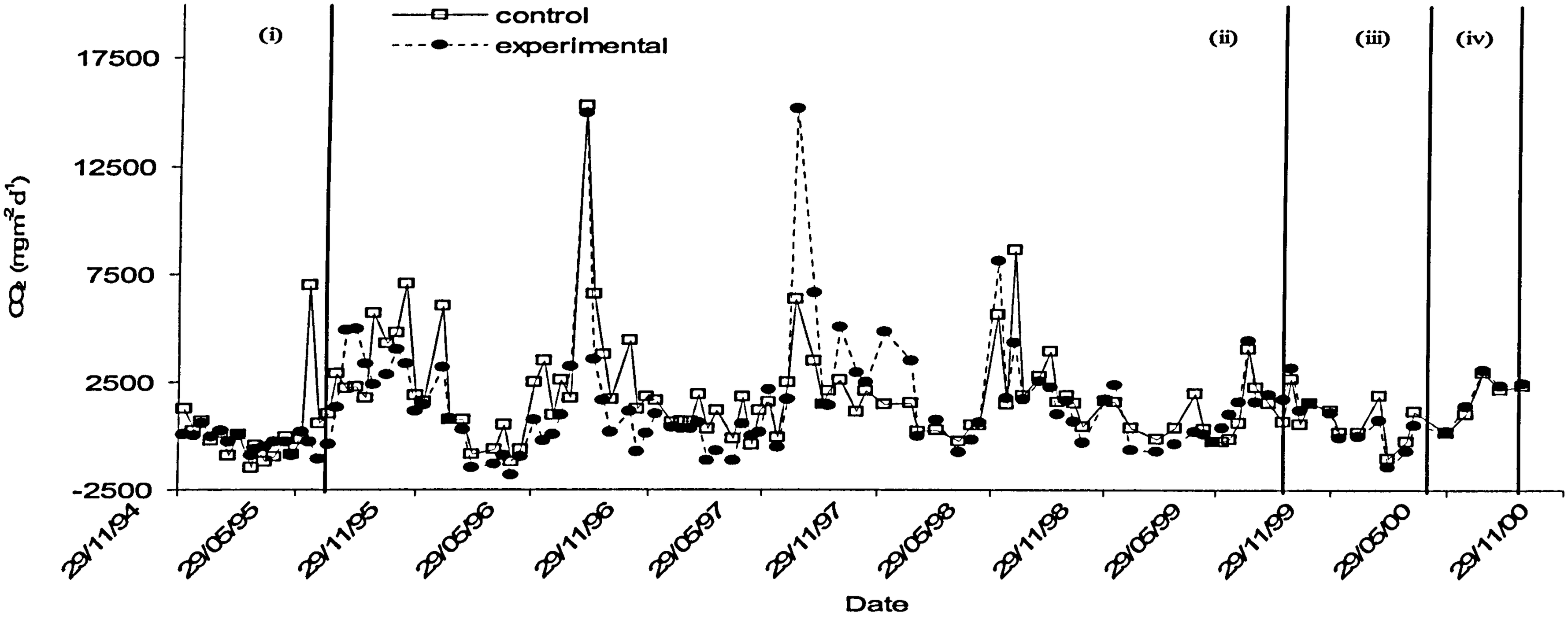

Figure 6.07 (a). Mean CO_2 flux in control & experimental wetlands. Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), (ii) & (iii) a recovery period, & (iii) & (iv) a shorter simulation. On average standard error of the mean for the control & experimental wetlands was ± 621.6 & ± 616.9mg $m^{-2}d^{-1}$ respectively, n=5.

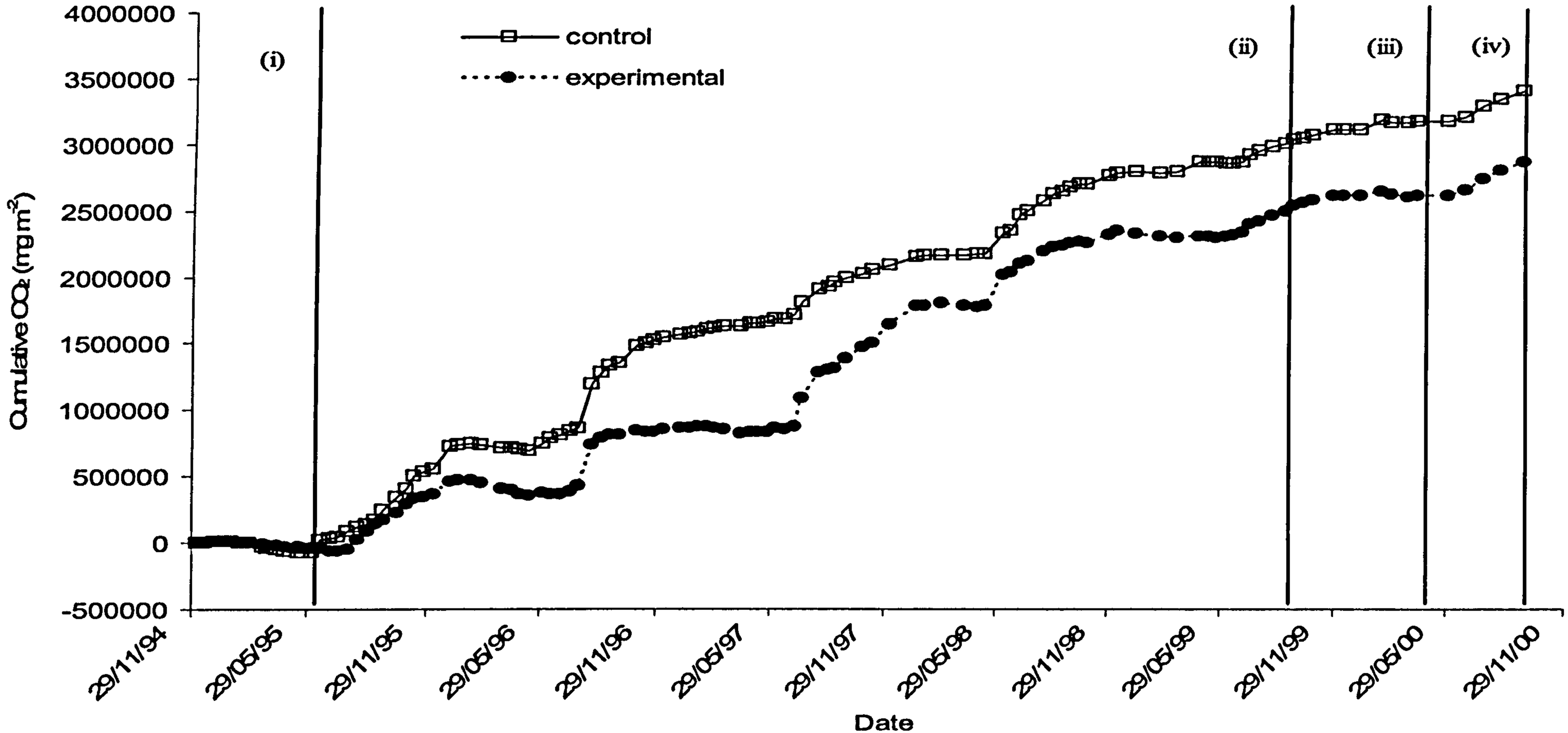

Figure 6.07 (b). Cumulative CO_2 in control & experimental wetlands. Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), lines (ii) & (iii) a recovery period, & lines (iii) & (iv) a shorter simulation.

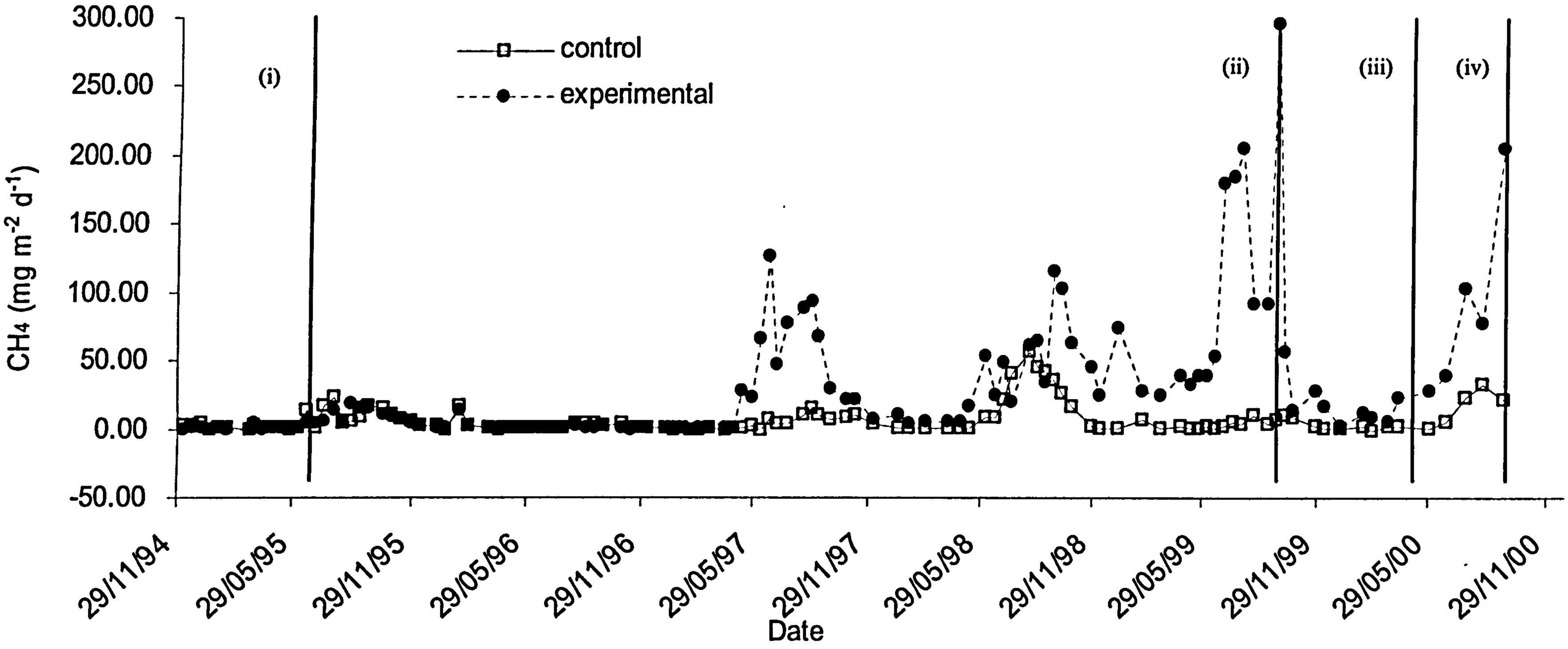

Figure 6.08 (a). Mean CH_4 flux in control & experimental wetlands. Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), (ii) & (iii) a recovery period, & (iii) & (iv) a shorter simulation. On average standard error of the mean for the control & experimental wetlands was ± 1.74 & ± 16.11mg $m^{-2}d^{-1}$ respectively, n=5.

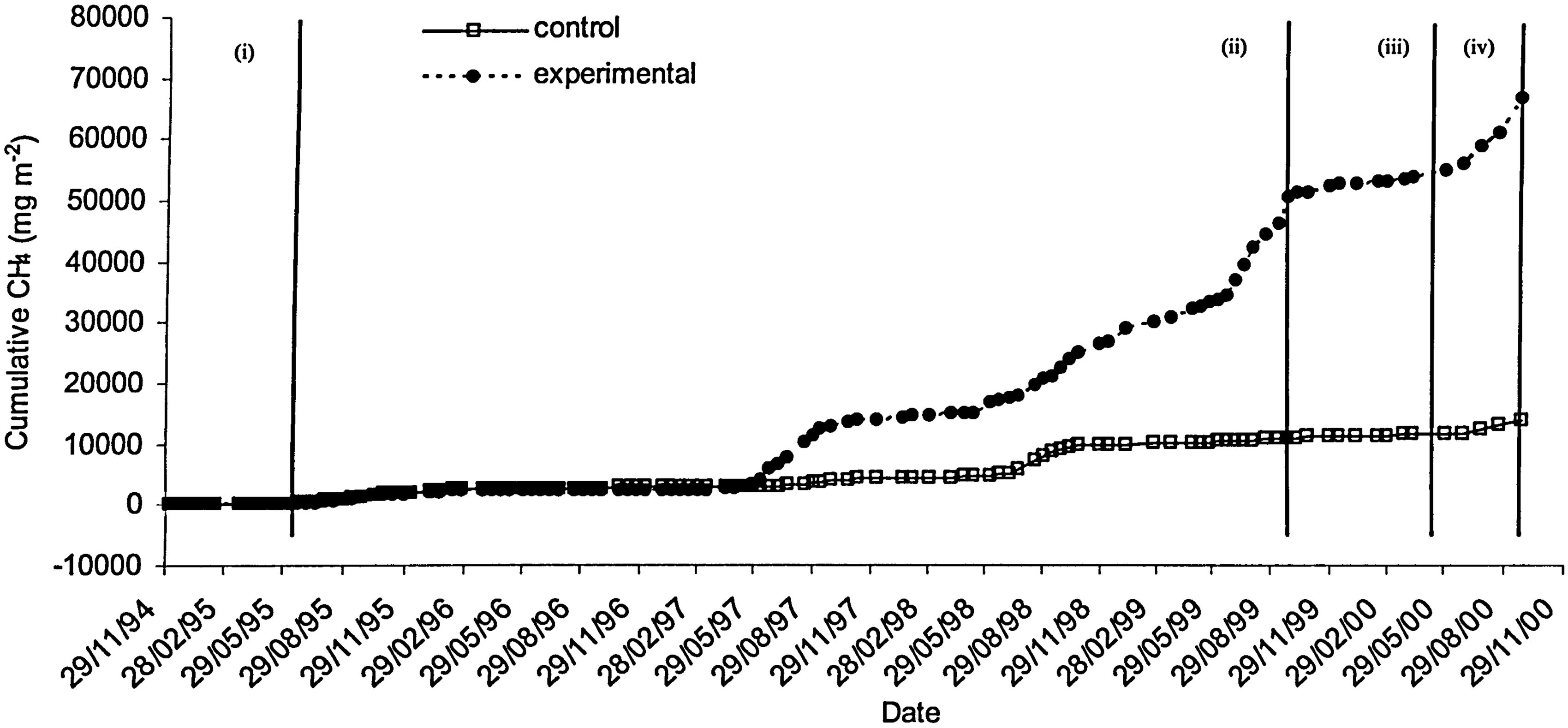

Figure 6.08 (b). Cumulative CH_4 in control & experimental wetlands. Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), lines (ii) & (iii) a recovery period, & lines (iii) & (iv) a second shorter simulation.

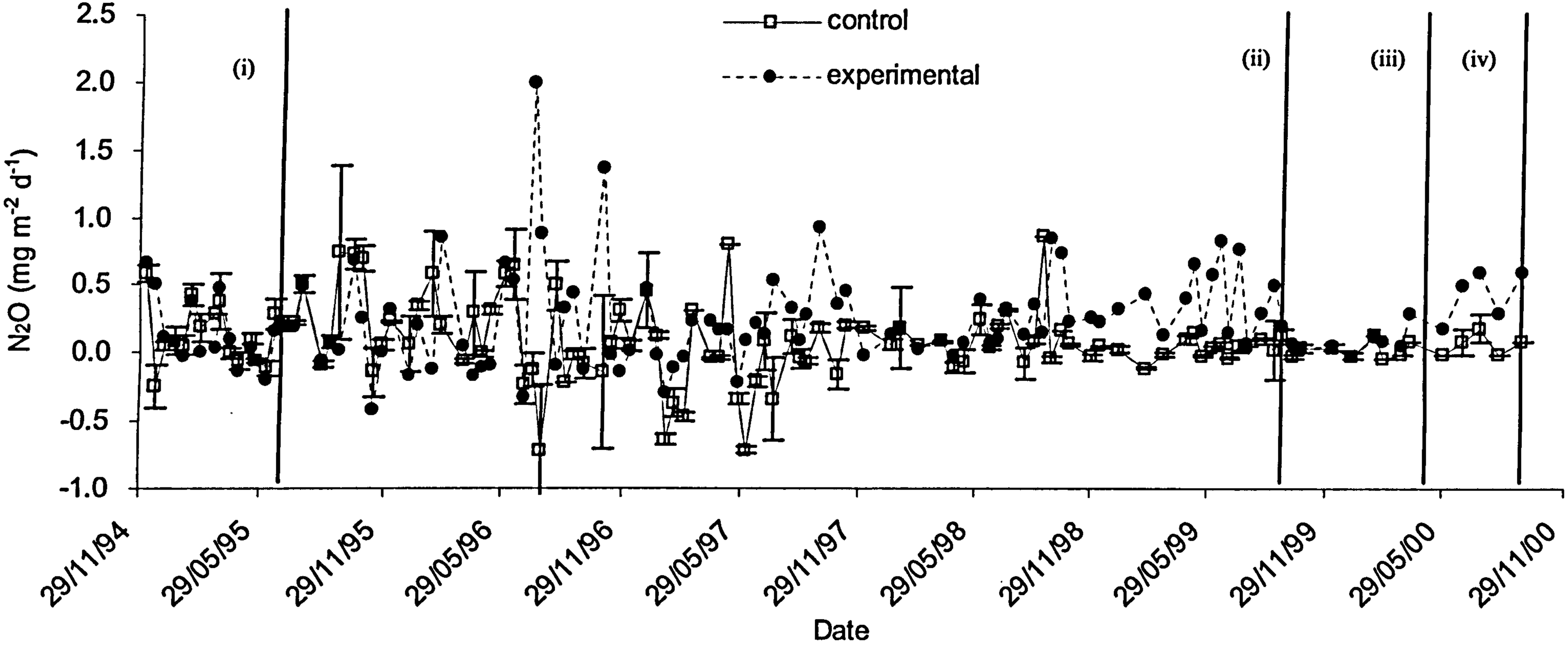

Figure 6.09. Mean N_2O flux in control & experimental wetlands. Vertical lines (i) & (ii) denote a continuous increased rainfall simulation (following a 6 month pre-treatment period), (ii) & (iii) a recovery period, & (iii) & (iv) a shorter simulation. On average standard error of the mean for the control & experimental wetlands was ± 0.1 & ± 0.2mg $m^{-2}d^{-1}$ respectively, n=5.

6.05 DISCUSSION

DOC concentrations and long term trends

The continuous rewet simulation during 1995-1999 produced dramatically increased concentrations of DOC and Fe (figure 6.01a, table 6.01, Fenner *et al.*, 2001; Hughes *et al.*, 1998). These remained elevated in relation to the control even after the increased rainfall simulation had ceased, suggesting that a change in carbon cycling (see below) may have been induced. Similarly, increases in DOC concentrations are also reported for the shorter simulation in 2000. Such increases were not as dramatic as those of the continuous simulation and this may have been due to the recovery period in the winter of 1999/2000, which would have allowed the drainage of the wetland, but also the short period over which the simulation was imposed. There is also the possibility that there has been some ecosystem acclimation to the imposed waterlogging (c.f. Hughes *et al.*, 1999). Nevertheless, the increased DOC concentrations both from continuous and periodic wetting clearly demonstrate a huge potential for reduced water quality in quantitative terms alone. This is likely to be most deleterious in summer/autumn when water quality is already compromised (c.f. Alarcon-Herrera *et al.*, 1994; Betts, 1998; Worrall *et al.*, in press). Seasonal peak concentrations of DOC and Fe can be attributed to increased biological activity in the summer (Hughes *et al.*, 1990; Worrall *et al.*, in press), leading to enhanced production of DOC and complexation of Fe in solution by organic ligands.

Increased DOC concentrations as a result of increased water inputs are consistent with other field, laboratory and modelling studies. Forsberg (1992) correlated increased rainfall with increased humic acid concentrations in Swedish lakes, and significantly increased DOM exports from upland moorland soils (brown earth and micropodzol) have also been reported as a result of additional water inputs in the field (Tipping *et al.*, 1999). Rain simulation experiments using intact soil cores have demonstrated that water draining through the upper horizons of a typical upland soil has a significantly higher DOC concentration than that from the whole core (Edwards, 1984). Rapid water movement through the upper horizon (due to increased water volumes) may allow DOC-rich water to by-pass adsorption sites in the deeper soil horizons (Moore, 1989). Based on modelling results from Canadian watersheds, Clair *et al.* (1999) also predict that DOC release will increase by around 14% with a doubling of atmospheric CO_2 concentrations, primarily due to increased runoff.

The anaerobic degradation pathway is said to favour the production of DOC compounds such as aldehydes and ketones, rather than rapid CO_2 production as is the case for the aerobic pathway (Ponnamperuma, 1972), and so increases in DOC concentration as a result of increased water inputs perhaps are intuitive. However, increased DOC concentrations (as a

function of depth) in marine and estuarine sediments have been attributed to a number of processes that also may be relevant here. These include *in situ* formation within sediments from the condensation of low AMW constituents to generate more stable macromolecules (Krom & Sholkovitz, 1977) and the partial selective mineralization of particulate organic matter (POM) by biotic and abiotic reduction processes (Burdige & Gardner, 1998; Canuel & Martens, 1993, 1996; Hatcher *et al.*, 1983). One other possible mechanism, mentioned below, is the release of sorbed DOC from the dissolution of metal oxide in sediments under reducing conditions (Chin *et al.*, 1998).

However, Worrall *et al.* (in press) reported that the 30 year increasing DOC trends observed in two out of the three Yorkshire catchments they studied were unlikely to be due to increased total rainfall as all catchments had been subject to similar conditions. But, the authors suggest that it was possible that such trends could have been due to some unobserved changes in climate, such as changes in rainfall intensity etc. When DOC concentrations in the pore waters of the experimental wetland were analyzed for trend, a surprisingly large increase of 5.2 mg $L^{-1}yr^{-1}$ was found. However, this was not statistically significant due to large natural variation and a data set of only five years must be interpreted tentatively. Perhaps of more concern is the significant increasing trend (0.87 mg $L^{-1}yr^{-1}$, $P<0.01$) detected in the control wetland, which again highlights the fact that DOC concentrations are already increasing, seemingly as a result of a changing climate, in line with the trends reported in numerous UK rivers (Freeman *et al.*, 2001a etc).

Phenolic compound concentrations (figure 6.01b) also increased dramatically under the continual increased rainfall simulation (1116.41%, $P<0.001$) and the effects remained in evidence during the recovery period (436.96%, $P<0.01$), suggesting a prolonged response is likely. During the 2000 simulation, phenolics concentrations remained higher relative to the control (424.77%, $P<0.1$ only) and although the previous wetting period may have contributed substantially, the pronounced peak in concentrations soon after the 2000 simulation began suggests that the latter had an impact on carbon processing. Moreover, this periodic waterlogging may be more realistic than the continuous simulation carried out previously at the site. Phenolic substances may be especially deleterious to water quality given their persistent nature and the fact that high ratios of phenolic compounds:DOC inhibit biofilm metabolism (Freeman *et al.*, 1990). The increased rainfall simulation produced a dramatic selective enrichment of phenolic materials, from which it could be inferred that DOC consumption by the biofilm in the recipient stream will be inhibited. A positive feedback to increased DOC concentrations could therefore be anticipated with retarded decomposition of DOC irrespective of origin, thus reducing water quality further.

DOC concentrations and enzyme activities

The mechanism for the enhanced peatland DOC and phenolic compound release may relate to altered carbon cycling enzyme activities. Phenol oxidase activities (figure 6.02, table 6.01) were significantly suppressed following the continuous rainfall simulation, during the recovery period of 1999/2000 (-63.08%, $P<0.01$) and as a result of the 2000 simulation (-103.33%, $P<0.001$), with activities being arrested at certain times. Such results support the original hypothesis and suggest that the biodegradation of phenolic compounds was inhibited by the increased waterlogging. This is consistent with the findings of McLatchey and Reddy (1998), who could not detect phenol oxidase activity under waterlogged conditions, and the work of Freeman *et al.*, (2001b) where activities were stimulated by aeration. Furthermore, the effect of such waterlogging on phenolic compound cycling can apparently be prolonged, since substantial concentrations of these materials were present even after the increased rainfall simulation had ceased.

From such results, it might be anticipated that the hydrolase enzyme activities would be accordingly inhibited due to the accumulation of phenolic materials (Freeman *et al.*, 2001b). However, this does not appear to be the case as ß-glucosidase activities were not suppressed during either the recovery period or the wetting phase of 2000 (figure 6.03, table 6.01). These results confirm the findings of Freeman *et al.* (1998) who found no change in ß-glucosidase activities at the same site. The authors suggested that the current natural levels of wetness were sufficient to limit activities and therefore that an increase in rainfall will cause no further decrease in decomposition. Given that anaerobic respiration produces low molecular weight aldehydes and ketones (Ponnamperuma, 1972) and hydrolases are not directly inhibited by waterlogging (Freeman *et al.*, 2001b), it may be that ß-glucosidase activities are sustained by the presence of the low molecular weight materials (substrate induction). Alternatively, ß-glucosidase may generate material from the higher molecular weight DOC (see chapter 3) that has been allowed to accumulate as a result of the suppressed phenol oxidase activities. A strong correlation was found between DOC and ß-glucosidase activities (0.8118, $P<0.05$), and between ß-glucosidase activities and phenolic compound concentrations (0.8889, $P<0.01$) in the experimental wetland. This suggests that ß-glucosidase plays an important role in carbon dynamics within the system, but does not elucidate the problem. However, the latter mechanism might explain why the DOC concentrations in this experiment are strongly correlated with phenolic compound concentrations (0.9419, $P<0.01$ and 0.8843, $P<0.01$ in the experimental and control wetland respectively). The fact that ß-glucosidase activities were not inhibited by the simulated increased rainfall, while phenol oxidase activities were significantly constrained may

serve to increase the phenolics:DOC ratio if the more labile material is utilized relatively rapidly.

Sulphatase activities, like those of ß-glucosidase, showed no evidence of suppression under the increased rainfall simulation (figure 6.04, table 6.01), in contrast to the work by Freeman *et al.* (1998) who reported a 44% ($P<0.001$) suppression of activities at the same site under the continuous simulation. This might mean that there has been some acclimation to waterlogging by the sulphatase producing communities, allowing sulphatase production and/or activity to be maintained in the 2000 simulation. Organic matter decomposition *via* the fermentation sulphate reduction pathway may therefore continue under conditions of increased waterlogging, generating peat decomposition products (e.g., DOC) and adding to those produced *via* the mechanisms discussed previously.

In contrast, phosphatase activities were consistently lower in the experimental wetland compared to the control site under both treatment and recovery conditions (figure 6.05, table 6.01). This may be due to inherent differences between the two sites, differences induced by five years of hydrological manipulation, or enhanced phosphate availability as a consequence of the increased water input to the site (meaning less phosphatase is required to acquire inorganic phosphate). A further possibility is that phosphatase is more sensitive to phenolic inhibition in comparison to the hydrolases above, and that current levels of saturation are not sufficient to limit activities.

DOC and Fe associations

High Fe contents are characteristic of many highly coloured rivers in peat dominated drainage basins (Wartiovaara, 1978) and Fe is carried into such waters complexed with humic substances (Ghassemi & Christman, 1968; Shapiro, 1964), other transport mechanisms (Gibbs, 1973) being of lesser importance (Heikkinen, 1990). Iron exists predominantly in a ferrous state in peatland water under anaerobic conditions (Takkar, 1969). The anoxic-oxic boundary layers in the hydrologically active surface peat are likely to be the most important sites for the formation of colloidal Fe-organic complexes (Heikkenen, 1990), as in bog lakes (Koenings & Hooper, 1976). There is also partial precipitation with OM in ochre formation at this boundary (Kuntze, 1982; Puustjärvi, 1953). The water table position and its fluctuations are closely connected with the location of this oxic/anoxic boundary in ombrotrophic (Damman, 1978) and minerotrophic peatlands (Heikkinen, 1990). Increased Fe concentrations from more waterlogged peat, as was found here, is supported by work done by Puustjärvi (1953) who attributed similar results to increased water flow from deeper anaerobic peats. Increased acidity is also known to reduce the Fe holding capacity of humic materials (Saar & Weber, 1982; Shapiro, 1964). However, this

seems unlikely to have caused the increase in Fe concentration here because the pH in the pore water of the experimental wetland did not change markedly following wetting, both sites fluctuating within the range of pH 3.9-4.8.

The strong correlation between Fe and DOM can clearly be seen in figure 6.01a and this has also been observed by Chin *et al.* (1998), among many others. They studied the abundance and properties of DOM in a wetland adjacent to Lake Erie (USA). Ferrous Fe and DOM were thought to co-accumulate in the pore fluid and it was hypothesized that reduction of Fe oxides coated with OM releases both DOM and Fe. Despite the fact that interactions between colloidal humic substances and macromolecular forms of Fe (along with the environmental factors governing them) are poorly understood (Mill, 1980), it is generally accepted that Fe has a key role in determining the nature of humic substances present (Heikkinen, 1990). It has also been assumed that the generation of the humic fraction is promoted by Fe (Shapiro, 1964). In organically coloured rivers with high Fe concentrations, changes in the Fe content of DOM might be of particular importance as this may be one factor controlling the degradation and abiotic particle formation processes of humic substances (Heikkinen, 1990). Such processes have been described by Armstrong *et al.*, (1966), Kuntze (1982), and Petersen (1986) in peat and freshwater systems.

Generally, the results of this study confirm the importance of DOC for colour formation and that there is seasonality in colour forming properties, both as a result of the varying nature of the DOC and due to its association with Fe, consistent with the work of Heikkinen (1990). Increasing Fe concentrations in the filtrates of the experimental wetland, especially in the summer/autumn, would reduce water quality, reinforcing that due to increased DOC concentrations at a time when demand and thus transportation from such catchments to areas of deficit is likely to be highest. The seasonal differences may be at least partially due to temperature-dependent microbial processes, such as anaerobic decay.

Figures 6.06a-c show a marked difference in the AMW spectra of DOC between the control and experimental sites, the latter having two additional peaks of high molecular weight material (corresponding to AMW ranges of >90000 to <200000 and >200000) in addition to selective enrichment of the >5000 to <90 0000 AMW fraction. The spectrum of DOC in the experimental site changed dramatically with season; the >90000 to <200000 AMW fraction could only be seen in spring (April/May). Concentrations of DOC and Fe in the control wetland were far lower and more consistent, with a small amount of DOC in the AMW ranges >5000 to <90000 and <5000 being detected throughout the year. Broadly similar fractions have

been reported in other studies of humic freshwaters (Alarcon-Herrera *et al.*, 1994; Lock & Ford, 1986; Shin & Lim, 1996).

The selective enrichment of the >5000 to <90000 AMW material under the increased rainfall simulation is of concern (even without the Fe association), since Alarcon-Herrera *et al.* (1994) suggest that removal of fractions distributed in this mid range of the DOC spectrum is less efficient than that of higher molecular weight material by conventional water treatment processes. Moreover, the results show that this fraction is responsible for complexing most of the Fe released from the wetland (table 6.02) as a result of leaching, in line with findings from other ecosystems (e.g., Goodman *et al.*, 1991), peatland and lake water studies (Ghassemi & Christman, 1968; Heikkinen, 1990; Koenings & Hooper, 1976). The >5000 to <90 000 AMW fraction is likely to consist of predominantly fulvic materials, which are said to be in the range of ca. 1000-30 000 Da and composed of highly oxidized aromatic rings with numerous side chains (Paul & Clark, 1989). Phenolic acids and benzene carboxylic acids are the fundamental units held together primarily by hydrogen bonding or van der Waals' forces and ionic bonding (Paul & Clark, 1989). X-Ray analysis, electron microscopy and viscosity measurements suggest that fulvic acids have a relatively open, flexible structure perforated with voids of various dimensions. Organic and inorganic compounds that fit into the voids can become trapped, provided the charges are complementary, perhaps accounting for the fact that this fraction showed the strongest Fe association in the AMW spectra. Sorbed OM present on Fe oxides is probably enriched with aromatic moieties (Gu *et al.*, 1996; Wang *et al.*, 1997) reinforcing the notion that such material will persist in the recipient waters. Humic substances, even in highly aerated waters, maintain increased Fe concentrations in solution as Fe (II) complexes (Theis & Singer, 1973), while reducing Fe (III). The potential for a substantial and persistent increase in colour is therefore considerable.

A high Fe content may, on the other hand, promote the sedimentation and decomposition of this relatively high molecular weight DOM in the recipient river in summer (Heikkinen, 1990), which may mitigate to some extent the adverse effects of waterlogging on water quality. The role of heterotrophic, Fe-organic colloid decomposing bacteria might be of importance in these processes because total river bacterioplankton density is said to increase with filtered Fe concentration (Heikkinen, 1990). Usually, heterotrophic and mixotrophic microorganisms capable of the mineralization of Fe-organic compounds are distributed in the water (Kuntze, 1982). Preferential UV oxidation of Fe-organic colloids may also occur (Armstrong *et al.*, 1966) and Shapiro (1964) reported that increased Fe content could make these colloids more susceptible to coagulation by electrolytes.

Humic acids are generally composed of higher molecular weight materials (ca. 10 000-100 000 Da) and these are likely to dominate the >90 000 to <200 000 ("spring peak") and the >200 000 AMW fractions that were only detected in the experimental wetland. Humic compounds, particularly humic acids, are the cause of much of the colour present in many rivers (Visser, 1984), bogs and wetlands (Thurman, 1985), therefore the increased input of such material is likely to have profound effects on water quality in the receiving aquatic system. The absorbance of humic acid over the wavelength range of 250-350 nm has been found to decrease with increasing fraction size in humic acid derived from peat (Tombácz, 1999). As the nominal size fractions decreased, the ratio of absorbance at 465 and 665 nm (the E4/E6 ratio) was also found to increase significantly from 6.6 to 14.7. This perhaps indicates a systematic change in chemical composition as this parameter is related inversely to the degree of condensation of aromatic group or the molecular weight (Rao & Choppin, 1995). Tombácz (1999) also looked at fluorescence emission spectra at several excitation wavelengths, finding significant differences in the emissions of the humic acid fractions; the smaller the size fraction the greater the fluorescence emission. Cross Polarization Magic Angle Spinning ^{13}C-Nuclear Magnetic Resonance (CPMAS ^{13}C-NMR) was also used to study the differences in chemical composition of the humic acid fractions. Aliphatic character was found to predominate in the unfractionated peat humic acid and became more pronounced in the largest sized fractions, while aromaticity increased with decreasing humic acid molecular size as did the abundance of functional (O-containing) groups (Tombácz, 1999). These studies suggest that there is probably a range of substances with differing properties within the AMW fractions separated here.

The 'spring peak' (>90 000 to <200 000 AMW fraction) represents a transient pool of carbon that is relatively rapidly transformed in, or transported from the site. The *in situ* processes involved in the formation and removal of this fraction are not clear, but may relate to increased production by plants and/or microbes as conditions become more conducive to growth at the start of the growing season. Another possibility is that this peak is formed as a result of a specific population of microbes that may, for example, only proliferate under these conditions. Such material may be important should rainfall and flooding events increase because this fraction appears to make a significant contribution to total DOC and Fe levels (figure 6.06d). Furthermore, Alarcon-Herrera *et al.* (1994) identify high TTHM concentrations in April and May, attributing this to the spring turnover in water bodies. The effects of this phenomenon may be exacerbated by the production of the 'spring peak' material in peat dominated catchments, due to climate change or land use practices.

The production of the two high AMW fractions in the experimental wetland are also of concern from a water quality perspective because such material is believed to be microbially inhibitory (Freeman *et al.*, 1990; Freeman & Lock, 1992) and thus, the possibility of reduced DOC decomposition of all molecular weights could be foreseen. Even more DOC and Fe would then persist until treatment, potentially increasing TTHM formation and further reducing water quality.

The shift in AMW spectrum with increased water input may relate to the change in predominant mode of metabolism (i.e., increased anaerobic respiration) and the altered enzyme activities observed in the experimental wetland. Suppressed phenol oxidase activities would allow the build up of phenolic and lignin derived compounds in the pore waters (Freeman *et al.*, 2001b), accounting for the higher molecular weight fractions and these may impair the breakdown of the lower molecular weight materials or provide a substrate for their production (e.g., *via* the action of ß-glucosidase). It is thought however, that much of the pore fluid DOM in reducing environments comprises partially decomposed, sediment derived carbohydrates (Hatcher *et al.*, 1983) and that lignin degradation (a potential source of aromatic moieties) is minimal (Chin *et al.*, 1998). The carbohydrate fraction is removed by aerobic microorganisms under oxidizing conditions and the aromatic content of the DOM pool increases as a result of lignin degradation (Benner & Hodson, 1985). Reducing conditions can cause even relatively labile non-aromatic organic molecules to be appreciably slow (Canuel & Martens, 1996). Their study may provide evidence to suggest that the degradation of more recalcitrant sediment OM (e.g., lignin components) does not occur to any great extent in reducing sediments and that it is not the source of the pore fluid aromatic DOM (Chin *et al.*, 1998). This may be true in this case, however, there may have been considerable aerobic degradation of lignin compounds before waterlogging occurred because of the relatively well drained nature of the peat. Such compounds would then persist under the reducing conditions imposed, possibly retarding the degradation of less refractory DOC allowing its accumulation. Similarly, the surface layer of peat might still support limited aerobic decomposition of ligniferous materials (c.f. figure 6.02) and physical or chemical release of organic materials is also likely. There is however, no evidence to rule out the generation of high molecular weight materials from lower molecular weight units under the experimental conditions.

Burdige and Gardner (1998) presented an alternative DOM pore water preservation and accumulation model. They observed that 60-90% of the organic carbon present in the pore waters of three different marine sediments passed through a 3000 Da ultra filter. It has been suggested (Amon & Benner, 1994, 1996; Guo *et al.*, 1996) that the high molecular weight compounds in marine surface waters are more labile than was previously thought and that much

of the refractory organic pool comprises complex but smaller organic compounds. Indeed, both the results of Burdige and Gardner (1998) and those of Chin *et al.* (1998) suggest that a significant portion of the OM in freshwater and marine sediments may also be composed of these recalcitrant 'polymeric low molecular weight' (p-LMW) substances (Chin *et al.*, 1998). Burdige and Gardner (1998) argue that larger macromolecules are broken down into two pools of organic carbon composed of rapidly mineralized simple organic compounds and p-LMW substances that will accumulate because of their refractory nature. This may be relevant in the production of the lower AMW materials (<5000 and >5000 to 90 000 Da) in this study. Furthermore, Alarcon-Herrera *et al.* (1994) found that the lowest AMW fraction of humic substances (3000 to 5000 Da) in the natural waters they studied showed the lowest percentage removal by conventional water treatment processes. Chin *et al.* (1998) proposed that the observed increases in pore water OM, polydispersity and molecular weights in the upper 3-5 cm of the sediments they studied are likely to be the result of both the of Burdige-Gardner model and the release of pre-sorbed terrestrial OM (during the reductive dissolution of Fe oxides in the pore fluids), as may be true in this situation. The latter perhaps explains the selective enrichment of the >5000 to <90 000 AMW fraction and associated increased Fe levels in the experimental wetland. Although the models discussed are not all from peat systems, such studies serve to illustrate that the mechanisms of DOM accumulation are likely to be complex and much work is required if we are to fully understand them. Humic substances show a huge variety of multifunctional source structures which, due to the randomness of formation, cannot be expected to behave uniformly and only trends can be predicted for their properties (Tombácz, 1999).

This simulation not only produced more total DOC with the potential to colour waters, but also a marked shift to the visible spectrum of fulvic material due to Fe-OM complexation. Highly coloured, rusty-brown pore water was clearly observed and a huge increase in the amount of material absorbing in the visible range. Thus, the aesthetic qualities of waters draining peatland catchments could be dramatically reduced in a future climate. The increased bromide concentrations reported by Hughes *et al.* (1998) are of further concern in relation to water quality. Naturally occurring bromide has received attention recently because, in association with humic substances in raw waters, it is readily incorporated into haloacetic acids (HAAs) in an organically bound form during water chlorination and chloramination (see Cowman & Singer, 1996; Hughes *et al.*, 1998). It is possible that DOC and Fe increases were mainly the result of a flush effect (reflecting increased solubility and leaching processes) but that the lag period of recovery is longer than for bromide (Hughes *et al.*, 1998). However, this seems unlikely given that the carbon cycling enzyme activities (phenol oxidase and ß-glucosidase) and the AMW spectra of DOC were significantly changed by the rainfall simulation.

Trace gas fluxes

Carbon dioxide fluxes during the 1999 and 2000 simulation remained similar to the control, consistent with the CEH long term data set (figure 6.07a). Cumulative figures show a slight decrease in CO_2 emissions from the experimental wetland (figure 6.07b), perhaps as a result of an increase in anaerobic metabolism and consequently a reduction in the rapid mineralization of DOC to CO_2.

Fluxes of CH_4 from the two wetlands over the first two years of the experiment were small (see figures 6.08a & 6.08b). However, with time a substantial increase in CH_4 release from the experimental site occurred. Seemingly, the methanogen populations had taken approximately two years to proliferate and become active following the initiation of experimental conditions. Increased CH_4 emissions were expected, since production is a strictly anaerobic microbial process in which methanogenic microorganisms reduce OM in the absence of other electron acceptors (Dasselaar *et al.*, 1999). And, increased DOC concentrations are likely to represent a substrate for methanogenesis (cf. van Veen *et al.*, 1989). Furthermore, CH_4 consumption is an aerobic microbial process in which CH_4 is oxidized by methanotrophs (Dasselaar *et al.*, 1999) and is thus likely to have declined under waterlogged conditions. The lag period may be due to the production of an aerobic hydrophobic layer at the surface of the peat as a result of the well drained nature of the site, which would retard saturation upon rewet. However, there are numerous factors which influence both CH_4 production and consumption thereby determining net fluxes (see e.g., Dasselaar *et al.*, 1999; Segers, 1998), including the type and amount of available OM (Dasselaar *et al.*, 1999). While cessation of the simulation during autumn 1999 resulted in a sharp fall in the water table and concomitant attenuation of CH_4 emissions from the experimental site, during the 2000 simulation the mean CH_4 emissions were rapidly and substantially increased. From such results it could be inferred that once established the methanogen population can respond more rapidly when anaerobic conditions return.

Most studies of N_2O fluxes have concentrated on either the effect of extreme drought (Freeman *et al.*, 1993b) or drainage (Martikainen *et al.*, 1993; Nykänen *et al.*, 1995). However, under the rainfall simulation N_2O emissions also eventually increased (figure 6.09). This may be related to increased denitrification, which is an anaerobic process, as opposed to nitrification which is aerobic (e.g., Dowrick *et al.*, 1999). The increased DOC concentrations in the mire may again provide substrates for increased N_2O production (Bandibas *et al.*, 1994), although there are numerous factors involving both nitrification and denitrification that may affect N_2O production that are beyond the scope of this study (see e.g., Dowrick *et al.*, 1999).

Peatlands are a major source of atmospheric CH_4, contributing an estimated 25-30% of the total released to the atmosphere each year (Cicerone & Oremland, 1988). It is estimated that CH_4 has approximately 21 times the atmospheric warming potential of CO_2 over a 100 year time period (Schimel *et al.*, 1996). Nitrous oxide is an even more potent greenhouse gas with an atmospheric warming potential of ca. 200 times that of CO_2 (Rodhe, 1990), and atmospheric concentrations are increasing at the rate of 0.2-0.3% per year (IPCC, 1996). The results from this study suggest that should rainfall events become more intense or occur more frequently, both CH_4 and N_2O emissions are likely to increase substantially from similar peatland systems. Positive feedback to climate change would therefore be predicted with implications for the management of peatlands and restored wetlands, not only from a DOC and water quality perspective but also as sources or sinks of trace gases.

6.06 CONCLUSIONS

An increased rainfall simulation on a previously well drained peat system produced dramatic increases in DOC concentrations, yielding high levels of Fe in the pore waters, and a pronounced shift in the AMW of this DOC to high molecular weight materials. Increased water inputs not only produced higher total DOC concentrations with the potential to colour waters, but also a marked shift to the visible spectrum of fulvic material due to Fe-OM complexation with highly coloured, rusty-brown pore water being clearly visible. A change in hydrological regime (increased water input) therefore has the potential to increase raw water colour and associated disinfection by-products following chlorination for drinking purposes. An unexpectedly large trend for increasing DOC concentrations was detected (5.2 mg $L^{-1}yr^{-1}$) but this was not significant due to considerable natural variation. Such reductions in water quality are likely to have extensive impacts because upland peat dominated catchments are major sources of potable water in the UK (Worrall, in press), and since peatlands are by definition areas of water surplus, they will be increasingly exploited as demand increases due to increased temperatures and water shortages. It is necessary to understand the biogeochemical processes at work in the catchment because it could be that a simple diversion of waters from rewetted areas or maintenance of a constant water table would significantly reduce the problem. Diverting waters with the onset of certain seasons may also improve water quality. Indeed, a simple turnout sequence of waterflow from various sub-catchments (in order to maximize colour reductions for the least water loss) has been successful according to McDonald *et al.* (1989), looking at a peatland catchment in the Yorkshire Pennines. Further investigations into the behaviour of various pollutants in relation to certain molecular weight fractions of DOC may also improve the efficiency of constructed wetlands, metal sequestration being an obvious area of importance where seasonal variations are likely. The increased emission of CH_4 and N_2O as

a result of increased water inputs also have implications for the management of wetlands as sources or sinks of trace gases, given the potential for positive feedback to climate change.

The autumn and winter of 2000/2001 were the wettest on record in England and Wales, causing widespread flooding (Marsh, 2001). There was a general sense of concern over this period that such heavy and prolonged precipitation was attributable to anthropogenic global warming. The probability of total boreal winter precipitation exceeding two standard deviations above normal will increase by five fold over parts of the UK during the next 50-100 years associated with a doubling of CO_2 (Palmer & Räisänen, 2002). Similar increases in probability were found for the Asian monsoon season in the boreal summer. These results were associated with enhanced storm track activity and 'wetter' storms'. The frequency of great floods has increased substantially during the twentieth century and a statistically significant positive trend for great flood risk is consistent with results from the climate model used by Milly *et al.* (2002). Their model suggests that the trend will continue, and given that rainfall in the UK is likely to become more intense (Hulme *et al.*, 2002), the results of this experiment could be indicative of future peatland exports and emissions from similar catchments.

6.07 REFERENCES

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CHAPTER 7: EFFECTS OF HYDROLOGICAL MANIPULATION ON THE DIVERSITY OF AROMATIC DEGRADING BACTERIA

7.01 INTRODUCTION

Recent climate change models predict both an increased frequency of summer droughts and increased rainfall in some regions (Hulme *et al.*, 2002; IPCC, 2001). It has been suggested that the former may contribute to increasing concentrations of dissolved organic carbon (DOC) in waters draining peat dominated catchments by increasing the enzymic mobilization of materials from the peat matrix (Hughes *et al.*, 1998). Chapter 5 provided evidence to support this (increased extracellular phenol oxidase activities under drought conditions and a continued increasing trend in DOC concentrations). However, in order to gain an insight into the effects of drought on the microbial community (the ultimate source of the majority of these enzymes (Ladd, 1978; Münster, 1991)), diversity studies have been conducted. Given that the existence of peat is said to be due to low rates of decomposition (a process mediated by microbial activity), which in turn are primarily attributed to the anaerobic, waterlogged conditions (e.g., Pind *et al.*, 1994), it was hypothesized that inducing drought would reduce constraints on the diversity of microorganisms capable of degrading phenolic compounds. Conversely, increased waterlogging would be expected to reduce this diversity, allowing the accumulation of anaerobic decomposition products (DOC) as was observed in chapter 6.

Aromatic compounds

The vast majority of naturally occurring aromatic compounds in the biosphere are derived from lignins (Elder & Kelly, 1994) and this is true of the humic loading to fresh waters (including wetlands) (Wetzel, 1992). Lignins are complex, polymeric examples of phenols (Steitwieser & Heathcock, 1985) sequestered in the second layer of the cell walls of plant tissues and are closely associated with cellulose (Elder & Kelly, 1994). They comprise ca. 25% of the land-based biomass on earth (Harwood & Parales, 1996) and can form 30% of the total dry mass of peat in the earlier stages of humification, rising to 50-60% in the later stages (Clymo, 1983). The mineralization and recycling of this, and other plant derived aromatic material, is dependent on microorganisms and is vital in the earth's carbon cycle. It is thought that the degradation of lignin, due to its abundance and recalcitrance, is the rate-limiting step in the carbon cycle (Colberg, 1988; Orth *et al.*, 1991). Lignins are made

up of carbon, hydrogen and oxygen as is cellulose, but with higher ratios of hydrogen and oxygen to carbon, and smaller ratios of oxygen to hydrogen (Krauskopf & Bird, 1995). They are produced by an enzyme-initiated dehydrogenative polymerization of three phenylpropane monomers, p-hydroxycinnamyl, coniferyl and sinapyl alcohols (Paul & Clark, 1989). However, like soil organic matter (SOM) lignin is not formed by a specific enzyme but a chemical reaction involving phenols and free radicals, it therefore does not show a specific order (Paul & Clark, 1989). The monomeric phenylpropane units in lignin are linked by several different carbon-carbon and ether linkages (rather than a single intermonomeric linkage), most of which are not readily hydrolysable. Thus, lignin is resistant to degradation by most microorganisms (Krauskopf & Bird, 1995). It is probable that only certain strains of bacteria and some fungi are capable of decomposing lignin, the most well known being the wood-decaying white-rot fungi. The white-rot fungi are the most active degraders of lignin (producing CO_2 and H_2O) but only in the presence of adequate oxygen (Paul & Clark, 1989). *Coriolus versicolor* decompose the ring, methoxyl, or longer side-chain components, and *Pleurotus ostreatus* as well as *Phanerochaete chrysosporium* also cause complete degradation (Paul & Clark, 1989). Brown-rot fungi (e.g., *Poria* and *Gloeophyllum*) decompose the polysaccharides associated with lignin, removing the CH_3 subgroups and R-O-CH_3 side chains, but leave phenol behind (Paul & Clark, 1989). In wet situations the soft-rot fungi are important, *Chaetomium* and *Preussia* being representative organisms (Paul & Clark, 1989). Actinomycetes such as *Streptomyces* and *Nocardia* along with aerobic, Gram negative bacteria such as *Azotobacter* and *Pseudomonas* (see later) also have the capacity to degrade lignin (Paul & Clark, 1989).

Lignin degradation involves the oxidative cleavage of side chains between a- and b-carbons leading to the formation of aromatic acids. Many benzoic acids, benzyl alcohol and benzaldehyde derivatives have been identified among the low molecular weight products of microbially-degraded lignins. Such monomeric compounds are potential carbon growth substrates for microorganisms but due to the resonance energy that stabilizes the carbon-carbon bonds of aromatic rings (see below), these compounds pose a formidable biochemical challenge (Harwood & Parales, 1996).

The stability of the benzene ring

Benzene is the parent hydrocarbon of numerous compounds (some of which have been mentioned above) that are referred to as aromatic because many have a characteristic

aroma. Compounds in which a hydroxy group is directly attached to a benzene ring (hydroxybenzenes) are known as phenols. There have been many attempts to describe the structure of benzene but the advent of modern wave mechanics was required before this was incorporated within a unified electronic theory (e.g., see Streitwieser & Heathcock, 1985). X-ray crystallography has shown that benzene has a regular hexagonal structure composed of six carbon atoms, each of which are bonded to a single hydrogen atom and two other carbons giving a symmetrical sigma-bonded framework, with a carbon-carbon bond distance of 1.40 Å (intermediate between that for a single bond (1.54 Å) and a double bond (1.33 Å)). Bond angles in a regular hexagon are 120° suggesting that $s\rho^2$-hybrid orbitals are involved in the structure of benzene (Streitwieser & Heathcock, 1985). The 'fourth valence' of the carbon atom is now recognized as being π-bonds from ρ-orbitals extending equally around the ring. A continuous system above and below the ring within the ρ electron circulate is generated because the individual ρ orbitals overlap. The experimentally derived value for the stability of the benzene ring shows that it is more stable than the derived value of the theoretical non-delocalized cyclohexatrene unit, i.e., the delocalization conferred by the delocalized ρ system has a stabilizing effect. Thus, the chemistry of benzene predominantly involves substitution reactions in which the products retain the ρ system and only the hydrogen atoms are replaced by other groups. Nevertheless, it is necessary for the benzene rings assimilated by plant secondary metabolism to be cleaved and mineralized in order to maintain the flux through the carbon cycle.

Aerobic aromatic degradation

Aromatic compound degradation can be achieved by both aerobic and anaerobic microorganisms, however, the aerobic pathway is thought to be much better understood (although there has been a recent upsurge in research on anaerobic aromatic metabolism (see Elder & Kelly, 1994)). Aerobic decomposition has been studied here because, of those changes anticipated as a result of climate change, drought and concomitant changes in redox potential are likely to increase the contribution of this pathway in wetland systems. Water is the single most important regulator of wetland biogeochemistry (Ponnamperuma, 1972) and attention has been focussed on the effects of drought due to the perceived dependence of wetlands on waterlogging for persistence and stability (Freeman *et al.*, 1998). Moreover, the enzymes able to degrade phenolic compounds and therefore mobilize the peat matrix (increasing DOC exports upon rehydration) are oxidases, the activity of

which is strongly stimulated by the presence of bimolecular oxygen (Freeman *et al.*, 2001). However, increased water inputs (as a result of climatic changes) may also have an important inhibitory effect on aerobic aromatic decomposition in the surface layers, which can be disproportionately important in terms of biogeochemical cycling (Mitsch & Gosselink, 2000), allowing DOC accumulation.

Generally, there are three phases of degradation in bacteria for the aerobic dissimilation of aromatic substrates (Dagley, 1986; Harayama & Timmis, 1992). First, the aromatic compound is prepared for ring cleavage by a variety of ring modification reactions. The substrate undergoes changes in its substituent groups, particularly through the introduction of hydroxyl groups by mono- or di-oxygenases (which is said to be a common step despite the many and diverse pathways), to produce dihydroxyaromatic metabolites. Dihydroxybenzenes, usually catechols (i.e., 1,2-dihydroxybenzenes), are metabolites in the aerobic catabolizm of most aromatic substrates (Williams & Sayers, 1994).

Phase two involves ring fission using the catechols as a substrate. This is catalyzed by dioxygenases that break one of the carbon-carbon bonds of the ring through adding molecular oxygen, producing an unsaturated aliphatic acid. Two families of ring-cleavage enzymes occur: the intradiol (or *ortho*) dioxygenases, which are Fe^{3+} enzymes and produce *cis, cis*-muconic acid (or a derivative), and the extradiol (or *meta*) dioxygenases, which are Fe^{2+} enzymes and produce 2-hydroxymuconic semialdehyde (or a derivative). Catechol 1,2-dioxygenase (C12O or pyrocatechase) (EC 1.13.11.1) is the archetypal intradiol dioxygenase, with the archetypal extradiol dioxygenase being catechol 2,3-dioxygenase (C23O or metapyrocatechase) (EC 1.13.11.2). The term *ortho*-cleavage is used when it occurs between hydroxyl groups (intradiol cleavage) and *meta*-cleavage when it occurs adjacent to one of the hydroxyls (extradiol cleavage). The gentisate pathway forms the third aerobic ring cleavage route. This is followed when the two hydroxyl groups on the aromatic ring are *para* to each other and cleavage occurs between the carboxyl-substituted carbon and the adjacent carbon (Dagley, 1971).

The third catabolic phase involves the conversion of ring cleavage products to small aliphatic compounds that can enter the central metabolism directly. Ring cleavage along with subsequent intradiol metabolic steps is commonly known as the ß-ketoadipate (or

ortho) pathway and extradiol cleavage as the *meta* pathway. ß-ketoadipate is a key intermediate of the *ortho*-cleavage pathway, hence the name.

The dissimilation of the archetypal ring cleavage substrates catechol and protocatechuate are each catalyzed by the *meta*- and *ortho*-cleavage pathways. Enzymes involved in *meta*-cleavage differ from those in the *ortho*-pathway because they have the ability to also catalyze the degradation of methalated catecholic substrates, meaning that they have been extensively studied in relation to the degradation of methylated aromatic hydrocarbons, such as toluene and xylene (Harwood & Parales, 1996). There are modified *ortho*-cleavage pathways which have evolved to utilize chlorinated substrates, with enzymes that are closely related to the ß-ketoadipate pathway. These apparently primarily dissimilate chlorinated catechols generated from chlorobenzoate, chlorobenzene and chlorophenoxyacetate metabolism, and are encoded on catabolic plasmids (Harwood & Parales, 1996). *Meta*-cleavage pathways specifying phenol, toluene, and naphthalene degradation have been described that are also plasmid encoded. These *meta*- and modified *ortho*-pathways have been the subject of several recent reviews due to their contribution to the degradation of environmental pollutants (e.g., Assinder & Williams, 1990; Powlowski & Shingler, 1994).

The biochemistry of the *meta* and *ortho* pathways is seemingly conserved in all genera of eubacteria in which they are found. Broadly, aerobic aromatic catabolizm therefore consists of a variety of pathways that converge on a limited number of common intermediates (dihydroxybenzenes), which are then further assimilated by a few common pathways (Williams & Sayers, 1994).

Bacterial diversity

The analysis and characterization of microbial communities can be problematic due to an inability to culture the majority of bacterial species present in an environmental sample and the large number of species encountered. Isolated bacteria may therefore account for a small proportion of the total bacterial diversity originally present. The problem is emphasized in oligotrophic habitats, such as ground water, where approximately 0.1-1% of the bacterial species are culturable (Amann *et al.*, 1995). Ambiguous morphological and physiological traits have proved to be a further difficulty in the identification of microbes.

More recently, with the development of culture-independent techniques that allow amplification and sequencing of DNA extracted from the environment, the genotypic analysis of naturally occurring assemblages has become a focus of attention in the field of microbial ecology. The ubiquitous prokaryotic 16S ribosomal RNA (16S rRNA) gene is frequently used in such studies. Within the 16S rRNA gene are conserved nucleotide sequence regions, and variable nucleotide sequence regions. By designing polymerase chain reaction (PCR) primers in conserved regions that flank a variable region of the gene, the variable stretch of DNA may be amplified and then sequenced. Alignment and relative identity determinations of variable regions from different bacterial sequences may then be used to infer bacterial phylogeny.

This section describes the use of Temporal Temperature Gradient Gel Electrophoresis (TGGE) for sequence variation studies. Denaturing Gradient Gel Electrophoresis (DGGE) is commonly used to separate PCR-amplified bacterial 16S rDNA fragments and is performed at high temperature in the presence of a chemical gradient. The main advantage of this method is the direct determination of bacterial genetic diversity in both a qualitative and semi quantitative way, simplifying analysis and making it superior to cloning and subsequent sequencing (Muyzer *et al.*, 1993). In TGGE a constant concentration of denaturant is used in the gel combined with a gradual increase in temperature over the course of the separation to provide the denaturing environment. Thus, the pouring of chemical gradient gels is avoided using this technique (Zoller *et al.*, 1998).

TGGE separates PCR products of similar length according to sequence variation. In theory, each discrete band within a profile differentiated by TGGE represents an individual DNA species from a microbial consortium, permitting a rapid insight into the genetic diversity *in situ*. The relative intensity of each band represents the probable relative abundance of a particular species (Muyzer *et al.*, 1993). However, care is required because molecular sequences (e.g., 16S rDNA) are not used to define species and strains belonging to a given species may have different sequences (Stackebrandt & Goebel, 1994). In this study, different bands as separated by TGGE will be referred to as DNA species. Described here is the application of TGGE to examine the diversity of bacteria capable of producing the enzyme C23O encoded by the *xylE* gene. C23O is the archetypal extradiol dioxygenase (see above) and the *meta* pathway provides the commonest route for catabolizm of toluene, naphthalene and biphenyl in the *Pseudomonads* (Williams & Sayers, 1994). The most

detailed investigations have been carried out on the catabolizm of these aromatic hydrocarbons in a relatively limited range of bacteria, mostly of this genus. Hence, C23O is the enzyme of choice here. Furthermore, cleavage of the catechol ring by this enzyme produces a yellow compound (2-hydroxymuconic semialdehyde), providing a method to determine whether activity is present in soil samples before further investigation.

Information regarding the potential effect of climate change upon the diversity of bacteria that possess this ecologically important enzyme is apparently scarce. The diversity of bacteria possessing *xylE* type genes has therefore been examined under contrasting hydrological regimes that have been imposed in order to simulate climate change scenarios. Peat samples were taken from both the wetlands exposed to simulated successive drought and simulated increased rainfall, along with their appropriate controls (referred to as upper and lower control respectively), as described previously in chapters 5 and 6 respectively. This study was intended as a preliminary investigation into a specific pathway in the carbon cycle of a peat accumulating wetland and the potential effects of climate change on the microbial population responsible for the recycling of aromatic compounds. It is beyond the scope of this experiment to provide an evaluation of the molecular/microbiological techniques available for diversity studies, but it was hoped this work would provide an example of the potential for more detailed evidence to support the results reported in chapters 5 and 6 regarding phenol oxidase activities and DOC concentrations. Both are broad terms, probably incorporating numerous types of enzymes and aromatic compounds which will be involved in many metabolic pathways. This section however, focuses on a well documented, specific catabolic pathway, the biochemistry of which appears to be conserved in all genera of eubacteria in which this reaction sequence is found (Williams & Sayers, 1994).

7.02 MATERIALS AND METHODS

Hydrological manipulations

Successive summer droughts and increased rainfall simulations were induced in wetlands within the Cerrig-yr-Wyn sub-catchment of Plynlimon, as described in chapters 5 and 6 respectively.

Principle of TGGE

TGGE is an electrophoretic method used to identify single base changes in a segment of DNA. Within a polyacrylamide gel, double stranded DNA is subjected to an environment of increasing denaturant (temperature) and will melt in discrete segments called 'melting domains'. The melting temperature (T_m) of these domains is sequence specific and two factors influence the temperature at which the DNA duplex melts:

1. The H bonds formed between complementary base pairs (GC rich regions melt at higher temperatures than regions that are AT rich).
2. The attraction between neighbouring bases of the same strand (or 'stacking').

Partial melting of the DNA will occur when the T_m of the lowest melting domain is reached, producing branched molecules and reducing mobility in the gel. The branched single stranded moiety of the molecule becomes entangled in the gel matrix and no further movement occurs. Since the T_m is sequence specific, different nucleotide sequences will generate different DNA melting profiles due to mobility shifts at different positions in the gel. Migration will again become a function of size if the fragment completely denatures but this is prevented by the presence of a high melting domain, usually artificially created at one end of the molecule by the incorporation of a GC clamp. This is achieved by using a PCR primer with a 5' tail consisting of a sequence of 40 GC.

TGGE exploits the principle on which denaturing gel electrophoresis (DGGE) is based without the need for a chemical denaturing gradient (Zoller *et al.*, 1998). This makes it simpler, faster and more reproducible (Myers *et al.*, 1985) so rapid high-throughput screening is possible in theory. PCR-amplified DNA from the gene of interest is loaded onto the acrylamide gel containing a constant concentration of urea. The temperature is then increased gradually and uniformly during electrophoresis, giving a linear temperature gradient over the length of the run. A denaturing environment is thus formed by the constant concentration of urea in the gel in combination with the temporal temperature gradient. The temperature ramp rate (measured in °C h^{-1}) can be controlled precisely using the Dcode™ system (Bio-Rad Laboratories Inc.). Control over the temperature range and ramp rate allows optimum denaturing conditions to be maintained.

Calculating run parameters

The addition of the 30-40 base pair GC clamp to one of the PCR primers ensures that the region screened is in the lowest melting domain. In order to calculate the temperature range over which to run the TGGE, it is necessary to generate a melting profile of the DNA using a DNA melting software program (for example MacMelt™ or WinMelt™ software, Bio-Rad). Such software is based on the melting algorithm for the original Melt87 software developed by Lerman & Silverstein (1987). The run parameters along with the temperature range for the gradient had conveniently been carried out previously (following the Bio-Rad TGGE manual, Bio-Rad) due to work on the *xylE* type gene in association with degradation of oil (Bangor Biodegradation Group, School of Biological Sciences, University of Wales, Bangor).

Designing primers

Previously identified prokaryotic *xylE* type nucleotide sequences were accessed from the National Centre for Biotechnology Information GenBank database (NCBI). Alignment of the *xylE* homologues using the Clustal 1.4 alignment algorithm (Bioinformer, The European Bioinformatics Institute) identified conserved nucleotide regions within the gene. These regions of homology were then used to design the following degenerate forward and reverse PCR primers, (5'-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CGA (GC)(CT)T GCT (GC)GG CCT GAT CGA-3' and 5'-CGT (AG)GT (AG)AA TCG CCT TGC C-3' respectively). The primers amplify a ca. 200 base pair fragment of the *xylE* type gene.

DNA extraction and PCR-amplification

Genomic DNA was extracted (3 replicates) from peat samples taken from the two control and two experimental sites at Cerrig-yr-Wyn (see chapters 5 & 6) using a Mo Bio UltraClean™ soil DNA kit and protocol (Mo Bio Laboratories Inc.). Extracted DNA was quantified by gel electrophoresis using a HyperLadder (Bioline, London). Undiluted DNA (6 ng μL^{-1}), 1/5 and 1/10 dilutions (using autoclaved ultra pure water) were used for the PCR reaction to determine optimal concentrations.

The final volume for the PCR reaction was 25 µL, containing 0.5 µM of each primer, various amounts of soil DNA template, and a 1:1 ratio of HotStarTaq Master Mix Kit (Qiagen Ltd. UK). The PCR reaction was performed using a TecheGene thermal cycler

(Techne, UK), according to the manufacturers' instructions. To increase the number of different soil *xylE* type genes amplified 'touchdown PCR' (involving the lowering of the annealing temperature by 1°C per cycle until a temperature equal to, or 2-5°C below, the T_m of the primers is reached) was used. Using the touchdown procedure reduces the formation of spurious by-products during the amplification process (Don *et al.*, 1991; Muyzer *et al.*, 1993).

After the initial activation step of 15 min at 95°C, a three stage cycling program was used including 35 cycles of 94°C for 1 min (denaturation), 1 min annealing temperature (as previously described) and 2 min 30 sec at 72°C (extension), followed by a final extension step of 10 min at 72°C. The presence of DNA amplified from the PCR reactions was visualized using agarose gel electrophoresis and EZload (Bio-Rad) molecular ruler.

Temporal Temperature Gradient Electrophoresis (TGGE)

Various amounts of PCR product (50, 100, 200, 400 ng) were mixed with 2x bromophenol blue loading dye and electrophoresed in a 6% polyacrylamide gel (acrylamide/bis 37.5:1) containing 7 M urea, 20% formamide and 2% glycerol in 1.25x TAE buffer (50 mM Tris-Cl, 25 mM acetic acid, 1.25 mM EDTA (ethylenediamine-N,N,N',N'-tetra acetic acid), pH 8.0) using the Dcode™ system (Bio-Rad). This was run at 100 V for 16 hours across a temperature range of 40-55°C and using a temperature ramp rate of 1°C h^{-1}. Following electrophoresis, gels were equilibrated in 1.25x TAE buffer for 15 min, stained in EtBr (25 mg/mL in TAE) for 20 min and destained in TAE before viewing and photographing under UV light (302 nm).

7.03 RESULTS

Figure 7.01 shows the TGGE generated *xylE* band profiles from the experimentally droughted and upper control wetlands. The upper control site (lanes 5-7) showed a simple band profile with only 4 faint DNA bands (representing the DNA species present possessing the *xylE* type gene) being detectable at positions A, D, F and G. In contrast, the experimentally droughted wetland (lanes 2-4) produced a complex profile composed of at least 10 distinct bands at positions A-J, along with less distinct bands beyond position J. In some cases the larger annotated bands may contain more than a single homologue of *xylE*. The intensity of the DNA bands (an indication of numerical dominance) is greater in the

experimentally droughted wetland, both for those common *xylE* homologues found at both sites and those detected only in the drought impacted wetland.

TGGE generated *xylE* band profiles from the lower control (lanes 5-7) and increased rainfall simulation (lanes 2-4) wetlands are presented in figure 7.02. In the case of the lower control wetland, the band profile is very similar to the experimentally droughted wetland, with *xylE* homologues that migrated to positions A-J to produce distinct bands, along with bands beyond and between these positions. Conversely, the increased rainfall simulation site produced a profile with only 3 distinct bands at positions A, B and D, detectable in one replicate only (lane 2). Lanes 3 and 4 showed one barely detectable band at position D and one distinct band at position D respectively.

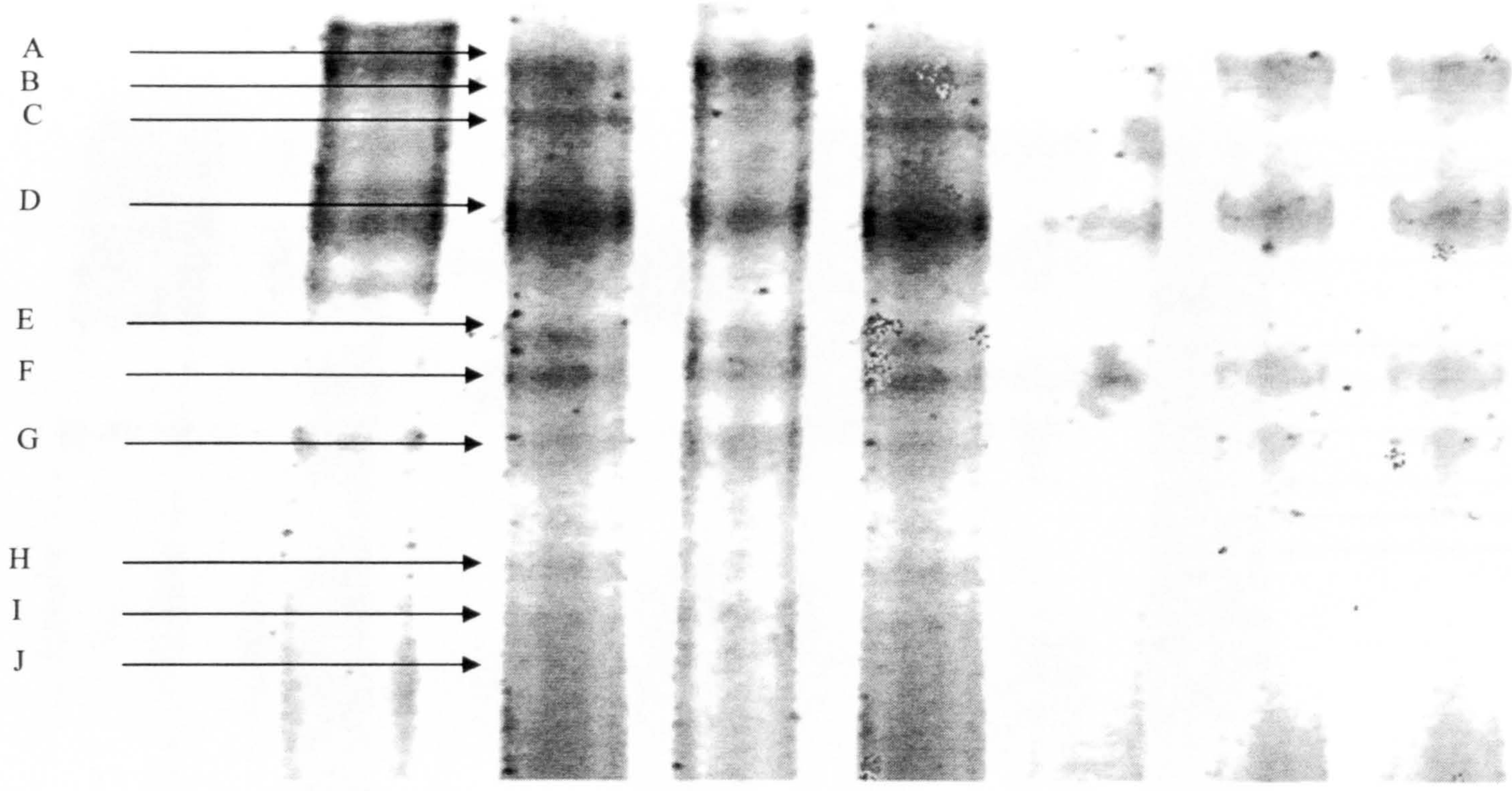

Figure 7.01. TGGE analysis of the amplified DNA fragments using primers specific for the *xylE* type gene, & DNA (200ng) extracted from the experimentally droughted (lanes 2-4) & control (lanes 5-7) wetlands as the template. TGGE running conditions were 100V for 16 hours across 40-55°C & using a ramp rate of 1°C h^{-1}. A molecular marker was run in Lane 1.

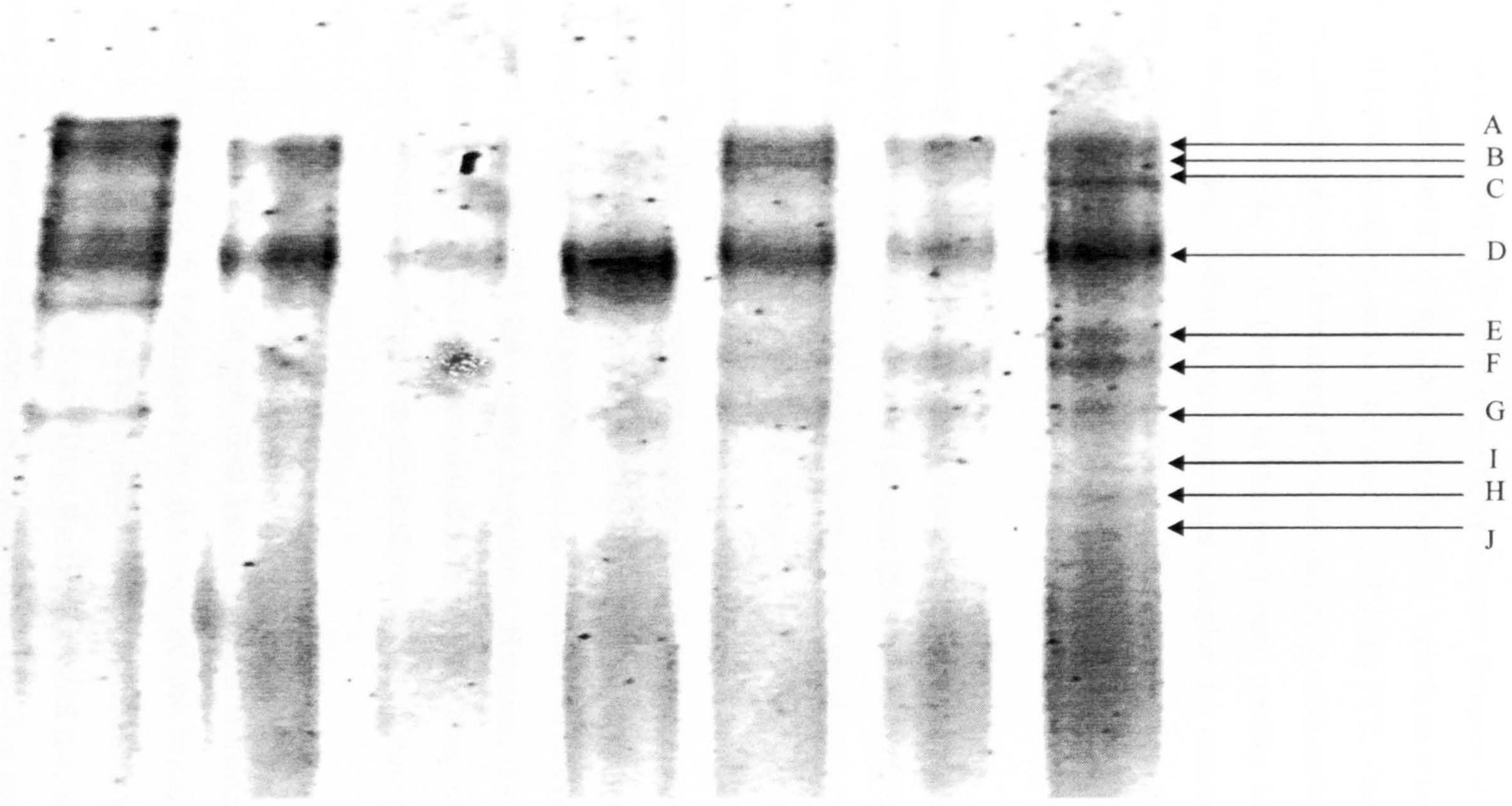

Figure 7.02. TGGE analysis of the amplified DNA fragments using primers specific for the *xylE* type gene, & DNA (200ng) extracted from the increased rainfall simulation (lanes 2-4) & control (lanes 5-7) wetlands as the template. TGGE running conditions were 100V for 16 hours across 40-55°C & using a ramp rate of 1°C h^{-1}. A molecular marker was run in Lane 1.

7.04 DISCUSSION

TGGE results should be interpreted with the knowledge that such a technique (as with DGGE) provides a relative measure of species diversity and abundance, allowing microbial populations to be profiled in a qualitative and semi quantitative manner (Muyzer *et al.*, 1993). As with many molecular techniques involving DNA amplification, there is an intimate dependency on the efficiency of DNA extraction and on unbiased high fidelity PCR. Despite the widespread use of PCR its quantitative use is less straightforward and three main methods are discussed in relation to TGGE by Felske *et al.* (1998). In this study band profiles have been compared, hence genetic diversity from different environmental samples, by running PCR products in adjacent lanes. Each band, in theory, represents a DNA species and the TGGE technique enables the resolution of genes differing by only one base pair over the fragment in question (Muyzer *et al.*, 1993).

Effects of simulated drought on the diversity of bacteria possessing the *xylE* type gene

The experimentally droughted wetland showed distinct DNA bands at positions A-J, indicating that the *xylE* type gene is present in numerous bacterial species at the site (figure 7.01). The complex profile represents the diversity of aromatic catabolizing bacteria under these conditions. In contrast, the upper control showed bands at positions A, D, F and G but very few others, suggesting that there is less species possessing the gene encoding for the production of C23O. Since C23O is involved in aerobic aromatic degradation, such increased diversity is probably the result of increased soil aeration, i.e., an increased diversity of aerobes and/or facultative aerobes, consistent with the original hypothesis. However, this technique does not provide evidence for a change in the total bacterial diversity and the microorganisms possessing these *xylE* type genes were not necessarily metabolically active at the time of sample collection (see chapter 9).

The relative quantity of DNA in the individual bands suggests numerical dominance by a number of DNA species (ca. 6, represented by the most intense bands in the profile i.e., positions A-G) that co-dominate in the drought impacted wetland. This compares to only two or three types (i.e., represented by bands A and D) in the upper control site. Seemingly, there is a greater diversity and abundance of bacteria possessing these *xylE*-like genes that are able to proliferate in the droughted wetland with the capacity to degrade aromatics when conditions become drier (perhaps more akin to those of a conventional terrestrial soil). This is consistent with the increased extracellular phenol oxidase activities and removal of pore water phenolic compounds in the short term (reported in chapter 5). These results also support the proposed mechanism for increasing DOC concentrations associated with drought events, whereby drying facilitates enhanced enzymic mobilization of the matrix and releases DOC products available

for export upon rehydration of the peat (chapter 5). Such evidence may mean an increased carrying capacity has been achieved in the experimental wetland, which was not anticipated because of the mild nature of the drought imposed. The potential for a radically altered microbial community structure and function with more severe or frequent drought, as a result of climate change, is therefore highlighted.

Of course the results above may only be true for the surface layer of peat, especially given the mild nature of the simulated droughts. However, the upper layers of the peat may be disproportionately important in terms of enzyme activities and hydrological processes (c.f. Freeman *et al.*, 1994; Mathur & Lavesqué, 1985; Mitsch & Gosselink, 2000). The diversity of bacteria possessing the *xylE* type gene may also be shifted with depth by the drought, and so it would be prudent to carry out further work on the peat profile to determine whether this was the case. Certain microorganisms may, for example, seek moisture or lower temperatures deeper within the peat profile under drought conditions, thus altering the community structure of the sub-surface layers.

Effects of simulated increased rainfall on the diversity of bacteria possessing the *xylE* type gene

The profile produced by the lower control wetland (figure 7.02) was very similar to that of the drought impacted site, showing several DNA species (*xylE* type homologues) throughout the gel profile (positions A-J). This is consistent with the well drained nature of the peat (based on the above hypothesis that reduced waterlogging relieves the constraints on microbial diversity). The simulated increased rainfall site produced one major DNA species (position D) and very few others (these being less numerically dominant from the relative quantity of DNA in the bands), supporting the original hypothesis that increased rainfall would reduce the diversity of bacteria possessing the *xylE* type gene. Furthermore, lanes 2 and 3 indicated low abundance within a particular DNA species, excepting that represented at position D, which appears to dominate *meta*-cleavage at the site. This gel profile may have been the result of rewet killing a large proportion of bacterial species when the simulation commenced, with the subsequent waterlogging maintaining relatively adverse conditions and allowing only a single or limited number of DNA species to dominate the extradiol pathway. According to Kieft *et al.* (1987), up to 50% of the microbial community can be killed in a single drying/wetting event but little is known about which organisms survive such events and the speed at which they recover. Populations of key degraders could be reduced for extended periods (Clein & Schimel, 1994), while other components of the microbial biomass are resistant to drying/rewetting stress (Bottner, 1985). In relation to litter, it has been hypothesized that in areas where episodic drying and rewetting become more severe, populations of cellulytic and lignolytic fungi could

be reduced to the point where litter decomposition would be suppressed to a greater extent than would be anticipated by changes in moisture levels only (Schimel & Gulledge, 1998). The enhanced DOC and phenolic compound concentrations produced under conditions of increased water input (chapter 6) may relate to the reduced aerobic mineralization of aromatics due to a limited diversity and abundance of bacteria able to metabolize DOC directly, or transform it into a form that another member of the community can utilize. In aerobic conditions (where there is ample free oxygen) organic carbon is used as an energy source and released primarily as CO_2, whereas under anaerobic conditions (in the absence or near absence of oxygen) fermentative metabolism is dominant, resulting in production of various low molecular weight organic acids (e.g., acetate and lactate), alcohols, and CO_2 (Ponnamperuma, 1972).

While there is an apparently reduced abundance and diversity of bacteria involved in *meta*-cleavage, an increase in those associated with other aerobic (e.g., *ortho*-cleavage) or anaerobic aromatic degrading pathways may occur, and thus an increase in total diversity cannot be precluded. Nevertheless, there is clearly the potential for altered carbon cycling in northern peatlands as a result of increased rainfall/flooding events. It should be noted though that such results would be unlikely if the wetland system was not naturally relatively well drained before treatment, since such a system would be predominantly saturated before such an increased rainfall simulation began. Information provided by studies of this type may also be of relevance to the restoration of drained wetlands; perhaps a gradual raising of the water table would reduce adverse effects on water quality, produced by pulses of DOC, associated with the death of large proportions of the aromatic degrading microbial community upon rewetting.

General features

Co-migrating DNA bands (positions A and D) were found in all four wetlands (figures 7.01 & 7.02), suggesting the presence of a predominant species of bacteria with the *xylE* type gene at the time of sample collection. These bands may represent *xylE*-like genes from species of facultative aerobe as they were present in all four wetlands, i.e., they are likely to be able to tolerate both aerobic and anaerobic conditions. The variable nature within the replicate samples for both the successively droughted and the increased rainfall simulation sites indicates that different microclimates exist within the peat, as was expected, and this was especially true for the simulated increased rainfall wetland. This supports the hydrochemical data, since replicates were found to be spatially variable (see chapter 6). It is therefore likely that peatlands can be considered as mosaics in terms of microbial diversity and microclimates as well as at larger scales such as topography (hummocks, hollows and lawns) and landforms etc. (Weltzin *et al.*, 2001). And, at each scale the response to climate change is likely to differ (Weltzin *et al.*, 2001).

In this study, only the bacterial population possessing the *xylE* type gene was examined because the enzyme (protein product) is known to be involved in the catabolizm of aromatics in the field sites. However, not all the microbes detected were necessarily active in that catabolizm within the soil. If TGGE was performed, for example, before a simulated drought and over a period of successive droughts, the band profile could be monitored as a different assemblage of bacteria became dominant due to their ability to survive/proliferate in the more 'terrestrial' edaphic conditions. This would aid our understanding of the diversity and function of bacterial consortia as it relates to changing factors in that particular environment. It would also be necessary to examine the diversity of bacteria possessing enzymes involved in *ortho*-cleavage, the other major pathway of aerobic aromatic degradation, in order to provide a more complete picture of the potential effects of climate change. Both C12O and P34O (protocatechuate 3,4-dioxygenase, which cleaves protocatechuate), in addition to C23O, would therefore need to be considered in the same way.

In isolation the TGGE results do not allow us to presume that there would be a change in function in the experimental wetlands, despite changes in the diversity of bacteria possessing the *xylE*-like gene, since different species may perform the same function within the two sites. Furthermore, diverse species may work essentially as a single organism in the catabolizm of aromatics. However, in conjunction with the hydrochemistry and enzyme activity data presented in chapters 5 and 6 respectively, it seems that both increased drought and rainfall events have the potential to alter the bacterial community structure and the carbon cycling function of the peat system, with implications for the quality receiving waters.

7.05 CONCLUSIONS

From the gel profiles presented, in conjunction with hydrochemical and enzyme activity data (chapters 5 and 6), both an increased frequency of summer drought and increased rainfall, as a result of climate change, have the potential to alter the bacterial community structure and the carbon cycling function of the northern peatland system. Simulated drought apparently allowed a greater diversity and abundance of bacterial species able to catabolize aromatics *via* the extradiol pathway, while the rainfall simulation reduced this diversity and abundance. Such community changes are likely to influence aquatic carbon exports from peatlands as well as other aspects of ecosystem function, such as trace gas fluxes to the atmosphere (Schimel & Gulledge, 1998).

Understanding accurately the function of bacterial consortia in the environment necessitates the measurement of microbial community structure and diversity as it relates to changing factors in

that environment. TGGE provides a 'tractable' way of assessing this diversity. Indeed, it has been found to be a reliable technique and a valuable tool for such studies (Felske *et al.*, 1998). However, this (and other molecular techniques in general) perhaps are not yet a panacea for all soil ecological analysis and need to be incorporated into a polyphasic approach (Ogram, 2000).

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CHAPTER 8: THE EFFECTS OF ELEVATED CO_2 & TEMPERATURE ON BIOFILM DOC PROCESSING

8.01 INTRODUCTION

The biofilm or epilithon is important in lotic organic matter (OM) processing because of the high wetted surface area to water volume ratio (Sinsabaugh & Linkins, 1988). Numerically, epilithic biofilm are dominant by two to three orders of magnitude (Geesey *et al.*, 1977; 1978) and metabolically they are an order of magnitude more active (Ladd *et al.*, 1979). It has been shown that as much as 75% of dissolved organic carbon (DOC) additions to a river can be removed within a 30m stretch (Hynes *et al.*, 1974). These assemblages of algae, bacteria, fungi and protozoa form the primary site for the processing of removed carbon (Mickleburgh *et al.*, 1984).

Terrestrially derived DOC is a major component of the riverine carbon pool and several studies identify wetlands as being the major contributor of organic carbon within a catchment where they exist (e.g., Hope *et al.*, 1997; Wetzel, 1992). This material is susceptible to further processing once within the lotic environment where a proportion will be converted to CO_2, a process recently recognized as being important in global carbon losses to the atmosphere (Richey *et al.*, 2002). Waters draining from peaty catchments are dominated by phenolic compounds that can inhibit microbial metabolism of DOC (Appel, 1993; Freeman *et al.*, 1990; Wetzel, 1992) and it is within these waters that the rising trend in DOC concentrations reported for UK rivers is particularly pronounced (Freeman *et al.*, 2001a). Phenol oxidase is one of the few enzymes able to degrade phenolic substances (McLatchey & Reddy, 1998) which are derived primarily from lignin, the most refractory component of vascular plant detritus (Sinsabaugh & Linkins, 1988). And phenol oxidase activity has been detected in biofilm from highly coloured waters (Sinsabaugh & Linkins, 1988, 1990). Given that this enzyme was recently recognized as a major control on carbon storage in organic-rich northern soils (Freeman *et al.*, 2001a), it may also be crucial in determining DOC concentrations in rivers.

Despite the wealth of studies demonstrating the importance of the epilithon in the removal of DOC from the water column (Hynes *et al.*, 1974; Mickleburgh *et al.*, 1984 etc.), little information exists on the potential effects of climate change on such assemblages. This is surprising given that the removal of DOC is often the largest recurrent water treatment cost in peat dominated upland catchments (Worrall *et al.*, in press) and that its incomplete removal leads to the production of carcinogenic disinfection by-products (DBPs) (e.g., Alarcon-Herrera *et al.*, 1994; Worrall *et al.*, in press). Temperatures are likely to increase as a result of elevated

CO_2 (eCO_2) (IPCC, 2001; Webb, 1996) and an increasing trend for water temperatures has already been reported in globally diverse localities, with up to 1°C being reported in Europe (see Webb, 1996). Temperature is arguably one of the most important physical properties of river water and exerts a strong influence on many physico-chemical characteristics of water. These include viscosity, density, surface tension (Stevens *et al.*, 1975), the solubility of oxygen and other gases (e.g., ASCE Committee on Sanitary Engineering Research, 1961), and chemical reaction rates (e.g., Brezonik, 1972). Lotic biota can be affected in numerous ways by thermal regime (Macman, 1974) with effects on growth, metabolism, life histories and tolerance to pollution to name but a few (Elliot, 1994). Since altered phenol oxidase activities may influence the removal of a potent enzymic inhibitor (namely phenolic compounds, Wetzel, 1992), we sought to test the hypothesis that that phenol oxidase would be stimulated under warmer temperatures allowing more rapid DOC processing.

The majority of studies involving natural biofilm seemingly have focussed on its degradatory processes and responses to a changing supply of allochthonous substrate, probably due to the fact that these attached communities play a major role in dissolved organic matter (DOM) decomposition. However, since light grown epilithon contains photoautotrophs and these organisms can entirely support the heterotrophic component (Haack & McFeters, 1982), elevated temperature (eTemp) may increase the autochthonous production of DOC from biofilm communities with implications for carbon cycling and water quality. It is widely accepted that increased atmospheric CO_2 levels have the potential to induce a fertilizer effect in certain species of terrestrial plants (provided there are sufficient nutrients), and thus it may be reasonable to hypothesize that an analogous situation could be found in illuminated biofilm. Increasing the carbon input may increase the carbon output into the water column *via* such mechanisms as increased algal exudation or lysis, for example, with a general stimulation of all components of the biofilm.

This study was intended to explore whether biofilm has the potential to contribute significantly to DOC concentrations in response to eCO_2 and/or eTemp. A factorial design simulation of climatic change was used that included a 3°C higher temperature (eTemp), 235 ppm elevated atmospheric CO_2 concentrations (eCO_2) and a combined eCO_2/eTemp treatment with natural biofilm coated stones as the sample material, collected from a mid Wales stream draining upland peat. In addition to aqueous DOC and phenolics concentrations, and biofilm phenol oxidase activity measurements, an indicator of both total metabolic activity (2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl) tetrazolium chloride, referred to as INT) and algal biomass (chlorophyll *a* concentration) was used. Hydrolase enzyme activities (ß-glucosidase and

phosphatase) and aqueous anion concentrations were also measured. All biofilm samples were assayed intact in the hope of obtaining meaningful measurements of microbial metabolism and allowing interrelated functions of the polysaccharide matrix (PSM) to continue undisturbed.

8.02 METHODS

Study site

Samples were collected from an unshaded, oligotrophic tributary of the river Wye, draining flush wetlands in the Plynlimon catchment (UK NGR SN 820 866), a gully system typical of the upland peat dominated catchments in Wales (Hughes *et al.*, 1996). It was therefore assumed that the photoautotrophs would be providing a large proportion of the heterotrophic requirements.

Sampling procedure

Biofilm covered stones were taken from open areas of the stream channel to allow four replicates of both natural light and dark incubations for each treatment. These were then carefully transported to a controlled temperature room (7°C) in river water where they were gently rinsed with defined synthetic river water (SRW) (see chapter 2) to remove loosely held particulates. Biofilm samples were allowed to equilibrate for one month after which pre-treatment sampling commenced. The epilithon colonized stones (and replicated 'blanks' containing only SRW) were then placed in each of the following treatments at the solar dome facility (CEH Bangor):

1. Ambient CO_2/ambient temperature (control)
2. Elevated (235 ppm above ambient, ca. 605 ppm) CO_2/ambient temperature (eCO_2)
3. Ambient CO_2/elevated (3°C above ambient) temperature (eTemp)
4. Elevated CO_2/elevated temperature (eCO_2/eTemp)

Light traps were constructed using black, opaque plastic and with a 1 cm gap at the base allowing air circulation. These also served to prevent unquantified allochthonous material entering the systems. Dark incubated samples were assumed to consist of predominantly heterotrophic communities (Lock & Ford, 1985; Rounick & Winterbourne, 1983). Light incubated samples also had similar constructions (except using colourless, transparent plastic) to prevent artefacts due to the alteration of airflow and entry of materials to one set of samples only.

Sampling was carried out at fortnightly, and then monthly intervals, at the same time of day to avoid artefacts due to diurnal fluctuation. Assays involving the biofilm were performed immediately after sample collection and water samples were filter sterilized and refrigerated.

Aqueous DOC concentrations

DOC concentrations were measured using a Shimadzu 5000 Total Organic Carbon Analyzer and phenolic compound concentrations using the Box assay, as described in chapter 2.

Algal biomass (indicated by chlorophyll a concentrations)

Biofilm covered stones were carefully immersed in 100 mL of 95% ethanol (Sigma-Aldrich Co. Ltd., Dorset). Large marbles were used as stoppers to prevent volume changes. Chlorophyll was extracted by boiling for 2 min in a water bath and was measured spectrophotometrically (Jespersen & Christoffersen, 1987) using a plate reader, following centrifugation.

Biofilm extracellular phenol oxidase activities

Phenol oxidase activities were determined as described in chapter 2, except that 5 replicates of intact biofilm covered stones (plus killed controls: 2% v/v formaldehyde for 1 hour, residual fixative removed prior to incubation by rinsing with SRW) were used instead of peat and proportionately increased solution volumes to ensure that the biofilm was fully submerged.

Biofilm extracellular ß-glucosidase activities

MUF-ß-D-glucoside (Sigma-Aldrich) was dissolved in 2 mL of Methylcellosolve: 2-Ethoxyethanol (Ethylene Glycol Monoethyl Ether) (Sigma-Aldrich) and made up to 100 mL with SRW to produce 0.3 mM or 0.5 mM solutions. Enzyme activities were determined by the protocol described in chapter 2, except that formaldehyde treated stones were used as killed controls.

Total metabolic activities (indicated by INT concentrations)

Biofilm metabolic activity was estimated by incubation with the electron transport acceptor INT, the reduction of which produces a water insoluble dye, INT-formazan (Trevors, 1984). The method was modified for use with freshwater biofilms by Blenkinsopp and Lock (1990). Colonized stones and killed controls were incubated in 100 mL bottles with 30 mL of 0.02% INT (Sigma-Aldrich) in SRW for 18 hours (Freeman *et al.*, 1994) at the appropriate water temperature taken at the time of sampling. The stones were then drained and the excess dye removed by rinsing with SRW. In order to terminate the reaction, 30 mL of 2% formaldehyde was added. INT-formazan was extracted from the biofilms using 30 mL of chilled methanol (Sigma-Aldrich, Spectroscopic Grade) for 2 hours at -20°C. The extracts were then filtered

(GF/F, Whatman, Kent, UK) to remove particulates and the absorbance of the filtrate measured at 480 nm against a 0-30 µg mL^{-1} INT-formazan (Sigma-Aldrich) standard range dissolved in methanol. Readings of the killed controls were subtracted from the samples to account for interference due to the extraction of chlorophyll, in addition to the INT-formazan, when using methanol. Results are expressed as µg INT-formazan cm^{-2} h^{-1}.

Surface areas

All stones used for chlorophyll, enzyme and INT assays were subsequently dried and wrapped in aluminium foil (of a known area to weight ratio), which was then trimmed around the stone to obtain a measure of surface area (Lamberti & Resh, 1985). This allowed activities to be expressed per unit surface area.

Anions

Fluoride, chloride, nitrate, phosphate and sulphate concentrations were monitored in the water surrounding the biofilm covered stones using a DIONEX 200i/sp system and AS4A column as described in chapter 2.

Scanning Electron Microscopy (SEM)

Following 24 weeks of treatment, replicate biofilm colonized stones were prepared for SEM by critical point drying and sputter coating.

8.03 STATISTICAL CONSIDERATIONS

Data was tested for normality using the Kolmogorov-Smirnov test and each treatment was compared against the control using ANOVA and Dunnetts simultaneous tests (Minitab version 13.32, Minitab Inc.). Enzyme and metabolic measurements were compared using actual activities rather than cumulative values.

8.04 RESULTS

8.04.1 Photoautotrophic (light grown) biofilm communities

Table 8.01. Range of determinands and significant differences for light grown biofilm under elevated CO_2 (eCO_2), elevated temperature (eTemp) and elevated CO_2/elevated temperature (eCO_2/eTemp) simulations in relation to the control

Determinand	Treatment	Minimum	Date	Maximum	Date	Δ%	P
DOC (mg L^{-1})	control	2.12 (1.26)	27/02/01	14.25 (0.92)	07/07/01		
	eCO_2	1.87 (1.15)	27/02/01	15.86 (0.11)	07/07/01	10.37	ns
	eTemp	2.13 (1.02)	05/03/01	14.16 (0.04)	07/07/01	1.98	ns
	eCO_2/eTemp	4.05 (1.87)	13/03/01	25.72 (2.16)	07/07/01	70.19	***
Phenolic compounds (mg L^{-1})	control	0.06 (0.01)	17/05/01	0.41(0.05)	13/03/01		
	eCO_2	0.06 (0.01)	09/06/01	0.43 (0.08)	13/03/01	3.62	ns
	eTemp	0.18 (0.02)	09/04/01	0.57 (0.06)	26/04/01	59.34	ns
	eCO_2/eTemp	0.26 (0.09)	09/04/01	0.74 (0.03)	14/08/01	85.13	**
Chlorophyll *a* (μg cm^{-2})	control	0 (0.11)	17/05/01	0.84 (0.15)	07/07/01		
	eCO_2	0.101 (0.11)	27/02/01	1.21(0.14)	07/07/01	53.89	ns (10%)
	eTemp	0.154 (0.11)	26/04/01	0.92 (0.1)	07/07/01	32.93	ns
	eCO_2/eTemp	0.2 (0.07)	27/02/01	1.79 (0.17)	07/07/01	98.75	**
Phenol oxidase activity (pmol dicq cm^{-2} min^{-1})	control	12.0 (2.2)	13/03/01	49.0 (2.1)	07/07/01		
	eCO_2	5.0 (3.6)	13/03/01	37.0 (2.4)	09/04/01	-21.28	ns
	eTemp	8.0 (5.2)	26/04/01	50.0 (1.3)	05/03/01	14.59	ns
	eCO_2/eTemp	0.0 (0.0)	05/03/01	3.3 (2.1)	09/06/01	-45.29	**
β-glucosidase activity (nmol MUF cm^{-2} h^{-1})	control	0 (0.35)	17/05/01	5.76 (0.41	07/07/01		
	eCO_2	0.96 (0.47)	17/05/01	6.4 (0.35)	07/07/01	-7.02	ns
	eTemp	2.3 (0.38)	05/03/01	8.8 (0.32)	07/07/01	30.70	ns
	eCO_2/eTemp	2.96 (0.39)	27/02/01	7.56 (0.41)	07/07/01	33.35	*
Metabolic activity (μg formazan cm^{-2} h^{-1})	control	0 (0.49)	17/05/01	5.76 (0.58)	07/07/01		
	eCO_2	0.90 (0.51)	17/05/01	9.01 (0.45)	07/07/01	11.11	ns
	eTemp	0.89 (0.59)	05/03/01	9.34 (0.68)	07/07/01	25.83	ns (10%)
	eCO_2/eTemp	1.85 (0.43)	27/02/01	11.66 (0.66)	07/07/01	72.17	***

Percentages changes (Δ%) in relation to the mean of the ambient control samples are shown and P values where ns (10%) denotes significant at the $P<0.1$ level only, * significance at the $P<0.05$, ** at the $P<0.01$ and *** at the $P<0.001$ level. Numbers in parentheses indicate standard error of the mean. These conventions apply throughout unless stated otherwise. n=4.

No significant difference ($P>0.05$) was found between the DOC concentrations of any of the treated samples in comparison to the control prior to treatment. Generally, DOC concentrations increased throughout the year from winter to summer and showed a broad summer peak between May and August under all treatments, corresponding to between 12 and 24 weeks of treatment (figure 8.01). Maximum concentrations in all treatments occurred on 07/07/01, ca. 19 weeks after treatments commenced. Elevated CO_2 did not significantly increase ($P>0.05$) mean DOC concentrations over the treatment period, although overall the concentration was 10.37%

higher than the control, and eTemp produced DOC concentrations that were very similar to the control. In contrast, the eCO_2/eTemp treatment produced unexpectedly high DOC levels throughout the study period and these were significantly higher than the control (70.19%, $P<0.001$).

Concentrations of phenolic substances were more variable than those of DOC with no discernible seasonal pattern (figure 8.02), perhaps due to greater percentage errors as a result of low concentrations. Substantially higher concentrations with respect to the control were observed in those treatments that included eTemp, with the eTemp treatment producing levels that were 59.34% higher than those of the control, though this was not significant. The combined effects of eCO_2/eTemp produced the largest increase in phenolic compound concentrations in relation to the control (85.13%), and this was significant ($p<0.01$).

Algal biomass followed a similar pattern to that of DOC, with the highest concentrations being observed in the summer months (figure 8.03). The greatest increases in algal biomass were found where eCO_2 was included in the simulation, although under eTemp algal biomass was also higher than the control (32.93%). However, only eCO_2/eTemp produced significant differences in comparison to the control (98.75%, $P<0.01$), with that under eCO_2 being significant at the $P<0.1\%$ level only (53.89%).

Extracellular degradative capacity can potentially be conserved within the PSM (Lock, 1981; Lock *et al.*, 1984) and so cumulative activities have been plotted. Significant differences in relation to the control are given in the table above (using actual rather than cumulative values). Phenol oxidase activities declined significantly (-45.29%, $P<0.01$) under conditions of eCO_2/eTemp, while no significant difference was observed in either the eCO_2 or eTemp samples (figure 8.04). There was however, a substantial decline in activities in the case of the former (-21.28%) and conversely a stimulation of activities in the latter (14.59%). ß-glucosidase activities were stimulated to a similar extent in both the eTemp treatments, whereas eCO_2 produced similar activities to the control (figure 8.05). The mean treatment effect was significantly different to the control under conditions of eCO_2/eTemp only (33.35%, $P<0.05$), while eTemp showed a substantial but insignificant increase in activities (30.70%). All treatments increased total metabolic activities in relation to the control (eCO_2 11.11%, ns; eTemp 25.83%, $P<0.1$ only) and this is illustrated in figure 8.06, but again only the eCO_2/eTemp treatment reached significance relative to the control, producing a dramatic response (72.17%, $P<0.001$). Phosphatase activities were measured at the end of the experiment only, i.e., following 24 weeks of treatment and are shown in figure 8.07.

Table 8.02. Phosphatase activities for light grown biofilm under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp simulations in relation to the control following 24 weeks of treatment (14/08/01)

Treatment	Phosphatase activity (nmol MUF cm^{-2} h^{-1})	Δ%	P
control	0.458 (0.071)		
eCO_2	0.59 (0.068)	28.75	ns
eTemp	0.679 (0.052)	48.06	ns
eCO_2/eTemp	0.814 (0.092)	77.52	*

Phosphatase activities were stimulated under all treatments with respect to the control (eCO_2 28.75%, eTemp 40.06%) but only those under eCO_2/eTemp were significantly different to the control (77.52%, $P<0.05$).

A summary of the correlations between determinands under each of the climate change simulations is shown in the following table.

Table 8.03. Correlations between light grown biofilm determinands under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp simulations under light conditions

DOC	Chlorophyll *a*	β-glucosidase
control	0.9239***	0.6491*
eCO_2	0.9654***	0.8414**
eTemp	0.8868***	0.9817***
eCO_2/eTemp	0.9963***	0.8913***
Chlorophyll a	β-glucosidase	INT
control	0.8200**	0.9631***
eCO_2	0.9355***	0.9776***
eTemp	0.8821***	0.8588**
eCO_2/eTemp	0.8882***	0.9823***
β-glucosidase	INT	Phenolics:DOC
control	0.7652**	-0.3640 (ns)
eCO_2	0.9225***	-0.4909 (ns)
eTemp	0.9768***	-0.7409*
eCO_2/eTemp	0.9213***	0.7580*
INT	Phenolics:DOC	
control	-0.4789 (ns)	
eCO_2	-0.6992 *	
eTemp	-0.6779*	
eCO_2/eTemp	-0.7737**	

Chlorophyll *a* is used here to indicate algal biomass and INT total metabolic activity. The ratio of phenolic compound concentrations to the total DOC pool is represented by the term phenolics:DOC and this convention will be used throughout unless stated otherwise. Non significant correlations are shown by the term (ns).

Numerous strong and significant correlations were found between measurements associated with the light grown biofilm. Algal biomass was a prominent feature of the data and was highly correlated (positively) with DOC, β-glucosidase and metabolic activities in particular.

Figures 8.08a-d show a representative scanning electron micrograph of biofilm taken from each of the climate change simulations following 24 weeks of treatment, i.e., towards the end of the growing season in late summer (14/08/01). Under the control conditions, bacteria were dominant (figure 8.08a), while this component was less abundant in the eCO_2 communities, which showed a limited number of flagellate-like organisms (8.08b). Communities maintained at eTemp showed a marked change in community structure in the form of prolific growth of filamentous organisms (possibly cyanobacteria and fungal hyphae) (8.08c). The combination of these two treatments produced an assemblage characterized by numerous diatoms and a thick PSM (8.08d) but less obvious bacterial component in comparison to the control.

8.04.2 Heterotrophic communities (dark grown biofilm)

Table 8.04. Range of determinands and significant differences for dark grown biofilm under elevated CO_2 (eCO_2), elevated temperature (eTemp) and elevated CO_2/elevated temperature (eCO_2/eTemp) simulations in relation to the control

Determinand	Treatment	Minimum	Date	Maximum	Date	Δ%	P
DOC (mg L^{-1})	control	1.08 (0.92)	05/03/01	7.85 (0.69)	17/05/01		
	eCO_2	1.03 (0.22)	05/03/01	4.87 (0.52)	17/05/01	-25.79	ns
	eTemp	1.19 (0.32)	09/04/01	3.86 (0.17)	17/05/01	-18.90	ns
	eCO_2/eTemp	1.48 (1.06)	13/03/01	4.69 (0.61)	07/07/01	-11.79	***
Phenolic compounds (mg L^{-1})	control	0 (0)	26/04/01	0.23 (0.06)	17/05/01		
	eCO_2	0.02 (0.09)	09/04/01	0.30 (0.04)	17/05/01	41.78	ns
	eTemp	0 (0.08)	09/04/01	0.62 (0.04)	09/06/01	87.55	ns
	eCO_2/eTemp	0 (0.04)	09/04/01	0.35 (0.04)	14/08/01	49.59	ns
Phenol oxidase activity (pmol dicq cm^{-2} min^{-1})	control	30 (5.9)	17/05/01	90 (4.8)	09/06/01		
	eCO_2	6 (6.2)	17/05/01	65 (2.4)	26/04/01	-38.54	ns
	eTemp	20 (4.1)	09/06/01	84 (3.9)	09/04/01	-10.06	ns (10%)
	eCO_2/eTemp	30 (6.8)	14/08/01	71 (3.1)	09/04/01	-21.83	ns
β-glucosidase activity (nmol MUF cm^{-2} h^{-1})	control	0.01 (0.009)	13/03/01	0.09 (0.01)	26/04/01		
	eCO_2	0 (0.02)	09/06/01	0.06 (0.01)	26/04/01	-58.43	ns
	eTemp	0 (0.16)	26/04/01	0.25 (0.10)	23/03/01	36.28	ns
	eCO_2/eTemp	0.016 (0.018)	13/03/01	0.074 (0.02)	09/04/01	-35.15	ns
Metabolic activity (μg formazan cm^{-2} h^{-1})	control	0 (0.78)	13/03/01	7.2 (0.81)	26/04/01		
	eCO_2	0 (0.74)	09/06/01	5.8 (0.81)	07/07/01	-20.95	ns
	eTemp	0 (0.85)	26/04/01	10.0 (0.81)	07/07/01	-12.29	ns
	eCO_2/eTemp	0.3 (0.86)	13/03/01	6.5 (0.68)	09/04/01	-24.86	ns

Heterotrophic DOC concentrations showed an acute summer peak in May (following 12 weeks of treatment) when all treatments produced their maximum concentrations (figure 8.09). In relation to the control, all treatments showed slightly increased DOC removal but only that under the combined treatment (eCO_2/eTemp) was significant (-11.79%, $P<0.001$).

Levels of phenolics (figure 8.10) tended to remain low in all treatments over the earlier part of the year and increase in the summer months. Despite substantially increased concentrations of phenolic compounds under eCO_2 (41.78%), eTemp (87.55%) and eCO_2/eTemp (49.59%), mean treatment effects did not achieve significance.

All treatments presented suppressed phenol oxidase activities with the most pronounced effect being observed under eCO_2 (-38.54%), although none were statistically significant at the $P<0.05$ level (figure 8.11). Substantially suppressed ß-glucosidase activities were also observed where eCO_2 was included in the treatment conditions and the most dramatic suppression was found under eCO_2 (-58.43). Conversely, under eTemp activities were stimulated (36.28%). However, no treatment was significantly different to the control. ß-glucosidase activities are illustrated in figure 8.12. Metabolic activities were not significantly different to the control under any of the simulations, although where eCO_2 was included within a treatment a suppression of over 20% was observed (figure 8.13). Similarly, none of the climate change simulations were found to exhibit significantly different phosphatase activities to those of the control following 24 weeks of treatment (figure 8.14).

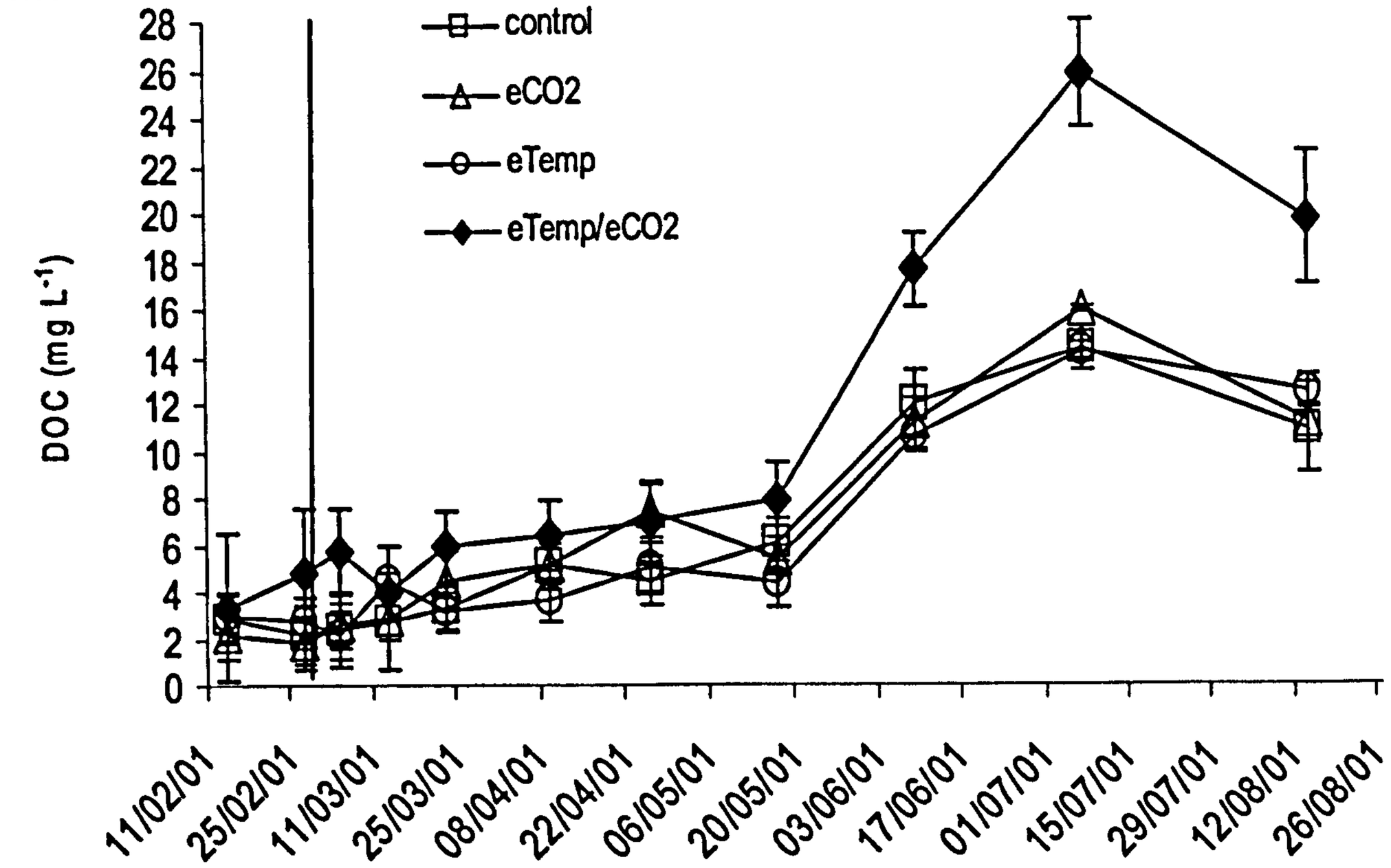
A summary of the correlations between determinands under each of the climate change simulations is shown in the following table.

Table 8.05. Correlations between dark grown biofilm determinands under elevated CO_2 (eCO_2), elevated temperature (eTemp) and eCO_2/eTemp simulations

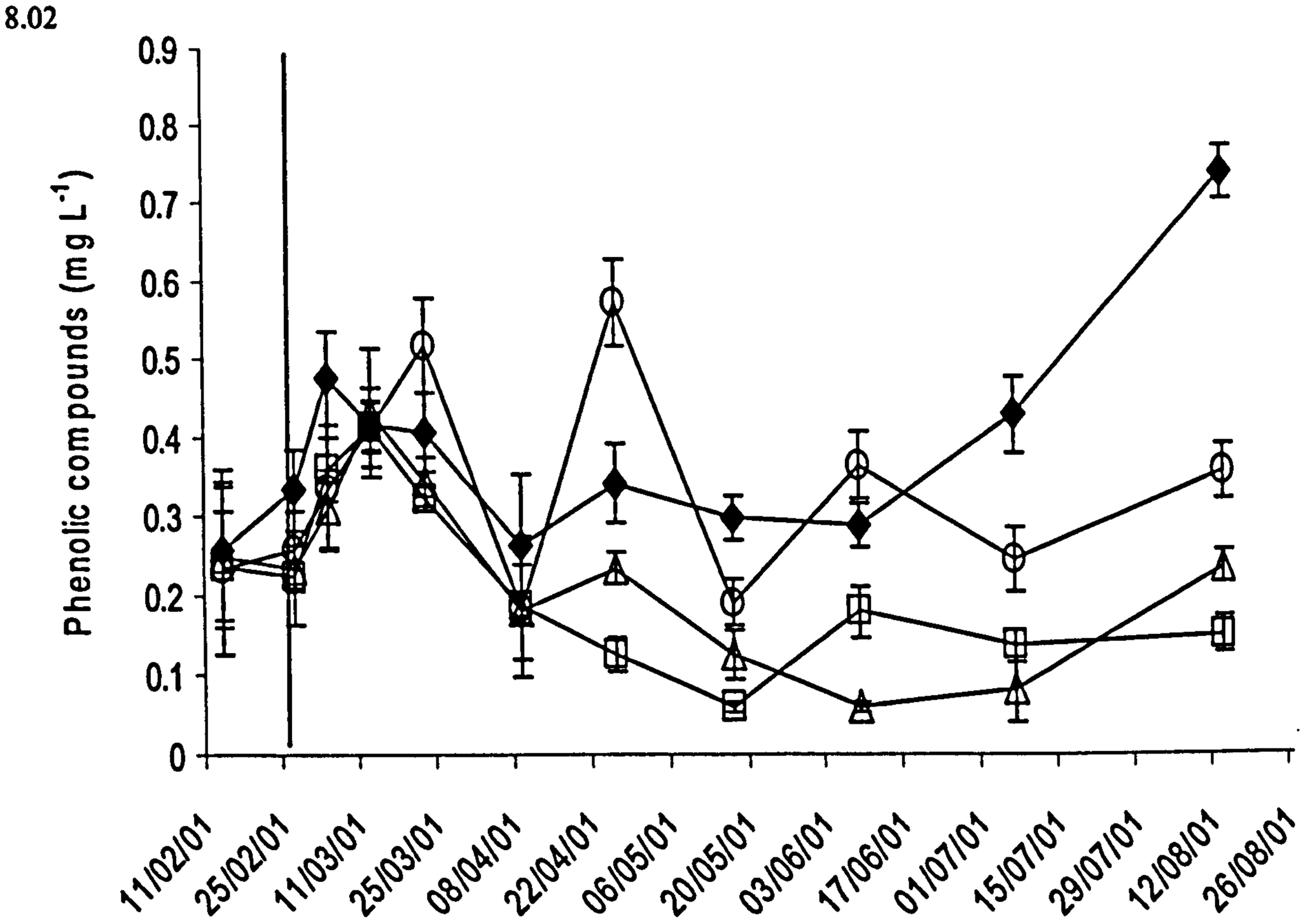
Phenolics:DOC	β-glucosidase	INT
control	-0.9545***	-0.9130***
eCO_2	-0.9069***	-0.9247***
eTemp	-0.5084 (ns)	-0.6563*
eCO_2/eTemp	-0.9262***	-0.8366**

The heterotrophic biofilm showed a limited number of strong negative correlations between phenolic compound:DOC ratios and both total metabolic activities and extracellular β-glucosidase activities.

8.01

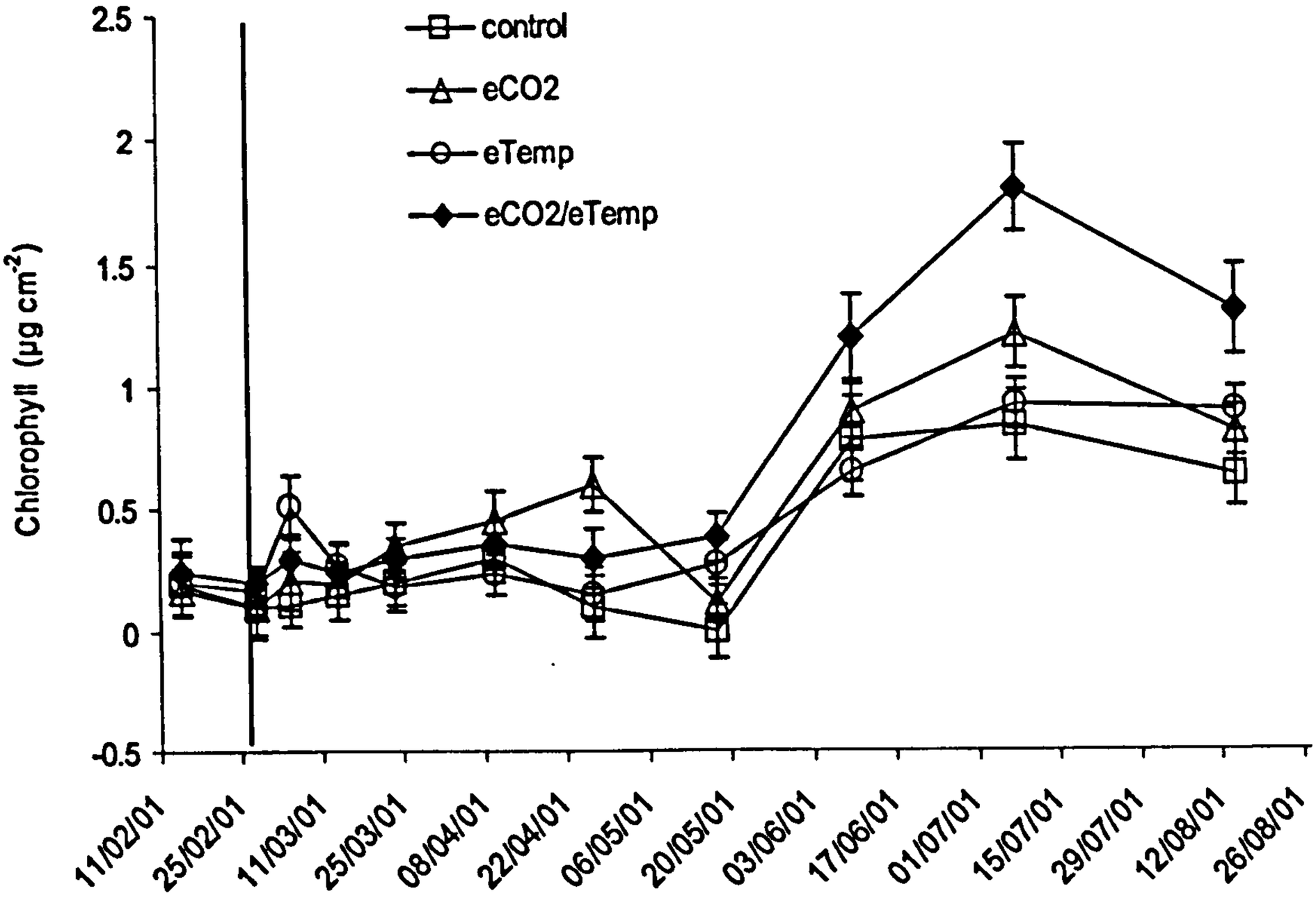


8.02

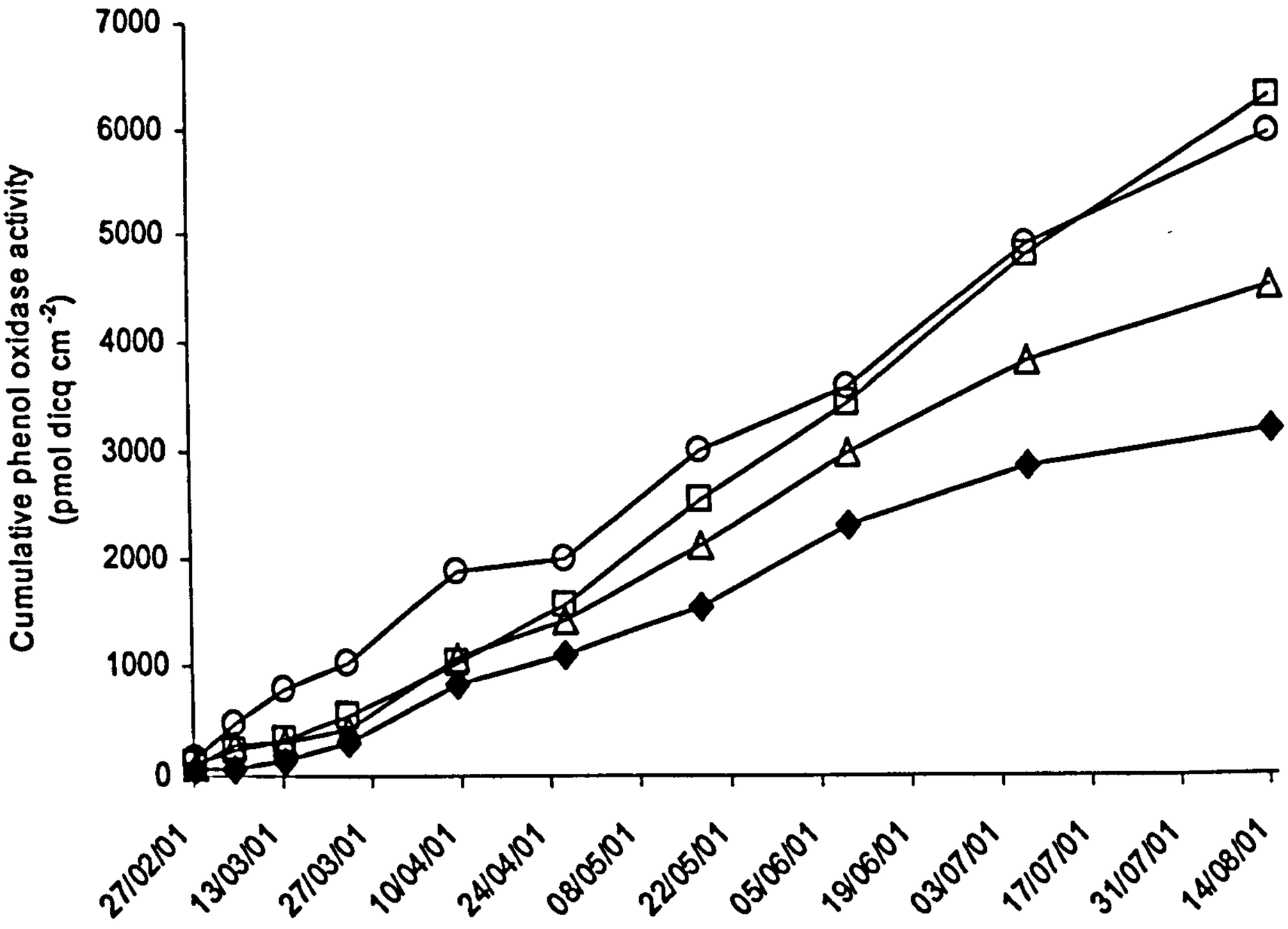


DOC (figure 8.01) & phenolic compound (figure 8.02) concentrations of waters overlying photoautotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. Vertical line denotes the start of treatments. Error bars represent standard error of the mean, n=4.

8.03

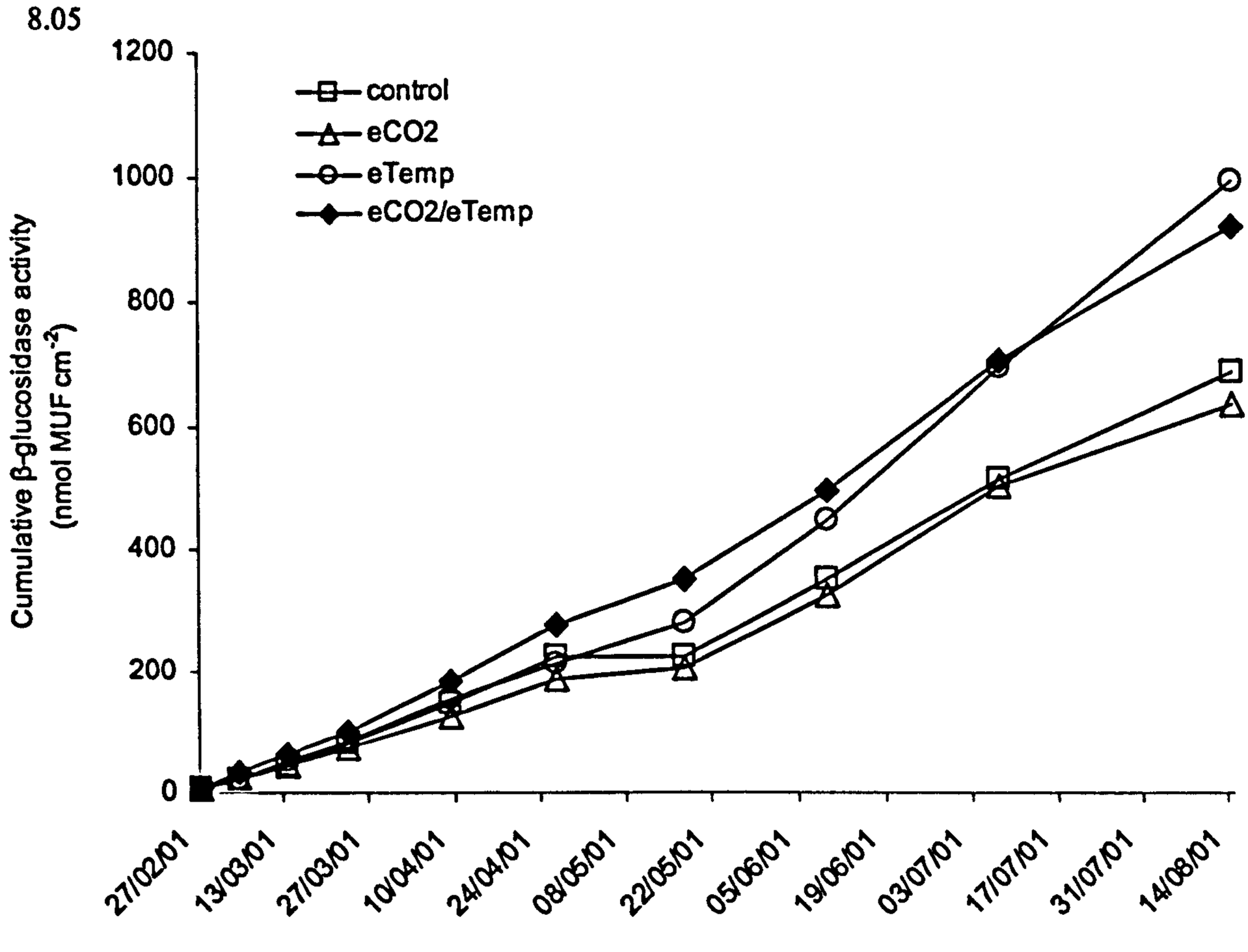


8.04

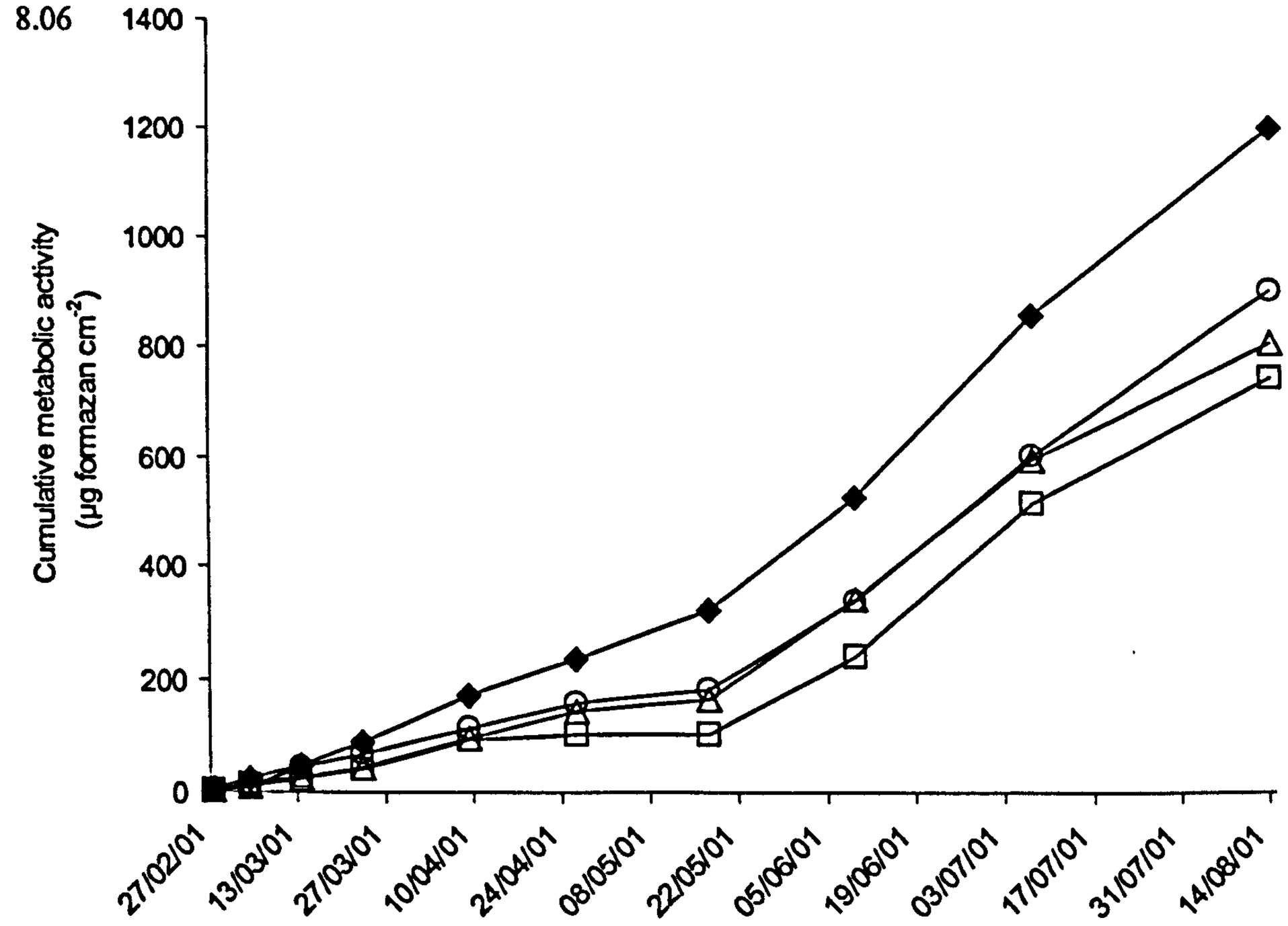


Algal biomass, indicated by chlorophyll *a* concentrations, (figure 8.03) & cumulative phenol oxidase activities (figure 8.04) of photoautotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. Vertical line denotes the start of treatments. Error bars represent standard error of the mean, n=4.

8.05



8.06



Cumulative β-glucosidase (figure 8.05) & cumulative total metabolic activities, indicated by INT reduction, (figure 8.06) of photoautotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. n=4.

8.07

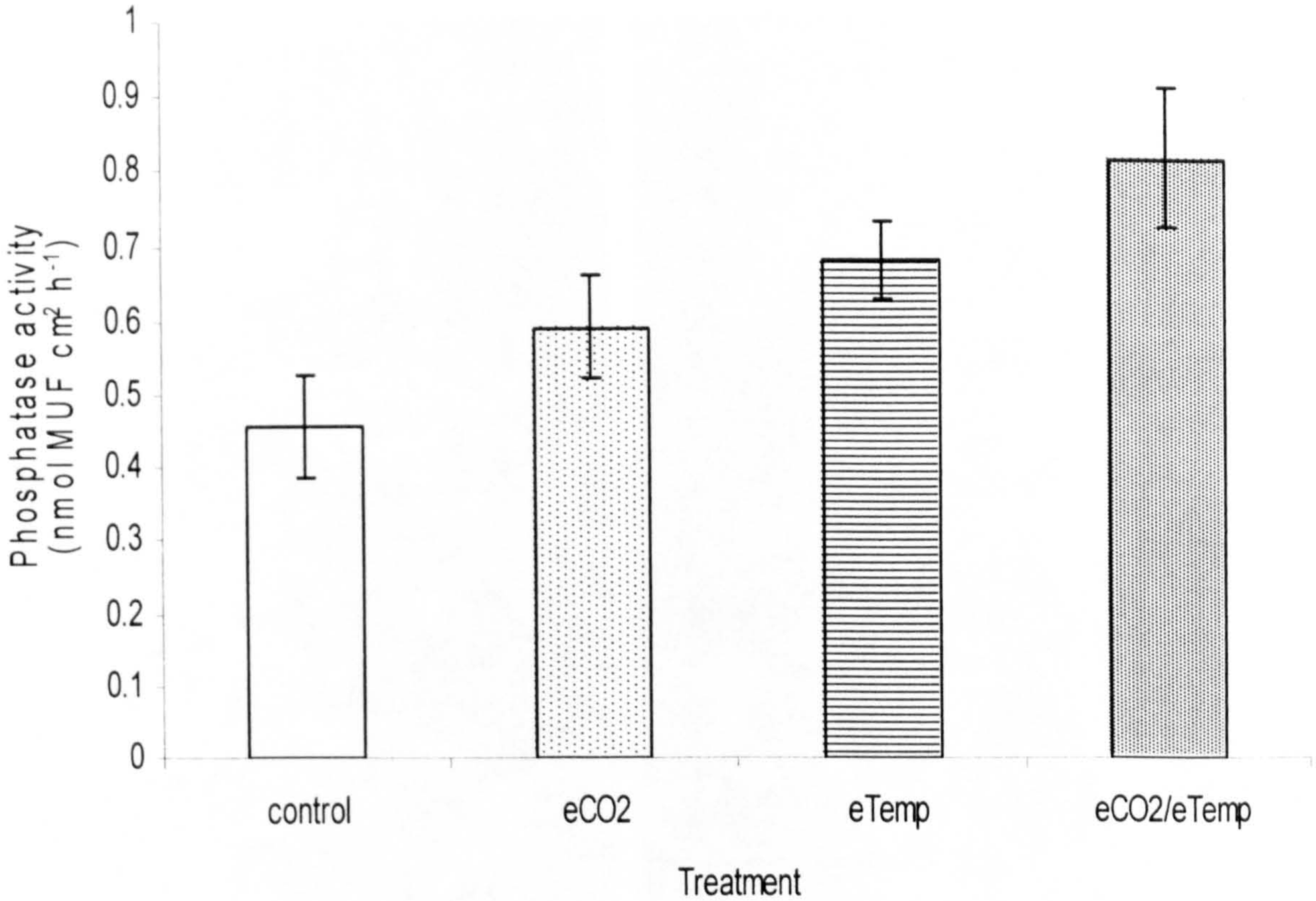


Figure 8.07. Phosphatase activities of photoautotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. Error bars represent standard error of the mean, n=4.

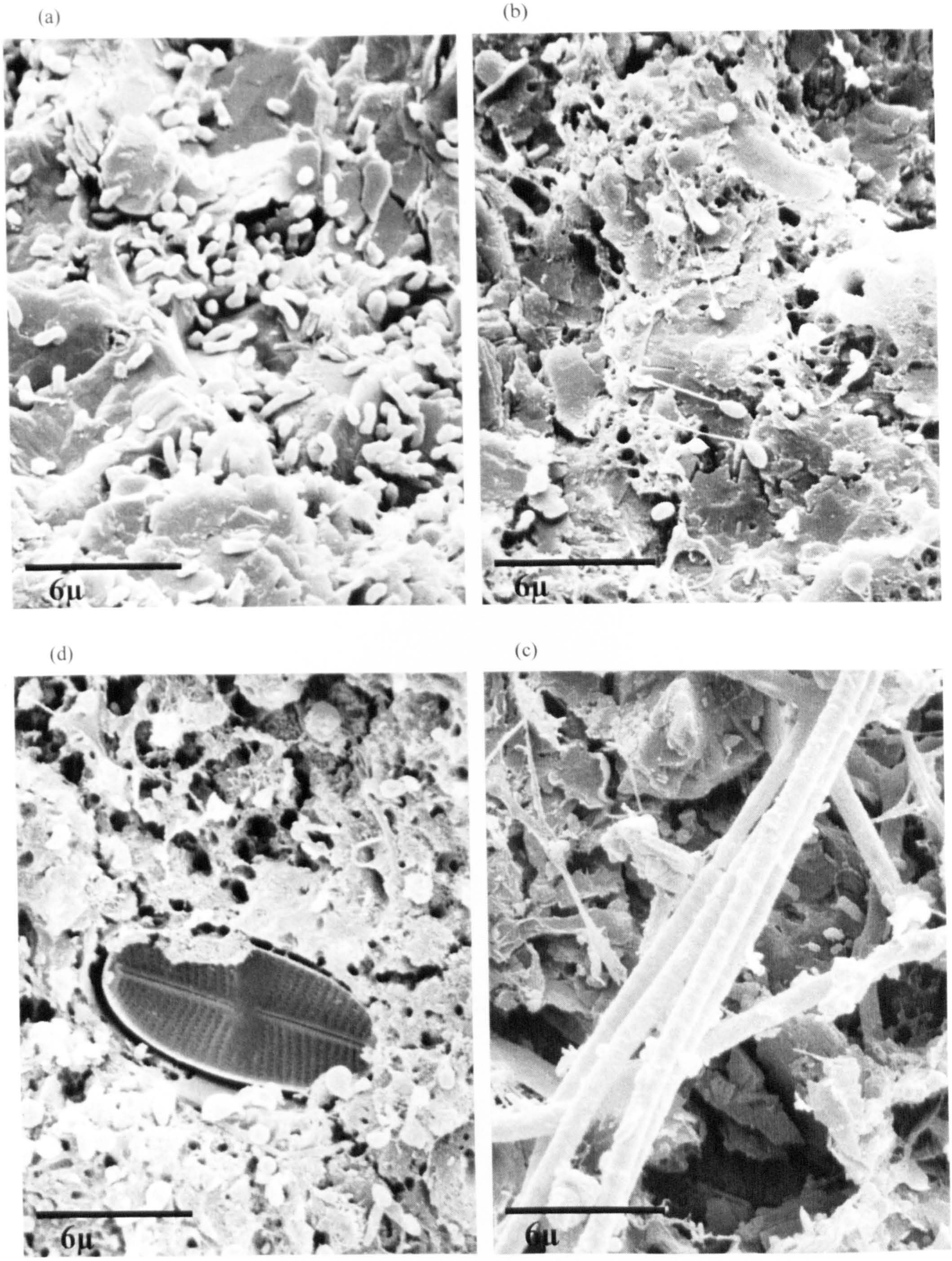
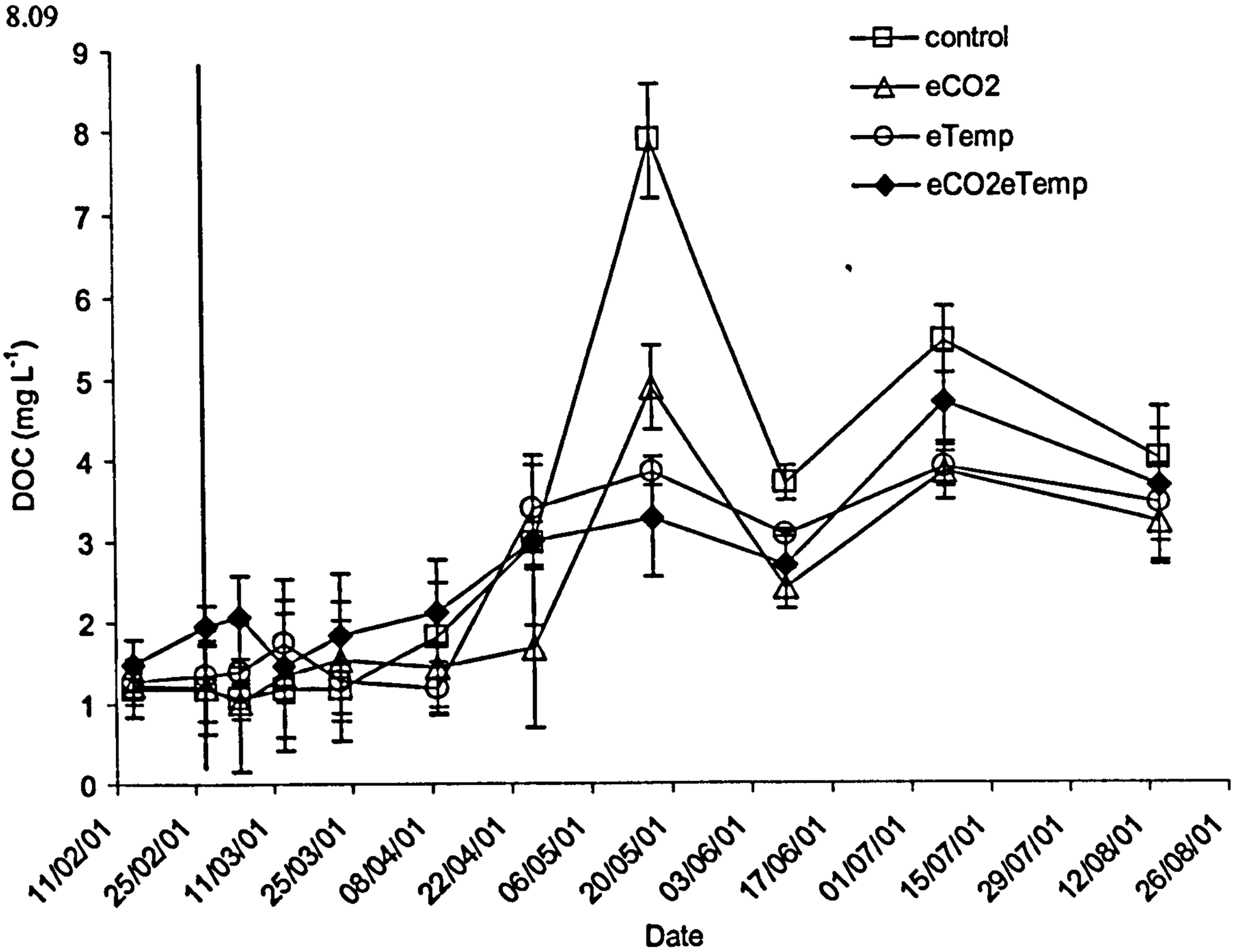
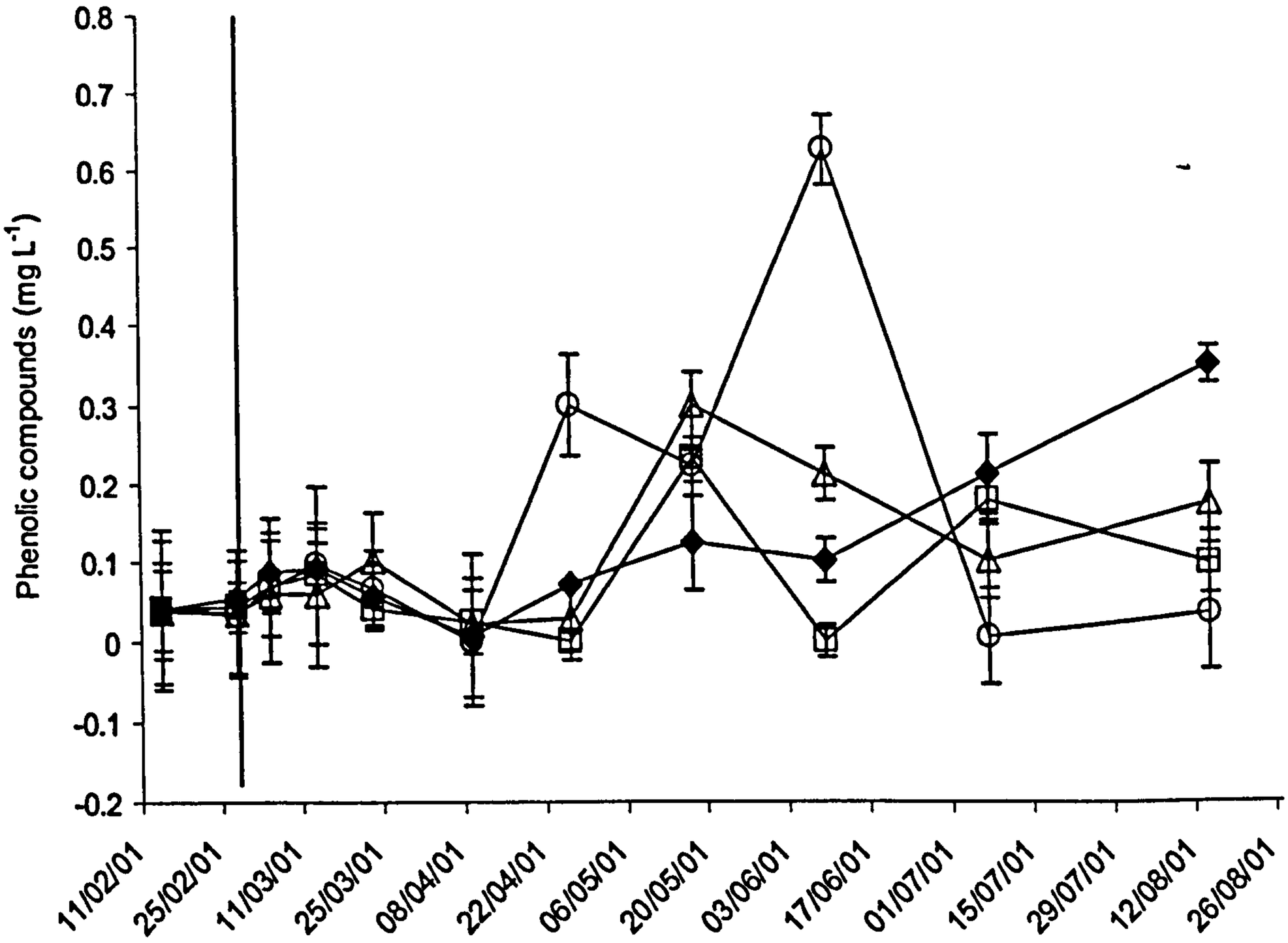


Figure 8.08. Scanning electron micrographs of biofilm coated stones maintained under (a) control, (b) elevated CO_2, (c) elevated temperature & (d) elevated CO_2/elevated temperature conditions.

8.09

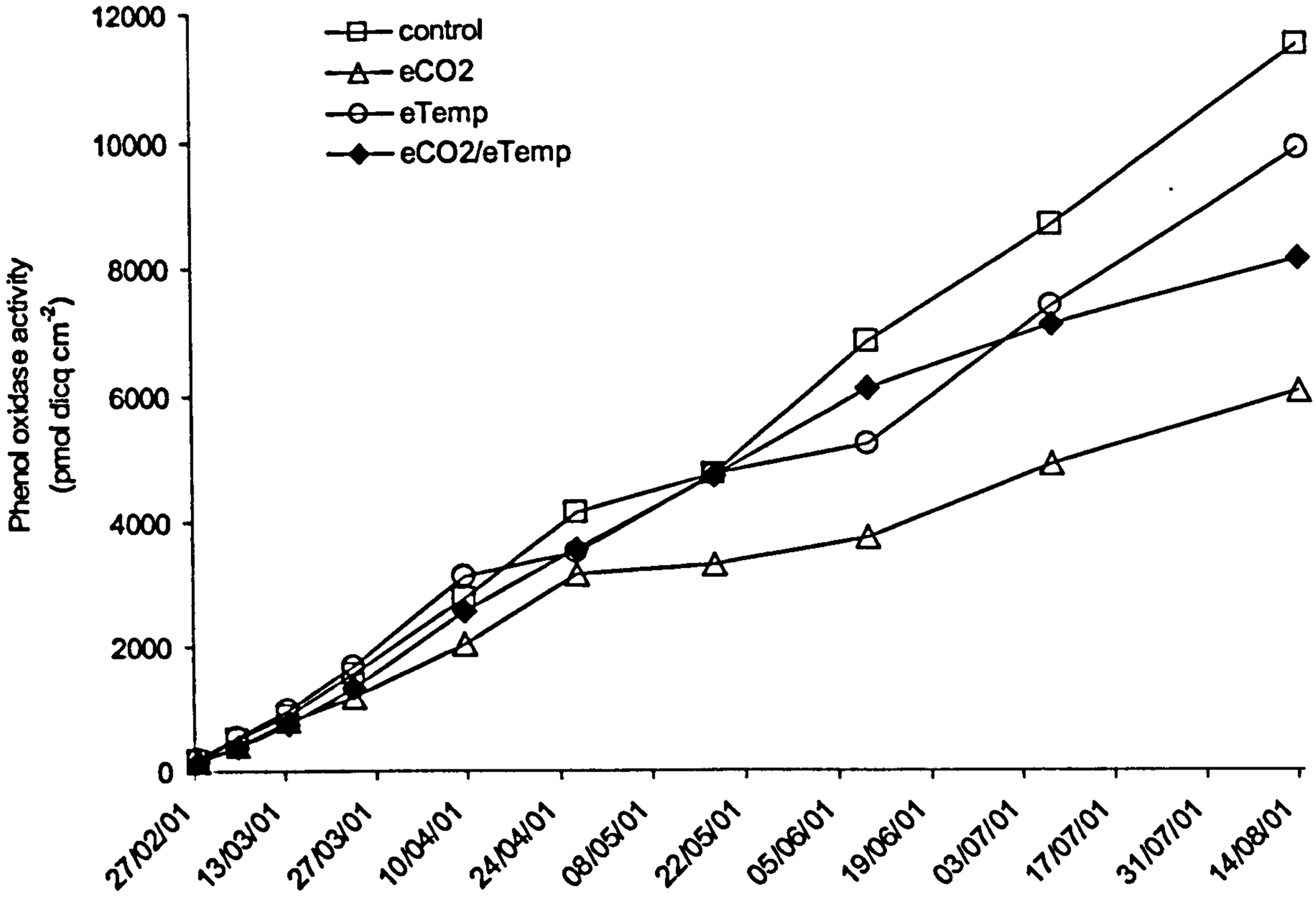


8.10

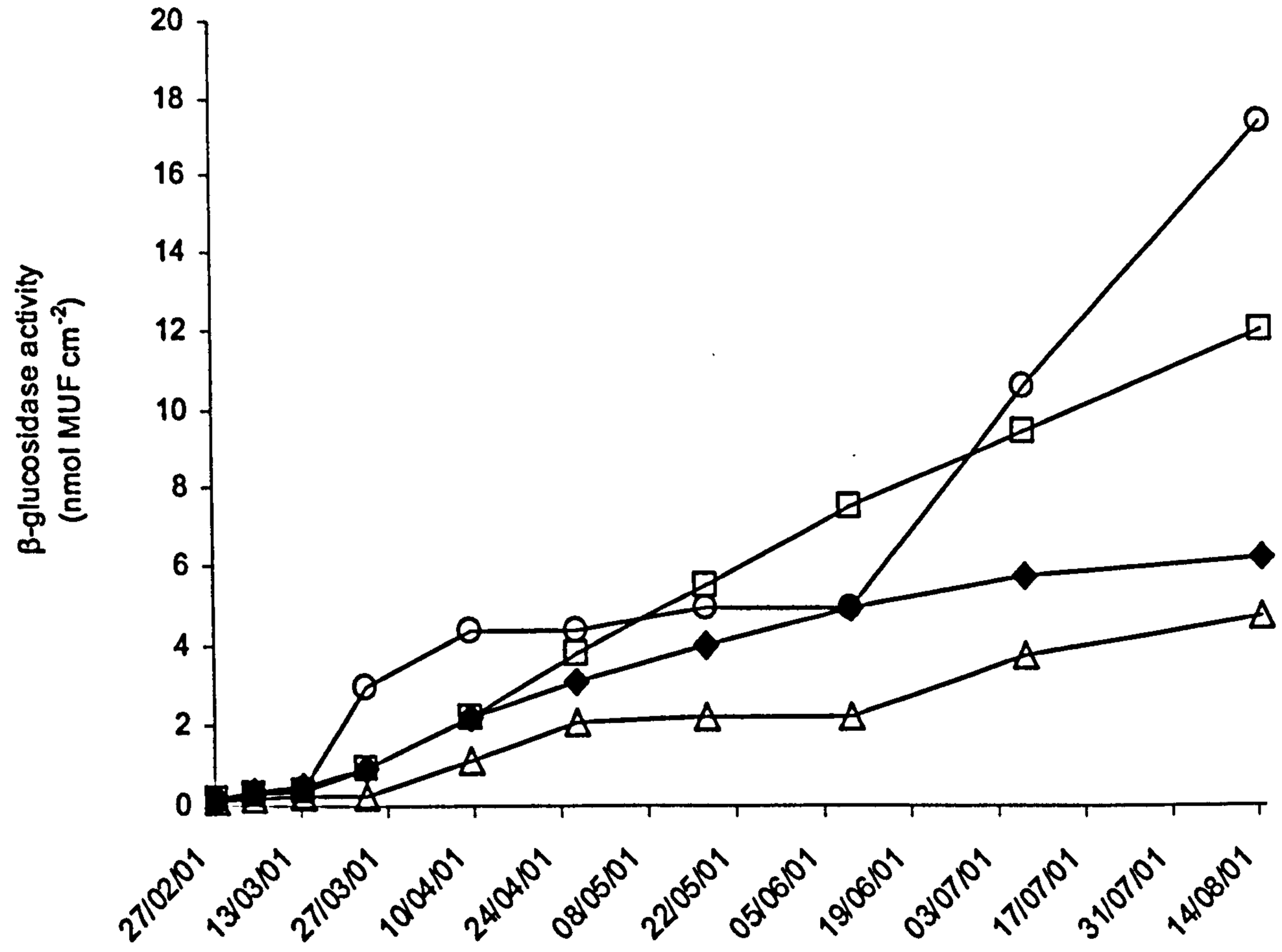


DOC (figure 8.09) & phenolic compound (figure 8.10) concentrations of waters overlying heterotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. Vertical line denotes the start of treatments. Error bars represent standard error of the mean, n=4.

8.11

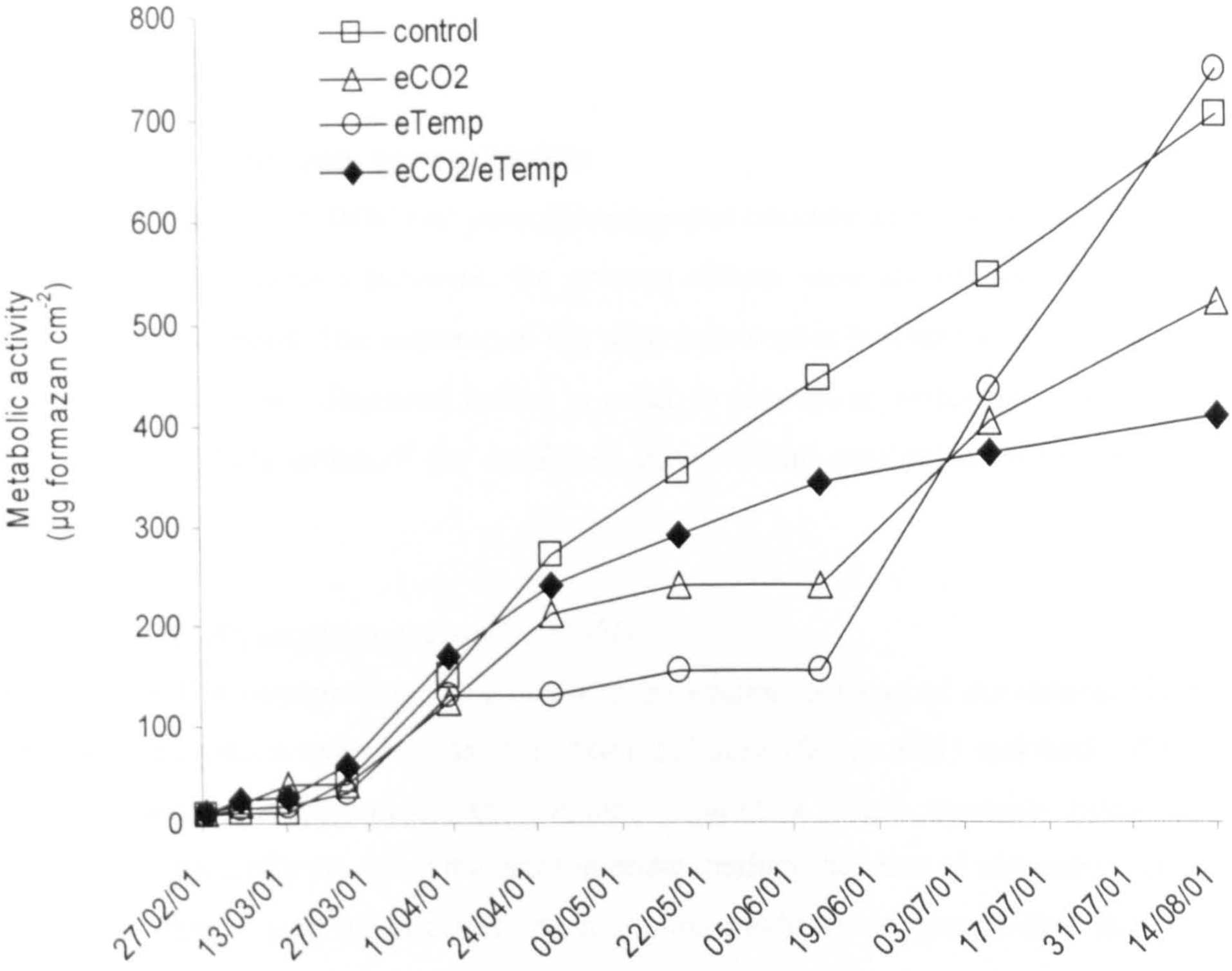


8.12

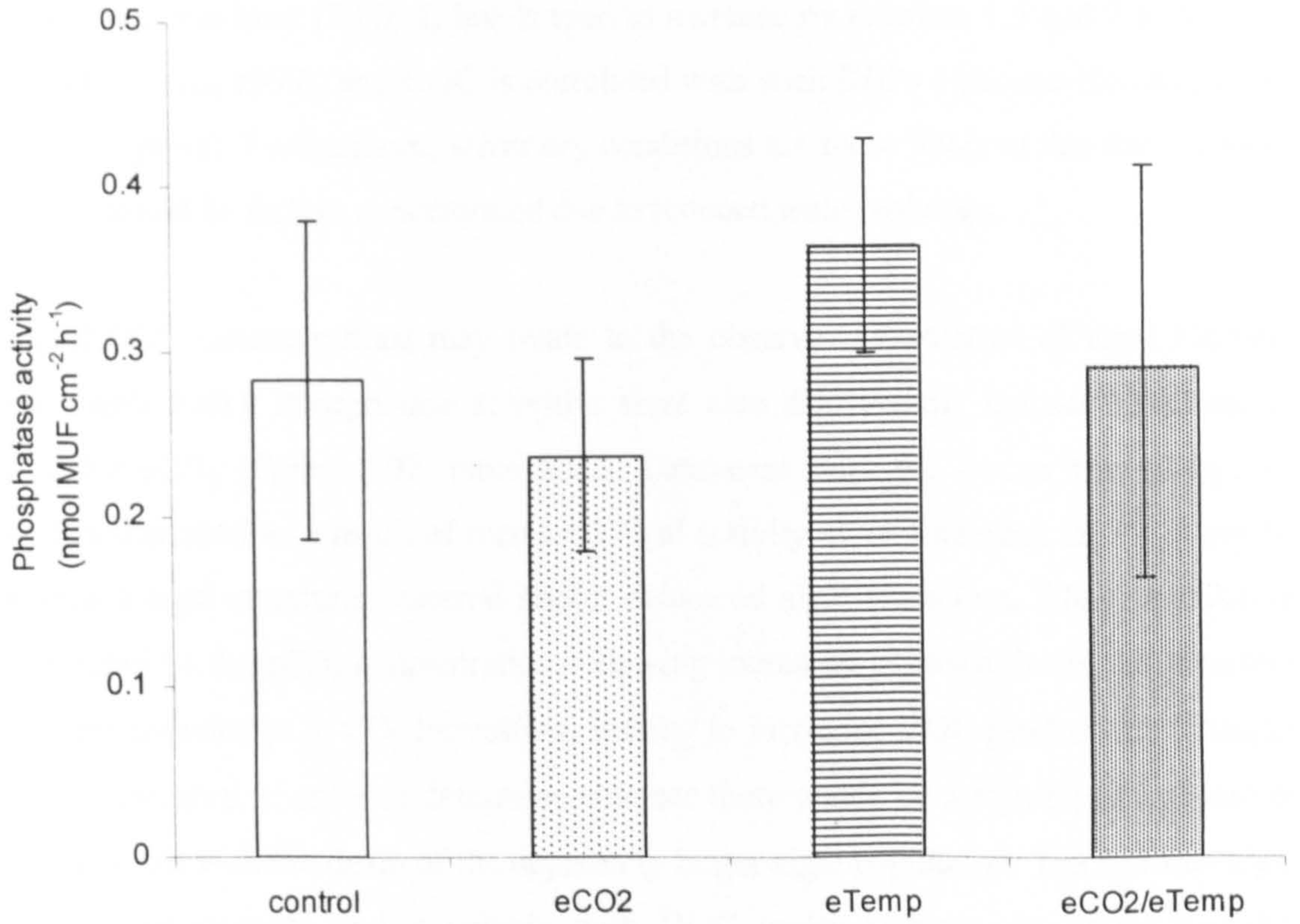


Cumulative phenol oxidase (figure 8.11) & cumulative β-glucosidase activities (figure 8.12) of heterotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. n=4.

8.13



8.14



Cumulative total metabolic activities, indicated by INT reduction, (figure 8.13) & phosphatase activities (figure 8.14) of heterotrophic biofilm under control, elevated CO_2 (eCO_2), elevated temperature (eTemp) & eCO_2/eTemp conditions. Error bars represent standard error of the mean, n=4

8.05 DISCUSSION

8.05.1 Photoautotrophic (light grown) biofilm

Substantial changes in mean DOC and phenolic compound concentrations were found under the eCO_2 and eTemp simulations however, the greatest effects were always found when these treatments were combined. The response of the photoautotrophic biofilm to the separate eCO_2 and eTemp treatments are discussed below in order to attempt to understand the processes occurring and the likely effect of the combined eCO_2/eTemp conditions on biofilm carbon processing.

Effects of elevated CO_2 on photoautotrophic biofilm

Under eCO_2, DOC concentrations were found to be similar to those of the control although maximum concentrations were increased in April and July (figure 8.01) and both DOC and phenolic compound concentrations on average showed a slight increase (table 8.01). Spring/summer apparently produced the greatest effect, perhaps because of increased biological activity at this time of year when conditions are more conducive to growth (and decay) and therefore the release of DOC. Increased spring and summer DOC maxima may be of concern because total trihalomethane (TTHM) levels tend to increase by between 1.5 and 2 fold in the summer months (Betts, 1998), and DOC is correlated with such DBPs (Alarcon-Herrera, 1994; Worrall *et al.*, in press). Furthermore, warm dry conditions are more likely at this time of year, meaning DOC would be further concentrated due to reduced water volumes.

The increased DOC concentrations may relate to the observed stimulation of algal biomass (figure 8.03, table 8.01). Phosphatase activities were also substantially but not significantly increased under eCO_2 (figure 8.07, table 8.02), consistent with the notion that phosphate acquisition has increased as a result of increased algal activity. Such increases in DOC may be due to increased algal structural material and/or enhanced algal exudation. Algal stimulation may be facilitated by the eCO_2 concentrations allowing increased photosynthesis and therefore greater resource investment in CO_2 harvesting, leading to increased DOC production. A longer study may be warranted in order to determine whether there would be a significant increase in DOC concentrations with the death of the apparently larger algal population. The fact that algal biomass correlated strongly and positively with DOC under ambient conditions (0.9239, $P<0.001$) suggests that the photoautotrophs already contribute the majority of DOC to the surrounding water, either by exudates or algal cell lysis. The original assumption that the photoautotrophic element would be important in these particular samples is seemingly affirmed. If this is the case, we might expect an increase in the correlation between algal biomass and DOC concentrations under the eCO_2 conditions should the photoautotrophs be stimulated.

Indeed, a correlation coefficient of 0.9654 ($P<0.001$) was found. However, these results do not preclude a heterotrophic mechanism for increased DOC concentrations, *via* the use of algal materials for example.

It is widely believed that heterotrophic components of the biofilm, in particular bacteria, are the major producers of extracellular enzymes (Münster, 1991), but it must be noted that other sources may also contribute to the decomposition potential of the system. The substantial, though non significant, decline in phenol oxidase activities under eCO_2 (figure 8.04, table 8.01) suggested that the biofilm degradatory capacity had been somewhat compromised. From this it can be inferred that no enhancement of allochthonous or autochthonous DOC degradation is likely as a result of increased labile photoautotrophic inputs. Reduced activities may be due to a decline in enzyme production or inhibition of pre-existing enzymes and the reduced bacterial presence in relation to the control provides evidence to support the former (figures 8.08b & 8.08a respectively). While metabolic activities were not suppressed (figure 8.06, table 8.01), indicating that enzyme production is likely to be sustained, INT reduction was used here as a measure of total intracellular and extracellular reductive capacity of both heterotrophs and autotrophs within the biofilm (Trevors, 1984). Thus, a stimulation of the former would mask any inhibition of heterotrophic metabolism. This study provided no means of determining such inhibitory mechanism(s) but these may include direct CO_2 inhibition (as a result of the extra burden of metabolic waste product), end product inhibition due to increased amounts of labile exudates, nutrient deficiency (as a result of enhanced photoautotrophic activity), species composition shifts or alterations to the chemical composition of photoautotrophic materials. All of these mechanisms have been proposed in relation to the terrestrial system (see chapter 2).

Seemingly, ß-glucosidase activities were not significantly affected by eCO_2 (figure 8.05, table 8.01), despite some increase in DOC concentrations and algal biomass. Thus, the potential for decomposition of this extra material has not been stimulated, which is not inconsistent with the idea that algal exudates may be providing all the *in situ* heterotrophic carbon requirements, in line with the findings of Haack and McFeters (1982). ß-glucosidase activity and DOC concentrations correlate strongly and positively under eCO_2 ($R^2 = 0.8414$, $P<0.01$) and in all treatments (table 8.03), supporting the results reported by Münster (1991) looking at lakes Mekkojärvie and Plussee. This perhaps suggests either a stimulation of activities due to, for example, algal exudates (labile or otherwise), or structural material (i.e., substrate induction). Another possibility is enzymic generation of DOC (Freeman *et* al., 2001a; see chapter 3) by the hydrolysis of low molecular units from high molecular weight, non-dissolved structural polymers. There is also a strong correlation between ß-glucosidase activities and algal biomass

under the control conditions (0.8200, P<0.01), consistent with the findings of Chappell and Goulder (1994) from three differing Yorkshire streams. The eCO_2 treatment reinforced this correlation (0.9355, P<0.001), but this does not elucidate whether there is an increase in exudation and/or algal biomass; exudation will probably be strongly related to chlorophyll concentration because the latter is an indicator of algal biomass (Chappell & Goulder, 1994) as would actual algal biomass i.e., structural material etc. However, these results underline the tight coupling between heterotrophs and photoautotrophs (provided that ß-glucosidase activities are not primarily due to release by photosynthetic organisms).

The findings above raise the question of competition between photoautotrophs and heterotrophs under eCO_2 conditions, as was postulated by Freeman *et al.* (1998) in relation to the terrestrial system, since when the former are stimulated the latter seem relatively less active (compared to the potential shown under eTemp for example (see below)). There may be some element(s) or co factor(s) that are required by both photoautotrophs and heterotrophs that become limiting under eCO_2, which the photoautotrophs are more efficient at acquiring. Perhaps then interspecies relationships within the biofilm are not as harmonious as the idea of algae producing labile carbon sources that the heterotrophs can then utilize (c.f. Haack & McFeters, 1982; Kaplan & Bott, 1982 etc.) may suggest. And, climatic changes may well alter any balance that did exist between photoautotrophic and heterotrophic carbon processing.

Metabolic activities correlated more strongly with algal biomass (0.9776, P<0.001) than in the control (0.9631, P<0.001), as might be expected from the other findings discussed. These correlations underline the importance of the photoautotrophic component to the metabolism of the type of biofilm assemblages studied here, either for contributing directly to the total metabolic activity or indirectly through transfer of resources to the heterotrophs, even before treatments are imposed. Metabolic activities also correlated more strongly with ß-glucosidase activities (0.9225, P<0.001) than in the control (0.7652, P<0.01), perhaps indicating that the extracellular cleavage of polysaccharides plays a more important role in the metabolism of the eCO_2 biofilm as a result of stimulated photoautotrophic production. A negative correlation between the phenolic compound:DOC ratios and metabolic activities was found (-0.6992, P<0.05), supporting the findings of Freeman *et al.* (1990). This was much stronger under eCO_2 than under control conditions (–0.4789, ns) and may suggest a potential problem from a water quality perspective because metabolic activities and therefore DOC consumption (by *in situ* and downstream heterotrophs) may be inhibited. Thus, not only would there be a tendency for increased photoautotrophic DOC production, but also reduced heterotrophic degradation of DOC both *in situ* and downstream.

Effects of elevated temperature on photoautotrophic biofilm

Under eTemp, DOC concentrations were similar to the control (figure 8.01, table 8.01) as was true for the eCO_2 treatment, however, a substantial enrichment of phenolic compounds was observed (figure 8.02, table 8.01). A positive feedback to increasing DOC concentrations and reduced water quality could therefore be foreseen. Shifts in phenolic compound concentrations may provide an indication that carbon processing is changing, despite the fact that total DOC concentrations remained similar. Furthermore, eTemp is likely to allow production over a longer growing season (Gian-Reto *et al.*, 2002; Menzel & Fabian, 1999; Roetzer & Chmielewski, 2000) when cooler temperatures would normally limit DOC production

A substantial though non significant increase in algal biomass was induced under eTemp (figure 8.03, table 8.01). This may be due to a general increase in growth rates under a warmer regime and could account for the increased phenolic compound concentrations. Though still significant, the correlation between algal biomass and DOC (0.8868, $P<0.001$) is reduced with respect to the control, suggesting that the photoautotrophic component is relatively less dominant in terms of DOC processing and export to the surrounding water. Heterotrophic processing may have become more important to some extent (see later) and this may be through the degradation of algal material, polymerization of low molecular weight compounds or heterotrophic exudation. Although a large proportion of the substrates utilized by the heterotrophs probably originated from the photoautotrophs, there may be some temporal separation between the transfer of algal materials to the heterotrophs and their subsequent release as DOC. The 'microbial loop' (the trophic system which was found to be separate from the typical photosynthetic organisms-grazers food chain) could be responsible for the processing of a considerable amount of OM (Azam *et al.*, 1983; Ducklow, 1994).

Phenol oxidase activities were not significantly different under an eTemp regime but tended to increase (figure 8.04, table 8.01). In view of the fact that heterotrophic organisms (especially bacteria (Münster, 1991) and fungi (Paul & Clark, 1989)) are said to be important in extracellular enzyme production, prolific cyanobacterial growth and the presence of fungal hyphae (figure 8.08c) are consistent with increased enzyme production. The increased abundance of phenolic substances may therefore reflect increased photoautotrophic production, with increased algal exudation and/or structural material supporting heterotrophic metabolism and therefore leaving more phenolic materials to accumulate. Enzymic generation of dissolved phenolics (Freeman *et* al., 2001a; see chapter 3) is also a possibility, by for example, cleavage of the terminal bonds in structural polymers to create oligomers or monomers that may or may not be less recalcitrant than the original compound. Phenol oxidases are also known to have

biosynthetic roles in cell wall and spore development along with other functions, in addition to degrading phenolic compounds (Sinsabaugh & Linkins, 1988). Thus, it is feasible that such enzymes may be actively generating phenolic substances from algal (or other photoautotrophic) inputs rather than allowing their accumulation, perhaps representing a different mechanism of increased DOC production than that under eCO_2. The nature of such compounds and the effects of this increased load of potentially recalcitrant material on the downstream heterotrophs require further study. Freeman *et al.* (1990) noted the inhibitory effect of an increased phenolic compound:DOC ratio upon biofilm metabolism and therefore downstream heterotrophic DOC consumption may decline, reducing water quality as a result.

The eTemp conditions produced considerably higher ß-glucosidase activities than the control (figure 8.05, table 8.01) from which a stimulation of the heterotrophic community can be inferred, either as a direct result of the increased temperature and/or increased photoautotrophic DOC production. This increased activity may be preventing a greater increase in DOC accumulation meaning potential inhibitors, such as phenolic substances from peatland systems (Freeman *et al.*, 2001a), may be important in determining water quality in a future climate. Increased activities may also allow accelerated consumption of labile DOC relative to phenolic compound removal, thus contributing to the observed selective enrichment of the latter. Enzyme activities may generate dissolved phenolic substances (e.g., from algal material) faster than they can be consumed by the microbes in the system, or again microbial intracellular metabolism is supported to a greater extent by algal exudates allowing the accumulation of organic carbon from other sources. The increased metabolic activities, or at least lack of suppressed activities (figure 8.06, table 8.01), perhaps suggest that the former is less likely. A further possibility is that there is production of phenolic compounds derived from the low molecular weight units produced by the action of ß-glucosidase (i.e., polymerization). The strongest most significant correlation between ß-glucosidase activities and DOC concentrations was produced under eTemp (table 8.03), further evidence to suggest that the heterotrophic component has become relatively more active. Seemingly, the warmer conditions allow less tight coupling between photoautotrophs and heterotrophs, in line with other factors discussed above, since the correlation between metabolic activities and algal biomass (0.8588, $P<0.01$) is lower than in the control but higher between metabolic activities and ß-glucosidase activities (0.9768, $P<0.001$) (table 8.03). The phenolic compound:DOC ratio under eTemp correlated negatively with ß-glucosidase and metabolic activities (-0.7409, $P<0.05$ and -0.6779, $P<0.05$ respectively) as with eCO_2. However, both are more strongly and more significantly correlated than in the control or eCO_2 treatment (table 8.03), perhaps due to the relative increase in inhibitory phenolic material as a result of either the photoautotrophic activity and/or

heterotrophic metabolism. Again the potential for inhibition of *in situ* and downstream heterotrophic DOC consumption is highlighted.

Effects of elevated CO_2/elevated temperature on photoautotrophic biofilm

DOC and phenolic compound concentrations were consistently and significantly higher under eCO_2/eTemp compared to the control (on average 70.19%, $P<0.001$ & 85.13%, $P<0.01$ respectively) and the separate treatments (figure 8.01, table 8.01), with selective enrichment of phenolic material. This demonstrates that there is the potential for substantially increased raw water colour due to biofilm production in upland rivers, should temperatures rise in conjunction with atmospheric CO_2 concentrations. The summer months as previously discussed are likely to pose the greatest threat.

The dramatic increase in DOC and phenolic compounds is probably at least in part due to the pronounced increase in algal biomass (on average 98.75%, $P<0.01$), again suggesting that it will be the photoautotrophs that are stimulated in a similar future climate. In some months (e.g., July) a synergistic effect was apparent (when increases in algal biomass under separate eCO_2 and eTemp treatments were compared) (figure 8.03), suggesting that the latter may facilitate optimal uptake of the primary photosynthetic resource (CO_2) and conversion to algal biomass. This might be mediated by higher rates of CO_2 diffusion into the water, due to an increased concentration gradient produced by rapid algal growth. Alternatively, warmer conditions may allow more rapid or efficient heterotrophic nutrient cycling, allowing increased photoautotrophic growth and uptake of CO_2, although this is unlikely as the heterotrophs appear to be inhibited by the eCO_2/eTemp conditions (see below). The correlation coefficient between algal biomass and DOC concentrations also supports the theory that there is a photoautotrophically mediated synergistic effect on biofilm production, this being the strongest and most significant of all the treatments (0.9963, $P<0.001$, table 8.03) indicating tighter coupling between algal biomass and biofilm DOC exports. Other photoautotrophs, in addition to algae, are also likely to be stimulated and the results from the SEM showed abundant diatoms and a thick PSM but less pronounced bacterial presence (although this may be masked by the PSM) (figure 8.08d), in agreement with the findings discussed.

A surprising reduction in phenol oxidase activity was exhibited under the eCO_2/eTemp simulation (-45.29%, $P<0.01$, figure 8.04), and this may allow the accumulation of the phenolic substances (Freeman *et al.*, 2001b). Such suppression may be caused by reinforced inhibition of heterotrophic metabolism as a result of the stimulation of the photoautotrophic component. In contrast, significantly higher ß-glucosidase activities were observed in comparison to the

control (33.35%, P<0.05, figure 8.05), which could contribute to the phenolic enrichment of the DOC pool.

Elevating both CO_2 concentrations and temperatures increased mean metabolic activities dramatically (72.17%, P<0.001) and these reached higher levels than the separate treatments (figure 8.06). Again this activity is highly correlated with algal biomass (0.9823, P<0.001), more so than any other treatment or the control, suggesting that the photoautotrophic activity has become even more dominant in the biofilm metabolism as a whole. ß-glucosidase activities correlate more strongly with metabolic activities (0.9213, P<0.001) than the control but not as strongly as under eTemp alone (table 8.03). This might be because there is an increase in heterotrophic activity to a certain degree (probably due to increased temperature and/or production of labile carbon *via* increased photoautotrophic activity) but also some limitation on heterotrophic production, as previously discussed.

The pronounced selective enrichment of phenolic compounds implies that these conditions in a future climate have the greatest potential to set up positive feedback to reduced biodegradation of DOC *via in situ* and downstream heterotrophs. This is supported by the strongest negative correlation between the phenolic compound:DOC ratios and both ß-glucosidase and metabolic activities (–0.7580, P<0.05 and –0.7737, P<0.01 respectively, table 8.03). It may be that both photoautotrophic and heterotrophic mechanisms are selectively enriching the DOC pool with phenolic materials under these conditions, although not necessarily at the same point in time.

The dramatic effect of eCO_2/eTemp (over and above that of eTemp) was unexpected because waters draining peat soils can be more saturated with respect to CO_2 than the atmosphere (Dawson *et al.*, 2001; Palmer *et al.*, 2001), and the biofilm colonized stones were removed from within ca. 50m of a peat deposit. Thus, it was assumed that the biofilm community would be relatively insensitive to changes in atmospheric CO_2 concentrations. The observed results might partially be explained by the fact that the eCO_2 concentrations in peatland and spring fed streams usually decrease quickly downstream as the water loses CO_2 to the atmosphere (Dawson *et al.*, 2001; Herman & Lorah, 1987 respectively). This phenomenon might mean that the samples collected were not usually exposed to CO_2 levels similar to those in the eCO_2 treatment. This seems unlikely to be the entire solution though, given the small distance downstream from the peat deposit and that not all the excess CO_2 is lost to the atmosphere immediately due to the kinetics of degassing (House *et al.*, 1984; Stumm & Morgan, 1981). However, upland rivers are exceptional as CO_2 invasion from the atmosphere to the river (e.g., in the Nidd, Swale and Tweed) often occurs (Neal *et al.*, 1998). In stream excess CO_2 partial

pressure ($EpCO_2$) relates to a) CO_2 generation by microbial respiration (associated with the breakdown of organic carbon in the bulk water and sediments) and respiration by photosynthesizing organisms at night, b) dissolved CO_2 uptake by benthic algae, phytoplankton and macrophytes in daylight, and c) loss or gain of CO_2 to or from the atmosphere at the air-water interface (Neal *et al.*, 1998). The stream used in this study was very shallow (<1m deep), unshaded and clear, with a streambed of predominantly gravel and stones, presumably allowing a high degree of colonization by photoautotrophs and therefore high levels of photosynthesis that would reduce the $EpCO_2$. In addition, the DOC draining into the field site is dominated by refractory humic and fulvic compounds rather than labile material. Less biodegradation could therefore be anticipated with less respiratory CO_2 being produced as a result (Neal *et al.*, 1998). Hence, the biofilm collected for study may have been exposed to relatively lower levels of CO_2 than initially expected for substantial periods. The largest and most statistically significant effects of eCO_2/eTemp were found in the summer months, coinciding with the period when northern upland rivers were observed to become sinks for CO_2 as a result of photosynthetic activity in the day time (Neal *et al.*, 1998). Such work implies that biofilm productivity has the potential to affect $EpCO_2$. It seems feasible though, that the reverse could be true; CO_2 concentrations may influence biofilm productivity since waters can be made under saturated with respect to the atmosphere due to photosynthesis. From the results observed here, the biofilm appears to be sensitive to changes in atmospheric CO_2 concentration, having the capacity to optimize productivity at the higher levels imposed, particularly when combined with eTemp. However, this may only be true for dissolved inorganic carbon limited systems and further work would be essential to determine whether this was the case.

Summary of the effects of climate change simulations on photoautotrophic biofilm DOC processing

Seemingly, both increasing atmospheric CO_2 concentrations and rising temperatures, as a result of climate change, have the potential to alter the carbon processing of photoautotrophic biofilms and reduce water quality but possibly *via* different mechanisms. However, only when these treatments were combined were the responses both dramatic and statistically significant. Elevated CO_2 apparently tended to stimulate photoautotrophic production of DOC while suppressing phenol oxidase activities, allowing phenolic compound accumulation. Under eTemp conditions, there was little evidence for phenol oxidase inhibition and the increased phenolic compound concentrations may have been produced *via* photoautotrophic processes and/or enzymic generation. Enhanced ß-glucosidase activities may effectively accelerate the selective enrichment of phenolic compounds. The effects of eCO_2/eTemp apparently interact to reinforce the increasing DOC concentrations and selective enrichment of relatively recalcitrant

phenolic materials. Such phenolic enrichment may set up positive feedback to increased DOC concentrations, given their enzyme inhibiting properties (Appel, 1993; Freeman *et al.*, 1990; Wetzel, 1992), leading to retarded decomposition of even the most labile DOC. Both the *in situ* biodegradation of DOC and that of other downstream heterotrophic populations may thus be inhibited.

8.05.2 Heterotrophic (dark grown) biofilm

Biofilm was collected on stones that were naturally illuminated at the field site. These stones were then maintained in the dark for the rest of the experiment, hence decaying algae and other biofilm materials would be the major source of carbon. The magnitude of acclimation shown by the biofilm to these heterotrophic conditions would depend on the original trophic importance of the algal component. In oligotrophic communities supported by primary production, as in this case, biofilm development on darkened substrata is greatly retarded (Lock & Ford, 1985; Rounick & Winterbourne, 1983), thus it is hoped that the heterotrophic biofilm used is representative of that found naturally. Since the stream bed was composed of gravel and stones, it is likely that the substratum will be constantly shifting due to water movements so perhaps it is quite natural for previously illuminated biofilm to become heterotrophic. However, due to the simplified nature of the experiment some discrepancy must be accepted between *in vivo* photoautotrophic or heterotrophic potentials of the natural biofilm and those populations used to assess activity here. Measurements of enzyme activities in the dark incubated biofilm represents the potential ability of the heterotrophic communities present to hydrolyse allochthonous organic compounds without end product inhibition from exudates produced by neighbouring photoautotrophic cells. It must be noted that because of the experimental protocol, the rates measured may not represent actual activities in the field as no account was taken of natural substrate concentrations.

Effects of elevated CO_2 on heterotrophic biofilm

Elevated CO_2 apparently failed to suppress total DOC consumption by the heterotrophs since concentrations of DOC were not significantly different to the control, tending to decrease (figure 8.09, table 8.04), while phenolic compound concentrations increased somewhat (figure 8.10, table 8.04). The relative contribution of phenolic substances to the DOC pool therefore increased, suggesting a limited potential for positive feedback to reduced water quality. Such selective enrichment may be due either to a reduction in phenolic biodegradation potential (meaning less phenolic materials can be degraded and only the more labile material is consumed), or increased heterotrophic phenolic compound production. The majority of evidence points to the former as phenol oxidase, ß-glucosidase and metabolic activities were all substantially reduced in the summer months in comparison to the control, though none of them

significantly (figures 8.11-8.13 respectively, table 8.04). This may be as a result of direct or indirect effects of eCO_2, as discussed previously in relation to the photoautotrophic biofilm in which the effect was more pronounced. Strong negative correlations between ß-glucosidase activities and phenolic compound:DOC ratios in both the control and eCO_2 treatment were found (-0.9545, $P<0.001$ and -0.9069, $P<0.001$ respectively), which implies that phenolic substances may compete with substrate DOC compounds thereby inhibiting enzyme activities (Freeman *et al.*, 1990). Inhibition of heterotrophic DOC removal *in situ* and downstream is therefore a possibility. The reduction in phenol oxidase activities may represent a reduced capacity for refractory DOC decomposition from autochthonous and allochthonous sources. Similarly, suppressed ß-glucosidase activities indicate reduced cellulose decomposition and carbon mineralization rates (McLatchey & Reddy, 1998; Sinsabaugh *et al.*, 1991), allowing even relatively labile DOC to persist. The relative amount of phenolic compounds clearly has an important regulatory effect on these heterotrophic biofilm systems (which are likely to have contributed to this ratio).

Effects of elevated temperature on heterotrophic biofilm

The eTemp treatment also did not significantly effect DOC concentrations with levels tending to decrease (figure 8.09, table 8.04), whereas phenolic compound concentrations (figure 8.10, table 8.04) and therefore phenolic compound:DOC ratios increased substantially but not significantly. This may be due to suppressed phenol oxidase activities (-10.06% $P<0.1$ only, figure 8.11) allowing phenolic compound accumulation (Freeman *et* al., 2001b), coupled with a rapid utilization of labile DOC (see figure 8.12, table 8.04) leaving the more recalcitrant older organic material. Another possibility is that certain heterotrophic strains are producing more phenolic materials (e.g., by polymerization), with the action of ß-glucosidase perhaps providing a source of low molecular weight moieties. Total metabolic activities were somewhat constrained (figure 8.13, table 8.04), perhaps as a result of the selective enrichment of phenolic materials generated by enzymic processes (the correlation coefficient being -0.6563, $P<0.05$)). Any increased generation of low molecular weight materials *via* the increased activity of ß-glucosidase is therefore unlikely to be offset by rapid consumption, given that metabolic rates were not significantly stimulated.

Effects of elevated CO_2/elevated temperature on heterotrophic biofilm

DOC concentrations were modestly reduced under the combined treatment (-11.79%, $P<0.001$). However, phenolic compound concentrations and therefore the proportion of such materials within the total DOC pool, increased (table 8.04). This suggests that adverse effects on water quality could occur and these results may have been due to the appreciable, though non

significant, suppression of phenol oxidase activities (figure 8.11, table 8.04) allowing the build up of such compounds. ß-glucosidase activities showed a marked reduction (figure 8.12, table 8.04) perhaps as a result of increased phenolic inhibition and reduced metabolic activities (figures 8.10 & 8.13 respectively, table 8.04), the latter presumably meaning reduced enzyme production. The decline in metabolic activities seemingly related to the increased phenolic compound:DOC ratio, as again there was a strong negative correlation between these two determinands (-0.8366, $P<0.01$).

Summary of the effects of climate change simulations on heterotrophic biofilm DOC processing

Although the results are less clear than those of the photoautotrophic community, there is a limited potential for altered heterotrophic carbon processing as a result of eCO_2/eTemp. In the heterotrophic biofilm community, selective enrichment of phenolic materials may be due to a reduction in degradatory capacity, since phenol oxidase was apparently somewhat inhibited under all conditions. This phenolic enrichment may have been further intensified under the eTemp simulation where ß-glucosidase activities were stimulated, perhaps leading to rapid removal of labile DOC. There is also the potential for positive feedback to reduced water quality as a consequence of increased phenolic compound concentrations compromising both *in situ* DOC consumption and that of heterotrophs downstream. Thus, from these results, heterotrophic DOC consumption is unlikely to mitigate the adverse effects of increasing DOC concentrations on potable water supplies.

8.05.3 Comparison of light and dark grown biofilm responses

Generally, in the light grown communities ß-glucosidase activities and total metabolic activities (figures 8.05 & 8.06 respectively) were higher than those of dark incubated, heterotrophically driven biofilms, particularly when photoautotrophic populations were active in the spring and summer months (figures 8.12 & 8.13 respectively). This is perhaps due to the oligotrophic nature of the ecosystem studied, meaning the growth of heterotrophic biofilm is likely to be severely retarded (Lock & Ford, 1985; Rounick & Winterbourne, 1983). ß-glucosidase activities appeared to be linked to the hydrolysis of algal carbohydrate and/or exudates because maximal algal biomass and ß-glucosidase activities coincided (c.f. table 8.03). In the heterotrophic system there is a much stronger inverse correlation between the phenolic compound:DOC ratios and ß-glucosidase activities than in the illuminated biofilm (tables 8.05 & 8.03 respectively), probably indicating the difference in trophic mode, i.e., the lack of labile exudates from living algae. In contrast, phenol oxidase activities were higher in the heterotrophic (figure 8.11) than the photoautotrophic (figure 8.04) biofilm. This might be expected when dealing with the utilization of higher molecular weight, relatively refractory,

allochthonous sources of OM and autochthonous algal structural material (as would be dominant in this experiment), rather than exudates continuously supplied by live algae. The increased phenol oxidase activities support the evidence from the phenolic compound:DOC ratio correlations, suggesting that heterotrophic biofilms have different dominant pathways of DOC processing. Generally, correlations were weaker between heterotrophic biofilm variables perhaps because of the lack of labile algal exudates and more uncertain nature of resource capture. It maybe that there needs to be a greater diversity of heterotrophic species (or functional groups) in order to degrade aromatic materials efficiently or prevent end product inhibition however, this is purely speculation.

The response of both phenol oxidase and ß-glucosidase activities to the various climate change simulations differed depending on the biofilm system (photoautotrophic or heterotrophic). The competition for non-carbon resources, presence of low molecular weight algal exudates and possibly the oxygenating effect of the algal component may account for the different enzymic responses seen in the two systems. A further possibility is that the enzymes are produced by different species of heterotroph in the two systems and therefore have different properties, which would explain the differing responses to environmental stimuli. Significant increases in DOC concentrations and suppressed phenol oxidase activities were found under eCO_2/eTemp conditions, but only in the presence of the photoautotrophic component. This suggests that the photoautotrophs enhance the inhibitory effects of treatment on the heterotrophic component, either directly (for instance through competition for resources, c.f. Freeman *et al.*, 1998) or indirectly (e.g., by production of inhibitory materials (Nishizawa *et al.*, 1985)).

In illuminated biofilms, high algal biomass was associated with peaks of total metabolic activities (c.f. table 8.03). In order to affirm that the energy for maintenance of heterotrophic communities was derived from algal exudates, rather than decaying structural carbohydrates, a measure of xylosidase activities may have been useful. A positive relationship between xylosidase and ß-glucosidase activities would be expected if the latter were true, since substrates for these enzymes coexist intimately with each other in plant and algal cell walls (Goodwin & Mercer, 1983). However, no significant correlation was found for illuminated biofilm in an oligotrophic North Wales stream (Jones, 1990). Furthermore, the use of algal exudates by biofilm heterotrophs is well documented in nutrient poor streams (Goulder, 1988; Haack & McFeters, 1982; Miller, 1987; Rounick & Winterbourne, 1983). Carbon flow experiments in an extremely oligotrophic mountain stream inferred a direct flux of soluble algal products to the bacterial population, with little heterotrophic utilization of dissolved organics from the overlying stream water (Haack & McFeters, 1982). In the absence of photoautotrophs,

dark-incubated biofilms exhibited maximum metabolic activities very early in the growing season and also in the late summer (August), unlike corresponding activities in light grown biofilms (which peaked in mid summer), perhaps reflecting differing times of population turnover.

Dark grown communities are said to differ from corresponding light produced biofilms in the response of the heterotrophs to allochthonous inputs from the water column. Artificial alteration of the OM pool available to such communities results in the acclimation of heterotrophs (Kaplan & Bott, 1985). Dark incubated, acclimated communities are reported to metabolize detrital OM more effectively than mixed communities. This perhaps suggests that the heterotrophs exposed to an altered DOC composition (through the effects of climate change) may eventually acclimate to regain most, if not all, of their degradative capacity. However, increased exudation from photoautotrophs and/or biomass may provide the *in situ* heterotrophs with all their requirements and possibly an increased proportion of the *ex situ* heterotrophs also, thus reducing degradation of any allochthonous material given that there is preferential utilization of labile OM (Meyer *et al.*, 1987). Therefore, even if heterotrophic activities are not inhibited by climate change in the future, or acclimate to such conditions, it would be prudent to recognize the potential for increased DOC concentrations as a result of photoautotrophically mediated changes in biofilm processing. Both the photoautotrophic and heterotrophic systems demonstrated the potential to inhibit not only the *in situ* heterotrophs, but also those downstream of such impacted communities.

8.06 CONCLUSIONS

The hypothesis that eTemp would significantly stimulate phenol oxidase activities, increasing the biofilms capacity for DOC removal, apparently does not hold true for the photoautotrophic or the heterotrophic community. Thus, any increase in phenolic compound inputs (autochthonous or allochthonous) is likely to reduce water quality. Moreover, the phenol oxidase activities of the photoautotrophic biofilm were significantly suppressed under the combined eCO_2/eTemp treatment (on average -45.29%, $P<0.01$) which is likely to further compromise removal efficiencies.

With regard to the hypothesis that under eCO_2 autochthonous DOC will assume greater importance and stimulate DOC production by all components of the biofilm, the light gown community was apparently somewhat stimulated (producing increased DOC concentrations), but this was not statistically significant. Furthermore, the *in situ* heterotrophs (within this illuminated biofilm) were not equally stimulated with a tendency for reduced phenol oxidase

activities in both trophic systems. However, in the case of the combination of eCO_2/eTemp treatments it can be inferred that increasing the carbon input (in the form of CO_2) enhances carbon output. The photoautotrophs produced more biomass (on average 98.75%, $P<0.01$) with DOC concentrations becoming even more tightly coupled with the indicators of photoautotrophic processing, while heterotrophic carbon consumption was inhibited. So, despite the presence of increased carbon inputs, the elevated DOC output could not be attributed to a general stimulation of all components of the biofilm but seemed to be due to enhanced photoautotrophic production coupled with suppressed heterotrophic consumption. Problems associated with DBPs are increasingly being encountered by water companies (Lightowlers, 2001) and as global warming intensifies there is likely to be increased algal primary production but potentially reduced autochthonous and allochthonous DOC removal by the biofilm heterotrophs, accelerating this decline in the quality of our water supplies. Furthermore, the problem could be extensive given that biofilm coats all wetted surfaces (Lock, 1993), but further research is necessary to determine the effects of climate change on systems with differing hydrochemical characteristics.

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CHAPTER 9: GENERAL DISCUSSION

9.01 DISCUSSION

During the examination of the potential effects of climate change on dissolved organic carbon (DOC) processing in peatlands, several issues have arisen which are discussed below.

Sensitivity of extracellular enzyme activity to climate change and enzymic mobilization

Microorganisms produce enzymes that catalyze the degradation of organic matter (OM) facilitating nutrient cycling in wetlands, and the extracellular hydrolysis of OM is considered to be the rate limiting step in decomposition (Sinsabaugh *et al.*, 1993). The work presented in this thesis demonstrates that the activity of these enzymes shows pronounced seasonality, increasing in summer and autumn (chapters 3, 5, 6), which strongly suggests that climate change is likely to have a considerable impact on decomposition and therefore carbon export from peatlands. Indeed, climate change simulations induced dramatic effects on such activities. Both laboratory (chapters 2 & 3) and sub-catchment scale experiments (chapter 5) raised the issue of 'enzymic mobilization' whereby the carbon cycling enzymes phenol oxidase and β-glucosidase may have the capacity to generate dissolved phenolics and DOC respectively by cleavage of OM from the peat matrix, rather than simply consuming pre-existing materials dissolved in the pore waters as was conventionally assumed. The effect of soil warming on this process was marked (chapters 2 & 3) and may provide a mechanism for increasing DOC concentrations in UK rivers (Freeman *et al.*, 2001a), but this is debated below.

Temperature as a predictor of DOC export

It seems unlikely that there is enough evidence for a simple and direct relationship between increased temperature and increased export of DOC (Tranvik & Jansson, 2002). While it is acknowledged that there are broad patterns in DOC concentrations in rivers between climatic zones (Meybeck, 1982), variation is said to be related to hydrology as well as biological productivity and the balance between the latter and decomposition (e.g., see figure 9.01). The proportion of the catchment that constitutes a wetland is the major variable affecting DOC yield from Northern Hemisphere catchments (Curtis, 1998), and hydrological variables both within and between sites can largely explain variations (Tranvik & Jansson, 2002). The increase in DOC concentrations in Sweden during the 1970s and 1980s was used as an illustration because annual temperatures decreased (in contrast to the British data (Freeman *et al.*, 2001a)). The effect being attributed to increased precipitation and runoff in these typical northern boreal areas (Forsberg, 1992). The headwater streams of the experimental lakes in north western Ontario showed an increase in DOC concentrations when increased temperatures were

accompanied by drier conditions (Tranvik & Jansson 2002). However, a drop in DOC concentration in the lakes, and therefore rivers downstream, was said to occur when a 2°C increase in temperature was accompanied by decreasing precipitation as drier conditions lead to longer retention times and hence greater in-lake DOC removal (Schindler *et al.*, 1997). These in-lake processes (microbial and photochemical) are capable of effective DOC degradation in northern lakes with high humic substance concentrations (Granéli *et al.*, 1996; Molot & Dillon, 1997). Thus, temperature alone may not be an accurate predictor of DOC concentration and DOC exports should be discussed considering transport as well as concentration, the former being the product of concentration and discharge. Since river discharge can often explain variations in DOC export (Thurman, 1985), Tranvik & Jansson (2002) found it difficult to believe that the British rivers did not show increased discharge.

In response to this questioning (on the basis of spatial patterns of DOC concentration confounding effects of hydrology and apparently conflicting observations from other regions), factors that control spatial variation and those that determine temporal variation at an individual site must be distinguished (Evans *et al.*, 2002). The example used was that a catchment wetland area is unlikely to change on a decadal time scale. Evans and co workers point out that the observation that DOC is higher in cooler regions, where low decomposition rates allow peat to accumulate, is entirely consistent with the proposed mechanism for peatlands in the UK. Rising temperature at an individual site will therefore increase peat decomposition, leading to greater DOC export. It is agreed that hydrological changes can significantly influence DOC export and so considering it as a two-stage process may be helpful: DOC being produced in the soil and then transported from the soil to the drainage network. Discharge may control the transport stage, with hydrology affecting short term fluctuations in riverine DOC export, but unless long term changes are accompanied by changes in DOC production a sustained increasing DOC flux cannot occur (Evans *et al.*, 2002). While it is thought possible that changes in flow path could affect DOC supply in mineral horizons, such changes are believed to be less important in peatlands. Soil moisture is thought to be the primary hydrological factor influencing peatland DOC production and long term trends, since decomposition will be enhanced under drier conditions (by a stimulation of enzyme activities (Freeman *et al.*, 2001b) and increased chemical oxidation (Worrall *et al.*, in press)) with the potential for increased DOC production over and above that experimentally observed. Both rainfall and temperature affect soil moisture and although temperature has increased in the UK in recent decades, regional rainfall patterns are heterogeneous (Evans *et al.*, 2002). Furthermore, Forsberg (1992) found that a substantial proportion (10-40%) of the increase in river transport of coloured material was independent of discharge. However, it is acknowledged that area specific increases in winter:summer rainfall ratios (Burt *et al.*, 1998) may have contributed to DOC increases by reducing soil moisture in

summer and increasing washout in winter. Tranvik and Jansson (2002) also pointed out that warmer, drier conditions in north western Ontario led to reduced DOC concentrations due to enhanced in-lake removal as a result of longer residence times (Schindler *et al.*, 1997). Evans *et al.* (2002) address this with the fact that soils in that region are thin with recently decomposed plant material providing a substantial, relatively labile DOC source (Schiff *et al.*, 1997). UK blanket peat DOC is said to be older and more recalcitrant with input/output data for British upland lakes (which typically have lower residence times) suggesting that in-lake DOC removal is minimal (Curtis *et al.*, 1998). The similar trends in DOC reported for stream sites also argue against in-lake factors. It is highlighted that DOC from peat dominated uplands persists into the lower reaches of UK rivers (Worrall *et al.*, in press), consistent with work suggesting that riverine DOC entering the oceans largely comprises aged refractory compounds (Raymond & Bauer, 2001).

The consensus is that climate change may considerably alter DOC exports, which are important for both freshwater and oceanic environments. Although other factors (such as hydrological change) are likely to be of great importance, it seems probable that increasing temperatures will enhance DOC export by stimulating decomposition of the peat matrix directly and as a result of drying. Indeed, changes in enzyme activities as a result of an altered thermal regime were found to significantly alter carbon processing throughout most of the year both qualitatively and quantitatively (chapters 2 & 3).

Potential for increased DOC export from peatlands as a result of drying and wetting cycles

As might be anticipated from the comments above, the increased rainfall simulation (chapter 6) produced dramatic (though not statistically significant) increases in DOC concentrations with a rising trend, and given that rainfall drives discharge (Tranvik & Jansson, 2002), there is the potential for elevated DOC concentrations in the receiving waters. Such information may be useful in terms of catchment management strategies to avoid pulses of DOC as a result of hydrological changes and restoration practices. Successive summer droughts (chapter 5) produced a smaller but significantly increasing trend in DOC concentrations over the nine year data set. From these results and those of the bacterial diversity studies at the same sites (chapter 7), it could be inferred that increased extremes of saturation may be more important in determining DOC exports from peatlands than a particular event in isolation. The radiative effects of anthropogenic changes in atmospheric composition are expected to cause an intensification of the global water cycle (Cubasch, 2001) through an increased frequency and severity of summer drought (Hulme *et al.*, 2002: IPCC, 2001), with more intense rainfall (Palmer & Räisänen, 2002) and flooding events (Milly *et al.*, 2002). Similarly, winter:summer

rainfall ratios are increasing (Burt *et al.*, 1998). Peatland systems, in the UK at least, traditionally could be considered as relatively stable in terms of environmental conditions (including soil moisture) compared to, for example, Antarctic soils where a much greater variation in water availability and temperature occurs. This is a further reason why peatlands may become increasingly vulnerable to climate change should our climate become more extreme.

A fluctuating water table will create alternate aerobic and anaerobic conditions, which may stimulate OM decomposition and nutrient release depending on the electron acceptor availability (D'Angelo & Reddy, 1994; Reddy & Patrick, 1975). The mechanisms responsible require further research. However, we could speculate that drying and wetting cycles may accentuate DOC export by rapid enzymic generation from the vast peat resource (exacerbating chemical oxidation) in the drier and therefore warmer period, combined with increased rainfall that would flush out these products into the recipient waters. The latter would then maintain conditions conducive to the production of anaerobic DOC end products and suppress the rapid aerobic mineralization of DOC to CO_2 (Ponnamperuma, 1972), a potential limit on the DOC concentrations available for export. Rapid water movement, as a result of intense rain events, through the upper horizon may allow DOC-rich water to by-pass adsorption sites in the deeper soil horizons (Moore, 1989). Drying and wetting is associated with pipe formation and cracking (Gilman & Newson, 1980 etc), therefore increased peat oxidation and even more rapid water flow could be foreseen, further reducing DOC adsorption. Moreover, hydrophobic properties induced in the surface layer as a result of drought (Gilman & Newson, 1980; Worrall *et al.*, in press) insulate the peat below from recharge by rainfall, effectively intensifying the drought and exacerbating DOC release.

Changes in the bacterial community composition and hence enzyme activity/production as a result of wetting and drying are likely not only to influence aquatic carbon exports from peatlands (chapter 7) but also trace gas fluxes to the atmosphere (Schimel & Gulledge, 1998), and correlations between carbon cycling enzymes and trace gas production have been found (e.g., chapter 3). Understanding accurately the function of bacterial consortia in the environment necessitates the measurement of microbial community structure and diversity as it relates to changing factors in that environment. However, molecular techniques in general perhaps are not yet a panacea for all the difficulties associated with soil ecological analysis and need to be incorporated into a polyphasic approach (Ogram, 2000). Methods developed specifically for analysis of microbial communities are likely to be needed before soil molecular genetics become completely quantitative for routine use in microbial ecology laboratories.

Analogies between the peatland system and stream biofilm

Analogies have been drawn between the soil properties of terrestrial systems and the polysaccharide matrix (PSM) found in epilithic biofilm communities; both are 1) a retention/trapping system for dissolved nutrients, 2) a medium for transport between cells, and 3) a support medium for maintaining extracellular enzymes in close proximity to the microbial cells (Lock, 1993; Lock *et al.*, 1984). It seems that analogous responses to climate change are also likely (chapters 2 & 8). Photoautotrophs within both the peatland and the biofilm community apparently mediated increased DOC concentrations under elevated CO_2 combined with elevated temperature (eCO2/eTemp), seemingly through increased biomass input and exudation with its associated effects on the heterotrophs *in situ*. Organic carbon input increased but also heterotrophic decomposition of that material appeared to be inhibited, implying competition between photoautotrophic and heterotrophic components for non-carbon resources. This has been proposed in relation to the terrestrial system under eCO_2 (Freeman *et al.*, 1998). Thus, some fundamental aspect of heterotrophic carbon catabolism may be compromised by a future climate, however the cause(s) of this phenomenon require further work.

There was a tendency for selective enrichment for phenolic compounds in both the wetland and the stream environment, providing a biological concentration mechanism and potentially enhancing the phenomenon of increasing recalcitrance with distance downstream as a result of biodegradation (e.g., Wetzel, 1992). Perhaps similarities are not surprising if these two systems are simply considered as extremes of one another. Indeed, it is likely that there is a continuous gradient of microenvironmental conditions between the wetland and the stream in this instance, with any delineation probably being purely artificial.

Importance of qualitative changes in the DOC pool

DOC concentrations were invariably positively correlated with phenolic compound concentrations (chapters 2, 3, 5, 6 & 8). Such results support the concepts put forward by Wetzel (1992) that phenolic and humic substances have a regulatory role in the ecosystem, controlling enzyme activities by allowing their storage, transport and reactivation at displaced sites. The relative proportion of phenolic compounds in the DOC pool was apparently particularly important in determining enzyme activities in the biofilm system (chapter 8) consistent with the findings of Freeman *et al.* (1990). Heterotrophic (dark grown) epilithon (i.e., lacking labile exudates derived from photoautotrophs) was especially inhibited by high phenolic compound:DOC ratios and so there may be a critical relative concentration of phenolic substances at which these materials exert a limitation on enzyme activities. Selective enrichment of high molecular weight fractions (associated with high iron (Fe) concentrations)

and phenolic compounds as a result of increased rainfall (chapter 6), or enzymic generation of phenolic substances due to warmer conditions (chapters 2 & 3), for instance, are therefore likely to be important factors constraining biofilm DOC removal in a future climate. In addition to the chemical nature, molecular weight and relative composition of the DOC pool, the shape of a compound may be important in determining its persistence. Recent fractal geometry concepts (reviewed by Senesi & Loffredo, 1999) may therefore provide a powerful, quantitative description of these natural, heterogeneous and random materials.

Humic substances are often said to have a key role in controlling the behaviour and mobility of metals in the environment, whether trace elements or pollution derived heavy metals. The solubility, plant availability and volatility of metals can be profoundly influenced by reactions with humic substances (Livens, 1991). Possible repercussions of an altered DOC load from wetlands therefore extend beyond water quality; for example, microbial DOC consumption is an important link in aquatic food chains and wetland derived organic carbon has been identified within such food chains (Schell, 1983). Phenolic rich materials generally dominate the DOC released from peatlands and these may represent nutrients (e.g., Tranvik, 1988) and/or microbial inhibitors (e.g., Appel 1993; Freeman *et al.*, 1990; Wetzel, 1992). Changes in both the quality and quantity of DOC released therefore have the potential to modify in-stream microbial metabolism, either suppressing or stimulating the demand for DOC from the water column (Freeman *et al.*, 1994a). This in turn may effect the flux of CO_2 to the atmosphere because rivers can represent a significant CO_2 source (Richey *et al.*, 2002). An estimated 20% of the total DOC exported from terrestrial ecosystems to the oceans is attributed to wetlands (Lugo *et al.*, 1989), thus any change in this flux could be of great significance to the nutrient budgets of coastal systems and in the global carbon cycle.

Difficulties associated with research on ecosystem response to climate change

Peatland plant and microbial communities are complex, dynamic, mosaics from the ecosystem to molecular scale leading to peat that is neither homogenous in space nor time. The organic carbon produced by such systems is also complex being derived from a myriad of sources. 'Dissolved organic carbon' is a term encompassing a huge variety of compounds, some of which will represent substrates others intermediates and products of enzyme reactions. Indeed, a continuum may be present at any one time that reflects the dynamic processing of DOC within the mire and recipient waters. This, along with the intricate relationships between the component organisms, makes it very difficult to determine exact causes and effects of the observed changes. The huge variety of multifunctional source structures leads to humic structures being considered as consisting mainly of cross-linked components giving rise to fractal-like entities (Tombácz, 1999). Due to the randomness of formation, a uniform behaviour

is not expected and only trends can be predicted for the properties of humic substances (Tombácz, 1999). The response of DOC exports to climate change is therefore unlikely to be consistent.

Climate change models are forecasting highly regionalized scenarios (Hulme *et al.*, 2002; IPCC, 2001). When considering the impacts of potential changes in climate, scientists are faced with an immense task not only due to the regional nature and inconsistency of predictions but also because of the complexity of ecosystems. Any ecological research involving the study of ecosystems and their response to changing environmental parameters is complex. Simulating these systems in a laboratory may simplify such studies, allowing particular situations to be modelled, but this approach does not account for other influences that cannot be readily replicated. The need for long term, field scale research to be conducted cannot be overstated and the acclimation of trace gas emissions to successive summer drought illustrates this (chapter 5), demonstrating that aspects of wetland function may show resilience to climate change as well as extreme sensitivity. Even if such studies can be conducted, the effects of climate change are likely to be region specific if not site and microsite specific. And, it may be that without knowledge of the thermal and hydrological history of a site we cannot accurately model such systems.

Studies have shown that hysteresis (the lagging of an effect behind the cause of an effect) can be important in controlling microbially mediated processes such as trace gas fluxes (Moore & Roulet, 1993) and changes in the microbial population may be a cause of this phenomenon. However, most ecosystem models use simple response functions between a process and environmental conditions rather than incorporating hysteresis (Schimel & Gulledge, 1998). In order to model wetland DOC and trace gas emissions in a changing environment, we must be able to predict how well anaerobic populations survive under aerobic conditions and the rate of regrowth following flooding. The degree of water table fluctuation and seasonal flooding may vary considerably with climate change, thus it may be impossible to predict how long term environmental changes will alter these processes if we lack a good understanding of microbial community dynamics. Similarly, Updegraff *et al.* (1998) suggest that our ability to predict the effects of climate change on trace gas fluxes may be constrained by hysteresis in the temperature response of CO_2 and CH_4 production in peat soils.

The Kyoto Protocol, agreed in December 1997, sets legally binding greenhouse gas objectives for each industrialized country as listed in its Annex B. A target of an average reduction of 5.3% in relation to 1990 values by 2080 was set at the third Conference of the Parties in Kyoto (De Leo *et al.*, 2001). The Protocol allows countries to meet their targets by planting forests to

sequester CO_2 rather than reducing output. It also sets the scene for the trading of carbon credits between nations and businesses. Given the regional nature of changes in climate and the uncertainties of ecosystem response, the prospect of carbon trading is of concern especially for areas such as Wales with large areas of peatland that have the potential to change from a sink to a source of carbon (chapter 2, 3, 5 & 6).

Common themes and feedbacks to reduced water quality

Despite the intricacies described, and in addition to the analogies between the terrestrial and aquatic system, further common themes emerged. Elevated atmospheric CO_2 concentrations are likely to be accompanied by higher temperatures (Hulme *et al.*, 2002; IPCC, 2001), and these conditions apparently induced not only increased DOC release from the peat (chapter 2) and biofilm systems (chapter 8) but also reduced removal of such matter. Treatment effects were generally more pronounced in the summer/autumn in all experiments, from the laboratory to the sub-catchment scale. This probably relates to biological production, with increased inputs from both plant and microbial photoautotrophs at this time of year coupled with increased heterotrophic decomposition of such material and the peat matrix. The enhanced DOC load would occur at a time when water flow is already likely to be lower (concentrating pollutants) and water quality is reduced (Betts, 1998). An increased frequency and/or intensity of summer droughts would exacerbate the situation both by reducing water volumes and increasing matrix mobilization (enzymic degradation and chemical oxidation), thereby generating DOC products for export from the peatland (chapter 5). Furthermore, increased abstraction (as a result of drought and/or increasing demand *per capita*) would intensify the problem directly, by reducing water volumes, and indirectly because water temperatures increase as a result of such reductions in volume (Webb, 1996). The latter may provide another positive feedback to increased levels of disinfection by-products (DBPs) in drinking water supplies given the potential for selective enrichment of phenolic compounds by the biofilm (chapter 8). Although Freeman *et al.* (1994b) found that drought induced a drop in the temperature of stream water draining a Welsh peatland (due to an increased contribution of cooler baseflow derived from deeper in the soil profile), which may offset some adverse effects of increasing temperatures in recipient waters. A further interacting factor requiring consideration is the increased rainfall intensity predicted in certain areas (e.g., Palmer & Räisänen, 2002). This promotes rapid export of materials generated in the catchment (c.f. Wetzel, 1992; Worrall *et al.*, in press) and the subsequent accumulation of phenolic-rich, high molecular weight and Fe associated DOC fractions (chapter 6) that are highly coloured and likely to persist in the recipient waters. Finally, to compound the situation, growing seasons have been lengthening and are predicted to continue to do so (Gian-Reto, 2002; Menzel & Fabian, 1999; Roetzer & Chmielewski, 2000), a

phenomenon that provides increased opportunity for biological DOC production in the summer months.

Multiple opportunities for system feedback apparently exist at every level, which could interact to produce dramatically increased DOC concentrations in receiving waters. Such feedbacks are not confined to the wetland but will potentially produce a cascade effect into adjacent ecosystems. We therefore need to view wetlands as part of a much larger, linked system if we are to understand the impact of climate change on carbon cycling. The reason for the dominance of feedback mechanisms may be because peat is situated at the interface between the biosphere, hydrosphere, geosphere and atmosphere. It owes its existence to the interactions that occur between such spheres and can be both especially affected by climate (see chapter 2) and influence climate by the production or consumption of trace gases (chapter 2, 3, 5 & 6). The importance of feedback to climate change is emphasized by the work of Cox *et al.* (2000) who calculated a global mean warming of 8°C over land, compared to 5.5°C without the carbon cycle feedback, by the year 2100. This increased warming may further exacerbate DOC release from peatlands with associated reductions in water quality.

9.02 CONCLUSIONS

We are only at an early stage in the projected trends of global warming, but this thesis exposes a clear potential for extensive reductions in water quality as a result of elevated atmospheric CO_2 concentrations, increasing global temperatures (especially as a result of the interaction between these two factors) and alterations to the hydrological regime (increased drought frequency and more intense rainfall events) of northern peatlands (summarized in figure 9.01). Similarly, catabolism of DOC by the biofilm in the receiving waters is also likely to be compromised when elevated CO_2 concentrations are accompanied by warmer temperatures, both as a direct result of changed environmental conditions and due to increasing concentrations of aged and recalcitrant organic compounds released from the peatlands. Furthermore, there is the potential for significantly enhanced photoautotrophic DOC production. Given that these communities are present on all wetted surfaces (Lock, 1993), there is considerable potential for problems of reduced water quality within the UK and beyond. These mechanisms could account for the rising trend in DOC concentrations reported in UK rivers spanning a variety of catchment types (Freeman *et al.*, 2001a) over a 30 year period (Worrall *et al.*, in press) and the increasing frequency with which problems associated with DBPs are being encountered by water companies (Lightowlers, 2001), despite the fact that classical studies demonstrated efficient DOC removal by the biofilm (Hynes *et al.*, 1974; Mickleburgh *et al.*, 1984). This DOC export is likely to increase if global warming intensifies, further reducing the quality of drinking water. Moreover, transportation to areas of deficit with

increasing temperatures/drought (IPCC, 2001) and increased use of relatively inexpensive upland waters is likely to expose more of the population to water of reduced quality. While chlorination has traditionally been accepted as a successful treatment for drinking water in the last century (Bull *et al.*, 1995), from a human health perspective research into a viable alternative to conventional treatment is required. Since water companies are already facing an increased frequency of DBP related problems (Lightowlers, 2001) and the removal of DOC often constitutes the largest recurrent water treatment cost in such upland peat dominated catchments (Worrall *et al.*, in press), there are also economic incentives to improve such treatment methods.

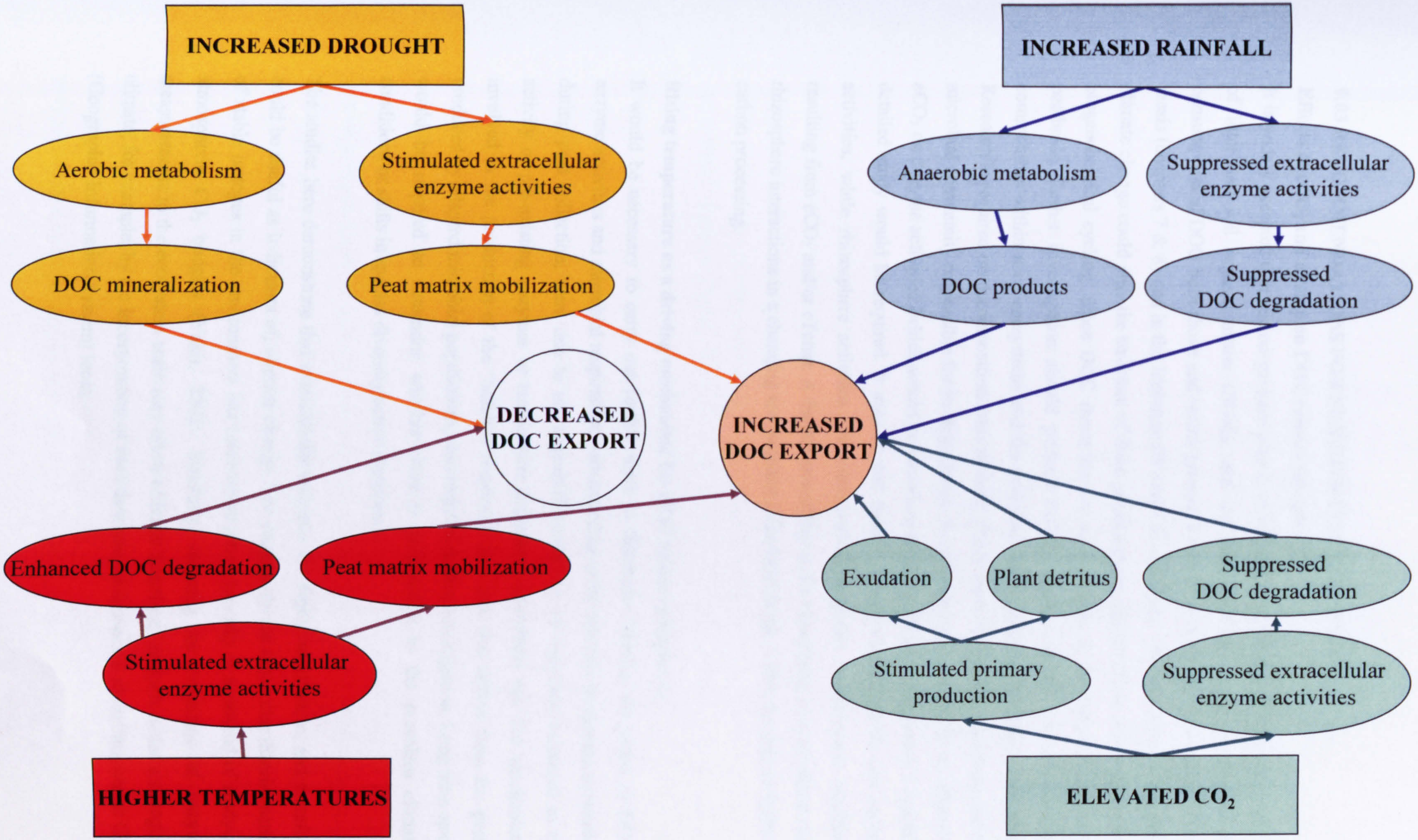

Figure 9.01. Flow diagram showing potential shifts in DOC processing as a result of predicted climatic changes for northern peatlands

9.03 RECOMMENDATIONS FOR FURTHER INVESTIGATION

Effects of eCO_2 and eTemp on DOC release (chapter 2)

It is widely accepted that microorganisms play a critical role in the biogeochemical processing of litter and soil organic matter (SOM), and are thus involved in the production and consumption of DOC both above and below ground. There is evidence from the results of this thesis (chapters 7 & 8) and in the literature (Schimel & Gulledge, 1998) to suggest that global climate change could alter the structure of these communities, and therefore induce changes in biogeochemical cycling. Since DOC dynamics are so important in the biogeochemistry of peatlands, further investigation should perhaps include studies on the role of microbial communities within such ecosystems and the potential effects of climate change upon them. Research is required on decomposition rates including both the nature of the plant litter and the microbial community responsible for its degradation. In order to understand fully the effects of eCO_2 on enzyme activities (which would be important in decomposition processes), a spatially detailed study would be required. It may be that there is a suppression of bulk soil enzyme activities, while rhizosphere activities are stimulated in response to increased exudation resulting from eCO_2 and/or eTemp. A further possibility is the uncoupling of specialized root-rhizosphere interactions in a changing environment (Van Noordwijk, 1998), leading to shifts in carbon processing.

Rising temperature as a driving mechanism for DOC release (chapter 3)

It would be necessary to carry out further work to determine whether the peaks in DOC, enzyme activities and microbial respiration, corresponding to the ambient temperature recorded during peat collection, were due to net microbial production of enzymes, increases in the activity of pre-existing enzymes or temperature dependent solubilities etc. The mechanisms involved in the production of the 'baseline response', and how this differs from the peaks produced by adapted microbial populations, also requires further investigation. Long term study would be required to determine whether there is acclimation to the prevalent climatic conditions or shifts in species diversity/active members.

The studies here demonstrate that extracellular enzymes are highly sensitive to, and therefore could be useful as indicators of, climate change. The enzyme-driven molecular discrimination of stable isotopes in the environment has received attention recently as a method of studying atmospheric CO_2 budgets (Yakir, 2002). Similarly, reducing the resolution of enzyme measurements to the ecosystem scale may allow efficient functional analysis under a changing climate, for example, by the incorporation of such data into mathematical modelling within GIS (Geographic Information System) tools.

Warmer soil temperatures may favour the growth of different microbial communities, causing changes in the properties of DOC exported from peatlands due to OM decomposition proceeding *via* differing catabolic pathways. Methods based on stable isotope labelling could be employed to link specific soil processes to the taxonomic identity of the microbial populations involved. Carbon labelled with stable isotope (^{13}C) is taken up by soil microbes and incorporated in their cell structures (DNA, Phospholipid Fatty Acids (PLFAs)), which are specific to the populations involved. The ^{13}C labelled components can be detected and separated from unlabelled material by stable isotope mass spectrometry or centrifugation because they would be expected to have a higher mass than those grown on unlabelled substrate. Analysis of ^{13}C labelled bacterial cell lipids (PLFAs) has been used to detect and classify atmospheric CH_4 oxidising bacteria, for example (Bull *et al.*, 2000). Similarly, RNA based molecular techniques (e.g., Temperature Gradient Electrophoresis) could also be used to study diversity shifts.

A further factor which may be of relevance to increasing DOC concentrations is that the fauna in UK upland systems is dominated by *Cognettia sphagnetorum* (Vejdovsky) 1877 (a species of enchytraeid), which can represent up to 95% of the soil animal biomass (Coulson & Whittaker 1987). This species is often concentrated in the upper horizons of such soils where OM has accumulated (Neilsen 1955a). They can also be found in deeper layers, where transient increases in numbers are attributed to vertical migration in response to adverse conditions (Neilsen 1955a) or to differential mortality and reproduction rates (Neilsen 1955b). Respiration and nutrient leaching in microcosm studies are strongly affected by enchytraeids (Haimi & Boucelham 1991; Setälä *et al.* 1991). Briones *et al.* (1998), also using a microcosm approach, investigated the effects of enchytraeids on DOC leaching in a cambic, stagnohumic gley soil along with the implications of potential climate change for this process. They found that leaching of DOC was significantly enhanced by *C. sphagnetorum*, with the greatest effect being seen in the upper soil layers (0-6 cm). The authors suggest that the enchytraeids mobilized carbon from OM with a low carbon to nitrogen ratio because the ratio of DOC to dissolved organic nitrogen (DON) in the leachate decreased in faunated systems. The vertical distribution of enchytraeids was found to effect DOC production, and this distribution is strongly affected by climate. Thus, Briones and co-workers proposed that increases in DOC found in a field soil warming experiment with the same soil were largely a result of changes in the vertical distribution of these organisms. Enchytraeids may have a role in producing the responses observed here, for example, by aerating the peat and therefore stimulating microbial enzyme activity or production with increasing temperature. Furthermore, Cole *et al.*, (2002) demonstrated that enchytraeids can influence the microbial community structure, and therefore work on how this relates to enzyme activities in peat may prove informative.

Stable isotope (^{13}C) labelling experiments (chapters 4A & B)

The ^{13}C pulse labelling studies demonstrated a considerable scope for applications of this approach to investigations of photosynthate carbon partitioning and its fate through plant and soil pools. Bryophytes have a unique and sometimes dominant role in net primary production and in delaying nutrient transfer in the peatland system. Given that they are central to the dynamics of carbon accretion (Clymo & Hayward, 1982; Gajawski *et al.*, 2001; Kuhry & Vitt, 1996) and that accurate modelling of carbon cycling requires the inclusion of such plants, they warrant further study. Labelled materials also offer considerable potential for studies requiring compound-specific $^{13}C/^{12}C$ analysis of plant, soil and other biological samples.

Hydrological manipulation studies (chapters 5 & 6)

Long term monitoring over both the early and late stages of drought response would be required to understand how this would affect the nature of DOC (e.g., phenolic content) and enzyme activities. As was found for trace gas emissions, responses may differ in direction and/or magnitude at different stages within a single drought and as a result of successive droughts (e.g., there may be acclimation to certain intensities of drought). Further work would be necessary to determine how the timing of a drought affects the system and whether there is a critical severity or frequency over which a response is induced.

In view of the fact that the development of peat is strongly dependent on climate (occurring where precipitation exceeds evapotranspiration and typically where annual precipitation is over 500 mm (e.g., Gignac & Vitt, 1994)), increased drought is likely to reduce peat accumulation rates and increase degradation rates. This would potentially further accelerate climate change since less atmospheric CO_2 could be sequestered. To gain further insight into the effects of drought on accretion rates, dating techniques would be necessary (e.g., natural abundance carbon isotope or Pb^{210} analyses).

As mentioned previously, a fluctuating water table may stimulate OM decomposition (D'Angelo & Reddy, 1994; Reddy & Patrick, 1975) and work is required to determine whether this process is important in inducing increasing trends in DOC concentrations, as well as the precise mechanisms involved.

Effects of hydrological manipulations on the diversity of aromatic degrading bacteria (chapter 7)

Culture-independent methods provide a valuable tool with which to increase our understanding of the diversity and function of peatland microbial consortia as they relate to changing factors

in the environment. Chapter 7 provided evidence that the diversity of bacteria possessing the catechol 2,3-dioxygenase (C23O) gene was affected by changes in hydrological regime. However, in addition to *meta*-cleavage, *ortho*-cleavage, the other major pathway of aromatic dissimilation (i.e., the enzymes catechol 1,2-dioxygenase (C12O) and protocatechuate 3,4-dioxygenase (P34O)), would require examination in order to provide a more complete picture of the potential effects of climate change upon aerobic decomposition. Temporal Temperature Gradient Gel Electrophoresis could be performed, for example, before a simulated drought and over a period of successive droughts. Monitoring the band profile may reveal a different assemblage of microbes gradually becoming dominant due to their ability to survive/proliferate in the prevailing conditions. Aromatic compound degradation can also be achieved by anaerobic microorganisms and there has been a recent upsurge in studies on anaerobic metabolism (see Elder & Kelly, 1994). This is another area of research that demands consideration in relation to the effects of climatic change on aromatic degradation.

Methods to examine the diversity and composition of the *metabolically active* members of the microbial community present in a soil may also prove useful in determining the potential effects of climate change on aromatic degradation and biogeochemical cycling. One such approach is the identification of the individual 16S ribosomal RNA (16S rRNA) sequences in clone libraries generated from reverse transcription-polymerase chain reaction (RT-PCR) amplification products, obtained from total RNA. This technique has been used to identify the metabolically active members of a bacterial community in a polychlorinated biphenyl-polluted moorland (Nogales & Moore 1999). Growing (i.e., metabolically active) bacteria contain more ribosomes and rRNA than resting or starved cells (Bremner & Dennis, 1996; Nomura *et al.*, 1984), therefore the 16S rRNA RT-PCR products generated from extracts of total RNA essentially reflect the metabolically active portion of the community (Nogales & Moore, 1999).

Effects of eCO_2 and eTemp on biofilm DOC processing (chapter 8)

In order to better understand the likely effects of climate change on biofilm carbon processing a long term, field scale experiment including a natural hydrological regime would be required. The response of lotic and lentic systems will probably differ and nutrient status is likely to have a profound effect (see Turner *et al.*, 1994). Ideally the integrity of samples would be maintained using, for example, an ISO°safe VTC™ (Verifiable Temperature Container, Basingstoke Hants.). This would allow the history of the sample conditions to be known at all times and allow issues of species change, alterations in chemical composition, and acclimation to be addressed with confidence. Further experimentation is necessary to determine the mechanism behind the different enzymic response induced in the photoautotrophic and heterotrophic communities under climate change simulations. And, challenge experiments (e.g., Freeman &

Lock, 1992) would allow an examination of the system response to the mobilization of peatland DOC, as is anticipated under climate change scenarios (Freeman *et al.*, 2001a, b).

Tracer experiments would be essential to determine the fate of CO_2 through the photoautotrophs into the heterotrophic component of the biofilm and the surrounding media (PSM or water). An example of this technique is provided by the work of Hall (1995), where ^{13}C was dripped as sodium acetate into a headwater spring at Coweeta Hydrologic Laboratory to assess the use of bacterial carbon by stream invertebrates. Stable isotopes can also be employed to study the use of terrestrial *versus* aquatic organic carbon by consumers (e.g., Huryn *et al.*, 2001). Such a technique may be useful in determining the effects of changes in DOC composition and processing with changes in climate. RNA-based methods could be employed to study the microbial composition of these biofilms and relative community shifts with climate change. The RT-PCR approach has been used to analyze sulphate reducing populations in multispecies biofilm (Amann *et al.*, 1992) and recently with electrophoretic techniques (e.g., DGGE and TGGE) to estimate the diversity of active bacteria in marine and soil samples (Felske & Akkermans, 1998; Teske *et al.*, 1996). Combining stable isotope (e.g., ^{13}C or ^{15}N) and molecular techniques may prove to be a powerful tool in future biogeochemical research.

The biofilm reactor as a method for humic substance removal has been studied in relation to the microbial decomposition of aquatic fulvic acid (AFA) (Shin & Lim, 1996). And, a mixture of chlorinated organic compounds has been successfully degraded to water soluble metabolic intermediates and CO_2 by a two stage anaerobic-aerobic biofilm reactor (Fathepure & Vogel, 1991). A reciprocating motion submerged biofilm process (RMSBP) was also developed for nitrogen, biodegradable carbon and trihalomethane (TTHM) precursor simultaneous removal, as a pre-treatment of polluted raw water for drinking water supply (Takasaki *et al.*, 1992). They aimed to obtain stable treatment water throughout the year without pre-sedimentation systems. A decrease in non purgeable DOC and UV absorbance was found but at around 2-10 times the cost of the traditional filtration process and simultaneous removal was only consistently achieved from autumn to spring when water temperatures were low. Although much work is required, such technologies provide huge scope for future research including, for instance, the use of molecular techniques to investigate microbial assemblages and optimize removal of particular DOC fractions by certain species or consortia.

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