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The effects of water table drawdown and catchment management on DOC export and water quality

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The effects of water table drawdown and catchment management on DOC export and water quality

A thesis submitted to Bangor University by:
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Abstract

The intention of this study was to investigate the two most significant sources of dissolved organic carbon (DOC) to drinking water supplies; peatlands and algae. For two drinking water reservoirs in north Wales, Llyn Cefni and Llyn Alaw, markedly different seasonal trends were recorded, and these were linked to contrasting aspects of the lake's catchment and the meteorological conditions experienced during the two surveys. For Llyn Cefni, the DOC signatures for the inflowing streams and lakes correlated significantly, Afon Erddreiniog (R^2=0.585, p<0.01) and Afon Cefni (R^2=0.784, p<0.001), indicating a strong flux of terrestrial DOC into the lake from a nearby peatland. Much less of a seasonal trend in DOC was recorded at Llyn Alaw, with concentrations averaging 10.7 mg L^{-1}, reflecting the absence of a peaty catchment. DOC derived from autochthonous production was also shown to be influential. Both lakes experienced nitrate depletion during the summer of the surveys, falling from 12.7 mg L^{-1} in Llyn Cefni and 6.5 mg L^{-1} in Llyn Alaw to undetectable levels, suggesting extreme eutrophy, although the lack of detectable phosphate at Llyn Alaw may be why the growth of algal blooms was much less prevalent than at Llyn Cefni. A new type of floating constructed wetlands (FCW) was shown to offer a potential solution to the production of algal blooms in the reservoirs, whereby over a four-week period, the FCWs were able to reduce algal growth by 80%, through sequestration of the key nutrients nitrate and phosphate and possibly due to the direct inhibitory properties of phenolic compounds on the algae. Furthermore, it was discovered that 8 *Phragmites australis* plants is the ideal number to maximise nutrient uptake and minimise algal growth in 70 litres of water.

Studies in Finland and Malaysia were undertaken to highlight the importance of peatland management practices, as vast areas of the world's boreal and tropical peatlands have been drained for agricultural purposes. The study in Finland showed that although there was no

consistent response in DOC export potential with drainage across the sites of contrasting nutrient status, a significant correlation was observed between DOC and the water content of the soil, $R^2 = 0.59$ ($p < 0.001$), indicating that as the water-table height falls the DOC export potential of the soil increases. It was also considered that the trend in DOC is driven by changes in soil pH and the resulting suppression of the key carbon regulating enzyme phenol oxidase; where acidity has increased following drainage phenol oxidase activity has declined and the concentration of DOC released has increased, conforming the view that phenol oxidase acts as an 'enzymic latch' in peatlands. The study in Malaysia recorded a 40% greater export potential of DOC from the oil palm soil and more than twice the DOC concentration in drainage waters compared to the undisturbed peat soil. The activity of the key hydrolytic enzyme β-glucosidase was 25% higher in the oil palm soil than the undisturbed peat. This may be due to an improved soil organic matter quality at the oil palm site and suggests that increased activity of this enzyme may have been crucial for mobilising DOC from the soil matrix. The repercussions of these studies are discussed with emphasis on the potential impacts of a changing climate.

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List of Abbreviations

C	Carbon
CH_4	Methane
$CHBr_3$	Bromoform
$CHCl_2Br$	Dichlorobromoform
$CHCl_3$	Chloroform
$CHClBr_2$	Chlorodibromoform
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
FCW	Floating constructed wetlands
GC-MS	Gas chromatography- mass spectrometry
HMW	High molecular weight
L-Dopa	L-dihydroxyphenylalanine
LMW	Low molecular weight
ME	Mesotrophic
MUF	Methylumbelliferone
N	Nitrogen
NEP	Net ecosystem production
NMR	Nuclear magnetic absorbance
NOM	Natural organic matter
OL	Oligotrophic
OM	Ombrotrophic
P	Phosphorous
SOM	Soil organic matter
SUVA	Specific ultra violet absorbance
THM FP	Trihalomethane formation potential
THMs	Trihalomethanes
UV	Ultra violet

List of Definitions

Eutrophic	Area of high primary productivity due to excessive nutrients
Hydrophilic	Charge polarized molecules capable of hydrogen bonding, enabling them to dissolved more readily in water
Hydrophobic	Non-polar molecules that are repelled from a mass of water
Mesotrophic	Bodies of water or soil with a moderate inherent fertility and productivity
Oligotrophic	Bodies of water or soil with very low nutrient levels
Ombrotrophic	Soil or vegetation which receives all their water and nutrients from precipitation
Transphilic	Molecules of intermediate polarity

Chapter 1

Introduction

1.1 Introduction

The act of turning on a tap and using the water it dispenses has become a natural and everyday occurrence in developed countries with the assumption taken that the water is safe for human consumption. However, there are many 'issues' that affect the quality of potable drinking water and water treatment works often face an uphill struggle to providing drinking water of the highest quality. One of the most important issues centres on the presence of natural organic matter (NOM) in freshwaters. During the disinfection stage of water treatment, NOM reacts with the disinfectant to produce a class of undesirable, harmful compounds known as disinfectant by-products (DBPs) (Kits *et al.*, 2002).

The principal aim of water treatment is to remove the harmful microbiological diseases that exist in untreated waters. A variety of disinfection methods are utilised worldwide to do this, however, the use of chlorine has emerged at the forefront due to the relative simplicity and low costs associated with the process (Schoenen, 2002). It is a process that has saved millions of lives (Craun *et al.*, 1994) However, when chlorine reacts with naturally occurring organic matter it produces a suite of DBPs, the principal type of by-product compound usually being trihalomethanes (THMs) (Villanueva *et al.*, 2006).

Thus, whilst the application of chlorine during the disinfection process mitigates the problem of pathogens in potable drinking water and improves the biological quality of the water, water companies must implement ways of minimising DBP formation to maximise the final water's chemical quality. This is best achieved by improving treatment facilities and reducing the concentration of organic matter in the water that is to be treated. The high costs of improving water treatment works mean this is not always viable, therefore water companies need to improve their understanding of processes occurring within the catchment of source waters that impact on the concentration of organic matter in the water and, if necessary, implement

appropriate management strategies. Observations that NOM concentrations in freshwaters are increasing (Freeman *et al.*, 2001b) and that this may be linked to the destabilisation of organic-rich peatlands, which are found with the catchments of many sources of drinking water in the UK, are placing an even greater emphasis on the need to improve the efficiency of water treatment processes.

1.2 Natural organic matter

Freshwaters throughout the world contain NOM, its existence a consequence of interactions between the hydrological cycle, the biosphere and the hydrosphere (Murray, 2003). Once NOM enters water systems from terrestrial sources, heterotrophic microorganisms, especially bacteria, are quantitatively the most important initial consumers of it, slowly altering its structure and chemical reactivity (Pusch *et al.*, 1998). Variations in sources denote a significant difference in the organic composition and physical forms of NOM among water types (Hedges *et al.*, 1994). NOM has been shown to be a complex mixture of organic material and to consist of quite diverse organic compounds ranging in size and structure from simple sugars to complex humic compounds. Humic compounds constitute approximately 25–50 % of the total NOM found in freshwater (Thruman, 1985), while the remaining non-humic fractions are composed of proteins, polysaccharides, and hydrophilic organic acids (Grasso, 1990). As the key constituent of NOM is carbon, and because it mostly exists in freshwaters in the dissolved form rather than particulate form (i.e. below 0.45 μM), NOM is more commonly referred to as dissolved organic carbon (DOC) and therefore DOC will be the term most frequently used to describe organic matter in freshwater in this study.

Within the DOC fraction humic compounds are principally coloured, non-polar, hydrophobic and have a high molecular weight. They are more stable than non-humic compounds and remain unchanged in the environment for thousands of years (Vaughan and Malcolm, 1985), due to their relative resistance to microbial degradation (Fukushima *et al.*, 1996). Furthermore, humic compounds are able to complex with metal ions and can interact with clay minerals and organic chemicals (Schnitzer and Khan, 1972). Conversely, non-humic compounds comprise uncoloured, polar, hydrophilic and low molecular weight structures. These compounds are more labile and may be easily broken down by microbes, therefore exhibiting rapid flux rates in lakes

(Schnitzer and Khan, 1972). Humic substances are generally more commonly occurring than non-humic substances in freshwaters.

Furthermore DOC in freshwaters can be divided into two distinct groups based on its origin. DOC derived from terrestrial sources is called allochthonous, whereas DOC generated directly by processes within the lake is termed autochthonous (Wetzel, 2001). Allochthonous organic matter is derived principally from the decay of dead plant matter within the terrestrial catchment of a freshwater body. It also arises from the eroding of topsoil during times of high wind and rain (Wetzel, 2001). DOC has been described as being a major source of energy and material for freshwaters and is consequently subject to significant microbial transformation as it is transported in waters (Wetzel, 1975). Autochthonous organic matter derives from photosynthetic inputs and the bacterial decomposition of organic matter leading to the subsequent release of DOC (Fukushima *et al.*, 1996). This difference in origin brings about a variance in the chemical characteristics of the DOC. In the case of allochthonous organic matter, it is generally nitrogen poor, optically dense, highly coloured, rich in aromatics and has a high molecular weight. Autochthonous organic matter is said to be rich in nitrogen, relatively transparent, low in aromatics and has a lower molecular weight (Curtis and Schindler, 1997; Gergel *et al.*, 1999). The concentration of lake water DOC depends on in-lake production and the influx from the lake's catchment. However, in the main DOC is composed of high molecular weight compounds such as humic and fulvic acids, with the more labile fractions, such as polysaccharides and low molecular weight acids, rapidly metabolised by bacteria (Wu, 1999).

1.3 Treatment of drinking water

The presence of DOC in surface waters is one of the most critical factors affecting the disinfection of drinking water. Disinfectants used to remove pathogens consist of highly reactive molecules that generate undesired compounds, known as DBPs, upon reaction with DOC. The existence of DBPs is an important public health matter with some compounds being identified as carcinogenic (Bull *et al.*, 1995) and more recent epidemiological studies indicating that they can also be associated with reproductive and development problems (Niewenhuijsen, 2000). The short-term health risk posed by DBPs is of relatively low magnitude when compared to the bacteriological risks of drinking untreated water. However, DBPs pose a much more long-term health risk due to continuous exposure to the compounds not only through drinking water but also through bathing (Xu *et al.*, 2002; Singer, 2006). They are considered to pose the greatest health risk to consumers of treated water.

Among the various disinfectants available to water companies, chlorine is the most frequently used (Richardson, 2003). This is largely due to its effectiveness against water borne pathogens, relative low cost and its ability to provide a residual protection against contamination in the distribution system (Rodriguez and Serodes, 2001). However, the use of chlorine as a disinfectant gives rise to THMs upon reaction with DOC. In the United Kingdom concerns about health risks resulting from exposure to THMs led to drinking water quality regulations which state that the concentration of THMs in drinking water must not exceed 100 $\mu g\ L^{-1}$(DWI, 1998).

THMs are a class of diverse, naturally occurring compounds called organohalides (Gribble, 2003) and are made up of four compounds similar in structure to methane. During the reaction with chlorine the hydrogen atoms of methyl groups found within DOC compounds are substituted with chloride and bromide ions or a combination of the two to form chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2Br$), chlorodibromomethane ($CHClBr_2$) and bromoform ($CHBr_3$)

(Baytak *et al.*, 2008). Chloroform is the most frequently detected compound (Rodriguez *et al.*, 2003), however depending on the bromide content of the raw water, the concentration of bromoform may be greater than chloroform (Westerhoffa *et al.*, 2004).

Whilst THMs are often the dominant type of DBP formed, drinking water may in fact contain a complex cocktail of DBPs. More than 500 DBP compounds have been reported in the literature for the major disinfectants currently used (Richardson, 2003), with each having their own chemical and toxicological properties (Villanueva *et al.*, 2003). Woo *et al.* (2002) divided the DBPs into seven different classes, and in addition to THMs it was reported that haloacitic acids, halonitriles, halokeytones and aldehydes may be present in final waters. It was noted that the variation in DOC composition and the use of different disinfectants and dose bring about the different types of DBPs. The formation of DBPs is also affected by several other factors including water temperature and pH, and the residence time of the water in the distribution system (Baytak *et al.*, 2008).

Obtaining an understanding of the composition of DOC in source waters is critical in understanding the potential formation of DBPs and for improving the efficiency of drinking water treatment processes (Leenheer *et al.*, 2003). Traditionally, the humic fractions of DOC consisting of compounds high in aromaticity have been considered the dominant precursors in THM formation (Imai, 2003). Effective removal of humic substances in DOC therefore emerged as a critical issue in the production of high quality drinking water from surface water (Qin *et al.*, 2006). The removal of DOC from the raw water is facilitated during the coagulation/ flocculation step, using an inorganic coagulant such as ferric chloride or aluminium chloride. The process removes DOC by adsorption onto "flocs" and can typically achieve 50 – 80% removal of DOC (Murray and Parsons, 2003). It is possible to increase the efficiency of DOC removal by increasing the coagulant dose, however, this will inevitably lead to an accompanying increase in the amount of chemical residuals generated and associated increases in costs and operators time

(Murray and Parsons, 2003). The incomplete removal of DOC by treatment works ensures DBPs are present in virtually all chlorinated water supplies throughout the world (Capece, 1998). It also means that DOC is present in the final water, which can lead to problems such as bacterial re-growth in the distribution system (Lehtola *et al.*, 2001).

Humic substances are principally derived from allochthonous, i.e. terrestrial sources. However, autochthonous DOC can be generated in large quantities and over short time scales during algal bloom formation associated with lake eutrophication. Such blooms can be extremely deleterious for lake water quality, as they can release toxic compounds, cause the blockage of treatment filters and produce non-humic DOC compounds which are virtually untreatable (Van Dolah *et al.*, 2001; Cheng and Chi, 2003; Knappe *et al.*, 2004). The reactivity of non-humic fractions has been shown to be comparable to that of humic fractions in relation to THM formation and should therefore also be considered when attempting to manage its production (Owen *et al.*, 1995). However, water treatment works have no real measure in place to facilitate their removal (Cheng and Chi, 2003) and therefore cannot truly control over their production of THMs.

1.4 Characterisation of dissolved organic carbon

The characterisation of DOC is fundamental in understanding its origin and structure. The structural characteristics may be regarded as fingerprints of DOC, reflecting their sources and formation history (Lu *et al.*, 2004). The complex and varied structure of DOC compounds can make this process complicated and challenging, although some of these obstacles have been overcome and reasonable success has been achieved in the isolation and fractionation of DOC into more homogeneous components (Kitis *et al.*, 2002). In the context of DBP formation, the specific precursor compounds involved in the reaction with chlorine are not well documented.

Historically, a variety of methods have been employed to isolate the organic fractions of DOC some, including precipitation, ultrafiltration, solvent extraction, resin adsorption chromatography and freeze drying (Thurman and Malcolm, 1981). Ultrafiltration for example is used to fractionate DOC into different molecular weight fractions. It is a pressure-driven membrane process where solutes are separated according to their molecular size. Ultrafiltration membranes are typically characterised by molecular weight cut-off values established by manufactures, who calibrated the membranes by measuring membrane rejection of macromolecules having known molecular weights (Benner *et al.*, 1997). There is however a disadvantage to this process in the fact that the macromolecules often have structural characteristics significantly different than DOC (Kits *et al.*, 2002). It is therefore unrealistic to directly relate the molecular weight of DOC fractions to manufacture-specific molecular weight cut-off values.

The development of macroporous resins for adsorption chromatography simplified the analytical technique to isolate and concentrate the fractions of DOC (Thurman and Malcolm, 1981). Resin fractionation of DOC in water is a technique which concentrates and categorises the water organic complex into structurally more specific, physiochemically more analogous subgroups. Various XAD resins have been used to isolate organic solutes (Thurman and Malcolm, 1981), the fractionation based on differences in DOC sorption efficiencies on these resins under acid or

base conditions (Chow *et al.*, 2005). The DOC is adsorbed onto a series of resins and eluted with a solvent, which isolates the DOC into three major operationally defined fractions; hydrophobic, transphilic and hydrophilic (Chow *et al.*, 2005)

Whilst ultrafiltration and fractionation allow the separation of DOC into individual groups based on their molecular weight and sorption efficiencies, it is preferable to analyse DOC as one entity so that characteristics that exist due to interactions between individual compounds are not excluded. The ideal method for analytical characterisation of DOC therefore examines the sample as a whole, allowing information on a molecular level to be obtained (Cook, 2004). The structural characteristics of DOC are most commonly examined by spectroscopic analysis, such as ultraviolet/visible absorbance (UV/VIS), infrared and fluorescence spectroscopy and nuclear magnetic resonance (NMR). The determination of UV/VIS absorbance provides an insight into the characteristics of DOM and its potential to form THMs (Chow *et al.*, 2005). The absorbance of UV (200-400 nm) and visible (400-800 nm) radiation by organic matter is caused by atomic electrometric vibrations, involving electrons in the s-, p- and h- orbitals. Absorbance at 254 nm is typically used as a surrogate parameter to estimate concentrations of DOC (Dobbs *et al.*, 1972; Jones, 2006), particularly when the aromatic content of the DOC is high (Chow *et al.*, 2005). NMR is one of the most promising techniques, providing useful recognition of the chemical structure of DOC compounds because, unlike many other techniques, its signal is directly related to chemical functional groups (Lu *et al.*, 2004). The method of choice for rapid screening of dilute DOC samples is proton (^{1}H) NMR (Cook, 2004). It can be used for qualitatively estimating the relative abundance of various humic substance functional groups and is especially useful for characterising their aromatic and aliphatic components. Typical ^{1}H-NMR spectra for DOC are complex and typified by broad signals with fine structures related to specific methylene, methyne and aromatic hydrogens (Cardoza, 2004). ^{1}H-NMR does have an undesirable limitation in that any resonance close to the water resonance will be affected, however, this can be overcome by using a deuterated solvent such as D_2O (Cook, 2004).

Whilst ^{1}H-NMR spectroscopy has been used for humic substance functional group characterisation, analysis by ^{13}C-NMR is generally preferred when sufficient sample quantities are available. ^{13}C-NMR produces greater chemical shift dispersion, allowing identification of ketone and carboxylate functional group and yielding a quantitative spectrum. Unfortunately, due to the low natural abundance of ^{13}C (1.1%), ^{13}C-NMR spectra is inherently insensitive and this problem coupled with limited sample solubility is a significant barrier to performing the measurements (Cordoza, 2004).

1.5 Agricultural practices and eutrophication

Modern agricultural practices can have a significant impact on environmental pollution. Nitrogen (N) and phosphorus (P) are frequently used in fertilisers to achieve sufficient yields of crops on agricultural land, however, the application of excess fertiliser can lead to surplus N and P being transferred to water bodies through runoff and leaching (Feng *et al.*, 2005). Freshwater systems receiving excess nutrients may experience a dramatic deterioration in water quality, due to enhanced eutrophication and the formation of algal blooms (Herath, 1997; Codd, 2000).

Global inorganic fertiliser use coupled with agroecosystem contributions, make up 75% (120 Tg N per year) of all anthropogenic inputs to the global nitrogen budget (Howarth, 2004). This has a dramatic effect on atmospheric N concentrations; it is estimated that roughly 85% of NH_3, 81% of N_2O and 35% of $NO+NO_2$ entering the atmosphere originates from agricultural activities. Moreover, agricultural contributions to N loads in watersheds are of great concern for the contamination of aquatic ecosystems and the regional effect of N loads on nutrient balances in water bodies (Bao *et al.*, 2006). Over time, the efficiency of N fertilizer as a crop nutrient has been shown to decrease while the transportation of N into water bodies increases (Bao *et al.*, 2006). This suggests that enhanced agricultural activity is increasing the amount of N leaching into the ground water and ultimately the surrounding water bodies (Feng *et al.*, 2005). A direct linear relationship between NO_3^- concentration in surface waters and the percentage of land devoted to crop agriculture within the watershed had been reported in some studies (e.g. Schiling and Libra, 2000).

Similarly to N, human activities are also having important effects on the fluxes of P to the environment. Large quantities of P-containing minerals are mined and processed to create fertilizers. These fertilizers are used extensively worldwide and in many cases the soil already contains adequate P reserves. As is true of N, substantial quantities of P are also added to the land in the form of animal manures (Smith *et al.*, 1999). In many areas, P inputs from fertilizers

and manures greatly exceed its outputs in farm produce and consequently P is accumulating in the soil (Foy and Withers, 1995). The total amount of P exported in runoff from the landscape to surface waters has been shown to increase linearly with the soil P content (Sharpley *et al.*, 1996). In an example from Northern Ireland, soil P reserves have accumulated at a rate of roughly 1000 kg P km^{-2} $year^{-1}$ over the past 50 years, and these increases have been accompanied by increases in the loss rates of inorganic P in runoff of roughly 2 mg m^{-3} $year^{-1}$ (Foy *et al.*, 1995). Although small, this rate of change in P runoff is ecologically significant when continued over periods of decades or more (Foy *et al.*, 1995).

Therefore, in the event of N and P being applied in excess of plant requirements for growth, surplus N and P may accumulate in soils, leach from the land into surface waters, compromise ground waters or enter the atmosphere via NH_3 volatilization and N_2 and N_2O production (Smith *et al.*, 1999).

The leaching of N and P into aquatic ecosystems stimulates the growth of algae and vascular plants in freshwater systems. In the event of excess nutrient loading a state of eutrophication may be induced, where the water body becomes nutrient enriched (Smith *et al.*, 1999). Waters having relatively large supplies of nutrients are termed eutrophic (well nourished), and those having poor nutrient supplies are termed oligotrophic (poorly nourished). Those waters having intermediate nutrient supplies are termed mesotrophic (Nurnberg, 1996). Table 1.1 summarises the variation in N and P concentration into freshwaters.

Table 1.1Average characteristics of the different trophic states of lakes based on N and P concentrations (Nurnberg, 1996):

Trophic state	**TN(mg m^{-3})**	**TP(mg m^{-3})**
Oligotrophic	<350	<10
Mesotrophic	350 – 650	10 – 30
Eutrophic	650 –1200	30 – 100

The concept of nutrient limitation may be considered to be a keystone of eutrophication, where the lack of one key nutrient is the primary limiting factor for plant growth in a given ecosystem. In lakes and reservoirs, P is considered to be the key limiting nutrient (Schindler, 1997) and the growth of plants in a given freshwater ecosystem should be proportional to the rate of supply of this nutrient. To minimise eutrophication, management practices within the catchment of rivers and lakes should therefore focus on ways of minimising the leaching of P.

N and P enrichment of freshwaters is typically accompanied by increases in the biomass of suspended and/or benthic algae. The nutrient enrichment of freshwaters can cause a variety of water quality problems through increased algal growth (Nurnberg, 1996; Johnk *et al.*, 2008):

- Increased biomass and changes in species composition of suspended algae and periphyton (algal concentrations may reach millions of cells per millilitre)
- Reduced water clarity
- A deterioration in the water's taste and odour quality
- Blockage of intake screens and filters
- Fouling of submerged lines and nets
- Disruption of flocculation and chlorination processes at water treatment plants
- Restriction of swimming and other water-based recreation

- In extreme cases, near complete depletion of dissolved oxygen concentrations, which can lead to mass mortalities of plant and fish life

From a water treatment perspective, it is actually when the algal bloom begins to die away that it poses the greatest problems for water treatment. As the algal bloom begins to senesce and decomposes, it releases low molecular weight carbon (LMW) that is 'virtually untreatable' (Cheng and Chi, 2003). The occurrence of freshwater algal blooms has increased over the last few decades in a number of locations around the world (Van Dolah *et al.*, 2001; Moore *et al.*, 2008) although the specific reasons for this are not well understood. Climate change may increase the formation of algal blooms through warmer temperatures, therefore greater emphasis should be made to minimise their formation. Some experiments have shown promise in reducing algal growth using barley straw (Welch *et al.*, 1990, Ridge and Pillinger, 1994). The anti-algal properties of barley straw are not fully understood, however it has been suggested that the algal growth inhibitors are derived from oxidised lignin (Ridge and Pillinger, 1994). An alternative hypothesis suggests that the fungi responsible for degrading the barley straw also produce anti-algal compounds. However, the general anti-algal effects of decomposing barley straw are unlikely to be explained by anti-algal properties of specific fungi (Pillinger *et al.*, 1992). Despite the possible benefits, the use of barley straw requires considerable management effort and the long term ecological safety is not known (Ball *et al.*, 2001; Martin and Ridge, 1999).

Wetlands offer an alternative approach for the prevention of algal blooms. A consequence of the high levels of biological productivity within wetlands is that pollutants which enter through run off are easily broken down into substrates for the plants and microorganisms (Mitsch and Gosslink, 2000). In addition to this they are also able to act as chemical sinks, storing vast amounts of carbon (Jenkinson *et al.*, 1991) and nutrients in the water (Vymazal, 2007). The characteristic of storing vast amounts of carbon is largely attributed to waterlogging of the soil, creating anaerobic conditions and inhibiting enzymic decomposition of organic matter through

an 'enzymic latch mechanism' (Freeman *et al.*, 2001a and 2004b). The enzymic latch mechanism is dependent upon the presence of plant derived enzyme-inhibitory phenolic material (Wetzel, 1992). Phenolic compounds are also a defence mechanism against microbial infection (Codignola *et al.*, 1989) and several studies have been undertaken to exploit these compounds to suppress algal blooms (Everall and Lees, 1997; Pillinger *et al.*, 1994; Ferrier *et al.*, 2005).

1.6 Peatland characteristics and properties

Peatlands are a unique wetland ecosystem, which are characterised by having soils of very high organic matter content but with large variations in hydrology, biogeochemistry and vegetation composition (Laiho, 2006). They are found principally in the boreal zone of the world, where precipitation is high, but there are also significant areas of peatland in the tropical region of south-east Asia. They play a key role in the global terrestrial carbon cycle (Matthews and Fung, 1987); whilst they occupy approximately 3% of the world's land surface they contain up to 455 Pg of C, equating to approximately 1/3 of the world's store of soil carbon (Gorham, 1991). In a natural and pristine state, peatlands are considered to be largely stable ecosystems, slowly sequestering carbon due to impaired decomposition within the soil. Although this means that they do not release CO_2 in large quantities to the atmosphere, they can be very important and unique sources of methane to the atmosphere and DOC to freshwaters (Clair *et al.*, 1999; Billett *et al.*, 2004).

The most important property of peat soil above all others is that the soil is waterlogged. This means that the soil is largely anaerobic, and as the decomposition of organic matter is much slower in the absence of oxygen (Moore and Bellamy, 1974; Clymo, 1983), there is a build up of organic material. Decomposition is also hampered by the pH of the soil, which is often low due to the build up or organic acids and the cation exchange properties of Sphagnum (Kuhry *et al.*, 1993), and low temperatures, which are characteristic of the boreal zone in which most of the world's peatlands are found. These characteristics also mean that the soil is of poor quality for plant growth and the productivity of peatlands is often low. However, it is the fact that decomposition of senescing vegetation is *even lower* that means there is a continuous build up of organic matter in the soil (Gore, 1983).

Peatlands have been separated into two distinct groups; fens and bogs. Bogs are an example of an entirely rain fed peatland, which is isolated from surface waters. Consequently bogs are

nutrient poor and acidic (usually below pH 4.5) and are only able to support the growth of acid-tolerant mosses. Conversely fens are a more open type of peatland that receive drainage from surrounding mineral soils and are comparatively more nutrient enriched than bogs, have a higher pH (usually greater than 4.5) and are able to support grasses, sedges and reeds (Mitch and Gosselink, 2000). This difference in the hydrology of bogs and fens and the resulting biogeochemical differences affect rates of decomposition within each type. The isolation of bogs from surface waters lowers the nutrient content of the soil and ultimately their potential to decompose organic matter (Mitch and Gosselink, 2000). The accumulation of organic matter lowers the soil pH further. The capacity of fens to receive nutrients from ground-waters enables a faster rate of decomposition within the soil and therefore a more productive environment (Farrish and Grigal, 1988).

The carbon storage capacity of peatlands is thought to be controlled by the activity of one type of enzyme, phenol oxidase, which has been described as an enzymic 'latch'. Phenol oxidase is the only enzyme capable of degrading phenolic compounds (Sinsabaugh *et al.*, 1991) which are a high molecular weight, recalcitrant organic compound derived from the decay of lignin and cellulose (Dickinson, 1983). However, the activity of phenol oxidase in peat soils is extremely low due to the absence of oxygen, for which it has a fundamental requirement (McLatchey and Reddy, 1998) therefore the concentration of phenolic compounds in peat soils is high. Phenolic compounds have been shown to be inhibitory to hydrolytic enzymes. This suite of enzymes are the dominant decomposers of organic matter in soils (Burns, 1978) and determination of their activity can generate information into the overall rates of decomposition in soil (Marx *et al.*, 2001). Therefore, inhibition of hydrolase enzymes, which occurs indirectly through the lack of phenol oxidase activity, is the reason why peat soils have traditionally sequestered such large amounts of C.

In the anaerobic environment of peat soil, decomposition of organic matter proceeds mostly to DOC and the porewaters of peatland soils therefore contain high concentrations of DOC (as high as 60 mg L^{-1}). As peatlands are found in areas of high precipitation, DOC effluxes to freshwaters can be high (Grieve, 1990), although the extent of this process varies dramatically throughout the year and is dependent to a large extent on the local hydrological flow regime (Worrall *et al.*, 2002). The release of C from peat is often considered to be largely controlled by water table depth (Holden, 2005). The majority of DOC production from peatlands occurs during the summer, when rates of decomposition are at their greatest (Tegan and Dorr, 1996). With rainfall typically low during the summer, this leads to a build up of DOC which is then washed out during the heavy rainfall that is characteristic of the autumn season (Scott *et al.*, 1998).

The extent of peatlands within the catchment is often the most influential determinant on the size of the allochthonous input of DOC to source waters. For example, Urban *et al.* (1989) reported that the single most important variable controlling the DOC yield from a catchment is the proportion of the area occupied by peatlands. The role of peatlands in contributing to the DOC concentration of source waters is therefore critical, particularly in the UK where they dominate the headwaters of many water supply catchments (Evans *et al.*, 1999).

Tropical peatlands cover an area of approximately 30-45 million ha (Verwer *et al.*, 2008) under waterlogged and acidic substrate conditions (Hirano *et al.*, 2007). They have traditionally been one of Earth's most efficient ecosystems for sequestering C due to high rates of above-ground biomass productivity and suppressed decomposition of organic matter in the anoxic soil (Jauhiainen *et al.*, 2008). Unlike boreal and temperate peatlands, those in the tropics are mostly forested and therefore contain significant C stocks above ground in addition to below ground. Peat deposits are usually thicker in tropical regions than those that exist in colder climates. The majority of the world's tropical peatlands are found in south-east Asia (67.1%; 27.1 million ha);

principally Indonesia (55%; 22.5 million ha) and Malaysia (7%; 2 million ha) (Hooijer *et al.*, 2006; Page *et al.*, 2008). Tropical peatlands are considered to be sites of high biological activity and play a significant role in the consumption of greenhouse gases (Bouwman, 1990).

1.7 Anthropogenic impacts on peatlands

Peatlands have traditionally sequestered large stocks of soil carbon. They have therefore acted to naturally reduce the concentration of CO_2 in the atmosphere. As the balance between low productivity and decomposition is small (Päivänen and Vasander, 1994), peatlands are an ecosystem particularly prone to climate change and environmental degradation (Gorham, 1991; Weltzin, 2003). Concern recently has focused on observations of rising concentrations of DOC in freshwaters. Originally reported by Freeman *et al.* (2001b) as a 65% increase in DOC over a 12 year period for 22 sites in the UK, observations of rising DOC have now been reported for many sites in the northern hemisphere (Evans *et al.*, 2005; Monteith *et al.*, 2007). As the greatest increases have been reported at sites with the most peat dominated catchments, it has been postulated that peatlands may be destabilising and starting to release some of the C they have sequestered since the end of the last ice age. The consequences of rising freshwater DOC concentrations for the quality of drinking water are of great concern for the water industry, given the potential for increases in DBP formation and the fact that DOC removal is already the costliest stage of the treatment process.

There has been much debate into the possible causes of this rising trend in DOC, with rising temperature and atmospheric CO_2, decreasing acid deposition and changes in land use and hydrology hypothesised to be causing the changes (Freeman *et al.*, 2001b; Tranvik and Jansson, 2002; Freeman *et al.*, 2004b; Worrall *et al.*, 2004a; Worrall *et al.*, 2006a; Monteith *et al.*, 2007). There is yet to be general consensus on the most likely driving mechanism, however.

Perhaps the most important anthropogenic impact to have occurred in peatlands worldwide has been land use change, which has often been preceded by drainage of the peat soil. As it is the waterlogged condition of the soil that is deemed to be responsible for maintaining the stability of peatlands (Freeman *et al.*, 1996), drainage has the potential to seriously unstabilise the C balance of a peatland. Peatland drainage by open cut channels has been a common land management

technique in many European countries and was undertaken in the hope of increasing agricultural productivity (Worrall *et al.*, 2007a). Due to basic scientific principles, such as that decomposition is much more rapid in aerobic than anaerobic soils, it was widely thought that the lowering of the water table in peatlands and a transition to more aerated conditions results in increased levels of microbial activity within the peat profile and enhanced decomposition, potentially changed peatlands from net C sinks into net C sources (Laiho, 2006). However, results from studies assessing the impact of drainage on decomposition in peatlands are mixed (e.g. Alm *et al.*, 1999; Minkkinen *et al.*, 2002), with decreases in pH, temperature and substrate quality potentially offsetting increases in oxygen content. In terms of DOC, there have been comparatively fewer studies compared to the assessment of changes in CO_2 release, but it is generally thought that peatland drainage will enhance DOC export to freshwaters. For example, Mitchell and McDonald (1995) observed a greater discolouration of freshwaters flowing from drained areas of a peatland catchment in northern England. In an effort to try and restore peatlands and reverse upland degradation many agencies in the UK have begun to block peat drains. However, water companies fear that drain-blocking could in fact increase DOC leaching in the short term; potentially to a level the water treatment works cannot process (Worrall *et al.*, 2007a). The waterlogged nature of peatlands may also be compromised in the future by lowering of the water table through processes associated with climate change (Mitchell and Warrilow, 1987). Roulet *et al.* (2006) have predicted a decrease of 14-22 cm in the height of the water-table in boreal fens for a doubling of the atmospheric CO_2 concentrations. This is similar to the water-table drawdown induced by forestry drainage in many European countries, so sites that have been drained for forestry could be used as a proxy for gaining insights into the effects of climate change.

Interest in tropical peatlands has intensified in the last few years due to the widespread environmental degradation that these ecosystems have undergone. Since the 1970's vast areas have been developed for large-scale agricultural plantations (Melling *et al.*, 2006), principally for

the growth of oil palm. Indonesia and Malaysia have been the countries most affected; Malaysia is now the largest producer and exporter of oil palm and its products (Yousoff and Hansen, 2007) and in 2007 more than 4.3 million ha were under oil palm cultivation occupying over one third of total cultivated area in Malaysia (MPOB, 2008). This has had serious repercussions for CO_2 emissions; drainage has lead to enhanced peat decomposition, whilst clearing the land of vegetation eliminates the strong C sequestering potential of the ecosystem. Peat fires have also had a devastating impact on tropical peatlands, especially in Indonesia (Jaenicke *et al.*, 2008) and the drainage of peatlands makes them particularly susceptible to fire damage. As a result, many degraded tropical peatlands have switched from strong C sinks to C sources through the release of thousands of tonnes of CO_2 (Page *et al.*, 2002). The C emissions from degraded peatlands in Indonesia alone accounts for 4% of the total global anthropogenic emission of greenhouse gases (Hooijer *et al.*, 2006) and sharp increases in atmospheric CO_2 concentrations have been associated with the burning of vast swathes of SE Asian peatlands (Page *et al.*, 2002). There is very little information as to the impact of converting pristine peatlands into agricultural land on the concentration and fluxes of DOC into freshwaters.

1.8 Overall summary

Providing safe drinking water for human consumption is becoming more of a challenge for water companies due to rising concentrations of DOC in freshwaters. This is because DOC reacts with disinfectants such as chlorine to produce undesirable and harmful compounds known as DBPs. The most common DBPs formed are THMs, which are known to be carcinogenic to humans.

A greater understanding is needed of processes occurring within the catchment of water bodies that influence the concentration of DOC in the water. Identifying the origin of DOC provides an insight into the potential formation of DBPs and to do this more thorough methods of DOC characterisation are needed. Peatlands are known to be one of the principle sources of DOC to rivers and lakes; traditionally these ecosystems have been responsible for locking up C but recent evidence suggests that they may be destabilising and beginning to release C back into the atmosphere and into freshwaters. Changes in land management in boreal and tropical peatlands have had perhaps the most devastating effects on peatlands as vast areas have been drained for the establishment of agricultural crop plantations. There is evidence that this has increased the export of DOC to freshwaters, but the effect has not been studies in great detail compared to impacts on the release of CO_2. The issue is of great significance to water companies as DOC removal is one of the principal stages of water treatment.

Agricultural practices also pose additional problems to water quality as the application of excess fertilisers may cause eutrophication of water bodies through the leaching of N and P into water sources. The enhanced level of nutrients can aid the development and growth of algal blooms which can cause problems for water treatment due to dramatically increased concentrations of organic matter, which can lead to the blockage of filters, an increase in the concentration of LMW DOC (which is virtually untreatable and leads to bacterial re-growth in the distribution system) and a greater formation of DBPs. As the formation of algal blooms may increase with

climate change, water companies need to improve ways of minimising their formation, principally through reducing the build up of N and P in the water.

1.9 Aims of the project

The aim of the project is ultimately to examine some of the most important processes that are known to influence the concentration of DOC in freshwaters, i.e. those occurring within both the catchment and the lake. This will be initiated by performing surveys of two drinking water reservoirs on the island of Anglesey, north Wales, where the treatment of water can, at times, be difficult. It is hoped that the survey will aid in the detection of dominant sources of DOC into the reservoirs and to what extent each is influenced by allochthonous and autochthonous inputs. The work will expand on that of Jones (2006) and will use various techniques to better characterise DOC (^{1}H-NMR and fractionation which will be cross-referenced against THM FP) and to generate an assessment of the flux of DOC from key inflows to each lake.

As each of these reservoirs is known to succumb to algal blooms, a new type of constructed wetland will be developed with the intention of reducing algal bloom growth. It is hoped that the installation of a floating structure that is able to sequester N and P would aid in the reduction of algal bloom densities and ultimately a reduction in LMW C generated in the water body.

Peatlands in the boreal and tropical zone have been subject to environmental degradation through drainage and relatively little is known about the impacts on DOC export to freshwaters over long time scale. This will be examined by sampling peatlands in Finland and Malaysia that have been subject to long-term drainage for the establishment of forestry and oil palm plantations respectively, facilitating the comparison of the effects of drainage on these two contrasting peatlands. In addition to assessments of DOC export potential and the repercussions for water quality, the activity of soil enzymes will also be determined due to their key role in the decomposition of organic matter and potential mobilising impact on DOC. It is hoped that findings from these experiments will also provide an insight into changes in DOC export that may occur with water-table drawdown caused by climate change.

Chapter 2

Contrasting influences on the biogeochemical characteristics of two shallow, eutrophic Welsh drinking water reservoirs

2.1 Abstract

Excess dissolved organic carbon (DOC) and inorganic nitrogen and phosphorus can be considered major pollutants in freshwater ecosystems, particularly those used as sources of drinking water. DOC reacts with chlorine used during water treatment to produce a suite of disinfection by-products (DBPs), some of which are known to be harmful to human health. Concern has arisen recently that rising DOC concentrations in freshwaters, linked to climate change, changes in soil acidity and peatland destabilisation, may increase the occurrence of DBPs in drinking water. This chapter describes the seasonal dynamics of DOC, nitrate and phosphate in two shallow, lowland, eutrophic drinking water reservoirs (Llyn Cefni and Llyn Alaw) in north Wales, UK with respect to drinking water quality. Markedly different seasonal trends were recorded, and these were linked to contrasting aspects of the lake's catchment and the meteorological conditions experienced during the two surveys. The presence of a large peatland within the catchment of Llyn Cefni led to a large flux of DOC to the lake during the autumn, 16.2 mg L^{-1} in October 2005 and 15.0 mg L^{-1} in October 2006, and provided a source of DBP precursor compounds. The greater size of Llyn Alaw is suggested to be the reason for the lack of a strong seasonal DOC trend, with concentrations averaging 10.7 mg L^{-1}. Both lakes experienced nitrate depletion during the summer of the surveys, falling from 12.7 mg L^{-1} in Llyn Cefni and 6.5 mg L^{-1} in Llyn Alaw to undetectable levels, suggesting extreme eutrophy, although the lack of detectable phosphate at Llyn Alaw may be why the growth of algal blooms was much less prevalent that at Llyn Cefni. Despite the high input of allochthonous DOC and growth of algal blooms at the Cefni, the thorough treatment processes employed at this works were sufficient to minimise trihalomethane (THM) concentrations to below 65 μg L^{-1} and lower than the maximum permissible limit.

2.2 Introduction

The presence of natural organic matter (NOM) in drinking water reservoirs poses a problem to water treatment plants that endeavour to provide clean, safe drinking water, due to its reactivity with disinfectants and the formation of harmful by-products. Disinfection is a vital stage in the treatment of water, ensuring the elimination of harmful microbes. The most commonly used disinfectant is chlorine, due to the relative simplicity and low cost associated with its implementation (Schoenen, 2002) and its effectiveness against a broad range of bacterial, viral and protozoan pathogens (Nazir and Khan, 2005). However, the presence of NOM in raw water supplies is a matter of concern because it is a potentially large source of carbon (C) compounds that contain methyl groups. These groups react with chlorine to produce a suite of disinfection by products (DBPs), many of which are known to be detrimental to human health in the long term (Singer, 2006). The most commonly occurring and well known class of DBPs are trihalomethanes (THMs) (Villanueva *et al.*, 2006), which are suspected to be carcinogenic to humans (Kits *et al.*, 2002). Chloroform is usually the dominant type of THM formed, although the presence of bromide ions in the raw water can induce the formation of predominately brominated THMs (Rodriguez and Serodes, 2001).

A key constituent of NOM in freshwater is C (Wetzel, 1975). Dissolved organic carbon (DOC) is generally recognised as the principle fraction of NOM (Yu *et al.*, 2003) and comprises particles less than 0.45 μm in size (Thurman, 1985). DOC is composed of recalcitrant high molecular weight compounds such as humic and fulvic acids, in addition to more labile polysaccharides and low molecular weight acids, which can be rapidly metabolised by bacteria (Wu, 1999). A number of factors affect the DOC concentration of freshwater lakes and rivers and one of the most important is the amount of organic rich soil, principally found in peatlands, that is present within the catchment (Urban *et al.*, 1989). Since the end of the last ice age, northern hemisphere peatland ecosystems have sequestered vast stores of C and are estimated to contain a third of the

world's soil C stock (Jenkinson *et al.*, 1991). This characteristic is largely attributed to waterlogging of the soil, creating anaerobic conditions and inhibiting enzymic decomposition of organic matter (Freeman *et al.*, 2004b). Recent evidence suggests that climate change may be reducing the C sink strength of peatlands, leading to an increase in the flux of DOC into freshwaters (Freeman *et al.*, 2001b). This may therefore impact upon drinking water quality through increased THM formation.

Freshwater DOC is complex and poorly understood; it is comprised of a virtually limitless number of different C compounds, all of varying molecular size, weight and structure. Methods to measure quality of DOC have tended to focus on determining basic structural information from measuring the sample as a whole, such as through UV-Visible and fluorescence spectroscopy (Her *et al.*, 2003; Uyguner *et al.*, 2004; Chow *et al.*, 2005). To better understand the sources and characteristics of DOC compounds, and how they might affect drinking water quality, it is essential to separate or isolate DOC and perform more complex analyses, such as nuclear magnetic resonance (NMR) and pyrolysis GC-MS (Kitis *et al.*, 2002). The development of macroporous resins enabled the separation of DOC into 'fractions', by isolating and concentrating DOC into structurally more specific and physiochemical more analogous subgroups (Thurman and Malcom, 1981). It has allowed for DOC to be separated into hydrophobic (fulvic and humic acid) and hydrophilic (carbohydrates with low molecular weight proteins and amino acids) groups. An additional class of compounds may also be identified based on their intermediate polarity between hydrophobic and hydrophilic compounds; these compounds are described as transphilic (Marhaba *et al.*, 2003).

In addition to DOC, the presence of nitrogen (N) and phosphorus (P) plays a key role in influencing the biogeochemical characteristics, and ultimately water quality, of freshwater ecosystems. N and P are frequently used in fertilisers to achieve sufficient yields of crops on agricultural land, however, the application of excess fertiliser can lead to surplus N and P being

transferred to water bodies through runoff and leaching (Feng *et al.*, 2005). Freshwater systems receiving excess nutrients may experience a dramatic deterioration in water quality, due to enhanced eutrophication and the formation of algal blooms (Herath, 1997; Codd, 2000). These blooms can turn freshwaters anoxic and dramatically increase dissolved organic matter (DOM) concentrations within the lake, whilst the algae themselves may be directly harmful to human health (Smith *et al.*, 1999).

Understanding processes affecting the dynamics of DOC, N and P is therefore critical for determining influences on THM formation during water treatment. This chapter reports measurements of seasonal changes in the chemistry of two drinking water reservoirs in north Wales, Llyn Cefni and Llyn Alaw, and describes potential implications to drinking water quality. The presence of a peatland ecosystem within the catchment of Llyn Cefni is expected to lead to a greater flux of allochthonous DOC to this lake, whilst large areas of agricultural land within the catchment of both lakes are expected to be significant sources of N and P.

2.3 Materials and Methods

2.3.1 Study area

The two study sites are shown in figure 2.1. Llyn Cefni (UK grid ref. SH4450077100) is a small reservoir in the centre of the island of Anglesey, north Wales, which was formed in 1950 by damming the river Afon Cefni and Cors Erddreiniog, a nearby peatland. It has a maximum depth of 4 m, a total surface area of 0.86 km^2, a maximum length of 2.3 km and a catchment area of 46.9 km^2. Two inflowing streams have previously been identified as important sources of allochthonous DOC to the lake, Afon Cefni to the south and Afon Erddreiniog to the north (Jones, 2006). Afon Cefni flows through a series of agricultural fields before entering the reservoir, while Afon Erddreiniog drains Cors Erddreiniog, a fen peatland north of the reservoir.

Llyn Alaw (UK grid ref. SH392866) is also a man-made reservoir on the island of Anglesey. It was formed in 1966 by flooding a peatland, Cors y Bol, to provide drinking water for the northern half of the island. It has a maximum depth of 5.2 m, a total surface area of 3.6 km^2, a maximum length of 4.3 km and a catchment area of 33.6 km^2. A few small streams feed into the lake, the largest being Afon Alaw. The lake's catchment is largely agricultural, and Afon Alaw flows through a succession of fields before entering the reservoir. There is an additional source of water to the lake; the Cors y Bol inflow. This is a subsidiary dam that mostly serves as an overflow from the reservoir, although infrequently some water is pumped back into the lake to prevent flooding of the catchment. A small peatland is present in the catchment of the Cors y Bol inflow. A summary of the limmological properties of both lakes can be seen in Table 2.1.

Table 2.1 - Summary of the limmological properties of Llyn Alaw and Llyn Cefni

	Llyn Alaw	**Llyn Cefni**
Mean annual temperature	12 °C	13 °C
Mean annual precipitation	85.5 mm	108.8 mm
Maximum lake depth	5.2 m	4 m
Maximum lake length	4.3 m	2.3 km
Lake surface area	3.6 km^2	0.86 km^2
Watershed	33.6 km^2	46.9 km^2

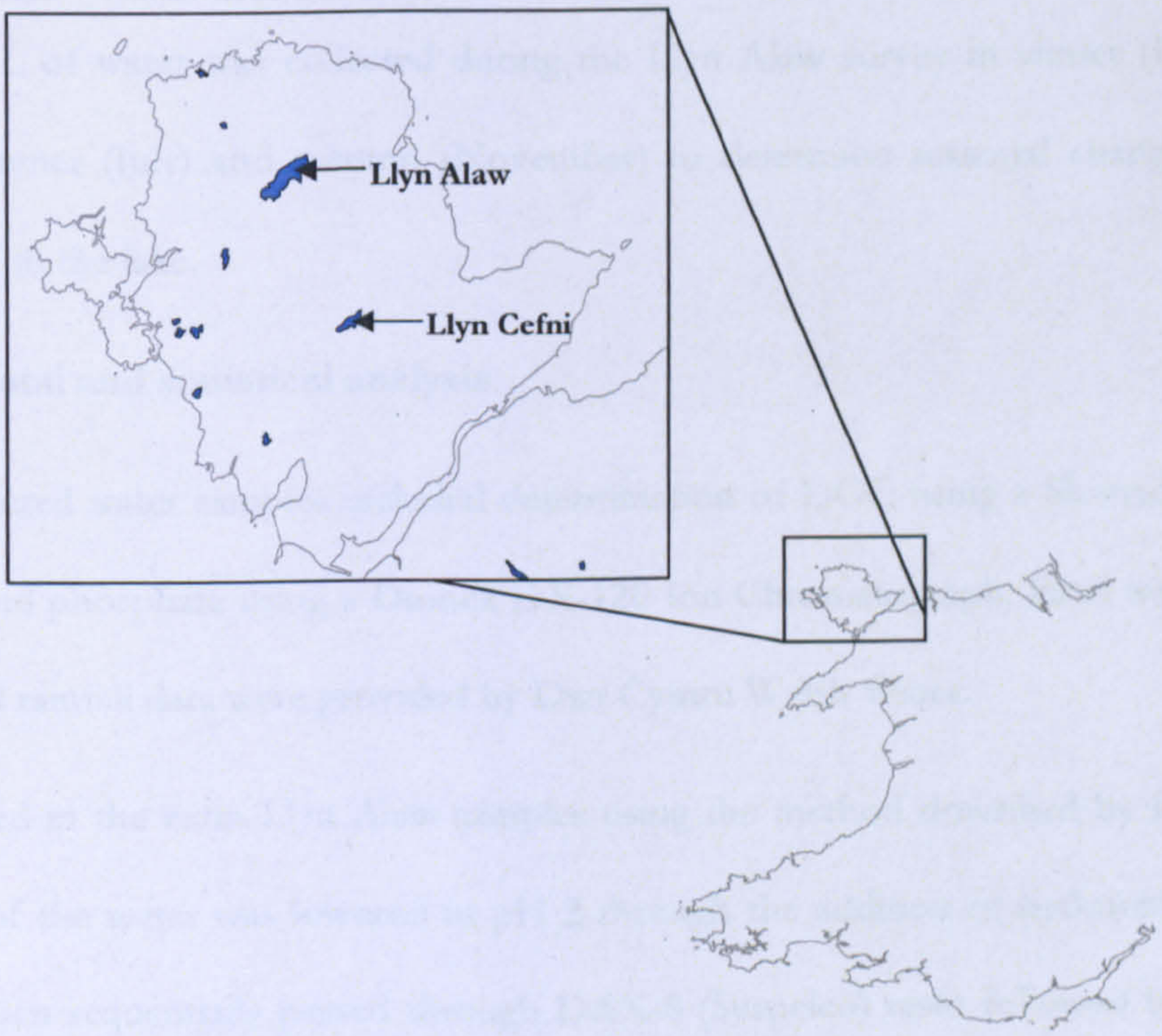


Figure 2.1 – The two study sites; Llyn Cefni and Llyn Alaw, on the island of Anglesey, Wales, UK

2.3.2 Sample collection

A twelve month survey of Llyn Cefni and Llyn Alaw was undertaken between October 2005 and October 2006 and January 2007 and December 2007 respectively. Seasonal changes in the DOC, nitrate and phosphate concentrations of inflowing streams to the lakes were measured, whilst the DOC flux was calculated for the inflowing streams. For the Llyn Cefni survey, routine samples (n=5) were collected every six weeks from four sampling points: Afon Erddreiniog and Afon Cefni, the raw lake water and the final treated water. For the Llyn Alaw survey, routine samples (n=5) were collected every four weeks from four sampling points: the Afon Alaw and Cors y Bol inflows, the raw lake water and the final treated water. The flow rate of the inflowing streams was measured to enable the calculation of their individual discharge into the reservoirs and, following collection, all samples were filtered through sterilised 0.45 μm filters membranes and stored at 4 °C in a refrigerator. Raw water temperature was also measured on each sampling visit. An additional 2.5 L of water was collected during the Llyn Alaw survey in winter (February), spring (April), summer (July) and autumn (November) to determine seasonal changes in the 'fractions' of DOC in the lake.

2.3.3 Experimental and statistical analysis

Analysis of the filtered water samples included determination of DOC using a Shimadzu TOC-5000 and nitrate and phosphate using a Dionex DX-120 Ion Chromatograph. Final water THM concentrations and rainfall data were provided by Dwr Cymru Welsh Water.

DOC was separated in the extra Llyn Alaw samples using the method described by Kim *et al.*, (2006a). The pH of the water was lowered to pH 2 through the addition of hydrochloric acid. The sample was then sequentially passed through DAX-8 (Surpelco) resin followed by XAD-4 (Amberlite) resin at a flow rate of 1.5 ml minute^{-1}, to isolate the DOC into three fractions; hydrophobic, transphilic and hydrophilic. The concentration of each fraction was calculated using the following equations:

Hydrophobic = [DOC] DAX-8 in – [DOC] DAX-8 out

Transphilic = [DOC] DAX-8 out – [DOC] XAD-4 out

Hydrophilic = [DOC] XAD-4 out

A series of correlations were performed on the datasets using Pearson correlation in SPSS (version 11). The correlations considered the interactions between rainfall and subsequent DOC concentrations entering both lakes and the effect of temperature and DOC on THM production. The standard error of each data point was calculated by dividing the standard deviation of the sample by the square root of the sample size.

2.4 Results

2.4.1 Llyn Cefni survey

The general trend of DOC at all four sampling sites was a minimum during the spring followed by a maximum during the autumn (Figure 2.2). Peaks in DOC concentration were also recorded in January and March. Afon Erddreiniog had a greater DOC concentration that Afon Cefni during all but one month (August), where its concentration fell to a minimum of 3.81 mg L^{-1}. The treated water consistently had lower DOC concentrations than the raw lake water, between 65% and 70%, however, it always followed the same seasonal trend. The calculated discharge of DOC from both inflows to the reservoir was almost identical throughout the year (Figure 2.3). Low levels of discharge were recorded during the summer and peaked during spring, to a maximum of 19 g s^{-1} and 22g s^{-1} for Afon Erddreiniog and Afon Cefni respectively.

The concentration of nitrate (Figure 2.4) had a general trend of peaking during early spring and steadily declining throughout the summer, however, peak in concentration was recorded in Afon Erddreiniog in August (15.02 mg L^{-1}). The concentration of phosphate (Figure 2.5) in Llyn Cefni was highest during late autumn and early winter (0.26 mg L^{-1} and 0.13 mg L^{-1}) and undetectable levels were recorded for the remainder of the year. Low concentrations of phosphate, below 0.12 mg L^{-1} were recorded in both Afon Erddreiniog and Afon Cefni with levels peaking in March and steadily declining during the summer.

THM concentrations (Figure 2.7) of the final treated lake water showed peaks both in October 05 and October 06, which were65 μg L^{-1} and 61 μg L^{-1}, respectively. Concentrations were at their lowest during the winter, corresponding to the coldest temperatures.

2.4.2 Llyn Alaw survey

Neither one of the four sampling sites displayed an obvious trend of DOC (Figure 2.2). Both inflows, Afon Alaw and Cors y Bol, produced a minimum in DOC concentration during late

winter and early spring, whilst early summer and late autumn produced the greatest DOC concentrations. The rate of DOC discharge from Afon Alaw (Figure 2.3) was generally low throughout the year, with the greatest rate being recorded during November, 111 g s^{-1}, and January 59 g s^{-1}. Llyn Alaw followed a similar but slightly dampened trend to that displayed by the Cors y Bol inflow, whilst the final treated water showed very little fluctuation and was largely constant, continuingly producing concentrations of below 8.22 mg L^{-1} regardless of the season. Fractionation of the DOC in Llyn Alaw (Figure 2.6) revealed that during the summer hydrophilic compounds dominated the DOC structure (76%) and hydrophobic compounds were shown to dominate during the autumn (55%). During the winter and spring, no one fraction was much more prevalent than the other.

Nitrate concentrations (Figure 2.4) were slightly higher in the Cors y Bol inflow when compared to the Afon Alaw inflow. The general trend exhibited by all four sites showed concentrations at their lowest during the summer and highest in the winter. However, both the Cors y Bol and Afon Alaw inflow produced peaks in concentrations in September before following the same trend as the raw and final treated lake water for the remainder of the year.

THM concentrations for the treated lake water (Figure 2.7) produced peaks both in April and August (67 μg L^{-1} and 57 μg L^{-1}), corresponding to warm lake water temperatures. As the temperature decreased so too did the total THM concentration, with concentrations below 30 57 μg L^{-1} recorded in January and February.

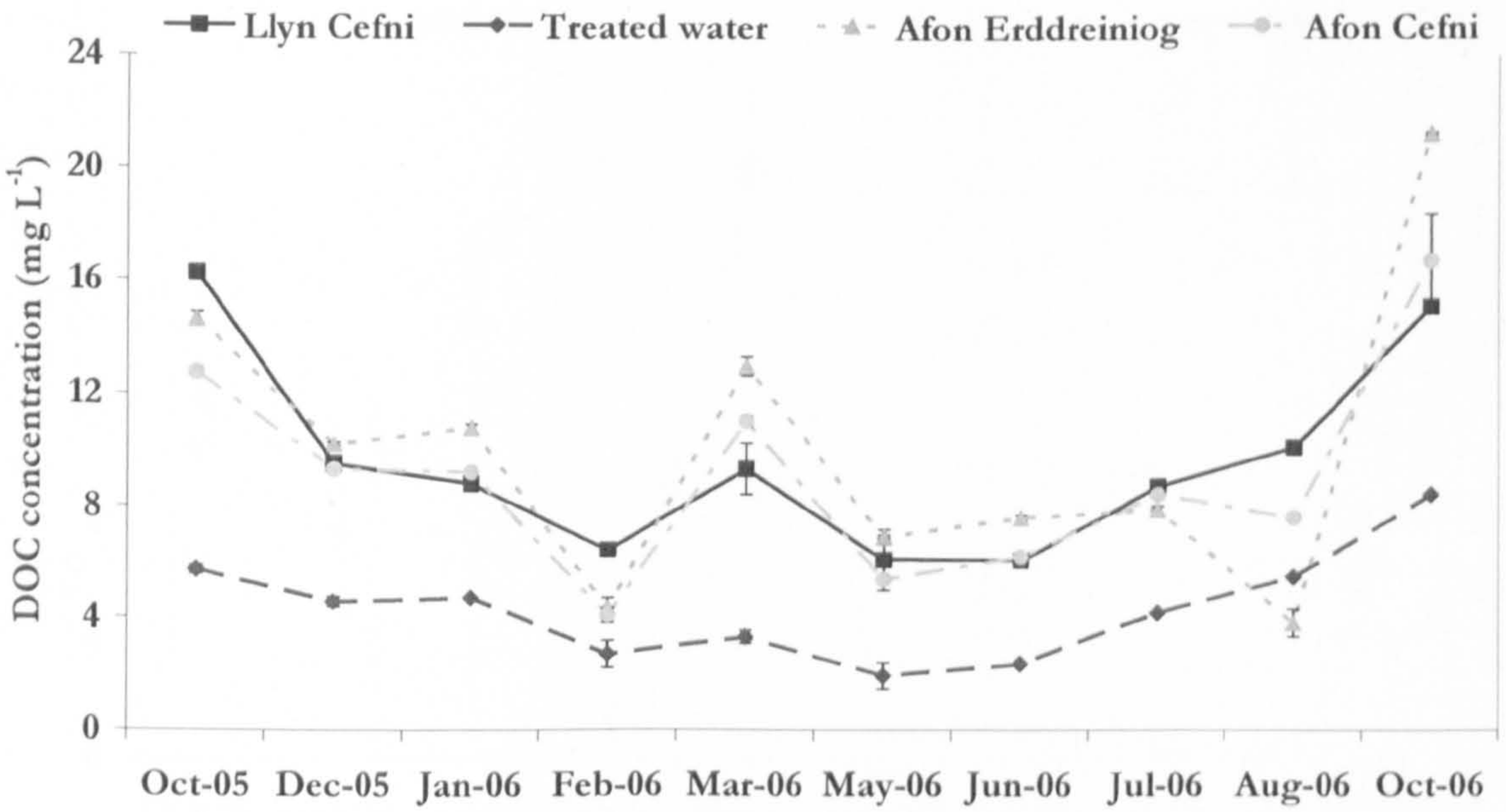
2.4.3 Correlations between measured parameters

The total volume of rainfall received in the 4 days prior to sampling correlated weakly but not significantly with the concentration of DOC in Llyn Cefni (R^2=0.115, p>0.05; Figure 2.8). Rainfall 4 days prior to sampling was considered as statistical tests showed this number of days correlated best with DOC concentrations.

The formation of THMs correlated significantly with the lake water temperature at both Llyn Cefni (R^2=0.444, p<0.05) and Alaw (R^2=0.479, p<0.05; Figure 2.9).

The formation of THMs correlated significantly with the raw water DOC concentration for Llyn Cefni (R^2=0.483, p<0.05; Figure 2.10) but not for Llyn Alaw (p>0.05).

The concentration of DOC in Llyn Cefni correlated significantly with the DOC concentration in both Afon Erddreiniog (R^2=0.585, p<0.01) and Afon Cefni (R^2=0.784, p<0.001; Figure 2.11). For Llyn Alaw, no significant correlations were recorded when comparing the concentration of the lake water DOC with that in Afon Alaw and the Cors y Bol inflow (p>0.05; Figure 2.12).



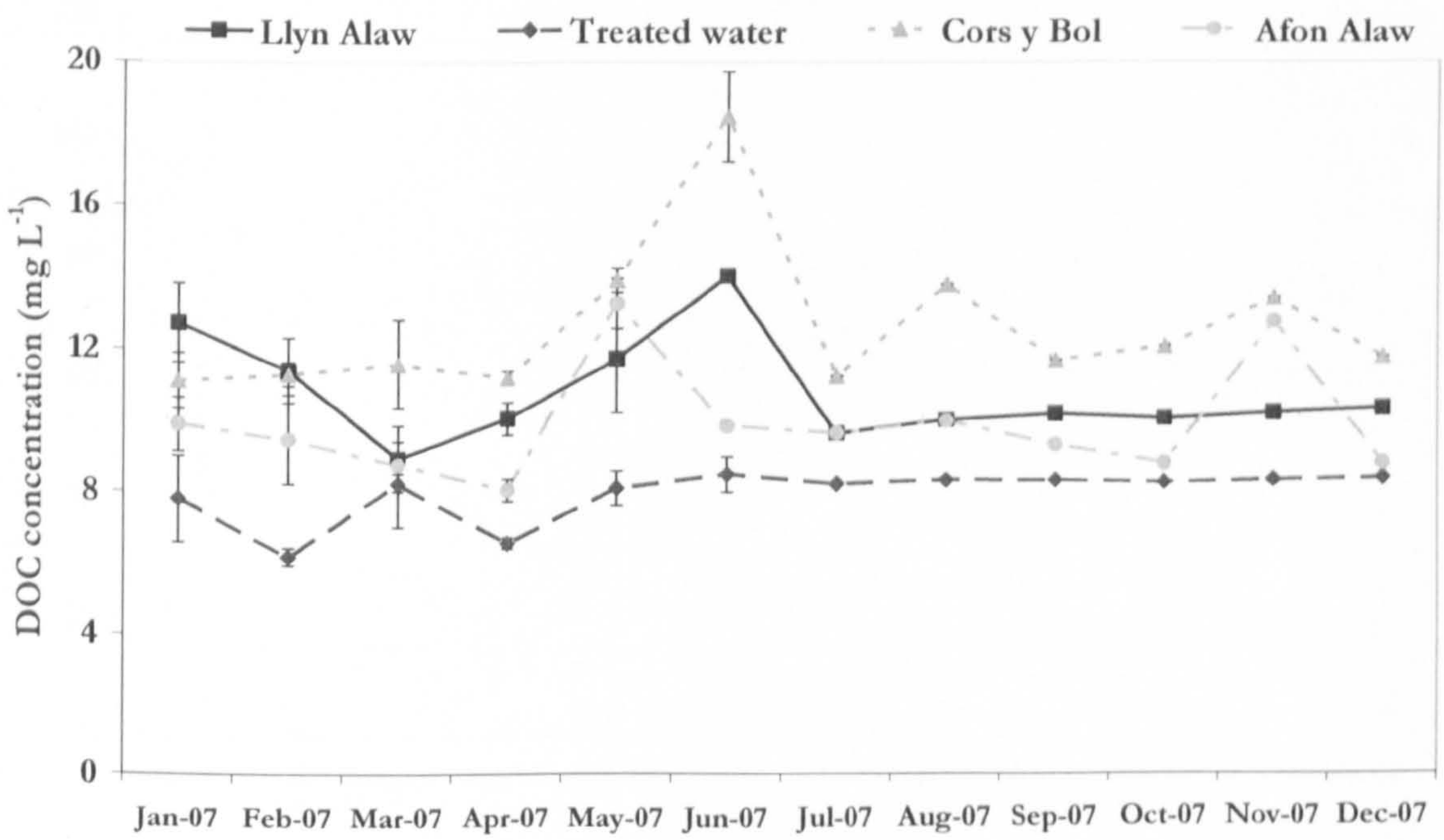


Figure 2.2 – Mean DOC concentrations of the four sampling sites for Llyn Cefni (top) and Llyn Alaw (bottom)

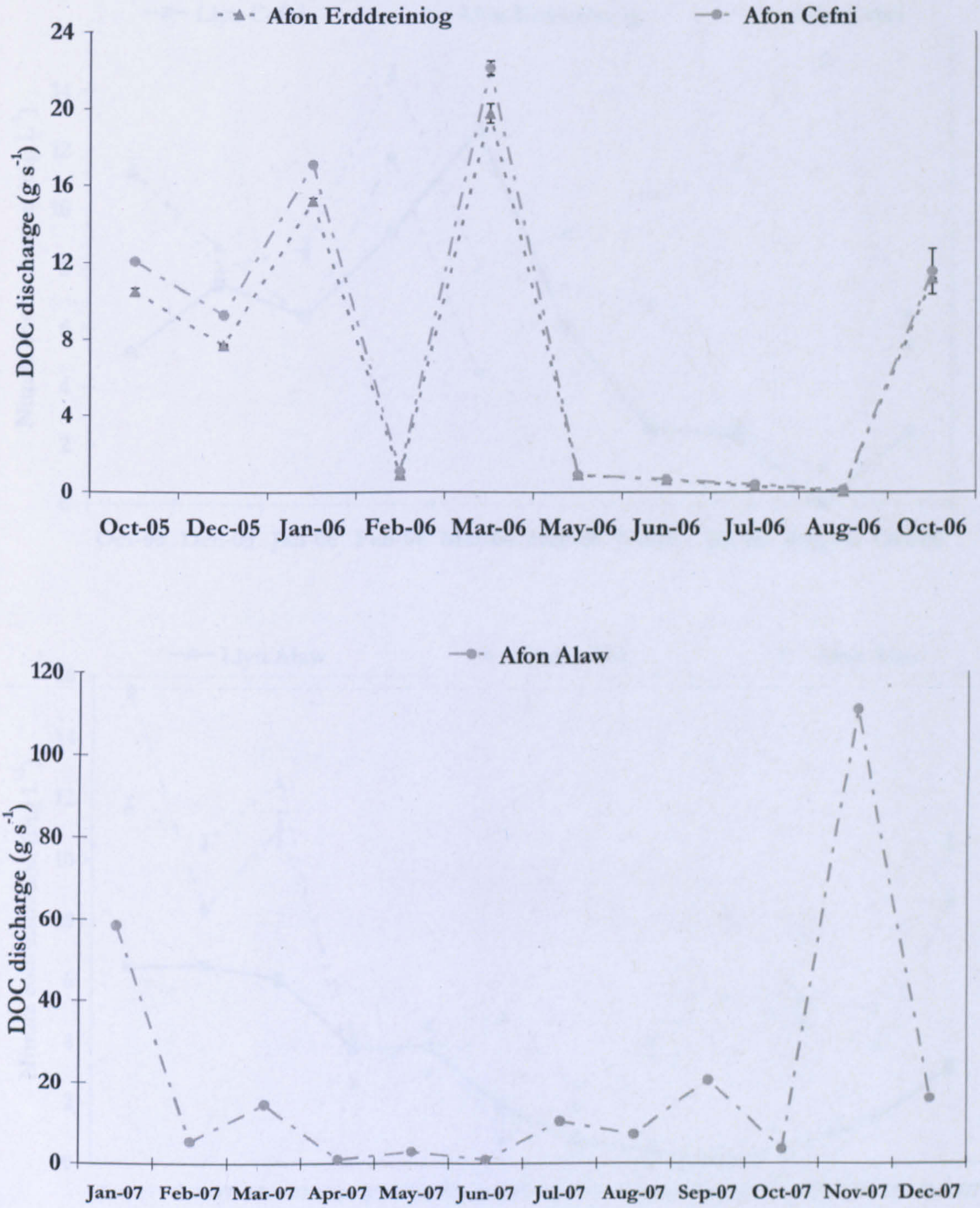
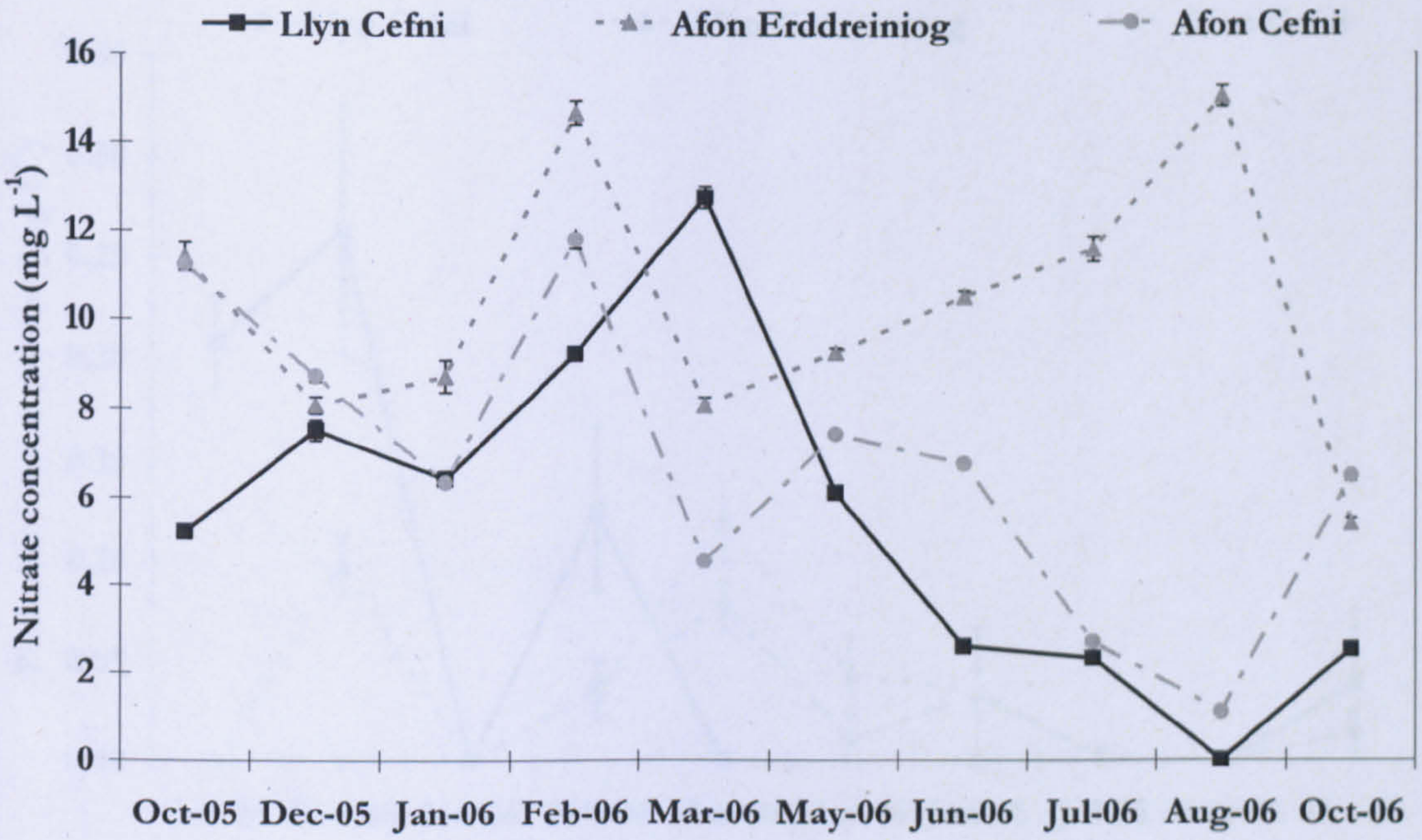


Figure 2.3 – Calculated flux of DOC of the major inflowing streams into Llyn Cefni (top) and Llyn Alaw (bottom)



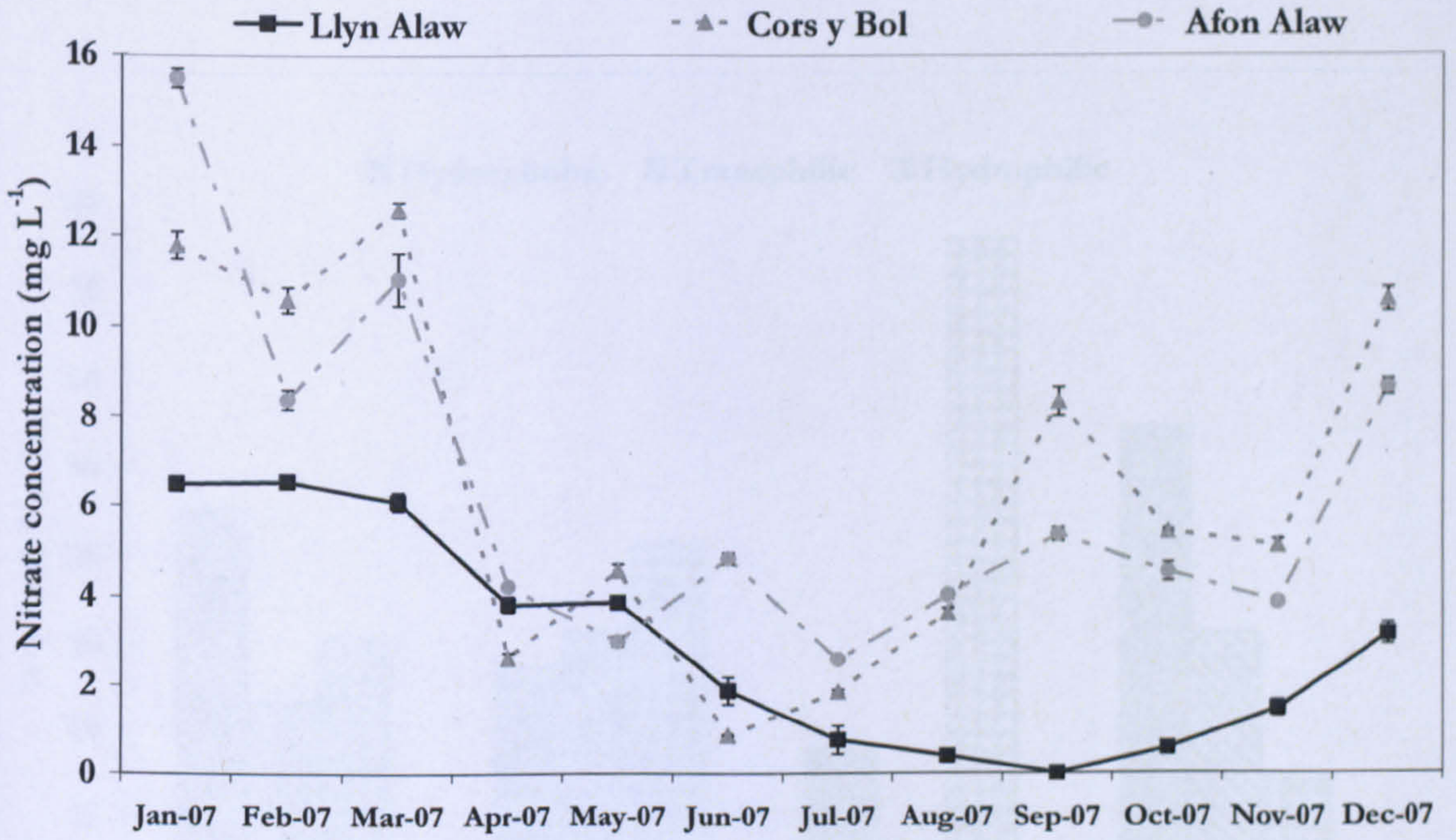


Figure 2.4 – Mean nitrate concentrations of Llyn Alaw, Afon Erddreiniog and Afon Cefni (top) and Llyn Alaw, Afon Alaw and Cors y Bol (bottom)

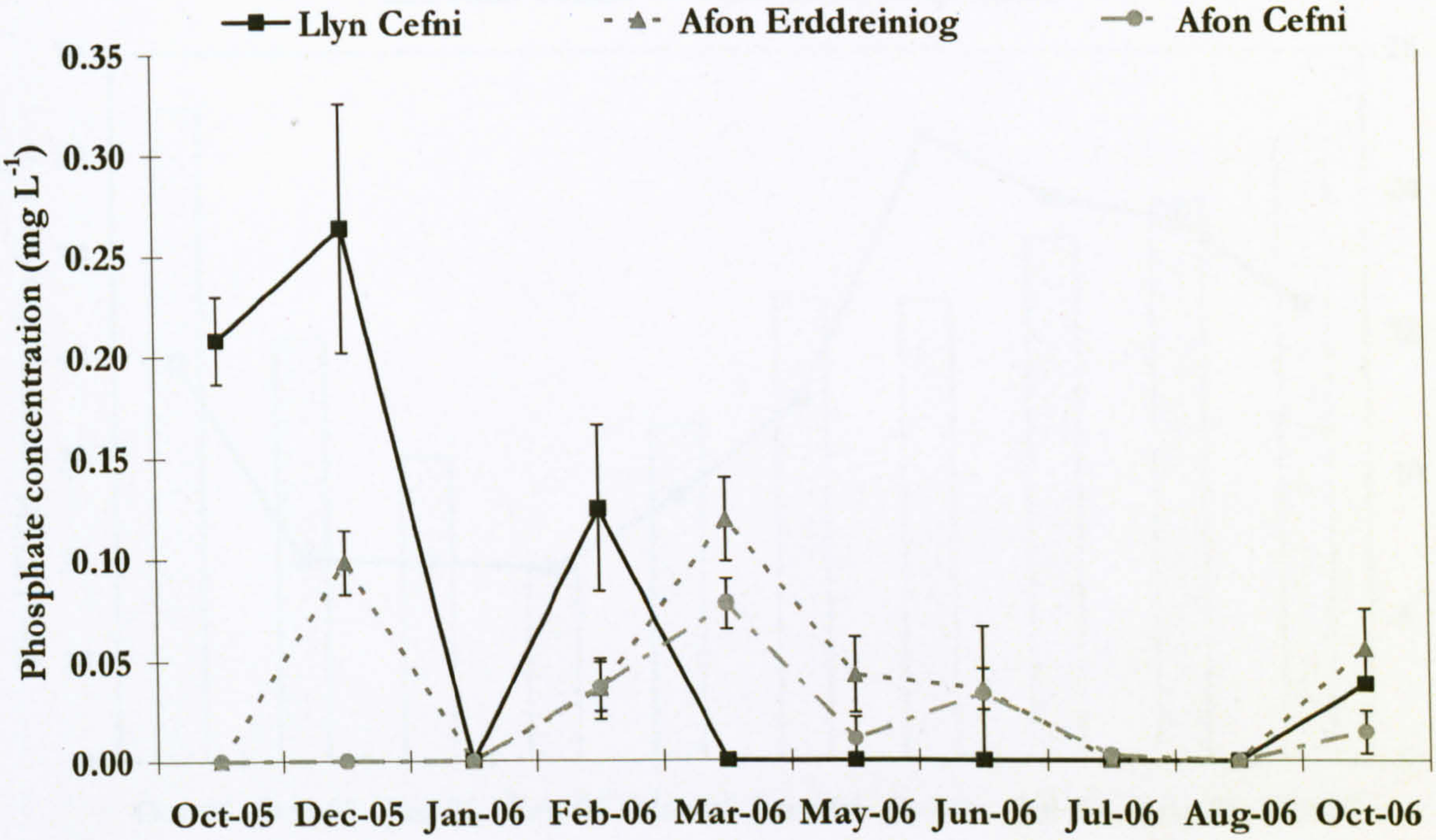


Figure 2.5 – Mean phosphate concentrations of Llyn Cefni, Afon Erddreiniog and Afon Cefni

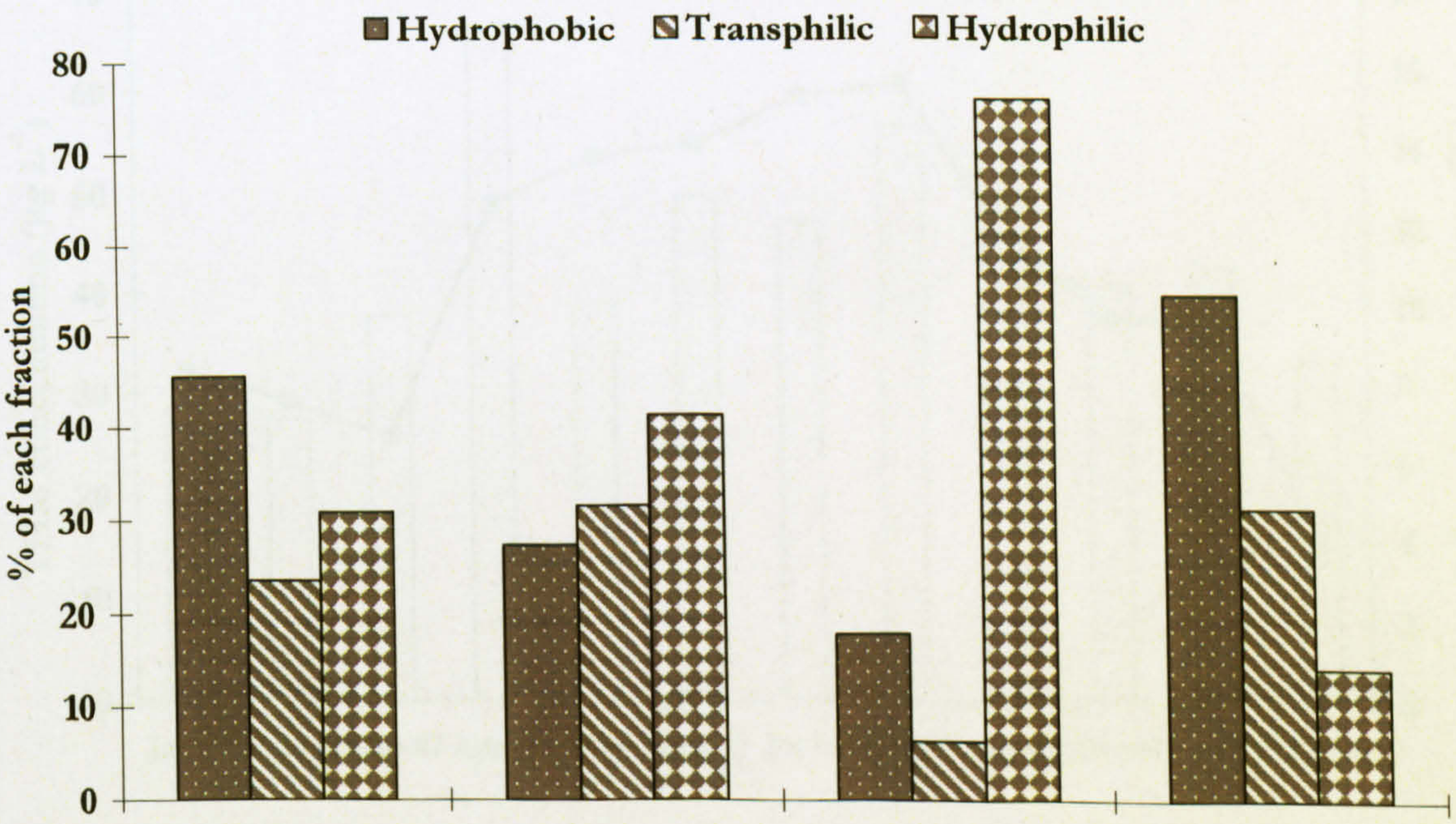


Figure 2.6 – Seasonal changes in DOC 'fractions' for Llyn Alaw

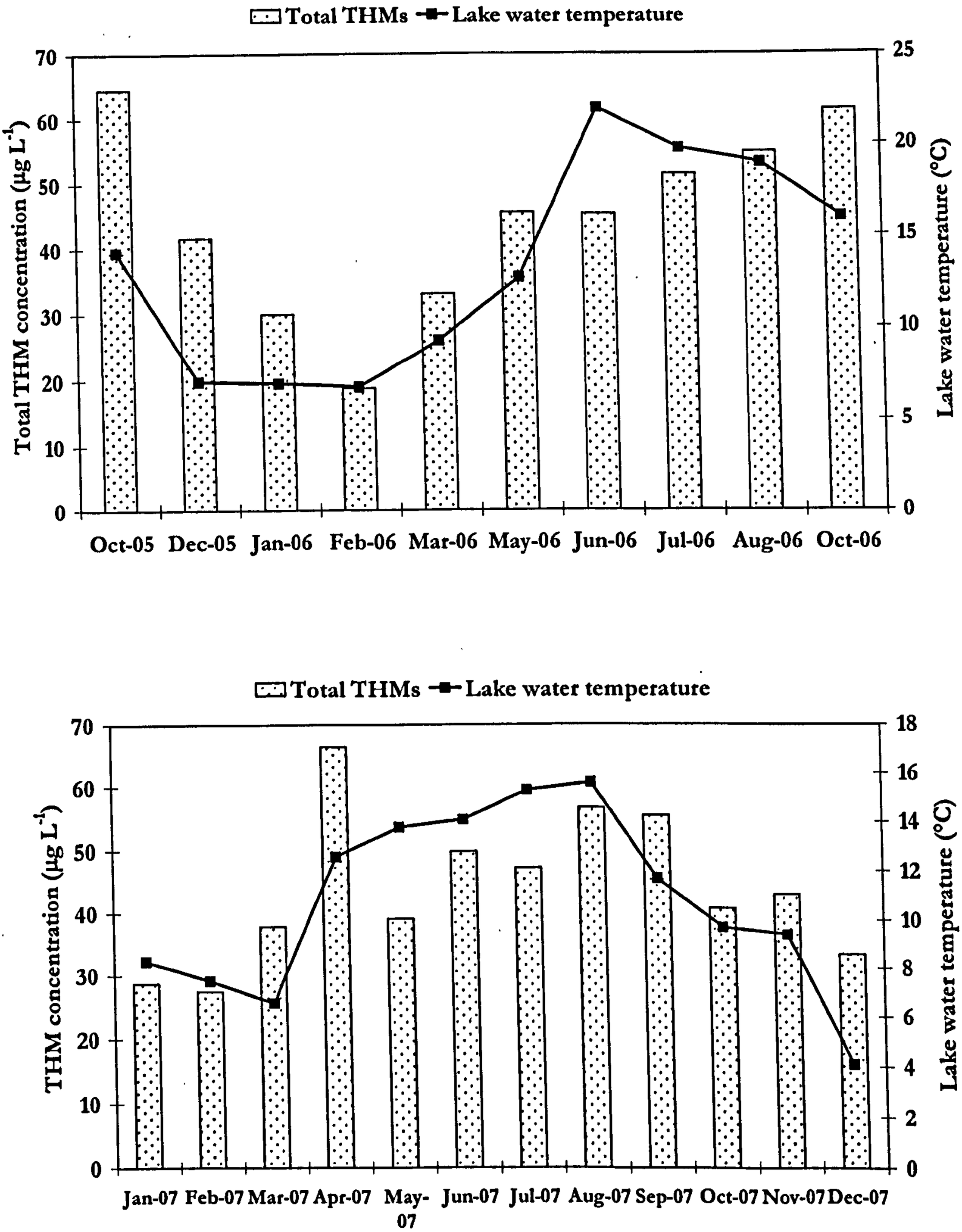


Figure 2.7 – Total THM concentrations and lake water temperature for Llyn Cefni (top) and Llyn Alaw (bottom)

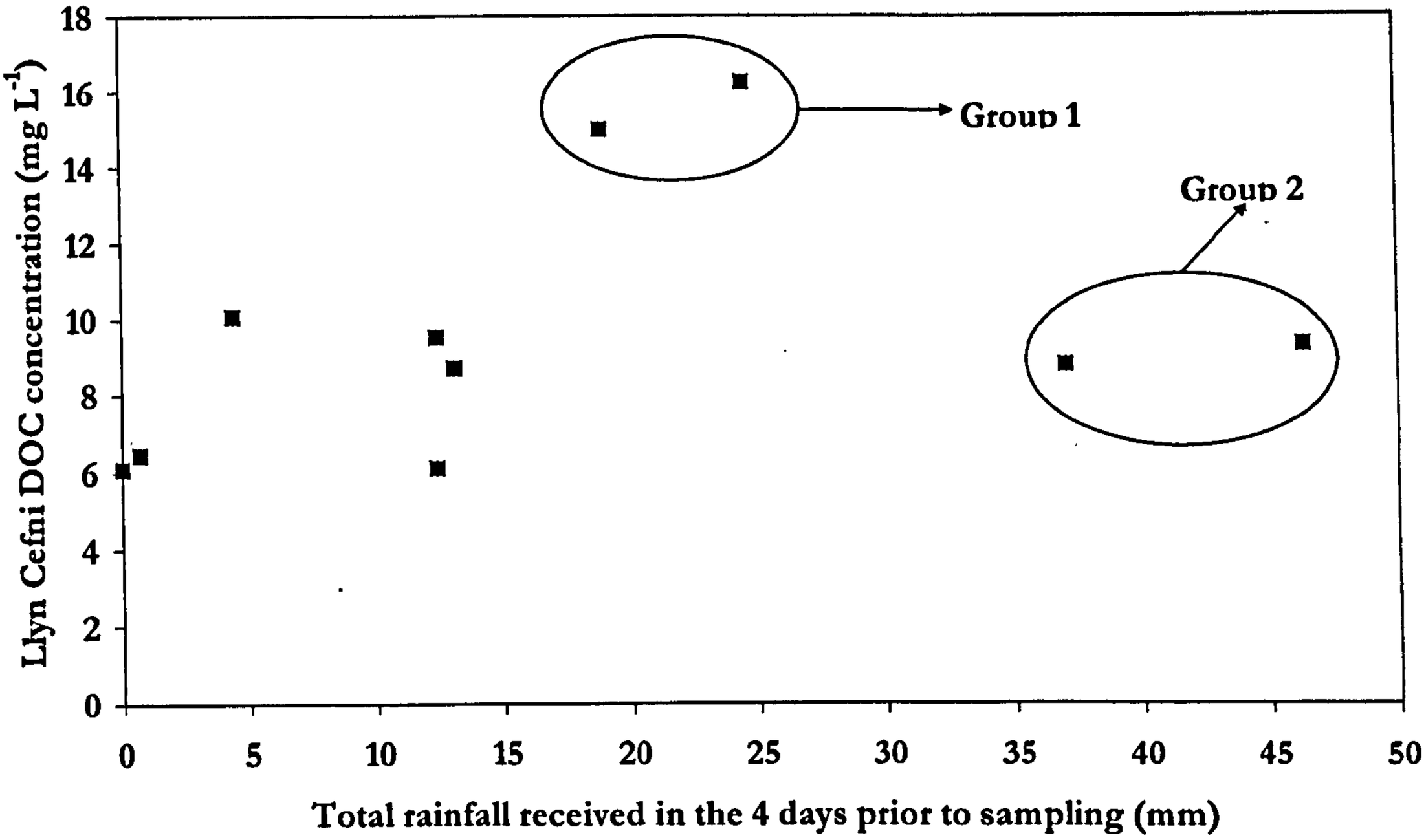


Figure 2.8 – Relationship between Llyn Cefni DOC concentration and total volume of rainfall at the lake 4 days prior to sampling.

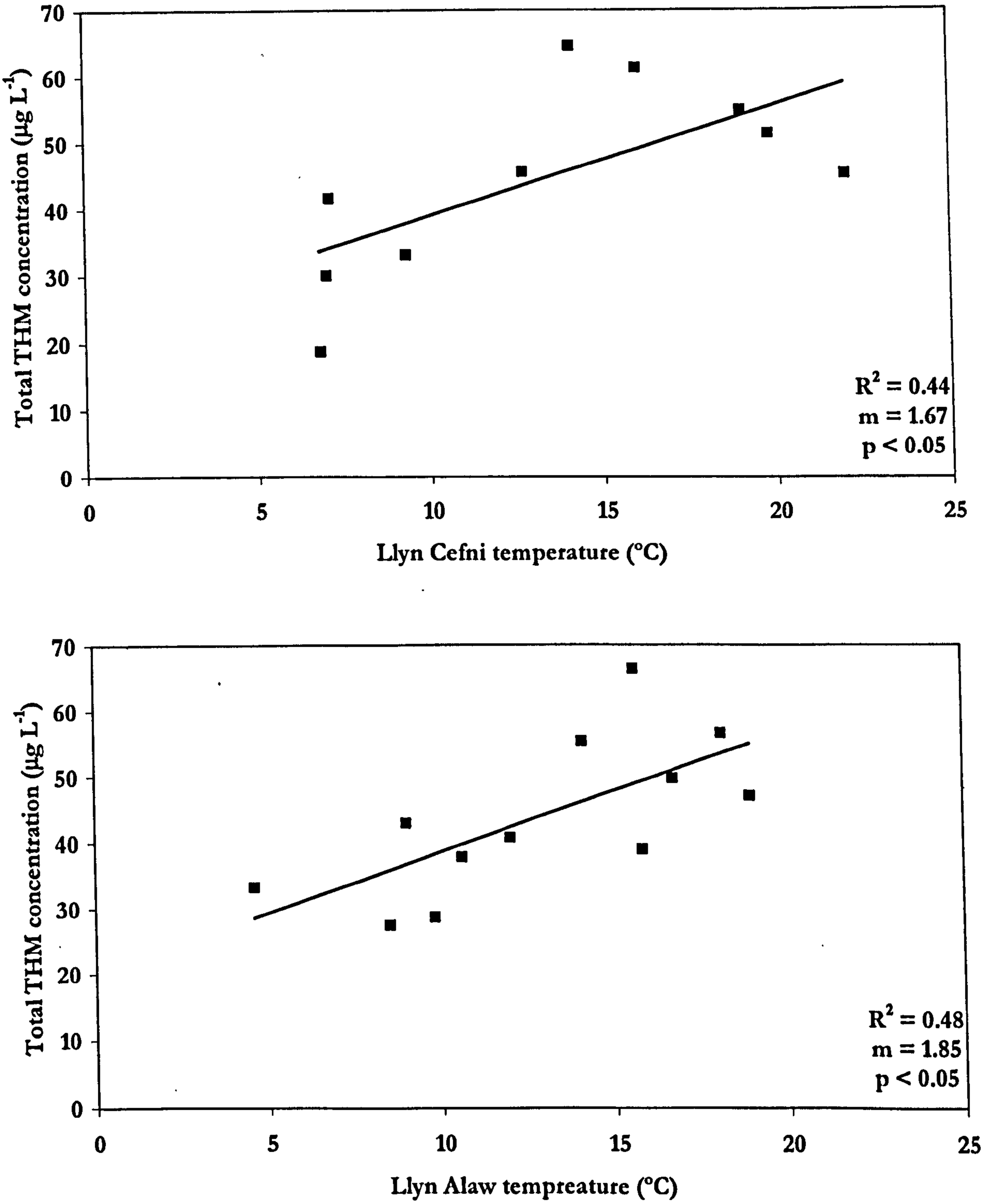


Figure 2.9 – Relationship between lake water temperature and final water total THM *concentration for Llyn Cefni (top) and Llyn Alaw (bottom). Significant linear relationships are indicated by slope lines and* R^2 *values are presented in the data.*

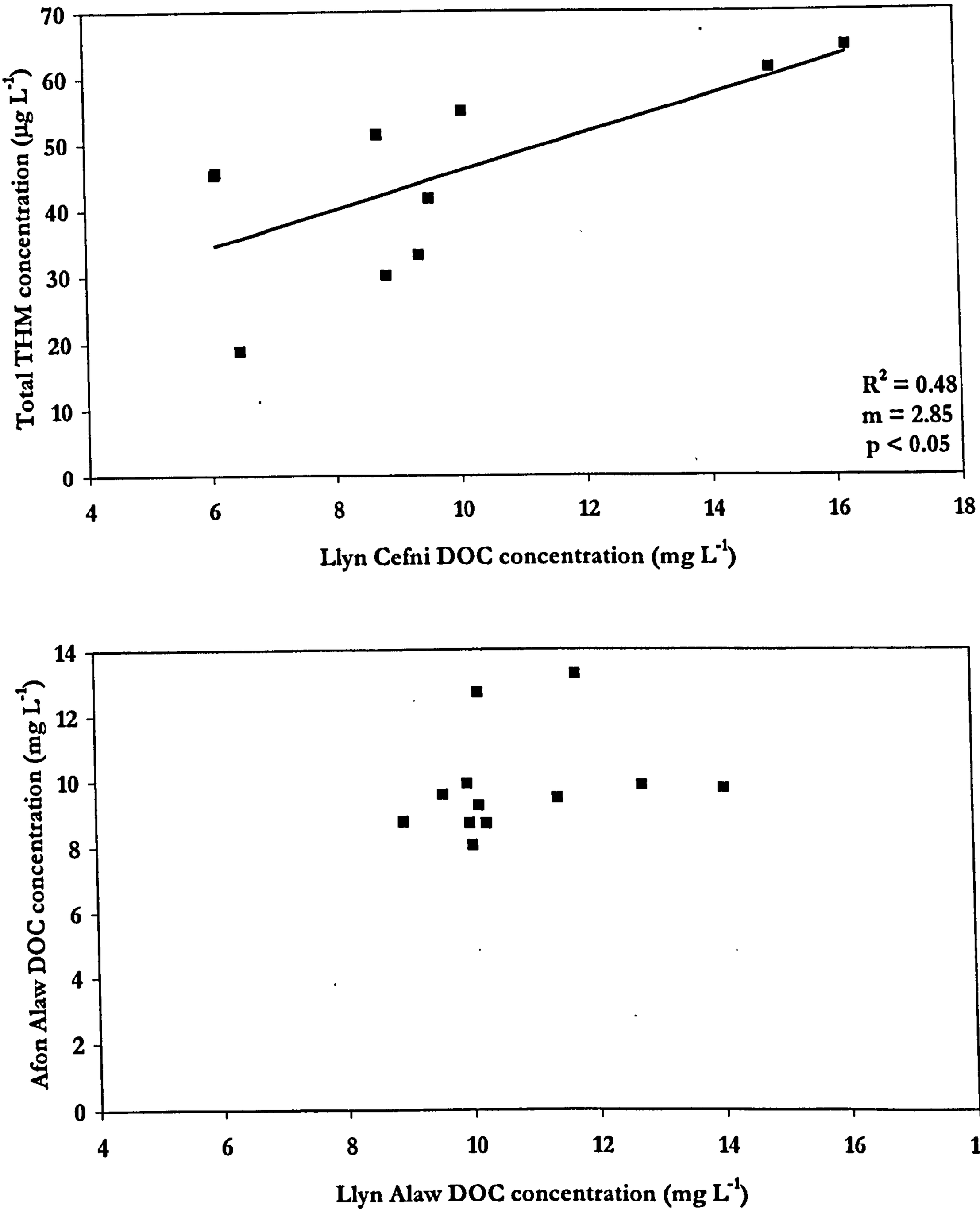


Figure 2.10 - Relationship between lake water DOC concentration and final water total THM concentration for Llyn Cefni (top) and Llyn Alaw (bottom). Significant linear relationships are indicated by slope lines and R^2 values are presented in the data.

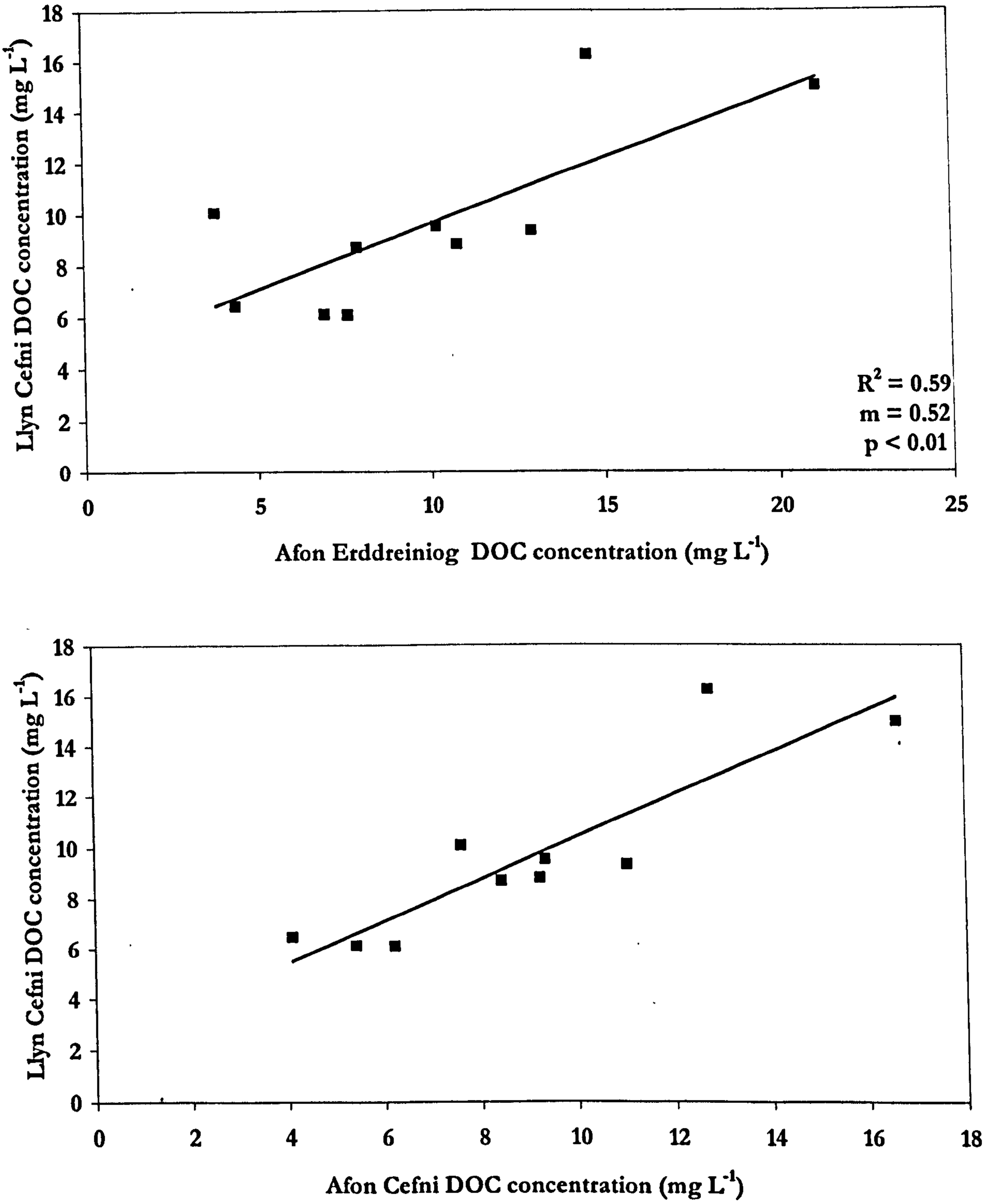


Figure 2.11 - Relationship between DOC concentrations for Llyn Cefni and Afon Erddreiniog (top) and Afon Cefni (bottom). Significant linear relationships are indicated by slope lines and R^2 values are presented in the data.

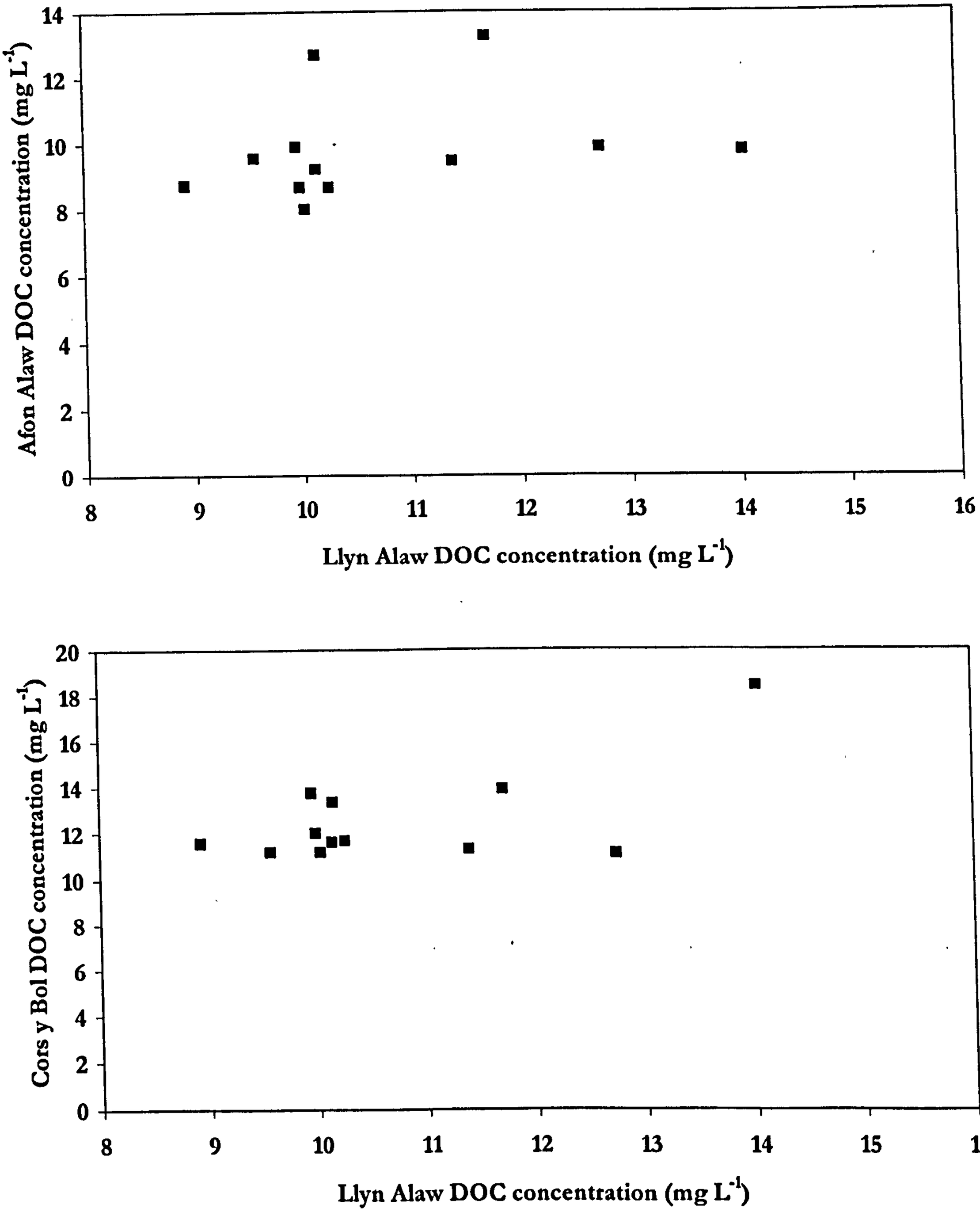


Figure 2.12 – Relationship between DOC concentrations for Llyn Alaw and Afon Alaw (top) and Cors y Bol inflow (bottom).

2.5 Discussion

Llyn Cefni and Llyn Alaw are both shallow, lowland lakes and, as is often typical for such ecosystems, both are occasionally subject to high inputs of C, N and P, which have detrimental impacts on their water quality. These inputs have entirely different origins (natural vs. anthropogenic), but both necessitate thorough and complex water treatment processes to ensure that drinking water from these lakes is fit for human consumption. Analysis of the data collected during the two surveys highlights the major influences on biogeochemical properties of Llyn Cefni and Llyn Alaw. The seasonal dynamics of DOM in both lakes varied markedly and can largely be attributed to differences in properties of the lake's catchments and meteorological conditions experienced throughout 2006 and 2007.

Concerning DOC, a strong seasonal trend was evident for Llyn Cefni, with higher maximum and lower minimum DOC concentrations than Llyn Alaw and the greatest concentrations measured in the autumn. These differences may reflect contrasting aspects of their size and catchment characteristics. Firstly, Llyn Cefni has a surface area four times smaller than that of Llyn Alaw. Lake size is known to be of great influence on hydrological and biogeochemical processes (Hanson *et al.*, 2007), and it has previously been reported that lake area can correlate inversely with DOC concentration (Xenopoulos *et al.*, 2003). Secondly, Llyn Cefni contains within its catchment Cors Erddreiniog, a 289 hectare peatland. It is well known that the proportion of peatlands within the catchment of temperate lakes is one of the most influential determinants on the size of the allochthonous C input (Dillon and Molot, 1997) and previous work has demonstrated Cors Erddreiniog to be an important source of DOC to Llyn Cefni (Jones, 2006). These attributes may explain why Llyn Cefni has a higher maximum DOC concentration, particularly during the autumn when temperate lakes typically experience a flush of peatland-derived DOC (Scott *et al.*, 1998; Mash *et al.*, 2004). The high concentrations of DOC in October 2005 and 2006 in Afon Erddreiniog, the stream draining the peatland, provide evidence of an

autumn time pulse of DOC into Llyn Cefni. The concentration of DOC in Afon Erddreiniog was almost always higher than Afon Cefni, reflecting the peatland influence, although the concentration would be higher were it not for breakdown of the DOC occurring within Afon Erddreiniog once it is mobilised from the peatland (Jones, 2006). The concentration of DOC in Llyn Cefni correlated significantly with the DOC concentration in both Afon Erddreiniog and Afon Cefni, indicating the influence of these streams as point sources of DOC to the lake. Once corrected for the discharge volumes of each stream, it is apparent that Afon Cefni and Afon Erddreiniog both contribute almost equal quantities of DOC to Llyn Cefni. Jones (2006) also reported that DOC from Afon Erddreiniog has a higher SUVA (Specific Ultra Violet Absorbance) than DOC from Afon Cefni, again due to the peatland origin. Such a characteristic will make the DOC more amenable to removal during treatment, but will also make it more liable to form DBPs during water treatment (Archer and Singer, 2006; Fabris *et al.* 2008).

For Llyn Alaw, the major inflowing stream, Afon Alaw, does not appear to contribute significantly to the DOC concentration of the lake and there was no significant correlation between the lake and stream water DOC concentrations. Most noticeably, the relatively large flux of DOC in November 2007 did not significantly increase the concentration measured in the lake in November or December 2007. Although the stream drains a relatively large area of land, its small size in relation to the volume of the lake and the lack of a peatland within its catchment is perhaps the reason why it is not an influential source of allochthonous DOC. This also explains the absence of an autumn-time pulse of DOC in both Afon Alaw and Llyn Alaw. Moreover, the concentration of DOC in the Cors y Bol inflow does not correlate significantly with the lake's DOC concentration. This is also probably due to its small size relative to the lake and because it is only intermittently used to pump water back into the reservoir to prevent flooding of the catchment. While the inflow flows through a small area of peatland, this is unlikely to contribute a significant amount of DOC to Llyn Alaw given that the water initially derives from Llyn Alaw and has only minimal contact with the peatland before being pumped back into the lake. Overall,

the data suggests that the dominant source of allochthonous DOC to Llyn Alaw is from run-off, rather than riverine point sources. The large volume of Llyn Alaw in relation to Llyn Cefni (at least 4 times greater) will significantly dilute large fluxes of terrestrial DOC and consequently dampen any seasonal trends. It may be possible that a significant source of DOC to Llyn Alaw derives from the lake bed due to its original construction taking place on a peatland. Although lake bed sediments are generally considered as significant sinks of organic C in boreal systems (Wachenfeldt, *et al.* 2008), this may be an important source in Llyn Alaw due to the lake's shallow depth which could allow wind-induced mixing to cause upwelling of organic C from the lake bed.

Perhaps the greatest influence on the seasonal dynamics of biogeochemical properties of freshwater lakes is fluctuating weather conditions, and there is ample evidence of its influence in the data obtained, particularly for Llyn Cefni. Rainfall is known to highly influence the amount of DOM in lakes due to increased allochthonous input associated with run-off (Li *et al.*, 2008). Therefore, it would be expected that an increase to the total amount of DOM in Llyn Cefni would occur soon after a rainfall event. This is evident in the seasonal trend for Llyn Cefni, with the three peaks in DOC concentration coinciding, but not correlating, with high rainfall totals in the days prior to sampling. The lack of a significant correlation may be due to the seasonal variability of the availability of DOC that can potentially be released from soils during rainfall. As highlighted on figure 2.8, there are two distinct regions, one of high DOC and intermediate rainfall (Group 1), and one of intermediate DOC and high rainfall (Group 2). The former is for October 2005 and 2006 and represents the typical autumn-time pulse for temperate lakes. The latter is for January and March 2006, demonstrating that even when rainfall is high, there is less potentially leachable DOC in catchment soils at this time of the year. This can be ascribed to the seasonal variation of decomposition processes occurring with the soil; during late summer and early autumn, soil temperatures and consequently microbial and enzymic organic matter decomposition are at their highest (Tate, 1987; Kang and Freeman, 1999). Coupled with the high

input of C from senescing vegetation, this creates a large pool of potentially leachable C in soils during this time of the year. During the winter, this pool of relatively labile C has been depleted, and there is less available to wash out of the soil (Freeman *et al.*, 2001*a*).

Periods of low rainfall lead to a reduction in the flux of terrestrially-derived DOC to lakes and there is evidence of this for Llyn Cefni. The period May to August 2006 was notably dry, with the total volume of rainfall recorded at the lake approximately half the long-term average. As a result, the flux of DOC from both Afon Erddreiniog and Afon Cefni did not exceed 0.9 g/s, over ten times less than the flux recorded after a rainfall event. This offers a reason for why the concentration of DOC in Llyn Cefni in the summer was two-thirds lower than during the autumn. During 2007, Llyn Alaw had a higher summertime concentration of DOC than Llyn Cefni in 2006. The summer of 2007 was much cooler and wetter than 2006; this will have increased the allochthonous input of DOC to Llyn Alaw during 2007 and suppressed decomposition of existing DOC within the lake. The sunny weather and high lake water temperature at Llyn Cefni during the summer of 2006 led to the formation of extensive toxic algal blooms. This can be seen in the measured concentrations of nitrate and phosphate, which both become undetectable in August 2006, an indication of extreme eutrophy (Wetzel, 2001). In terms of DOC, it has been described by Tittle and Kamjunke (2004) how a mixotrophic algal bloom may initially deplete DOC concentrations during early summer as the bloom develops. However, during late summer the algae become a source of DOC as the blooms die away due to a lack of nutrients and falling temperatures and sunlight levels. In the case of Llyn Cefni, this may have provided an additional source of DOC during autumn 2006, supplementing the autumn pulse of terrestrially-derived DOC. The data for nitrate in Afon Erddreiniog also confirms the trend reported by Jones (2006); this stream contributes more nitrate to Llyn Cefni than Afon Cefni, probably due to greater use of fertiliser in the fields within the stream's catchment. For Llyn Alaw, the depletion of nitrate during the summer also suggests the growth of algae. However, data from Dwr Cymru Welsh Water indicate that there was no growth of

significant algal blooms at the lake during 2007. This may have been due to phosphate limitation; none was recorded for the lake or the two inflowing streams and P has recently been identified as the key nutrient for algal growth (Schindler *et al.*, 2008).

Although there was little variation in DOC concentrations in Llyn Alaw throughout the year, fractionation revealed marked seasonal variations in DOC quality. The seasons showing the greatest contrasts in DOC characteristics were summer and autumn. During the summer the hydrophilic (76 %) fraction dominated, with relatively little hydrophobic material (18 %), however, in the autumn the opposite was true, with 14 % hydrophilic and 55 % hydrophobic. These findings appear to corroborate the suggestions of allochthonous and autochthonous influences on the lake. The autumn flush of terrestrially-derived DOC will contain high-molecular weight compounds leached from soil and consequently will contain mostly hydrophobic compounds (Imai *et al.*, 2001). The proliferation of algal blooms during the summer will lead to a large input of lower molecular weight, hydrophilic structures (Kritzberg *et al.*, 2006). A reduction in the percentage of hydrophobic material in the lake would occur during the winter and spring as the supply of senescing sources of C within the catchment diminishes, whilst the formation of algal blooms would be expected to proceed rapidly during spring as water temperature and sunlight increase. The winter and spring seasons appear to act as transitional stages between these opposing scenarios.

The formation of THMs correlated strongly with the lake water temperature at both Llyn Cefni and Alaw, which agrees with previous studies reporting that the formation of DBPs is temperature dependent (Rodrigues *et al.*, 2007). At Llyn Cefni, the formation of THMs correlated slightly more strongly with the raw water DOC concentration, with the peaks in THMs in October 2005 and 2006 coinciding with the highest DOC concentrations. This emphasises the importance of the autumn-time pulse in DOC and the influence of peatlands in negatively affecting the quality of drinking water through leaching excess C to freshwaters. For Llyn Alaw,

the lake water DOC concentration did not correlate with the formation of THMs. This emphasises the lack of significant fluxes of terrestrially derived DOC to this lake and suggests that understanding processes affecting DOC dynamics within this lake is not important for minimising THM formation. Due to the thorough treatment processes employed at both treatment works, THM concentrations were always below the current limit of 100 μg L^{-1} set in the UK (DWI, 1998).

The seasonal variations of treated water DOC concentrations mirrored the raw water DOC for both lakes. At times of high lake water DOC, it would therefore be expected that, in addition to THMs, high concentrations of DOC would also exist in the final water. This may lead to opportunistic bacterial re-growth of organisms such as *Serratia fonticolain* in the distribution system and may contribute to degradation of the drinking water through the occurrence of taste and discolouration problems (Niquette *et al.*, 2001). The removal of DOC was generally much more efficient at the Cefni than the Alaw water treatment works and this is due to the use of different treatment regimes. Whilst both treatment works use coagulation as a means of removing DOC there is some variation in the actual application and removal of the flocs. Llyn Cefni treatment work employs dissolved air floatation, whereby small bubbles of air are pushed up through the water causing the flocs to rise up the surface before being scrapped off by mechanical means. Conversely Llyn Alaw adopts a method of clarification, whereby the flocs are left to settle to the bottom, a method commonly adopted to treat river sources as it is better adapted to the flashy nature of rivers. However, this study has demonstrated that Llyn Alaw shows very little seasonal variation, therefore clarification is not the most appropriate.

2.6 Conclusions

This study demonstrates the influence of climate, lake size and catchment properties on biogeochemical characteristics of two similar lowland, eutrophic lakes in Wales, UK. The concentration of DOC in the two major inflowing streams to Llyn Cefni correlated with the DOC concentration in the lake, suggesting they are major point-sources of DOC. Conversely, neither of the major inflows to Llyn Alaw are influential sources of DOC. It was demonstrated that the presence of a peatland within the catchment of Llyn Cefni led to a large autumn-time flux of DOC to the lake; the absence of a peatland within the catchment of Llyn Alaw meant there is a low input of allochthonous DOC to this reservoir. The concentrations of THMs correlated significantly with lake water temperature for both sites and for Llyn Cefni the formation of THMs also correlated with the lake water DOC, suggesting that the inflowing streams are important sources of THM precursors. Llyn Cefni frequently experiences toxic algal blooms, which together with undetectable concentrations of nitrate and phosphate during the summer of 2006 suggests extreme eutrophy. The absence of P in the catchment of Llyn Alaw may be why this lake does not experience algal growths to a similar extent.

Chapter 3

Identification of disinfection by-product precursors and their potential to form trihalomethanes in drinking water

3.1 Abstract

The presence of dissolved organic carbon (DOC) creates problems during water treatment processes due to the formation of potentially harmful disinfection by-products (DBPs). Water treatment companies need to better understand the sources of DOC to reservoirs in order to understand seasonal variations in its characteristics and implications for its 'treatability'. ^{1}H-NMR spectroscopy has shown promise in gaining basic structural information of natural DOC and for fingerprinting signatures from different sources. In this study, DOC isolated from a eutrophic reservoir and its two major inflows was characterised. The signatures for the streams and lakes correlated significantly with the greatest correlation occurring in December, $R^2 = 1.00$ between Afon Cefni and Llyn Cefni and $R^2 = 0.95$ between Afon Alaw and Llyn Alaw, indicating a strong flux of terrestrial DOC into the lake, except when DOC derived from autochthonous production was recorded in the lake. The inflow draining a major peatland was also shown to be an important source of aromatic compounds for three of the four months, which consequently has implications for the formation of DBPs.

3.2 Introduction

Dissolved organic carbon (DOC) is a large and dynamic global reservoir of carbon and it plays a critical role in many aspects of biogeochemical cycling. Its ubiquitous presence in freshwaters has important implications for water treatment processes and ultimately affects the quality of drinking water. Disinfectants used to remove potentially harmful microorganisms contain highly reactive molecules that can generate disinfection by-products (DBPs) upon reaction with compounds of DOC. Some DBP species have been identified as carcinogens (Villanueva *et al.*, 2006) and some have been associated with reproductive problems (Nieuwenhuijsen *et al.*, 2000). The most effective way of reducing the formation of DBPs and, concurrently, treatment costs is to minimise DOC concentrations in source waters. To achieve this, water treatment works need to gain a greater understanding of the origins of DOC within the drinking water reservoir's watershed, its structural attributes and the characteristics that affect its 'treatability' and reactivity.

Freshwater DOC is composed of a mixture of complex organic compounds and determining its chemical structure can be a time-consuming process. It is often separated into broad groups based on hydrophobicity, aromaticity or molecular weight. Humic substances, which constitute approximately 25–50% of DOC typically found in freshwaters (Thurman, 1985), are large, hydrophobic, aromatic compounds and have been identified as the principal precursors in the formation of DBPs (Liang *et al.*, 2003).

The basic structural characteristics of DOC may be characterised without the need for isolation and separation of DOC by spectroscopic analysis, such as ultraviolet/visible absorbance, infrared and fluorescence spectroscopy, and nuclear magnetic resonance (NMR). NMR is one of the most promising techniques, as it provides a useful recognition of the chemical structure within DOC because its signal is directly related to specific chemical functional groups (Lu, 2004). It has been argued that NMR has proved the most useful technique for investigating the structure of DOC (Lam and Simpson, 2008). The method of choice for rapid screening of dilute samples

of DOC is ^{1}H-NMR (Cook, 2004). It is used for qualitatively estimating the relative abundance of various functional groups and especially for characterising their aromatic and aliphatic components. Typical ^{1}H-NMR spectra for humic substances are complex and typified by broad signals with fine structures related to specific methylene, methyne and aromatic hydrogens (Cardoza, 2004). The chemical composition and structural nature of DOC varies with its origin and usually carries structural signatures related to the characteristics of its parental material. These characteristics may be regarded as 'fingerprints' of DOC, reflecting their sources and formation history, thus potentially enabling identification of one source of DOC from another (Lu, 2004). Fingerprinting DOC from different sources is where ^{1}H-NMR has become particularly useful (Ma *et al.*, 2001; Lu *et al.*, 2004).

This chapter describes how ^{1}H-NMR spectroscopy was used to characterise DOC isolated from two streams flowing into a eutrophic drinking water reservoir in north Wales. The aim was to identify if any streams made dominant contributions of DBP precursor compounds. Water samples were collected from the two principal inflows entering the reservoir, in addition to the raw lake water. ^{1}H-NMR spectra was obtained for each sampling point in conjunction with establishing their trihalomethane formation potential (THMFP). It was expected that the inflow draining from a nearby peatland would contain the greatest abundance of aromatic compounds and would consequently have the highest THM formation potential. The other inflow drains mostly agricultural land and is therefore expected to have a lower aromatic DOC content and THM formation potential.

3.3 Materials and Methods

3.3.1 Study Area

The focus of this study was Llyn Cefni, a small reservoir in the centre of the island of Anglesey, north Wales, UK. Detailed site information is presented in Chapter 2. Two inflowing streams have previously been identified as important sources of allochthonous DOC to the lake; Afon Erddreiniog to the north and Afon Cefni to the south (Jones, 2006). Afon Erddreiniog drains Cors Erddreiniog, a fen peatland north of the reservoir, whilst Afon Cefni flows through a series of agricultural fields before entering the reservoir.

3.3.2 Sample collection

Samples were taken from Llyn Cefni and the two inflowing streams during the four months from October 2005 to February 2006, to identify the structural composition of the DOC and to determine if either inflow made a more significant contribution to the potential to form THMs at Llyn Cefni. Two litres of water were collected in glassware prewashed with Milli-Q grade deionised water. All samples were filtered through sterilised 0.45 μm filter membranes and refrigerated at 4 °C in the laboratory. Daily rainfall data for the sampling period was provided by Dwr Cymru Welsh Water.

3.3.3 ^{1}H-NMR spectroscopy

DOC was analysed by ^{1}H-NMR similar to the methods described by Gillam and Wilson (1985) and Ma *et al.* (2001). DOC was isolated by freeze-drying 1 litre of the sampled water and the extract dissolved in 0.5 ml NaOD. The sample was then centrifuged at 10,000 rpm for 5 minutes and the supernatant transferred to an NMR tube. Spectra were recorded on a Bruker 500 MHz superconducting NMR spectrophotometer, each one run for 512 scans to ensure a sufficiently high signal: noise ratio. A line broadening of 0.3 Hz was applied during the data processing and the chemical shifts were integrated using the D_20 signal as a reference. The ^{1}H-NMR spectra

were quantitatively analysed based on the general chemical shift assignments used by Ma *et al.* (2001); Kim and Yu (2005), and divided into four distinct regions (Table 3.1).

Table 3.1 – ^{1}H-NMR chemical shift regions and the types of compounds detected

Region	Chemical Shift (ppm)	Nature of bound protons
I	0.0-1.6	Aliphatic methyl and methylene protons.
II	1.6-3.2	Protons of the methyl and methylene groups α to aromatic rings, protons on carbons in α position to carbonyl, carboxylic acid, ester or amino acids.
III	3.2-4.3	Protons on carbon of hydroxyl, ester and ether, and protons on methyl, methylene and methyne carbons directly bonded to oxygen and nitrogen.
IV	6.0-8.5	Protons bound to aromatic rings.

3.3.4 Trihalomethane formation potential and Specific Ultraviolet Absorbance

THM concentrations were measured using an adaptation of procedure 5710 in 'Standard Methods for the Examination of Water and Wastewater' (American Public Health Association, 1992) and the method used by Goslan (2003). Samples were chlorinated using a volume of sodium hyporchlorite solution adjusted to the amount of DOC previously measured in each sample using a Shimadzu TOC-5000. Phosphate buffer was added to the samples to ensure pH compatibility and they were left to incubate for 7 days at 25°C. THM concentrations were then determined using a Hewlett Packard 5890 Gas Chromatograph fitted with a Restek MXT-1 crossband ® 10090 dimethyl polysiloxane column (30 m length, 0.53 mm i.d., 7.0 μm d.f.). The concentrations of THMs were calculated using a standard curve with concentrations of 1, 10, 100 and 1,000 μg L^{-1} of the four individual THMs ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$).

Absorbance of each sample at 254 nm was measured on a Camspec M330 UV-Visible spectrophotometer and used with DOC concentrations to determine values of Specific Ultraviolet Absorbance (SUVA).

3.3.5 Statistical analysis

A series of correlations were performed on the datasets using Pearson correlation in SPSS (version 11). The correlations considered the interactions between the two significant inflows into the lake, Afon Erddreiniog and Afon Cefni, and DOC concentrations and the effect rainfall had on the abundance of region IV DOC in the lake.

3.4 Results

3.4.1 ^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra obtained for all samples were similar in their overall shape to previously published spectra of DOC (e.g. Figure 3.1), with broad peaks in the upfield region representing complex mixtures of organic structures interspersed with sharp peaks indicating specific functional groups (Ma *et al.*, 2001). The large peak at 4.9 ppm is the HOD peak from the D_2O solvent, whilst that at 8.4 ppm is formate, a decomposition product of humic acids dissolved in NaOH (Gillam and Wilson, 1985). For all samples and for each month, the dominant fraction of DOC was found in region I (Figure 3.2). Some subtle differences between the samples were observed for the other regions of the spectra, however. For three out of the four months, Afon Erddreiniog had the highest composition of aromatic compounds (region IV), especially for the samples taken in October and December. In these two months this stream also had the highest composition of region II compounds, which can also indicate aromaticity. When comparing the two streams, in addition to the greater aromaticity of Afon Erddreiniog, the stream generally contained less DOC compounds from region I than the DOC isolated from Afon Cefni.

For three out of the four months, compounds resonating in region III were most commonly found in the sample taken from Llyn Cefni. The contribution of region III compounds for Llyn Cefni was particularly high in October 2005. Another notable aspect of the spectra was the high percentage of region I compounds for the two streams in February 2006.

3.4.2 SUVA and THMFP values

Averaged over the four months of sampling, DOC in Afon Erddreiniog had the largest contribution to the SUVA of the three samples, followed by Afon Cefni and Llyn Cefni (Figure 3.3). The same trend was recorded for THMFP values (Figure 3.4), with the difference between Afon Erddreiniog and Llyn Cefni greater than that for SUVA.

3.4.3 Correlations of DOC compositions for Llyn Cefni with Afon Erddreiniog and Afon Cefni

When comparing the structure of the DOC isolated from Llyn Cefni with that from the two inflowing streams, significant correlations ($p<0.05$) were observed for three out of the four months for Afon Cefni and two of the four for Afon Erddreiniog (Figure 3.5). The strongest correlation between the structures of DOC isolated from Afon Cefni and Llyn Cefni occurred in December 2005 ($R^2=1.00$), followed by February 2006 ($R^2=0.94$) and January 2006 ($R^2=0.92$). A correlation for October produced a strong correlation ($R^2=0.64$) however it was not significant. The strongest correlation between the DOC of Afon Erddreiniog and Llyn Cefni also occurred in December ($R^2=0.95$) followed by February ($R^2=0.92$). The correlations in October ($R^2=0.90$) and January ($R^2=0.86$) were strong but not significant at $p>0.05$.

3.4.4 Correlations of total rainfall and percentage abundance of region IV DOC.

A strong, positive correlation ($R^2=0.74$) was observed between total rainfall 4 days prior to sampling and the percentage of region IV DOC for Llyn Cefni, however it was not significant at $p>0.05$ (Figure 3.6). Similar correlations for DOC from Afon Erddreiniog and Afon Cefni were weak and insignificant. Rainfall 4 days prior to sampling was considered as statistical tests showed this number of days correlated best with DOC concentrations.

3.4.5 Correlations of DOC structures with THMFP values

No significant correlations were recorded between any of the DOC regions and the sample's overall THMFP value (Table 3.2). The only notable correlation was for Afon Erddreiniog THMFP vs. region II DOC, which was very strong ($R^2=0.88$). It is worth highlighting that the correlations of THMFP values with region IV DOC produced only very weak correlations for all samples.

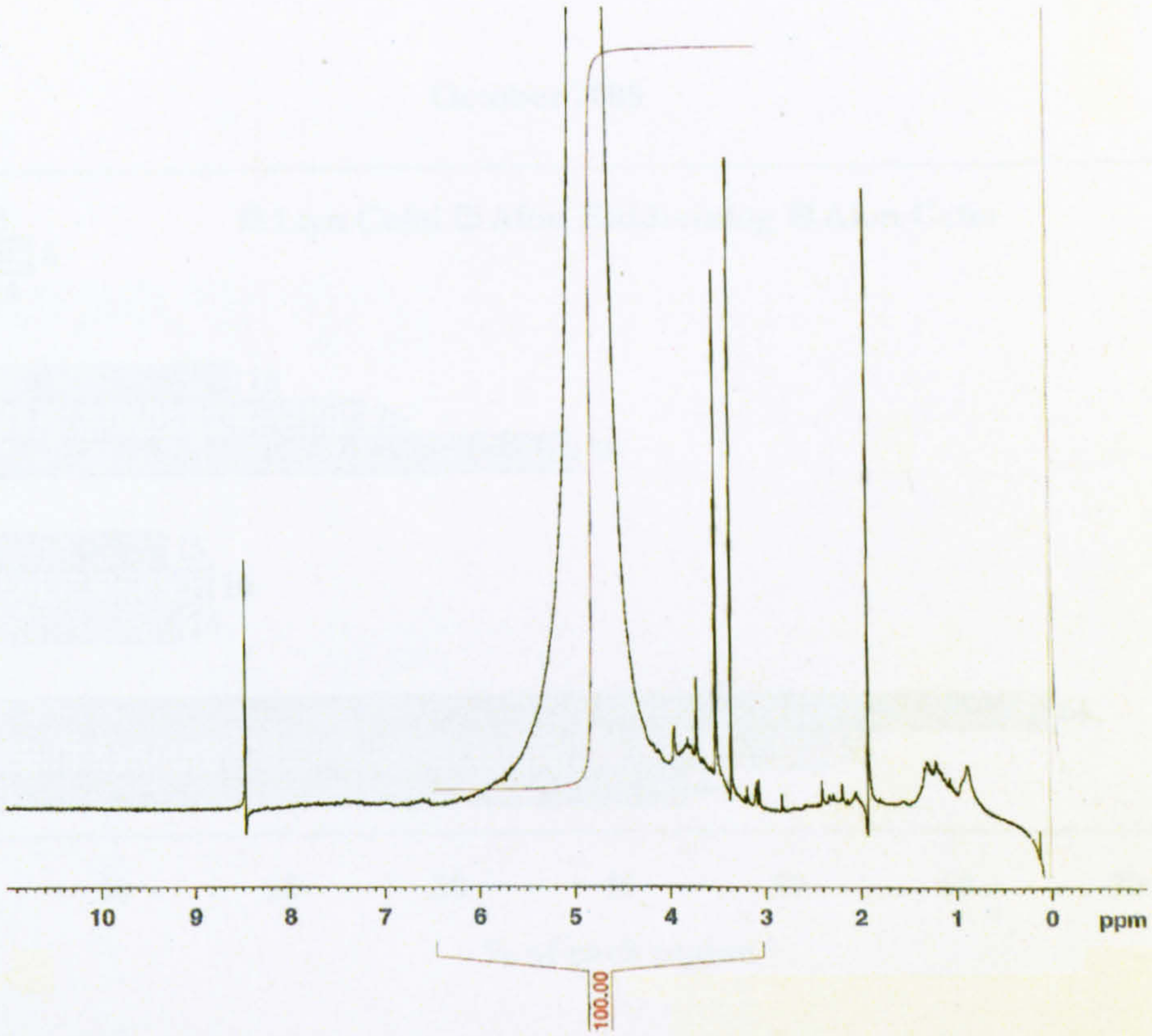
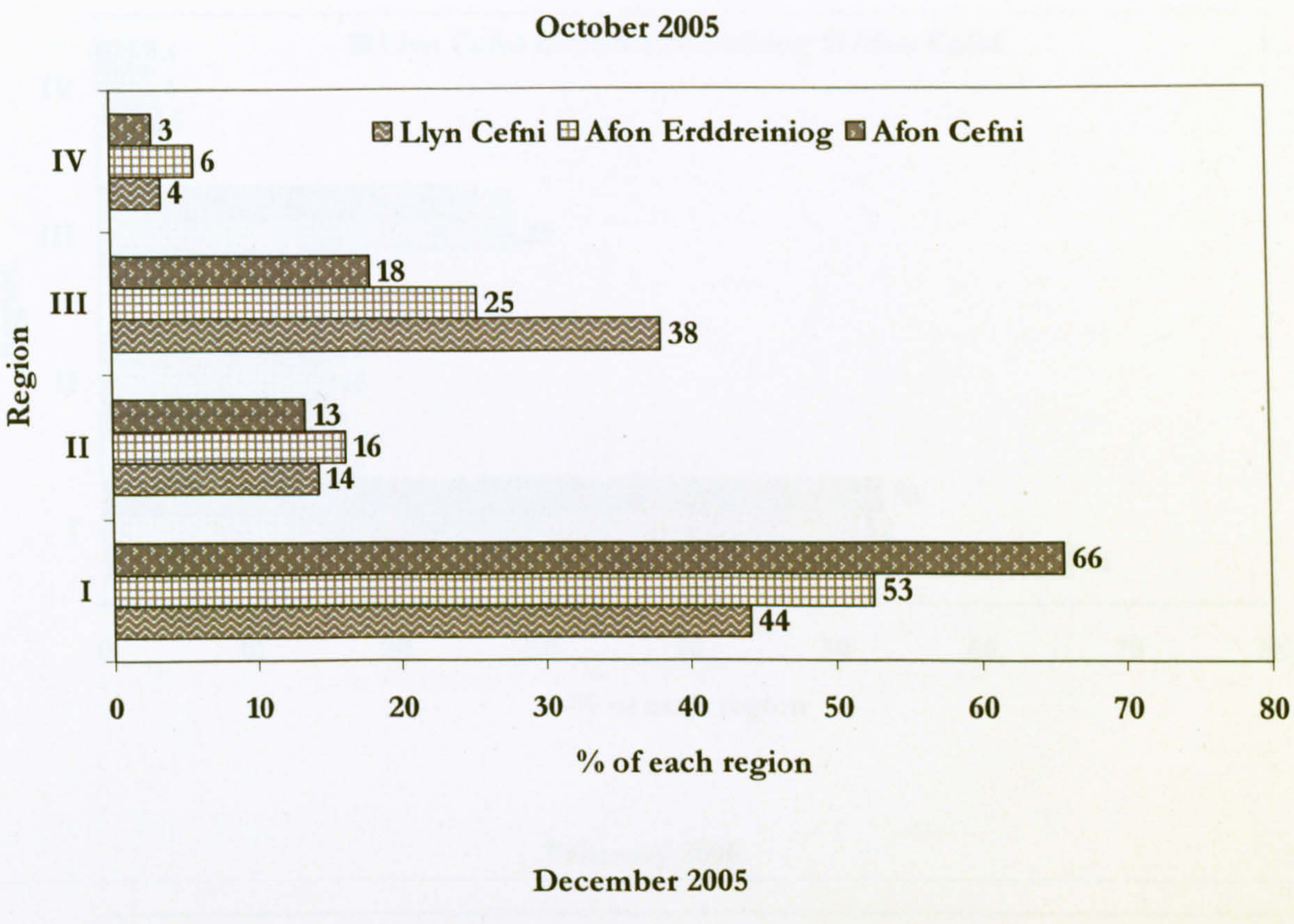
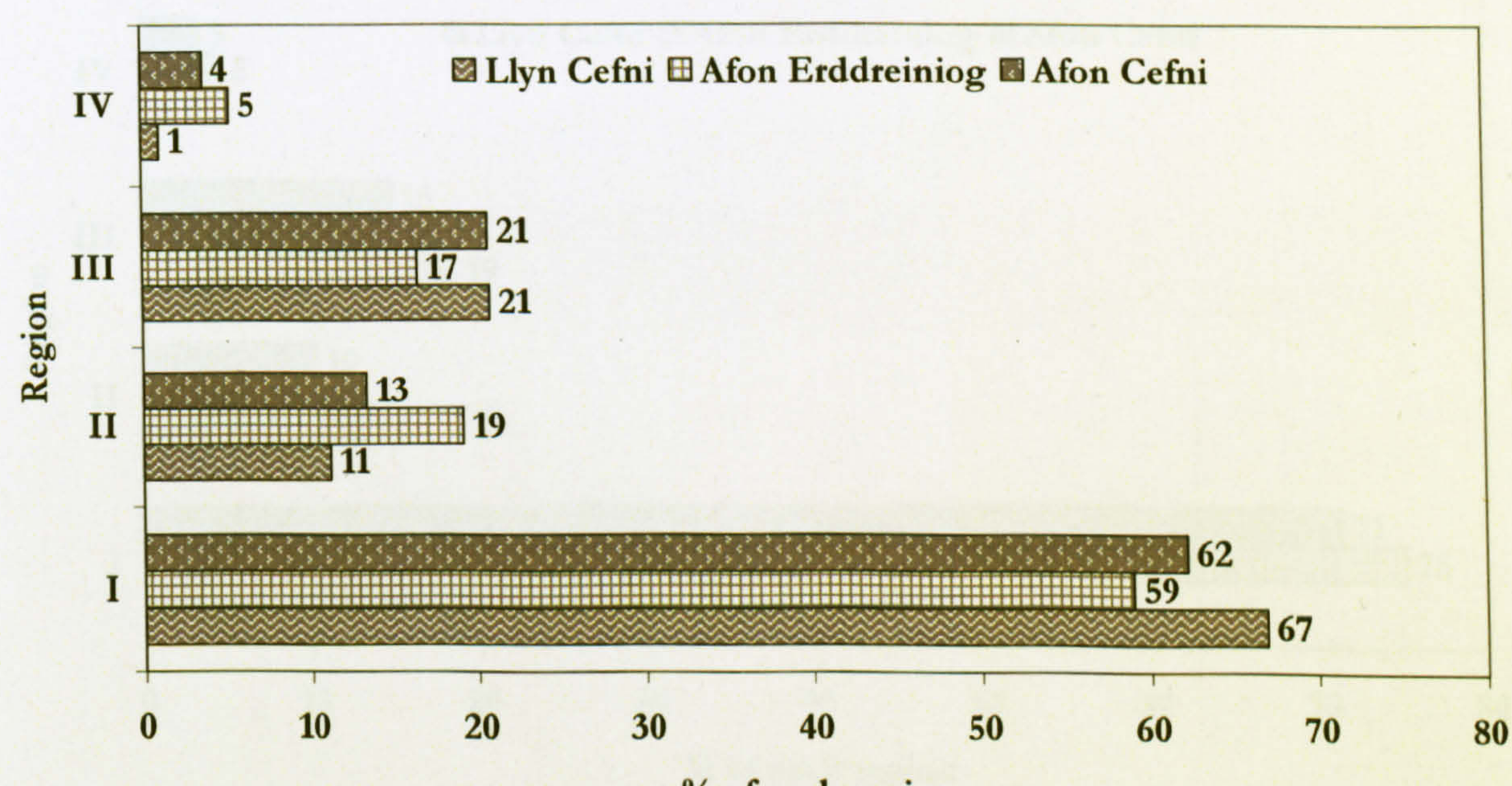


Figure 3.1 – ^{1}H-NMR spectra of DOC extracted from Llyn Cefni in October 2005

Table 3.2 – Correlations between sample THMFP values and the ^{1}H-NMR determined DOC structures

		I	II	III	IV
Llyn Cefni	Correlation	0.001	-0.311	0.754	-0.289
	Significance	*0.999*	*0.611*	*0.141*	*0.637*
Afon Erddreiniog	Correlation	-0.914	0.935	0.569	0.397
	Significance	*0.086*	*0.065*	*0.431*	*0.603*
Afon Cefni	Correlation	-0.722	0.795	0.710	0.157
	Significance	*0.278*	*0.205*	*0.290*	*0.843*


October 2005
Llyn Cefni
Afon Erddreiniog
Afon Cefni
Region
IV
III
II
I
3
6
4
18
25
38
13
16
14
66
53
44
0
10
20
30
40
50
60
70
80
% of each region


December 2005
Llyn Cefni
Afon Erddreiniog
Afon Cefni
Region
IV
III
II
I
4
5
1
21
17
21
13
19
11
62
59
67
0
10
20
30
40
50
60
70
80
% of each region

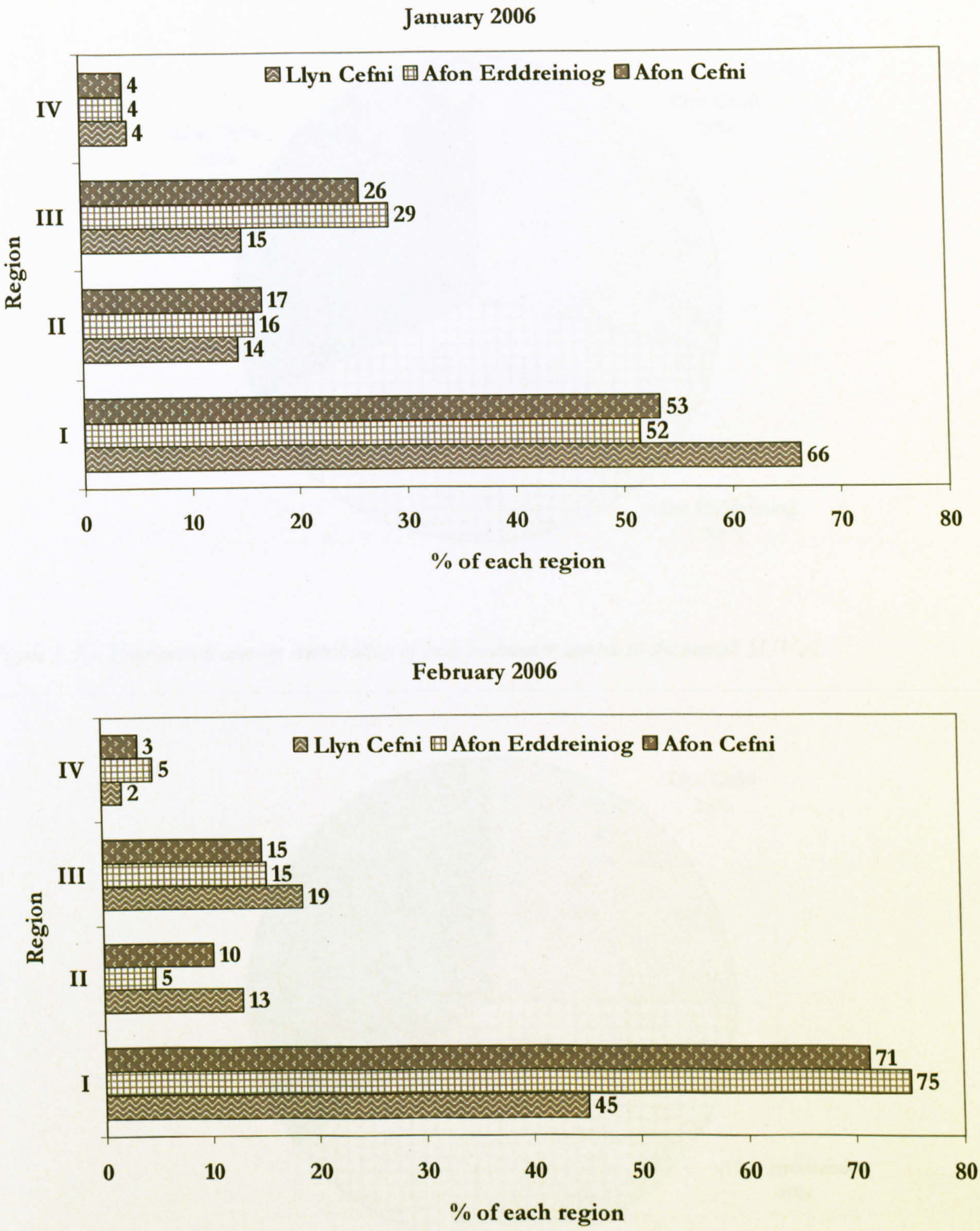


Figure 3.2 – Structural composition of the DOC isolated from the three freshwater samples, as analysed by ^{1}H-NMR *spectroscopy. Values shown indicate the percentage of DOC found in each region.*

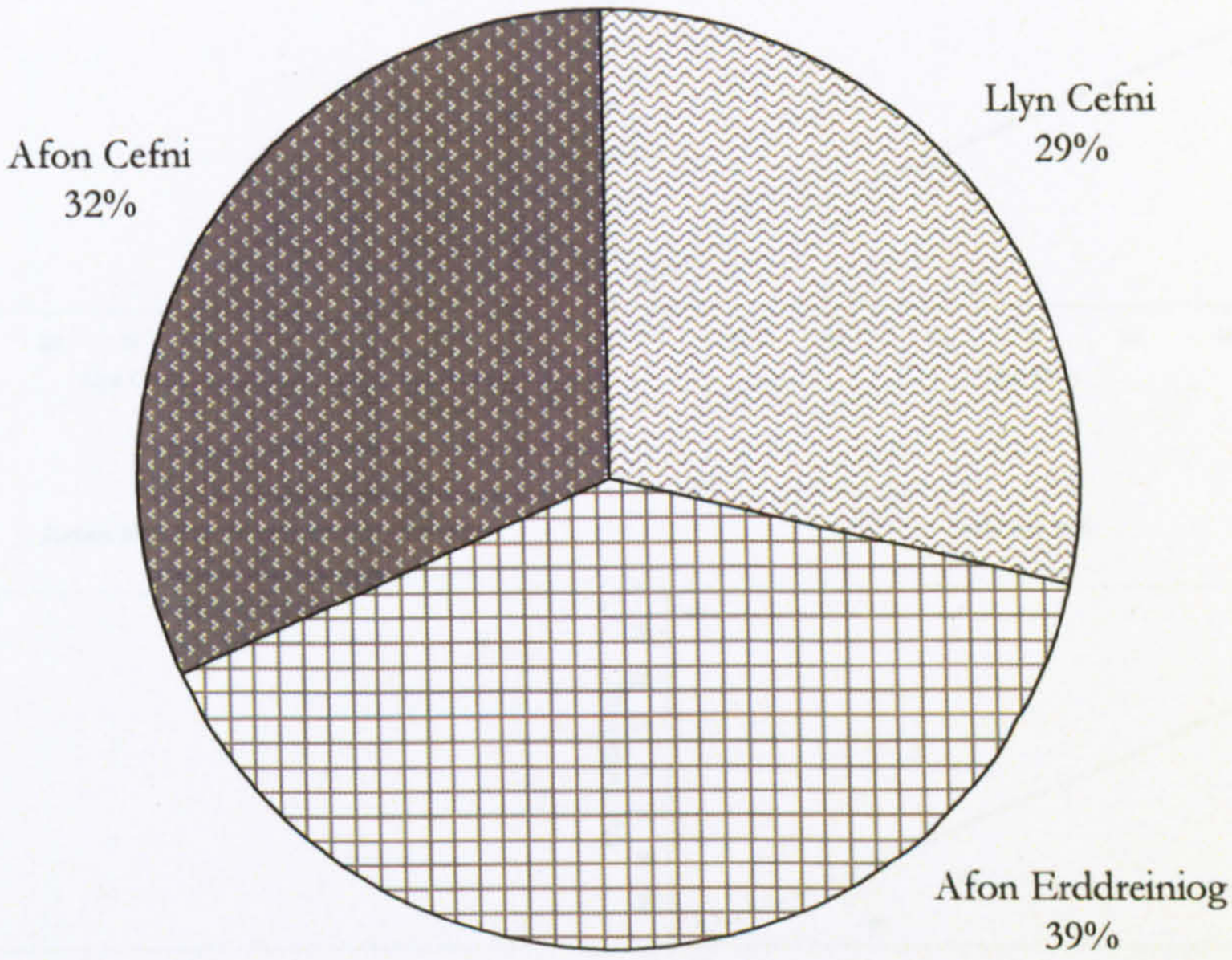


Figure 3.3 – Four-month average contribution of each freshwater sample to the overall SUVA

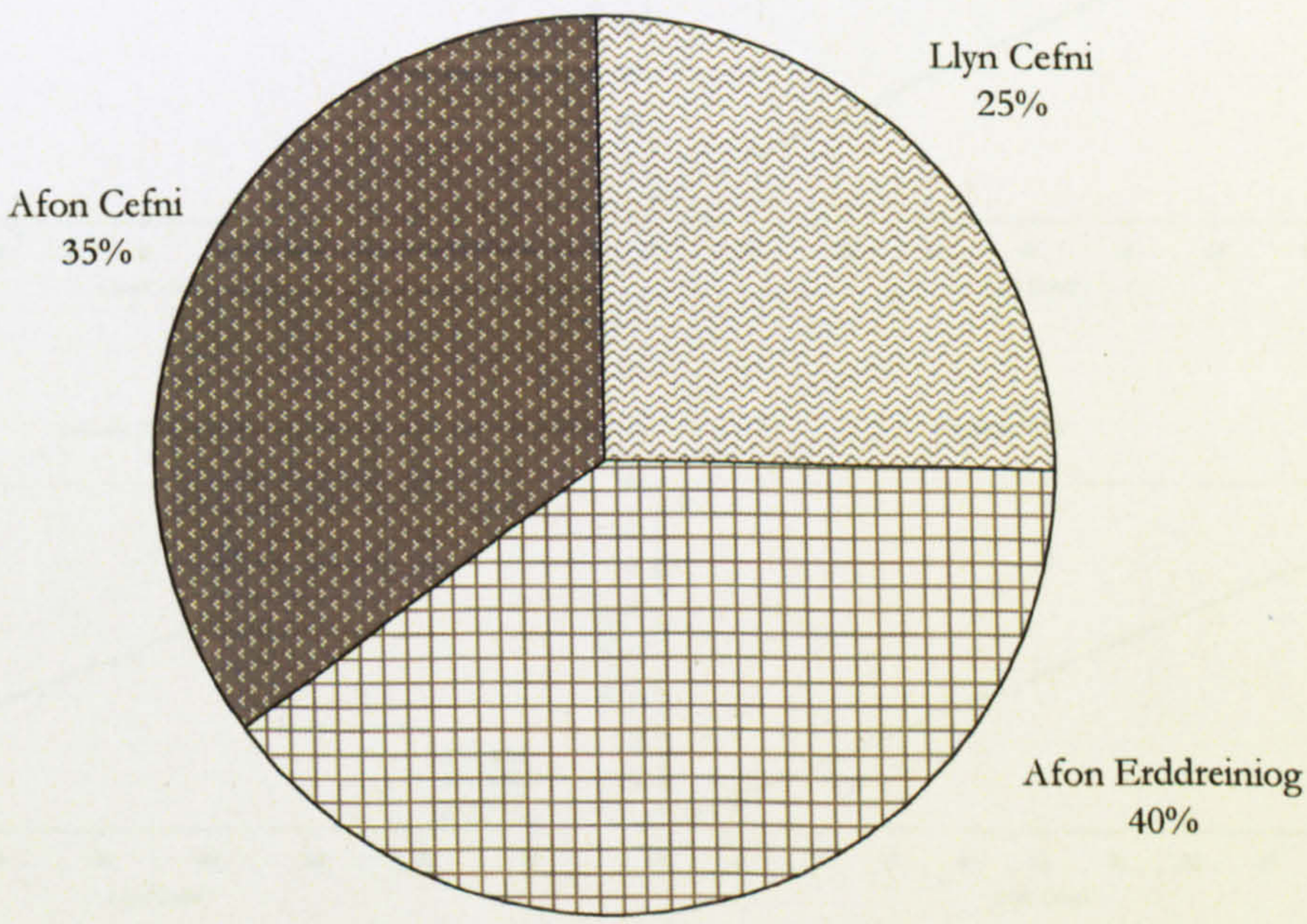


Figure 3.4 – Four-month average contribution of each freshwater sample to the overall THMFP

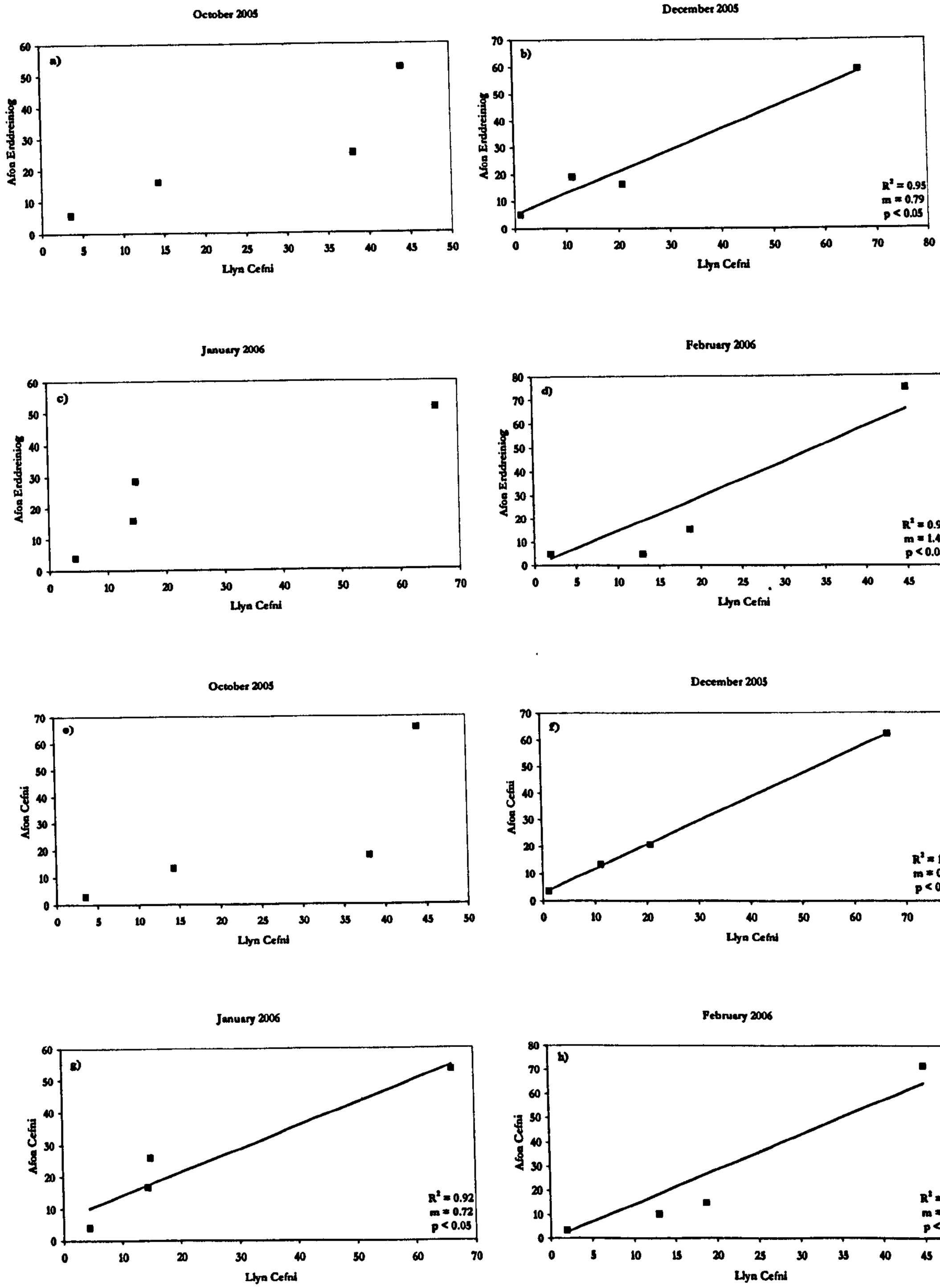
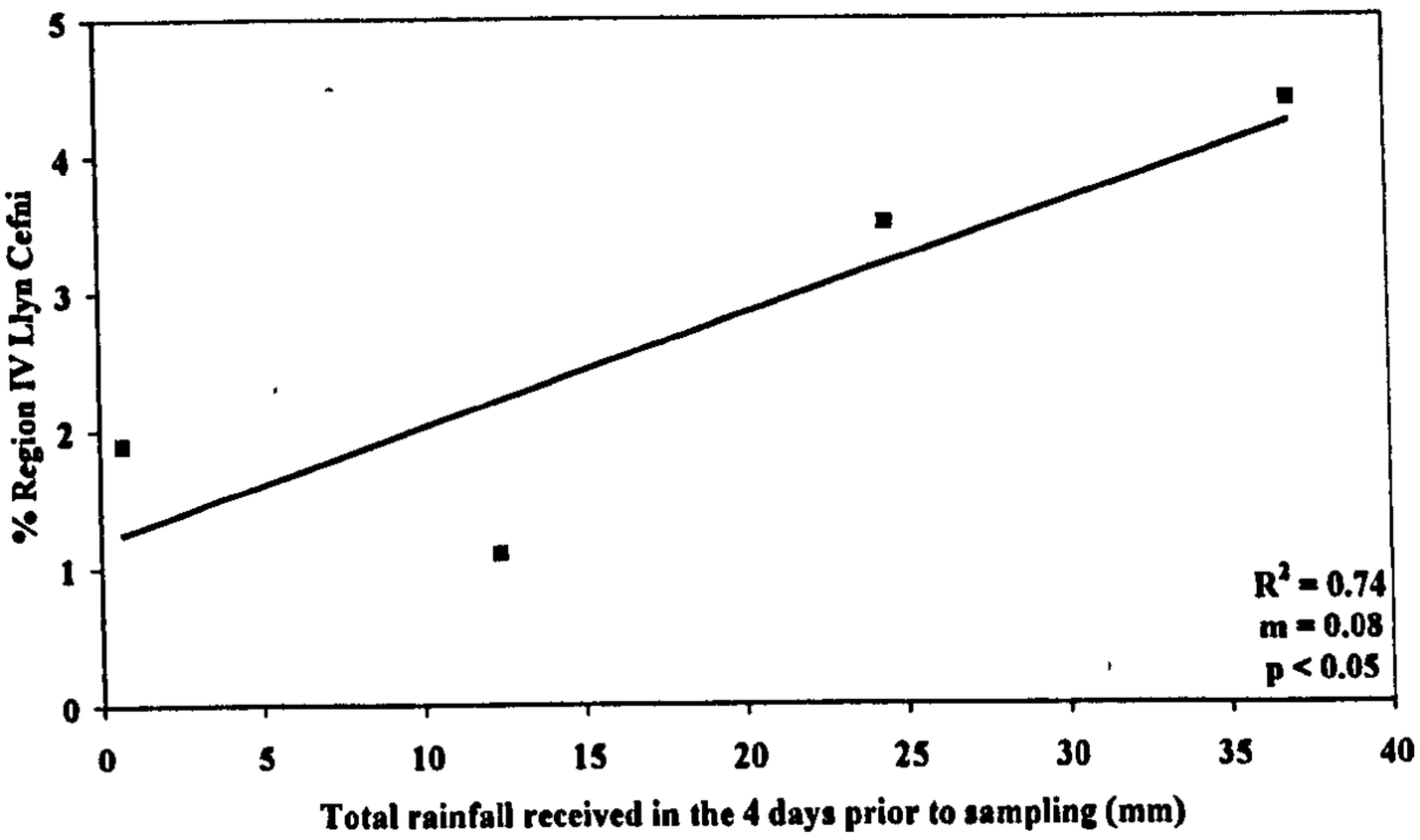
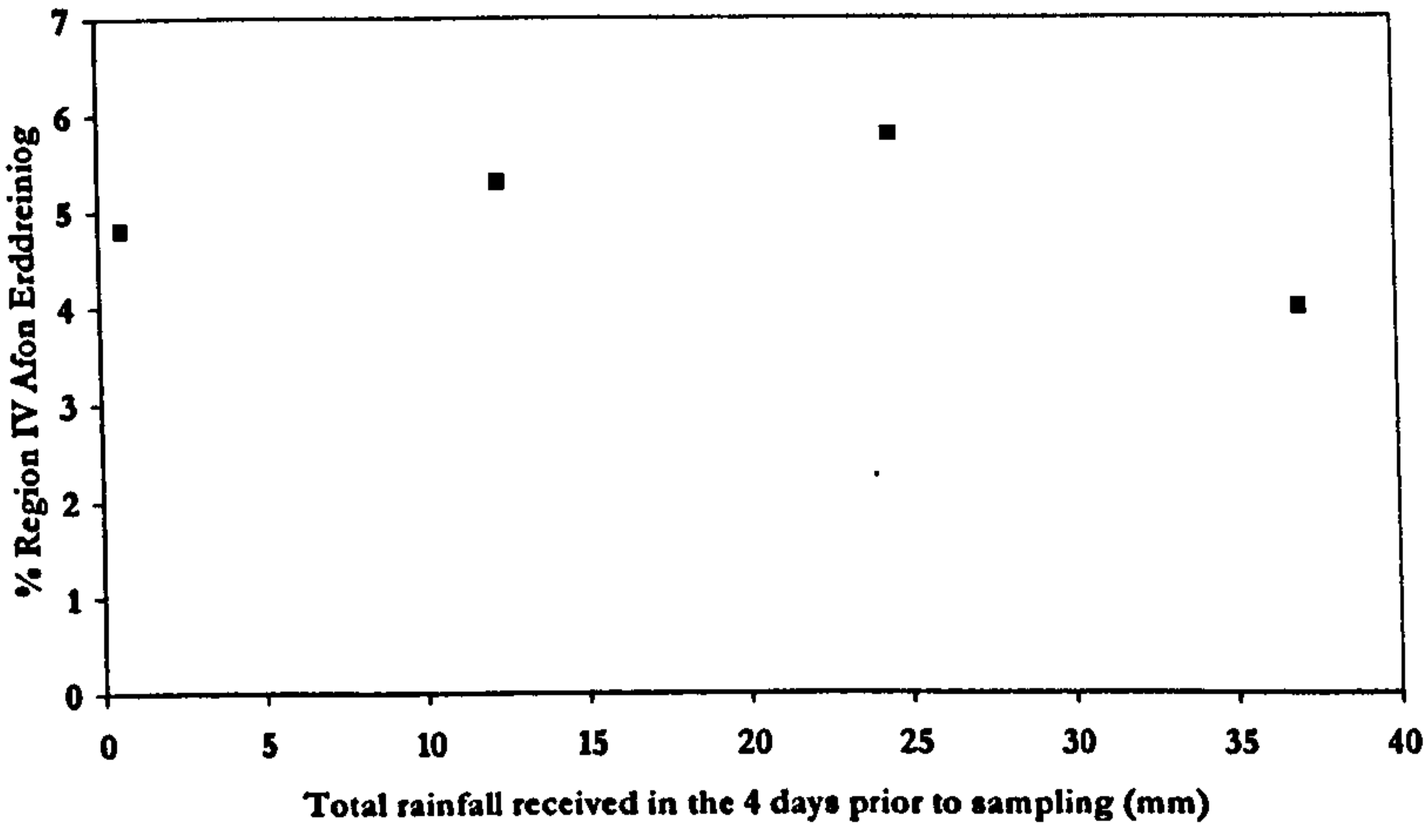


Figure 3.5 - Relationship between the ^{1}H-NMR *determined DOC structures of Llyn Cefni and Afon Erddreiniog (a-d) and Afon Cefni (e-h) for the four sampled months. Significant linear relationships are indicated by slope lines and* R^2 *values are presented in the data.*





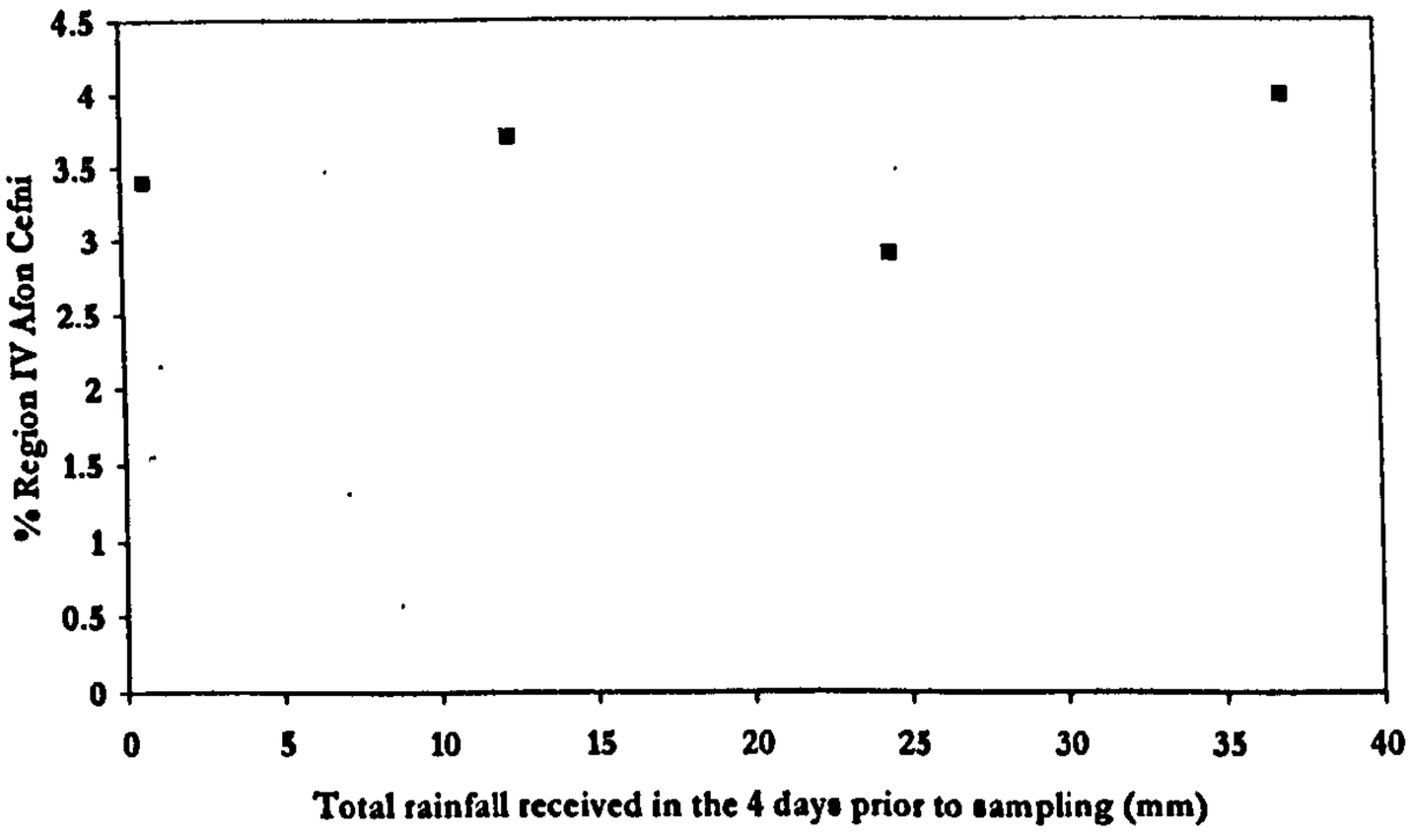


Figure 3.6 - Relationship between the percentages of ^{1}H-NMR determined region IV DOC in a) Llyn Cefni, b) Afon Erddreiniog and c) Afon Cefni and rainfall 4 days prior to sampling for the 4 months of sampling. No significant relationships were found, but the correlation for Llyn Cefni was strong.

3.5 Discussion

According to ^{1}H-NMR spectroscopy, the most commonly occurring DOC compounds for all samples during the four months were those found in region I i.e. mostly aliphatic. This is consistent with other studies of the ^{1}H-NMR characterisation of DOC, which show compounds typically contain a molecular skeleton dominated by aliphatics (Malcolm, 1990; Lu *et al.*, 2004). In terms of water quality and the potential to form disinfection by-products, the aromatic region is potentially of most interest given the numerous studies that have shown compounds with such structures to be significant DBP precursors (e.g. Reckhow *et al.*, 1990; Singer, 1999; Kim and Yu, 2005; Fabris *et al.*, 2008). In this study, the recorded percentages of aromatic compounds in the samples analysed were of a similar magnitude to those measured by Hatcher *et al.* (1980) for aquatic humic acids and for DOC isolated from rainwater (Santos *et al.*, 2009), but lower than that recorded for standard humic substances by Kim *et al.(* 2006b). The latter may have been due to the use of a greater number of scans during the spectroscopic analysis. In all but one month, Afon Erddreiniog was found to have the highest percentage of aromatic compounds. It also had the highest SUVA and THMFP of the three samples; this reflects the influence of the peatland, Cors Erddreiniog, at the source of the stream. Chapter 2 previously demonstrated that this peatland is a significant source of DOC to Llyn Cefni via Afon Erddreiniog, and, given that the DOC is more aromatic and higher molecular weight than that isolated from Afon Cefni, indicates the peatland is an important source of THM precursors. In addition to region IV, DOC from Afon Erddreiniog also had the highest percentage of DOC compounds from region II during October and December and a strong correlation was recorded between the abundance of DOC from this region and the THMFP of Afon Erddreiniog. The resonance in region II can be associated with carbons adjacent to aromatic, double bonds or carbonyls (Grasso *et al.*, 1990); therefore this may support Afon Erddreiniog as a source of aromatic, peatland-derived carbon compounds.

Although aromatics have been implicated as the principal structures of THM precursor compounds, we recorded no significant correlations between the percentage abundance of region IV DOC and the THMFP of any of the three samples. This represents the inherent difficulty of characterising DOC accurately, particularly the compounds which are large in molecular size and weight and which contain molecules that can react readily with chlorine and highlights the lack of knowledge concerning the specific chemical structures associated with DBP formation following chlorination of natural waters (Hua and Reckhow, 2007).

A particular weakness of ^{1}H-NMR spectroscopy may be why no significant correlations were observed. The aromatic region was consistently the lowest contributor to the structure of the DOC for all the samples analysed; however, it is likely that the recorded percentages are somewhat of an underestimation. Specifically, a masking effect can occur, where sites on the aromatic structure can be substituted by elements other than hydrogen, for example halogens or larger functional groups (Grasso *et al.*, 1990). This can mean large portions of the aromatics contained within a sample do not resonate and remain invisible using this method. To allow better characterisation of the aromatic region, analysis by ^{13}C-NMR spectroscopy is recommended (Cordoza, 2004), although it is often unsuitable due to the extremely long run times involved to obtain meaningful spectra. Despite this limitation of ^{1}H-NMR, is extremely useful in gaining basic information on the general structure of DOC and comparing the signatures of samples from different sources.

The highest concentration of aromatic compounds in Afon Erddreiniog was recorded in October and is typical of the autumn flush of DOC into freshwaters that typically occurs when peatlands are present within the catchment (Scott *et al.*, 1998; Mash *et al.*, 2004). Chapter 2 described two distinct periods of potential DOC release from Cors Erddreiniog during periods of heavy rainfall; a high quantity of DOC leached in the autumn during moderate rainfall and a lower leaching of DOC during the winter, even during high rainfall. Data from this study is

consistent with that observation, as the Afon Erddreiniog DOC composition had 2% less aromaticity in January 2006 compared to October 2005, despite the much higher rainfall experienced in January. The occurrence of vegetation senescence within the catchments of freshwaters during the autumn mobilises a large reservoir of coloured, aromatic DOC, and the principal transport route for this to water bodies is rainfall. It was interesting that a strong correlation, albeit insignificant at $p>0.05$, was recorded between the total amount of rainfall four days prior to sampling and the percentage of region IV DOC in Llyn Cefni, but not for the two inflowing streams. Figure 3.6 appears to suggest that even during low rainfall the amount of aromatic compounds in the streams is similar to when rainfall is high, but during times of low rainfall, there are much less aromatic compounds in the lake than during high rainfall (approximately -50%). As aromatic DOC is recalcitrant and not readily decomposed in lakes (Wetzel, 2001), it is unlikely that the aromatic DOC in Llyn Cefni will have been decomposed, so this cannot explain the observed reduction during times of low flow. It is likely to be simply due to the reduced flux of terrestrially-derived organic matter into the lake during low rainfall. In a shallow, eutrophic lake such as Llyn Cefni, a reduced flux may be insufficient to maintain the heightened terrestrial DOC signature induced in the lake following heavy rainfall against a background DOC composition dominated by autochthonous compounds. DOC in rivers and streams tends to be much more allochthonous (Imai *et al.*, 2001), as it flows directly from soils and the continual mixing minimises algal growth. This may explain the difference in the percentage of aromatic DOC in the lake and the streams flowing into it with changes in rainfall.

Another important observation from the ^{1}H-NMR spectra was that during October 2005 there was a much larger presence of region III DOC in Llyn Cefni than for any other month of sample. Llyn Cefni is a eutrophic lake and often experiences algal bloom formations during the summer months; 2005 was no exception. The presence of a significant amount of region III DOC in October appears to coincide with the senescence phase of the algal bloom cycle. Grasso *et al.* (1990), Biber *et al.* (1996) and Giroldo *et al.* (2007) described how DOC released by

phytoplanktonic organisms is principally composed of amino acids, peptides, proteins and carbohydrates. These types of compounds are typically found in region III in ^{1}H-NMR spectra. Therefore, DOC deriving from senescing algal blooms significantly impacts upon the total DOC pool in Llyn Cefni during this period. This type of DOC is relatively labile, explaining the lower abundance of region III from December onwards. Llyn Cefni had the lowest SUVA and THMFP of the three samples. This probably reflects the greater contribution of autochthonous DOC; it is well known that this type of DOC is lower in molecular weight and aromaticity than terrestrially-derived DOC and studies have shown it to have a much lower THMFP per unit carbon (Nguyen *et al.*, 2005)

When comparing the DOC signatures of the inflowing streams with that of the lake, the correlations were always positive (R^2>0.64). The correlations were significant for two months for Afon Erddreiniog and three months for Afon Cefni. The lack of a correlation in October may be because of the large amount of algal-derived region III DOC in Llyn Cefni, and the lack of such an autochthonous DOC production in the streams. This data confirms the observations in Chapter 2, that the two streams are significant sources of DOC to the lake and substantiates that view that ^{1}H-NMR spectroscopy can be used for 'fingerprinting' the chemical structures of different sources of DOC. Although there were stronger correlations of Llyn Cefni DOC with Afon Cefni DOC, the similar signatures of both the streams DOC, coupled with the flux measurements reported in Chapter 2, suggest that neither of the streams is a dominate source of DOC to Llyn Cefni. It is worth noting that the percentage of region I (i.e. aliphatic) DOC in both streams was higher in February than any other month. This month was characterised by much lower rainfall than any of the previous three months and the increase in region I is likely to be due to a reduced flux of terrestrially-derived DOC.

3.6 Conclusions

This study emphasised the potential for ^{1}H-NMR spectroscopy to be used as a tool for 'fingerprinting' samples of DOC and for distinguishing different structural characteristics for samples from diverse sources. Whilst aliphatic compounds were identified as the dominant component of DOC in all the samples analysed, there was a greater abundance of aromatic compounds in the stream draining into the reservoir from a nearby peatland. However, the lack of a correlation with THM formation potentials for the aromatic region and previously reported work on allochthonous fluxes of DOC into Llyn Cefni, suggest that this inflow does not necessarily dominate as a source of THM precursors to the reservoir. Spectra obtained by ^{1}H-NMR also identified compounds associated with autochthonous DOC production, further highlighting the status of Llyn Cefni as a eutrophic lake.

Chapter 4

The potential use of floating constructed wetlands to reduce freshwater algal bloom formation

4.1 Abstract

The formation of algal blooms in freshwaters can be extremely detrimental to the overall health of an ecosystem and in reservoirs used as sources of drinking water they can be difficult to remove during water treatment processes. With the frequency of algal bloom formations increasing, possibly due to climate change, the need to minimise excess algal growth has never been greater. This study incorporates theories of peatland carbon (C) sequestration (the 'enzymic latch'), nutrient sequestration and algal inhibition to create a floating constructed wetland (FCW) capable of reducing the growth of algae. Over a four-week period, the FCWs were able to reduce algal growth by 80%, through sequestration of the key nutrients nitrate and phosphate and possibly due to the direct inhibitory properties of phenolic compounds on the algae. Furthermore, it was discovered that 8 *Phragmites australis* plants is the ideal number to maximise nutrient uptake and minimise algal growth in 70 litres of water. Although there are concerns about the leaching of dissolved organic carbon (DOC) from the FCWs, this may be more than offset by the beneficial suppression of algal growth and the resulting reduced input of 'untreatable' low molecular weight DOC.

4.2 Introduction

The physical and chemical treatment of drinking water is vital to ensure it is safe for human consumption. With rising populations, particularly in developing countries, the demand for water has never been greater and providing access to clean, safe sources to an ever expanding global population is likely to be one of the greatest challenges of the 21st century. In developed countries, the majority of water treatment works use a multi-step process of chemical addition, coagulation, flocculation, sedimentation and disinfection, usually with chlorine (Xia *et al.*, 2004). However, the effectiveness of water treatment processes can vary with changes in the chemistry and biology of the source waters and consequently the procedure can be costly. Freshwaters throughout the world contain dissolved organic carbon (DOC), its existence a consequence of interactions between the hydrological cycle, the biosphere and the hydrosphere (Murray and Parsons, 2003). Effective removal of DOC has emerged as a critical issue in the treatment of surface waters (Qin *et al.*, 2006), which can be prone to high inputs of autochthonous and allochthonous DOC. The source of organic components responsible for the composition of DOC often varies substantially throughout the year, resulting in differences in the ease with which DOC can be removed from water and how it reacts with chemical disinfectants (Goslan *et al.*, 2002).

The relative size and structure of the individual compounds comprising DOC is crucial in understanding its removal efficiency. Whilst coagulation and flocculation is reasonably successful in removing the high molecular weight (HMW) DOC, the fraction responsible for the production of most disinfection by-products (DBPs), it has been shown to be less than adequate in the removal of low molecular weight (LMW) carbon (C) (Matilainen *et al.*, 2002, Volk *et al.*, 2000). The presence of this fraction of DOC in treated water is thought to aid undesirable microbial re-growth in the water distribution system (Qin *et al.*, 2005).

HMW DOC is principally terrestrially derived (allochthonous), whilst LMW DOC is considered to primarily originate from within lake (autochthonous) processes (Kritzberg *et al.*, 2006). The most significant autochthonous source of DOC is generally considered to be algae (Cheng and Chi, 2003). The presence of algae is a crucial component of food webs within freshwater ecosystems; however, 'excessive' algal growth can become a problem. Algal 'blooms' are primarily caused by excess nutrients (eutrophication), particularly phosphate and sometimes nitrate (Herath, 1997). The extent of eutrophication is also dependent on physical factors such as the size of the water body, extent of mixing, depth of light penetration and water temperature (Herath, 1997).

The occurrence of algal blooms in lakes and reservoirs is potentially hazardous as it can cause a severe decline in the level of dissolved oxygen within the lake water, which may lead to mass mortalities of fish and present a serious health threat to animals and humans (Johnk *et al.*, 2008). Eutrophication can often lead to blooms of cyanobacteria and eukaryotic phytoplankton (Davies and Koop, 2006); these can have a significant effect on human health and may cause acute intoxication (Van Dolah *et al.*, 2001). They can also severely hamper water treatment processes and the quality of drinking water by clogging filters, raising coagulant and chlorine demand, increasing disinfection by-product (DBP) formation, producing undesirable taste and odours and increasing microbial re-growth potential in distribution systems (Knappe *et al.*, 2004).

It is when the algal bloom begins to die away that it poses the greatest problems for water treatment. As the algae enter the senescence phase, the decomposing cells release LMW C which is virtually untreatable (Cheng and Chi, 2003). Therefore, minimising the growth of algal blooms is essential in reducing the amount of LMW C in sources of drinking water.

The occurrence of freshwater algal blooms has increased over the last few decades (Van Dolah *et al.*, 2001; Moore *et al.*, 2008) although the specific reasons for this are not well understood. Climate change may increase the formation of algal blooms, therefore it is important that efforts

should be made to minimise their formation. Some experiments have shown promise in reducing algal growth using barley straw (Welch *et al.*, 1990; Ridge and Pillinger, 1994). The anti-algal properties of barley straw are not fully understood, but it has been suggested that the algal growth inhibitors are derived from oxidised lignin (Ridge and Pillinger, 1996). An alternative hypothesis suggests that the fungi responsible for degrading the barley straw produce anti-algal compounds. However, the general anti-algal effects of decomposing barley straw are unlikely to be explained by anti-algal properties of specific fungi (Pillinger *et al.*, 1992). Despite the possible benefits, the use of barley straw requires considerable management effort and the long term ecological safety is not known (Ball *et al.*, 2001, Martin and Ridge 1999).

Wetlands offer an alternative approach for the prevention of algal blooms. A consequence of the high levels of biological productivity within wetlands is that pollutants which enter through run off are easily broken down into substrates for the plants and microorganisms (Mitsch and Gosslink, 2000). In addition to this they are also able to act as chemical sinks, storing vast amounts of C (Jenkinson *et al.*, 1991) and nutrients in the water (Vymazal, 2007). The characteristic of storing vast amounts of C is largely attributed to waterlogging of the soil, creating anaerobic conditions and inhibiting enzymic decomposition of organic matter through an 'enzymic latch mechanism' (Freeman *et al.*, 2001a; 2004b). An additional benefit of wetland soil is the presence of plant derived phenolic material (Wetzel, 1992). Phenolic compounds are a defence mechanism against microbial infection (Codignola *et al.*, 1989) and several studies have been undertaken to exploit these compounds to suppress algal blooms (Everall and Lees, 1997; Pillinger *et al.*, 1994; Ferrier *et al.*, 2005).

It is therefore possible that the nutrient absorbing capabilities of wetland plants and microbes in conjunction with their ability to store large amounts of soil phenolic C may provide a unique method for controlling algal blooms. While a static constructed wetland would offer initial respite from algal blooms, in the long term it may well pose an additional threat to water quality

by leaching previously used substrates. Therefore, a floating wetland may be more practical; a structure that could easily be removed once nutrients have been sequestered, thus preventing them from leaching back into the water during plant senescence outside the growing season. Furthermore, a floating wetland can be placed in the lake exactly when and where it is required and, compared to traditional constructed wetland used for water treatment, would not need to have water diverted to it from the reservoir.

This chapter describes how a small-scale pilot experiment was carried out using floating constructed wetlands (FCWs) as a means of controlling algal blooms in artificially eutrophic ponds. The FCWs were designed to create an ecosystem that would help reduce algal bloom densities based on the combination of several different theories, plant selection, growth medium and phenolic supply.

4.3 Materials and Methods

4.3.1 Phase 1

A small-scale pilot experiment was undertaken for five weeks during the summer of 2007, in an attempt to gauge the effectiveness of FCWs in reducing algal growth in freshwater. The experiment comprised five control and five treatment ponds, each filled to 40 L capacity with de-chlorinated tap water. The water was then artificially altered to a eutrophic state through the addition of "Long Ashton nutrient solution", with concentrations taken from Wetzel (2001) and scaled up to generate an extremely eutrophic environment. Algae was then added to the 10 ponds in equal quantities following its culture from water collected from Llyn Penrhyn on Anglesey, Wales, a naturally eutrophic lake. One FCW was placed in each treatment pond, with each FCW consisting of two parts; a growth medium, comprising coya, peat and shredded heather litter, and several *Phragmites australis* plants. *Phragmites australis* was the chosen plant species due to its ability to sequester nitrate and phosphate and its common use in remediation wetlands (Massacci *et al.*, 2001). The planted FCWs were conditioned prior to use; they were prepared two weeks before the experiment commenced to allow the plants to grow and washed daily with water to minimise the build-up of C, nitrogen (N) and phosphorus (P) that could potentially leach from the FCWs once they were placed in the ponds. In the control ponds, FCWs without growth medium and plants were used. The ponds were placed in an elevated position on the roof of the Memorial Building, Bangor, Gwynedd, UK, where they could receive full sun, for a period of four weeks.

Water samples were collected weekly from the day the FCWs were placed in the ponds. From each pond, 250ml was extracted and all samples were filtered through a 0.45 μm Whatman cellulose acetate filter and stored at 4°C until analysis. Water in the ponds was mixed daily to mimic natural conditions, whilst additional water was added to replace that which had evaporated. Additional nutrients were added to each pond on the third week to replenish those

nutrients which had been utilised. The analyses carried out on each sample included the determination of DOC concentrations using a thermalox TOC analyser. The concentration of phenolic compounds was determined according to the method of Box (1983). A Unikon 943 double beam UV/ VIS spectrometer was used to measure the absorbance at 254, 665 and 750 nm. Specific ultraviolet absorbance (SUVA) (L mg^{-1} m^{-1}) was calculated as a ratio of UV absorbance at 254 nm (m^{-1}) to DOC (mg L^{-1}), the higher the value the more aromatic and higher molecular weight the DOC (Volk *et al.*, 2002). The concentrations of nitrate and phosphate were measured using a Dionex DX-120 ion chromatograph. Algal densities were measured using a method adapted from Golterman (1978), using chlorophyll a as an indicator.

4.3.2 Phase 2

The second experiment, carried out in the summer of 2008, involved the determination of the appropriate number of plants required to achieve a reduction in algal bloom densities in a given volume of water. The experiment comprised six ponds; one control (no plants) and five treatment (planted) ponds, each filled to 70 L capacity and altered to a eutrophic state as described for phase 1. In the planted ponds, each had a different number of *Phragmites australis* plants (2, 4, 6, 8 and 10) grown hydroponically in the water. This experiment was run for five weeks in the same location. Water samples were collected weekly from the day the plants were placed in the ponds. All other methodological approaches were identical to those described for phase 1.

4.3.3 Statistical analysis

A series of t tests were performed on the datasets to enable the comparison of the means for all the analyses at each sampling time point between the different treatments. Pearson correlation was used to determine the relationship between the number of plant and corresponding chlorophyll concentration in the Phase 2 experiment. Both statistical tests were performed SPSS

(version 11). The standard error of each data point was calculated by dividing the standard deviation of the sample by the square root of the sample size.

4.4 Results

4.4.1 Phase 1

In all of the analyses undertaken, significantly different trends were recorded for the control and planted treatments. For chlorophyll (Figure 4.2), the mean concentration in the control ponds increased significantly, from 9.5 μg L^{-1} in week 0 to 128.1 μg L^{-1} in week 4 (p<0.001). In the planted ponds, the mean concentration increased significantly from 9.4 μg L^{-1} in week 0 to 29.1 μg L^{-1} in week 1 (p<0.001), but then did not change significantly for the remaining three weeks (p>0.05). After having almost identical concentrations of chlorophyll in week 0, the planted ponds had approximately 80% less chlorophyll than the control ponds by week 4 (Figure 4.1a and 4.1b).

The mean concentration of DOC (Figure 4.3) within each pond also increased over the 4-week period. The rise was greatest for the planted ponds, increasing significantly from 6.5 mg L^{-1} in week 0 to 16.0 mg L^{-1} in week 4 (p<0.001), an average rise of 2.4 mg L^{-1} per week. DOC in the control ponds increased significantly from 4.7 mg L^{-1} in week 0 to 10.0 mg L^{-1} in week 4 (p<0.001), a rise of 1.3 mg L^{-1} per week.

The mean concentrations of phenolic compounds (Figure 4.4) followed a similar trend to DOC, increasing from week 0 to week 4 and at a greater rate for the planted ponds. In the control ponds, the concentration rose significantly from 0.54 mg L^{-1} in week 0 to 2.13 mg L^{-1} in week 4 (p<0.001); in the planted pond from 0.73 mg L^{-1} in week 0 to 3.76 mg L^{-1} in week 4 (p<0.001).

Values of SUVA (Figure 4.5) showed markedly different trends for each treatment. For the control ponds, SUVA declined from 2.71 L mg^{-1} m^{-1} in week 0 to 0.42 L mg^{-1} m^{-1} in week 3 (p<0.001) and did not change significantly in week four (p>0.05). In the planted pond, the SUVA did not change significantly throughout the experiment (p>0.05), although the mean value declined slightly from 3.20 L mg^{-1} m^{-1} in week 0 to 2.37 L mg^{-1} m^{-1} in week 4.

The mean concentrations of nitrate (Figure 4.6) measured in the both treatments varied significantly from week to week ($p<0.001$). In the control ponds, after an initial increase from week 0 to week 1, the concentration fell to 4.6 mg L^{-1} in week 2, rose to 17.2 mg L^{-1} following nutrient replenishment in week 3 and fell to undetectable levels in week 4. In the planted pond, the concentration of nitrate fell from 11.6 mg L^{-1} in week 0 to below the detection limit in weeks 1 and 2. It then increased to 15.0 mg L^{-1} in week 3 following replenishment and then back to an undetectable level in week 4.

The mean concentration of phosphate (Figure 4.7) in both treatments declined from 2.6 mg L^{-1} to below the limit of detection over the 4 weeks, only increasing when the nutrient was added in week 3. The decline in phosphate was greatest for the planted pond, falling to undetectable levels by week 2.

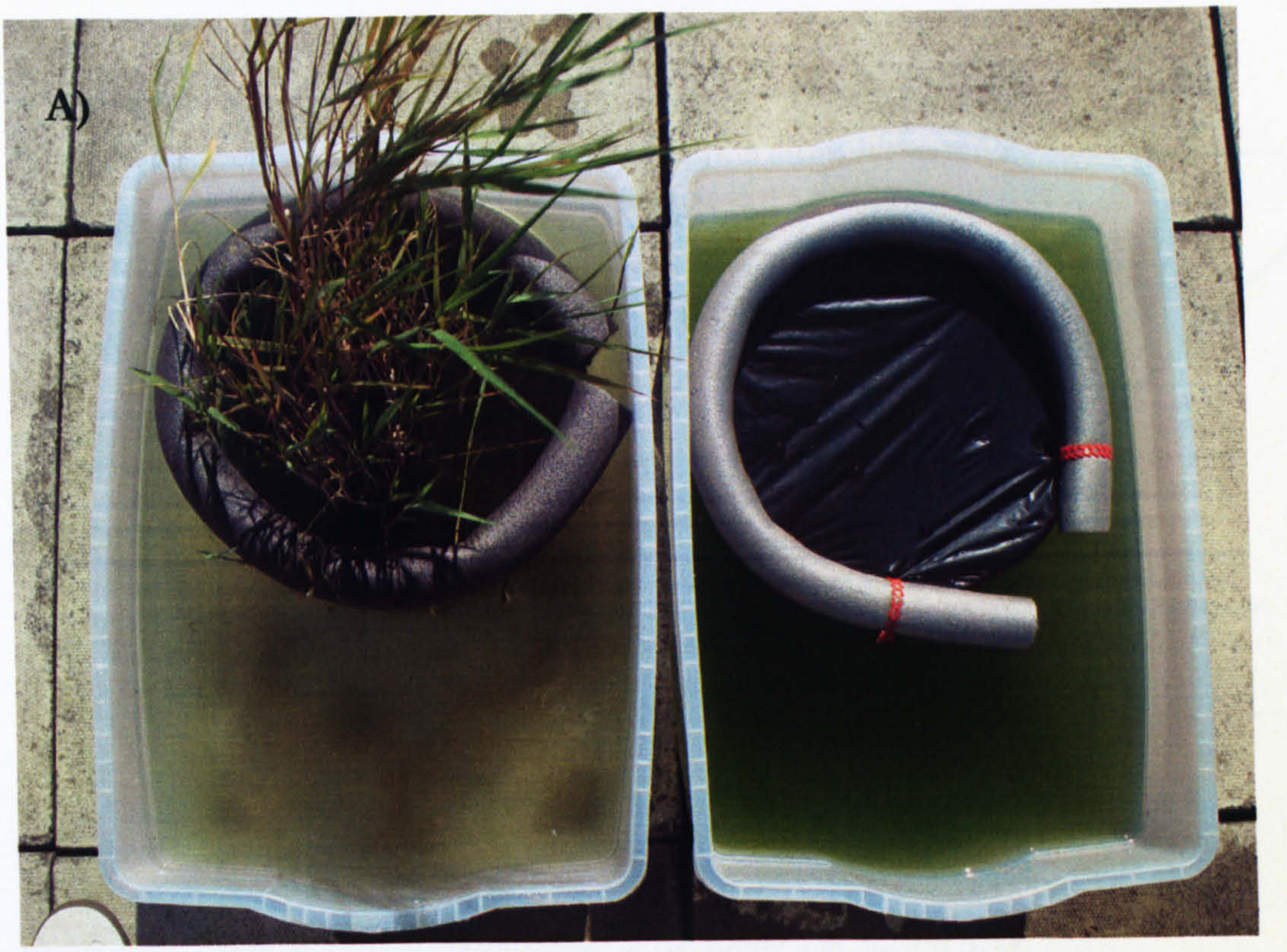
4.4.2 Phase 2

The chlorophyll concentrations (Figure 4.8) measured for the control and each of the planted treatments displayed similar trends, increasing from week 0 to week 3 and then declining in week 4. The greatest increase in chlorophyll was recorded for the control system, in which the concentration rose from 7 μg L^{-1} in week 0 to 133 μg L^{-1} in week 3. The systems containing 8 and 10 plants contained the lowest concentrations of chlorophyll; 35 and 32 μg L^{-1} respectively. For week 3, when the greatest differences in chlorophyll concentrations between the 6 treatments was recorded, there was a strong and significant negative correlation between the number of plants in the pond and the measured chlorophyll concentration (Figure 4.9; $R^2=0.83$, $p<0.05$). The pond with 8 plants had approximately 74% less chlorophyll than the control pond by week 3.

The concentration of DOC (Figure 4.10) within each pond increased over the 4 week-long experiment. Initially each of the planted systems showed similar trends; the DOC concentrations declined from week 0 (average 4.8 mg L^{-1}) to week 1 (average 3.7 mg L^{-1}) and increased in week 2

(average 8.4 mg L^{-1}). By week 3 there was more divergence between treatments, with the control having the highest concentration (10.0 mg L^{-1}). For the planted treatments, a strong but not quite significant negative correlation was recorded between the DOC concentration and the number of plants (R^2=0.74, p<0.1), with the treatment with 10 plants having the highest DOC concentration. By week 4, the treatment effect was less clear, with the 6 plant treatment having the highest DOC concentration (9.32 mg L^{-1}), the 4 plant treatment having the lowest (6.60 mg L^{-1}), and the control being in the middle (7.7 mg L^{-1}).

The trend for phenolic compound concentrations (Figure 4.11) largely mirrored that for DOC, with an increase from week 0 to week 3 and no further increase by week 4. Similarly to DOC, by week 3 the control had the highest concentration (2.48 mg L^{-1}) and out of the planted treatments, that with 10 plants had the highest concentration (2.28 mg L^{-1}) whilst that with 2 had the lowest (1.41 mg L^{-1}).



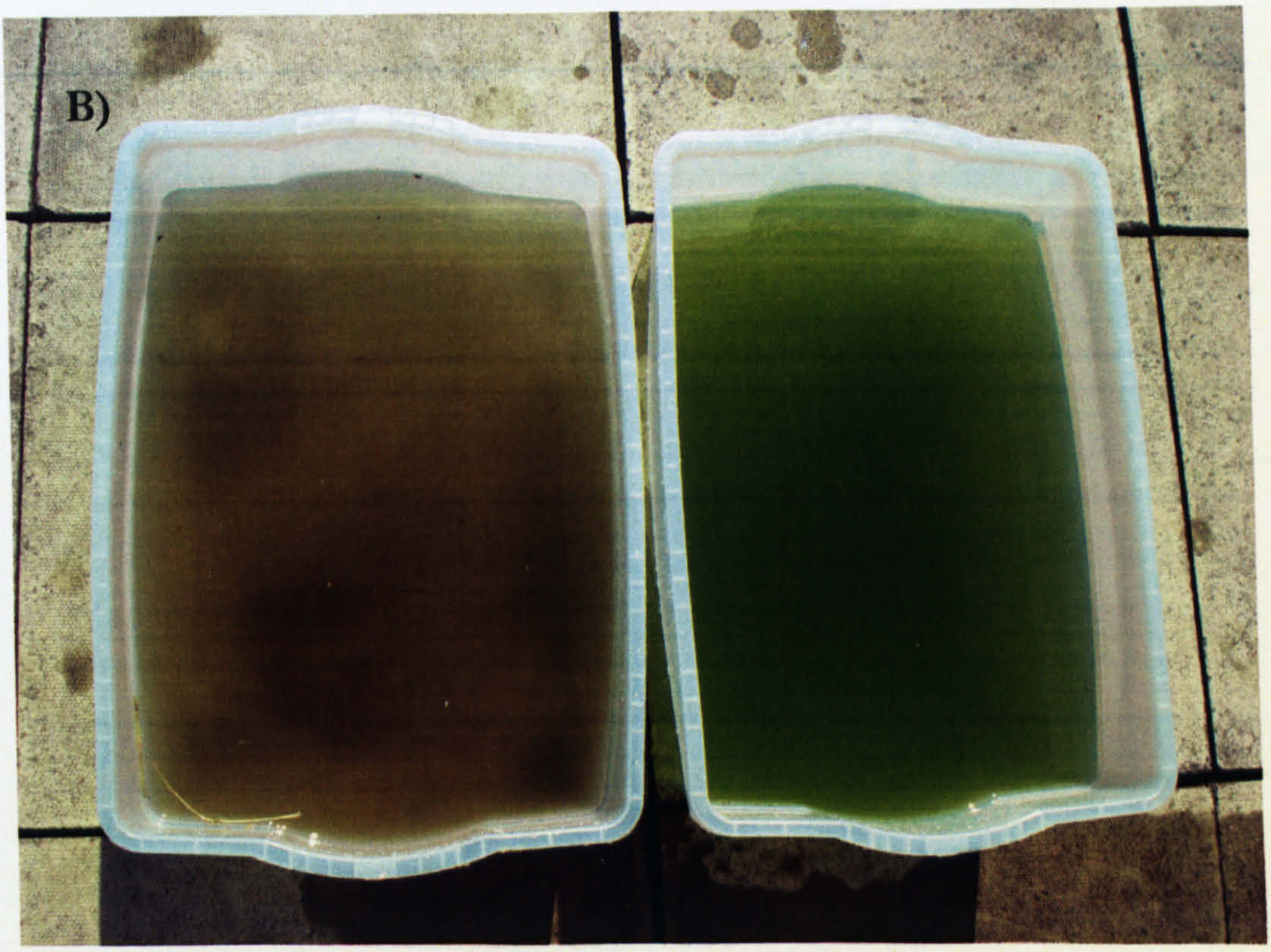


Figure 4.1 a) and b) – Phase 1 planted (left) and control (right) ponds after 4 weeks

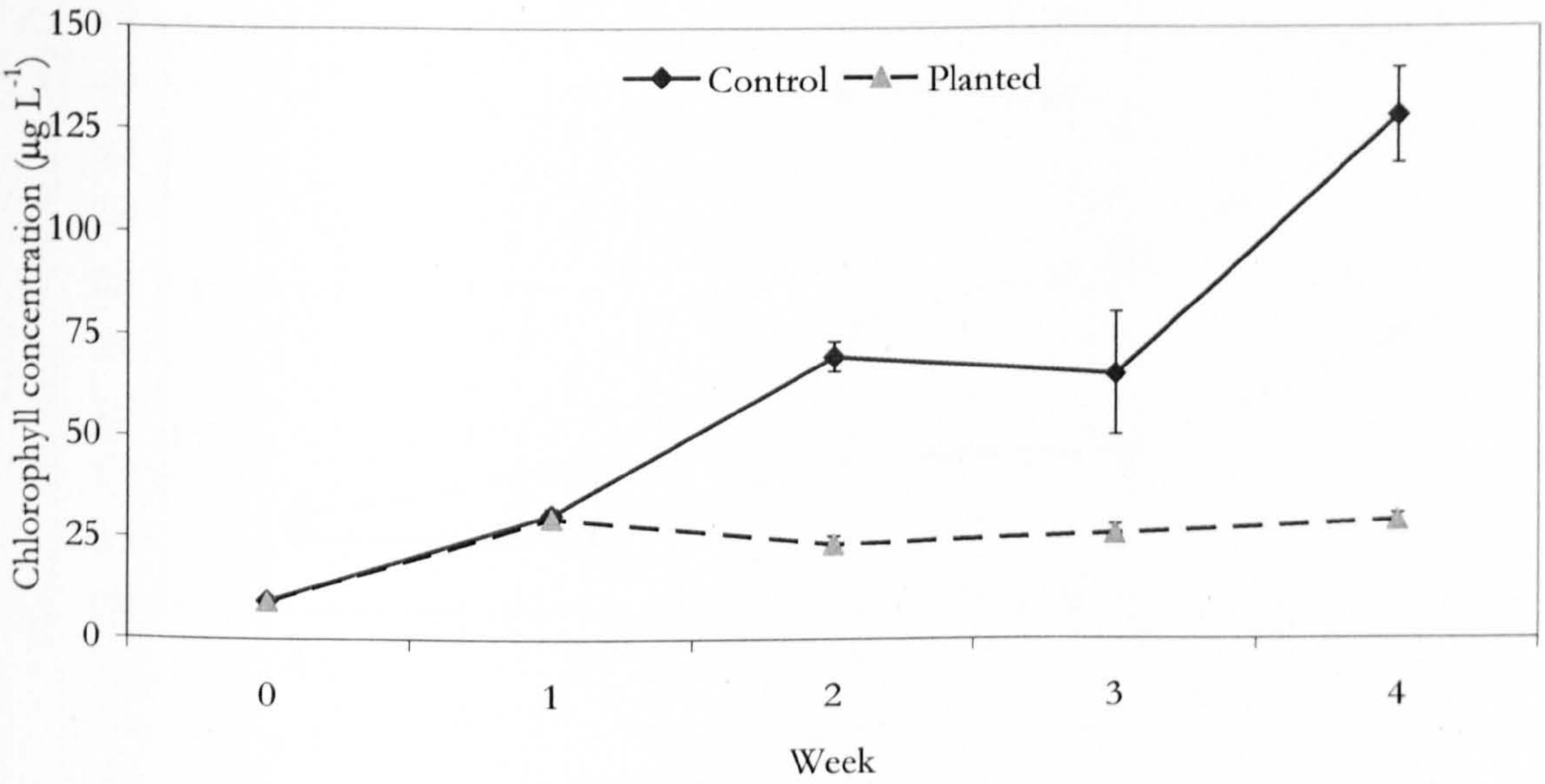


Figure 4.2 – Mean chlorophyll concentrations of the control and planted ponds

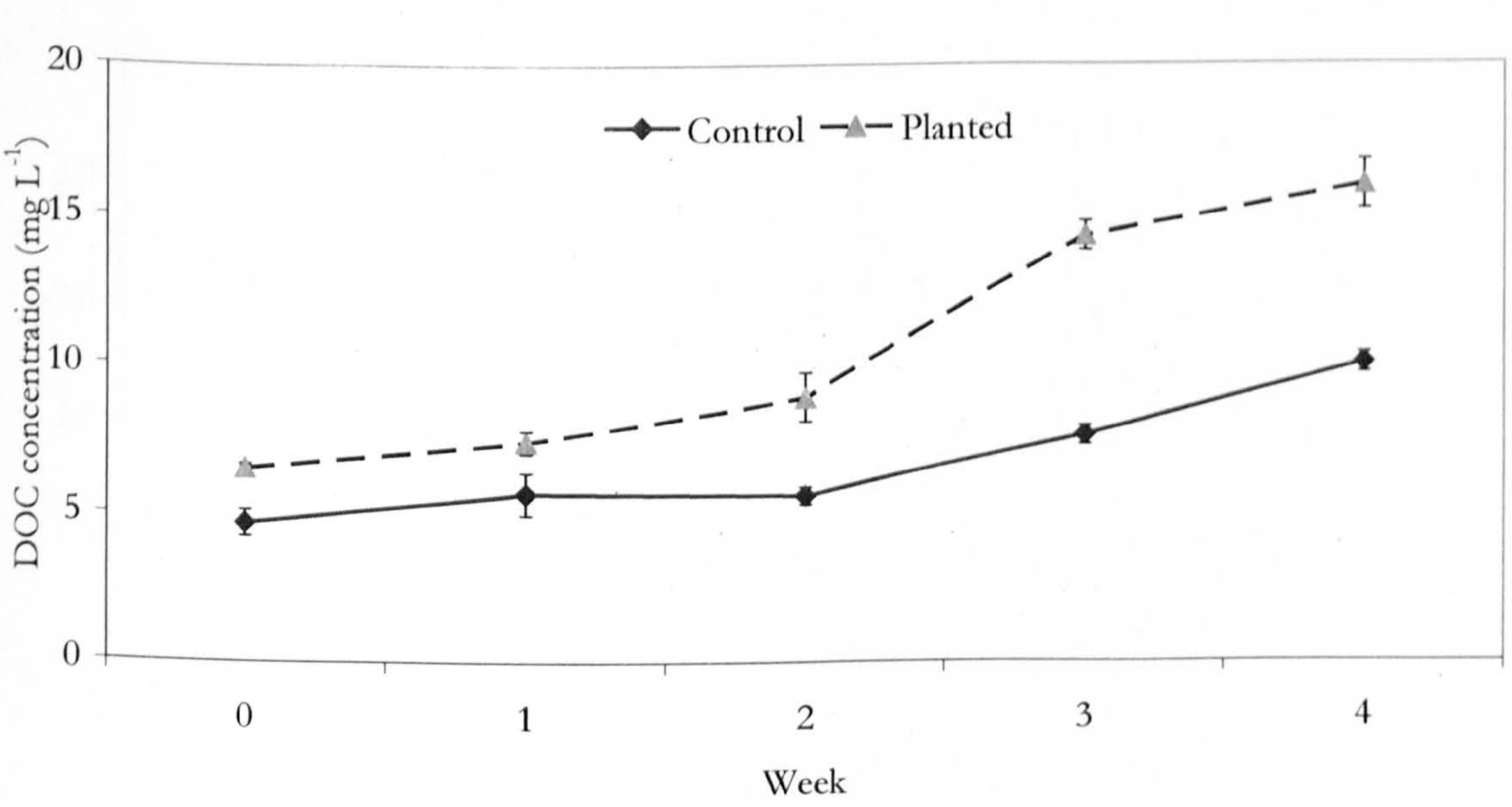


Figure 4.3 – Mean DOC concentrations of the control and planted ponds

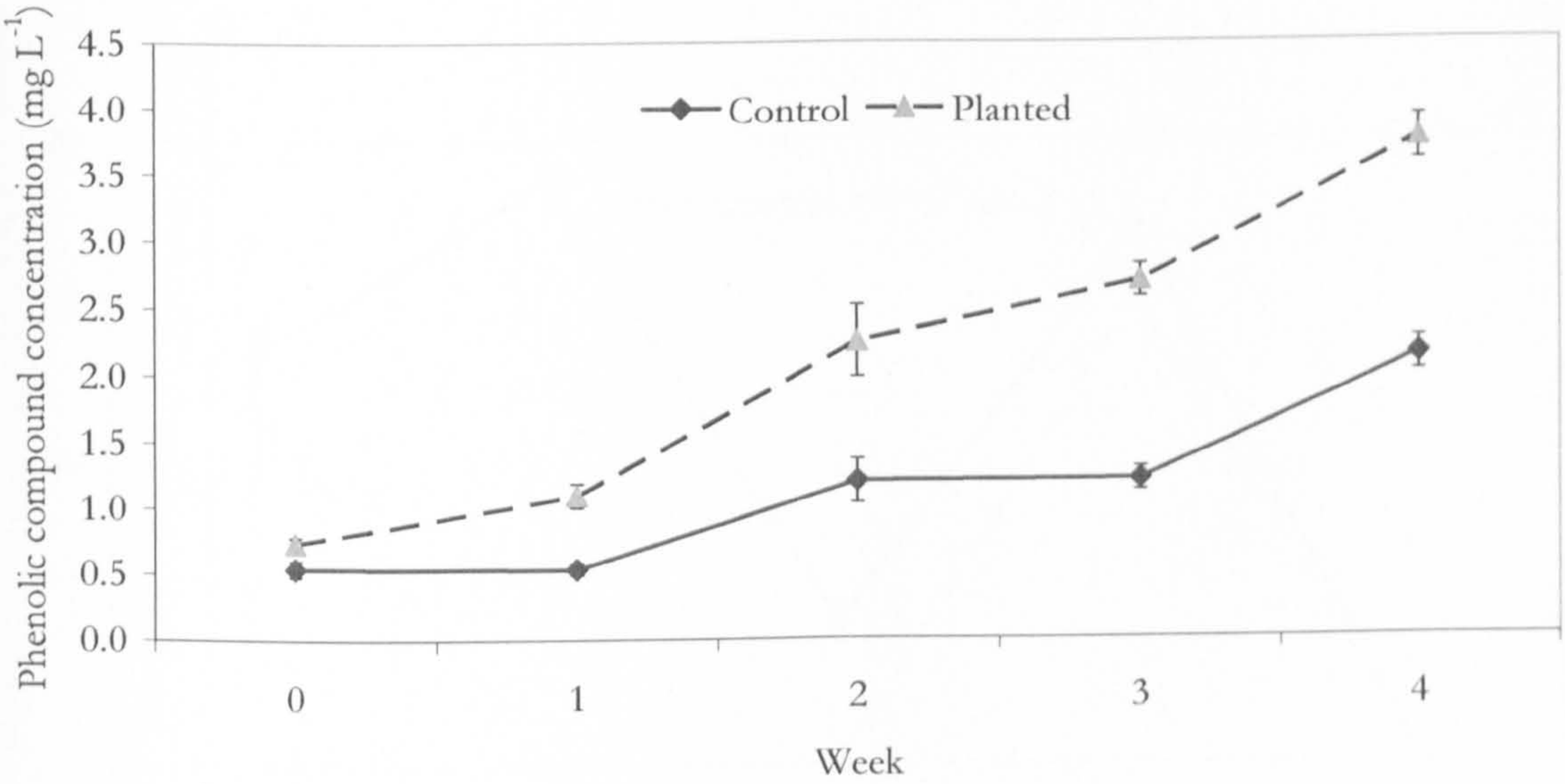


Figure 4.4 – Mean phenolic compound concentrations of the control and planted ponds

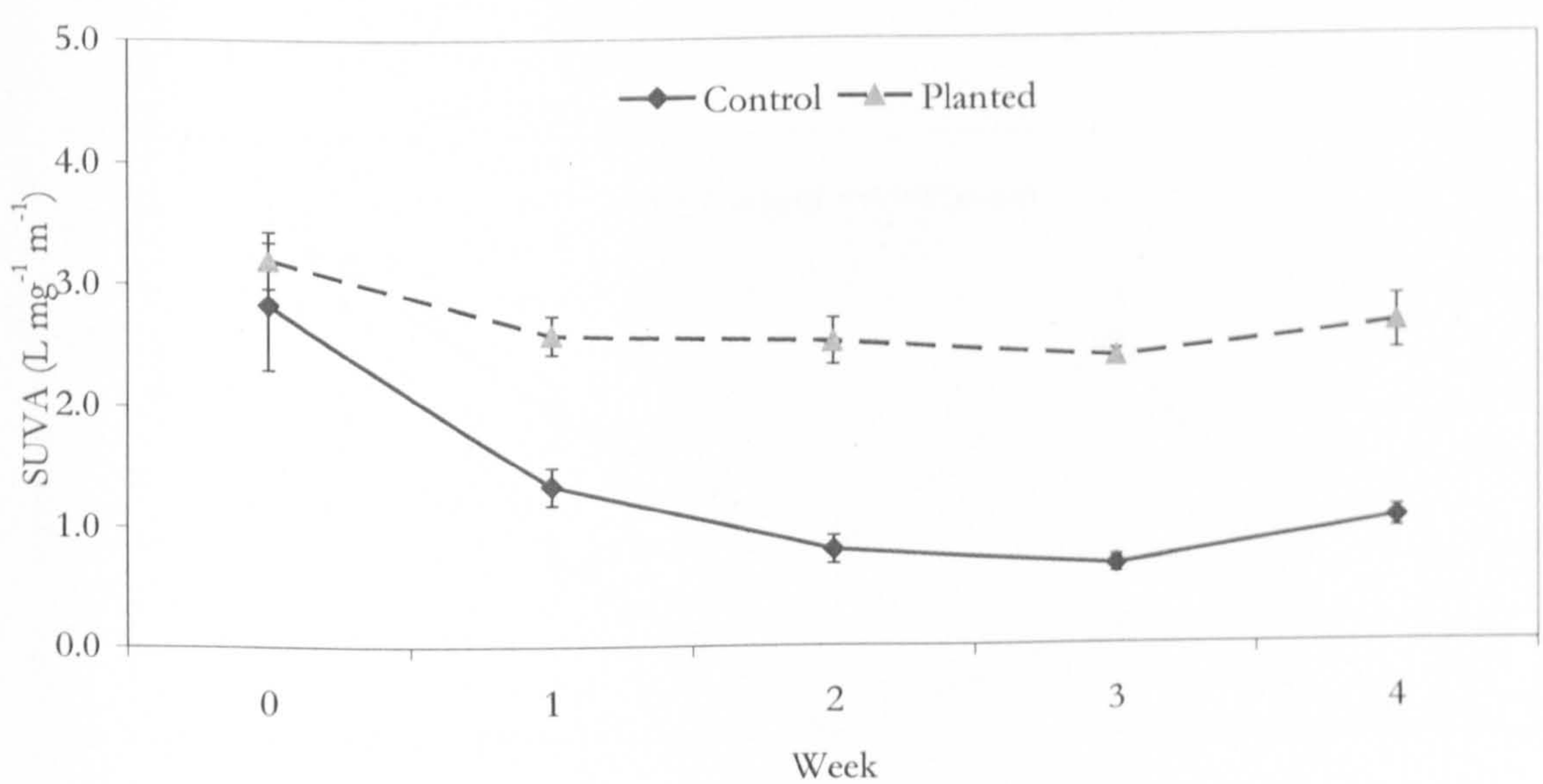


Figure 4.5 – Mean SUVA values of the control and planted ponds

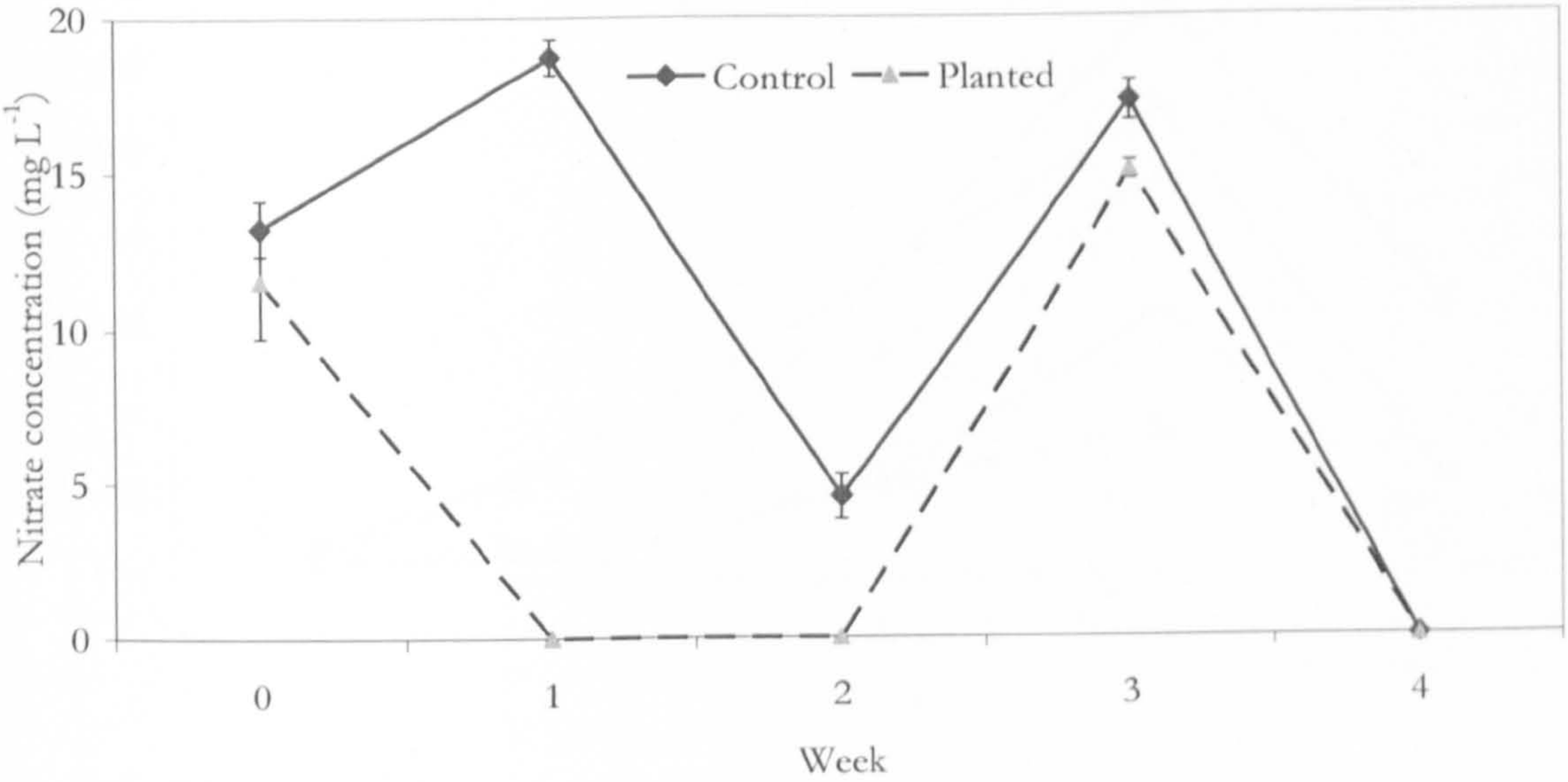


Figure 4.6 – Mean nitrate concentrations of the control and planted ponds

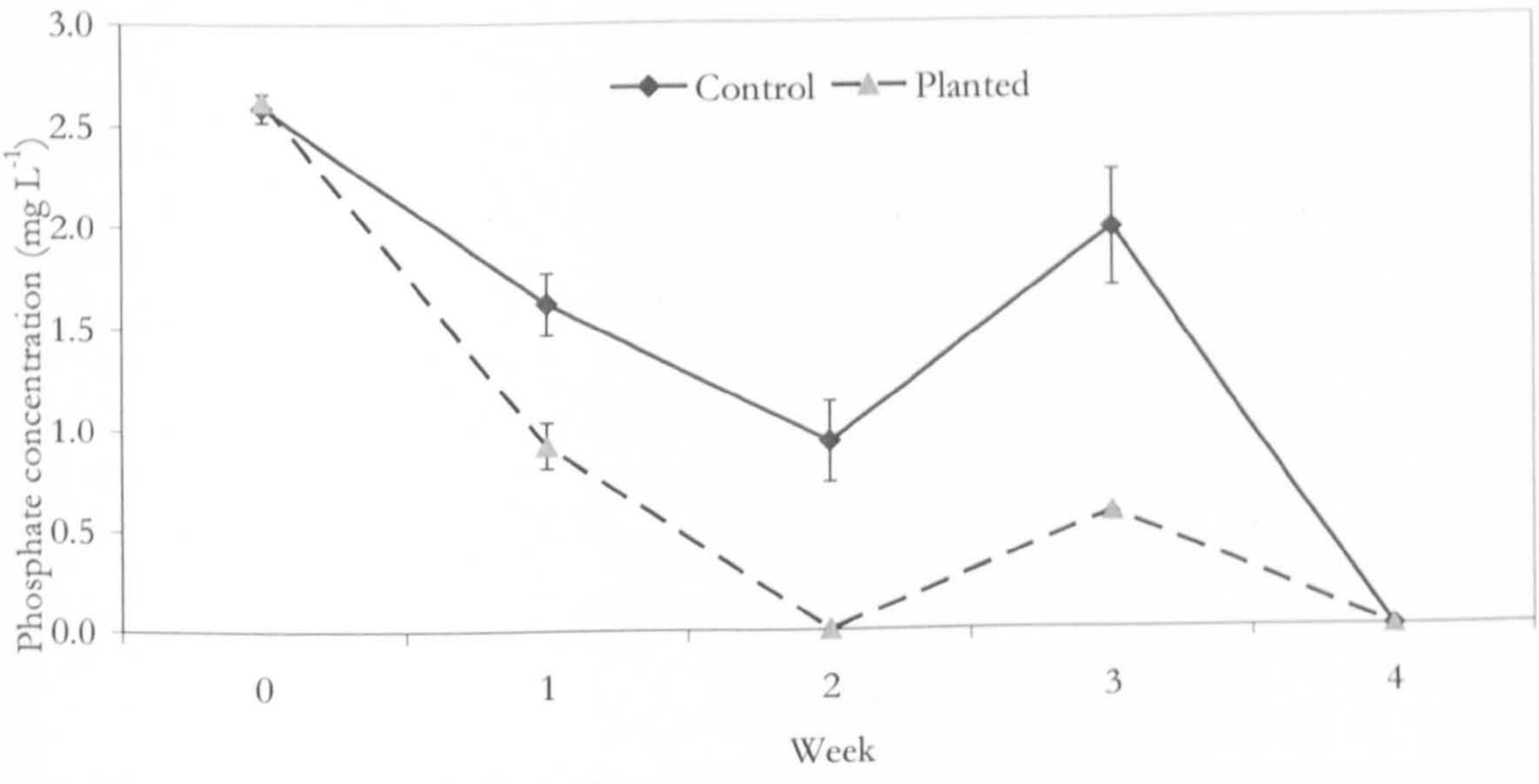


Figure 4.7 – Mean phosphate concentrations of the control and planted ponds

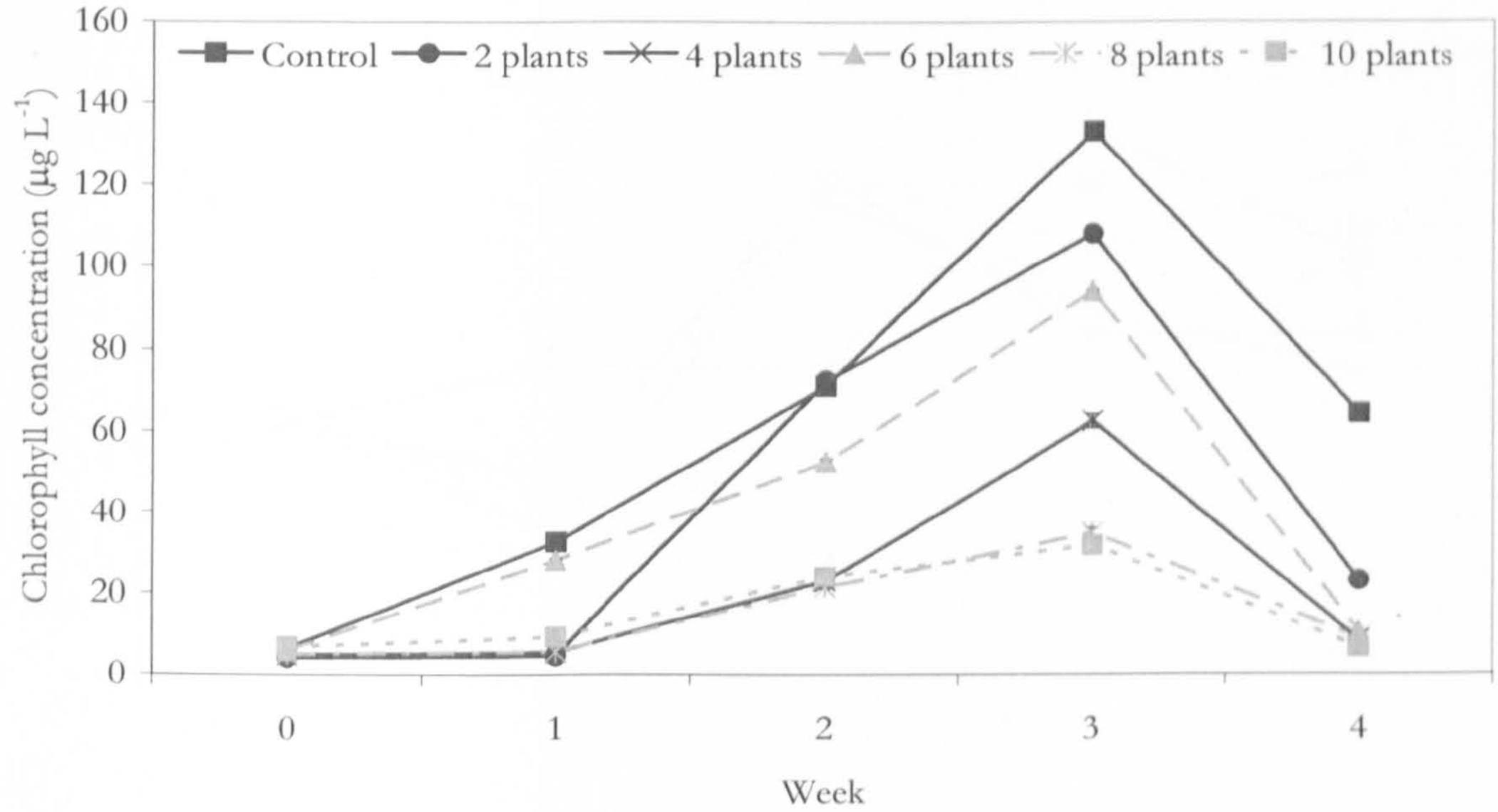


Figure 4.8 – Mean chlorophyll concentrations of the control and planted ponds

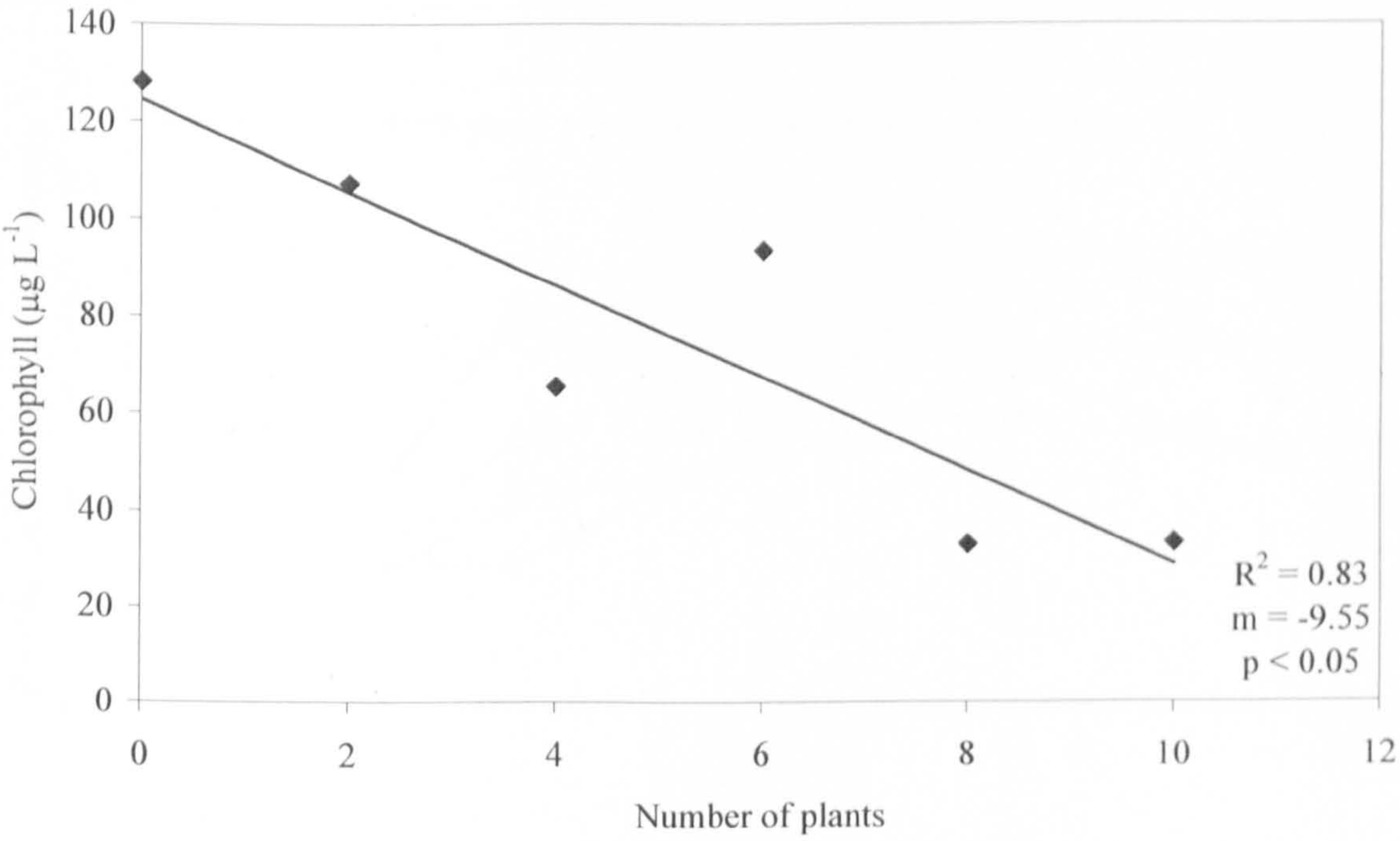


Figure 4.9 - Relationship between the number of Phragmites australis plants and the concentration of chlorophyll in artificially eutrophic ponds. The linear relationship is significant and the R^2 value is presented in the data

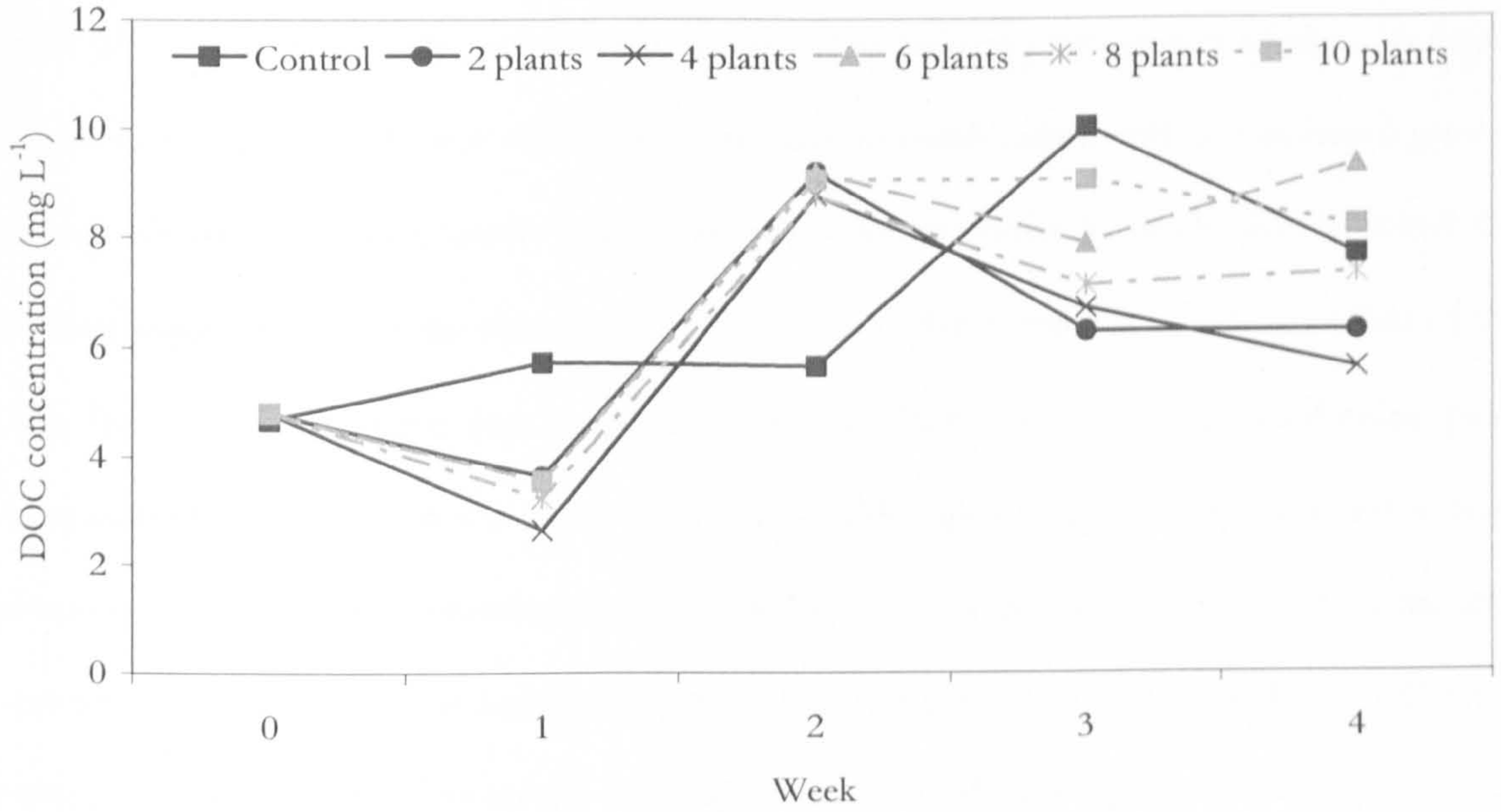


Figure 4.10 – Mean DOC concentrations of the control and planted ponds

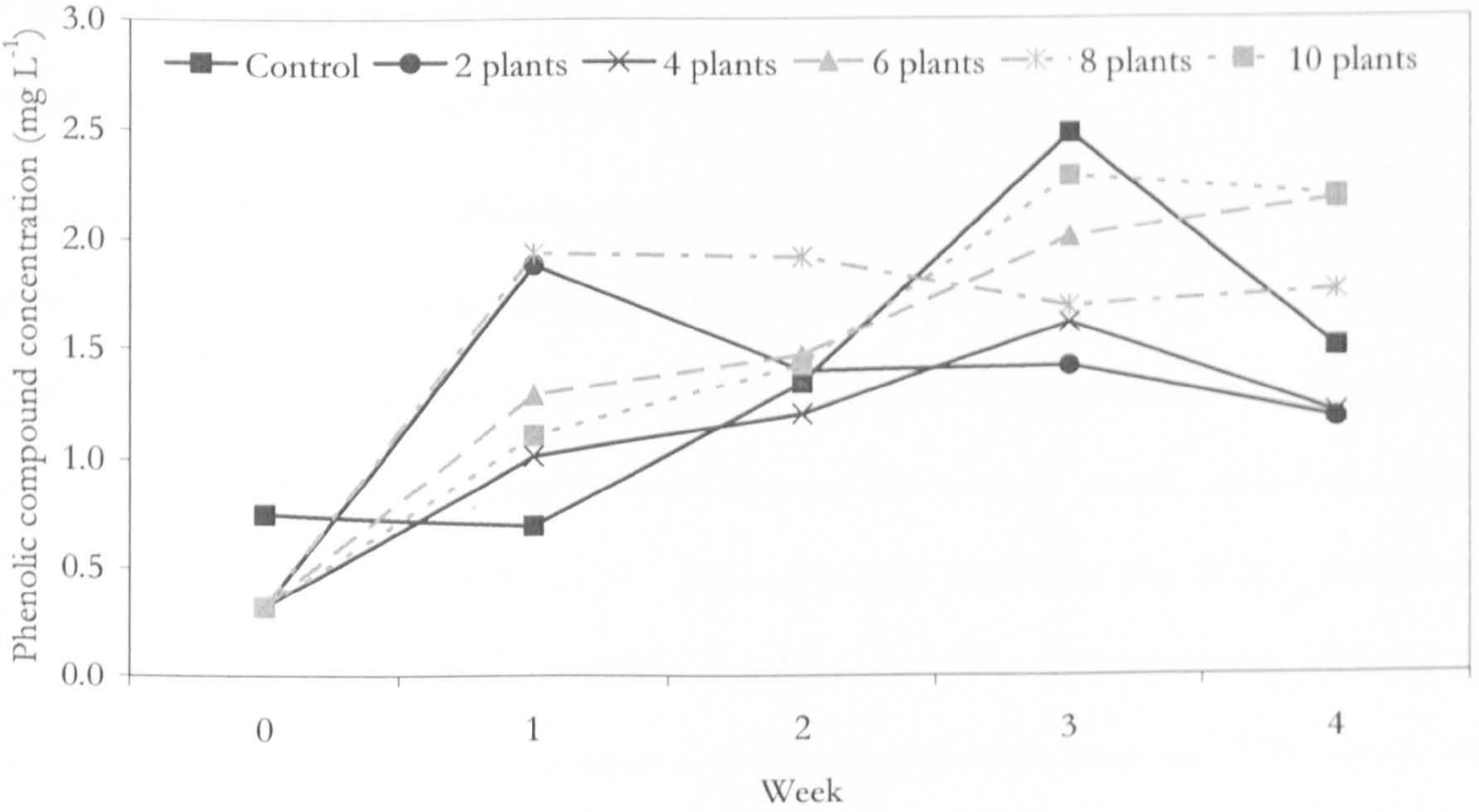


Figure 4.11 – Mean phenolic compound concentrations of the control and planted ponds

4.5 Discussion

The FCWs used in this study proved very successful at reducing the growth of algae in small-scale freshwater ponds. The use of *Phragmites australis*, in combination with a peat-based growth medium, efficiently reduced nitrate and phosphate concentrations to levels that inhibited the growth of algae. Data from the phase 1 experiment shows that there was initially no effect of the FCWs, but once the systems had become established after a week, they reduced chlorophyll concentrations to 80% below the control treatment. Although not significant, there was a small decrease in the chlorophyll concentration in week 3 for the control system; this can be attributed to nutrient limitation and some algal senescence. Once nutrient levels were replenished following sampling in week three, chlorophyll concentrations rose sharply again in the control, but continued to be suppressed in the planted treatment. Overall, this data suggests that if these systems are to be used to combat algal blooms in real freshwater lakes, the initial period of FCW establishment needs to be factored into predictions of the length of time required to reduce nutrient concentrations and algal densities.

Although the FCWs proved efficient at reducing algal bloom formation, this benefit must be balanced against the negative aspect of additional DOC leaching from the systems. This DOC would principally originate from the peat. Using phase 1, week 4 experimental data, algal growth contributed approximately 5.3 mg L^{-1} of DOC in the control ponds, whilst the FCWs contributed 9.5 mg L^{-1} of DOC in the planted ponds. Therefore the FCWs contributed approximately an extra 4.2 mg L^{-1} of DOC. If it is assumed that this experiment achieved the maximum reduction in nutrient and algal concentrations possible from an FCW set-up, this suggests that the use of these systems in a real freshwater lake should not contribute more than 5 mg L^{-1} of extra DOC. The occurrence of algal blooms in freshwater lakes or reservoirs typically occurs during the summer months, when water temperatures and sunlight levels are highest (Johnk *et al.*, 2008). This is also a time of year when DOC concentrations in lakes tend to be low,

due partly to a reduction in the flux of allochthonous DOC as demonstrated in Chapter 2. This can be attributed to lower rainfall, lower availability of leachable C and greater water usage by vegetation in the lake's catchment (Roberts, 1998). The increased input of DOC from the FCWs may therefore occur at a time when DOC concentrations of the lake in which they are utilised are naturally low. Chapter 2 described that when algal concentrations were elevated, the DOC concentration of Llyn Cefni was low, about 6 mg L^{-1}. A FCW set-up in Llyn Cefni may therefore raise the summertime DOC concentration to approximately 11 mg L^{-1}, still significantly lower than the 16 mg L^{-1} recorded in the lake during the autumn, when there is usually a flux of DOC from a nearby peatland. The water treatment works at Llyn Cefni is able to efficiently treat the water during this autumn peak; therefore the FCWs would not be expected to compromise treatment processes during the summer. In a real situation, the amount of DOC released by the FCWs is likely to be much lower than 5 mg L^{-1}; this experiment was designed to test the efficiency of FCWs to reduce nutrient concentrations in a small volume of water over a short period of time. Based on their success, in a lake or reservoir the FCWs would have a longer time period over which to sequester nutrients and could therefore be used at a much lower FCW:water volume ratio. This would minimise the total amount of DOC they release.

Although the FCWs would cause a greater increase in the total concentration of DOC in a freshwater lake compared to one where algal blooms are allowed to form, analysis of the structure of this DOC suggests this change in the source of the DOC would actually favour its removal during water treatment processes. In the planted treatment, although there was a significant increase in phenolic compound concentrations, there was no significant change in the SUVA of the DOC. This suggests the overall molecular weight and size of the DOC pool changed little over the course of the experiment and that the FCWs would not alter the quality of DOC present in a freshwater lake. In the control treatment, a large decrease in SUVA was recorded. This is indicative of the growth of algae and coupled with the increase in DOC recorded for this treatment by week 4, confirms that algae can input large quantities of LMW

DOC into freshwater lakes. This type of DOC is more difficult to remove during the physical and chemical treatment of water (Cheng and Chi, 2003), therefore is likely to persist in the final water that is distributed to consumers. In the distribution phase, LMW C is a substrate for harmful bacterial growth (Volk *et al.*, 2000).

The aim of the phase 2 experiment was to establish whether there are an optimal number of plants for an individual FCW system and to determine whether the plants make a significant contribution of DOC to the water. If the number of plants can be minimised, the amount of plant derived C leaching into the water and the costs of an FCW system will be reduced. Focusing on the data from week 0 and week 3 only, the latter being when the chlorophyll concentrations were highest, it is apparent that, in terms of reducing chlorophyll concentrations, eight plants are optimal for 70 L of water, with 10 offering no additional benefit. The DOC concentration of the ponds increased from week 0 to week 3, due to both C derived from algal growth and C leached from the *Phragmites australis*. The most significant source appears to have been from the algae, as the control pond had the highest DOC concentration. For the planted ponds, there was a general trend of less plants equating to lower DOC concentrations in the water. The plants do therefore contribute some DOC to the water, however, it is still less than if algal blooms are allowed to form. Furthermore, it is likely that most of the DOC originating as root exudates from the *Phragmites australis* would be utilised as substrate DOC by microorganisms living in the peat of the growth medium (Bonkowski *et al.*, 2000). The concentration of phenolic compounds also increased from week 0 to week 3, and, proportionally, to a greater extent than the total DOC concentration. This suggests that both algae and the plants can be important sources of phenolic compounds. The leaching of phenolics by the plants may be of additional benefit to reducing algal growth; these compounds have been demonstrated to have inhibitory properties towards algae (Pillinger *et al.*, 1994; Ferrier *et al.*, 2005).

This experiment has demonstrated that the FCW setup we employed was capable of minimising the growth of algae in freshwater, but it is important to ensure that the individual FCWs are removed from the lake at the end of the growing season. Further algal growth outside the growing season is unlikely, due to unfavourable temperatures and levels of sunlight. However, if the FCWs were left *in situ* throughout the winter, the senescence of the *Phragmites australis* vegetation would likely input large amounts of C, N and P into the lake (Polomski *et al.*, 2009).

There is also potential for these systems to also have value outside of the lake, when they are not in use. Once removed from the lake, the vegetation could be cut back and used to create environmentally sustainable products (Kiviat and Hamilton, 2001). As *Phragmites australis* is a perennial plant, it could be used in an FCW over a number of years without needing to be replaced. Another additional benefit could be as a *source* of nutrients. The movement of the FCWs out of submerged conditions would created an aerobic environment in the peat substrate, initiating the 'enzymic latch' (Freeman *et al.*, 2001a), stimulating decomposition and causing the release of sequestered inorganic nutrients at a site where they would have value as a fertiliser.

4.6 Conclusions

This study demonstrated the potential of a FCW to sequester nitrate and phosphate in a small-scale freshwater pond and it was therefore effective at reducing the growth of algae. Over a period of 4 weeks, the concentration of chlorophyll, an indicator of algal growth, increased to 128.1 μg L^{-1} in the unplanted control treatment, whilst in the treatment that contained FCWs the chlorophyll concentration only reached 29.1 μg L^{-1}. Furthermore, it was discovered that 8 *Phragmites australis* plants is the ideal number to maximise nutrient uptake and minimise algal growth in 70 litres of water.

If the FCW set-up employed in this pilot study is to be scaled up for use in freshwater lakes which are prone to algal bloom formation, the issue of C leaching from the systems needs to be addressed further. The release of C from the growth medium into the water will increase DOC concentrations in the lake, somewhat offsetting the beneficial inhibition of algal growth. However, we have shown that the type of C leached from these systems in much more 'treatable' than algal-derived DOC, so may not be completely undesirable. The benefits of algal suppression against changes in the DOC concentrations and characteristics could be assessed better in a larger scale study.

Chapter 5

Long-term drainage of a boreal peatland; Impacts on soil enzymes and the release of DOC across a nutrient gradient

5.1 Abstract

The drainage of peatland ecosystems has been widespread in many northern hemisphere countries. In Finland, only 38% of peatlands can still be classified as pristine, with the majority of disturbed peatlands having been drained for the establishment of forestry plantations. The implications of this for the overall carbon (C) balance are not well understood, especially regarding the flux of dissolved organic carbon (DOC) to freshwaters. Therefore, a study was carried out at Lakkasuo peatland in southern Finland, where areas of ombrotrophic, oligotrophic and mesotrophic peat were drained 9 and 48 years ago, in addition to areas that have been left undisturbed, to assess the effect of drainage on DOC export potential. Although no consistent response in DOC export potential with drainage was recorded across the sites of contrasting nutrient status, a significant correlation was observed between DOC and the water content of the soil (80 μg cm^{-3} increase in DOC for a 5% decrease in soil water content), presumably because increased aeration stimulates decomposition of the peat matrix. An alternative explanation is that the trend in DOC is driven by changes in soil pH and the resulting suppression of the key C regulating enzyme phenol oxidase; where acidity has increased following drainage phenol oxidase activity has declined and the concentration of DOC released has increased, conforming the view that phenol oxidase acts as an 'enzymic latch' in peatlands. In addition to leading to an increase in DOC concentrations, measurements of SUVA demonstrated that the overall molecular weight of the leachable DOC also increased (SUVA increased on average by 1.117 L mg^{-1} m^{-1} due to drainage across the 3 sites). The results of this study have implications for both C storage in drained peatlands and for the quality of freshwaters; for the latter it is likely that the treatment of drinking water will be more difficult and enhanced disinfection by-product (DBP) formation will occur if source waters contain drained peatlands within their catchments.

5.2 Introduction

Peatland ecosystems have traditionally sequestered vast stores of carbon (C) and play a key role in the global C cycle. They are characterised by having soils that are mostly waterlogged and anoxic; this suppresses the decomposition of organic matter leading to net C accumulation. Despite only occupying approximately 3% of the Earth's land surface area, peatlands are estimated to contain up to 455 Pg C as peat, or 30% of the world's global soil C store and the equivalent of half of the amount of atmospheric CO_2-C (Gorham *et al.*, 1991).

As the imbalance between net primary production and decomposition is relatively small (Päivänen and Vasander, 1994), peatlands are particularly sensitive to environmental changes that may affect their ability to continue sequestering C. Probably the greatest environmental disturbance to have affected peatlands worldwide has been land use change. Peatlands are 'economically poor' and many millions of hectares in the boreal and temperate zones have been converted to areas of agriculture and forestry. Drainage of the peat is usually the first stage of this process; it is achieved with the installation of open cut drainage ditches and the resulting reduction in the height of the water table seriously undermines the ability of the peatland to continue to sequester C. As the waterlogged conditions of peatlands are deemed to be responsible for maintaining their stability (Freeman *et al.*, 1996), a persistent lowering of the water table has the potential to destabilise the vast reservoir of C that peatlands contain (Laiho, 2006) given that aerobic decomposition of organic matter is about 50 times faster than anaerobic decomposition (Clymo, 1983). The enzymic 'latch' has been proposed as a regulator of C storage in peatlands due to the absence of oxygen inhibiting the key enzyme phenol oxidase (Freeman *et al.*, 2004b); water-table drawdown therefore has the potential to activate this enzyme through increased oxygenation of the soil, which will ultimately increase organic matter decomposition.

Despite this, laboratory and field studies on the effects of water-table lowering on changes in C dynamics of peatlands suggest the issue is not so straightforward. Whilst a number of published

studies have demonstrated net losses of C following water-table drawdown (e.g. Glenn *et al.*, 1993; Alm *et al.*, 1999; Jaatinen *et al.*, 2008), some have actually reported increases in C storage in peat (e.g. Minkkinen and Laine, 1998; Minkkinen *et al.*, 2002; Laiho *et al.*, 2004). Laiho (2006) provided an excellent synthesis of contrasting results concerning the impact of a lowered water-table on peat decomposition. It is apparent that whilst water-table drawdown increases oxygen content which ultimately favours decomposition, this can be offset by decreases in pH (Laine *et al.*, 1995), soil temperature (Minkkinen *et al.*, 1999) and substrate quality (Laiho and Laine, 1996). Domish *et al.* (1998) suggested that increased organic C flows from tree stands can account for increased below-ground C storage following drainage for forestry. The balance between peat decomposition (C outflow) and litter input (C inflow) is therefore crucial for determining C storage in peat following water-table drawdown (Minkkinen and Laine, 1998) and the response of different peatlands to water-table drawdown ultimately depends on their nutrient status, local climatic conditions and hydrology.

In comparison to gaseous forms of C (principally CO_2 and CH_4), dissolved organic carbon (DOC) is an often overlooked component of the terrestrial C cycle, despite its importance in the overall C balance of peatland catchments in the world's temperate and boreal zones (Billett *et al.*, 2004). DOC is released from peatlands due to the incomplete decomposition of organic matter under anaerobic conditions and in many catchments the concentration of DOC in streams and rivers can be explained by a simple linear relationship with the percentage of peatlands in the catchment (Dillon and Molot, 1997). DOC is an important flux of C between terrestrial and aquatic systems, with an average 0.1 to 1 Tg C $year^{-1}$ ultimately transported to the world's oceans via streams and rivers (Hope *et al.*, 1994). As a large proportion of riverine DOC is decomposed once it reaches the ocean (Hedges *et al.*, 1997), ultimately increasing atmospheric CO_2 concentrations, it is important to understand processes influencing the flux of DOC from terrestrial systems. Widespread observations of rising freshwater DOC concentrations during the

past two decades (Evans *et al.*, 2006; Monteith *et al.*, 2007) are placing a greater emphasis the DOC component of the terrestrial C cycle. Increasing DOC in freshwaters has been linked to peatland destabilisation due to, for example, increasing temperature (Freeman *et al.*, 2001b), atmospheric CO_2 concentrations (Freeman *et al.*, 2004a), precipitation (Tranvik and Jansson, 2002) and decreasing sulphate deposition (Monteith *et al.*, 2007).

Compared to gaseous forms of C (CO_2 and CH_4) there have been fewer studies into the influence of water-table drawdown on the production of DOC in peatlands, especially in the long-term. Laboratory experiments with peat cores have demonstrated that a lowering of the water-table can lead to increased DOC release (e.g. Mitchell and McDonald, 1992; Freeman *et al.*, 1993*a*), assumed to be due to the increased aerobic breakdown of soil organic matter. Clark *et al.* (2009) reported that water-table drawdown led to a highly significant increase in DOC production between 1 and 40 cm below the soil surface. They also highlight the role of secondary biogeochemical changes, most notably falling pH driven by increasing sulphate, in masking effects on DOC that are driven by climatic changes. Sulphate driven acidity is known to have a strong influence on DOC solubility in peatlands, particularly during and after periods of drought (Clark *et al.*, 2005). Data from a limited number of field studies have reported that peatland drainage increases the flux of DOC to freshwaters. For example, Mitchell and McDonald (1995) observed a greater discolouration of freshwaters flowing from drained areas of a peatland catchment in northern England. Wallage *et al.* (2006) recorded significantly higher DOC concentrations in the porewaters of artificially drained peat compared to intact peat. Worrall *et al.* (2007b) have modelled the effects of peat drainage on DOC export and reported DOC flux values more than twice as high from drained peat compared to pristine peat. Drought/re-wetting events are known to cause large episodic increases in DOC export, as carbon compounds mobilised through aerobic decomposition during periods of drought accumulate and can be washed out of the soil during periods of high flow. For example, at a site

of experimental hydrological manipulation, Plynlimon, mid-Wales, UK, it was reported that concentrations of DOC leaching from a drained peatland were 20 times higher under rewetting conditions (Fenner *et al.*, 2001).

In Finland, peatlands occupy approximately 30% (30.5 million ha) of the country's land area (Tomppo and Henttonen, 1996), but a large proportion can no longer be classified as pristine. Turunen (2008) presented a comprehensive review of C storage in Finnish peatlands. The study showed that drainage for forestry has been the most dominant land use activity to take place, with 55% of mires having undergone this change as of 2000. Only 38% of peatlands are now regarded as pristine. The sites where drainage of peatland for forestry have occurred have enabled studies into the impacts of the long term water-table draw down on peatland C storage and some of the most detailed studies on the impact of peat drainage on the dynamics of C have been performed in Finland. Turunen (2008) reported that 5304 Tg of C was stored in peat in 2000, a decrease of 73 Tg from 1950. Given the extensive and widespread drainage that has occurred on Finnish peatlands since 1950 this could be considered a relatively small loss of C. Few studies have addressed the effects of water-table drawdown on DOC production in a Finnish peatland. The only known study is that of Sallantaus (1992), who reported fluxes of 8.0 and 14.1 g m^{-2} a^{-1} for pristine and drained fen catchments and 12.4 and 16.6 g m^{-2} a^{-1} for pristine and drained bog catchments, indicating a greater magnitude of DOC release for the drainage of more nutrient poor sites. Drainage of peatlands for forestry in Finland has typically occurred to a depth of 10-40 cm (Laine *et al.*, 2004). According to Wallage *et al.* (2006), this is the zone of greatest DOC production in a peatland, therefore past peatland management practises in Finland have potentially mobilised a large store of leachable C.

This chapter describes the implications of long-term drainage on the export potential of DOC from pristine and artificially drained peatlands in Finland. The study took place across a nutrient gradient and in areas of peatland drained 9 and 48 years ago. Thus, we have addressed DOC

release due to long-term anthropogenically caused water-table drawdown, which has been achieved through an extensive network of drainage channels to enable forest growth. Climate change is expected to lower soil water-tables in northern hemisphere peatlands due to increased evaporation and reduced precipitation (Mitchell and Warrilow, 1987; Gitay *et al.*, 2001), although predictions of the potential magnitude of this change are limited. Roulet *et al.* (2006) have predicted a decrease of 14-22 cm in the height of the water-table in boreal fens for a doubling of the atmospheric CO_2 concentrations. This is similar to the water-table drawdown induced by forestry drainage, therefore sites that have been drained for forestry could be used as a proxy for gaining insights into the effects of climate change. In addition to measurements of DOC export potential, analyses of soil enzymes were also undertaken given their role as key regulators of organic matter decomposition and their potential to mobilise DOC from the organic matrix of peat soils (Fenner *et al.*, 2005).

5.3 Materials and Methods

5.3.1 Study site and sampling regime

The study was carried out at Lakkasuo mire (61°48'N, 24 °19'E, ca. 150m a.s.l), a raised bog complex in central southern Finland. Detailed site descriptions are given in Turunen and Moore (2003) and Jaatinen *et al.* (2007). Lakkasuo is an eccentric, 'raised', bog which exists on a nutrient gradient from ombrotrophy to mineratrophy; 56% of the mire is ombrotrophic bog, the remainder is minerotrophic fen. Within the fen exists both oligotrophic and mesotrophic areas of peat. Half of the peatland was drained in 1961 to promote forest growth, which has reduced the water-table height by 10 cm (bog) to 40 cm (fen) below that for the pristine part (Jaatinen *et al.*, 2007). In the undrained part, a series of experimental water-table manipulations took place in 2001 to mimic water-table drawdown that is predicted for peatlands this century due to climate change (Laine *et al.*, 2004). Drainage ditches 40 cm deep have lowered the water table by approximately 10 cm (bog) to 20 cm (fen) at these sites (Jaatinen *et al.*, 2007).

This study therefore compared pristine (P) mires from each of the three nutrient types; ombrotrophic bog (OM), oligotrophic fen (OL) and mesotrophic fen (ME) and assessed the impact of 9 years and 48 years of drainage. Sampling was undertaken for 3 days in April 2007. At each site, 4 replicate soil samples were collected from a depth of 12 cm, the top 10 cm was discarded and the remaining 2 cm (approximately 100 ml) was sealed in plastic bags. All samples were transported back to the UK, where they were stored at field temperature until further analysis. Water homogenates of each soil sample were prepared within one week of sample collection by adding 27 ml of ultra-pure water to 3 cm^3 of soil, homogenising the sample in a stomacher and centrifuging at 10,000 rpm for 5 minutes. The pH of an aliquot of the soil waters was determined before the remaining supernatant was filtered through 0.45 μm filters.

5.3.2 DOC characteristics and ion concentrations

DOC concentrations, UV absorbance and Specific UV absorbance (SUVA) were determined on the water-extractable solutions. DOC was measured using an Analytical Sciences Ltd Thermalox TOC analyser. UV absorbance (UV-254) was measured at 254 nm through a 1 cm quartz cell on a Uvikon 943 UV/Vis spectrophotometer, with ultra-pure water used to zero the baseline. SUVA was calculated as (UV-254 × 100)/DOC, with the units L mg^{-1} m^{-1}. Nitrate and total base cation (Na^+, K^+, $Mg2^+$ and $Ca2^+$) concentrations were determined on a Dionex DX-120 ion chromatograph, using AS14A and CS12 columns.

5.3.3 Soil enzyme activities

The activities of five extracellular hydrolase enzymes (β-glucosidase, arylsulphatase, phosphatase, β-xylosidase and N-acetyl-β-glucosaminidase) were assayed using a modified version of the methods developed by Freeman *et al.* (1995) and Kang and Freeman (1999). MUF-free acid (4-Methylumbelliferone) and MUF enzyme substrates (400 μM MUF-β-glucopyranoside, MUF-sulphate, MUF-β-xylopyranoside, MUF N-acetyl-β-glucosaminide and 200 μM MUF-phosphate) were dissolved in methyl cellosolve (ethylene glycol monomethyl ether) overnight and diluted in deionised water. For each sample, 1 cm^3 samples of peat were prepared into two separate stomacher bags and 7 mL of deionised water added to one (standard) and 7 mL of enzyme substrate added to the other. The samples were then homogenised for 60 seconds (Seward Colworth model 400) and left to incubate at field temperature for 1 hour (45 minutes for phosphatase). Following incubation, 1.5 mL of solution from stomacher bag was centrifuged at 10,000 rpm for 5 minutes. For the standards, a standard curve was prepared by transferring 250 μL of the supernatant from each vial into six separate wells on a black fluorescence microplate. Fifty μL of varying concentrations of MUF free acid solution were added to the supernatant to create a concentration curve of 0-100 μM, from which the enzyme activity was determined. For the samples with added substrate, 300 μL of supernatant was transferred to the microplate. The

fluorescence of the samples was measured at 450 nm emission and 330 nm excitation, with a slit setting of 2.5, on a BMG Fluostar Galaxy fluorimeter. Values of enzyme activity were automatically calculated from the standard curve by the software program. Enzyme activities were expressed as MUF produced ($\mu M\ g^{-1}\ min^{-1}$), normalising to the dry weight of the soil. Soil dry weight, gravimetric water content and soil organic matter (loss on ignition) were determined by heating replicates of 1 cm^3 of peat at 105°C and 550°C for 24 hours each in a Carbolite muffle furnace and calculating the weight loss.

Phenol oxidase was assayed using a modified version of the method developed by Pind *et al.* (1994). For each sample, 1 cm^3 of peat were placed in six separate stomacher bags, 9 ml of deionised water was added and the contents homogenised for 60 seconds. Of this homogenate, 300 µL was transferred to separate centrifuge vials and 750 µL of deionised water was added to 3 (blanks) and 750 µL of 10 mM L-DOPA (dihydroxyphenylalanine) solution was added to the other 3 (substrate). The samples were incubated at field temperature for 9 minutes and then all vials were centrifuged at 10,000 rpm for 5 minutes. Of the supernatant, 300 µL was transferred to wells of a clear microplate, and the absorbance read at 460 nm. The activity of phenol oxidase was calculated by subtracting the mean blank value from the mean substrate value and using Beers Law, the molar absorbency coefficient for the L-DOPA product 3-dihydroindole-5,6-quinone-2-carboxylate (dicq) (3.7×10^4) and the dry weight of each sample. Phenol oxidase activity is expressed as $\mu mol\ dicq\ g^{-1}\ min^{-1}$.

5.3.4 Statistical analyses

The data was tested for significant differences between a) the pristine mires from the three contrasting nutrient types, to determine the effects of nutrient content, and between b) the undrained mires and those subject to 9 year and 48 year drainage for each nutrient type, to determine the effects of drainage. Significant differences *between* nutrient types for 9 and 48 years of drainage were not considered. One-way ANOVA with Tukey post-hoc was used to test for

differences between soil types, with p values of <0.05 deemed statistically significant. Pearson correlation was used to test for significant relationships between various soil parameters. Both statistical tests were performed using SPSS (version11). The standard error of each data point was calculated by dividing the standard deviation of the sample by the square root of the sample size.

5.4 Results

5.4.1 A comparison of soil parameters for pristine mires across an increasing nutrient

Table 5.1 provides a statistical summary of the results described below, with a number of significant differences recorded.

The pH of the water extracts differed substantially and increased significantly from 4.29 (OM) to 5.03 (OL) to 5.44 (ME) ($p<0.001$ across the three types), consistent with the increasing nutrient status of the soils (Figure 5.1).

The concentration of water-extractable DOC declined with increasing nutrient status, from 120 (OM) to 104 (OL) to 72 μg cm^{-3} (ME), although differences were not significant (Figure 5.2).

The calculated SUVA (indicator of the aromaticity of the DOC) was greatest in both the OM and ME sites (approx. 1.5 L mg^{-1} m^{-1}), compared to 1.1 L mg^{-1} m^{-1} for the OL site (Figure 5.3). However, differences were not significant.

The activity of five hydrolase enzymes, β-glucosidase, arylsulphatase, β-xylosidase, N-acetyl-β-glucosaminidase and phosphatase, all displayed contrasting trends for each site. For β-glucosidase the greatest activity was measured in the OL and OM sites (16.0 and 16.2 μmol g^{-1} min^{-1} respectively) (Figure 5.4). In the OM site the activity was approximately 50% lower, at 8.3 μmol g^{-1} min^{-1}, but this was not significantly lower than the other sites.

The activity of arylsulphatase was greatest in the OL site at 20.1 μmol g^{-1} min^{-1}, which was not significantly greater than the ME site (15.8 μmol g^{-1} min^{-1}), but was compared to the OM site (7.5 μmol g^{-1} min^{-1}) (Figure 5.5).

For β-xylosidase, the greatest activity was recorded in the OM site (4.0 μmol g^{-1} min^{-1}), followed by the ME site (3.5 μmol g^{-1} min^{-1}), with the activity lowest in the OL site (2.7 μmol g^{-1} min^{-1}), although differences were not significant (Figure 5.6).

The activity of N-acetyl-β-glucosaminidase was greatest in the OL site (10.1 μmol g^{-1} min^{-1}) (Figure 5.7). This was not significantly higher than the ME site (6.1 μmol g^{-1} min^{-1}), but was compared to the OM site (4.3 μmol g^{-1} min^{-1}).

For phosphatase, the activity increased significantly with increasing nutrient status ($p<0.01$) (Figure 5.8), from 24.3 to 34.5 to 87.3 μmol g^{-1} min^{-1} for the OM, OL and ME sites respectively.

The activity of phenol oxidase was greatest in the ME and OL sites (0.4 μmol dicq g^{-1} min^{-1}), both being significantly higher than the OM site (less than 0 - undetectable) ($p<0.01$) (Figure 5.9).

Concentrations of nitrate were greatest in the OM site (24.5 μg cm^{-3}), followed by the ME site (15.5 μg cm^{-3}) and the OL site (4.9 μg cm^{-3}), however, differences were not significant (Figure 5.10).

The concentration of base cations increased with increasing nutrient status, with mean values of 9.3, 15.6 and 10.3 μg cm^{-3} for the OM, OL and ME sites respectively (Figure 5.11).

5.4.2 The effect of 9 and 48 year drainage on soil parameters

Table 5.2 provides a statistical summary of the results described below, with a number of significant differences recorded. The pH of the OM peat did not change significantly with drainage for either site compared to the pristine mire. For the OL and ME peat, 9 year drainage significantly reduced the pH by approximately 0.2 and 1.0 units respectively. 48 year drainage of the ME peat did not change the pH significantly, but for the OL peat the pH was 0.8 lower than the pristine.

The concentration of water-extractable DOC from the OM site also did not change significantly with drainage. For the OL site, there was no effect of 9 year drainage, however, 48 year drainage significantly increased the DOC concentration to 373.5 μg cm^{-3}, compared to 104.3 μg cm^{-3} for the pristine, an almost 4-fold increase. Conversely, for the ME site, there was no effect of 48 year

drainage, but 9 year drainage significantly increased the DOC concentration to 272.8 μg cm^{-3} compared to the pristine peat, 72.1 μg cm^{-3}, also a near 4-fold increase.

For the OM site, the SUVA was not significantly different for the 9 year drained site compared to the pristine, but was significantly greater for the 48 year drained site (2.2 L mg^{-1} m^{-1}). The SUVA of the OL peat was significantly higher for the 9 year drained site (2.3 L mg^{-1} m^{-1}) compared to the pristine and significantly higher again for the 48 year drained site (3.2 L mg^{-1} m^{-1}). In the ME site the SUVA of the 9 and 48 years sites were both significantly higher than the pristine site, at 2.8 L mg^{-1} m^{-1} and 2.2 L mg^{-1} m^{-1} respectively.

The activity of the five hydrolase enzymes again displayed contrasting trends for each site. For β-glucosidase in the OM site, the activity in the 48 year drained site was significantly greater (+85%) than in the pristine and 9 year drained sites. In the OL and ME sites the opposite was true, with the activity in the 48 year drained site being significantly lower than that in the pristine peat (50% and 60% respectively). The 9 year drained site also had a significantly lower activity compared to the pristine for the ME peat.

Drainage of the OM site significantly reduced the activity of arylsulphatase for 9 year drainage compared to the pristine peat (-28%), but not for the 48 year site. Both 9 and 48 year drainage significantly reduced the activity for the OL and ME sites (approximately -75%), although there were no significant effect of 48 over 9 years of drainage.

The only significant effect of drainage on the activity of β-xylosidase was for the OM site, where 48 years of drainage significantly increased the enzyme's activity compared to the pristine and 9 year drained peat (+65%).

For N-acetyl-β-glucosaminidase, there was no effect of drainage on the activity in the OM site. However, for the OL and ME sites, both 9 and 48 year drainage significantly reduced the enzyme's activity by approximately 50%.

The activity of phosphatase showed the greatest variance amongst drainage regimes and nutrient availability. For the OM site, there was no significant effect of drainage. For the OL site, 9 year and 48 year drainage significantly reduced the activity of phosphatase by approximately 60%. The activity in the ME site was significantly reduced by 70% and 93% in the 9 and 48 year drainage sites respectively.

The activity of phenol oxidase in the OM site did not change significantly with drainage, with the enzyme generally being undetectable. In OL site, the enzyme was strongly affected by drainage, but only significantly for the 48 year drained site (approximate 95% reduction). There was no significant effect of drainage on phenol oxidase activity in the ME site.

For all three sites the concentration of nitrate decreased with the increasing length of drainage, but none of the effects were significant. In the case of the OM and ME sites, the concentration of nitrate decreased by approximately 50-80%. For the OL site, the decrease was a little greater overall, at 65-90%.

The total concentration of base cations decreased by approximately 45-50% for the OM and ME sites with 9 year drainage; the decrease was approximately 20% for the OL site. Drainage for 48 years reduced the base cation concentration by approximately 45% for the OM site and 30% for the ME site, with little overall change for the OL site.

5.4.3 Correlations between soil parameters

The soil parameter that best explained the variation in DOC export potential across all soil types was the water content of the soil (Figure 5.12). Pearson correlation gave a correlation coefficient of R^2=-0.587 (p<0.001). There was also a significant relationship between the soil water content and the SUVA of the water-extractable solution (Figure 5.13; R^2=-0.630; p<0.001). In addition to the soil water content, soil pH also correlated significantly, but weakly, with the DOC concentration of the extracted solution (Figure 5.14), with an R^2=-0.305 (p<0.001). Although

there was a lot of variability in the replicate data, especially for the more acidic pH values, the 25% highest DOC concentrations were recorded for a pH below 4.5. Soil pH also correlated significantly with the activity of phenol oxidase (Figure 5.15); with an R^2=0.533 (p<0.001). There was no significant correlation between the water content of the soil and the activity of phenol oxidase, although the enzyme appears to be most active between 85-90% water content (Figure 5.16).

Table 5.1 – Statistical comparison of the pristine Ombrotrophic (OM), Oligotrophic (OL) and Mesotrophic (ME) peat sites for a variety of soil parameters.

Soil parameter	Nutrient status comparison	Significant?	Significance level
pH	OM v OL	Y	<0.001
	OM v ME	Y	<0.001
	OL v ME	Y	<0.01
DOC	OM v OL	N	
	OM v ME	N	
	OL v ME	N	
SUVA	OM v OL	N	
	OM v ME	N	
	OL v ME	N	
β-glucosidase	OM v OL	N	
	OM v ME	N	
	OL v ME	N	
Arylsulphatase	OM v OL	Y	<0.05
	OM v ME	N	
	OL v ME	N	
β-xylosidase	OM v OL	N	
	OM v ME	N	
	OL v ME	N	
N-acetyl-β-glucosaminidase	OM v OL	Y	<0.05
	OM v ME	N	
	OL v ME	N	
Phosphatase	OM v OL	N	
	OM v ME	Y	<0.001
	OL v ME	Y	<0.01
Phenol oxidase	OM v OL	Y	<0.01
	OM v ME	Y	<0.01
	OL v ME	N	
Nitrate	OM v OL	N	
	OM v ME	N	
	OL v ME	N	

Table 5.2 – Statistical comparison of 9 and 48 year drainage with pristine peat for Ombrotrophic (OM), Oligotrophic (OL) and Mesotrophic (ME) peat sites for a variety of soil parameters.

Soil parameter	Nutrient status	Drainage history comparison (vs pristine)	Significant?	Significance level
pH	OM	9 year	N	
		48 year	N	
	OL	9 year	Y	<0.05
		48 year	Y	<0.001
	ME	9 year	Y	<0.001
		48 year	N	
DOC	OM	9 year	N	
		48 year	N	
	OL	9 year	N	
		48 year	Y	<0.001
	ME	9 year	Y	<0.01
		48 year	N	
SUVA	OM	9 year	N	
		48 year	Y	<0.05
	OL	9 year	Y	<0.001
		48 year	Y	<0.001
	ME	9 year	Y	<0.05
		48 year	Y	<0.05
β-glucosidase	OM	9 year	N	
		48 year	Y	<0.01
	OL	9 year	N	
		48 year	Y	<0.05
	ME	9 year	Y	<0.05
		48 year	Y	<0.05
Arylsylphatse	OM	9 year	Y	<0.05
		48 year	N	
	OL	9 year	Y	<0.01
		48 year	Y	<0.05
	ME	9 year	Y	<0.001
		48 year	Y	<0.001
β-xylosidase	OM	9 year	N	
		48 year	Y	<0.01
	OL	9 year	N	
		48 year	N	
	ME	9 year	N	
		48 year	N	

Table 5.2 (cont...) – Statistical comparison of 9 and 48 year drainage with pristine peat for Ombrotrophic (OM), Oligotrophic (OL) and Mesotrophic (ME) peat sites for a variety of soil parameters.

N-acetyl-β-glucosaminidase	OM	9 year	N	
		48 year	N	
	OL	9 year	Y	<0.01
		48 year	Y	<0.05
	ME	9 year	Y	<0.001
		48 year	Y	<0.01
Phosphatase	OM	9 year	N	
		48 year	N	
	OL	9 year	Y	<0.01
		48 year	Y	<0.05
	ME	9 year	Y	<0.001
		48 year	Y	<0.01
Phenol oxidase	OM	9 year	N	
		48 year	N	
	OL	9 year	N	
		48 year	Y	<0.01
	ME	9 year	N	
		48 year	N	
Nitrate	OM	9 year	N	
		48 year	N	
	OL	9 year	N	
		48 year	N	
	ME	9 year	N	
		48 year	N	

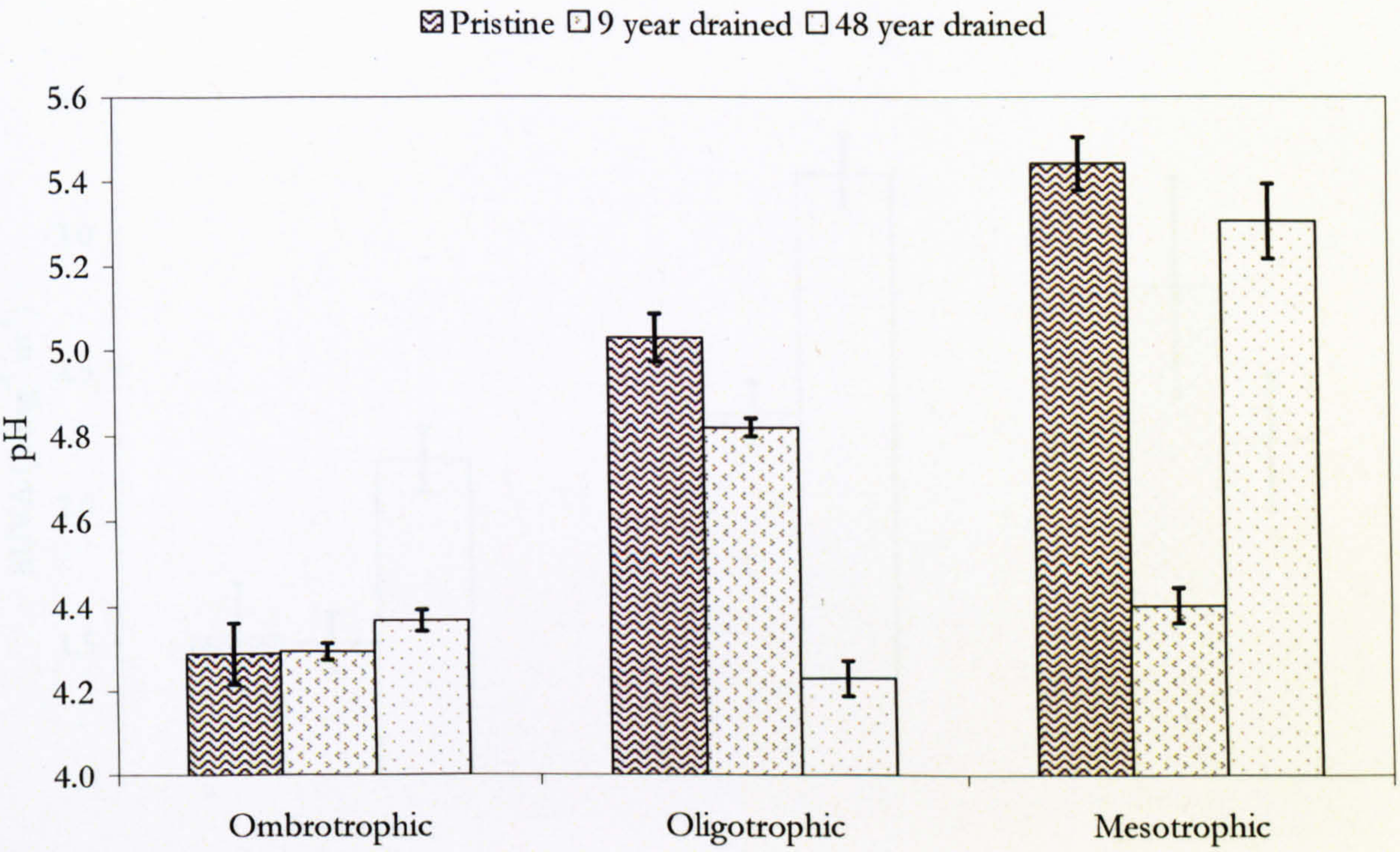


Figure 5.1 – Comparison of the water-extractable pH from the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

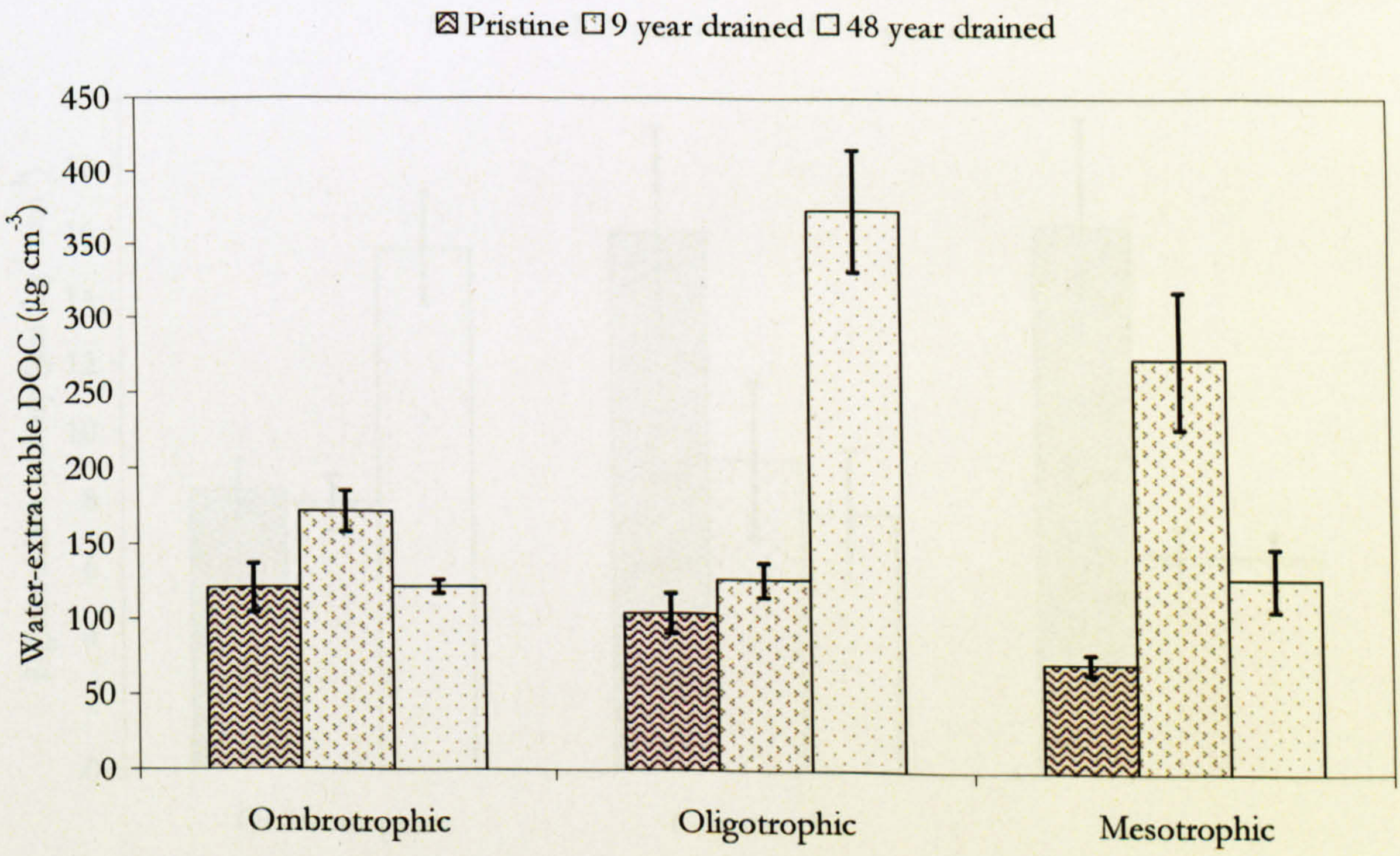


Figure 5.2 – Comparison of the water-extractable DOC concentrations from the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

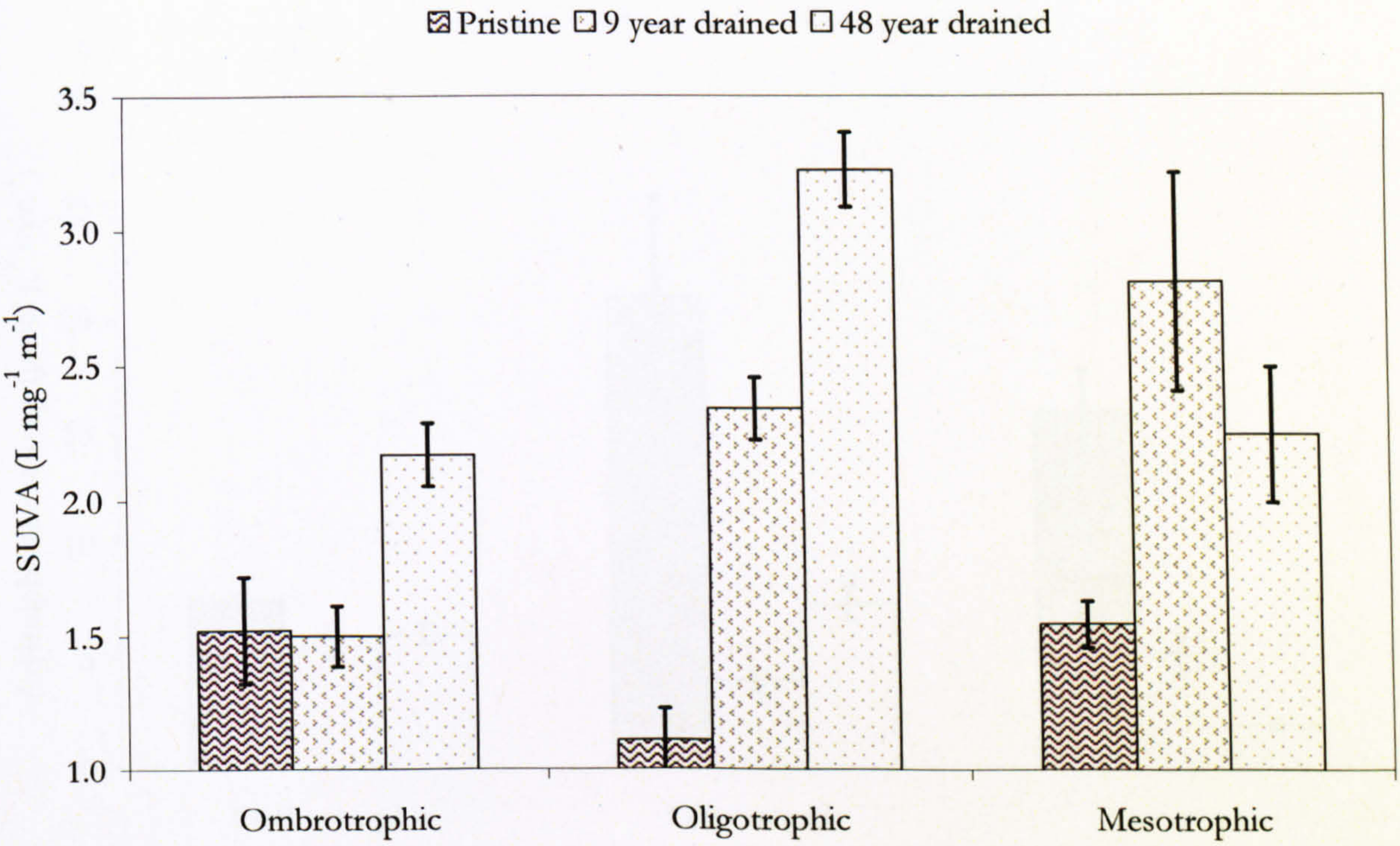


Figure 5.3 – Comparison of the SUVA of the water-extractable solutions from the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

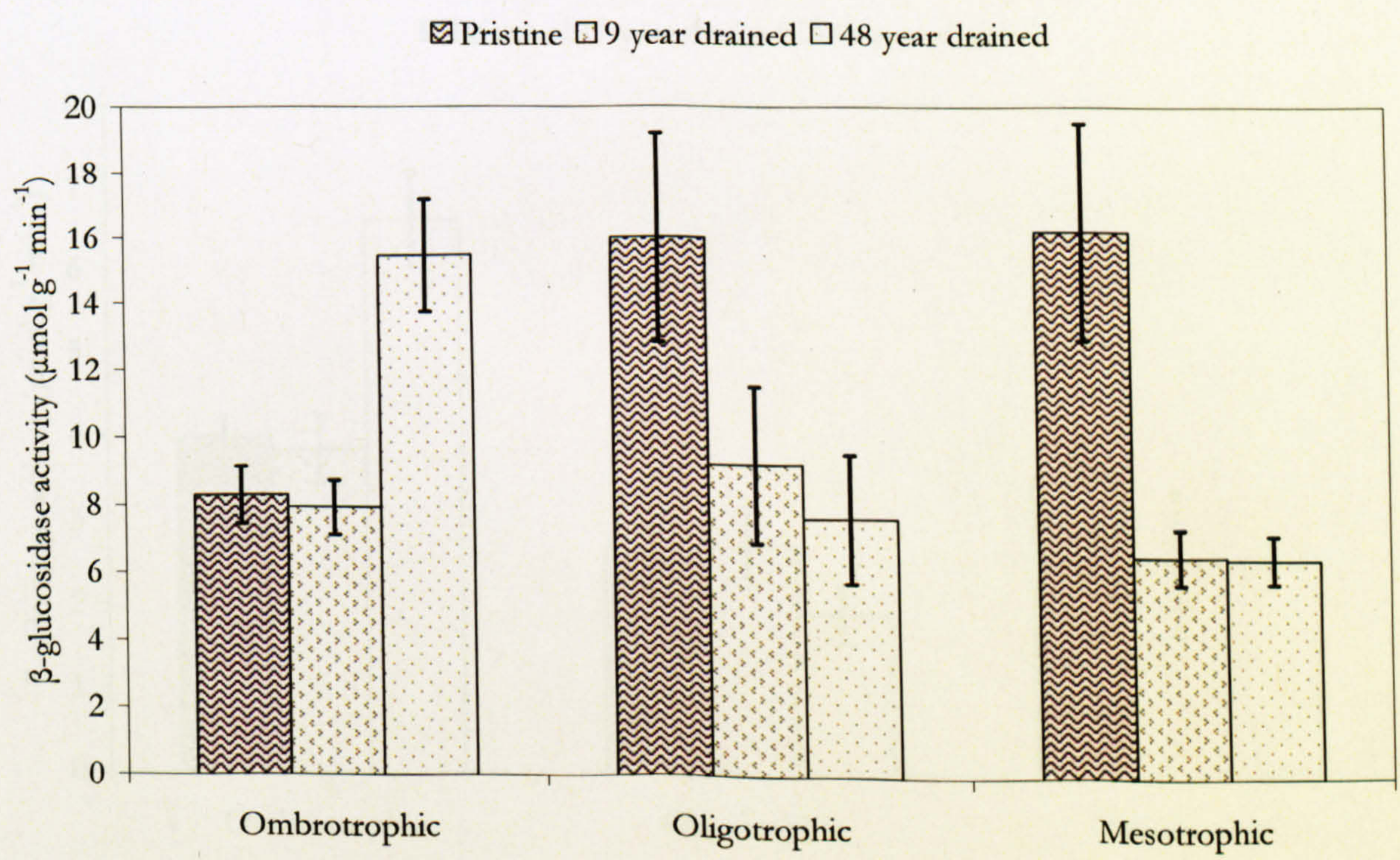


Figure 5.4 – Comparison of β-glucosidase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

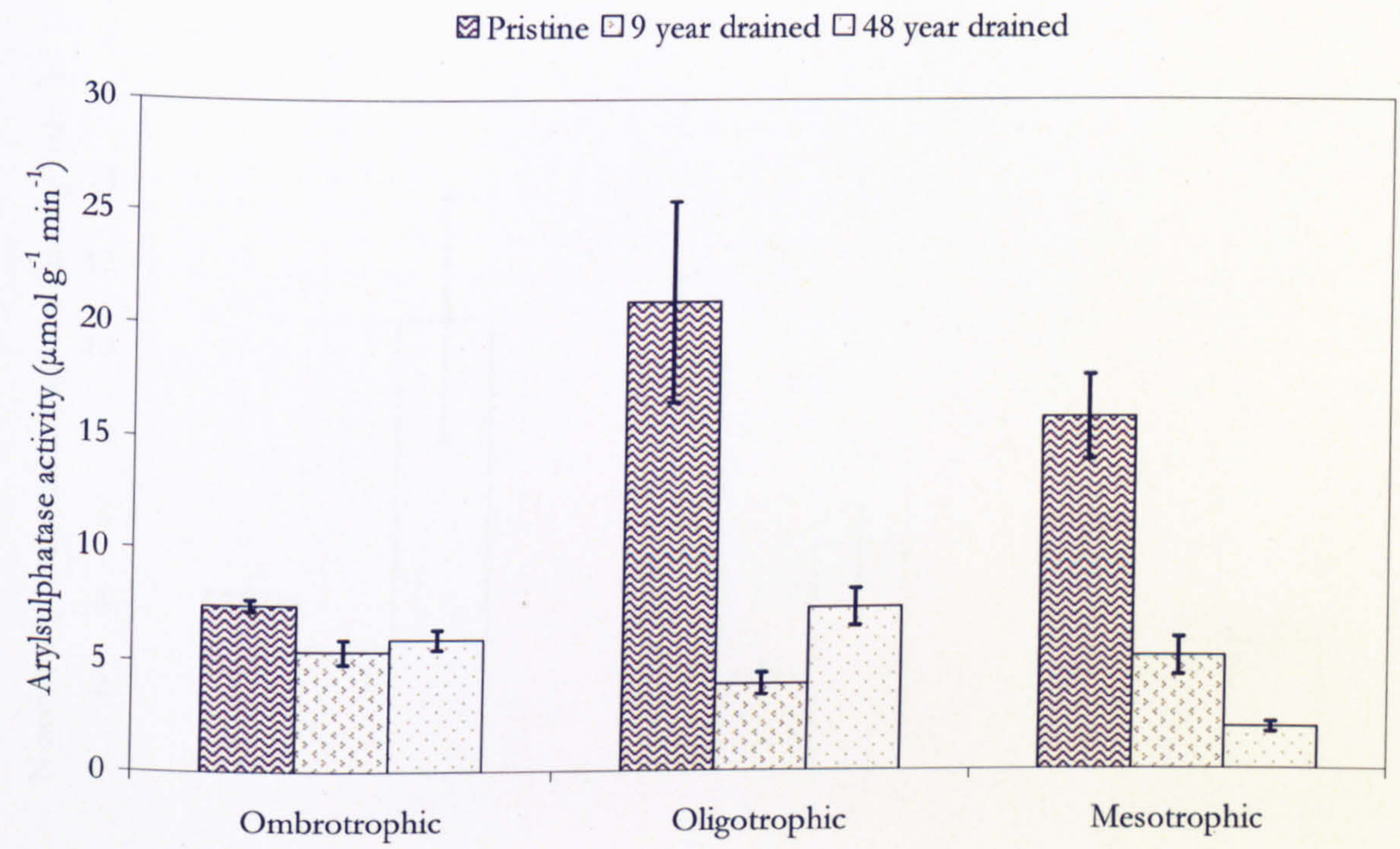


Figure 5.5 – Comparison of arylsulphatase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

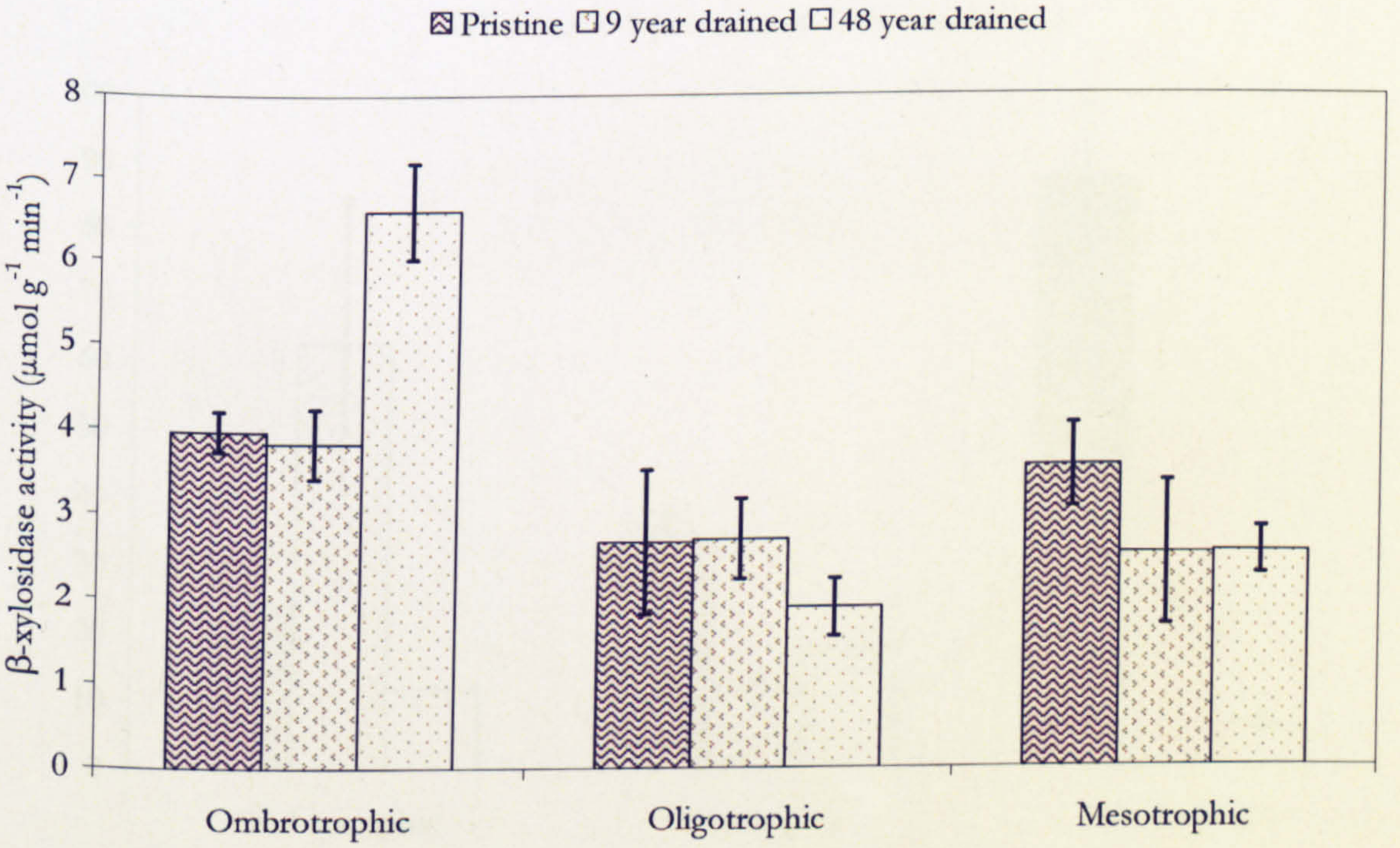


Figure 5.6 – Comparison of β-xylosidase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

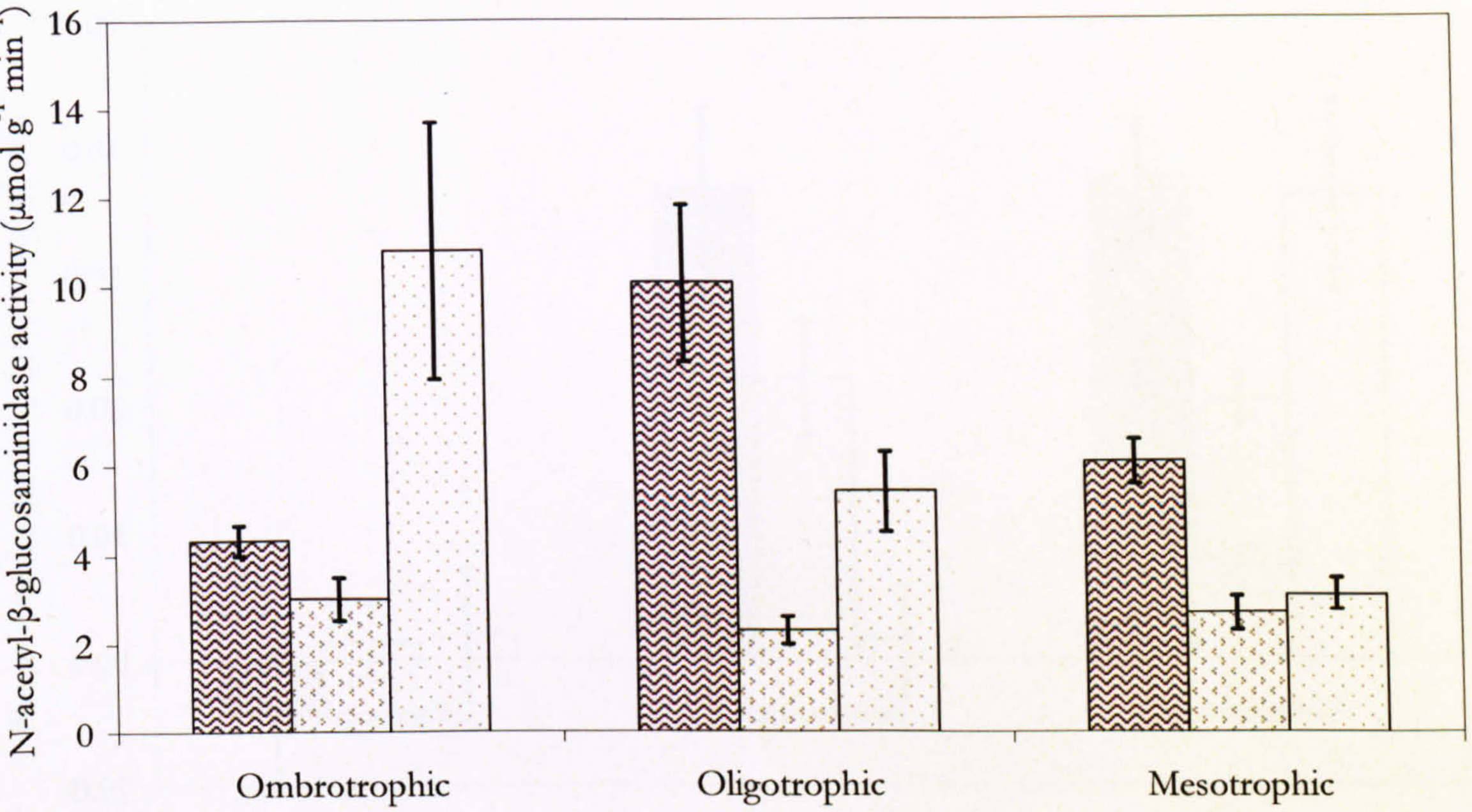


Figure 5.7 – Comparison of N-acetyl-β-glucosaminidase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

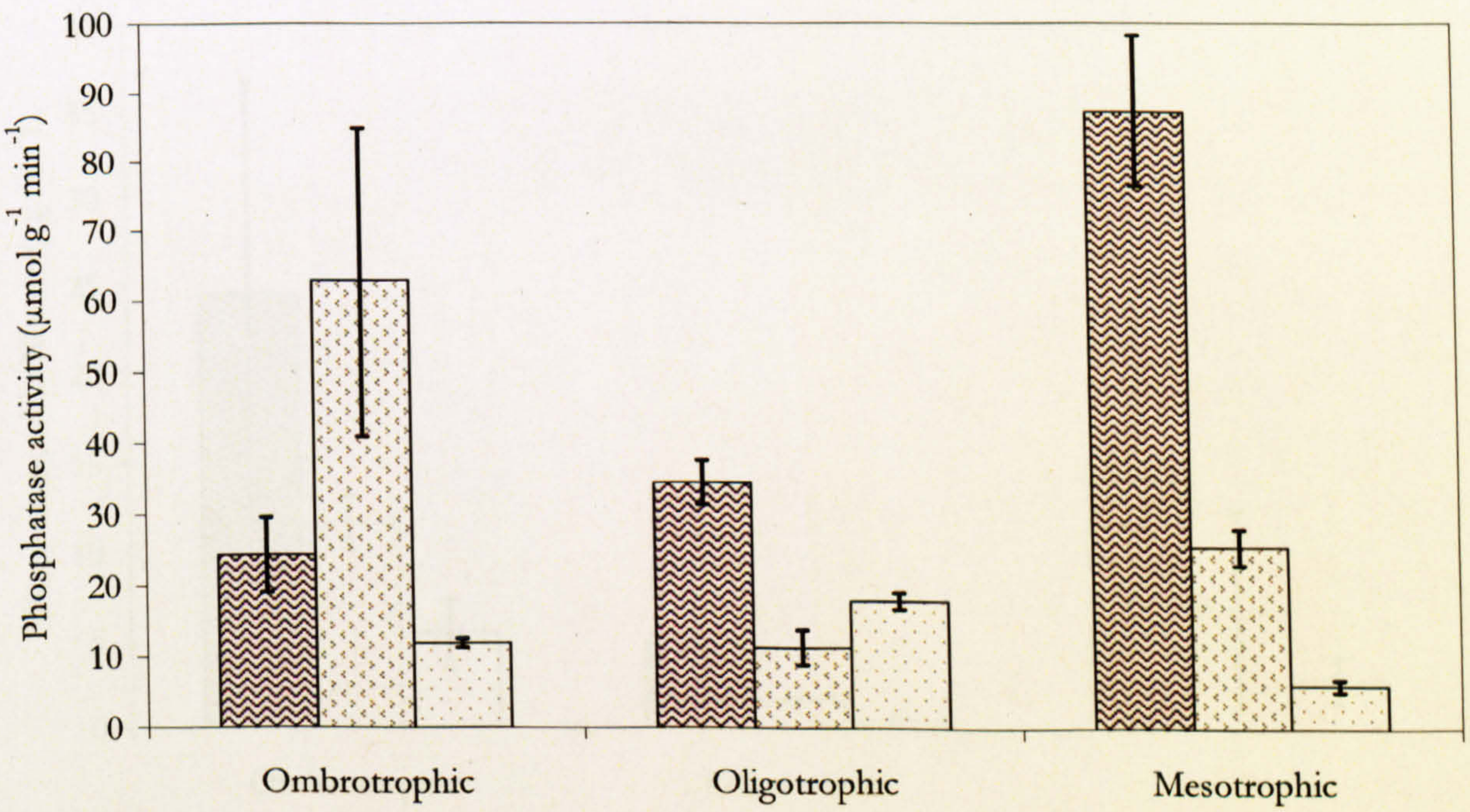


Figure 5.8 – Comparison of phosphatase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

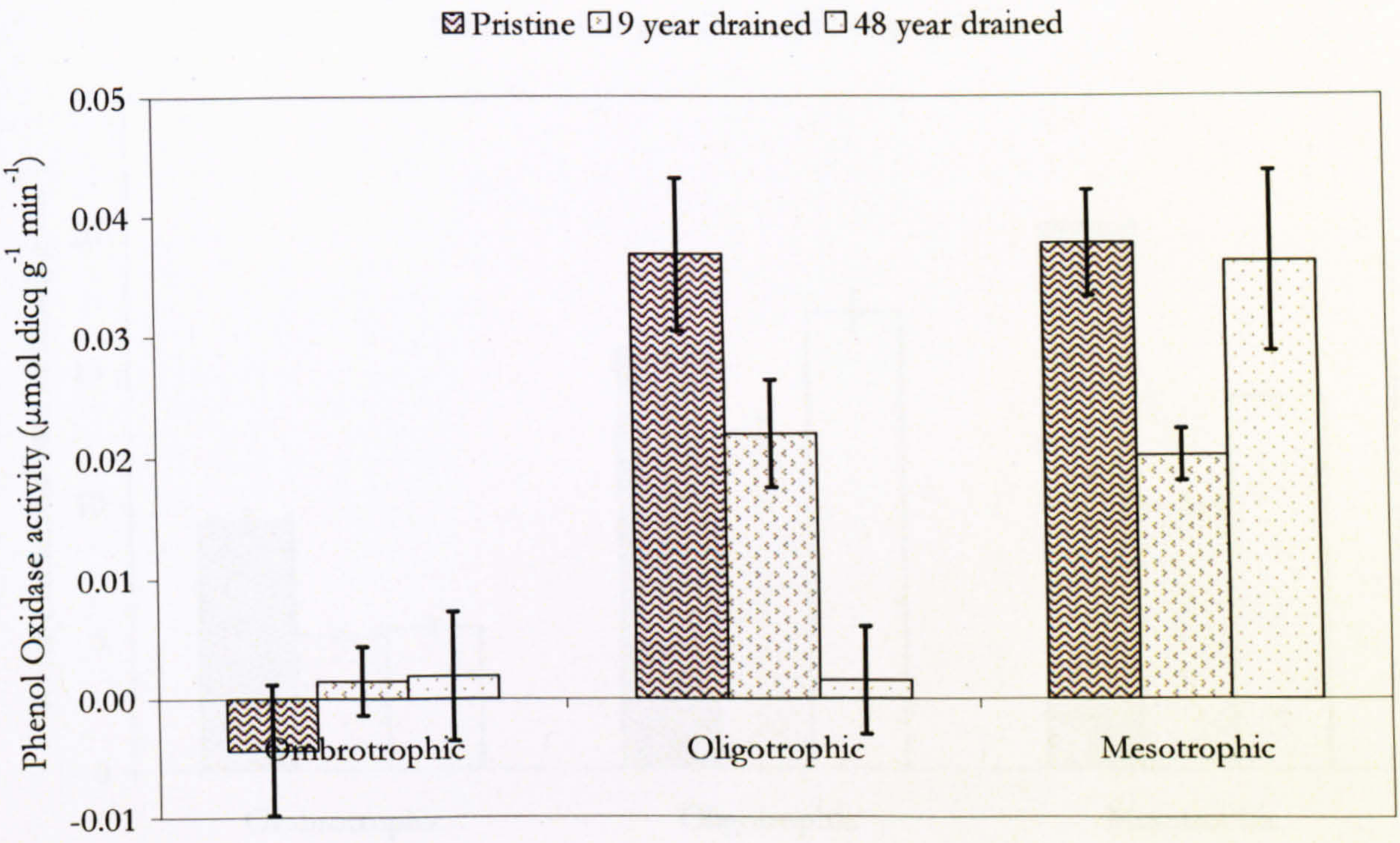


Figure 5.9 – Comparison of phenol oxidase activity at 10 cm depth in the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

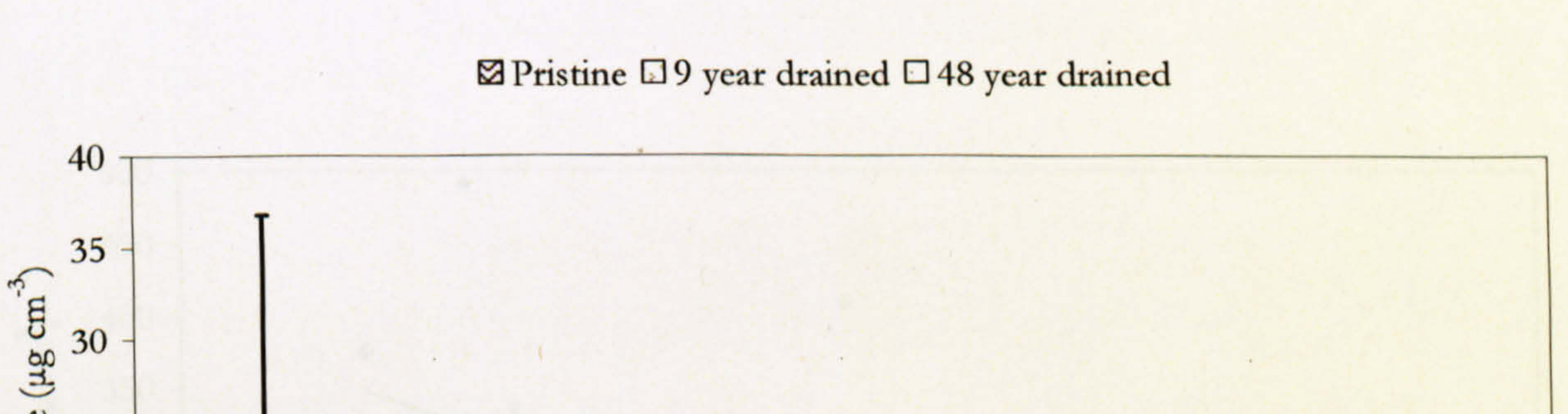


Figure 5.10 – Comparison of water-extractable nitrate concentrations from the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

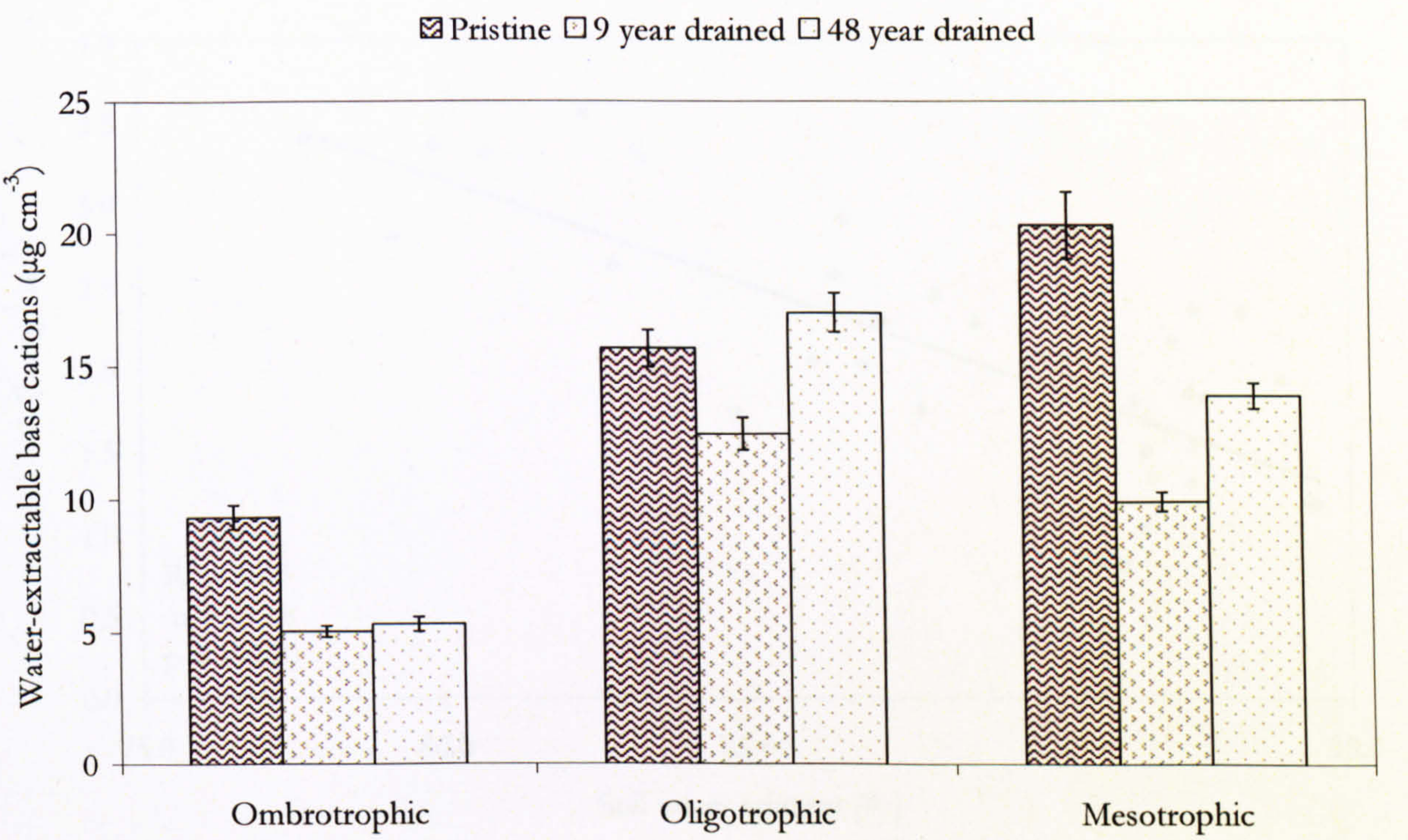


Figure 5.11 – Comparison of water-extractable base cation concentrations from the three contrasting nutrient and drainage regimes. Values are means of the samples for each site ± standard error (n = 4)

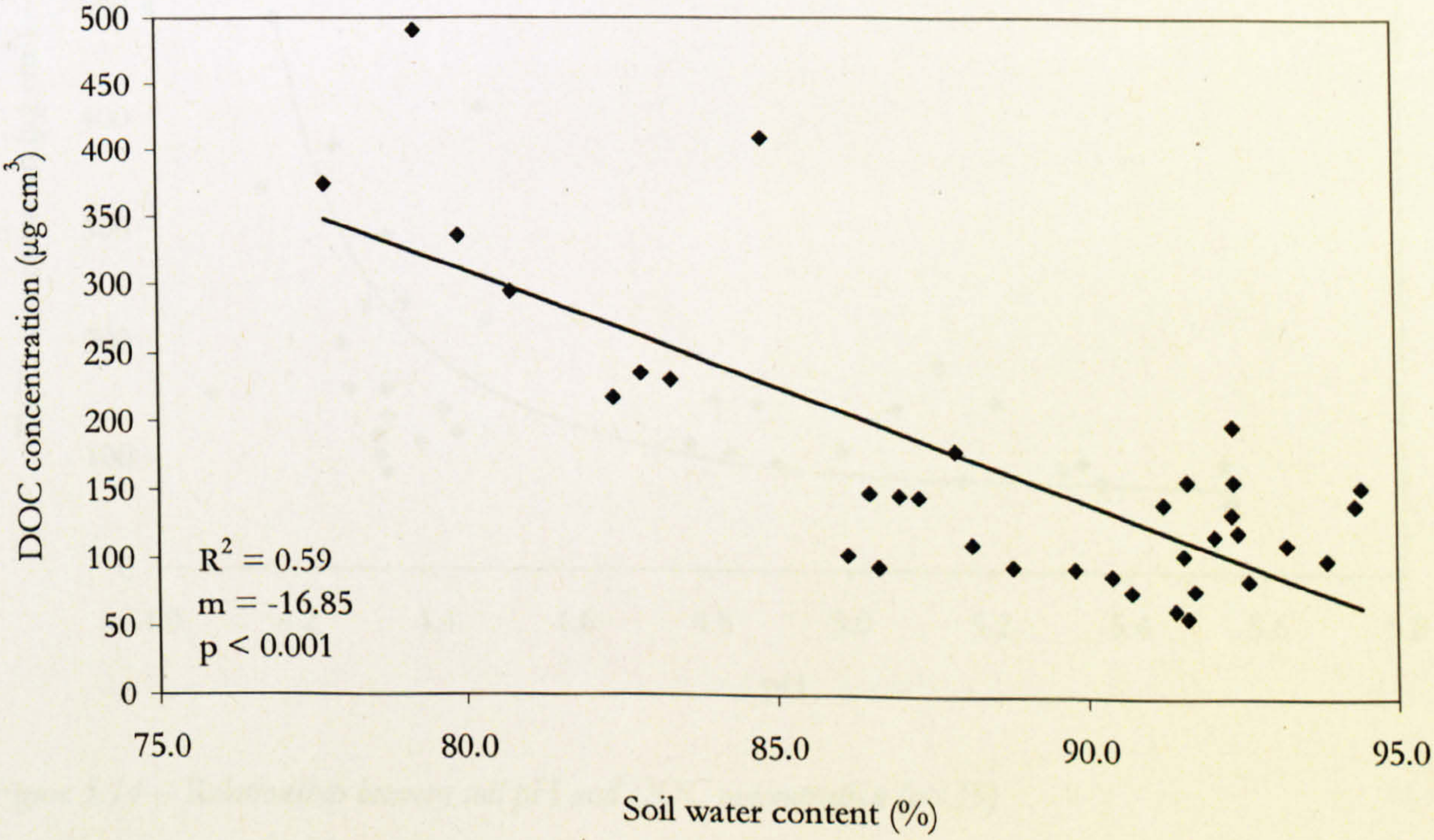


Figure 5.12 – Linear relationship between soil water content and DOC concentration (n=35)

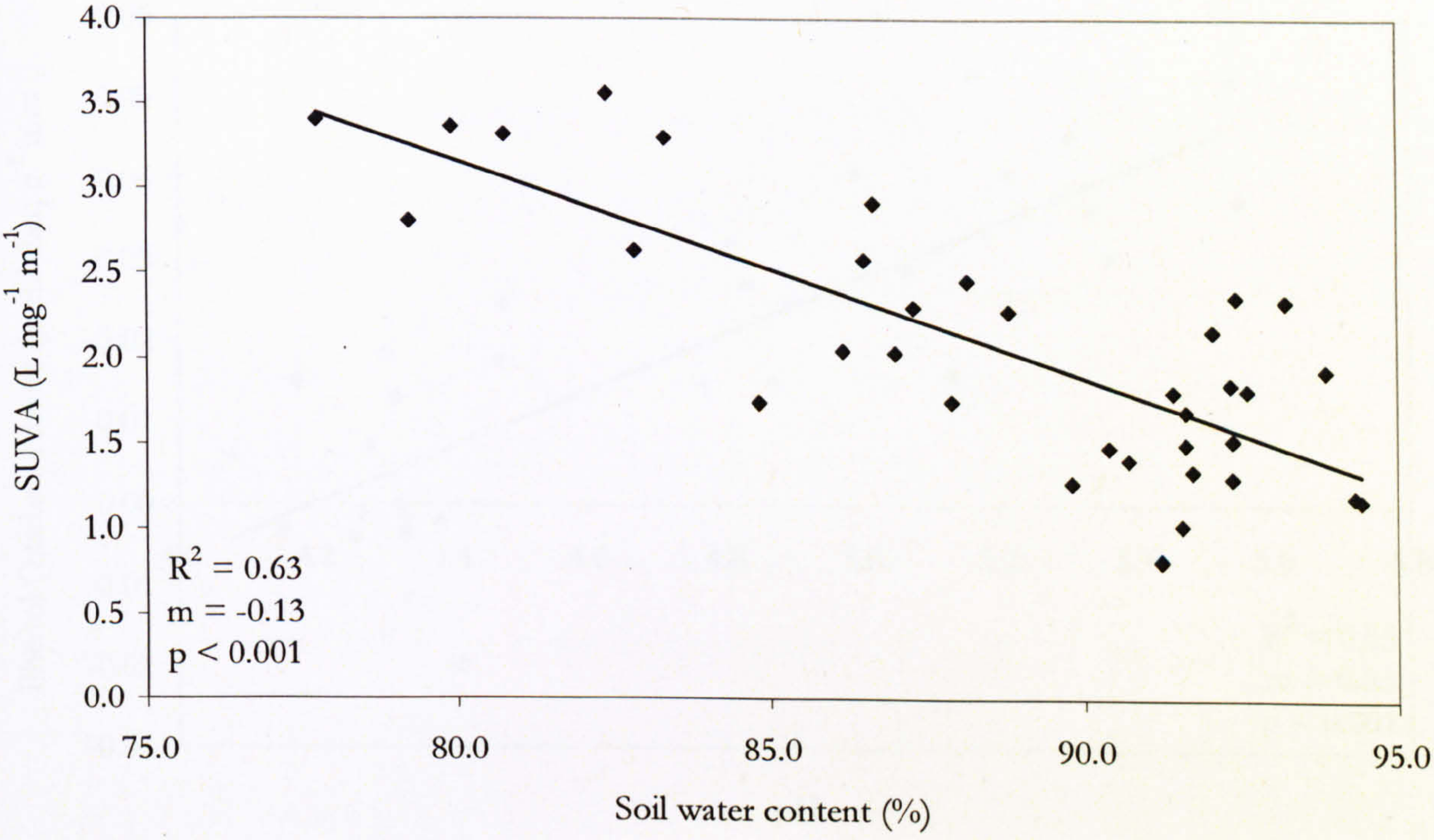


Figure 5.13 – Linear relationship between soil water content and SUVA (n=35)

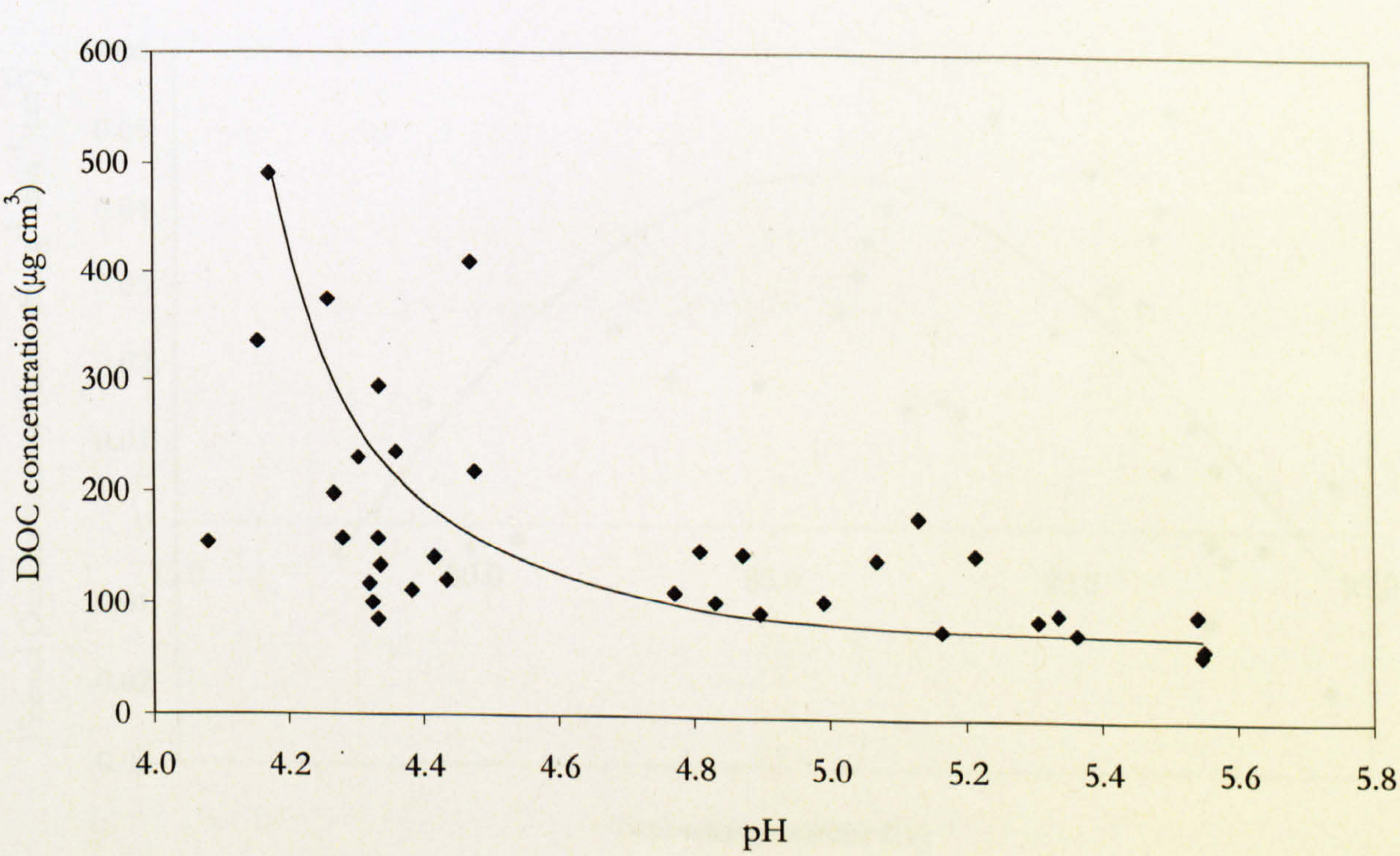


Figure 5.14 – Relationship between soil pH and DOC concentration (n=35)

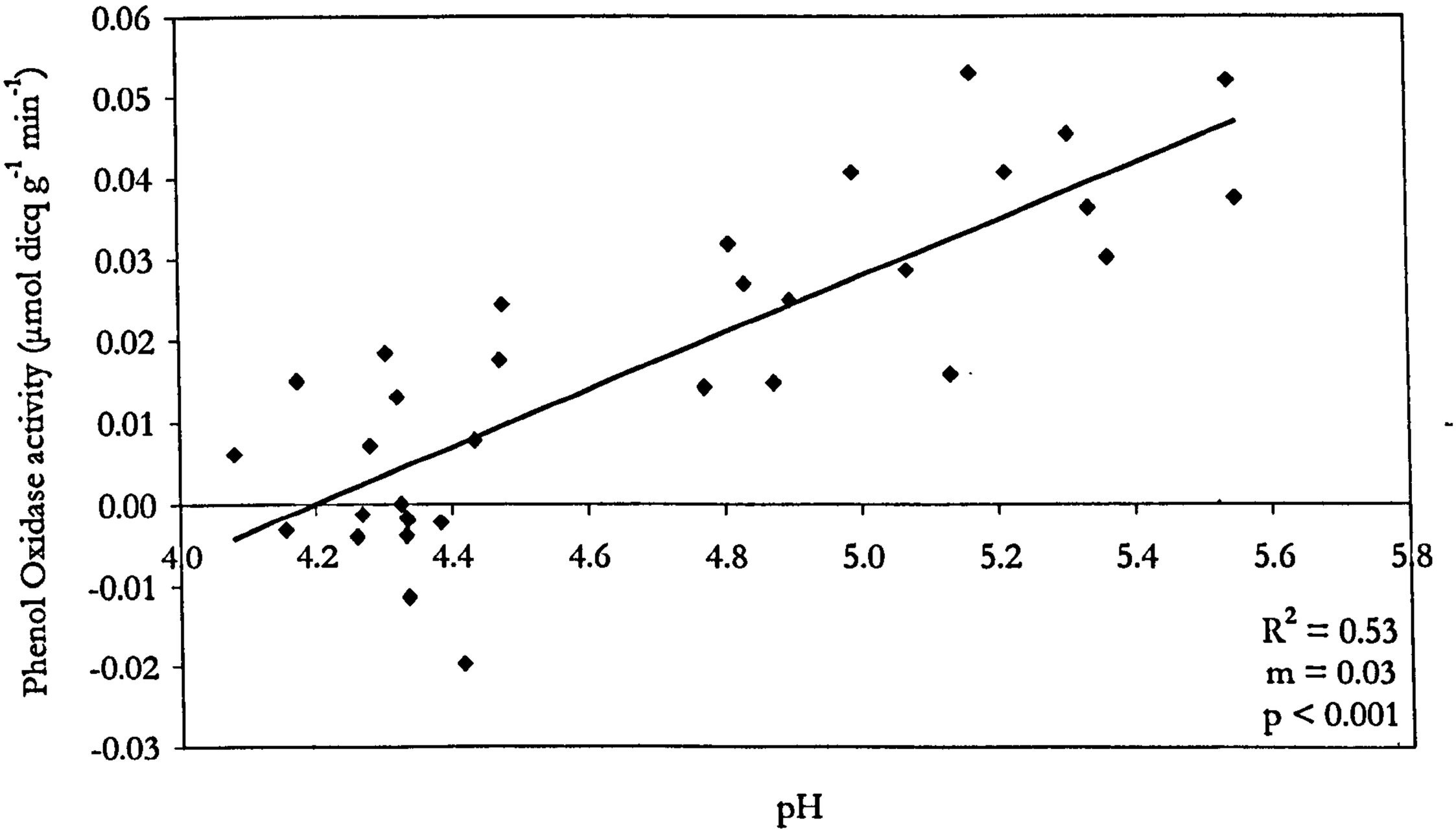


Figure 5.15 – Linear relationship between soil pH and phenol oxidase activity (n=35)

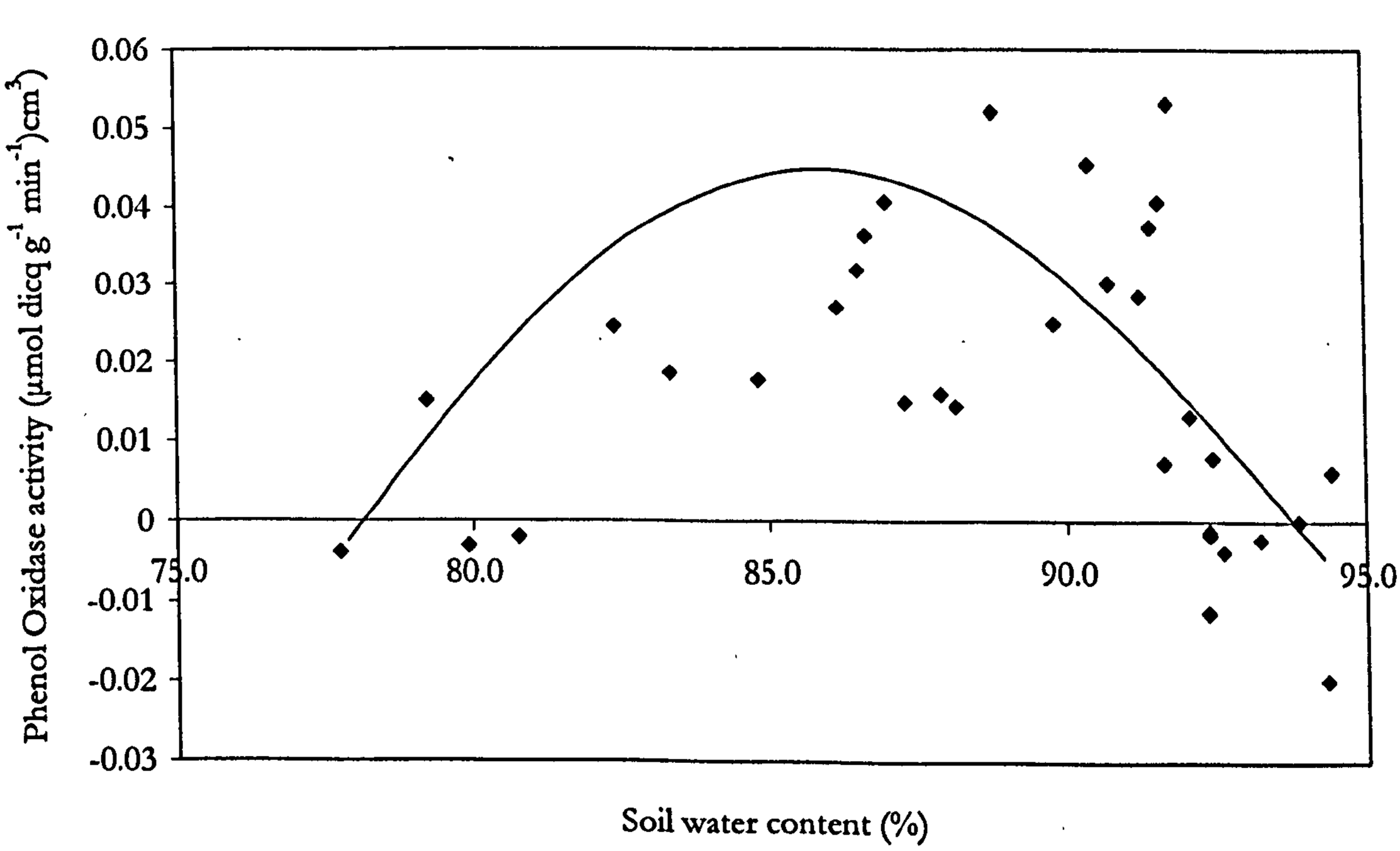


Figure 5.16 – Relationship between soil water content and phenol oxidase activity (n=35)

5.5 Discussion

The principle aim of this study was to determine the impact of long-term drainage on the potential export of DOC from a typical boreal peatland across a nutrient gradient. Despite the widespread occurrence of peatland drainage in many northern hemisphere countries, relatively few studies have addressed the issue of DOC release over long time scales. As peatlands in a natural state are known to be significant sources of DOC to freshwaters (e.g. Mattsson *et al.*, 2005); a greater understanding of processes influencing DOC export from drained peatlands is needed. No significant difference in the DOC export potential of the pristine mires across the nutrient gradient was recorded, suggesting the availability of nutrients, and resulting influences on other soil characteristics (such as pH), did not influence processes which release DOC at this study site. The greatest DOC export potential was found in the OL 48 year drained site, with a high value also recorded for the ME 9 year drained site. This suggests that the forestry and experimental drainage that has been undertaken in Lakkasuo peatland has increased the leaching of DOC to freshwaters, but the effect has not been consistent with nutrient status or drainage history (i.e. there was no significant effect on DOC export potential in the OL 9 year and ME 48 year sites). Rather, the parameter of greatest influence on DOC export potential suggested by this study was the water content of the soil, i.e. the lower the water-table, the greater the potential for DOC release from the peat matrix. Lowered watered-tables have been directly associated with increased CO_2 release due to a stimulation of peat decomposition (e.g. Moore and Knowles, 1989; Freeman *et al.*, 1993*c*; Dinsmore *et al.*, 2009), but there is less evidence of the impacts on DOC release. This study has demonstrated that the lower the water-table the greater the potential for DOC release and confirms the findings of Sallantaus (1992) that the drained peatlands at Lakkasuo are releasing more DOC than the areas of the peatland that have been left undisturbed. A 5% drop in the water content of the soil lead to an approximate 80 $\mu g\ cm^3$ increase in the DOC export potential of the soil. Increased release of DOC with reduced water

content of the soil is entirely plausible given water-table drawdown should also lead to increased aeration of the soil, a reduction in anoxia and stimulated decomposition of the peat matrix. The activity of enzymes were measured to assess rates of decomposition in the soil; the hydrolytic enzymes are the main mediators of organic matter degradation in soils and control the rate of both decomposition and the release of substances that can become available for microbial and plant uptake (Marx *et al.*, 2001), whilst phenol oxidase has been proposed as a regulator of C storage in peatlands (Freeman *et al.*, 2004*b*). Freeman *et al.* (2001a) proposed that phenol oxidase acts as an 'enzymic latch' in peat soil, whereby a lack of oxygen inhibits the enzyme, leading to a build up of inhibitory phenolic C compounds, which suppress the hydrolytic enzymes that can mobilise C from the peat matrix (Fenner *et al.*, 2005).

Regarding phenol oxidase, the enzyme's activity was influenced more by soil pH than the water content of the soil, confirming previous findings that low pH inhibits the activity of this enzyme (Ruggiero and Radogna, 1984; Pind *et al.*, 1994) and that of Williams *et al.* (2000), who reported pH to be a more important regulator of phenol oxidase activity than changes in aeration associated with change in water-table in a North American field site. Therefore, at locations where drainage has occurred and the pH has fallen, the enzymic latch is likely to operate by suppressing organic matter decomposition through acidity constraints, rather than by stimulating decomposition due to increased aeration. Previous studies have reported that drainage of peatlands can reduce the soil pH (Laine *et al.*, 1995), due to a reduction in inflowing groundwater, oxidation of organic and inorganic compounds and enhanced base cation uptake by trees (Laiho *et al.*, 2006). We did not observe a reduction in pH with drainage in the OM peat, but did for the more nutrient-rich sites, similar to that reported by Laine *et al.* (2005). The three mechanisms given above could explain this difference, as i) the OM sites are, by definition, influenced less by groundwater than the OL and ME sites, so a reduction in groundwater inflow due to drainage would not be as relevant at this site; ii) oxidation may be greater in more nutrient-rich sites if

nutrients stimulate decomposition (Verhoeven and Toth, 1995); iii) tree growth in the drained areas of the peatland has been greater at the more nutrient rich sites at Lakkasuo (Laine *et al.*, 2004). The large fall in pH with 48 year drainage at the OL site and 9 year drainage at the ME site could therefore explain the suppression of phenol oxidase, whereas the lack of a pH change at the 48 year drained ME site could explain the absence of phenol oxidase suppression. The sites of phenol oxidase suppression correspond to the sites where the DOC export potential is highest, whilst a weak but significant relationship between soil pH and DOC export potential was observed. This supports suggestions that phenol oxidase plays a critical role in C storage in peatlands and that changes in pH with drainage are critical. When phenol oxidase activity is suppressed by acidity, it is hypothesised that overall decomposition will be inhibited, leading to a net release of C.

Despite this suggestion of a key role for phenol oxidase, we did not observe a consistent response for any of the hydrolase enzyme activities at the drained sites that corroborates with the activity of phenol oxidase. This demonstrates the difficulty in isolating specific soil parameters that are associated with the activity of soil enzymes. Previous lab experiments investigating water-table drawdown on peatland C cycling, such as that by Freeman *et al.* (1996), have shown hydrolase enzyme activities to increase following water-table drawdown, but this was a short term experiment and the results probably reflect the initial rapid degradation of a small pool of easily decomposable organic compounds. This study suggests that over longer time scales, hydrolase enzyme activities tend to decrease following drainage. A plausible mechanism for this may be a decrease in substrate quality, which is a key factor in controlling rates of organic matter decomposition (Laiho, 2006). Substrate quality is known to decrease following drainage as more recalcitrant organic compounds exist in the deeper layers of peat (Hogg *et al.*, 1992).

This study confirms previous findings of the influence of nutrients on rates of organic matter decomposition and highlights their role in determining whether a peatland can continue to

accumulate C following drainage. Microbes responsible for decomposition in soil require nutrients to function (Swift *et al.*, 1979) so it would be expected that greater rates of organic matter decomposition would occur in the more nutrient-rich peat. For example, Verhoeven and Toth (1995) state that decomposition tends to be greater in base-rich compared to base-poor fens due to more favourable litter quality and water chemistry. Tolonen *et al.* (1992) reported up to 6 times greater C accumulation in Finnish ombrotrophic compared to minerotrophic peatlands, indicating impaired decomposition in the former. Thomas and Pearce (2004) presented data demonstrating that the strong cation exchange capacity of peat, especially where *Sphagnum* is the dominant vegetation species, reduces the availability of cations for microbial uptake and is therefore an important constraint on decomposition in peatlands. The nutrient content of the three sampled areas of peat in this study may explain differences in soil enzyme activities, at least for the pristine peatlands. Activities were generally lowest for the OM site, especially for phenol oxidase, β-glucosidase and arylsulphatase. The difference in activities between the three sites were similar to the measured base cation concentrations, with the greatest difference being between the OM and OL site, and less of a difference between the OL and ME sites. Previous studies at Lakkasuo have shown that the drainage undertaken at the site has increased CO_2 fluxes from the soil at a greater rate in the OL and ME sites compared to the OM, with the ME site being converted to a net C source and net C accumulation occurring in the OM site (Laine, 2004).

It is likely that contrasting results from the 9 and 48 year drained sites are not due to the 'extra' 39 years of drainage. In terms of C cycling, the greatest changes occur in the short term due to the decomposition of readily utilisable C; within 9 years it is likely that this pool will have been depleted. For example, Hargreaves *et al.* (2003) reported that drained Scottish peat acted as a source of CO_2 for 2-4 years following drainage, but after 4-8 years they reverted back to a sink again. In this study, any differences between the 9 and 48 year drained sites are likely to be due to

changes in vegetation composition. Probably the greatest change to occur on a drained peatland is the growth of new plant species and an increase in biomass (Laine *et al.*, 1995; Weltzin *et al.*, 2003). Tree growth can be rapid following drainage and greatest on the most nutrient rich peat (Laine *et al.*, 1995). At Lakkasuo, drainage has resulted in stark contrasts in the growth of new vegetation across the nutrient gradient (Minkkinen, *et al.*, 1999). At the OM site, where nutrients are in short supply, the vegetation type, which is dominated by *Sphagnum* mosses, has remained virtually unchanged since the onset of experimental drainage 9 years ago. At the OM site of 48 year drainage there is a small but significant presence of tree species, compared to the undrained portion where trees are absent. An example of how this could have been relevant is shown in the enzyme activity data; the only significant increase in hydrolase enzyme activities with drainage was for β-glucosidase and β-xylosidase in the 48 year drained OM site. These enzymes degrade cellulose and the more recalcitrant hemicellulose respectively. The increase in activity may be due to the growth of new tree stands. On the pristine OM site there will be an absence of root exudates due to the dominance of *Sphagnum*. The growth of trees in the drained portion will have generated an inflow of root exudates to the soil. These are a source of easily metabolised C for microbes and can lead to enhanced organic matter decomposition through a 'priming' effect (Kuzyakov, 2002; Fontaine et al., 2007). At the OL and ME sites, the growth of sedges, shrubs and trees is much more prevalent, especially for the 48 year drained peat but also to a lesser extent at the 9 year drained peat. The growth of such plant types will generate important new carbon flux pathways between the vegetation and the soil.

The dominance of Sphagnum at the OM site also has an important influence on decomposition through the release of phenolic compounds into the soil matrix. These compounds have been shown to inhibit decomposition in freshwaters and soils (Freeman *et al.*, 1990; Wetzel, 1992) and to play a part in suppressing the decomposition of organic matter in peatlands (Freeman *et al.*, 2001*a*). The presence of *Sphagnum* and the resulting release of phenolic compounds at the OM

site, together with the extremely low activity of the phenolic-degrading enzyme phenol oxidase, suggest a mechanism for suppression of organic matter linked to the above ground vegetation. In accordance with the enzymic latch, β-glucosidase, the key enzyme for cellulose degradation in soil, was approximately half as active in the pristine OM site compared to the OL and ME sites. This offers an explanation for why the OM portion of Lakkasuo may have continued to accumulate C following drainage. Another reason why the OL and ME sites appeared to respond to water-table drawdown more might be due to changes in dissolved oxygen. Lower water-tables mean less of a continuous flush of oxygenated waters (Freeman *et al.*, 1996). In their natural state, the more nutrient rich areas of the peatland receive more water from lateral flow, so they will also be receiving more of a flush of oxygen-rich waters. With drainage, the more nutrient rich sites may therefore experience a greater change in oxygen concentration due to drainage.

In addition to the soil pH and abundance of nutrients, it is apparent that the soil water content may also significantly influence the activity of phenol oxidase. Toberman *et al.* (2008) demonstrated that phenol oxidase is sensitive to water content and proposed an optimal value for the enzyme of approximately 85%. Above this value, low oxygenation may inhibit activity, and below it, moisture limitation may be a major stress factor. In this study, a similar trend was observed, with an optimal water content of approximately 85-90%. The lack of a stimulation of phenol oxidase activity in the drained peat may therefore be due to moisture limitation in addition to increased acidity, whilst the low values observed for the OM site may be due to the soil's exceptionally high water content (92-95%).

As well as leading to an increase in DOC, we also observed that, compared to the pristine peat, drainage significantly increased the SUVA of the DOC for all but the OM 9 year site, with the highest SUVA values associated with the lowest soil water contents. SUVA correlates well with the molecular weight and aromaticity of DOC (Volk *et al.*, 2002), indicating that drainage increases the leaching of the more complex C compounds, presumably suggesting mobilisation

of the peat matrix. This is a concern for freshwaters receiving run off from drained peatlands, particular where the water may form part of a source of drinking water. SUVA has been shown to be correlated well with the formation of disinfection by-products (DBPs), such as trihalomethanes (THMs) during water treatment (Reckhow *et al.*, 1990; Kitis *et al.*, 2001). Kitis *et al.* (2002) demonstrated that a doubling of SUVA values can lead to a 50-100% increase in DBP formation. In this study it was observed that drainage increased the SUVA by 2 to 3 times. This finding is supported by some previous studies. For example, Moore and Clarkson (2007) recorded 35% higher SUVA values for waters leaching from a drained peatland compared to an undrained site. Jaatinen *et al.* (2008) showed that an increase in the oxygen content in peatlands may preferentially stimulate fungal communities that degrade simple organic compounds, leading to an increase in the overall molecular weight of the DOC available for leaching.

Although the data was not significant, for all three peatland types, drainage has resulted in reduced concentrations of leachable nitrate. In a laboratory mesocosm experiment, Freeman *et al.* (1993*a*) showed that short-term water-table drawdown can increase nitrate concentrations through the mineralisation of nitrogenous organic compounds. This study showed that over much longer time periods available nitrate will decrease due to drainage. The most likely explanation for this is the increase in above-ground biomass and the resulting increase in the uptake of nitrate from the soil by the vegetation. From a water quality perspective, this data suggests that long-term drainage of peatlands decrease nitrate leaching to freshwaters.

5.6 Conclusions

Our results suggest that the leaching of DOC from peatlands generally increases with drainage for oligotrophic and mesotrophic peat, but not for ombrotrophic peat, with the molecular weight of the leached DOC (indicated by measurements of SUVA) also increasing. This has implications for the quality of freshwaters with drained peatlands in their catchments and suggests the treatment of drinking water might be more difficult and also lead to enhanced DBP production. The most influential soil parameter affecting DOC concentrations was the water content of the soil, with DOC export potential being higher the lower the water content of the soil. Assuming water content decreases and aeration of the soil increases as the water-table falls, the increase in DOC can be explained by a stimulation of organic matter decomposition in a more oxic environment. The lack of a significant increase in the DOC export potential of the OL 9 year and the ME 48 year drained sites may therefore be due to the fact that we did not observe a reduction in the water content of the soil. As the water-table at these sites is known to have fallen (Laine, 2004), it suggests spatial heterogeneity of the hydrology of the soils and a more thorough sampling regime is needed to determine DOC export potential across a more expansive area of the drained portion of the Lakkasuo.

An alternative explanation for the observed trends in DOC export potential is linked to the 'enzymic latch' mechanism. Soil pH correlated significantly with the activity of phenol oxidase and DOC, suggesting that at sites where drainage has induced an increase in acidity there has been an inhibition of phenol oxidase activity. As phenol oxidase is a regulator of C storage in peatlands (Freeman *et al.*, 2004b), suppression of this enzyme may lead to enhanced DOC release due to impaired rates of organic matter decomposition.

Phenol oxidase also displayed an optimal activity at a soil water content of 85-90%, corroborating the findings of Toberman *et al.* (2008) and offering a reason as to why the activity of the enzyme was virtually undetectable at the OM site.

In addition to demonstrating that the drainage of peatlands for the growth of forestry leads to increased leaching of DOC even decades after initial drainage of the soil, this study also highlights the potential for water-table drawdown driven by climate change to lead to increased DOC leaching in the long-term.

Chapter 6

Conversion of tropical peatland to oil palm plantation; Impacts on the activity of soil enzymes and release of DOC

6.1 Abstract

Tropical peatlands cover 30-45 million hectares and are one of Earth's most efficient ecosystems for sequestering carbon (C). In the last two decades many of the world's tropical peatlands have undergone significant environmental degradation, mainly through drainage and conversion to agricultural land. The establishment of oil palm plantations has been one of the commonest land use changes that tropical peatlands have undergone. Drainage causes rapid peat decomposition and has converted many peatlands in the tropics from C sinks to C sources due to the release of thousands of tonnes of CO_2. The loss of C in fluvial form, as dissolved organic carbon (DOC), has received very little attention. The aim of this study was to compare the DOC export potential of soils from a peatland and an oil palm plantation in Malaysia. For the oil palm soil, we recorded a 40% greater export potential of DOC and more than twice the DOC concentration in drainage waters compared to the peat soil. Activity of the key C cycling enzyme, β-glucosidase, was 25% higher in the oil palm soil. Given that this enzyme can mobilise C from the soil matrix, it may be critical in regulating C leaching from these soils. This study demonstrates that the conversion of peatlands to oil palm plantation will increase DOC leaching, typically from 233.7 μg cm^3 to 372.5 μg cm^3, which will have detrimental consequences for water quality. Measurements of changes in DOC flux resulting from the conversion of peatlands to oil palm plantation are now needed to enable greater quantification of this important C loss pathway.

6.2 Introduction

Peatland ecosystems cover just 3% of the Earth's land surface area but contain 20-30% of the global reservoir of soil carbon (C) and the equivalent of 70 times the current annual global C emissions from fossil fuel usage (Gorham, 1991; Hooijer *et al.*, 2006). Tropical peatlands cover an area of approximately 30-45 million ha (Verwer *et al.*, 2008) and have traditionally been one of Earth's most efficient ecosystems for sequestering C, due to both high rates of above-ground biomass productivity and suppressed decomposition of organic matter in the anoxic soil (Jauhiainen *et al.*, 2008). Unlike boreal and temperate peatlands, those in the tropics are mostly forested and therefore contain significant C stocks above ground in addition to below ground. Estimates of the total surface area and C storage capacity of tropical peatlands are subject to great uncertainty, but recent estimates suggest they constitute 9-14% of the global peat C store and 3.7% of the global soil C pool (Page *et al.*, 2008). The majority of the world's tropical peatlands are found in south-east Asia (67.1%; 27.1 million ha); principally Indonesia (55%; 22.5 million ha) and Malaysia (7%; 2 million ha) (Hooijer *et al.*, 2006; Page *et al.*, 2008).

The world's peatlands are of critical importance to climate change, as they have built up a vast store of terrestrial C and can continue to naturally sequester atmospheric C, but they are particularly vulnerable to environmental degradation. Whilst boreal peatlands have been the subject of numerous studies into the potential destabilisation of their C sequestering properties (e.g. Freeman *et al.*, 2001*b*; Worrall *et al.*, 2004), much less attention has traditionally been paid to tropical peatlands. Interest has intensified in the last few years, largely due to the widespread environmental degradation that these ecosystems have undergone. The most pervasive impacts have been logging and drainage, principally for the establishment of crop plantations. This has had serious repercussions for carbon dioxide (CO_2) emissions; drainage leads to enhanced peat decomposition, whilst clearing the land of vegetation eliminates the strong C sequestering potential of the ecosystem. Peat fires have also had a devastating impact on tropical peatlands,

especially in Indonesia (Jaenicke *et al.*, 2008) and the drainage of peatlands makes them particularly susceptible to fire damage. As a result, many degraded tropical peatlands have switched from strong C sinks to C sources through the release of thousands of tonnes of CO_2 (Page *et al.*, 2002). The C emissions from degraded peatlands in Indonesia alone accounts for 4% of the total global anthropogenic emission of greenhouse gases (Hooijer *et al.*, 2006) and sharp increases in atmospheric CO_2 concentrations have been associated with the burning of vast swathes of SE Asian peatlands (Page *et al.*, 2002).

Perhaps the most rapid land use change to have occurred on tropical peatlands in recent years has been the planting of oil palm (*Elaeis guineensis*), a raw material used in the biofuel, vegetable oil, pulp and paper industries (Rieley and Page, 2008). In Malaysia, oil palm plantations have grown rapidly and the country is now the largest producer and exporter of palm oil and its products (Yusoff and Hansen, 2007). The total area of oil palm plantation has increased from 300,000 to 2,500,000 ha in the last 3 decades and oil palm now occupies over one third of total cultivated land area (Germer and Sauerborn, 2008). As of 2004, 58% of the country's peat swamp forest had been converted to agricultural cultivation (Lee, 2004), of which approximately half was for the growth of oil palms (Hooijer *et al.*, 2006). Oil palm production is expected to expand in the next few years for use as a biofuel, which is considered a sustainable means of directly offsetting fossil fuel consumption.

Peatland must be drained before plantations can begin as oil palms cannot survive in waterlogged conditions. The optimum water table depth for oil palm cultivation is approximately 60-80 cm below the surface (Rieley and Page, 2005); this represents a significant deepening of the aerobic zone in previously waterlogged, anoxic peat soil. In theory this should dramatically increase the emissions of CO_2 from the soil through improved oxygenation of the soil stimulating decomposition of organic matter. A recent review of soil efflux studies on tropical peatlands that have undergone various land use changes suggests this is the case (Hooijer *et al.*,

2006). The limited number of published studies addressing this issue, and the high variability of the data gathered has however made it difficult to quantify the impact of converting peatland to oil palm with respect to C emissions. An example of the variability can be seen in two studies published in 2005. Furukawa *et al.* (2005) made monthly chamber measurements of soil respiration and found emissions were 3 times higher in a drained tropical peatland forest compared to an undrained site, whilst Melling *et al.* (2005) measured soil CO_2 emissions of an oil palm plantation that were 25% lower compared to an intact forested peat swamp. However, variability such as this can usually be explained by a more thorough assessment of the sites sampled in each of the studies; for example, subsequent aerial land surveys of the intact forested peat swamp sampled by Melling *et al.* (2005) showed that it may have undergone some disturbance and drainage that would have increased peat decomposition and given higher values of CO_2 emissions from the soil than a truly pristine tropical peatland (Hooijer *et al.*, 2006). The study by Hooijer *et al.* (2006) and more recent data, such as that reported by Reijnders and Huijbregts (2008) showing that the emission of CO_2 from the drainage of tropical peat soils for oil palm plantations results in CO_2 emissions two orders of magnitude greater than the drainage of non-peaty soils, is convincing in suggesting that the use of peatlands for oil palm growth is extremely detrimental to the storage of C in tropical peatlands and, consequently, has important repercussions for atmospheric CO_2 concentrations. During a year of good yield, a plantation can generate 3-6 tonnes of palm oil per hectare, which prevents 9-18 tonnes of CO_2 emissions from fossil fuels (Hooijer *et al.*, 2006). However, the emissions from drained peatlands due to peat decomposition is around 70-100 tonnes of CO_2 per hectare per year, or 10-30 tonnes of CO_2 per tonne of palm oil. It has been calculated that when oil palm is grown on previously undisturbed peatlands, it would take more than 420 years to repay the C debt caused by the enhanced CO_2 emissions caused by the drainage of the peat soil (Fargione *et al.*, 2008).

Measurements of CO_2 emissions from decomposing peat can quantify a large C flux, however in order to estimate the overall C balance, i.e. the change in Net Ecosystem Production (NEP) of a

peatland conversion to oil palm plantation, it is necessary to measure more of the carbon fluxes affected by the change in habitat (Verwer *et al.*, 2008). One component that has received almost no attention is the potential loss of C in fluvial form, as dissolved organic carbon (DOC). DOC is a critical and often overlooked component of the global C cycle. As a large proportion of riverine DOC is decomposed once it reaches the ocean (Hedges *et al.*, 1997), ultimately increasing atmospheric CO_2 concentrations, it is important to understand processes influencing the flux of DOC from terrestrial systems into freshwaters. An understanding of processes influencing DOC is also critical for assessing water quality, especially concerning water used for human consumption, given the role of DOC in leading to the formation of carcinogenic trihalomethanes (THMs) following water treatment (Singer, 2006). In the tropics, water derived from peatlands is often used as a source of raw water to supply households with drinking water. For example, in the Sarawak region of Malaysia, approximately 7700 mega litres of raw peat water serves as the water source for a population of 140,000 people (Sim and Murtedza, 2007).

Concentrations and fluxes of DOC within boreal peatlands and the streams that drain them are frequently reported (e.g. Urban *et al.*, 1989; Freeman *et al.*, 2001*b*) and studies have emphasised that DOC makes up a significant fraction of total C losses from these ecosystems (Billett *et al.*, 2004). However, equivalent data for tropical peatlands is much scarcer, particularly those that have undergone drainage for oil palm plantation (Verwer *et al.*, 2008). It was recently reported that a major river in Indonesia, the Siak, contains high concentrations of DOC due to effective leaching from tropical peat swamps within the river's catchment (Baum *et al.*, 2007). The flux of DOC was reported to be higher in catchments with a greater percentage of peat cover, and to increase with precipitation, indicating the DOC within the peatlands is relatively mobile. Another study has demonstrated that there are large losses of dissolved CO_2 and methane (CH_4) from rivers draining tropical peatlands (Ueda *et al.*, 2000). Sim and Murtedza (2007) characterised the chemical composition of samples of tropical peat and reported lower overall molecular sizes of isolated humic acids compared to temperate peat, which may be attributable to greater aerobic

decomposition in tropical soils. They also recorded higher THM formation potential of humic acids in rivers draining tropical peatlands, compared to examples published for temperate regions. The potential for the drainage of peatlands to mobilise THM-forming DOC therefore needs to be addressed. It is also relevant to point out that long-term monitoring data in boreal ecosystems have shown that DOC concentrations in freshwaters have been rising for the last two decades in large parts of Europe and North America (Monteith *et al.*, 2007), with changes in land use having been postulated as a possible driving mechanism (Worrall *et al.*, 2003).

This chapter reports data from a study focussing on the impact of converting tropical peatland to oil palm plantation on the release of DOC into freshwaters. In addition to measurements of the DOC export potential of different soil types and concentrations of DOC in drainage waters, we also report activities of soil enzymes. Measurements of enzyme activities can yield important information on the biological quality, fertility and productivity of soil (Dick, 1994) and are intrinsically linked to environmental conditions. As such, they are sensitive to disturbance and changes in land use (Shi *et al.*, 2006). Hydrolytic enzymes are the main mediators of organic matter degradation in soils and control the rate of both decomposition and the release of substances that can become available for microbial and plant uptake (Marx *et al.*, 2001). In peat soils in temperate regions, enzyme activities have been shown to correlate with DOC concentrations due to organic matter degradation (Fenner *et al.*, 2005). Consequently enzyme activities may be critical in controlling rates of DOC release to freshwaters.

6.3 Materials and Methods

6.3.1. Field sites and sample collection

The study was carried out at three separate sites located within a 10 km radius in the Selangor region of Malaysia (2°56'N, 101°37'E), representative of different land use types relating to the conversion of tropical peatland to oil palm. The separate soil types sampled during this study were peat, oil palm plantation and, for comparative purposes, a non-peaty forest. The peat site was a failed oil palm plantation, where drainage channels were dug through an area of peat in 1999 and the site was deforested, but the drainage was insufficient and the water table too high for the oil palms to grow. As a result, little decomposition of peat occurred and the soil is highly organic. This site was chosen to represent conditions similar to a tropical peat swamp, although cannot be considered as a truly pristine peatland. The oil palm plantation was established in 2000 on a successfully drained peatland. The water table at this site was approximately 75 cm below the surface at the time of sampling, with the oil palms on average about 4 m tall and planted at a density of approximately 50 palms ha^{-1}. The forest site was a dipterocarp forest on mineral soil that had undergone drainage but not deforestation. Sampling was undertaken for 3 days in June 2008, during the dry season. At each site, 5 replicate soil samples were collected from a depth of 10cm and sealed in plastic bags. For each drainage ditch, five 250ml samples of water were extracted directly into acid-washed polypropylene bottles at 5 points along a 20 m length of each ditch. All samples were transported back to the UK, where the soil samples were stored at field temperature until further analysis. The pH of the drainage water samples was determined and the samples filtered through 0.45 μm filters. Water homogenates of each soil sample were prepared within one week of sample collection by adding 27 ml of ultra-pure water to 3 cm^3 of soil, homogenising the sample in a stomacher and centrifuging at 10,000rpm for 5 minutes. The pH of an aliquot of the soil waters was determined before the remaining supernatant was filtered through 0.45 μm filters. A summary of the basic soil properties for each site is given in Table 6.1

Table 6.1 – Basic chemical and physical characteristics of the 3 sampled soil types (Mean ± SD)

	Forest	**Peat**	**Oil Palm**
Water-table depth (cm)	60	5	75
pH	5.00 (0.14)	4.07 (0.08)	4.19 (0.10)
Conductivity (μS cm^{-1})	22.3 (7.5)	70.8 (6.1)	113.4 (25.5)
% moisture	32.4 (5.6)	79.4 (2.8)	31.6 (8.2)
% soil organic matter	14.3 (2.5)	75.1 (9.2)	43.7 (13.9)

6.3.2 DOC and phenolics

Total DOC and phenolic compound concentrations were determined on the water-extractable and drainage water solutions. DOC was measured using an Analytical Sciences Thermalox TOC analyser. Phenolic compound concentrations were measured using a method similar to that described by Box (1983). For the soil samples, concentrations of total DOC and phenolics were expressed in μg cm^3 wet soil and for the waters as mg L^{-1}. The water-extractable method was chosen for determining dissolved C concentrations because it gives an indication of the potential losses of C from soils during rainfall, which in the tropics is frequent and often intense. Furthermore, a recent study has demonstrated that yields of DOC mobilised from tropical peat soils are highly influenced by rates of precipitation (Baum *et al.*, 2007).

6.3.3 Soil enzyme activities

The activities of three extracellular hydrolase enzymes (β-glucosidase, arylsulphatase and phosphatase) and phenol oxidase were assayed using a modified version of the methods developed by Freeman *et al.*, (1995) and Pind *et al.*, (1994). MUF enzyme substrates were used to detect hydrolase enzyme activities by measuring changes in fluorescence of samples at 450 nm

emission and 330 nm excitation on a BMG Fluostar Galaxy fluorimeter. The activity of the hydrolase enzymes is expressed in μmol g^{-1} min^{-1}.

Phenol oxidase was assayed using 10 mM L-DOPA (dihydroxyphenylalanine) solution as the enzyme substrate and detecting changes in absorbance at 460 nm. The activity of phenol oxidase is expressed in μmol dicq g^{-1} min^{-1}.

Soil bulk density (dry weight), gravimetric water content and soil organic matter (loss on ignition) were determined by drying replicates of 1 cm^3 of peat at 105°C and 550°C for 24 hours each in a Carbolite muffle furnace and calculating the weight loss.

Soil (basal) respiration is commonly used to assess microbial activity in soils (Vanhala, 2002). It was measured using a method similar to that described in Bonnett *et al.* (2006). One cm^3 of wet soil was placed into a darkened 30 ml bottle, sealed and stored at field temperature. After 2 hours of incubation, 5 ml of gas was withdrawn from each bottle and CO_2 concentrations were measured on an Ai Cambridge model 92 gas chromatograph, with a Porapak QS column at 350°C and N_2 carrier gas at a flow rate of 30 ml min^{-1}.

6.3.4 Statistical analysis

One-way ANOVA with Tukey post-hoc was used to test for differences between soil types. Pearson correlation was used to test for significant relationships between the analyses undertaken. Both statistical tests were performed SPSS (version 11). The standard error of each data point was calculated by dividing the standard deviation of the sample by the square root of the sample size.

6.4 Results

6.4.1 Dissolved organic carbon

Total concentrations of water-extractable DOC were significantly higher in the soil sampled from the oil palm plantation ($p<0.05$), with 372.5 μg cm^3 of DOC compared to 233.7 μg cm^3 for the peat and 84.9 μg cm^3 for the forest soils (Figure 6.1). The concentration of DOC in the drainage channels was also significantly highest in the water draining the oil palm plantation at 13.3 mg L^{-1} (Figure 6.2). In the peat and forest drainage channels the concentrations were 6.0 and 4.0 mg L^{-1}, respectively.

The concentration of phenolic compounds displayed a somewhat different trend to total DOC (Figure 6.3), with the peat soil having the highest concentration at 29.2 μg cm^3, although this was not significantly higher (at $p<0.05$) than the phenolics in the oil palm (26.5 μg cm^3). The amount of phenolics in the forest soil was significantly lowest, at 14.3 μg cm^3 ($p<0.05$). The phenolics:DOC can indicate the overall molecular weight of the DOC. The forest, peat and oil palm soils had values of 0.169, 0.125 and 0.071 respectively.

6.4.2 Soil enzymes and CO_2 emissions

The activity of three major hydrolytic enzymes, β-glucosidase, arylsulphatase and phosphatase, all displayed contrasting trends for each soil analysed. The activity of β-glucosidase correlated positively and significantly with the soil organic matter (SOM) content of the soil ($R^2=0.757$, $p<0.001$; Figure 6.4), suggesting SOM is critical in regulating β-glucosidase activity. The activity of β-glucosidase, arylsulphatase and phosphatase were normalised to dry weight. The highest activities of β-glucosidase were in the peat and oil palm soils, at 1.18 and 1.01 μmol g^{-1} min^{-1} respectively, both being significantly higher than the forest soil, 0.46 μmol g^{-1} min^{-1} ($p<0.001$ and $p<0.01$ respectively) (Figure 6.5).

6.4 Results

The activity of arylsulphatase was lower for all samples compared to activities of β-glucosidase (Figure 6.6). The activity of arylsulphatase in the forest soil was significantly higher than in any of the other samples, at 0.37 μmol g^{-1} min^{-1} ($p<0.05$) Much lower values were recorded in the peat (0.18 μmol g^{-1} min-1) and oil palm soils (0.08 μmol g^{-1} min^{-1}).

Activities of phosphatase were higher than β-glucosidase for all samples, however it is worth noting that the phosphatase activity in the oil palm, 0.86 μmol g^{-1} min^{-1}, was significantly lower than the other soils ($p<0.01$; Figure 6.7) The peat sample had by far the highest phosphatase activity, at 26.48 μmol g^{-1} min^{-1}, which was significantly higher than both the forest and oil palm soils ($p<0.001$).

The activity of phenol oxidase was highest in the peat soil and lowest in the oil palm soil, although differences between soil types were not significant at $p<0.05$ (Figure 6.8). This was probably due to the high variability of the replicate data.

The efflux of CO_2 from the soil, measured as basal respiration, also had high variability and consequently was not significantly different between soil types at $p<0.05$ (Figure 6.9). The peat soil had the highest CO_2 efflux, with the mean emission from the oil palm approximately 85% lower. A significant but weak positive correlation was recorded between the efflux of CO_2 and the percentage water content of the soils sampled ($R^2=0.352$, $p<0.05$).

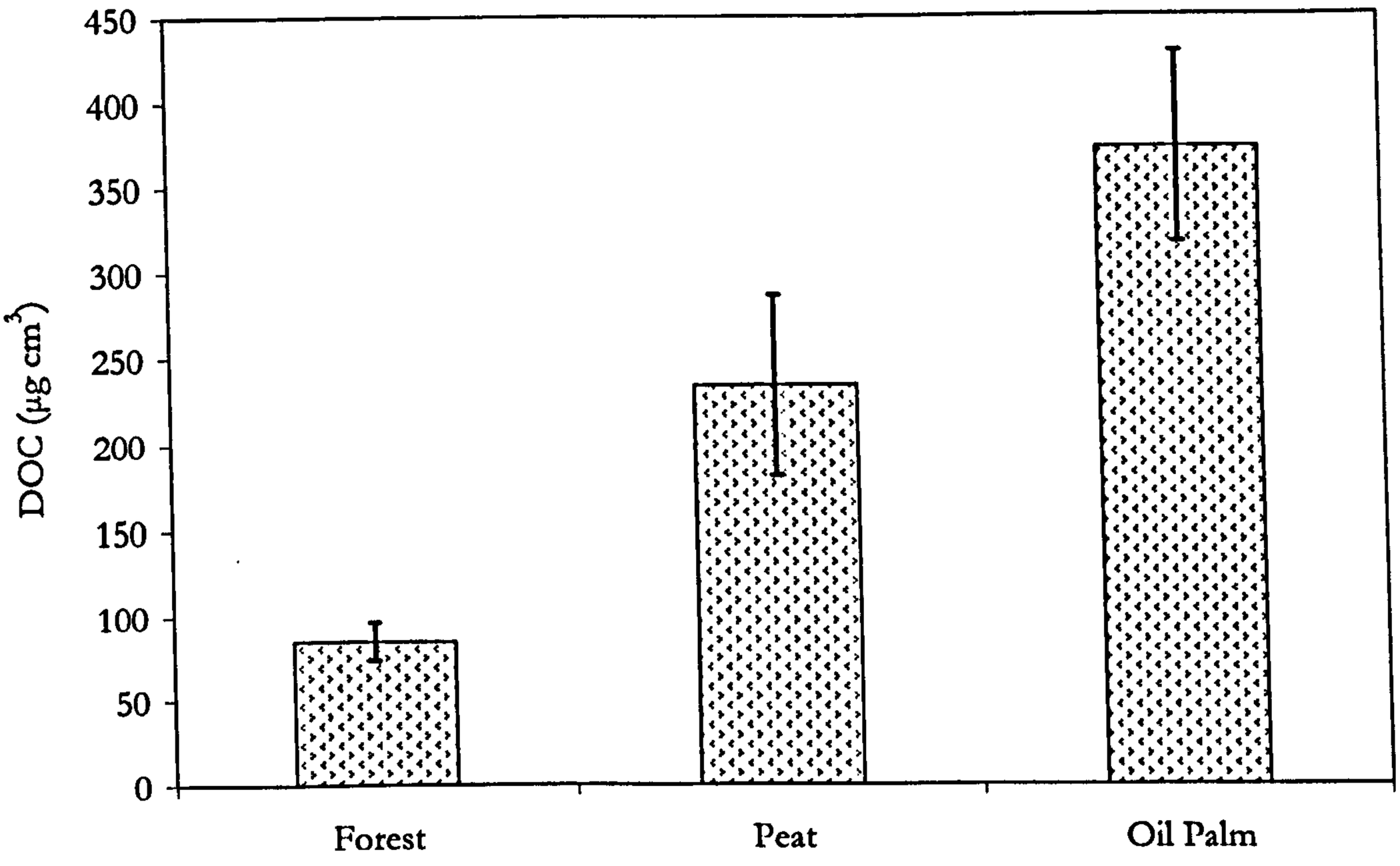


Figure 6.1 - Water-extractable DOC concentrations of the forest, peat and oil palm soils

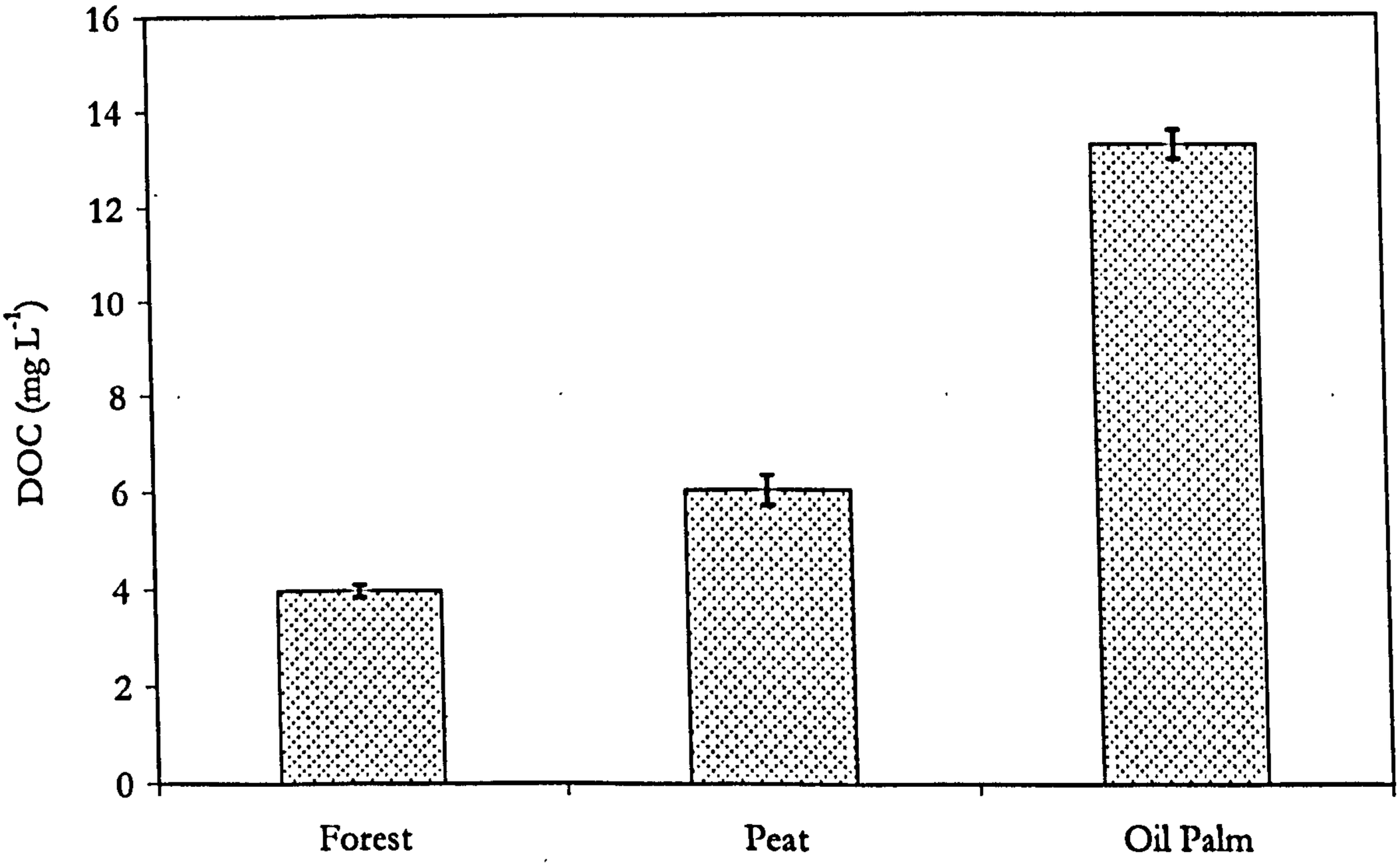


Figure 6.2 – DOC concentrations of the drainage channels within the forest, peat and oil palm sites

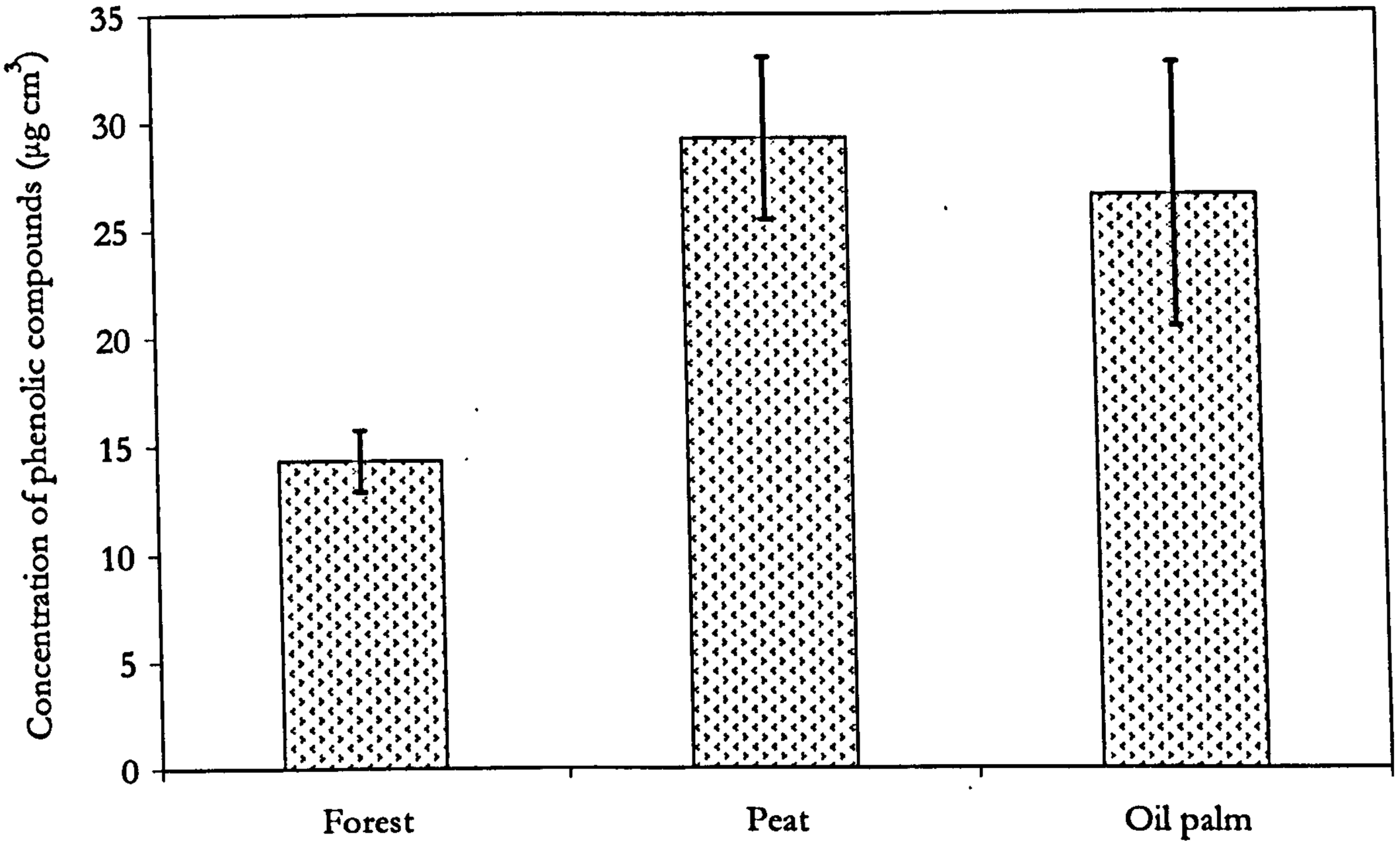


Figure 6.3 - Water-extractable phenolic concentrations of the forest, peat and oil palm soils

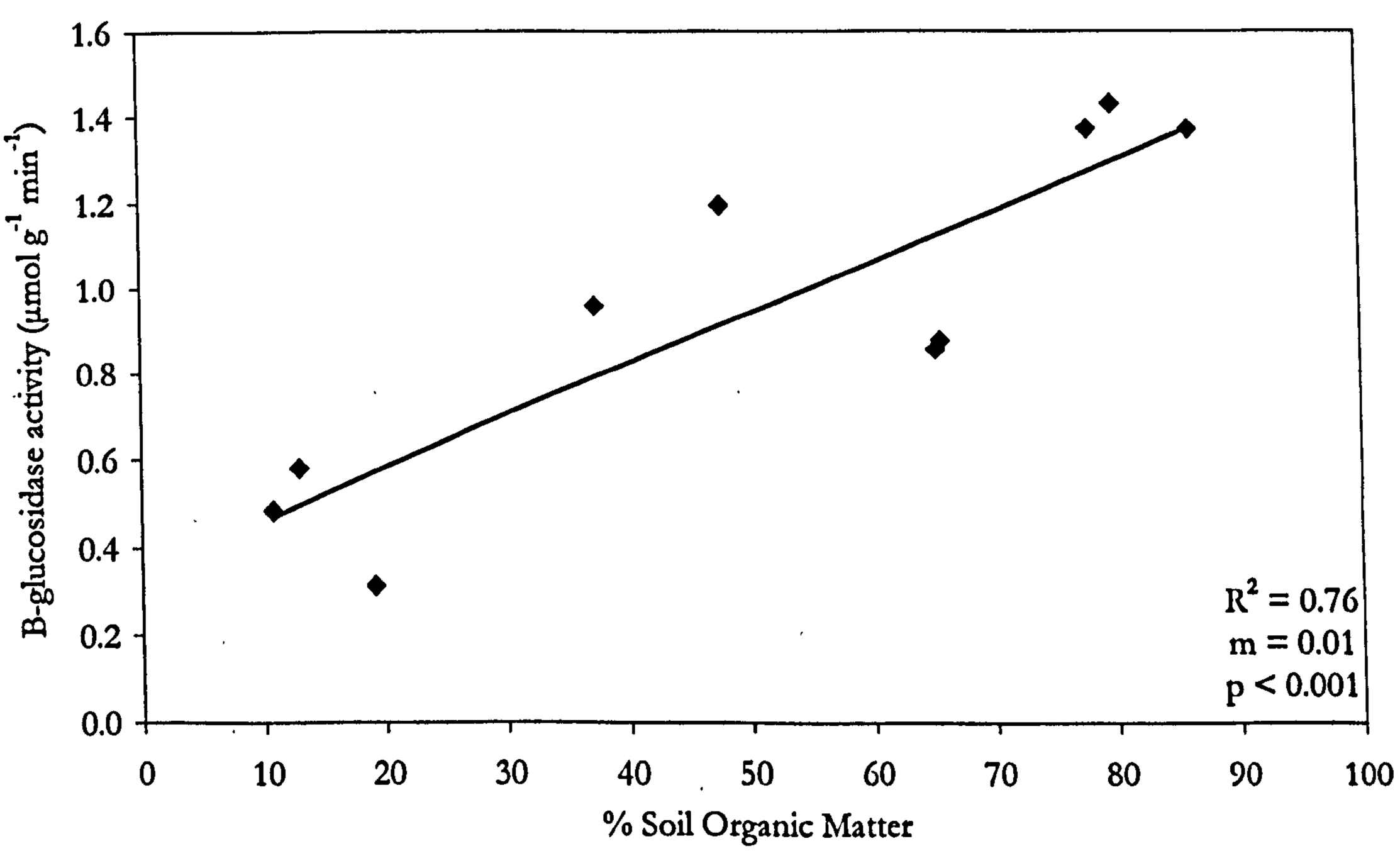


Figure 6.4 – Significant linear relationship between % Soil organic matter and the activity of β-glucosidase

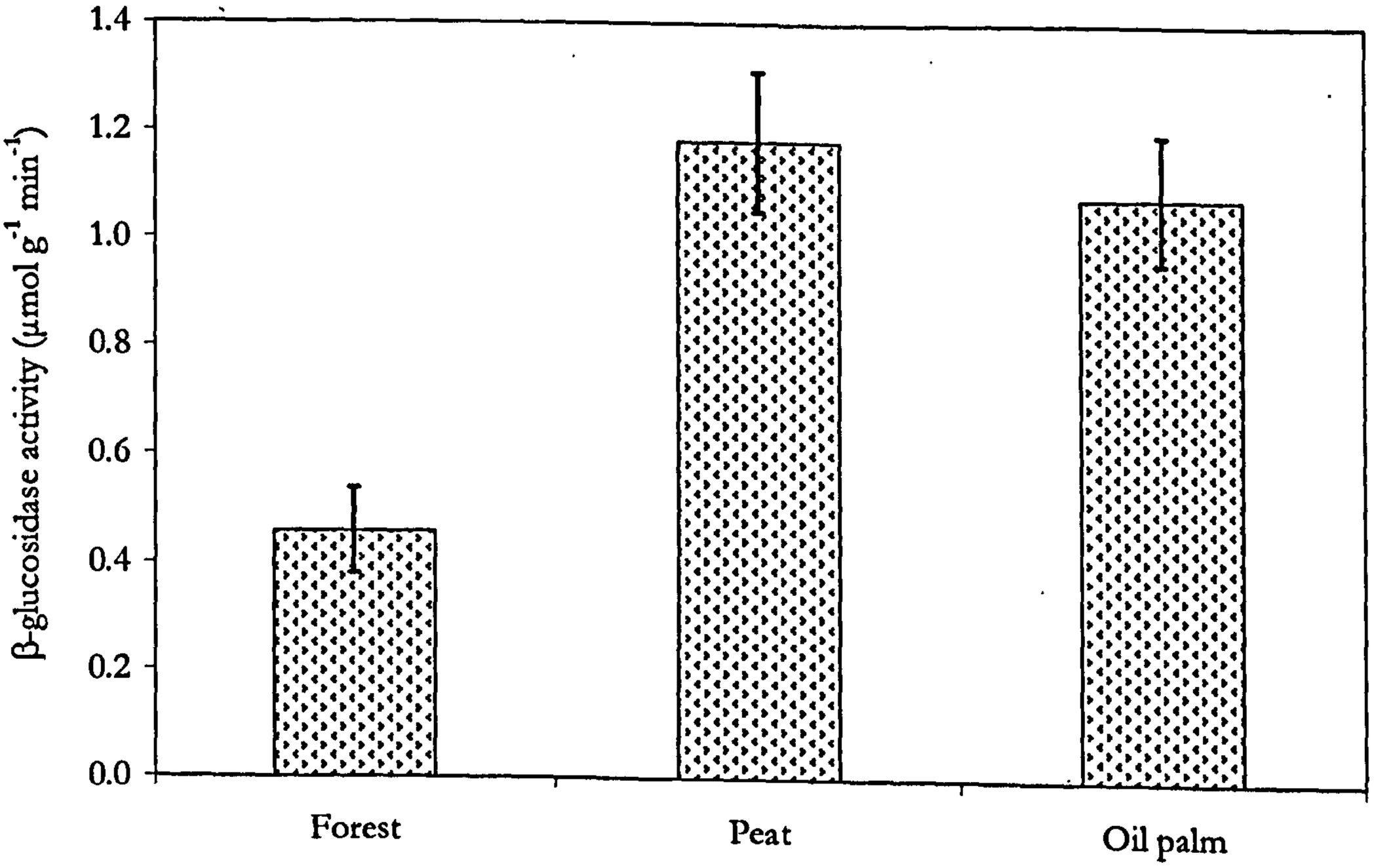


Figure 6.5 – β-glucosidase activity of the forest, peat and oil palm soils

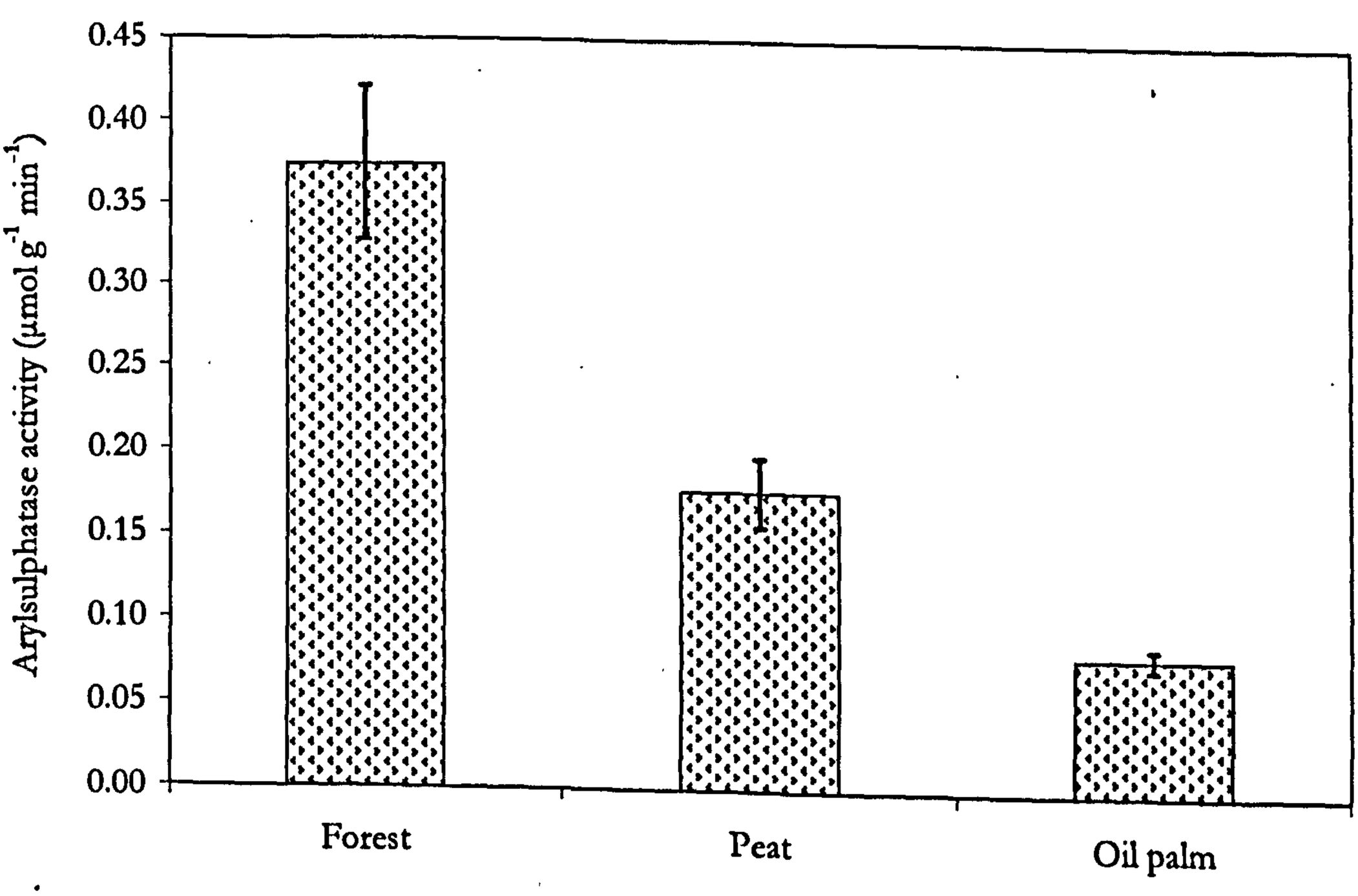


Figure 6.6 – Arylsulphatase activity of the forest, peat and oil palm soils

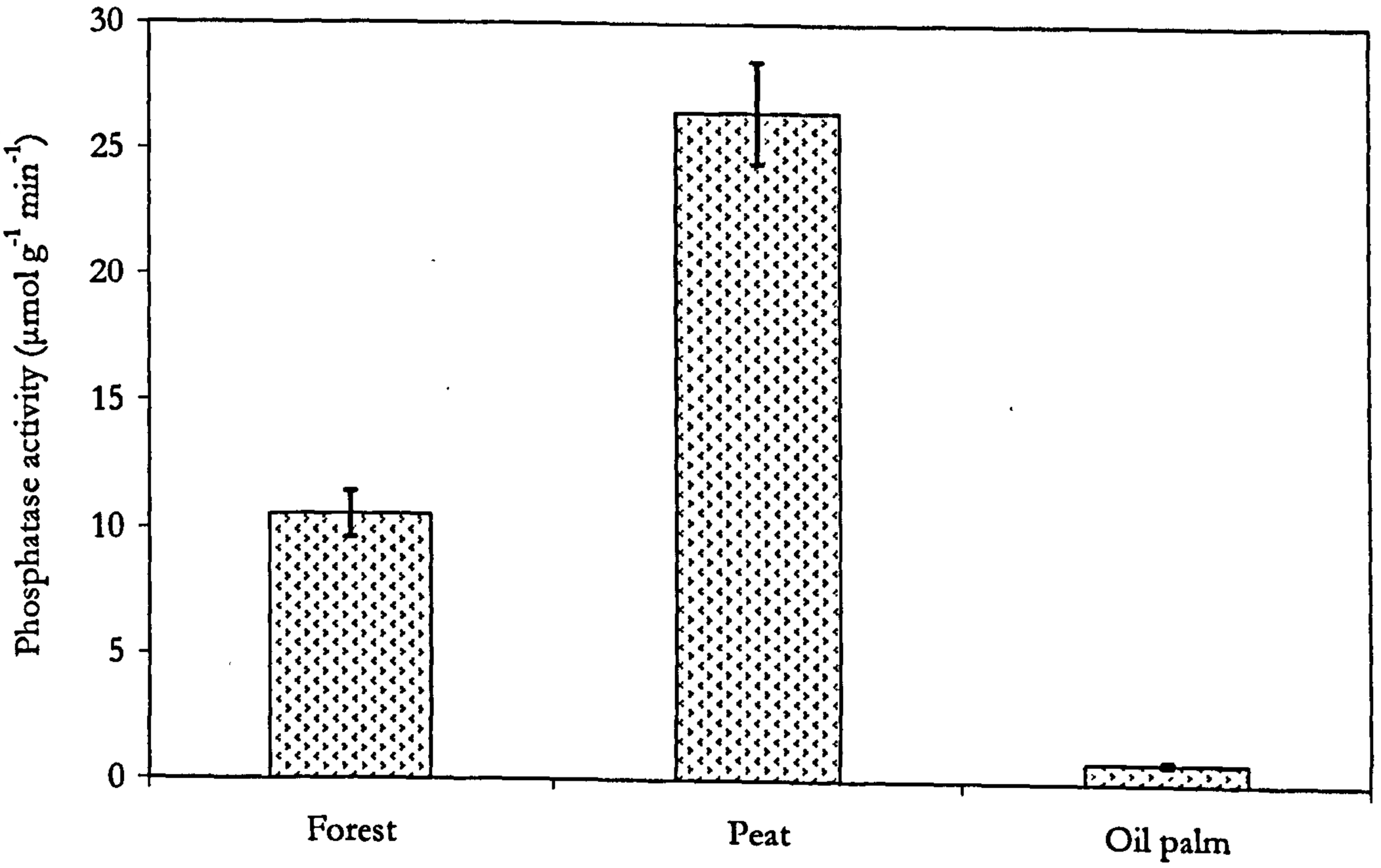


Figure 6.7 – Phosphatase activity of the forest, peat and oil palm soils

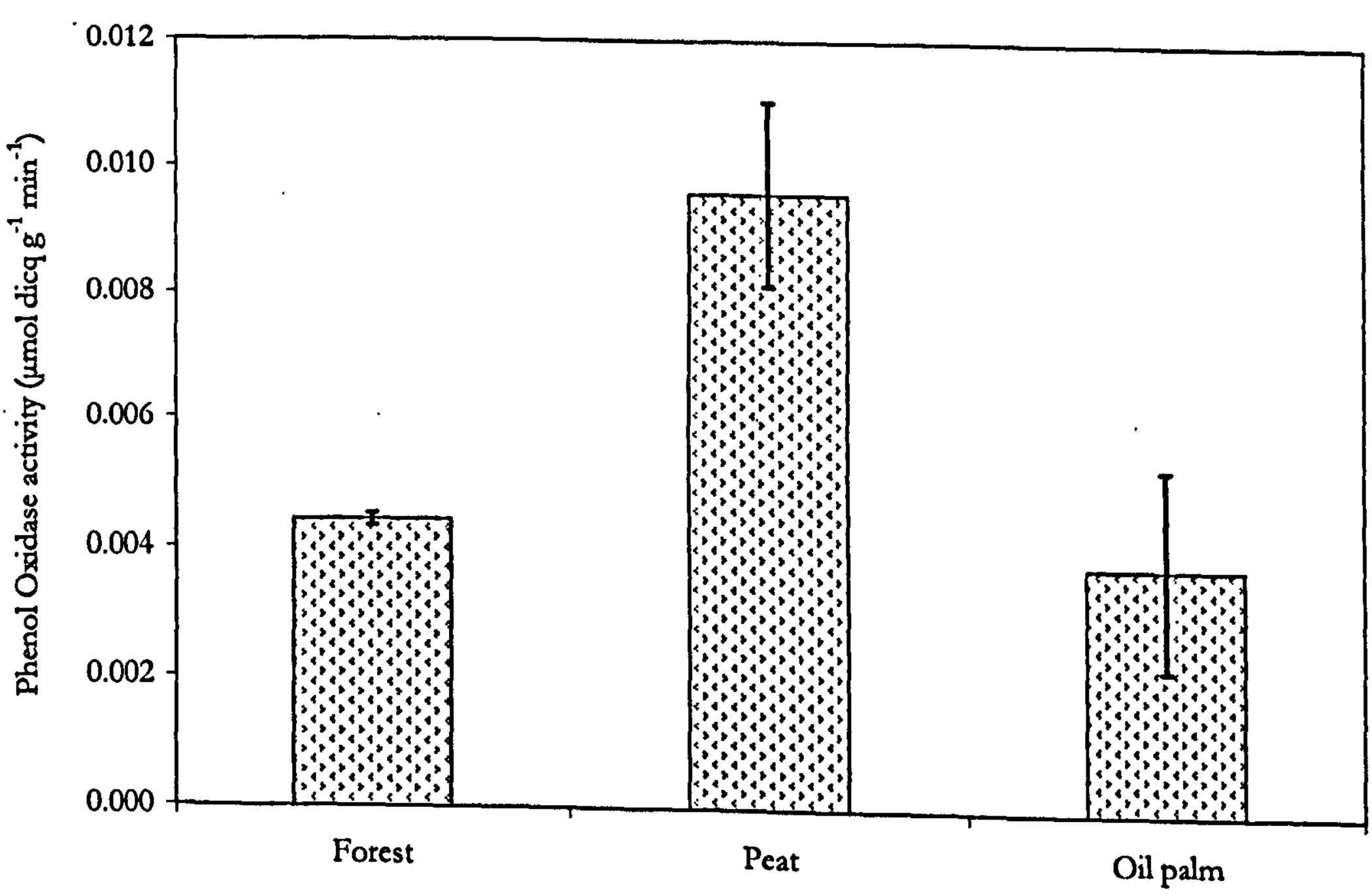


Figure 6.8 – Phenol oxidase activity of the forest, peat and oil palm soils

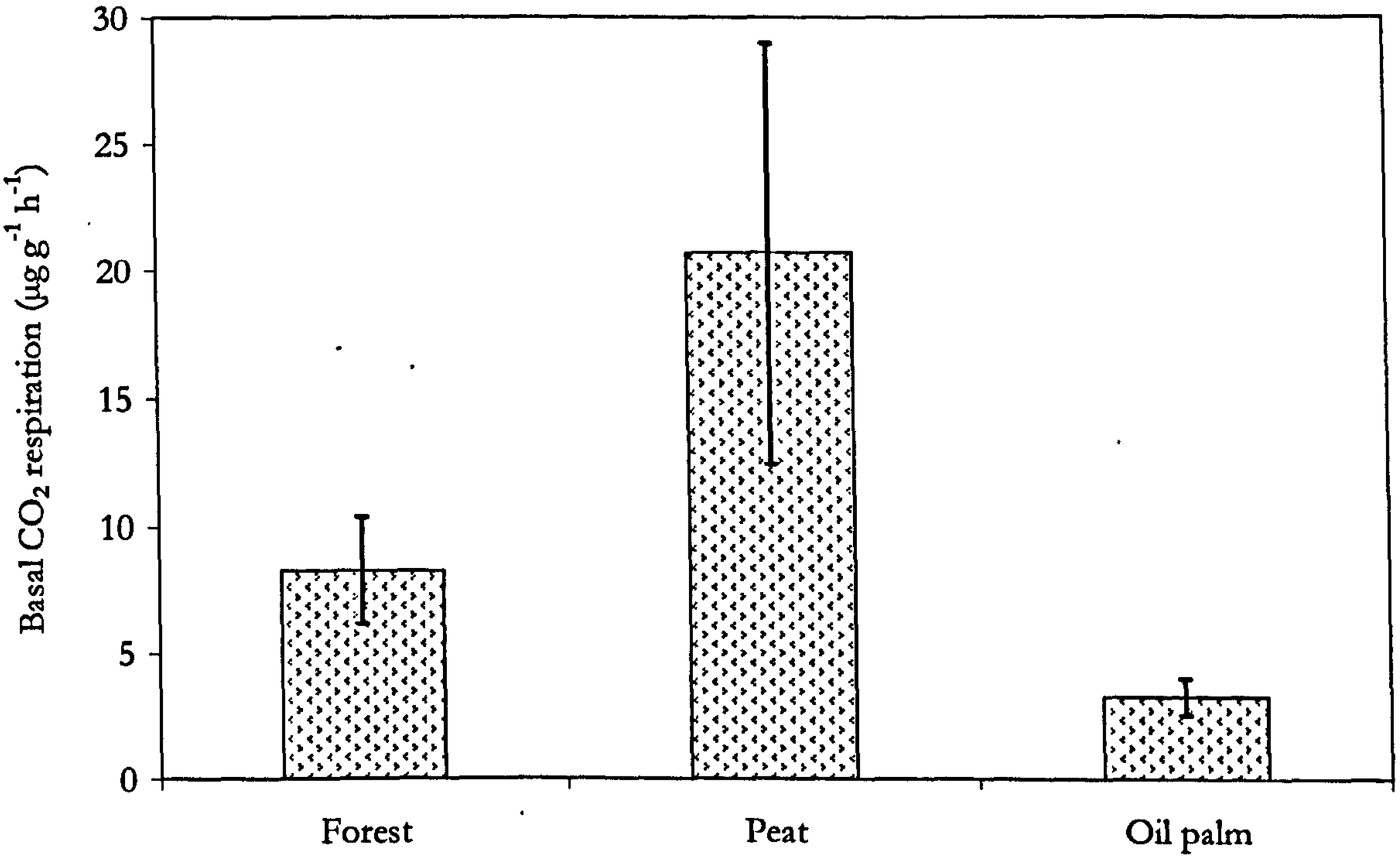


Figure 6.9 – Basal respiration of the forest, peat and oil palm soils

6.5 Discussion

The data obtained in this study demonstrates that the drainage of a tropical peatland and its conversion to an oil palm plantation can have important repercussions for soil C cycling processes and the leaching of DOC. The establishment of an oil palm plantation on a drained peatland will almost certainly convert the land from a net C sink to a net C source, certainly in terms of CO_2 emissions (Hooijer *et al.*, 2006) but until now there has been virtually no data available for the impact of such a land use conversion with respect to the flux of C in fluvial form. Although there was high variability in the data, a 40% greater concentration of leachable DOC was recorded in the oil palm plantation soil than the less disturbed peat soil, despite the much higher SOM content in the latter. Furthermore, the drainage channel within the oil palm plantation contained more than twice the concentration of DOC than the channel draining the peat. This suggests that DOC is an important pathway for the loss of C from oil palm cultivations grown on former peatlands. The initial drainage of a tropical peatland will probably lead to extremely high rates of DOC release. Dissolved organic matter (DOM) is the most mobile portion of organic matter in peat (Glatzel *et al.*, 2003) and in the tropics high temperatures can ensure high rates of organic matter decomposition (Rieley and Page, 2005), which can lead to mobilisation of the peat matrix. However, the oil palm sampled in this study was 8 years old and the high concentrations of DOC in the drainage ditch dissecting the plantation demonstrates the potential for long-term DOC releases following initial peatland drainage.

This may simply be due to the vast reservoir of C in tropical peat soils. A water-table drawdown of tens of centimetres to a metre in a formerly waterlogged peatland can create a large source of potentially leachable C. The peat site sampled had a high organic C content, measured at 0.13 g C cm^3. Assuming this is uniform with depth, this can mean that the drainage of a peatland to a depth of 70cm could potentially result in approximately 91 kg C m^2 becoming available for

aerobic decomposition. As the oil palm plantation sampled in this study was established on a former peatland, the much lower SOM content of the soil compared to the peat site indicates that a large loss of SOM will have occurred following drainage of the peatland due to enhanced decomposition in a less anoxic soil. The magnitude of the loss of SOM was similar to that reported by Detwiler (1986) for the conversion of tropical forests to agricultural land.

Enzymic degradation of SOM can lead to DOC release from peatlands through mobilisation of C from the soil matrix (Fenner *et al.*, 2005). In this study, the activity of β-glucosidase, an enzyme involved in degrading cellulose to glucose (Sinsaburgh, 1991), had equivalent activities in the peat and oil palm soils, therefore the activity of this enzyme cannot explain the higher DOC export potential of the oil palm soil. However, we observed a significant positive correlation between β-glucosidase activity and the SOM content of the soil, highlighting the role of SOM in acting as a substrate for enzymes that mediate organic C decomposition. Similar observations have been reported previously (e.g. Eivazi and Tabatabai, 1990; Waldrop *et al.*, 2000). Shi *et al.* (2006) measured a significant correlation between soil organic C and β-glucosidase, although not for different soil types, indicating other factors influence rates of enzymic decomposition e.g. microbial community composition. The phenolic:DOC ratio gives an indication of SOM composition with respect to enzyme activities. Phenolic compounds are known inhibitors of hydrolytic enzymes (Freeman *et al.*, 1990; Wetzel, 1992) and therefore suppress organic matter degradation (Freeman *et al.*, 2001*a*). The peat soil had a phenolic:DOC ratio twice that of the oil palm, consistent with the build up of phenolics in anoxic soil. The lower phenolics:DOC of the oil palm soil therefore suggest the SOM quality make it more favourable for decomposition, perhaps explaining the greater DOC export potential.

The differences in CO_2 effluxes between a peatland and an oil palm plantation recorded in this study agreed with those of Melling *et al.* (2005), who recorded lower fluxes from oil palm soil compared to a peat swamp. The high CO_2 flux in that study was later attributed to disturbance of

the 'pristine' peatland (Hooijer *et al.*, 2006) and the known disturbance of the peatland sampled in this study would also seem to be responsible for the high CO_2 emissions. Whilst the CO_2 flux from the peat site is not completely reliable for this reason, the emission of CO_2 from the oil palm soil was certainly low. Melling *et al.* (2005) stated the reason for the low CO_2 flux from the oil palm they studied was due to a lack of surface litter and the prevalence of recalcitrant peat (i.e. poor substrate quality). However, we have shown that the soil from the oil palm is rich in water-soluble C substrates which should aid microbial metabolism (Linn and Doran, 1984). Instead, the low effluxes in this and the Melling *et al.* (2005) study may be due to low water content of the soil. A significant correlation between soil water content and CO_2 respiration was observed in this study and has been published previously (e.g. Davidson *et al.*, 2000 and Vanhala, 2002). The dry weather experienced around the time of sampling may have lowered the soil water content sufficiently to suppress soil respiration.

The soil sampled from the forest had the lowest rate of DOC export potential, consistent with its non-peaty classification and low SOM content. The most distinguishing feature of the forest soil data was the high arylsulphatase activity. Arylsulphatase catalyses the release of sulphur bound to organic molecules. The high activity of this enzyme in the forest soil may be due to both a high sulphur demand from the above-ground vegetation and the soil pH. Previous work in a boreal forest has demonstrated the optimum pH for arylsulphatase to be 4-5, similar to that recorded for this forest soil (Wittmann *et al.*, 2004). For comparison, the same study found the pH optimum for β-glucosidase to be 3-4, perhaps helping to explain why the activity of this enzyme was more than 50% lower for the forest soil than the peat and oil palm soils (when normalised to soil dry weight).

There were also some interesting trends for phenol oxidase and phosphatase activities. The activity of phenol oxidase was generally lower than that reported for boreal peatlands (e.g. Fenner *et al.*, 2005). This can probably be attributed to the lower pH of the tropical peat soil, as

6.5 Discussion

pH is known to be an important regulator of phenol oxidase activity (Williams et al., 2000). Given the role of phenol oxidase in degrading recalcitrant and inhibitory phenolic compounds (Freeman *et al.*, 2004b), the enzyme may therefore be important in influencing SOM quality in tropical soils, which we have shown ultimately influences the activity of the key enzyme β-glucosidase (although we did not observe a negative correlation between phenolics and β-glucosidase). The activity of phosphatase was much higher in the peat than the oil palm soil. Phosphatase activity is typically high in wetland soils (e.g. Kang and Freeman, 1999) as phosphate is a key nutrient for microbes and plants and concentrations are low in waterlogged soils due to minimal release of phosphate from the mineralization of organic matter. The very low activity of phosphatase in the oil palm soil is probably due to the use of fertilisers providing sufficient phosphate. The amount of fertiliser typically applied to oil palm plantations is high and has been reported to be between 500 and 1000 kg ha^{-1} (Caliman, 2002). The use of fertiliser may offer an alternative explanation for the higher DOC export potential. Although the role of fertilisers in affecting the leaching of DOC from soils is unclear (Chantigny, 2003), some studies have reported increases in the leaching of organic matter with fertilizer use (e.g. Cambell *et al.*, 1999). The use of fertilisers and its possible impacts on the dynamics of SOM in oil palm plantations therefore warrants further investigation.

6.6 Conclusions

The data obtained in this study suggests that the planting of oil palm on tropical peatland can increase the leaching of DOC into freshwaters. This has important consequences for the global C cycle and for freshwaters, particularly for water quality and the provision of safe drinking water for human consumption. Although more studies are needed to elucidate the causes of the increased DOC export potential, the quality of SOM and its influence on enzyme activities may be crucial. A greater knowledge of the flux of C from peatlands converted to oil palm plantations is now needed to quantify the loss of C into freshwaters and how this compares to pristine peatlands. This study provides further evidence that the planting of oil palms on peatlands converts an ecosystem capable of naturally sequestering large quantities of C into one that becomes a major source of C.

Chapter 7

General Discussion

7.1 Drinking Water Quality

The production of safe drinking water is a multi-faceted process and one of the most critical aspects are the quality of the raw water and processes occurring within the catchment of the water body. In the past, water companies largely disregarded the quality of raw water, providing that the final water was considered safe. However, with stricter guidelines and regulations being introduced this is no longer the case and water companies now have a duty to look at the quality of raw water and any hazards identified within its catchment that may be affecting it.

Llyn Cefni and Llyn Alaw are two shallow, lowland lakes on the island of Anglesey, north Wales, used as drinking water reservoirs that serve the island's population of nearly 70,000. Both reservoirs are subject to high inputs of carbon (C), nitrogen (N) and phosphorus (P) and each have been shown to have a detrimental impact on quality at certain times of the year (Chapter 2 and 3). Analysis of the data collected during the two surveys revealed how the biogeochemical properties of both reservoirs varied not only by the origin and nature of the contaminant but also by season.

There are a multitude of processes that can occur within a freshwater catchment which can be detrimental to water quality and the most important, certainly in terms of disinfection by-product (DBP) formation, are those that influence the concentration and characteristics of dissolved organic carbon (DOC). DOC poses perhaps one of the greatest problems for water treatment and its removal from the water is often the most costly part of the treatment (in terms of chemical usage, labour and waste removal). Unless the removal of DOC is fully effective, any remaining DOC can react with the disinfectant to produce harmful DBPs (Kits *et al.*, 2002) and can also stimulate microbial re-growth in the distribution system (Niquette *et al.*, 2001). Both lakes displayed very different DOC characteristics. A strong seasonal trend was evident for Llyn Cefni (Figure 2.2, p 39), with the greatest concentrations recorded in the autumn, whilst for Llyn Alaw there was no obvious seasonal trend in DOC concentrations and no autumn time flux into

the lake (Figure 2.2, p 39). These differences reflect properties of the lakes themselves and contrasting characteristics of their catchments. One of the most important reasons for the observed differences between Llyn Cefni and Llyn Alaw is the presence of a peatland within the catchment of Llyn Cefni. The peatland, Cors Erddreiniog, was shown to influence the concentration and characteristics of DOC in the reservoir and to ultimately affect the water quality. This is in agreement with previously reported studies that the proportion of peatlands within the catchment of temperate lakes is one of the most influential determinants of the size of the allochthonous C input (Dillon and Molot, 1997). Jones (2006) reported that DOC entering Llyn Cefni via the Afon Erddreiniog inflow has a higher SUVA (Specific UV Absorbance) than that in Afon Cefni, which can be directly related to the leaching of higher molecular weight (MW) DOC from the organic-rich, anaerobic peat soils of Cors Erddreiniog. In Chapter 3, higher SUVA values were also recorded for the Afon Erddreiniog inflow and through ^{1}H-NMR spectroscopy it was also shown that the DOC has a greater proportion of aromatic compounds compared to the DOC isolated from Afon Cefni (Figure 3.2, p 66, 67). These characteristics meant that the DOC from Afon Erddreiniog had a greater THM formation potential (Figure 3.4, p 68).

Rainfall is known to highly influence the amount of DOC in lakes as increased allochthonous input occurs during run-off (Li *et al.*, 2008) and there is ample evidence of this being an important process, particularly for Llyn Cefni. It was demonstrated that the timing of heavy rainfall events on a seasonal basis is of particular importance as the availability of DOC throughout the seasons is different. It is highest during the late summer/early autumn period as rates of microbial and enzymic decomposition of organic matter are at their greatest and there is fresh input of C to the soil from senescing vegetation above ground (Tate, 1987; Kang and Freeman, 1999). During the winter, there is less DOC export from catchment soils as the pool of potentially leachable DOC has been depleted (Freeman *et al.*, 2001*a*). Thus, there is a much

greater potential for large influxes of DOC to freshwaters during the autumn and this was demonstrated for Llyn Cefni (Figures 2.3 and 2.8, p 40, 44). Taken together, these data emphasise that Cors Erddreiniog is of critical importance in influencing the DOC characteristics of Llyn Cefni.

Whilst Llyn Alaw also has a small peatland within its catchment, due to its relative size it did not appear to influence the raw water quality to the extent exhibited by Cors Erddreiniog for Llyn Cefni. It is hypothesised that a significant source of DOC to Llyn Alaw derives from the lake bed. This is due to its original construction taking place on a peatland and due to the lake's shallow depth; this would allow wind-induced mixing to cause upwelling of organic C from the lake bed. Although there was no real seasonal trend in the total DOC concentration of Llyn Alaw, further investigations into the structure of DOC within the lake did reveal a marked seasonal variation. The seasons showing the greatest contrasts in DOC characteristics were summer and autumn. During the summer, the hydrophilic (76%) fraction dominated, with relatively little hydrophobic material (18%), but in the autumn the opposite was true, with 14% hydrophilic and 55% hydrophobic material (Figure 2.6, p 42). These findings demonstrate that the lake was influenced by both allochthonous and autochthonous DOC. The autumn peak in hydrophobic DOC is most likely to be due to the leaching of terrestrially-derived high MW DOC, principally originating from soil (Imai *et al.*, 2001). During the summer the proliferation of algal blooms will lead to a large input of lower MW, hydrophilic structures (Kritzberg *et al.*, 2006). Despite this evidence of seasonality in the DOC characteristics, the processes causing these changes do not appear to be large enough to make significant impacts on total DOC concentrations in Llyn Alaw.

Autochthonous DOC production was also shown to be very important at Llyn Cefni. Algal growth in the lake during the summer and early autumn is known to be intense and visible mats of green algae can frequently be seen. Grasso *et al.* (1990), Biber *et al.* (1996) and Giroldo *et al.*

(2007) described how DOC released by phytoplanktonic organisms is principally composed of amino acids, peptides, proteins and carbohydrates. In Chapter 3, ^{1}H-NMR spectra demonstrated the presence of these compounds in the lake water. Indeed, the signal for autochthonous DOC in the NMR spectra was greater than the terrestrial aromatic signal in October, which is thought to be due to decomposing algal cells from the previous summer. Although the growth of algae does also occur in Llyn Alaw, it is not as intense as that which takes place in Llyn Cefni. This may be due to the absence of significant quantities of P in the lake's catchment (Figure 2.5, p 42), as phosphate is considered to be the key nutrient for algal growth (Schindler *et al.*, 2007).

In addition to the influences of peatlands and algal DOC, the differences between Llyn Cefni and Llyn Alaw can also be explained simply by their size. Lake size is known to affect the extent to which hydrological and biogeochemical process can be influential (Hanson *et al.*, 2007). It has been reported that lake area can correlate inversely with DOC concentrations (Xenopoulos *et al.*, 2003) and with the surface area of Llyn Alaw being four times larger than that of Llyn Cefni (Chapter 2, p 49), this offers an explanation as to why it was difficult to detect dominant processes affecting DOC concentrations at Llyn Alaw.

Although THM concentrations in the final water from Llyn Cefni and Llyn Alaw water treatment works were always below the current UK limit of 100 μg L^{-1} (DWI, 1998), strong seasonal variations in the extent of allochthonous and autochthonous inputs ensure constantly shifting DOC characteristics of the raw waters. This makes treating the water difficult and necessitates regular alterations to the coagulant and chlorine dose. In terms of DOC removal, the higher MW and more aromatic compounds, i.e. those from terrestrial sources such as peatlands (Curtis and Schindler, 1997), are generally more easily removed (Murray and Parsons, 2003), but also have a greater propensity to form DBPs (Archer and Singer, 2006; Fabris *et al.*, 2008). Water treatment facilities at Llyn Cefni must therefore always be operated efficiently due to lake's greater terrestrial DOC influence. DOC derived from algae is lower in MW and aromaticity (Jones,

2006). Although these characteristics ensure it is generally less reactive with chlorine, it is much more difficult to remove by conventional water treatment processes (Knappe *et al.*, 2004) and because it may pass through treatment works it can ultimately provide a substrate for microbial re-growth in the distribution system (Lehtola *et al.*, 2001)

The most successful treatment process for DOC removal is coagulation and this is in place at both treatment works. Based on differences between the raw and final water DOC concentrations, treatment processes at the Cefni works were shown to be more successful than those at the Alaw. This may be because of the greater relative contribution of terrestrially-derived (i.e. more easily removable) DOC at this lake. Although DOC removal was more efficient at the Llyn Cefni treatment works there was a greater formation of THMs when the lake water DOC concentration was high in the autumn; this emphasises the importance of the pulse in DOC at this time of year.

Both lakes were shown to have high concentrations of DOC during the summer. This is when lake water temperatures are highest and as the reaction between organic matter and chlorine is strongly temperature dependent (Knocke *et al.*, 1986), it is unfortunate that the two most influential factors affecting DBP formation peak at almost identical times. This places great emphasis on the need to minimise DOC concentrations during the late summer/early autumn period.

7.2 Influential sources of DOC

The remainder of this thesis focused on the most influential sources of DOC that lead to high lake water concentrations; peatlands and algae.

7.2.1 Peatlands

The health of many of the world's peatlands is one of the most important considerations for water companies in the next few decades, especially given that climate change may be causing destabilisation of peatlands and increased release of DOC to freshwaters (Freeman *et al.*, 2001b). Of all anthropogenic impacts on the world's peatlands, changes in land use have had the most widespread and severest impacts. This is because, for mainly economic reasons, vast areas have been drained for the growth of crops. In the two countries where experiments were undertaken, Finland and Malaysia, large proportions of the country's landmasses are occupied by boreal and tropical peatlands respectively, and the extent of drainage has been particularly pronounced. In Finland, it is estimated that 62% of the countries peatlands have been drained, principally for the growth of forests (Turunen, 2008), whilst in Malaysia, 58% of the countries tropical peatland has been intensively drained mainly for the growth of oil palm (Lee, 2004). The studies undertaken in both countries revealed that even many years after the peatlands had been drained, the soils continued to have a comparatively greater DOC export potential than undisturbed sites (Chapter 5 and 6).

In Finland, peatlands have been shown to highly influence freshwater DOC concentrations (Mattsson *et al.*, 2005) and the extensive drainage of peatlands in this country will have enhanced this effect. It may, therefore, have partly been the cause of observed increases in freshwater DOC concentrations reported in this country (Monteith *et al.*, 2007). However, the effect we observed was not consistent with nutrient status or drainage history, rather, the parameter of

greatest influence on DOC export potential was the water content of the soil. This suggests that the lower the water-table, the greater the potential for DOC release from the peat matrix.

We are living in a time of unprecedented importance in terms of the global environment. There is constant discussion about climate change and the consequences of a warming planet. As it seems unlikely that a 'quick fix' solution will be found for rapidly rising concentrations of CO_2, increased soil temperatures and a lowering of the water-table, for many peatlands is likely to occur to some degree in the next few decades. The repercussions for the C store of peatlands and for DOC export to freshwaters is certainly worrying and there is little that can be done to counteract this 'natural' drying. However, there is a lot that can be done about the artificial drainage of peatlands. This is certainly imperative in Malaysia and other countries with significant areas of tropical peatland. The draining of tropical peatlands in Malaysia to facilitate the production of oil palm is entirely a consequence of human development and the desire for economic growth. But it is happening at an alarming rate and at great environmental cost. For example, CO_2 emissions from degraded peatlands in neighbouring Indonesia alone accounts for 4% of the total global anthropogenic emission of greenhouse gases (Hooijer *et al.*, 2006). The data presented in Chapter 6 has demonstrated that as well as leading to increased CO_2 release, the conversion of tropical peatlands to oil palm plantations increases the leaching of DOC to freshwaters. This is the first known study to report such findings. The extent to which peatlands influence freshwaters in the tropics is not as well known compared to boreal and temperate ecosystems, therefore the results of this study cannot be interpreted in the context of known examples of drinking water sources in Malaysia. However, similar to the results obtained in the Finnish study, treatment of water for the purposes of drinking will become more difficult if drained peatlands are present in the catchment of the source water.

Regarding the role of soil enzymes, for the Finnish site pH correlated with the activity of phenol oxidase, which in turn was suppressed in the sites where significant increases in DOC export

potential was recorded. This emphasises the key role of phenol oxidase in peatlands and suggests that pH may be more important than aeration in controlling its activity at certain sites. This has been reported previously for a peat bog in north America (Williams *et al.* 2000). In Malaysia, the activity of phenol oxidase was lower when comparing activities in the pristine sites for both countries (Figures 5.9, p 121 and 6.8, p 149). This can probably be attributed to the lower pH of the tropical peat soil. In Malaysia, the drained peat (i.e. the site of oil palm growth), which had the highest DOC export potential of the three sampled soil types, also had suppressed activities of phenol oxidase compared to the undisturbed peat soil. Unlike for the Finnish site, this was not caused by an increase in acidity, as no significant change in pH was recorded (Table 6.1, p 142). Instead, the lower activity of the enzyme may have reflected the much lower water content of the soil. Data from Chapter 5 for the Finnish site suggested that phenol oxidase activity is highest when the soil water content is approximately 85-90% (Figure 5.16, p 124). This agrees closely with previous findings (Toberman *et al.*, 2008) and suggests that phenol oxidase activity may not always increase during drought as moisture limitation effects may prevent a response to increased oxygen.

In the Malaysian sites the activity of β-glucosidase was shown to be significantly influenced by the soil organic matter (SOM) content (Figure 6.4, p 147), demonstrating the importance of SOM as a substrate for hydrolytic enzymes. The peat soil had a phenolic:DOC twice as high as the oil palm soil, whilst the β-glucosidase activity was 25% lower. The phenolic:DOC ratio has also been reported to influence β-glucosidase in rivers (Freeman *et al.*, 2000) As it has been demonstrated that β-glucosidase can mobilise DOC from the soil matrix (Fenner *et al.*, 2005), the SOM quality may have been critical in controlling this effect at the Malaysian sites. It may be that the SOM quality was higher for the oil palm soil due to either the preferential past leaching of higher MW DOC, or the greater input of C from above-ground by the oil palm vegetation. The oil palm soil is known be fertilised, this may influence the mobility of DOC and requires further

investigation. For the Finnish study, a decrease in enzyme activities was generally recorded following drainage and it was suggested that, in direct contrast to the Malaysian soil, this may be due to a decrease in SOM quality. Further work is needed to assess the differences in this important soil parameter between boreal and tropical peat to determine its influence on key enzyme activities. It was interesting to note that the change in vegetation and the growth of trees caused an increase in β-glucosidase and β-xylosidase activity in the 48 year drained OM site, possibly due to a stimulation of decomposition caused by previously absent, easily metabolised root exudates. This represents an improvement in SOM quality and emphasises that the dominance of *Sphagnum* in pristine ombrotrophic peatlands can be critical for inhibiting decomposition *via* the enzymic latch (Freeman *et al.* 2001*a*) due to the release of phenolic compounds and a lack of root exudates.

The data from these two studies emphasise the importance of preserving peatlands located within the catchments of water sources and highlight the need to maintain current management practices at Cors Erddreiniog. In Chapters 2 and 3, the peatland was demonstrated to be enhancing the concentration of DBP-forming DOC in Llyn Cefni. If this effect was to intensify due to deterioration in the processes currently locking up C in the peatland, it could seriously undermine the ability of the Llyn Cefni treatment works to produce drinking water that meets current regulations. Due to the SSSI designation given to Cors Erddreiniog, it is unlikely that it will ever be artificially drained. However, a lowering of the water table due to climate change is entirely plausible. Roulet *et al.* (2006) have predicted a decrease of 14-22 cm in the height of the water-table in boreal fens for a doubling of the atmospheric CO_2 concentrations. This is similar to the level of water-table drawdown induced by the forestry and experimentally drained sites at Lakkasuo peatland in Finland. As reported in Chapter 5, water table drawdown led to a significant increase in DOC export potential and the effect was most pronounced the lower the water content of the soil. The effect was observed for the oligotrophic and mesotrophic areas of

peat, and as Cors Erddreiniog is oligotrophic, it suggests that if climate change lowers the water-table of this peatland the export of DOC to Llyn Cefni may increase.

7.2.2 Algal Blooms

Whilst the management of peatlands is deemed to be crucial for minimising the input of terrestrially-derived DOC to freshwaters, agricultural practises within a water body's catchment are crucial for determining the production of DOC within the water. The application of fertiliser to arable land to stimulate crop growth is a common agricultural practice; however, if it is poorly managed, excess nutrients (principally N and P) can leach from the soil into freshwaters. This may be severe enough to induce a state of eutrophication in the water and to lead to the growth of potentially toxic algal blooms. From a drinking water perspective, these blooms can severely hamper treatment processes and diminish the quality of drinking water by clogging filters, raising coagulant and chlorine demand, increasing DBP formation, producing undesirable taste and odours and increasing microbial re-growth potential in distribution systems (Knappe *et al.*, 2004).

The aim of Chapter 4 was to assess the feasibility of a floating constructed wetland (FCW) to reduce the potential formation of algal blooms. The intention was for the system to sequester N and P, depriving the algae of the key nutrients needed for growth and therefore inhibiting their formation. This was achieved through the use of *Phragmites australis* in combination with a peat-based growth medium. The results from this pilot study were encouraging as the FCWs reduced nitrate and phosphate concentrations to undetectable levels (Figure 4.6 and 4.7, p 91) and reduced chlorophyll concentrations in the water to 80% below that of the control system (Figure 4.3, p 89). FCWs may therefore offer an interesting and promising new technique for controlling algal bloom formation, and would have advantages over both traditional fixed constructed wetlands, which are prone to leaching organic matter and nutrients outside of the growing season, and barley straw bales, for which only limited success has been reported (Ball *et al.*, 2001). In order to gain a complete assessment of the potential success of FCW systems in reducing algal

growth formation, the experiment must be up-scaled and tested in a real freshwater lake prone to nitrate and phosphate inputs. This will answer the most important unknown regarding the systems; the potential for increased DOC release. Overall, algae contributed approximately 5.3 mg L^{-1} of DOC to the control ponds, whilst the FCWs contributed 9.5 mg L^{-1} of DOC to the planted ponds; the FCWs therefore contributed approximately an additional 4.2 mg L^{-1} of DOC (Figure 4.3, p 89). If it is assumed that this experiment achieved the maximum reduction in nutrient and algal concentrations possible from an FCW setup, this suggests that the use of these systems in a real freshwater lake should not contribute more than 5 mg L^{-1} of additional DOC. Both Llyn Cefni and Llyn Alaw were shown to be significantly influenced by DOC derived from algal blooms, as they are lowland lakes surround extensively by farmland. The FCWs have the potential to offer a solution to this problem. Algal blooms typically occur during the summer when water temperatures and sunlight levels are at their highest (Johnk *et al.*, 2008) and when terrestrial inputs of DOC concentrations tend to be low due to a reduction in the flux of allochthonous DOC (Chapter 2). However, during late summer/early autumn, as sunlight levels and water temperatures start to decrease, the algae start to die away and release large amounts of DOC, coinciding with the time of year when terrestrial inputs of DOC generally are at their highest. The increased input of DOC from the installation of the FCW would therefore occur at a time when DOC concentrations of the lake are not usually high, and will reduce the input of DOC from algal sources at a time when they are high. The DOC concentration measured in Llyn Cefni during the summer was approximately 6 mg L^{-1} (Figure 2.2, p 39). If a FCW system was to be introduced into Llyn Cefni the concentration of DOC may increase to 11 mg L^{-1}, which is still significantly lower than the peak concentration recorded during the autumn, 16 mg L^{-1}. The water treatment works was able to treat the water during the autumn efficiently, therefore any extra DOC added to the water by the FCWs would not be expected to hamper treatment processes. Furthermore, the amount of DOC released from the FCWs in a real life situation is anticipated to be much lower than 5 mg L^{-1} as the original experiment was carried out

in a small volume of water over a short period of time. In a real situation, the FCWs could be installed at a much lower density (i.e. a lower FCW:water volume ratio) and allowed to sequester nutrients over longer time periods, therefore minimising any DOC release from the systems. A further point to recognise is that although the FCWs may contribute additional DOC into the reservoirs, analysis of the structure of the DOC arising from these systems revealed that it actually favours removal during the treatment process. An increase in the amount of phenolic compounds was measured in the planted system and these high MW structures are more easily removed during water treatment. The DOC characteristics of the control system was indicative of a much lower MW, consistent with the growth of algae, and this type of DOC is more difficult to remove during the physical and chemical treatment of water (Cheng and Chi, 2003). On a per unit of C basis therefore, the systems would improve the efficiency with which the treatment works were able to treat the water.

7.3 Conclusions

Overall, the work presented in this thesis has highlighted the importance of the key allochthonous and autochthonous sources of DOC to freshwaters; peatlands and algae. Central to the issue and critical for minimising DOC concentrations in freshwaters are the implementation of good catchment management practices. Imperative is the preservation of peatlands; they contain vast stocks of C and any disturbance to their ability to hold on to these C stocks could render some sources of freshwater untreatable. Perhaps the most important disturbance to avoid is drainage, as this was shown to increase DOC export potential even over long time scales (Chapters 5 & 6). Also of great importance is for the leaching of excess fertiliser from agricultural land to be minimised in order to prevent algal blooms. In a very recent paper, Oulehle and Hruska (2009) conclude that observed freshwater DOC increases in the last two decades have been due to recovery from acidification and they suggest that catchment management should therefore not be given such great emphasis by water companies. This is a potentially dangerous recommendation. Although the case for recovery from acid deposition driving recent DOC increases is relatively strong (Monteith *et al.*, 2007) the debate is far from settled and there are recent publications that demonstrate that acidity is not the only influence (Worrall *et al.*, 2007a). Numerous past studies demonstrating that aspects of climate change, such as drought (Freeman *et al.*, 2001a), elevated CO_2 (Freeman *et al.*, 2004a), temperature (Fenner *et al.*, 2005) should not be discounted. The variation in water quality in Llyn Cefni and Llyn Alaw reported in Chapter 2 can largely be attributed to the differing catchments, the influence of more than one source of DOC and nutrients and the effect of seasonal changes in climatic conditions.

Climate change is expected to alter average temperatures and precipitation patterns, which may lead to even more intense and frequent floods and droughts (IPCC, 2001). In fact changes in the frequencies of extreme events, such as floods and droughts, may be one of the most significant consequences of climate change (Lenher, 2006). Consequently, it is considered that climate

change will affect the availability of water, as well as its quality, distribution, the complex infrastructure, and systems in place to manage water and existing climate variability (Şen, 2008). General future trends in Europe suggest that increases in average precipitation and its variability are expected for northern regions, suggesting higher flood risks, while less rainfall, prolonged dry spells and increased evaporation may increase the frequency of droughts in southern areas (Lenher, 2006).

This emphasises the importance of processes occurring within the catchment of freshwaters that influence the water quality. As many sources of drinking water contain significant quantities of peatland and agricultural land within their catchments, it would be prudent for water companies to make even greater efforts to improve catchment management and to keep abreast of ongoing research into the potential impacts of climate change.

7.4 Further work

A greater understanding is required of the potential to form DBPs with varying water quality. Whilst we were able to show that the presence of the peatland, Cors Erddreiniog, did lead to a large autumn flux of DOC into Llyn Cefni, which coincided with elevated THM concentrations, we did not explore the potential to form DBPs of varying reservoirs, from upland lakes to boreholes and aquifers. By combining assessments of the characterisation of DOC from varying water types by fractionation and ^{1}H-NMR, it may aid water companies when forecasting predictions of DBP formation. This should also provide a useful insight when deciding on the best type of treatment processes a water treatment works should employ to maximise organic matter removal.

Perhaps the most interesting future experiment is to determine the feasibility of an FCW setup for minimising algal growth in freshwaters. An ideal site in which to up-scale the experiment would be Llyn Cefni, as it is naturally divided into two narrowly linked sections. A series of FCWs could be added to one side and the other left as a control to enable accurate determination of the ability of the systems to sequester nitrate and phosphate and their potential to release DOC.

In Chapters 5 and 6 it was demonstrated that drainage of peatlands in Finland and Malaysia increased the DOC export potential of the soil. However, this is only important if it can be shown that the drained sites actually have a greater rate of DOC export to freshwaters. In order to determine this it is therefore vital to measure the DOC flux occurring from these drained peatlands. Additionally, further work is necessary to determine the age of the C being released from the peatlands. This would provide a valuable insight as to whether the additional C being released from these systems due to drainage is a consequence of increased plant productivity ('new' carbon) or the degradation of C stocks ('old' carbon).

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