# Biologically-relevant characteristics of dissolved organic carbon (DOC) from soil

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# Statement of Originality

I hereby confirm that this research was carried out by the undersigned and that all research material has been duly referenced and cited.

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# Acknowledgements

# Abstract

Of the organic matter in soils typically < 1% by weight is dissolved in the soil solution (dissolved organic matter; DOM). DOM is a continuum of molecules of various sizes and chemical structures which has largely been operationally defined as the fraction of total organic carbon in an aqueous solution that passes through a 0.45 µm filter. Although only representing a relatively small proportion, it represents the most mobile part of soil organic carbon and is probably enriched with highly labile compounds. DOM acts as a source of nutrients for both soil and aquatic micro-organisms, influences the fate and transport of organic and inorganic contaminants, presents a potential water treatment problem and may indicate the mobilisation rate of key terrestrial carbon stores. The objective of this research was to ascertain some of the biologically relevant characteristics of soil DOM and specifically to determine: (1) the influence of method and time of extraction of DOM from the soil on its biochemical composition and concentration; (2) the dynamics of DOM biodegradation; and, (3) the effects of repeated applications of trace amounts of DOM on the rate of soil carbon mineralization.

To examine the influence of method and time of extraction on the composition and concentration of DOM, soil solution was collected from a raised peat bog in Central Scotland using water extraction, field suction lysimetry, and centrifugation techniques on a bimonthly basis over the period of a year (Aug 2003 – Jun 2004). Samples were analysed for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), protein, carbohydrate and amino acid content. For all of the sampled months except June the biochemical composition of DOC varied with extraction method, suggesting the biological, chemical and/or physical influences on DOC production and loss are different within the differently sized soil pores. Water-extractable DOC generally contained the greatest proportion of carbohydrate, protein and/or amino acid of the three extraction methods. Time of extraction had a significant effect on the composition of water- and suction-extracted DOC: the total % carbohydrate + protein + amino acid C was significantly higher in Oct than Dec, Feb and Jun for water-extracted DOC and significantly greater in Dec than Aug, Apr and Jun for suction-extracted DOC. There

was no significant change in the total % carbohydrate + protein + amino acid C of centrifuge-extracted DOC during the sampled year. Time of extraction also had a significant effect on the % protein + amino acid N in water- and centrifuge-extracted DON: Oct levels were significantly higher than Feb for water-extracted DON and significantly higher in Aug and Apr for centrifuge-extracted DON. Concentrations of total DOC and total DON were also found to be dependent on time of extraction. DOC concentrations showed a similar pattern of variation over the year for all methods of extraction, with concentrations relatively constant for most of the year, rising in April to reach a peak in Jun. DON concentrations in water- and centrifuge-extracted DON peaked later, in Aug. There were no significant seasonal changes in the concentration of suction-extracted DON. A lack of correlation between DOC and DON concentrations suggested that DOC and DON production and/or loss are under different controls.

Laboratory-based incubation experiments were carried out to examine the dynamics of DOC biodegradation. Over a 70 day incubation period at $20^{o}C$, the DOM from two types of peat (raised and blanket) and four samples of a mineral soil (calcaric gleysol), each previously exposed to a different management strategy, were found to be comprised of a rapidly degradable pools (half-life: 3 – 8 days) and a more stable pool (half-life: 0.4 to 6 years). For all soil types/treatments, excepting raised peat, the total net loss of DOC from the culture medium was greater than could be accounted for by the process of mineralization alone. A comparison between net loss of DOC and loss of DOC to $CO_2$ and microbial biomass determined by direct microscopy suggested that at least some of the differences between DOC mineralised and net DOC loss were due to microbial assimilation and release. Changes in the microbial biomass during the decomposition process showed proliferation followed by decline over 15 days. The protein and carbohydrate fractions showed a complex pattern of both degradation and production throughout the incubation.

The effects of repeated applications of trace amounts of litter-derived DOC on the rate of carbon mineralization over a 35 day period were investigated in a laboratory based incubation experiment. The addition of trace amounts of litter-derived DOC every 7 and 10.5 days appeared to 'trigger' microbial activity causing an increase in $CO_2$

mineralisation such that extra C mineralised exceeded DOC additions by more than 2 fold. Acceleration in the rate of extra C mineralised 7 days after the second addition suggested that either the microbial production of enzymes responsible for biodegradation and/or an increase in microbial biomass, are only initiated once a critical concentration of a specific substrate or substrates has been achieved. The addition of 'DOC + nutrients' every 3.5 days had no effect on the total rate of mineralization.

To date DOC has tended to be operationally defined according to its chemical and physical properties. An understanding of the composition, production and loss of DOC from a biological perspective is essential if we are to be able to predict the effects of environmental change on the rate of mineralization of soil organic matter. This research has shown that the pools of DOC extracted, using three different methods commonly used in current research, are biochemically distinct and respond differently to the seasons. This suggests some degree of compartmentalisation of biological processes within the soil matrix. The observed similarities between the characteristics of the decomposition dynamics of both peatland and agricultural DOC suggests that either there is little difference in substrate quality between the two systems or that the microbial community have adapted in each case to maximise their utilisation of the available substrate. The dependency of the concentration and biochemical composition of DOC on the seasons requires further work to ascertain which biotic and/or abiotic factors are exerting control. Published research has focussed on factors such as temperature, wet/dry cycles, and freeze/thawing. The effect of the frequency of doses of trace amounts of DOC on increasing the rate of soil organic C mineralization, evident from this research, suggests that the interval between periods of rainfall may be relevant. It also emphasises how it can be useful to use knowledge of a biological process as the starting point in determining which factors may be exerting control on DOC production and loss.

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# Chapter 1: An Introduction to Dissolved Organic Matter in Soils

## 1.1. Organic Matter in Soils

Terrestrial soils contain some $1500 \times 10^3$ Mt of organic carbon, roughly double that in plants or in the atmosphere (Buringh, 1984). The amount of carbon in the soils of Great Britain is estimated to be 9838 Mt (6948 Mt in Scotland and 2890 Mt in England and Wales), over 80 times greater than the 113.8 Mt estimated to be stored in the vegetation (Milne and Brown, 1997). This organic matter ranges from the living organisms in the soil, the soil biomass, through undecayed plant and animal tissues and intermediate products of decomposition, to a fairly stable, homogenous brown to black material, referred to as humus, which shows little trace of the structures from which it was derived.

Soil organic matter typically accounts for 5% of the total soil volume, although this can be highly variable (Sylvia et al., 1998). Values for total organic carbon in two Swedish agricultural systems, one typical of an agricultural crop and the other typical of a perennial crop, equated to approximately 100 000 kg C ha$^{-1}$. Of this total, shoots and surface litter accounted for 3.72 – 4.02 % of the total, the soil microbial biomass 3.14 %, roots 0.68 - 4.65 %, and metazoan fauna (arthropods, nematodes, enchytraeids and earthworms) 0.01 – 0.06 % (Paul and Clark, 1996). The actual amount of organic C contained in a particular soil is a function of the balance between the rate of deposition of plant residues in or on the soil and the rate of mineralisation of the C residue by soil biomass. Losses by erosion or leaching may be significant in some cases (Baldock and

Nelson, 2002). With the exception of peatland and wetland soils, which accumulate 0.1-0.3 Pg C yr$^{-1}$ globally (Post et al., 1990), organic C levels do not increase indefinitely but tend toward equilibrium (Baldock and Nelson, 2002).

Plant residues provide the primary resources for organic matter formation in soil. The amount of plant litter, its composition and its chemical properties are essential controlling factors for the formation and decomposition of soil organic matter in terrestrial ecosystems (Swift *et al.*, 1979). A considerable proportion of the organic material becomes incorporated into the soil below-ground as root litter or through the process of rhizodeposition (Kögel-Knabner, 2002). In forest soils the contribution of root litter to the input of organic matter in the forest floor in cool-temperate climates varies between 20-50%, depending on the tree species and the life form (evergreen or deciduous) (Vogt *et al*., 1986). The contribution of animal residues to soil organic matter formation is relatively small (Kögel-Knabner, 2002), although soil animals, particularly invertebrates, do play an essential role in controlling litter decomposition in soils (Wolters, 2000).

As soil organic C is the main substrate for soil microorganisms, the turnover of organic carbon in the soil is determined by the activity of the soil microbial biomass. Although representing just 1-2 % of the total organic carbon in soil (Killham, 1994), the saprophytic soil microbes are the driving force of the carbon cycle (Paul and Voroney, 1980). When organic residues are added to soils, the  simple organic constituents e.g. amino acids and simple sugars, are either directly taken up by the microbes and oxidised during the process of respiration to $CO_2$ or assimilated for the biosynthesis of

new cellular material. More complex polymers such as cellulose are also microbially broken down by microbes to release readily oxidised or assimilated monomers. As decomposition progresses, the more resistant components tend to accumulate and reactive compounds are generated, some resulting from microbial modification of decomposing plant constituents and others from the production of microbial metabolites (Sylvia et al., 1998). Reactive aromatic compounds e.g. phenolics, enter into condensation reactions to form new polymeric materials often more resistant to decomposition than the original plant tissues (Sylvia et al., 1998). This process is called humification and the relatively stable (recalcitrant) material referred to as humus.

## 1.2.  Dissolved Organic Matter (DOM) in Soils

### 1.2.1  What is DOM?

Dissolved organic matter (DOM) of soils is usually operationally defined as the fraction of total organic matter in an aqueous solution that passes through a 0.45 µm filter. That which remains on the filter is defined as particulate organic matter. As the boundaries between dissolved, colloidal and particulate matter are not clear, particularly at the larger, less polar end of the range of organic molecules (Baldock and Nelson, 2002), DOM is regarded as a continuum of organic molecules of various sizes and structures (Kalbitz and Geyer, 2002), which may include colloidal suspensions (Moore, 1998). It includes both low molecular weight molecules such as simple carbohydrates and amino acids, as well as more complex, high molecular weight compounds largely derived from microbial metabolites and decomposition products of lignin and lignocellulose (Qualls and Haines, 1992)(Guggenberger *et al.*, 1994; Koivula and Hänninen, 2001; Küsel and Drake, 1999)

### 1.2.2  Extraction Methods

In the field, methods of extraction of soil water include the use of tension or zero–tension porous cups, percolation trays or wick lysimeters. In the laboratory DOM has been extracted by leaching undisturbed cores or repacked columns, by forcing soil solution through a porous membrane using a vacuum or centrifugal force or by shaking air-dried or field-moist soil with water, dilute salt or alkali solutions followed by separation of the solution by centrifugation or filtration. These different methods frequently used in current research may each be sampling different biochemical

compartments of the soil DOM. Criticisms of these methods and reasons why each may only be sampling a fraction of whole soil DOM are discussed in Chapter 2 (section 2.1).

### 1.2.3  Chemical Composition

Most analyses of the chemical composition of DOM have focused on quantification of the total organic carbon (dissolved organic carbon: DOC) and/or the relative amounts and characteristics of fractions separated by solubility in dilute solutions of NaOH and HCl or sorption chromatography. Classically, alkali extracted soil organic matter was fractionated into humic and fulvic acids based on its solubility in dilute solutions of NaOH and HCl. Characterisation by liquid state $^{13}$C-NMR spectroscopy, however, showed that water-soluble organic matter is significantly different from alkali-extracted humic and fulvic acids (Herbert and Bertsch, 1995): water soluble organic matter has a higher proportion of O-alkyl-C, reflecting the presence of polysaccharides and aliphatic acids and a lower proportion of aromatic C than fulvic and humic acids (Novak and Bertsch, 1991).

Sorption chromatography separates DOM according to the charge and hydrophilic/hydrophobic tendencies of its constituents, using non-ionic and ionic exchange resins (Leenheer, 1981). The components of DOM are therefore defined by their hydrophobic acid, hydrophobic base, hydrophobic neutral, hydrophilic acid, hydrophilic base and hydrophilic neutral properties, properties that regulate their interaction with soil surfaces (Qualls and Haines, 1991). This technique has shown DOM to be typically dominated by hydrophobic and hydrophilic acid fractions, for example 75 % total DOM in a forest floor under spruce stands (Qualls and Haines,

1991) and 79-86 % total DOM in the organic horizons of podzols (Cronan and Aiken, 1985; Vance and David, 1989) had hydrophobic and hydrophilic acid properties.

Various methods have been used to characterise fractionated and un-fractionated DOM at the molecular level. Some have involved pyrolysis or chemical or catalytic depolymerisation e.g. acid-hydrolysis of polysaccharides and CuO oxidation of lignin, followed by separation and quantification of the derivatives using a gas chromatograph (GC) equipped with a flame ionisation detector (e.g. (Kaiser *et al.*, 2001)) or mass spectrometer (GC/MS) (e.g. (Huang *et al.*, 1998)) to measure quantities of specific molecules.   Other methods have included liquid-state nuclear magnetic resonance spectroscopy (NMR) of [1]H (Kaiser *et al.*, 2002; Kalbitz *et al.*, 2003a) and ^{13}C isotopes (Guggenberger *et al.*, 1994; Kaiser *et al.*, 2001) to assess the quantity and distribution of specific functional groups within a sample and UV absorbance at 280 nm to estimate changes in aromaticity (Kalbitz. *et al.*, 2003b). Examples of the types of information on the chemical structure of DOC elucidated using these techniques is illustrated in Figure 1.

Several studies have shown that the both the chemical characteristics and concentrations of DOC show seasonal variation (Kaiser *et al.*, 2001; Marschner and Kalbitz, 2003; Nelson *et al.*, 1994), however there is little information on the seasonal variation exhibited by biologically significant components such as total dissolved organic nitrogen (DON), carbohydrates, amino-acids and proteins.

**Bulk DOM**

- consists of carbohydrate, aromatic, aliphatic and carbonyl structures (Novak and Bertsch, 1991; Guggenberger et al., 1994).
- summer and autumn samples have larger abundances of aromatic and aliphatic structures, carboxyl groups and less carbohydrate than winter and spring samples (Kaiser et al., 2001).

**Hydrophilic fraction**

- dominated by O-alkyl C, indicating largely composed of carbohydrates, and carboxyl C indicating oxidative transformation of organic matter. Little aromatic C therefore little contribution of lignin-derived compounds (Kaiser et al., 2001)

**Hydrophobic fraction**

- large proportions of aromatic C and small proportions of O-alkyl C. Large contribution of carboxyl C suggesting strongly oxidised m,aterial;. Evidence of O-aryl C, substitutred aromatic C and methoxyl C indicate that parts of the fraction likely to be derived from lignin decomposition (Kaiser et al. 2001)

**Hydrophillic Acid Fraction**

- higher degree of oxidative biodegradation than hydrophobic acids and higher ratio of microbially released polysaccharides versus plant-derived polysaccharides – therefore possibly intermediates in the decomposition of the hydrophobic acids (Guggenberger et al., 1994).

**Hydrophillic Neutral Fraction**

- enriched in carbohydrates mobilised by enzymatic cellulose and hemicellulose breakdown, as well as from microbial origin (Guggenberger et al., 1994).

**Hydrophobic Acid Fraction**

- high conc of carboxyl and hydroxyl groups + high ratio of vanillic acid to vanillin, both indicating a high degree of biodegradation of plant-derived compounds (Guggenberger et al., 1994).

**Hydrophobic Neutral Fraction**

- less degraded dimeric 'condensed' lignin sub-units and relatively high contents of non carbo aliphatics (Guggenberger et al., 1994).

**Figure 1** Example analyses of the chemical composition of DOM

## 1.2.4 Significance and Importance

DOM represents only a relatively small proportion (< 1 %) of soil organic matter (Killham, 1994), however it represents the most mobile part of soil organic carbon and is probably enriched with highly labile compounds. This has many far reaching consequences for both terrestrial and aquatic ecosystems:

- DOM provides a potential source of carbon for microbial metabolism (Meyer et al., 1987) both in the soil and in aquatic environments (Herbert and Bertsch, 1995). DOC from terrestrial sources forms the major component of the annual carbon budget in many headwater streams, providing the major source of energy for heterotrophic biological activity (Brooks *et al.*, 1999) and aged terrestrial sources of carbon are important contributors to the oceanic carbon budget (Raymond and Bauer, 2001).

- DOM is a major controlling factor in soil formation through the translocation of oxides, humus and silicate clays in soils (Dawson et al., 1978);(Jenny, 1980; Lundström et al., 1995)

- The transport and fate of organic and inorganic contaminants, including polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, pesticides, herbicides and metals in the environment can be influenced by the presence of DOM (Herbert and Bertsch, 1995). In some cases the contaminant may have a particularly high affinity for DOC, as may be the case for some PAHs (Marschner, 1998) and halogenated hydrocarbons (Kalbitz and Popp, 1999), or the DOM may occupy sites within the soil that would otherwise bind contaminants (Zsolnay, 2003).

- Aromatic compounds present in DOM absorb visible light at the blue end of the spectrum resulting in a characteristic brown coloration. This colouring affects the

penetration of light into surface waters (Schindler et al., 1996) and has to be removed from water extracted from rivers for aesthetic reasons before it can be used for domestic and industrial supply. Organic acids found in DOM react with HOCl (hypochlorous acid), used in water treatment processes, to form trihalomethanes (THMs). The 1998 European Drinking Water Directive sets limits to the concentrations of THMs because of their possible health effects (WHO, 1998).

- The oxidation of DOM in microsites may cause an increase in production of the greenhouse gases methane and nitrous oxide by depleting oxygen in microsites and acting as an electron donor [reviewed by Zsolnay, 1996].

- The production and export of DOM may be influenced by climate change and/or declining acid deposition. An average increase of 91 % in DOC concentrations has been observed over 15 years (1983-2003) in all of 22 acid-sensitive catchments, i.e catchments containing grazed moorland or significant areas of coniferous plantation forestry, that form the UK Acid Waters Monitoring Network (UKAWMN) (Evans *et al.*, 2005; Freeman *et al.*, 2001). During this time temperature trends across the UK showed an increase in mean temperature of $0.75^{\circ}C$ compared to 1960-87 data. Older datasets suggest that DOC increases were occurring at least as early as the 1980s (Harriman et al., 2001) and possibly as far back as the 1960s and 70s where water supply company data of water colour from peaty catchments in North East England showed several significant increases in colour and no decreases (Watts et al., 2001: Worrall et al., 2003). Similar trends have been observed in Northern Europe (Hejzlar et al., 2003; Skjelkvale et al., 2005) and North America (Driscoll et al., 2003; Stoddard *et al.*, 2003) suggesting a response to one or more external drivers.

- Analysis of the UKAWMN data suggests that the effects of both climate change and decreased acid deposition, and not hydrological change, nitrogen enrichment or

atmospheric $CO_2$ enrichment, may be significant (Evans *et al.*, 2005). Increasing temperatures have been shown to cause an increase in DOC production in the laboratory (Andersson and Nilsson, 2001; Christ and David, 1996) and in leached DOC in the field (Tipping *et al.*, 1999). Soils release greater quantities of organic acids in response to decreasing inputs of mineral acids (Krug and Fink, 1983) and increasing pH has been shown to increase DOC release in organic soils in the laboratory (Tipping and Hurley, 1988).

## 1.2.5  Sources and Losses

### 1.2.5.1    Introduction

DOM enters the soil water from precipitation, throughfall, stemflow, and surface and sub-surface litter leachate, and is generated within the soil through the processes of excretion from organisms, root exudation, exoenzymatic hydrolysis, microbial lysis and the abiotic processes of desorption and dissolution (Figure 2). It is removed from the soil water through microbial assimilation, root and/or mycorrhiza uptake, respiration, sorption to the solid phase, precipitation, and as leachate to surface watercourses and groundwater (Figure 3).
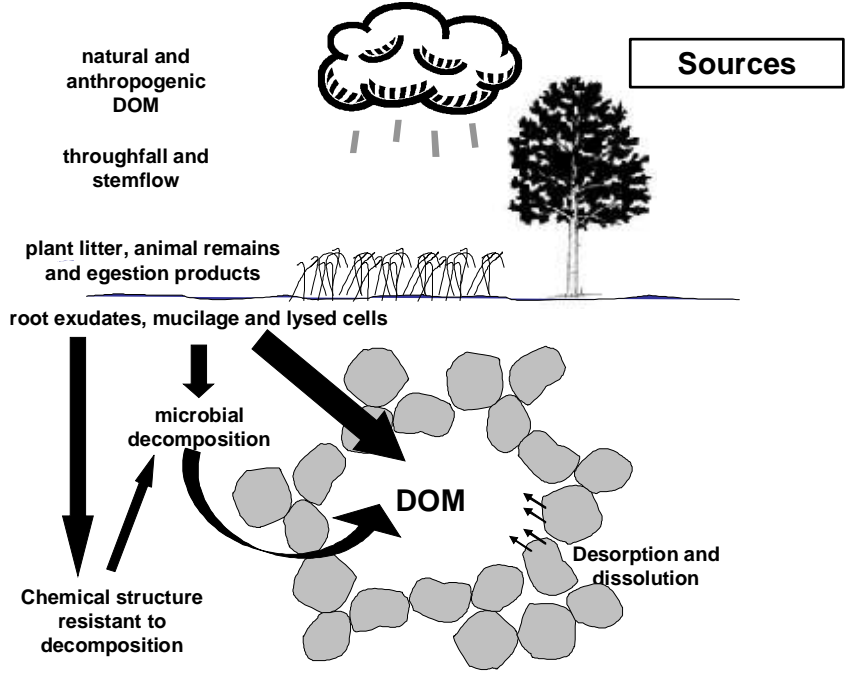
**Figure 2** Schematic representation of soil DOM sources**.**
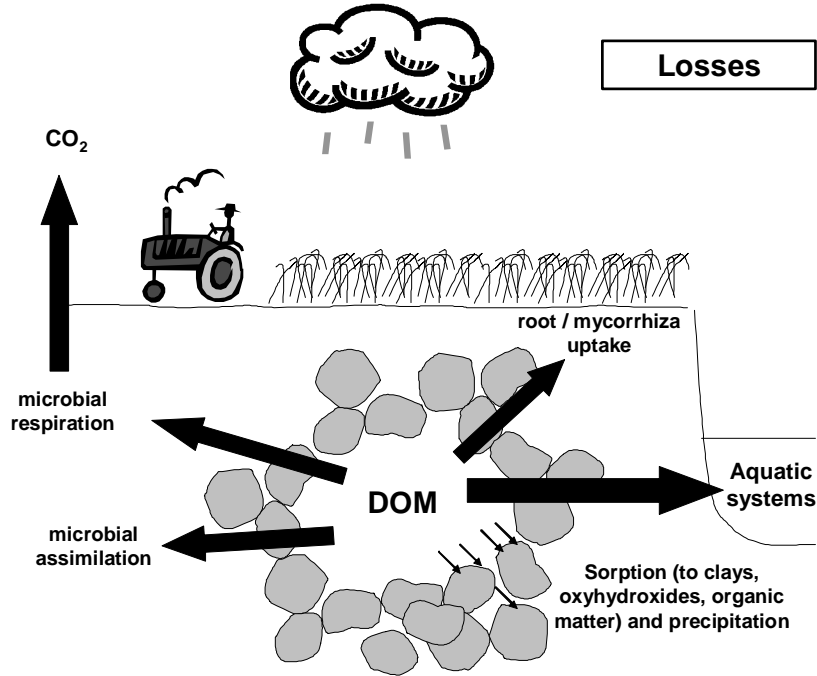


**Figure 3** Schematic representation of soil DOM losses.

**1.2.5.2     Leaching from Surface and Sub-surface Litter**

As plants senesce and begin to decompose, a proportion of their constituent organic matter is solubilised and rapidly leached from particulate material (Tukey, 1970). Although a number of investigations have shown that in forest ecosystems, solutions passing through the organic soil horizon are enriched with both DOC and DON (e.g. (McDowell and Likens, 1988; Qualls and Haines, 1991) (Currie *et al.*, 1996)), it is still unclear whether the dominant source of DOM is recent litter and/or microbial metabolites or relatively stable organic matter in the lower part of organic horizons (reviewed by Kalbitz *et al.*, 2000). The results of some research have suggested that DOM predominantly originates from the freshly fallen litter layer (Oi) and/or the partially decomposed layer (Oe) of the organic horizon (Michalzik and Matzner, 1999; Qualls and Haines, 1991). Studies on the carbohydrate composition of DOC from the Oa horizon of forest soils showed relatively high galactose + mannose to arabinose + xylose ratios of 0.67 – 6.78 (Guggenberger *et al.*, 1994). Arabinose and xylose are quantitatively important constituents of plant polysaccharides but not of microbes (Oades, 1984) and therefore such ratios indicate the dominance of microbially derived carbohydrates in DOM. Gregorich et al. (Gregorich *et al.*, 2000) on the other hand found that water-soluble organic $^{13}$C isotope signal in agricultural soils more closely resembled whole soil C than the microbial biomass, and the microbial biomass more closely resembled recent $C_4$ maize residues. This suggested that microbial biomass or recently deposited plant residues are not a major source of water soluble carbon in agricultural systems.


While most DOM leached from surface horizons is attenuated in the soil profile, a proportion will be transported to ground- or surface-waters e.g. (Wassennar et al., 1991)

found 20% of the mean annual flux of DOC from the A horizon, underlying a mixed boreal forest, was transported to the ground-water flow zone of a shallow silty-sand aquifer. The quantity of DOM exported to ground- or surface waters depends primarily on the rate of production of DOM in the organic horizon, the rate of DOM adsorption in the mineral horizons, and, hydrological conditions (McDowell and Likens, 1988) (Cronan and Aiken, 1985; Grieve, 1994; Moore, 1998).

### 1.2.5.3    Microbial Assimilation, Biodegradation and Production

Soil DOM is a substrate for micro-organisms (Zsolnay and Steindl, 1991); (Qualls and Haines, 1992; Zsolnay, 1996). Since soil microbes are basically aquatic (Metting, 1993) and their metabolism depends on the uptake of low molecular weight compounds (organic acids, sugars, amino acids) in solution across the cell membrane, DOC may be their most important C source (Marschner and Bredow, 2002). Carbon originating from the DOC will be mineralised to $CO_2$ through  the process of respiration, used to synthesise new cellular material or used to synthesise metabolites which are then released into the extracellular environment. Released metabolites may include enzymes, simple organic acids, polysaccharides and polyaromatic melanoid pigments (Sollins et al., 1996).

Biodegradation of DOM is quantified by the disappearance of DOC, the evolution of $CO_2$ or the disappearance of $O_2$. Quantifying disappearance of DOC measures both mineralization and assimilation whilst quantifying the disappearance or the evolution of $CO_2$/disappearance of $O_2$ measures mineralisation only. The dynamics of DOC

decomposition show a rapidly decomposable fraction with a turnover time of the order of a few days or less, and a slowly decomposable fraction with a turnover time of the order of several years (Qualls and Haines, 1992; Zsolnay and Steindl, 1991). The process of biodegradation itself induces changes in the properties of the DOM (Kalbitz *et al.*, 2003a). For instance, over a 90 day incubation period the relative abundance of recalcitrant lignin-derived aromatic compounds, such as phenols and lignin monomers, and microbially-derived carbohydrates and peptides were observed to increase at the expense of lignin dimers (Kalbitz *et al.*, 2003a). Although several studies have examined the kinetics of DOM biodegradation and Kalbitz et al. (2003) looked at changes in relative abundance of a number of DOM components, including carbohydrates and peptides, before and after a 90 day incubation period, there is little information available on the pattern of change of biological molecule concentrations and microbial population size during the decomposition process.

Several factors have been linked to the rate and extent of DOM biodegradation. These include its chemical composition, physical accessibility, the availability of N and $O_2$, temperature, soil water potential and sorption to soil surfaces. The influence of each of these factors is outlined below:

### (i)    Chemical Composition of DOM

Biodegradability of soil organic matter is enhanced by a high carbohydrate, organic acid and protein content (Marschner and Kalbitz, 2003) and reduced by the presence of more recalcitrant compounds such as alkyls and aromatics which accumulate during the decomposition process (Baldock et al., 1992; Kögel-Knabner *et al.*, 1992). Phenolic, lignin degradation products and some of the other products of residue decomposition can be considerably modified by microbial reaction and/or through chemical, oxidative

condensations (Killham, 1994) stabilising them against further biodegradation (Huang *et al.*, 1999). Soluble polyphenols, phenolic acids and plant-derived tannins have been shown to inhibit the activity of various enzymes (Benoit et al., 1968; Benoit et al., 1968; Gianfreda et al., 1995; Gianfreda et al., 1995). High litter quality materials have been found to produce greater amounts of biodegradable DOM (Boyer and Groffman, 1996). For example, DOC extracted from agricultural soils was found to have a higher percentage of degradable C and a higher rate constant, than DOC extracted from forest soils (Boyer and Groffman, 1996).

### *(ii)    Physical Accessibility*

Aggregation of soil particles may influence the accessibility of substrate to microbes and rates of diffusion of reactants and products of extracellular synthesis reactions, although direct evidence for this is limited (reviewed by Sollins et al., 1996). Aggregation may serve as much to protect microbes from predation as to protect substrate from degradation (Sollins et al., 1996).

### *(iii)    N availability*

Micro-organisms need a C source for both energy and growth. The utilisation of C for energy depends on the size of the microbial population. The utilisation of C for growth will not occur unless important nutrients such as N and P are present therefore nutrient limitations for the degradation of DOM will only arise when the ratio of biodegradable DOM to microbial biomass is wide and when DOM is poor in N or P. Elevated N inputs have been linked to increased DOM production (Guggenberger and Zech, 1993) possibly through enhanced microbial activity resulting in an increased release of DOM as intermediate degradation products and microbial metabolites (Zech *et al.*, 1994) or N-induced suppression of complete lignin degradation (Haider, 1986).

### (iv)     $O_2$ Availability

Decomposition is less complete and slower under anaerobic conditions than under aerobic conditions (Jenkinson, 1981), producing a higher proportion of water-soluble intermediate metabolites (Otsuki and Hanya, 1972) than aerobic conditions. When soils are so wet that the larger pores are water-filled, decomposition is limited by the rate at which oxygen can diffuse to the sites of microbial activity. No organic plant or animal component is completely resistant to decomposition under moist aerobic conditions, however, under strictly anoxic conditions aromatic structures such as components of lignin compounds can accumulate (Mathur and Farnham, 1985) as cleavage of the aromatic ring structure requires molecular oxygen.

### (v)     Temperature

Within the physiological range (0 - 35$^o$C) temperature stimulates microbial activity (Paul and Clark, 1996) and extracellular enzyme activity (McClaugherty and Linkins, 1990). As a consequence DOC production may increase at higher temperatures due to enhanced microbial breakdown of larger insoluble compounds to smaller soluble molecules (Christ and David, 1996). On the other hand, increased microbial activity also results in enhanced biodegradation and mineralisation of DOM. If this process dominates over the enhanced production, the net effect of microbial activity will be DOM depletion.

### (vi)     Soil Water Potential

Microbial activity is strongly influenced by soil water potential. As water potential reaches large negative values, microbial activity and hence biodegradation of DOM ceases (Jenkinson, 1988). Conversely, under the anaerobic conditions caused by saturation biodegradation is less efficient

### (vii)    Sorption to Soil Surfaces

Sorption to mineral surfaces can stabilise the organic matter against decomposition through complexation of functional groups, changed 3-D conformations and/or reducing its physical accessibility to microbes. On the other hand sorption to iron hydroxides within microbe-rich biofilms may lead to a concentration of organic matter at the microsite level (Lünsdorf *et al.*, 2000) enhancing the degradation of potentially soluble organic molecules (Guggenberger and Kaiser, 2003).

The importance of the role of the soil microbial community in the production of DOM is still a topic of debate. Guggenberger et al. (1994) and Huang et al. (1998) found microbial metabolites to be components of DOM, yet Park et al. (Park *et al.*, 2002) found only a weak correlation between $CO_2$ and DOC production. Gregorich et al. (Gregorich *et al.*, 2000) found in an agricultural soil that water-soluble organic $^{13}$C isotope signal more closely resembled whole soil C than the microbial biomass, and the microbial biomass more closely resembled recent $C_4$ maize residues, suggesting that the microbial biomass is not a major source of water soluble carbon. Microbial activity is undoubtedly important in the decomposition of macromolecules in the soil, such as cellulose, hemicellulose, proteins and lignin. It is possible that the turnover time of the relatively labile breakdown products of some of these molecules is so fast, e.g. half-life of amino acids in L, O and Ah horizons in permafrost-dominated taiga soils = 4, 6 and 14 h respectively (Jones and Kielland, 2002), that they do not cause a measurable change in the concentration of the labile organic molecules in the soil water (McDowell, 2003).

The role of microbial activity in the formation of the more refractory organic matter that appears to comprise a large bulk of the soil solution has rarely been investigated and may be too difficult to detect against the background of a large pool of adsorbed soluble matter (Qualls, 2000). However, it is known that microbes can mediate extracellular synthesis reactions by releasing extra-cellular peroxidases and phenoloxidases that oxidise phenols to quinines, which then react with O, N, and S nucleophiles in other phenols, amino acids, or peptides to yield aromatic polymers (Stevenson, 1982; Stott and Martin, 1990).

### 1.2.5.4    Sorption and Desorption

DOM inputs to mineral soils generally greatly exceed outputs and this in the past has been largely attributed to DOM sorption to mineral phases in subsoil horizons (Guggenberger *et al.*, 1998; Qualls and Haines, 1991; Qualls and Haines, 1992). Sorption and desorption are the main abiotic processes controlling transfer between the potentially soluble but sorbed matter in the soil and the DOM pool. The chemical interactions between a dissolved organic molecule and its sorbant usually lead to a change in the 3-d conformation of the organic molecule (Guggenberger and Kaiser, 2003) such that it no longer fits the active site of hydrolase enzymes and is thus effectively chemically stabilised against biodegradation. The degree of sorption depends on soil properties, such as the amount of organic C, Al and Fe oxides/hydroxides present, the mineralogy of the clay fraction (Kaiser *et al.*, 1996) and the chemical character of the DOM (Moore, 1998). The capacity for the solution to equilibrate with sorbing surfaces in some horizon can be influenced by hydrological conditions e.g. rate of sorption relative to the hydrologic residence time; rate of

diffusion relative to the hydrologic residence time; preferential flow short circuiting exposure of entire soil surface; flow paths such as surface runoff, lateral flow paths and perched water tables by-passing a strongly adsorbing mineral horizon (Qualls, 2000).

In inorganic horizons DOM is strongly adsorbed in the mineral soil to Al and Fe oxides/hydroxides and clay minerals, especially if the surface has low pre-existing levels of adsorbed carbon (Kalbitz *et al.*, 2000). Dissolved organic matter, due to its complex nature, can sorb to natural surfaces, such as Al and Fe oxides/hydroxides and clay minerals, through several different mechanisms including anion and ligand exchange{Tipping 1981 #198} (Jardine *et al.*, 1989; Murphy et al., 1990), cation bridging (Moore, 1998), hydrogen bonding, van der Waal's forces, and physical adsorption (Jardine *et al.*, 1989). In addition to sorption to the mineral components of the soil itself, sorption is thought to take place to Fe hydrous oxides present in biofilms. Biofilms form along preferential flow paths (Bundt, 2001) enriched in carbon (Guggenberger and Kaiser, 2003) and consist of bacterial cells growing within an organic matrix of extracellular polysaccharides (Lewandowski et al., 1994). Within the matrix, microgranular clusters of iron hydrous oxides (Lünsdorf *et al.*, 2000) may remove DOM from the soil water and transport it to the decomposers (Guggenberger et al., 2003).

While the adsorption/desorption of DOM to specific clays and oxides have been extensively investigated, the adsorption of soluble organic matter to solid soil organic matter has not. Work by Qualls (Qualls, 2000) suggests that H-bonding, and perhaps

van der Waal's forces, are the most likely mechanism of sorption of soluble organic matter to solid organic matter.

### 1.2.5.5    Dissolution/Precipitation

Factors such as pH, ionic strength and the presence of polyvalent cations, can affect the solubility of DOM.  The solubility of DOM will depend on its charge density (Tipping and Woof, 1990), which in turn depends on the pKa value of the organic matter and the pH of the soil solution. Changes in pH affect the protonation of functional groups involved in intramolecular ionic bonds. This leads to breakage of these bonds and loss of 3-D conformation. pH can also affect the solubility of $Al^{3+}$ ions (increasing pH causes decreased solubility of $Al^{3+}$) which cause DOM to flocculate (Andersson and Gahnstrom, 1985).

High ionic strength solutions may reduce charge density and therefore lead to coagulation (Tipping and Hurley, 1988). Chemical reactions between anionic functional groups of organic molecules and solution cations can reduce the surface charge density which in turn will alter the structural conformation of the adsorbed species, and consequently reduce solubility (Kalbitz *et al.*, 2000). Polyvalent cations, such as $Al^{3+}$, $Fe^{3+}$, $Ca^{2+}$ or $Mg^{2+}$, and trace metals, such as $Cu^{2+}$, $Mn^{2+}$, $Pb^{2+}$, $Cr^{3+}$ or $Cd^{2+}$, affect the solubility of organic matter (Baham and Sposito, 1994)(Guggenberger and Zech, 1994). Polyvalent cations can link negatively charged functional groups or organic molecules together and reduce their solubility by causing flocculation.

### 1.2.5.6    Precipitation, Stemflow and Throughfall

Dissolved organic matter present in rainfall can arise from anthropogenic sources e.g. herbicides, fungicides, insecticides, PCBs, PAHs, petroleum hydrocarbons, surfactants, solvents (Schwab, 2000) and/or natural sources, however this input of DOM into the soil is considered to be of minor quantitative importance (Zech and Guggenberger, 1996). Natural DOM in rainfall results from organic matter initially released as dust from the canopy of stands and subsequently washed out by rainfall (Guggenberger and Zech, 1994) and the washing of dry-deposited organic carbon, leaf leachate and the metabolic products of microbial activity (Zech and Guggenberger, 1996).

### 1.2.5.7    Rhizodeposition

Soluble organic matter exuded by roots comprises about 0.09% of the net primary production of a forest (Smith, 1976). Root exudates are largely very labile substances with first order decay constants in the order of $50 \times 10^{-3}$ per day (Reid and Goss, 1983). Most analysis of exudates have found that they are predominantly simple carbohydrates, amino acids and fatty acids (Kraffczyk et al., 1984; Kraffczyk et al., 1984; Smith, 1976). Labile material is also released into the rhizosphere as sloughed root cells lyse and polysaccharide mucilage and mucigel are secreted by root cells (Paul and Clark, 1996).

### 1.2.5.8    Uptake by Roots

While the largest source of N uptake by roots has been regarded as $NO_3^-$ and $NH_4^+$, Meitinnen (Meitinnen, 1959) showed that plant roots can take up low molecular size

amino-acids. It was generally considered that this uptake was minor compared with inorganic N. However in several tundra plant species, on organic soils, organic N uptake by roots accounts for 10-80% of total N uptake (Chapin et al., 1993; Kielland, 1994) and in several plant species, in typical boreal forest species (*Picea sylvestris*, *Picea abies*, *Vaccinium myrtillus* and *Deschampsia flexuosa*), rates of glycine uptake were similar to those of $^{15}$N –ammonium (Näsholm *et al.*, 1998).

Some ectomycorrhizal fungi can also take up organic forms of nitrogen (Abuzinadah and Read, 1986; Kielland, 1994; Wallander et al., 1997) or have the ability to release nutrients from litter layers by producing enzymes involved in the mineralization of organic matter (Abuzinadah and Read, 1988).

## 1.2.6  DOC Turnover

Quantifying gross rates of DOM production and loss is difficult because any bulk analyses reflect the net result of competing processes. Also it is unclear what fraction of the DOM produced by plants and microbes is actually measured in soil solution and extractions, and how much turns over so rapidly that it is effectively immeasurable (McDowell, 2003). The relatively small quantities of dissolved organic matter found in the general soil solution (< 1 % of soil organic carbon (Killham, 1994)) may disguise much greater rates of dissolution and turnover, due to microbial decomposition and assimilation. Extracellular lysis occurring near the microbial cell surface, with rapid uptake of monomers and mineralization, means C and N released through decomposition is rarely distributed far enough in to the soil solution to be lost by leaching (Qualls, 2000). The movement of the dissolved products of exoenzymatic hydrolysis will depend on the distance to which the enzymes diffuse from the cell

surface, the diffusion rate away and toward the cell surface, the rate of uptake at the cell membrane and the velocity and content of the pore water (Qualls, 2000).

Rates of DOM production will depend upon many variables including plant type, soil type, amount of primary production, nutrient dynamics, temperature (Burke *et al.*, 1989; Buyanovsky et al., 1987; Jenkinson and Rayner, 1977) moisture and management factors (Herbert and Bertsch, 1995).

### 1.2.7  Size of Soil DOM Pool

Most observations of soil solution DOM concentrations have focussed on the concentration of DOC and have been made in forest soils, especially Podzols where the complexing of Al and Fe with dissolved organic acids plays an important role in the mineral content of the different horizons. DOC concentrations in these soils typically decrease from 5 to 50 mg C $l^{-1}$ in the O and A horizons to about 1 to 5 mg C $l^{-1}$ in the C horizon. A table of average DOC concentrations as a function of soil order and horizon is given in Herbert and Bertsch, 1995.

The pool of potentially soluble organic matter in both mineral and organic horizons is much larger than the amount dissolved by any one leaching event (Qualls, 2000). Qualls (2000) found that that the most likely explanation for persistent leaching of DOC was equilibrium desorption. The pool of potentially soluble organic carbon was estimated at about 2.4% of the organic carbon of forest floor litter.

Temporal variations in DOC concentrations occur seasonally and by storm event. In general, DOC in soil solution is higher in the summer than in winter (Chittleborough *et al.*, 1992) although Tipping et al. (Tipping *et al.*, 1999) and Grieve (Grieve, 1994) found that exported loads of DOC per unit area were greatest during winter, due to a combination of high concentrations and high volumes. During storm events the shifting of dominant flow paths shift toward preferential flow through macropores, runoff, and lateral flow together with high pore water velocity reduce the sorption of DOC to mineral horizons (McDowell and Likens, 1988; Moore, 1998).

Some of the seasonal variation in DOC concentrations has been attributed to various environmental factors, including temperature, precipitation intensity and freeze-thaw cycles (reviewed by Kalbitz et al., 2000). No studies have attributed any seasonal variation to rainfall frequency. Rainfall contains small quantities of DOC from both natural and anthropogenic sources (Zech and Guggenberger, 1996). Trace amounts of DOC have been shown to be sufficient to trigger the biomass into a relatively short-lived increase in metabolic activity (De Nobili *et al.*, 2001). High frequency of rainfall could therefore lead to higher sustained biomass activity and consequently greater biodegradation of DOC.

## 1.2.8    Summary of Main Research Issues relating to DOC from a Biological Perspective

- The loss of soil DOM, and soil derived DOM in aquatic systems, to $CO_2$ is a biological process. Likewise biological processes play a role in the production and transformation of soil DOC.  Whilst a large body of research exists on the characteristics of soil DOM, it is largely based on operationally derived fractions. There is a lack of data on the biochemical composition of DOM in relation to season, its location within the soil structure, soil type, and ecosystem type, making any elucidation of its interaction with the soil microbial community difficult.

- There is a continuing debate, which has yet to be resolved, over the relative importance of biological processes, such as microbial metabolism, over abiotic processes, such as desorption/sorption, in controlling the size and composition of the soil DOM pool. Additionally there is a lack of understanding of both the relative contribution of each of the many biological processes relevant to DOM production and transformation and the net effect of microbial production versus depletion of DOM.

- Peatlands are an important terrestrial store of carbon, representing around a third to a half of the global soil carbon store, yet much of the biologically-relevant research to date has focussed on agricultural and forest ecosystems where the

biological characteristics of soil DOM are of economic significance. A greater focus on the biotic factors controlling DOM bioavailability, production, transformation and loss in peat soils is essential in predicting the long-term effects of environmental change on this globally important carbon store.

## 1.2.9    Research Objectives

This research project was designed to make a contribution to some of the main research issues outlined above (section 1.2.8) through the elucidation of:

(1) the biochemical composition of DOM in relation to both season and location within the structure of peatland soil;

(2) the decomposition dynamics of peatland and agricultural soils in relation to kinetics, biochemical composition and microbial community size;

(3) the influence of trace amounts of DOM on the rate of microbial mineralization of peatland organic carbon.

Within this context the following primary objectives were set:

- To evaluate the influence of method of isolation of DOM from peat soil on its biochemical composition.

- To quantify the changes in biochemical composition and concentration of peat DOM over the period of a year.

- To study the dynamics of total DOM; soluble carbohydrates, proteins and amino acids; and, the microbial population during decomposition of soil DOC in both peat and agricultural ecosystems.

- To investigate the effects of repeated applications of trace amounts of litter DOM on the activity of peat soil microbial biomass.

# Chapter 2: Influence of Method and Time of Extraction on Biochemical Composition of Peat DOM

## 2.1.   Introduction

A wide range of methods has been developed for isolating DOM from the soil matrix. In the field, DOM has been extracted by the use of tension or zero–tension porous cups, percolation trays or by wick lysimeters. In the laboratory DOM can be extracted by forcing solution out of the soil through a porous membrane using a vacuum or centrifugal force or by leaching undisturbed cores or repacked columns.  Many studies have used extracts made by shaking air-dried or field-moist soil with water or dilute salt solutions, followed by separation of the solution by centrifugation or filtration. Others have used aqueous solutions of alkaline-extracted soil organic matter as analogues of soil solution DOM.

All of these methods have been criticised to some degree in the past. The ceramic cups of tension (suction) lysimeters have the potential to retain analytes or to contaminate the sample (Wood, 1973). Centrifugation yields high concentrations of DOM in rhizosphere soil extracts, possibly due to disruption of roots or micro-organisms (Lorenz *et al.*, 1994) and major differences in soil solution chemistry result from using different centrifuge speeds and different filter media (Grieve, 1996). The quantity and quality of DOM extracted by shaking soil with a solution, followed by centrifugation or filtration, is dependent on the solution chemistry of the extractant (David *et al.*, 1989; Vance and David, 1989) temperature, extraction time, and extractant volume (Zsolnay, 2003). The process of filtration itself can result in cavitation with the formation of small

gas bubbles. Surface active DOM can then adsorb on the bubble surfaces, and when the bubbles collapse this adsorbed DOM unites as particulate organic matter (Zsolnay, 2003) which may alter its ecological function (Maurer, 1976). Organic filters may also leach dissolved organic carbon during the filtration process (Norrman, 1993). Hot solution extractions (Davidson *et al.*, 1987; Gregorich *et al.*, 2003) hydrolyse some of the organic material and kill vegetative microbial cells, resulting in the creation of DOM which did not exist *in situ* (Sparling et al., 1998; Zsolnay, 1996) . Alkali extraction releases materials unlikely to be found naturally dissolved in soil solution.

There are further complications. DOM is distributed among different pore sizes within soils and different methods are probably therefore sampling different fractions of the soil DOC. Zsolnay (Zsolnay, 1996) suggests that a proportion of the DOM in the soil is held within pores smaller than 0.2 μm, at a tension of less than −1500 kPa. Since under −1500 k Pa would be required to extract this material from the soil matrix, it would not be expected to be present in most DOM extracts. Realistically most centrifugation and suction techniques are effectively sampling from macropores (> 6 μm, water tension − 50 k Pa (Zsolnay, 1996), and to a greater or lesser extent depending upon the force applied, from intermediate sized pores. Organic matter extracted by agitation of soil with aqueous solutions is considered to include DOM present in the macropores and some DOM located in smaller pores, since it results in disturbance of soil structure (Chantigny, 2003).

There is growing evidence that different fractions of the soil DOM may actually be biochemically distinct. Macropores, for instance, are often associated with preferential

flow. Research by Bundt et al. (Bundt, 2001) showed that these preferential flow paths act as biological 'hot spots' in the soil with a higher soil organic carbon and nitrogen content than the matrix and a significantly larger, and probably more diverse microbial biomass. (Guggenberger and Kaiser, 2003). In such areas of high microbiological activity the turnover rate of DOM may be high enough to effectively lower the measurable concentration of its constituent molecules. It is possible that microbial community structures differ between preferential flow paths and the matrix, although evidence for this is conflicting (Bundt, 2001). A more diverse community would enable a more rapid and complete mineralisation of complex organic molecules. Strong et al. (Strong et al., 2004) on the other hand found that organic matter in large air-filed pores decomposed more slowly than in intermediate sized pores possibly due to decreased organism motility or decreased diffusion of solutes. If the DOM is present in very small pores or within soil aggregates it may be physically inaccessible to the microbial population and therefore remain protected to some degree from biodegradation. Biodegradation of DOM in these pores would depend on the outward diffusion of the DOM into larger water-filled pores or the inward diffusion of extracellular enzymes released by microbes in larger pores (Marschner and Kalbitz, 2003).

Although peat soils may significantly differ in structure to mineral soils, heterogeneity in the structure still exists. Dense well-decomposed peats have a fine matrix with small pore paces whilst less compact peats with larger plant fragments tend to have larger pore spaces and, if vascular tissue is present i.e. the remains of angiosperm or gymnosperm material, undegraded or partially degraded xylem vessels and/or tracheids may effectively form hollow tubes. In less decomposed peats, the chemical nature of the lining of such vascular tissue may lead to an additional distinguishing factor

between pore sizes to those discussed above: that of sorption capacity. Thus, as for mineral soils, it is possible that different methods used to isolate peat soil DOM may be sampling different biochemical compartments of the soil DOM.

The results of many field studies have shown that DOC concentrations show seasonal variation (Cronan and Aiken, 1985; McDowell and Likens, 1988; Tipping *et al.*, 1999). In general, DOC concentrations in soils are higher in summer than in winter (Chittleborough *et al.*, 1992; Guggenberger *et al.*, 1998; Scott *et al.*, 1998; Tipping *et al.*, 1999), although Tipping et al. (Tipping *et al.*, 1999) and Grieve (Grieve, 1994) found that exported loads of DOC per unit area were greatest during winter, due to a combination of high concentrations and high volumes. Guggenberger et al. (Guggenberger *et al.*, 1998), Tipping et al. (Tipping *et al.*, 1999) and Kaiser et al. (Kaiser *et al.*, 2001) have attributed seasonal changes in DOC concentrations to changes in microbial activity. Kaiser et al. (2001) hypothesise that varying microbial activity throughout the year may not only affect the amount of released organic matter but also its composition which in turn may lead to changing bioavailability. Some studies have shown higher DOC degradabilities in winter/spring than in summer/autumn (Kaiser *et al.*, 2001; Marschner and Kalbitz, 2003; Nelson *et al.*, 1994), whilst Yano et al (Yano *et al.*, 2000) determined DOC biodegradability as high as 40% in summer forest floor soil solutions while during winter, degradability was only 10-20%.

Very little research has been carried out on the seasonal variation exhibited by DON concentrations by comparison with DOC. The dynamics of DON, like DOC, are affected by decomposition processes, mineralisation, immobilisation, leaching and plant

uptake (summarised by (Murphy et al., 2000)). The specific relationship between DON and DOC is unclear. Currie et al. (1996) found that DON extracted from just beneath the organic horizons of pine and hardwood forest showed the same seasonal pattern as DOC. However, Williams and Silcock (Williams and Silcock, 2000) reported a higher seasonal variability of DON compared with DOC extracted from a raised peat bog, and Michalzik and Matzner (Michalzik and Matzner, 1999) found that DON concentrations in the partially decomposed  layer of a forest spruce ecosystem were more influenced by temperature than were DOC concentrations.

The objectives of this study were to determine: (1) the influence of method extraction on the composition of DOM from a raised peat bog in Central Scotland, with respect to the relative proportions of carbohydrate, protein and amino acids; (2) the effect of season on the composition of DOC and DON, with respect to carbohydrate, protein and amino acids; (3) the effect of season on total DOC and DON concentrations and (4) the influence of season on DOC/DON ratios. Carbohydrates, proteins and amino acids were singled out for this study, from the wide range of possible DOM components, as the concentrations of these DOM components should give an indication of relative biodegradability. This assumes that: the remainder of unidentified DOM is relatively recalcitrant and only a negligible proportion of carbohydrates, proteins and amino acids are detected by biochemical assays when chemically protected through complexation with molecules resistant to degradation. It also assumes that these molecules are bioavailable in situ, i.e. not sorbed to inorganic or organic material or contained within soil pores so small that they are physically inaccessible to microbes so that their degradation depends on the rate of diffusion of extracellular enzymes into the pore and the rate of diffusion of the breakdown products out.

## 2.2.    Hypotheses

- The proportion of proteins carbohydrate and amino acid carbon, as a fraction of the total organic carbon content in soil DOM, will vary according to the method used to isolate the DOM from the soil.

- The total proportion of protein, carbohydrate and amino acid carbon as a fraction of DOC (% protein + carbohydrate + amino acid C) and total proportion of protein and amino acid nitrogen as a fraction of DON (% protein + amino acid N) extracted in the autumn/winter will be higher than in the spring/summer.

- The seasonal changes in DOC and DON concentration will oppose changes in the proportion of protein, carbohydrate and amino acid carbon as a fraction of DOC (% protein + carbohydrate + amino acid C) and total proportion of protein and amino acid nitrogen as a fraction of DON (% protein + amino acid N) respectively, with higher concentrations in summer than winter.

- DON concentration is independent of DOC concentration.

## 2.3.    Materials and Methods

## 2.3.1      Soil Description

Soil DOM was sampled from the Of horizon of a drained, raised peat bog in area of the Carse of Forth ($4^o$ 01' W, $56^o$ 08' N, 15 m above sea level), near Stirling, known as Ochtertyre Moss Wood (Figure 5, 6 and 7). Ochtertyre Moss Wood is a mixed wood of silver birch (*Betula spp*) and Scot's Pine (*Pinus sylvestris*) with a ground cover dominated by bracken (*Pteridium aquilinum*) and moss (*Rhytidiadelphus loreus*). All samples were taken from within an area of 25 $m^2$ under a silver birch canopy. A description of the soil profile at the field site is given below (Table 1).

## 2.3.2  DOC Extraction

Over the period of a year beginning on 19th August 2003, DOM was extracted from the Of horizon (see) every two months using three different methods of extraction (see below). Three replicate samples were taken on each sampling occasion for each method. Soil moisture content was determined on each extraction date. An attempt was made to record soil temperature (25 cm depth) at the field site every 2 hours using a Squirrel data logger. On a number of occasions, however, the logger was tampered with leading to an incomplete set of data. Monthly rainfall (mm) and maximum/minimum air temperature ($^o$C) at 09:00 hours data was made available from Stirling University weather station, 5 km east of the field site and at the same altitude.
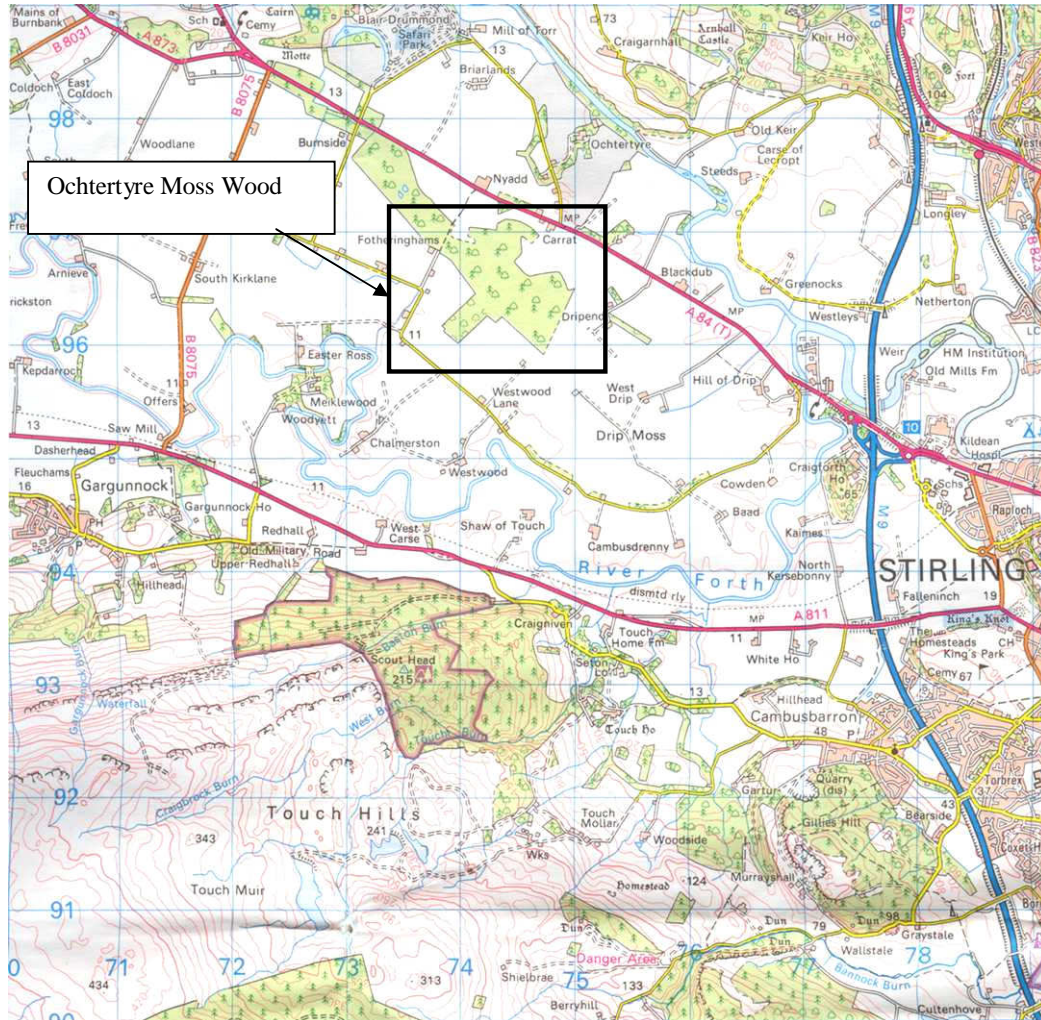
**Figure 4** Reproduction of extract from 1:50000 Ordnance Survey Map 57, showing the location of Ochtertyre Moss Wood, Stirling, Scotland. ©Crown copyright Ordnance Survey. All rights reserved.
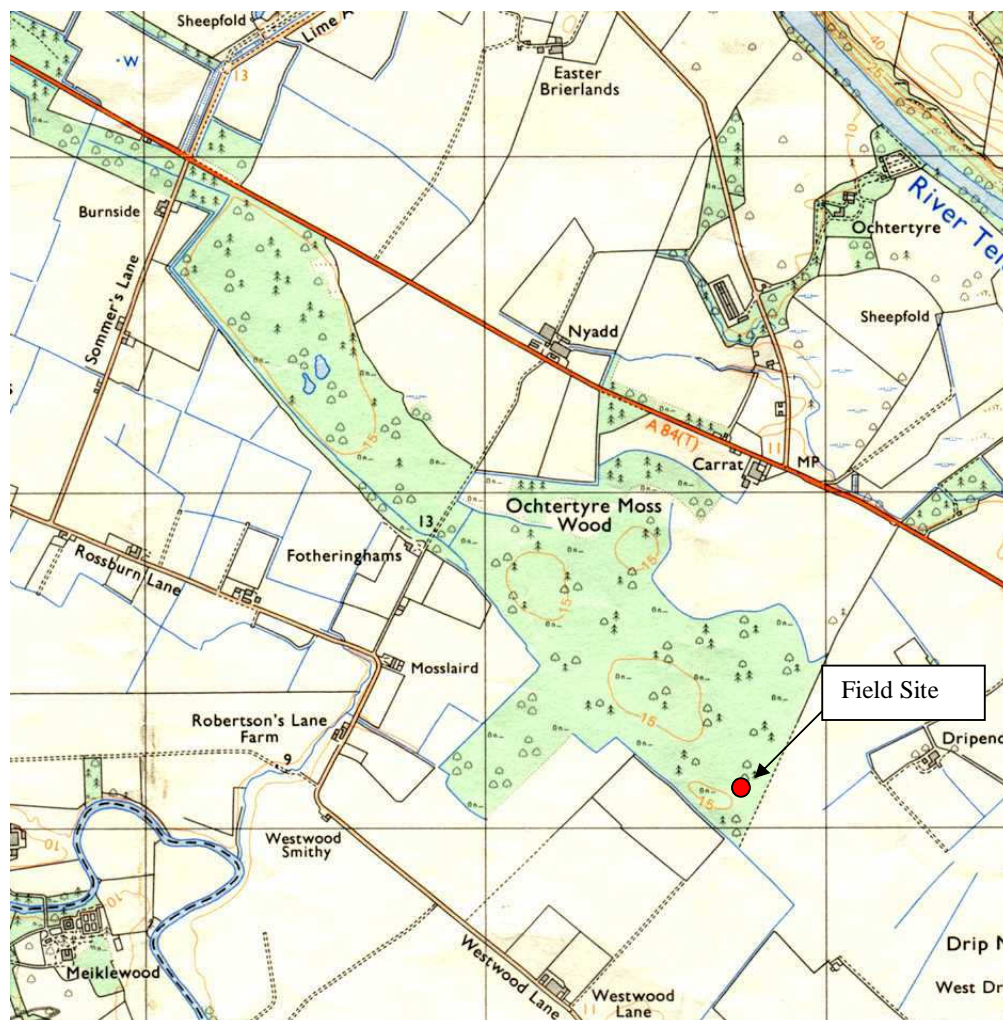
**Figure 5** Reproduction of extract from 1:25000 Ordnance Survey Map, showing the location of the field site. © Crown copyright Ordnance Survey. All rights reserved.

**Figure 6** View, looking northeast, of the southern end of Ochtertyre Moss Wood.



**Figure 7** View of sample site within Ochtertyre Moss Wood, looking south.

**Table 1** Description of soil profile, according to the Soil Survey Description Handbook (Hodgson, 1976) and the 'von Post Humification Scale' (Damman and French, 1987) at field site ($4^o$ 01' W, $56^o$ 08' N). Standard error of $H^+$ concentration and value of n follow pH values in brackets.

Soil Type: Peaty Soil

| Horizon | Depth (cm) | Description |
| --- | --- | --- |
| H | 0-5 | Dark reddish brown, 5 YR, 2.5/2. Fibrous and semi-fibrous litter layer with many fine and very fine roots. H5. |
| Oh | 5-19 | Reddish Black, 2.5 YR, N2.5/1. Amorphous peat. Fine and very fine roots common. H9. pH ($H_2O$) = 3.2 (0.0006, 10). pH($CaCl_2$) = 2.4 (0.004, 10). |
| Of | 19-100+ | Dark reddish brown, 5 YR, 3 /4. Fibrous peat containing recognisable remains of sphagnum. Fine and very fine roots common. H3. pH($H_2O$) = 3.2 (0.0006, 10). pH($CaCl_2$) = 2.4 (0.0004, 10). C/N ratio = 45/1 |

### 2.3.2.1   Method 1: Suction / Filtration

Field suction-lysimeters (Figure 8) consisting of a length of PVC tube (45 mm diameter) glued to a porous ceramic cup (pore size 3.375 μm) were buried into the raised peat to a depth of 50 cm. The top of each tube was fitted with a rubber bung and two air lines which remained above the soil surface (Figure 9). The first air line, extending to the bottom of the cup, was sealed. The second air line was used for drawing a vacuum of –70 kPa. This line was also then sealed and the lysimeter allowed to draw the soil solution into the ceramic cup. The radius of the recharge area of a suction cup i.e. the space in which water flows towards the cup, lies between 0.1 m and 0.5 m, depending on the capillary pressure in the soil, the suction pressure in the cup and its diameter, the pore size distribution of the soil and the depth of the groundwater surface in relation to installation depth (Grossman and Udluft, 1991). The collected sample was retrieved by drawing a vacuum on the first air line.

**(a)**                                                    **(b)**
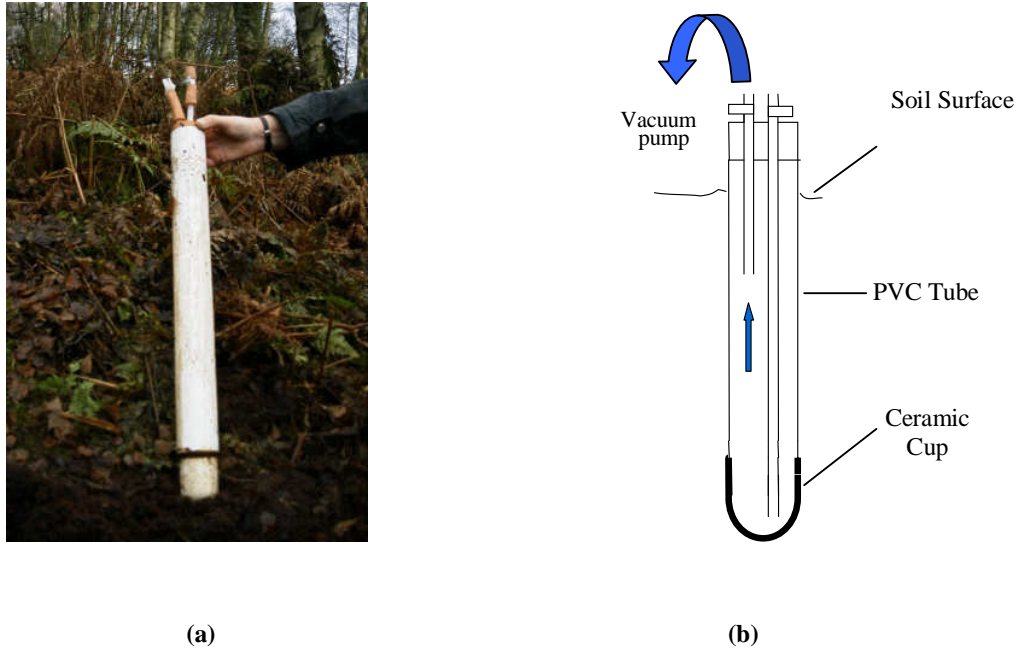
**Figure 8** External (a) and longitudinal section (b) views of a field suction-lysimeter.



**Figure 9** Field suction-lysimeters in place at field site

To ensure good hydraulic contact between the porous cup and the surrounding peat, a

soil auger of a smaller diameter than the sampler was used to create a vertical hole into

which the sampler is pushed to the required depth. The installation of the sampler was followed by a month long stabilisation phase during which water was sampled and discarded to minimise any sorption by the cup (Grossman and Udluft, 1991). After the stabilisation phase any samples retrieved were transferred to Nalgene® HPDE bottles and stored at 4$^{\circ}$C. Within 48 hours each sample was filtered through a pre-rinsed 0.45µm cellulose nitrate filter. The filtrate was transferred to Eppendorf® polypropylene tubes and frozen for biochemical analysis at a later date.

### 2.3.2.2   Method 2: Centrifugation / Filtration

Bulk soil samples of the Of horizon were collected, thoroughly mixed, sorted to remove root material greater than 1 mm and stored in sealed polythene bags at 4$^{\circ}$C. Soil solutions were extracted within 72 hours of sampling in a two-part centrifuge tube, the upper part of which drains into the lower part through a 0.45 µm cellulose nitrate filter (Figure 10). The filters were pre-rinsed three times with 10 ml double-distilled de-ionised water, acidified to pH 3.5 using $H_2SO_4$. 40 g field moist soil was packed into the upper part of the tube and spun at a relative centrifugal force (RCF) of 160 $g$ for 60 min, which equates to an extraction pressure of –71.7 kPa (Kinniburgh and Miles, 1983).
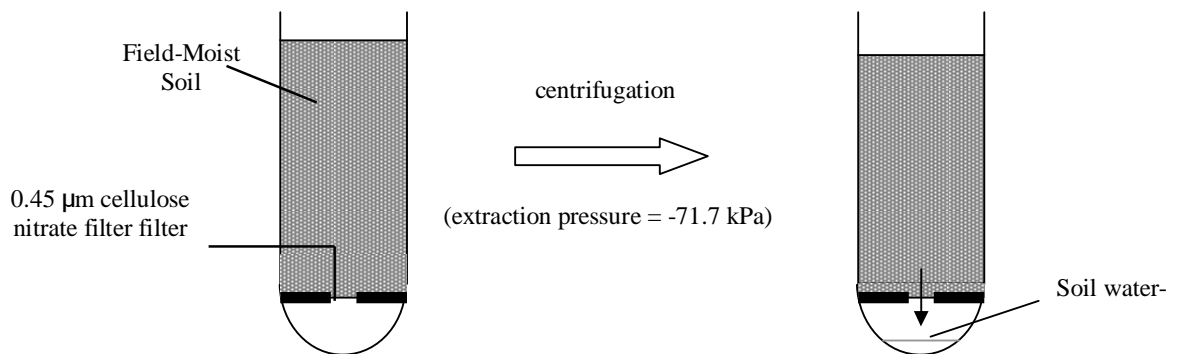


**Figure 10** Centrifugation and filtration procedure using a two-part centrifuge tube.

The extractants were transferred to Eppendorf® polypropylene tubes and frozen until required for analysis.

### 2.3.2.3   Method 3: Water- Extraction / Filtration

Bulk soil samples were obtained from the Of horizon, thoroughly mixed, sorted to remove root material greater than 1mm, and stored in sealed polythene bags at $4^oC$. Within 48 h of sampling, 50 g of field moist soil was added to 300 ml double-distilled, de-ionised water. After shaking for 1 h, the suspension was allowed to stand for 24 h at $4^oC$ before filtration through, 0.45 µm cellulose nitrate filters. The filters were pre-rinsed three times with 10 ml double-distilled de-ionised water, acidified to pH 3.5. The filtrate was transferred to Eppendorf® polypropylene tubes and frozen for biochemical analysis at a later date.

## 2.3.3  Biochemical Analyses

For all extractants DOC and DON concentrations were quantified. DOC was determined using a Shimadzu TOC-VCSN® Total Organic Carbon Analyser, with correction for inorganic-C. TON was determined as the difference between total nitrogen, measured using the Shimadzu TOC-VCSN® Total Organic Carbon Analyser, and mineral nitrogen ($NH_4^+ + NO_3^-$).  Protein concentration was measured using the micro Lowry, Ohnishi and Barr modification method (Ohnishi and Barr, 1978); carbohydrate concentration measured using a colorimetric method based on the use of anthrone reagent (Allen, 1989); and amino acid concentration measured as the difference between  ninhydrin determined N concentration (Moore and Stein, 1951) and ammonium concentration (Allen, 1989). Technical details for all these methods can be found in Appendix A.  Resulting values for all analyses were converted into mg C $l^{-1}$ or

mg N l$^{-1}$ as appropriate. Conversion of protein and amino acid concentrations to mg C or mg N was based on the assumption that plant proteins contain on average 16 % N (Allen, 1989) and a N-to-C ratio of 0.36 (Meli et al., 2003).

## 2.3.4  Data Analysis

ANOVAs and Tukey's post-hoc statistical tests (family error rate = 0.05) were used to ascertain the significance of differences in the composition of DOM in relation to both method of extraction and month (population from which data set collected was assumed to be normally distributed). Pearson's product-moment correlations were used to ascertain the degree and significance of the association between: (i) the protein, carbohydrate and amino acid fractions of DOC and DON; (ii) DOC and DON concentrations; and, (iii) DOC composition/concentration and soil moisture content.

## 2.4    Results

## 2.4.1  Influence of Method of Extraction on DOC Composition

The mean total % carbohydrate, protein and amino acid C fraction of DOC over the whole year was 10.0 % ($\pm$ SE 1.0) for water extraction, 8.3 % ($\pm$ SE 2.0) for centrifuge extraction and 4.8 % ($\pm$ SE 0.6) for suction extraction. The mean total % protein and amino acid N of DON over the whole year was 65.8 % ($\pm$ SE 6.6) for water extraction, 44.4 % ($\pm$ 5.9) for centrifuge extraction and 43.9 % ($\pm$ SE 4.5) for suction extraction.

Although time of extraction of DOC from the peat soil affected its composition over the time period August 2003 – June 2004 for all three types of extracted DOC (see section 2.4.3), its effects were dependent on extraction method (Figure 11). In late summer/autumn (August/October) 2003 and spring (February/April) 2004, method of extraction had a significant effect on the individual carbohydrate and protein, and combined carbohydrate + protein + amino acid, proportions of DOC (Table 2). Generally, where differences existed, water-extracted DOC contained the greatest proportion of protein, carbohydrate and amino acid. However, in February 2004 the centrifugation method extracted a greater proportion of total carbohydrate + protein + amino acid than the water-extraction method and a greater proportion of carbohydrate than both the water-extraction and suction-lysimeter methods. Significant differences in amino acid content with method of extraction were only detected for the month of December.

Mean annual concentrations of DOC were 32.4 mg C l$^{-1}$ ($\pm$ SE 3.1) for water-extraction, 77.6 mg C l$^{-1}$ ($\pm$ SE 6.7) for suction-extraction, and 108.2 mg C l$^{-1}$ ($\pm$ SE 8.8) for centrifuge–extraction. Mean annual concentrations of DON were 1.1 mg N l$^{-1}$ ($\pm$ SE 0.3) for water-extraction, 2.5 mg N l$^{-1}$ ($\pm$ SE 1.4) for suction-extraction, and 5.9 mg C l-1 ($\pm$ SE 2.5) for centrifuge–extraction. For reasons discussed in section 2.5.1, a statistical analysis of the significance of the differences in DOC and DON concentrations between methods of extraction was not carried out.

**Table 2** Significant differences (adjusted p-value < 0.05) between carbohydrate, protein and amino acid composition of DOC in relation to method of extraction and time of year (W = water-extracted DOC, C = centrifuge-extracted DOC and S = suction- lysimeter extracted DOC).

| DOC Component | Aug | Oct | Dec | Feb | Apr | Jun |
|---|---|---|---|---|---|---|
| carbohydrate | | W > C<br>W > S<br>C > S | | C > S<br>C > W | W > S | |
| protein | W > C<br>W > S | W > C<br>W > S | | | | |
| amino acid | | | W > C<br>W > S | | | |
| total carbohydrate + protein + amino acid | W > C<br>W > S | W > C<br>W > S | | C > W | W > S | |
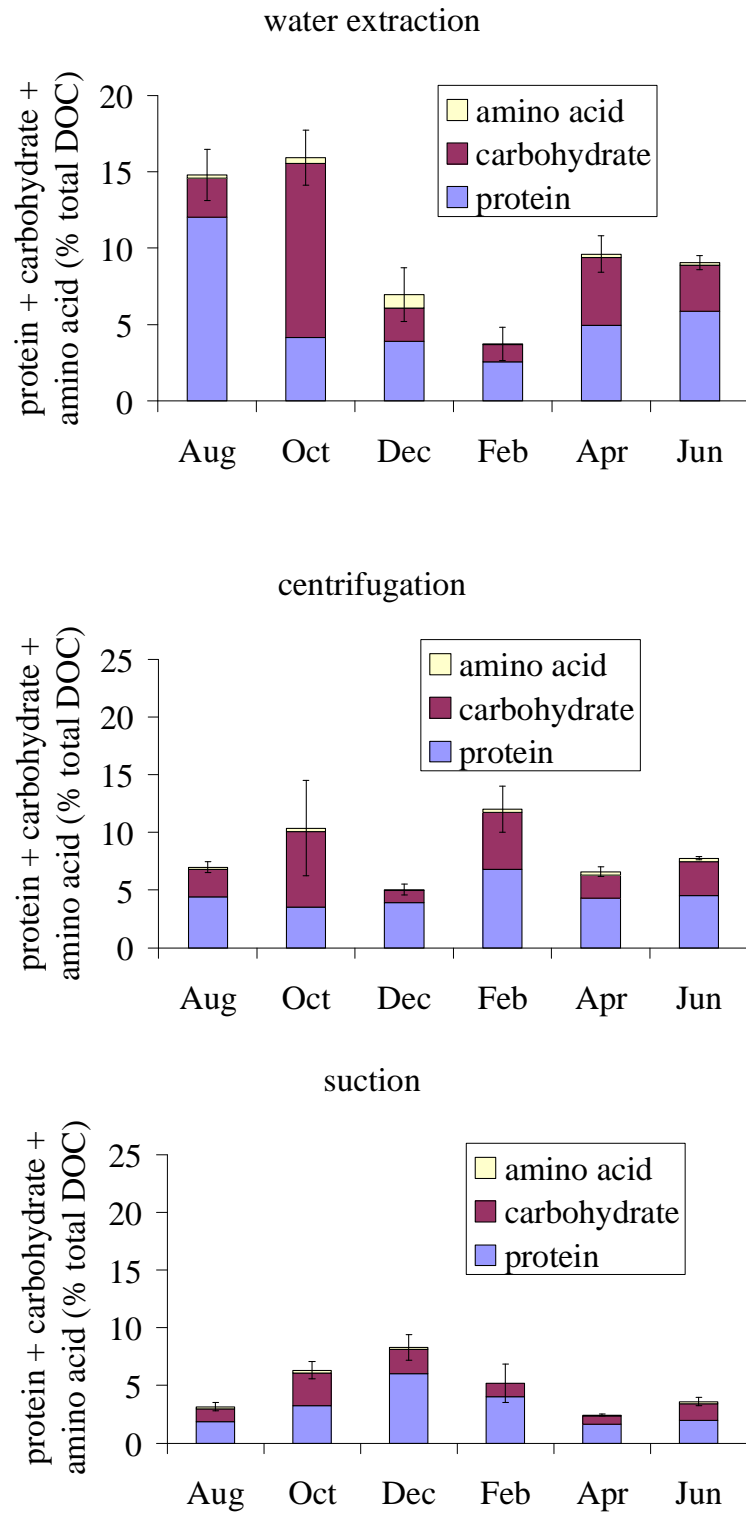
**Figure 11** Proportions of protein, carbohydrate and amino acid C in DOC extracted from the same peat soil using three different extraction methods: water-, centrifuge- and suction extraction. Error bars represent ± 1 SE of the combined total of carbohydrate + protein + amino acid (n = 3).

## 2.4.2  Influence of Time of Extraction on  DOC and DON Concentrations

DOC concentrations appeared to remain more or less constant from August 2003 until February 2004 with no significant changes, for all three methods of extraction (Figure 12). Levels then began to rise in April 2004, reaching a peak in June 2004 (50, 166 and 125 mg C $l^{-1}$ for water-, centrifuge- and suction-extracted DOC respectively). These DOC concentrations in June 2004 were significantly higher ($p < 0.05$) than December for water-extracted DOC, significantly higher than August, October and February for centrifuge-extracted DOC, and significantly higher than all months sampled apart from April for DOC extracted using a suction-lysimeter. No other statistically significant differences in DOC concentration existed between months of sampling. No significant correlations ($p < 0.05$) were observed between mean minimum or maximum daily temperature for the 4 weeks preceding sampling (Figure 14) and DOC concentration and between soil moisture content at time of sampling (Figure 15) and DOC concentration.

For DON the seasonal patterns in concentration are less clear cut than that for DOC, varying according to the method used to extract the DON (Figure 13). Water-extracted and centrifuge-extracted DON concentrations were highest in August 2003 (2 and 14 mg N $l^{-1}$ respectively). In the subsequent months water-extracted DON concentrations fell to significantly lower levels in Oct (0.5 mg N $l^{-1}$) and December (0.6 mg N $l^{-1}$). Whilst centrifuge-extracted DON concentration remained significantly lower than the August level for the remainder of the year (ranging from 2 to 7 mg N $l^{-1}$), there was

## water extraction



## centrifugation



## suction
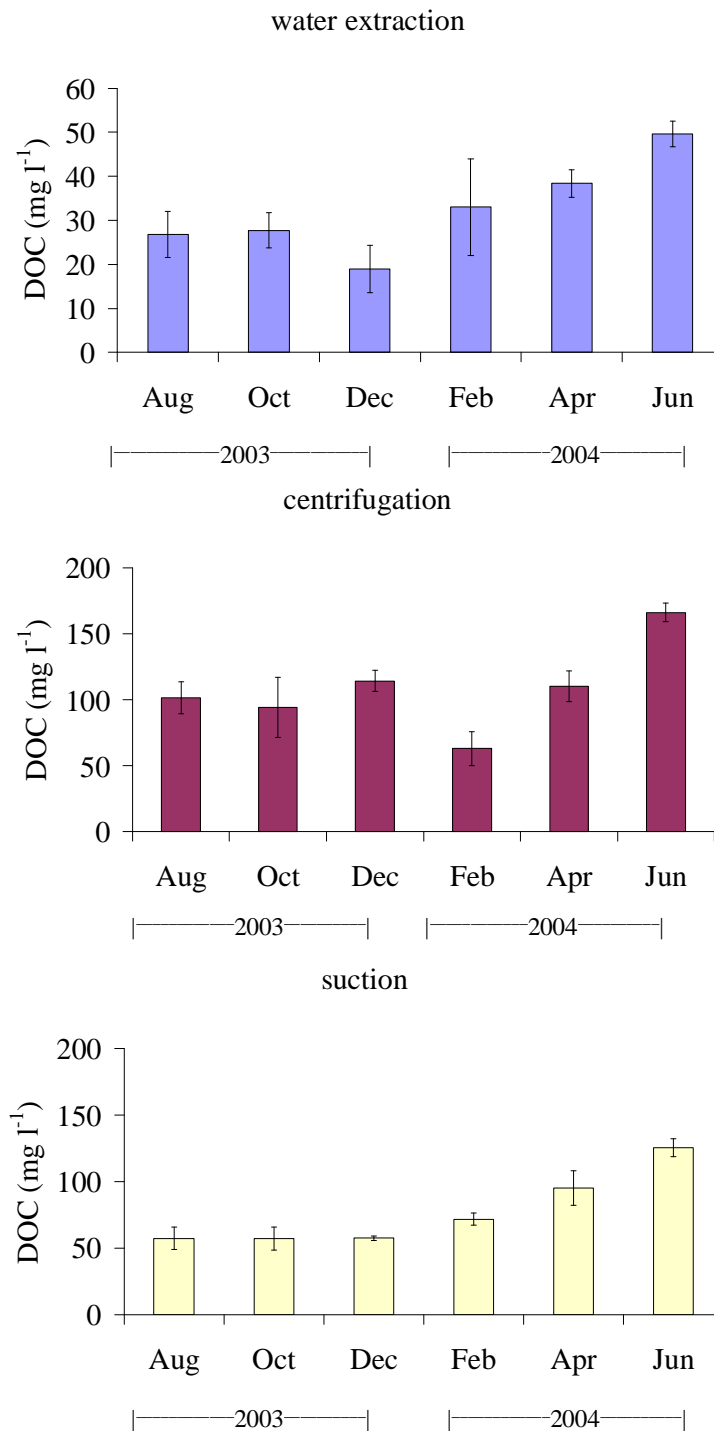


**Figure 12** Concentration of DOC (mg l$^{-1}$) extracted using three different methods of isolation over the period August 2003 to June 2004. Error bars represent ± 1 S.E. (n = 3) and reflect spatial variation.

another smaller but statistically significant rise in April. No significant changes in DON concentrations (ranging from 1 to 6 mg N l$^{-1}$) were observed at any time of the year for DON extracted using suction-lysimeters. No significant correlations ($p < 0.05$) were observed between mean minimum or maximum daily temperature for the 4 weeks preceding sampling (Figure 14) and DON concentration, with one exception: a positive correlation between the concentration of water-extracted DON and maximum temperature ($r = 0.837$, $p = 0.038$). There were no significant correlations between soil moisture content at time of sampling (Figure 15) and DON concentration.

water extraction

centrifugation

suction
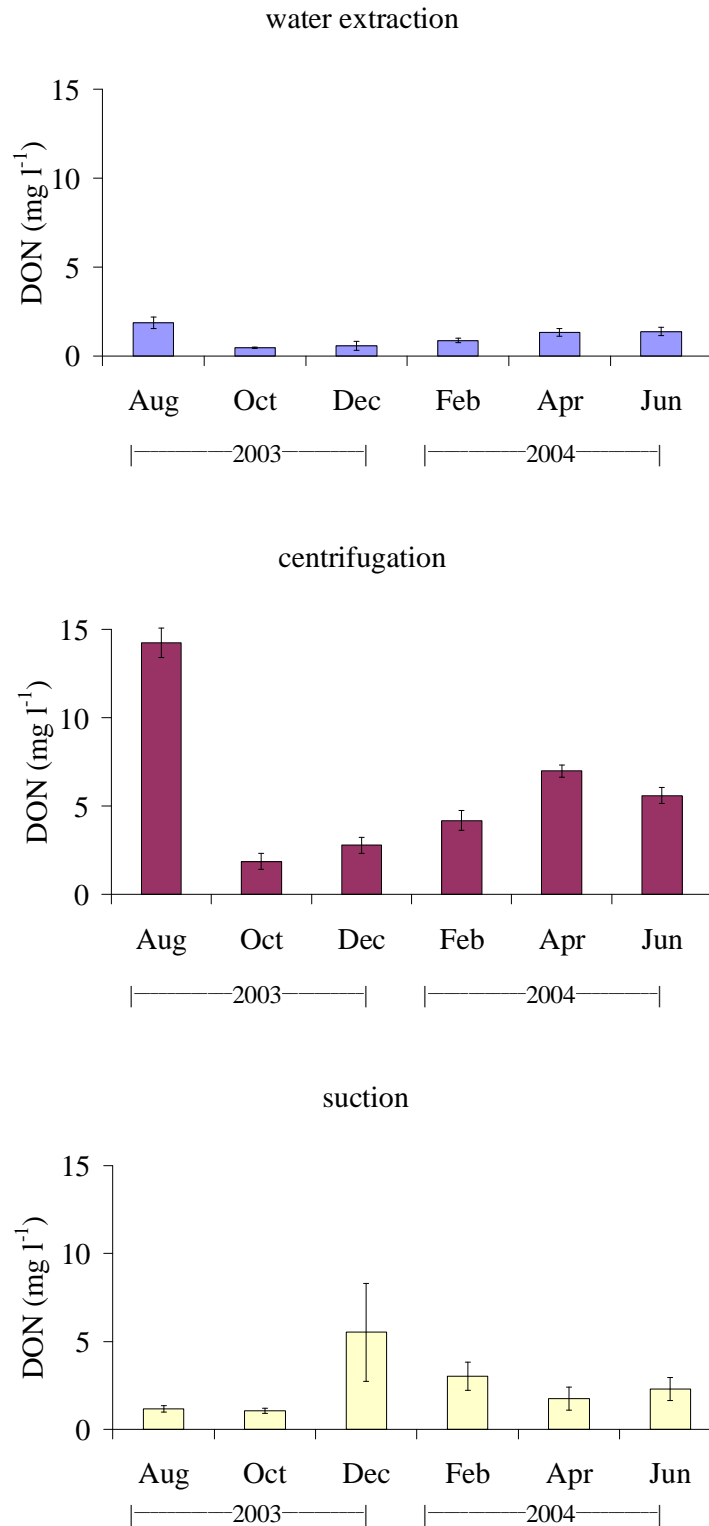


**Figure 13** Concentration of DON (mg l$^{-1}$) extracted using three different methods of isolation over the period August 2003 to June 2004. Error bars represent ± 1 S.E. (n = 3) and reflect spatial variation.
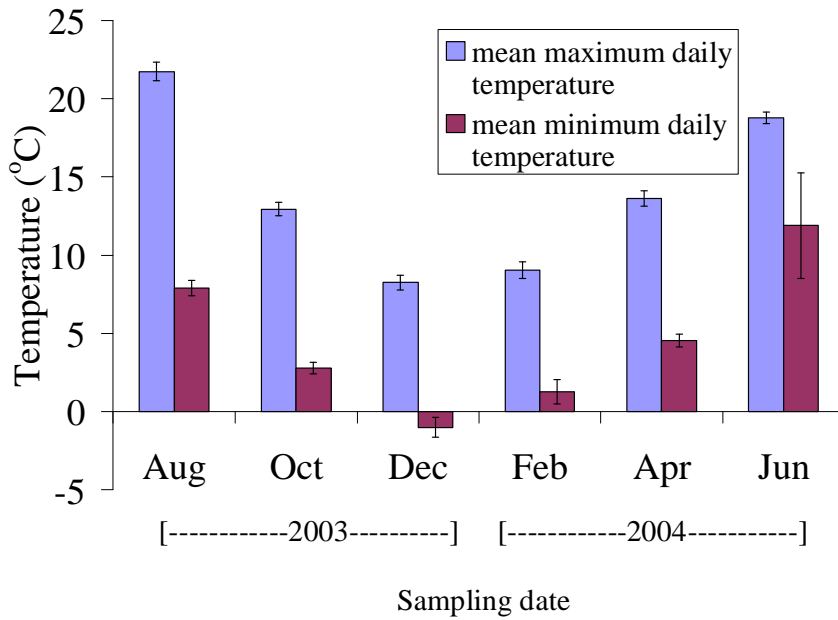
**Figure 14** Mean daily maximum and minimum air temperatures, recorded at Parkhead weather station, University of Stirling, for the four weeks preceding the sampling date in the specified month.
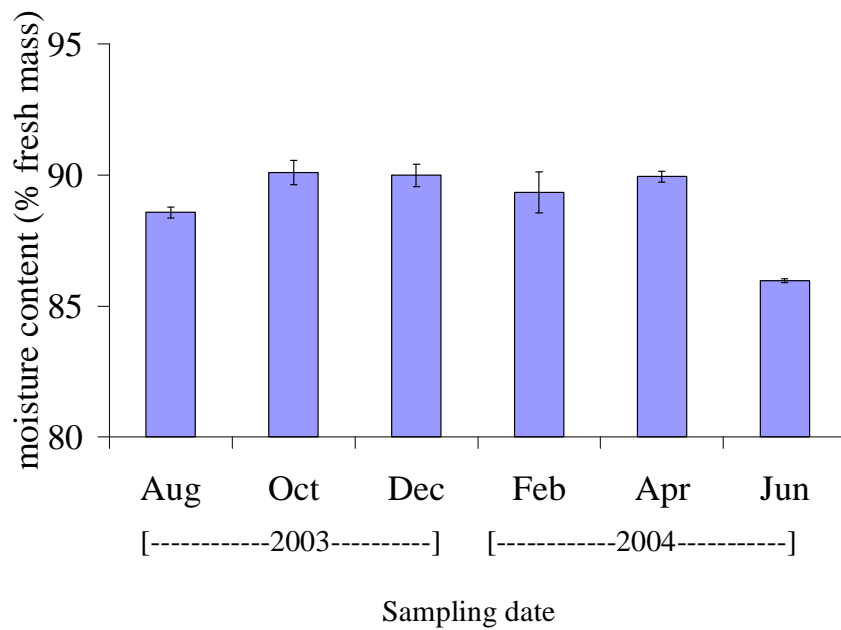


**Figure 15** Soil moisture content (% fresh mass) of soil at time of sampling (n = 3).

### 2.4.3 Influence of Time of Extraction on DOC and DON Composition

Time of extraction of DOC from the peat soil affected its composition over the time period August 2003 – June 2004 for all three types of extracted DOC. The % protein, carbohydrate and amino acid C was highest for the year in October for DOC extracted by water-extraction: October levels (16 %) were significantly higher ($p < 0.05$) than December, February and June (7, 4 and 9 % respectively), and August levels (15 %) significantly higher than December and February (Figure 11). For DOC extracted using a suction-lysimeter the total % protein, carbohydrate and amino acid C peaked in December (8 %) at levels significantly higher than in August, April and June (3, 2 and 4 % respectively). For centrifuge extracted DOC, this fraction peaked in February (12 %) but no one month was significantly different to another for the sampled year. Other fluctuations were observed throughout the year but the patterns of change were not consistent between methods of extraction (Figure 11). The variation in the proportions of protein + carbohydrate + amino acid C reflected was a function of fluctuations in protein, carbohydrate and unidentified DOC concentrations (Figure 16, Figure 17 and Figure 18). Changes in concentrations of these components generally appeared to be independent of one another: no significant correlation in seasonal variation was found between the concentrations of protein, carbohydrate and amino acid for both water- and suction-extracted DOC. For centrifuge–extracted DOC there was a significant correlation between protein and unidentified DOC concentrations only ($r = 0.847$, $p = 0.033$). The contribution of amino acids was generally relatively small (0.1–0.2; 0.1–0.5; and 0.00–0.3 mg C l$^{-1}$ for water, centrifuge and suction extraction methods respectively). No significant correlations ($p < 0.05$) were observed between % carbohydrate + protein + amino acid C and mean minimum or maximum daily

temperature for the 4 weeks preceding sampling and between % carbohydrate + protein

+ amino acid C and soil moisture content at time of sampling.



(a)



(b)
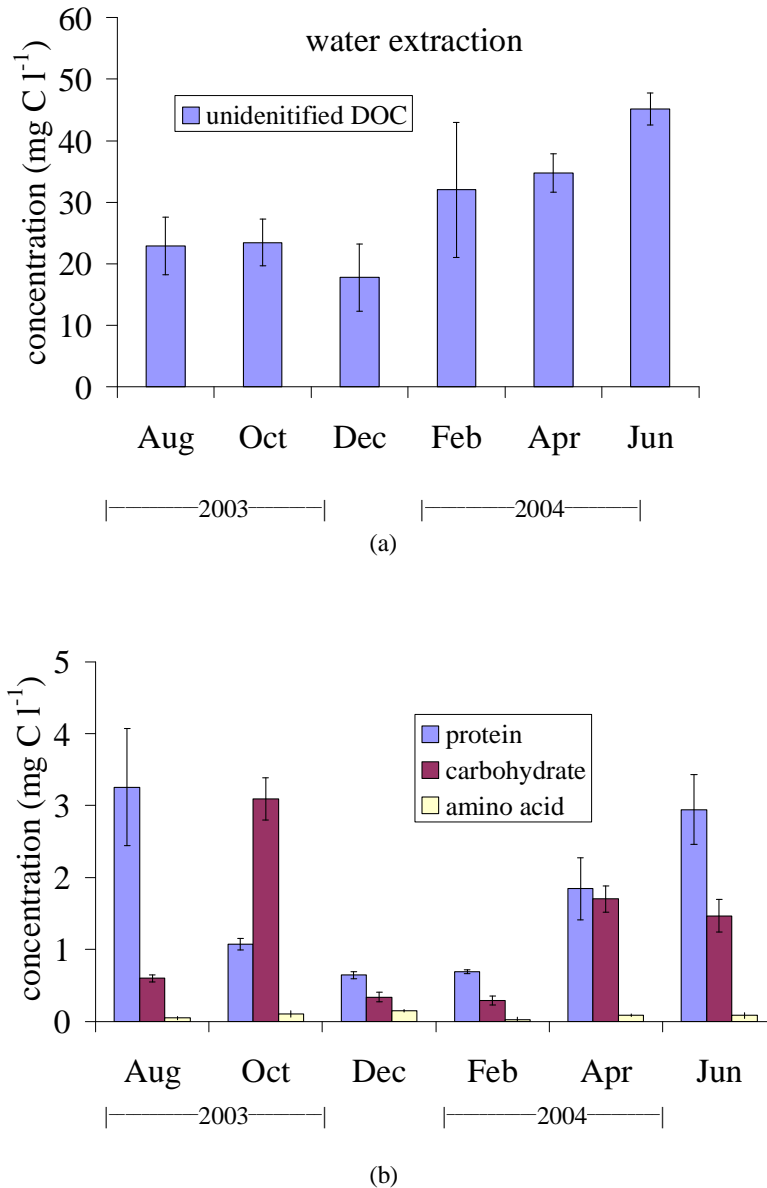
**Figure 16** Concentrations of unidentified (a) and identified (b) components of DOC (mg C l$^{-1}$) isolated from peat soil using a water-extraction method over the period August 2003 to June 2004. Error bars represent ± 1 SE (n = 3).

(a)



(b)
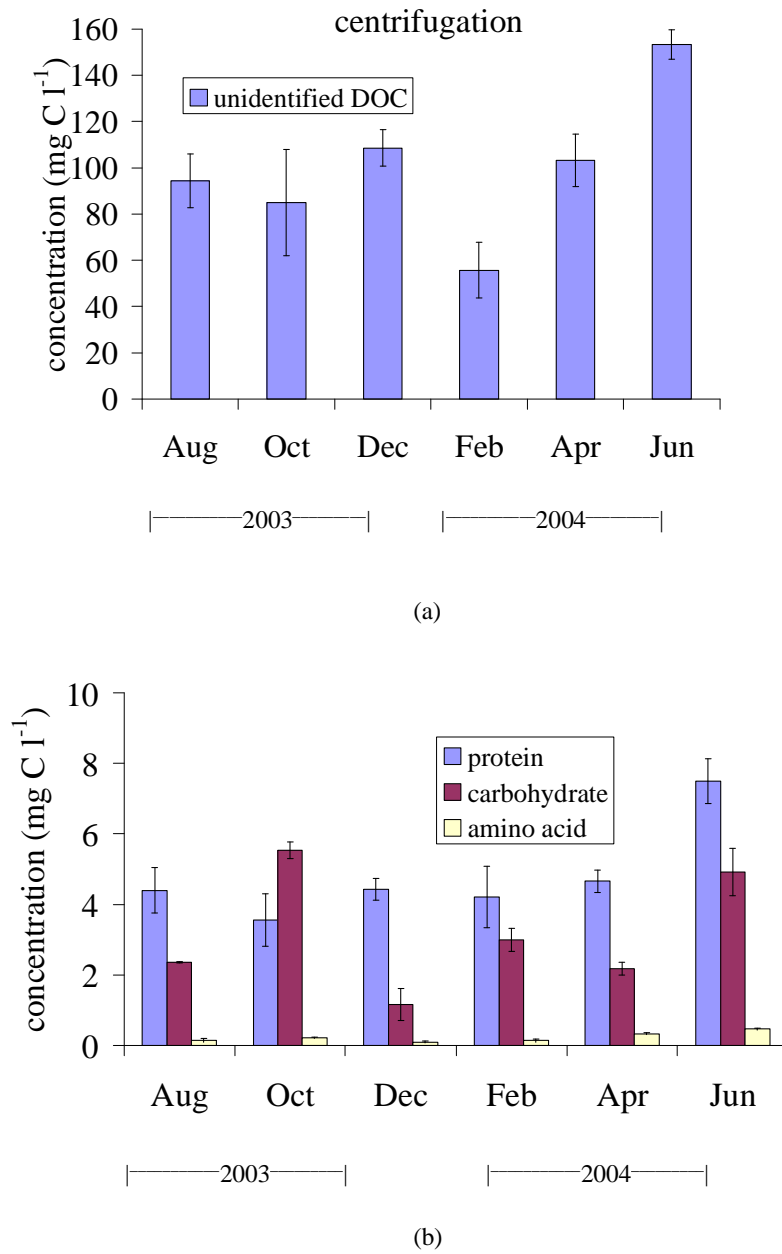
**Figure 17** Concentrations of unidentified (a) and identified (b) components of DOC (mg C l$^{-1}$) isolated from peat soil using a centrifuge-extraction method over the period August 2003 to June 2004. Error bars represent ± 1 SE (n = 3).
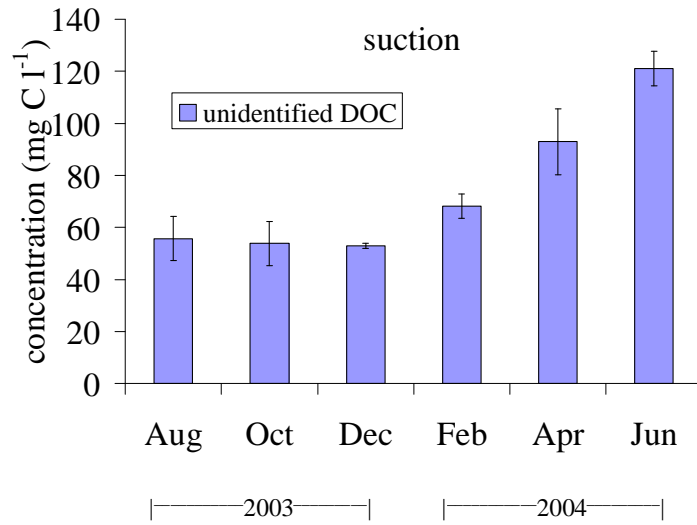
(a)
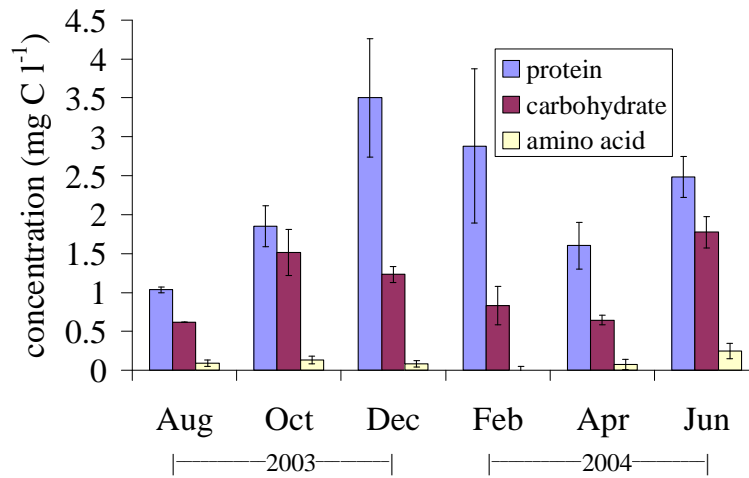


(b)

**Figure 18** Concentrations of unidentified (a) and identified (b) components of DOC (mg C l^{-1}) isolated from peat soil using a suction-extraction method over the period August 2003 to June 2004. Error bars represent ± 1 SE (n = 3).

The total protein + amino acid N as a fraction of DON (% protein + amino acid N) also showed variation with season (Figure 19). This fraction reached its highest levels in October irrespective of method of extraction. October levels for water-extracted DON (94%) were significantly higher than February (30%). October levels for centrifuge–extracted DON (74%) were higher than April (26%) and August (12%). Whilst October levels for suction-extracted DON were highest in October (68%), this value was not significantly higher than any other month sampled. The variation in the proportion of total protein and amino acid was a function of both fluctuating protein concentrations and fluctuating unidentified DON concentrations (Figure 20): concentrations of amino acids were relatively negligible, with values less than 0.17 mg N $l^{-1}$ for all three extraction methods. The fluctuations in protein, amino acid and unidentified DON appeared to be largely independent of one another: the only significant correlation between any of these components was between protein and unidentified DON in suction-extracted DON ($r = 0.840$, $p = 0.036$). No significant correlations ($p < 0.05$) were observed between % protein + amino acid N and mean minimum or maximum daily temperature for the 4 weeks preceding sampling and between % protein + amino acid N and soil moisture content at time of sampling.
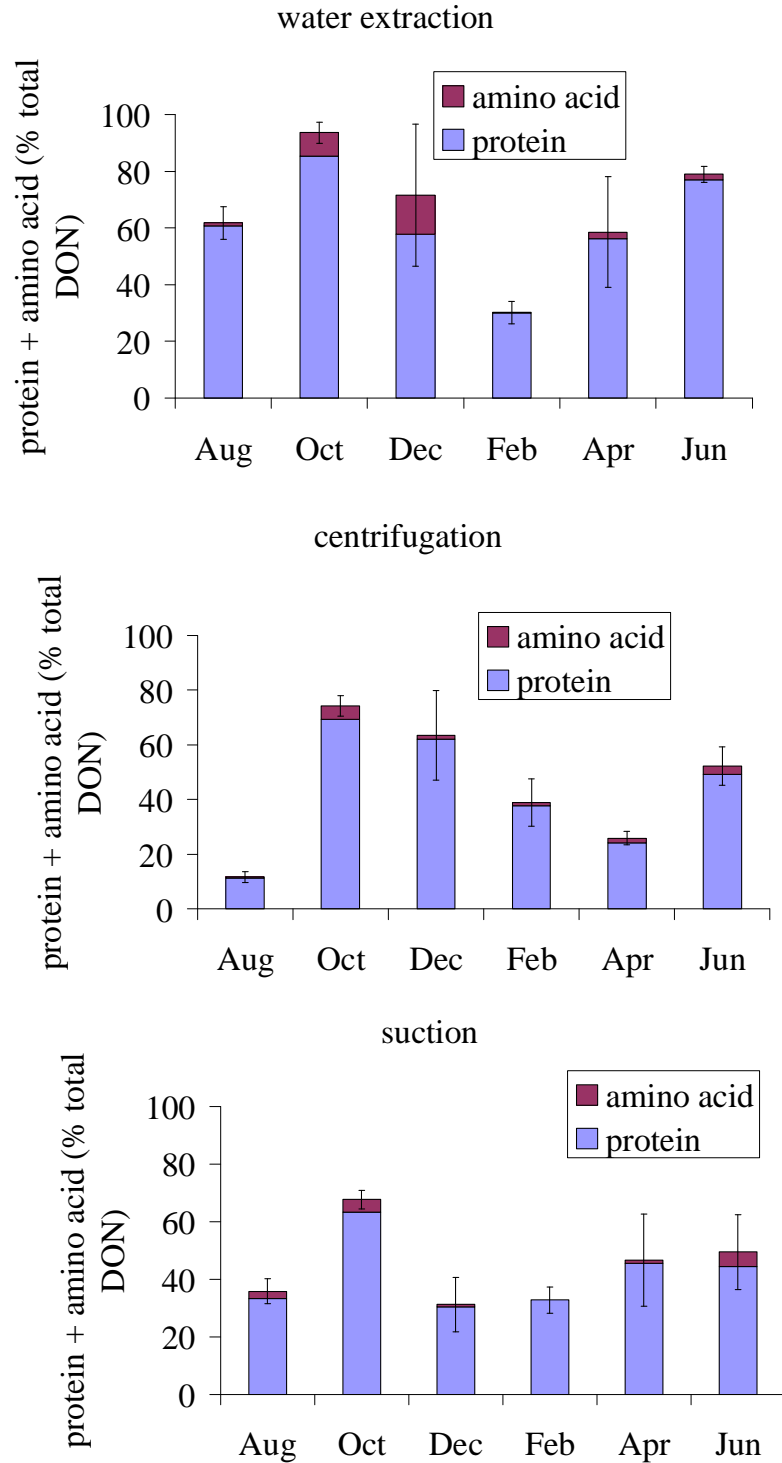
**Figure 19** Proportions of protein and amino acid N in DON extracted from the same peat soil using three different extraction methods: water-, centrifuge- and suction extraction. Error bars represent ± 1 SE (n = 3) of the combined total of protein + amino acid N.
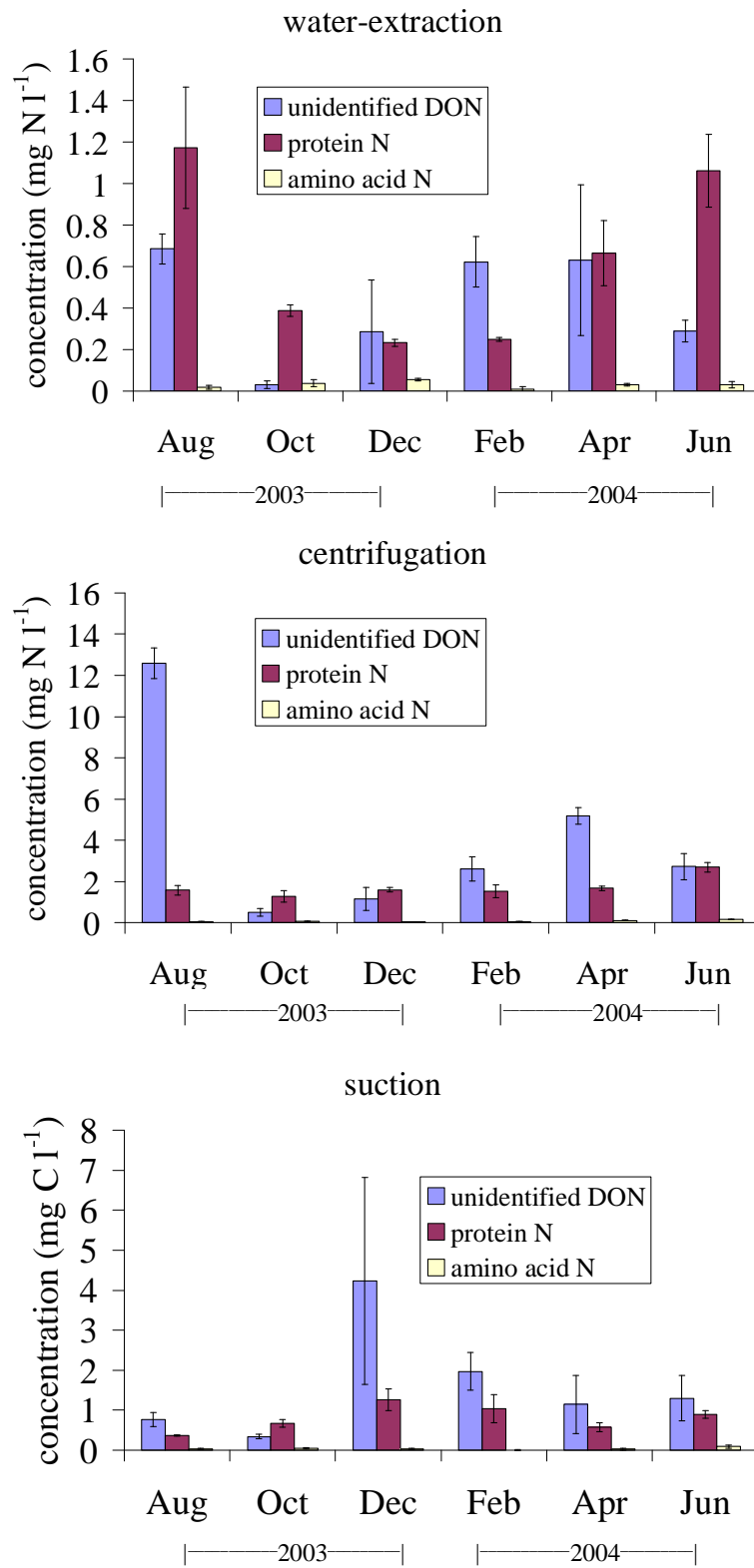
**Figure 20** Concentrations of unidentified DON and identified components (protein and amino acids) of DON (mg C l^{-1}) isolated from peat soil using water-, centrifugation- and suction-extraction methods over the period August 2003 to June 2004. Error bars represent ± 1 SE (n = 3).

## 2.4.4  DOC/DON ratios

In water extracted DOC, concentrations of DOC and DON were significantly correlated ($p < 0.05$), but the correlation was weak ($r = 0.474$). There was no significant correlation between DOC and DON (Figure 22) in centrifuge and suction extracted DOC ($r = 0.010$ and $-0.063$ respectively).

DOC/DON ratios varied with respect to both method of extraction and time of sampling (Figure 21). Whilst the average DOC/DON ratios for water-, centrifuge- and suction-extracted DOC were 35:1, 22:1 and 41:1 respectively individual values ranged from as little as 7:1 to as high as 61:1 (Table 3). Lowest DOC/DON ratios for the year were observed in August 2003 and highest just two months later in October for both water- and centrifuge-extracted DOC. For DOC extracted using a suction-lysimeter the seasonal pattern was somewhat different: October 2003 and April and June 2004 were similarly high whilst in December 2003 DOC/DON was at its lowest.

**Table 3** DOC to DON ratios of peatland soil water isolated using three different methods of extraction over the period August 2003 to June 2004. Standard errors ($\pm 1$ S.E.) are given in brackets ($n = 3$) and represent spatial variation.

| Sampling Date | DOC/DON ratio | | |
|---|---|---|---|
| | water- extraction | centrifugation | Suction |
| August | 14 ($\pm1$) | 7 ($\pm1$) | 49 ($\pm1$) |
| October | 61 ($\pm12$) | 50 ($\pm19$) | 54 ($\pm1$) |
| December | 33 ($\pm7$) | 41 ($\pm11$) | 10 ($\pm5$) |
| February | 38 ($\pm9$) | 15 ($\pm2$) | 24 ($\pm11$) |
| April | 29 ($\pm3$) | 16 ($\pm1$) | 55 ($\pm25$) |
| June | 36 ($\pm4$) | 30 ($\pm1$) | 55 ($\pm23$) |
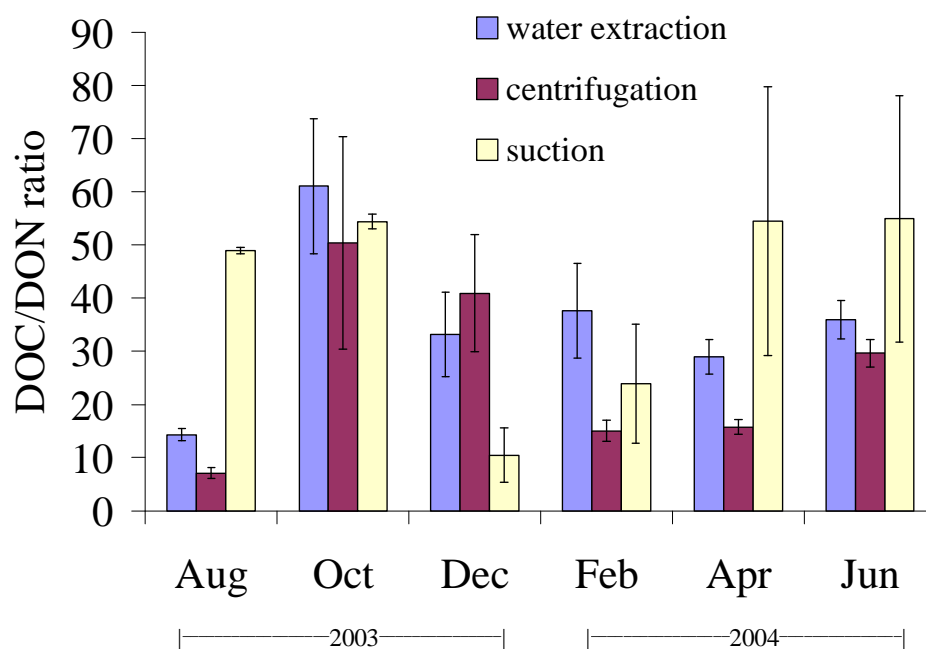
**Figure 21.** Mean effects of time and method of extraction on DOC/DON ratios of peat soil for the year Aug 2003 – Jun 2004 (n = 3).

## Water extraction

r = 0.474
( p = 0.047)

## Centrifugation

r = 0.010
(p = 0.968)

## Suction

r = 0.063
(p = 0.803)
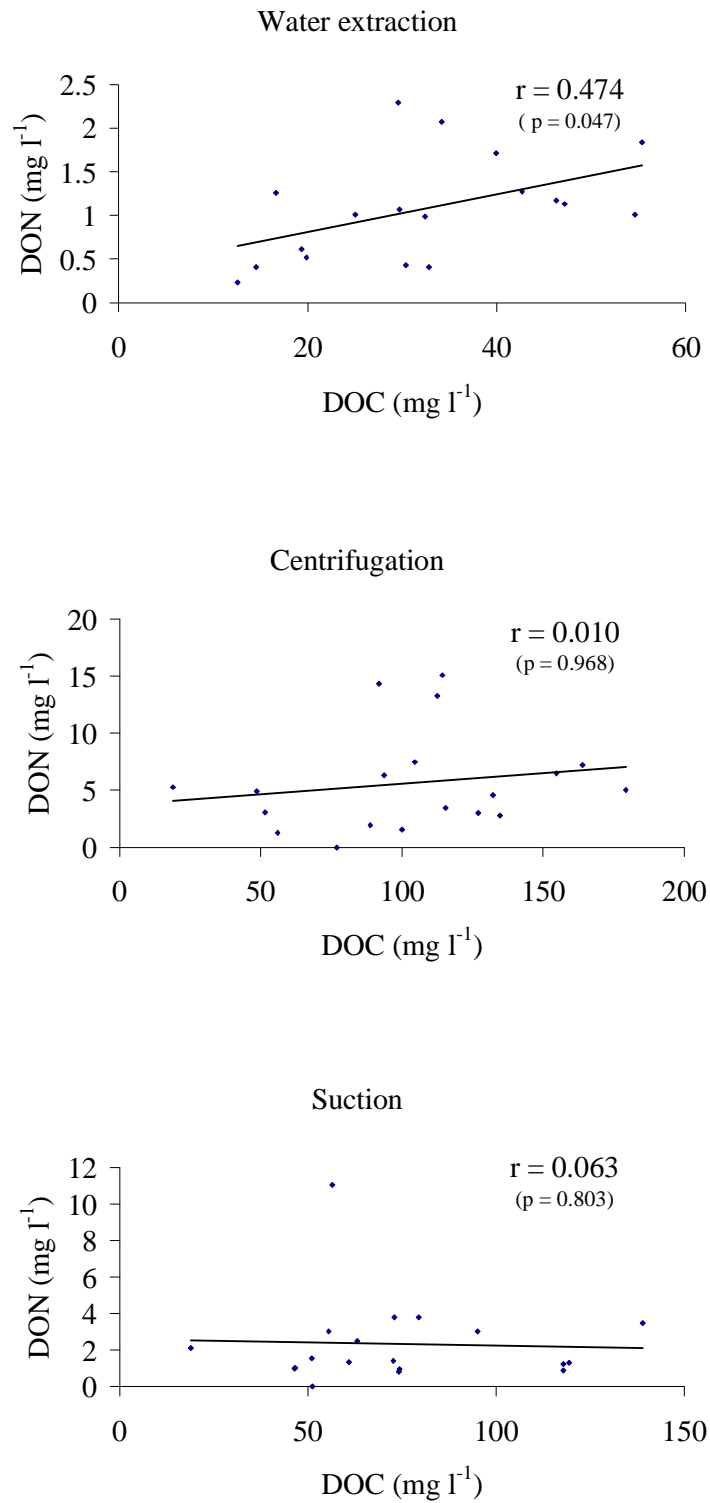
**Figure 22** Relationship between mean DOC and DON concentrations of DOM extracted from peatland soil every two months over the period of a year (n = 3). r values represent the value for Pearson's product-moment correlation, the p value for this correlation is given in brackets

## 2.5    Discussion

## 2.5.1  Influence of Method of extraction on DOC Composition

At certain times of the year the different methods of extraction were found to be sampling biochemically distinct fractions of DOC. In late summer/autumn (Aug/Oct 2003) and spring (Feb/Apr 2004) the size of the total protein, carbohydrate and amino acid fraction of peatland DOC varied with the method used to extract it. Where differences existed, DOC extracted using the water-extraction method generally contained a greater proportion of one or more of these types of molecules than that obtained using the centrifugation or suction methods. Only in February was this situation altered with centrifuge-extracted DOC containing a larger proportion of carbohydrate than either water- or suction-extracted DOC.

The water-extraction method involves the greatest degree of destruction of the soil structure and therefore would be expected to release DOC from within the smaller pores/structures as well as the larger macropores (Chantigny, 2003). If macropores associated with preferential flow paths have a significantly larger biomass than the matrix (Bundt, 2001), then the proportion of labile DOC, such as carbohydrates, proteins and amino acids, within larger pores would be anticipated to be lower than in smaller pores, assuming replenishment of fresh labile DOC to the macropores is a limiting factor. DOC in the micropores ($< 0.2$ µm) is also likely to be physically protected because of its lack of accessibility to microorganisms (Zsolnay, 1996). Biodegradation of DOC in these pores would depend on diffusion of the DOC into larger water-filled pores or the inward diffusion of extracellular enzymes from larger

pores (Marschner and Kalbitz, 2003). In dry conditions, where diffusion of DOC through the soil is impossible, labile material may effectively be trapped in micropores. A further contributory factor may be an artefact of the water-extraction process. The differences in the chemical characteristics of double-distilled, deionised water and soil water may be responsible for solubilising and desorbing molecules that would otherwise be insoluble or sorbed in the field.

The centrifugation method also involves some disturbance of soil structure and therefore small pores, however it is likely that micropores ($< 0.2$ µm) within aggregates still retain their structure and the strength of the centrifugal force applied (equivalent to -70 k Pa) would not be sufficient to extract DOC from within these micropores, typically held at a tension of less than -1500 k Pa (Zsolnay, 1996). The suction-filtration method carried out *in situ*, does not involve an extractant or destruction of the soil structure from which it is sampling, and, using an applied vacuum force of –70 kPa, is effectively only sampling from the larger pores present in the soil and therefore, for the reasons described above, is only sampling DOC low in biodegradable molecule content.

The seasonal dependence of the composition of DOC with respect to the different methods of extraction emphasises the spatial functional compartmentalisation of the soil structure. Possible causes of the influences of season on the varying composition of these compartments may be a consequence of any factor that may influence differing pore sizes to differing degrees e.g. hydrological conditions, the spatial location and relative activity of fungal and microbial populations; and, the activity of plant roots. For instance, changes in hydrological conditions during the year may affect the rate of

replenishment or loss of fresh DOC from the macro- and intermediate pores; the diffusion of DOC and enzymes in/out of micropores; and, the degree of aeration of the soil.

The existence of biochemically distinct fractions of DOC within the soil indicates that the biological, chemical and/or physical influences on DOC production and loss are different within the differently sized soil pores. The apparent ability of each of the three extraction techniques here to isolate three different fractions means that conclusions of previous research carried out using just one technique cannot be assumed to apply to whole soil DOC or any fraction of DOC extracted using a different technique. However, with the understanding that these fractions are probably spatially distinct it does allow previous research data to be looked at in a new light.

Comparisons between the absolute concentrations of DOC components extracted of all three different methods, as opposed to their proportions, was impossible because (1) the soil:solution ratio used in the water-extraction technique affects the concentration of soil water components extracted (Chapman et al., 1997; Zsolnay 2003) and (2) the influx of seepage water through large pores in the soil during the sampling process (Grossman and Udluft, 1991) may dilute the concentration of suction-extracted DOC.

## 2.5.2 Influence of Time of Extraction on DOC and DON Concentrations

Results here confirm those of previous studies (Chittleborough *et al.*, 1992) that DOC concentrations are higher in summer than in winter. This was found to be the case irrespective of method of extraction. This suggests that the factors controlling the production and/or loss of total DOC are the same regardless of the locality of the DOC within the soil i.e net production and/or loss are being driven by factors external to the specific conditions within the differently sized pores. Factors causing relatively high concentrations in June may possibly be: an increase in release of litter leachate into the soil as a result of both an increase in litter material and an increase in its decomposition rate; increased rhizodeposition; increased aeration due to increased invertebrate activity; and/or an increase in desorption rates, solubility or microbial activity due to increased temperatures. The general lack of correlation between mean minimum and maximum temperatures in the 4 weeks preceding sampling and DOC concentrations suggest that, at least on this time-scale, that temperature alone is not the controlling factor. The high levels of rainfall in June 2004, second only to December for the whole of the sampled year (Figure 23), and lack of correlation between moisture content and DOC concentration suggest that controlling factors that one may predict for summer months such as reduced dilution of soil DOC or increased aeration, are unlikely.
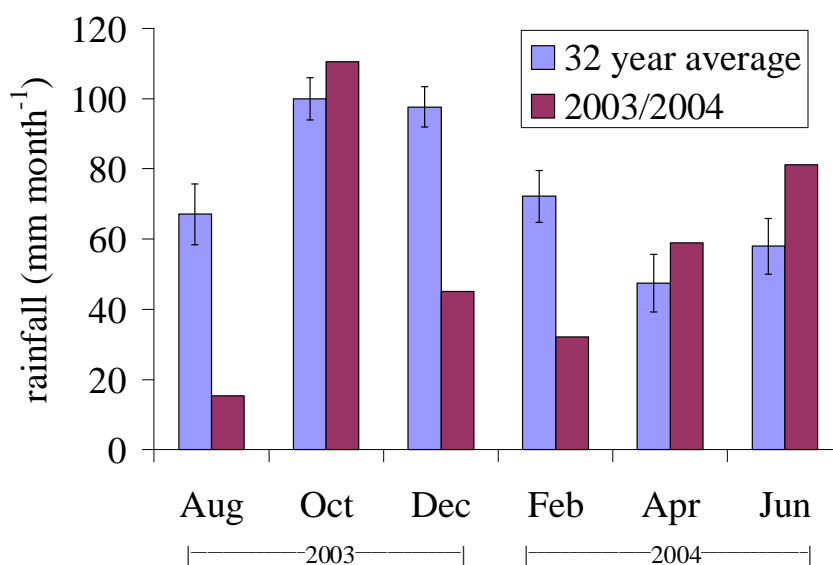
**Figure 23** 32 year and 2003/2004 mean monthly rainfall (mm month$^{-1}$). Data recorded at Parkhead weather station, University of Stirling.

In contrast to DOC concentrations which peaked in June, DON concentrations for water- and centrifuge-extracted DON peaked later in the summer, in August. High concentrations of DON in late summer may reflect an increase in N-rich decomposition products. Higher concentrations of summer DON relative to winter DON were also observed by Chapman et al (2001) in streams of four upland areas in Scotland, although the authors of this report speculate that this reflects an increase in the production of DON within the stream channel rather than an increase in allochthonous inputs. The reason for the absence of any significant seasonal changes in suction-extracted DON concentration is unknown and again highlights the influence of method of sampling on DOC properties.

## 2.5.3  Influence of Time of Extraction on DOC and DON Composition

Seasonal changes in the composition of DOC were evident. The changes in concentration over the course of the year described above (section 2.5.2) did not therefore simply represent a bulk increase or decrease in all the components of DOC. Instead, the seasonal dependency of DOC composition suggests that the relative importance of each of the sources and losses of DOC changes with the seasons.

The % carbohydrate + protein + amino acid of water extracted DOC, DOC assumed to represent DOC from macro, intermediate and micropores (see section 2.5.1), was higher in autumn (Oct) than summer (Jun). This suggests that in autumn soil DOC is more biodegradable than the same fraction in summer, assuming that only a negligible proportion of these molecules are detected when chemically protected through complexation with molecules resistant to degradation and the proportion of highly labile material in the unidentified DOC is relatively small. This agrees with Hongve et al. (2000) who compared the biodegradability of leaf litter percolates collected in October with those collected in May. However, the % carbohydrate + protein + amino acid in October, and in August, were also higher than winter levels (Dec/Feb). This contradicts other studies which suggest higher DOC biodegradabilities in winter/spring than summer/autumn (Kaiser *et al.*, 2001); Nelson et al., 1994).

The 'biodegradable' fraction of centrifuge extracted DOC responded differently to water-extracted DOC, showing little change in % carbohydrate + protein + amino acid with season. Thus the combined composition of intermediate and macro pores, with respect to this fraction, appears to remain relatively constant throughout the year. The

effect of season on the % of 'biodegradable' material in suction-extracted DOC, assumed to be largely derived from macropores (see section 2.5.1), fitted more clearly with the pattern observed in previous studies (Kaiser *et al.*, 2001); Nelson et al., 1994), with levels in December significantly higher than in summer (Aug/Jun). However, December levels were also significantly higher than spring (Apr).

A lack of any negative or positive correlation between the concentrations of the various fractions throughout the year, for all but protein and unidentified DOC in centrifuge-extracted DOC, suggests that the net production and/or loss of each of these components is under different controls e.g. carbohydrate concentrations might be determined by rates of rhizodeposition whilst protein concentrations may be dependent on microbial extracellular enzyme activity. Alternatively their production/loss may be part of a sequential process which is measurable on a timescale of months.

Similar to DOC the % protein + amino acid N as a fraction of DON also showed variation with season and the response to season varied according to method of extraction. Highest levels of this fraction were found in autumn (Oct) for all methods, significantly higher than February for water-extracted DON and higher than April and August for centrifuge-extracted DON. The mean % protein and amino acid N content of DON (44-66 %) is comparable to the mean values of 64–65 % observed by Michalzik and Matzner (1999) over a 2 year period for amino N in forest floor percolates following hydrolysis. As for DOC, the general absence of correlation between protein N, amino acid N and unidentified DON, suggests that each constituent is under different controls.

Discrepancies between the exact time of year when high biodegradabilities are observed i.e autumn/winter vs. winter/spring and differences in the degree of biodegradability of DOC and DON reported by different research teams may be due to the different compositions of leaf litter leaching degradable fractions at different times, differences in temperature affecting the rate of decomposition of the leaf litter, differences in the size and structure of microbial community, and/or differences in the pattern of precipitation affecting the rate of leaching. Generally, autumn/early winter DOC appears to have a greater % carbohydrate + protein + amino acid. In the autumn leaching of DOC from the plentiful leaf litter, resulting from senescence after the growing season, and desorption of sorbed material would be expected to take place with each rainfall but low temperatures would keep microbial activity and, consequently, the rate of mineralization and assimilation of labile DOC relatively low, causing an accumulation of labile DOC. As winter progresses the amount of leachable material from the leaf litter would decline so that by April biodegradability would be relatively low. In the summer months higher temperatures would increase microbial activity and the rates of hydrolysis and diffusion so that the rate of mineralization and assimilation of labile DOC by microbes is greater than the gain through leaching, throughfall, desorption, microbial production and/or root exudation. This theory however is clearly an oversimplification as the biodegradable fraction of DOC/DON isolated here from different physical compartments of peat soil was influenced differently by time of year.

## 2.5.4  DOC/DON Ratios

The lack of correlation between DOC and DON concentrations for centrifuge- and suction-extracted DOC and only a weak correlation for water-extracted DOC, along with the wide range of DOC/DON ratios observed (7:1 to 61:1), suggests DON and DOC concentrations are under different controls. These results are similar to those of Williams and Silcock (Williams and Silcock, 2000) who reported a higher seasonal variability of DON compared with DOC and Michalzik and Matzner (Michalzik and Matzner, 1999) who found that DON concentrations in the partially decomposed litter layer were more influenced by temperature than were DOC concentrations. The ratio of DOC to DON is likely to depend on many factors including the relative concentrations present in throughfall and litter leachate, and the relative rates of production and loss through microbial decomposition/assimilation, plant uptake, sorption/desorption and hydrologic flushing. The likelihood is that the net effect of all of these factors influences DOC and DON concentrations to differing degrees at different times of the year. For example, in peat soils, the C:N ratio of the solid phase is very high (>40:1) and the utilisation of C for growth will not occur unless important nutrients such as N are present, thus at times of the year when there is a more than sufficient supply of labile DOC to sustain the population e.g. inputs of labile DOC from fresh litter in the autumn, N-containing compounds may be preferentially hydrolysed by microbes causing the relatively high C:N ratios. The low DOC/DON ratios observed in August for water- (14:1) and centrifuge- (7:1) extracted DOC may indicate the presence of dissolved organics that are more highly decomposed and produced at a greater rate in summer when temperatures are higher (Melillo et al., 1982). This does not, however, account for the high DOC/DON ratio (49:1) of suction-extracted DOC at this time. This may possibly be due to more frequent hydrological flushing of these pores removing N-

rich decomposition products and bringing in fresh, relatively undecomposed DOC. Again, it seems that factors controlling DOC inputs and outputs in the larger macropores are different to those in the intermediate and smaller micropores.

## 2.6    Conclusions

- different methods of extraction of peatland DOC (water-, centrifugation- and suction-extraction) are sampling biochemically distinct fractions, in terms of carbohydrate, protein and amino acid composition, at certain times of the year. Where differences existed, DOC extracted using the water-extraction method generally contained a greater proportion of one or more of these types of molecules than that obtained using the centrifugation- or suction-extraction methods.

- The concentrations of peatland DOC and DON show seasonal variation. Results here show DOC concentrations in summer (June) to be higher than in winter, irrespective of method of extraction. In contrast DON concentrations for water- and centrifuge-extracted DON peaked later in the summer, in August. There were no significant seasonal changes in suction-extracted DON concentration.

- The proportion of carbohydrate + protein + amino acid carbon in DOC extracted from peatland showed seasonal variation, indicating that the processes controlling the production and/or loss of DOC are under seasonal control. The % carbohydrate + protein + amino acid carbon was higher in late summer/autumn than both winter and early summer for water-extracted DOC; showed no significant change for

centrifuge-extracted DOC; and, was higher in December than spring and summer for suction-extracted DOC.  Similarly, the proportion of protein + amino acid N as a fraction of DON also showed variation with season. Highest levels of this fraction were found in autumn (Oct) for all methods indicating, significantly higher than February for water-extracted DON and higher than April and August for centrifuge-extracted DON. There was a general absence of correlation between the concentrations of each of the constituents of both DOC and DON (protein, carbohydrate, amino acid and unidentified C) throughout the sampled year, suggesting that the production and/or loss of each is under different controls.

- There is no good correlation between peatland DOC and DON concentrations in water-, centrifuge- or suction-extracted DOC, suggesting that DOC and DON production and/or loss are under different controls.

# Chapter 3: Decomposition Dynamics of DOC

## 3.1.    Introduction

Dissolved organic carbon is an important substrate for soil microorganisms (Marschner and Bredow, 2002). Several laboratory incubation studies (Zsolnay and Steindl, 1991);(Boyer and Groffman, 1996; Gregorich *et al.*, 2003; Kalbitz *et al.*, 2003a; Nelson *et al.*, 1994; Qualls and Haines, 1992) have shown that a considerable proportion of DOC (10 – 88 % depending on source of DOC and experimental approach) is biodegradable within a period ranging from a few days to months.  Detailed studies of the dynamics of mineralization of DOC, from both forest and agricultural soils, indicate two kinetically distinct pools: a generally smaller, rapidly degradable pool with a half-life of the order of days and a larger, more stable pool with a half-life of the order of months and years (Gregorich *et al.*, 2003; Kalbitz. *et al.*, 2003b; Qualls and Haines, 1992). The rapidly degradable pool is believed to largely consist of labile carbohydrate monomers, low molecular weight organic acids and amino sugars whereas the more stable pool consists of relatively recalcitrant and complex microbial metabolites and the decomposition products of lignin and lignocellulose (Guggenberger *et al.*, 1994; Koivula and Hänninen, 2001; Küsel and Drake, 1999; Qualls and Haines, 1992).  As a consequence of the differing composition of these DOC pools, biodegradation may be mediated by two different microbial communities adapted to utilising different substrate material. Fontaine et al (Fontaine *et al.*, 2003) proposed that 'r-strategist' microorganisms, adapted to rapidly proliferate in response to an abundance of suitable substrate (Killham, 1994), decompose the most energetic compounds of fresh organic matter whilst 'K-strategists', dominate only in the latter stages of decomposition when the highly energetic compounds have been exhausted and only polymerised compounds

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remain. This hypothesis may also be applied to the pattern of microbial decomposition of DOC: the r-strategists rapidly proliferate in response to the availability of the readily-decomposable labile pool present in the DOC, assimilating soluble organic carbon as they do so, and then, once this pool is exhausted, the size of the microbial biomass declines releasing soluble organic material. Subsequently K-strategist organisms proliferate in response to reduced competition and begin to dominate the degradation of the more recalcitrant molecules that constitute the stable pool. The growth rate of the K-strategists would be expected to be slower than that of the r-strategists because of declining substrate quality and the increased allocation of energy to extracellular enzyme production and defence against predation (Tate, 1995). If such changes take place then not only will a proportion of DOC be mineralised during the process of respiration but some will be assimilated by the microbes during growth phases. Likewise DOC may be released as the biomass declines and cell lysis occurs. Thus the differences between observed mass loss of DOC and predicted loss due to measured $CO_2$ production could give an indication of the pattern of changes in the size of the microbial biomass during the decomposition of DOC.

Several studies have examined the kinetics of DOC biodegradation (Gregorich *et al.*, 2003; Kalbitz. *et al.*, 2003b; Qualls and Haines, 1992) and Kalbitz et al. (2003b) looked at changes in relative abundance of a number of DOC components, including carbohydrates and peptides, before and after a 90 day incubation period. There is little information available, however, on the pattern of change of biological molecule concentrations and microbial population size during the decomposition process

The objectives of this study were to compare the mineralization dynamics, as $CO_2$ released, with the loss of residual DOC during its utilisation by microorganisms for soils from different ecosystems, to examine the dynamics of protein, carbohydrate and amino acid fractions in DOC during microbial utilization, and to quantify any changes in the size of the microbial population during DOC utilization.

## 3.2.    Hypotheses

- The mineralization dynamics of peatland DOC will fit a double exponential decay model, indicating the presence of two kinetically distinct pools.

- The mass loss of total residual DOC will be greater than that predicted by the rate of mineralization due to assimilation of DOC by the microbial population and the pattern of loss will reflect changes in the size of the microbial population.

- Analyses of the composition of the DOC during the decomposition process will show a rapid decline in carbohydrate, protein and amino acid content within the first few days, in accordance with the rapid degradation of the hypothesised labile pool.

## 3.3    Materials and Methods

### 3.3.1  Soil Descriptions

Two contrasting soil types, organic peat soils and mineral agricultural soils were used. Peat soils were sampled from two bogs in Central Scotland. The first was a drained, raised peat bog on the Carse of Forth known as Ochtertyre Moss Wood (see section 2.3.1 for site description). Samples were taken from a depth of 20-50 cm at an altitude of 15 m above sea level. The second was a blanket peat bog in Loch Ard Forest ($4^o$ 28' W, $56^o$ 8' N), a Sitka spruce (*Picea sitchensis*) forest with *Calluna vulgaris*, *Erica tetralix* and *Molinia caerulea*. Samples were taken from 20-50 cm depth at an altitude of 175 m above sea level.   All samples were stored field-moist at $4^o$C for 1 week until use. The raised peat (pH($H_2O$) 3.2, pH($CaCl_2$) 2.4, C:N ratio 45:1) was classified as H3, according to the von Post humification scale. The blanket peat (pH($H_2O$) 3.6, pH($CaCl_2$) 2.6, C:N ratio 40:1) was classified as H5.

Four agricultural soils of the same soil type, classified as Orthic Humic Gleysol under the Canadian Soil Taxonomy system, approximately equivalent to Calcaric Gleysol under the FAO system), undergoing different management strategies were sampled from four different plots on the Agriculture Canada's Central Experimental Farm, Ottawa, Canada ($45^o$ 22' N, $75^o$ 43' W). The plots had been treated differently for 10 years at the time of sampling. The treatments were unamended, a monoculture of maize with no fertilizer or manure, a monoculture of maize fertilized with 100 t $ha^{-1}$ (wet weight) $year^{-1}$ dairy manure, a maize/soybean rotation fertilized with 50 t $ha^{-1}$(wet weight) $year^{-1}$ dairy manure, and a maize/soybean rotation fertilised with 100 kg

mineral N ha$^{-1}$ year$^{-1}$. The samples were taken from a depth of 0-15 cm at four locations within each plot and then bulked, air-dried for storage and wetted up to field capacity one week prior to use. Across all sites (Ap horizon) soil had a clay loam texture with 32% sand, 34% silt, and 34% clay and a pH of 6.0.  The C:N ratios of each plot are given in Table 4.

**Table 4** C:N ratios of  the soils from 4 different plots undergoing different crop management and soil amendment on the Agriculture Canada's Central Experimental Farm, Ottawa, Canada.

| Crop Management | Soil Amendment | C:N ratio |
|---|---|---|
| Maize monoculture | unamended | 13.3 |
| | 100 t ha$^{-1}$ (wet weight) year$^{-1}$ dairy manure | 11.7 |
| Maize / soybean rotation | 50 t ha$^{-1}$(wet weight) year$^{-1}$ dairy manure | 12.7 |
| | 100 kg mineral N ha$^{-1}$ year$^{-1}$ | 12.8 |

## 3.3.2  DOC Extraction

Soil suspensions were created by shaking field moist samples with distilled/deionised water for 1 hr and then centrifuged at a RCF of 900*g* for 10 minutes. To ensure similar final concentrations of DOC for all soil types a soil/water ratio (w/w) of 0.08 was used for the peat soils and a ratio of 0.33 for the agricultural soils. CaCl$_2$ was added to each suspension, to give final concentration of 1mM, prior to centrifugation to flocculate particulate organic matter. The supernatant solutions were decanted and filtered through sterile 0.22 µm cellulose nitrate membranes under aseptic conditions to separate the

DOC from the particulate matter and remove microorganisms. Finally $(NH_4)_2SO_4$ and $KH_2PO_4$ additions were made to all the suspensions to ensure a C:N:P:S:K ratio of < 5:1:1:1:1.

### 3.3.3 Incubation

To determine the biodegradability of each type of DOC, a composite inoculum containing indigenous microorganisms from all sites was obtained by adding 4 g dry weight equivalent of each of the mineral soils and 2 g dry weight equivalent of each peat soil to 250 ml of distilled/deionised water. The suspension was left at $20^oC$ for 18 hours, occasionally swirled and then left to allow particulate matter to settle out before being used as the source of the inoculum.

350 ml of each filter-sterilised DOC sample were added to 600 ml glass conical flasks and inoculated with 100 µl of the microbial inoculum.  Identical flasks containing 350 ml glucose solutions (20 mg $l^{-1}$) plus inoculum and 350 ml distilled/deionised water plus inoculum were also prepared. Three replicates of each of the different culture mixtures were made . Three equally-sized pieces of glass fibre filter (Millipore AP25 47 mm, each of area 2.8 $cm^2$, was placed in each flask to provide a surface for microbial growth (Qualls and Haines, 1992). All the flasks were stoppered with a rubber bung and incubated in the dark at $20^oC$ for 70 days. Mineralisation of DOC was quantified on days 1, 3, 7, 15, 42 and 70 by measuring the $CO_2$ concentration in the headspace of each incubation flask using gas chromatography (Varian Aerograph 90-P). A hypodermic needle was used to pierce a resealing rubber seal in the centre of each stopper, remove 1 ml of air from the headspace and inject it in to the chromatograph. Calculations of soluble $CO_2$, based on Henry's law (Kessler and Harvey, 2001;

Plummer and Busenberg, 1982), showed that in all cases dissolved $CO_2$ was negligible relative to gaseous $CO_2$.

A 40 ml sample was removed from each flask on days 0, 1, 3, 7, 15, 42 and 70, filtered through a 0.22 μm cellulose nitrate membrane filter (Whatman 47 mm) and then analysed for DOC, DON, proteins, amino acids, carbohydrates, $NH_4^+$ and $NO_3^-$ . The flasks were left open for 30 mins after liquids samples had been removed to aerate the headspace for 30 minutes before the bungs were replaced.

To determine microbial dynamics a second set of flasks were incubated and the microorganism population sizes on days 3, 7, 15, and 70, were directly enumerated using a filtration–epifluorescent microscopy technique. 40 ml of sample and one piece of glass fibre filter were removed from each flask and sonicated for 20 seconds to help dislodge microorganisms from the filter. 40 ml of sample from each incubation flask were stained for 2 min. with 5 ml of 1.0 μg ml$^{-1}$ DAPI (in 10 mM tris, 1 mM EDTA, pH 8) and then filtered manually using a syringe, through a 0.2 μm black Whatman Cyclopore® polycarbonate membrane filter (Porter and Feig, 1980; Hobbie et al., 1977). The membrane filter was immediately mounted on a slide with low fluorescence immersion oil and viewed under X1000 magnification using a Zeiss Axiovert 135 microscope and DAPI filter set (exciter 365 nm, dichroic 395 nm, barrier 420 nm). The number of cells falling within 5 fields of view on each membrane filter were counted.

### 3.3.4  Biochemical Analyses

Biochemical assays for DOC, protein, carbohydrate and amino acids were carried out as outlined in section 2.3.3 and detailed in Appendix A. Mean results of biochemical analyses of the inoculum only cultures were deducted from mean results of DOC-containing cultures (n = 3).

### 3.3.5  Assessing Microbial Release and Uptake of DOC

Differences between net loss of residual DOC and predicted loss due to measured $CO_2$ production were calculated to also give an indication of the microbial biomass release and uptake of DOC during the incubation period. Solubility changes were assumed to be negligible. Mean values obtained for the inoculum only cultures were deducted from mean values for the DOC-containing cultures.

### 3.3.6  Statistical Analysis of Mineralisation Data

A repeated measures ANOVA and a Tukey's post-hoc test were used to ascertain the significance of any differences between the extent of mineralization on any particular sampling date for any of the soil types/treatments .(data transformed to 'log10 (C +3)': where C = cumulative $CO_2$ production (% of initial DOC)).

An F-test was used to determine whether a single or double exponential decay curve was the best fit for each data set using GraphPad Prism®. The simpler equation was chosen unless the more complicated fit significantly better with $P < 0.05$. The time taken to mineralise half of the initial DOC substrate (the half-life) was calculated using the equation: $t_{1/2} = 0.693/k$ for each kinetically distinct pool of DOC.

## 3.4    Results

### 3.4.1    DOC mineralisation

After 70 days an amount equivalent to 5-39% of the initial soil DOC was mineralised and 48% of the dissolved glucose (Table 5). There were no lag phases in the mineralization of any of the DOC samples or glucose, indicating that the number and activity of microorganisms did not limit C mineralization. The cumulative $CO_2$ released by the blanket peat DOC was significantly higher ($p < 0.05$) than raised peat on day 7 and thereafter of the incubation period. Otherwise no significant differences existed between the extent of mineralization on any particular sampling date for any of the different soil types.

The dynamics of DOC mineralization for all the soil types fitted a double exponential first order decay model (Figure 24 andFigure 25) with an $r^2 > 0.99$ (Table 5).

i.e.            % of initial DOC mineralized = $[(100 - a)\, e^{-k1t}] + [a\, e^{-k2t}]$

where: t = time (days);  100 – a = proportion of DOC that is rapidly mineralised; a = proportion of DOC that is slowly mineralised; $k1$ = rate constant of the labile pool of DOC; and, $k2$ = rate constant of the more stable pool of DOC.

This model provided a better fit than a single exponential model. This indicates that the DOC was composed of two kinetically distinct pools: a readily decomposable, labile pool and a more stable pool, relatively resistant to decomposition. The mineralisation rate in the first few days was between 1 and 3 orders of magnitude higher than in the latter stages. The half-life of the labile pools was between 3 to 8 days compared with 0.4 to 6 years for the more stable pools (Table 5).

**Table 5** Mean quantitative measures of the extent and dynamics of the mineralization of DOC during a 70 day incubation period ($k$ = rate constant; $r^2$ = goodness of fit of double exponential decay model). Figures in brackets represent one S.E. of the mean (n = 3), except for * where figures in brackets represent the calculated effect of $\pm$ 1 S.E. of the rate constant on the half-life. It should be noted that due to repeated measures any errors observed are cumulative.

| DOC solution | Mineralised DOC (% total DOC) | Labile Pool Size (% total DOC) | $k$ (day$^{-1}$) | Stable Pool Half-life* (days) | Size (% total DOC) | $k$ (day$^{-1}$) | Half-life (years) | $r^2$ |
|---|---|---|---|---|---|---|---|---|
| Raised peat | 4.7 (3.0) | 2.7 (0.7) | 0.1046 (0.0417) | 6.6 (4.7-11.0) | 97.5 (0.7) | 0.0003 (0.0001) | 6.2 (4.7-9.5) | 0.998 |
| Blanket peat | 38.5 (18.7) | 12.5 (2.9) | 0.2452 (0.1314) | 2.8 (1.8-6.1) | 87.5 (2.9) | 0.0047 (0.0007) | 0.4 (0.4-0.5) | 0.997 |
| Maize (unamended) | 23.0 (9.9) | 13.2 (5.0) | 0.1172 (0.0723) | 5.9 (3.7-15.4) | 87.4 (5.2) | 0.0017 (0.0010) | 1.1 (0.1-2.4) | 0.995 |
| Maize-soybean rotation (manured at 50t ha$^{-1}$ year$^{-1}$) | 19.3 (5.6) | 14.4 (1.3) | 0.1426 (0.0250) | 4.9 (4.1-5.9) | 85.4 (1.3) | 0.0008 (0.0003) | 2.4 (1.7-3.8) | 0.999 |
| Maize (manured at 100t ha$^{-1}$year$^{-1}$) | 11.4 (3.9) | 7.5 (0.4) | 0.2382 (0.0370) | 2.9 (2.5-3.4) | 92.5 (0.4) | 0.0006 (0.0001) | 3.2 (1.7-3.8) | 0.999 |
| Maize-soybean rotation (fertilised at 100kg N ha$^{-1}$year$^{-1}$) | 28.8 (21.6) | 16.6 (7.7) | 0.0830 (0.0523) | 8.4 (5.1-22.6) | 84.2 (7.9) | 0.0023 (0.0015) | 0.8 (0.5-2.4) | 0.997 |
| Glucose | 47.7 (0.99) | 49.2 (7.0) | 0.2238 (0.0752) | 3.1 (2.3-4.7) | 54.5 (6.83) | 0.0004 (0.0025) | 4.9 (0.7-∞) | 0.993 |
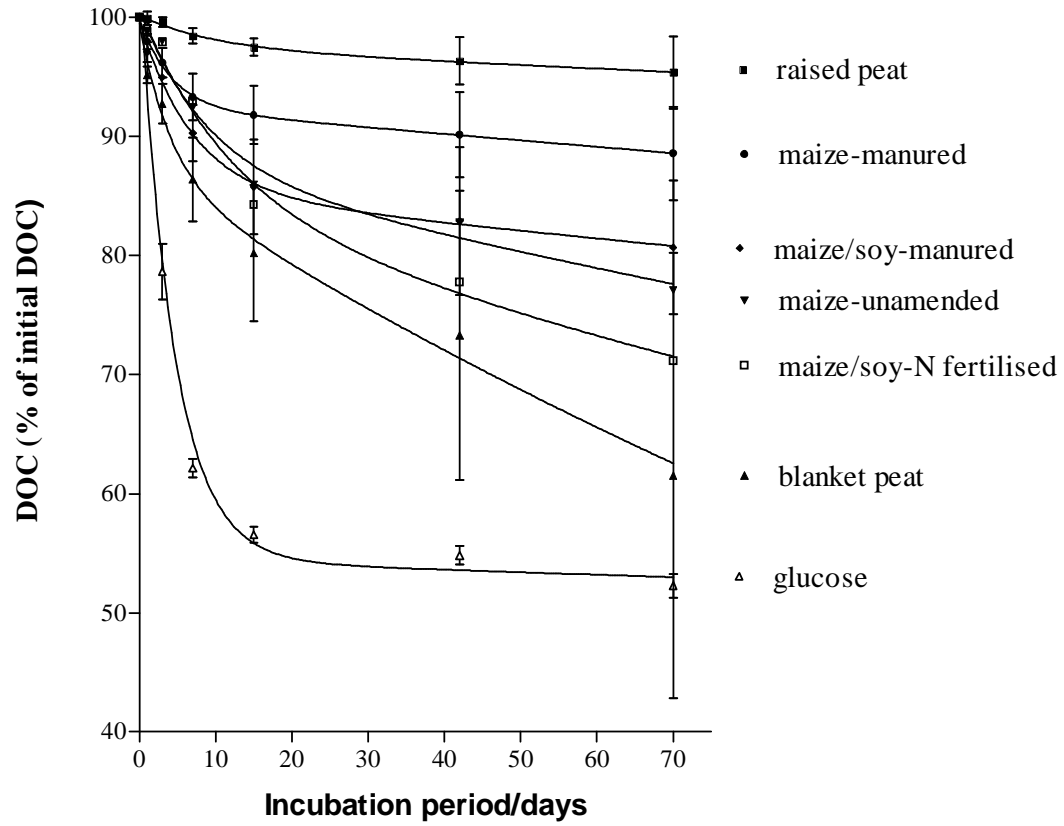
**Figure 24** Loss of soil DOC as a result of mineralization with error bars displayed (± 1 SE of the mean (n = 3)). Each curve represents a fitted non-linear regression based on a double-exponential decay curve ($r^2$ > 0.99 for all data sets)..
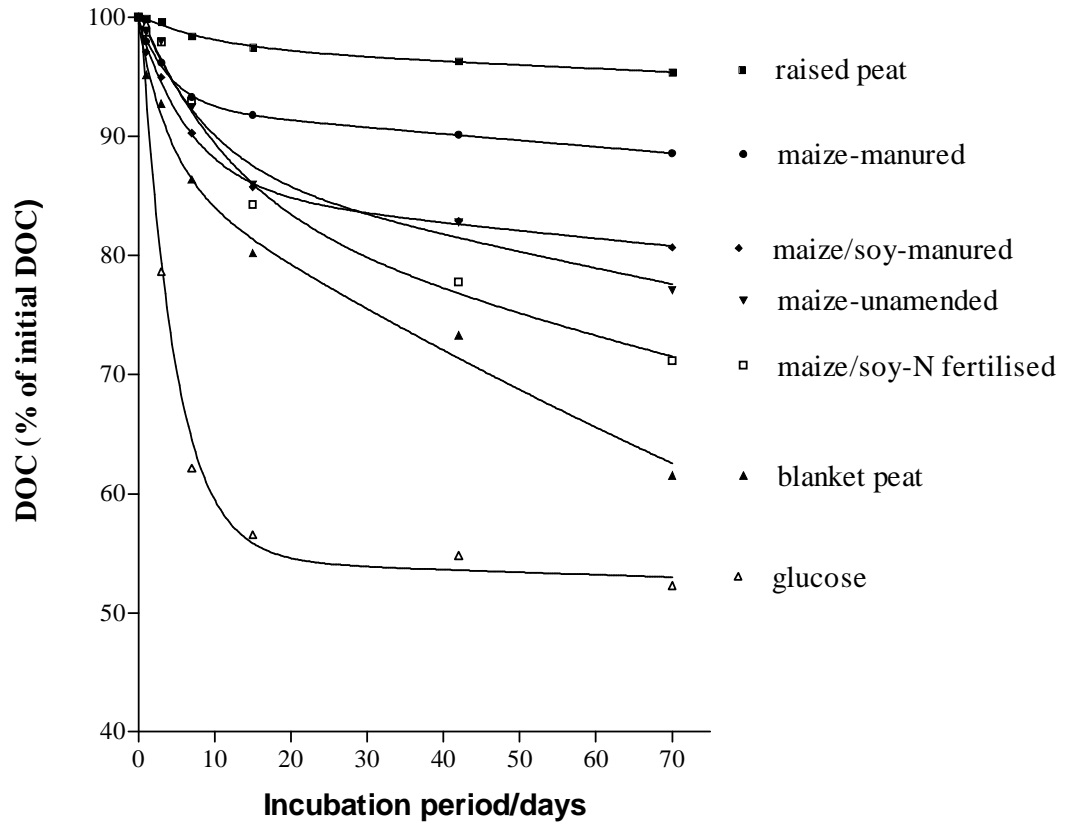
**Figure 25** Loss of soil DOC as a result of mineralization (error bars not displayed). Each curve represents a fitted non-linear regression based on a double-exponential decay curve ($r^2 > 0.99$ for all data sets).

## 3.4.2    Net loss of DOC

After 70 days the net loss of initial soil DOC was 4-44 % for the soil solution samples and 96% for the glucose sample. Net changes in DOC concentrations in all the cultures during the incubation were different to that predicted by the loss of DOC through mineralization, both in terms of extent and dynamics (Figure 26). For all soil types/treatments, except for raised peat where loss to $CO_2$ matched net loss, the net loss of DOC for the majority of the incubation period was greater than could be accounted for by the process of respiration/mineralization. In contrast to the double exponential decay pattern of decline in DOC during the incubation period based on mineralization rate, the pattern of net DOC loss was much more complex. For example, for all soil types/treatments except 'unamended maize', net DOC actually increased by 4 – 35% following an initial loss of between 12 – 35% initial DOC within the first 15 days (Figure 26). Overall, however, the proportion of net DOC loss between days 0 and 15 exceeded that lost between days 15 and 70, consistent with the pattern of loss of DOC to $CO_2$.

## 3.4.3    Protein, Carbohydrate and Amino Acid Fractions – Relative Changes in Composition

The composition of the DOC prior to incubation (day 0) varied according to soil type/treatment with respect to soluble protein and carbohydrate carbon (Table 6). No amino acid carbon was detected for any soil type/treatment initially or at any time during the 70 day incubation period. After 70 days of incubation there was a net increase in carbohydrate C for every soil type/treatment and, in all but two cases, a net decrease in soluble protein C. In the glucose control no carbohydrate C was present at

the end of the 70 day period (Table 6). These net changes between day 0 and day 70, however are somewhat arbitrary, as constant fluctuations in the amounts of carbohydrate, protein and unidentified C took place throughout the incubation. For four of the soil type/treatments carbohydrate C concentrations increased from day 0 (0.1 – 1.9 mg C / l), reaching an initial peak of between 1.2 – 2.7 mg C / l on day 1 raised peat, unamended maize and maize/soy manured) or day 3 (blanket peat) (Figure 28). By day 7 these concentrations had declined more than 10 fold (0.0 – 0.1 mg C / l). Subsequently this initial peak was followed by a second increase in carbohydrate C of similar magnitude to the first (1.0 – 2.5 mg / l), evident by day 15, followed by a second decline to concentrations ≤ one quarter of the peak value by day 42. However, once

**Table 6** Mean values (± 1 S.E.) for carbohydrate C and protein C as percentage of initial DOC (n = 3).

| Soil Type / Treatment | Day 0 | | Day 70 | |
|---|---|---|---|---|
| | Carbohydrate C (% initial DOC) | Protein C (% initial DOC) | Carbohydrate C (% initial DOC) | Protein C (% initial DOC) |
| Raised Peat | 1.7 ±1.0 | 61.3 ±10.1 | 14.2 ±2.5 | 66.4 ±1.0 |
| Blanket Peat | 1.1 ±1.4 | 35.8 ±9.7 | 13.1 ±1.2 | 5.9 ±3.0 |
| Maize (unamended) | 14.6 ±3.5 | -1.7 ±1.5 | 23.0 ±3.5 | 20.7 ±3.9 |
| Maize/Soy (manured) | 2.4 ±1.0 | 73.9 ±12.9 | 14.5 ±3.0 | 53.2 ±7.3 |
| Maize (manured) | 8.9 ±1.5 | 22.3 ±4.2 | 11.7 ±0.7 | 3.0 ±1.8 |
| Maize/Soy (N fertilised) | 5.7 ±2.3 | 11.4 ±2.8 | 17.6 ±0.6 | 9.4 ±8.1 |
| Glucose | 99.6 ±1.5 | 1.6 ±1.6 | 0.0 ±0.0 | 1.02 ±0.9 |

again carbohydrate C concentrations began to recover again reaching concentrations similar to, or greater than previous peak values (1.1 - 2.2 mg C $l^{-1}$) by day 70. Carbohydrate C concentrations in the maize/soy N-fertilised and maize manured cultures showed different patterns both to the above and each other. Carbohydrate C concentration in the maize/soy N-fertilised cultures peaked on day 3 (1.9 mg C $l^{-1}$) but no second peak occurred on day 15. Instead concentrations continuously declined to 0.0mg C $l^{-1}$ by day 42, before increasing to 1.6 mg $l^{-1}$ by day 70. For the maize-manured culture the fluctuations in carbohydrate C concentrations were of the same order as observed for all the other soil types/treatments, however like the previous example, just one peak in levels occurred within the first 15 days (day 7 – 1.6 mg C $l^{-1}$) but atypically followed a significant decline of 1.2 mg C $l^{-1}$ in the first day of the incubation. In the glucose only culture, the relatively high levels of carbohydrate (19.4 mg C $l^{-1}$) had declined to 0.5 mg C $l^{-1}$ by day 3 and 0.07 mg C $l^{-1}$ by day 7. From this point

concentrations of carbohydrate C remained relatively very low but showed a rise to 0.2 mg C / l by day 42, before returning to 0.0 mg C / l by day 70.

The magnitude and pattern of change in protein C concentrations was dependent on soil type/treatment. For all types/treatments, except maize/soy manured, a peak in concentration of between 1.7 – 11.3 mg C / l occurred in the first three days. However, in the blanket peat and maize-manured cultures this peak followed an initial decline of (0.5 and 1.8 mg C / l respectively) during the first day of the incubation. After the first 3 days each type/treatment generally responded differently to the incubation conditions (Figure 28). However, in all cases, except for maize manured, protein C concentrations tended towards a minimum on day 42 before increasing again in the latter stages of the incubation. The concentration of protein C in the maize–manured cultures, having reached a second peak on day 15, continuously declined throughout the remainder of the incubation period. In the glucose only cultures protein C concentrations rose from 0 mg C / l on day 0 to 2.3 mg C / l by day 3, but then declined back to 0.4 mg C / l by day 7. A second rise and fall of the same magnitude was observed between days 7 and 42, with concentrations dropping to just 0.2 mg C / l by day 42.

Changes in the concentrations of the remainder of the DOC levels ('unidentified C') also fluctuated throughout the incubation period. For all the cultures except for maize/soy N-fertilised and maize/soy manured, there was a peak in concentrations on day 7 or 15 (2.7 – 13.7 mg C / l) following an initial decline from initial concentrations over the first 1-3 days. Concentrations then either remained relatively steady (blanket peat and maize manured) or reached a second peak on day 42 before declining in the latter stages of the incubation (raised peat and maize unamended). For maize/soy

manured, peaks in concentration did occur on day 7 and 42 but the first was not preceded by an initial decline as for the aforementioned. For maize/soy N-fertilised no early peak was observed but instead a continuous decline between days 0 and 15, before concentrations rose to peak on day 42.

The relative contribution of each of the protein, carbohydrate and unidentified C fractions to total DOC fluctuates throughout the incubation period for all of the soil types/treatments (Figure 27). For instance protein C dominates the maize/soy bean N-fertilised DOC composition on day 15, but only contributes a relatively minor amount by day 70. Generally, protein C or unidentified C dominate, with carbohydrate C making a relatively minor contribution.
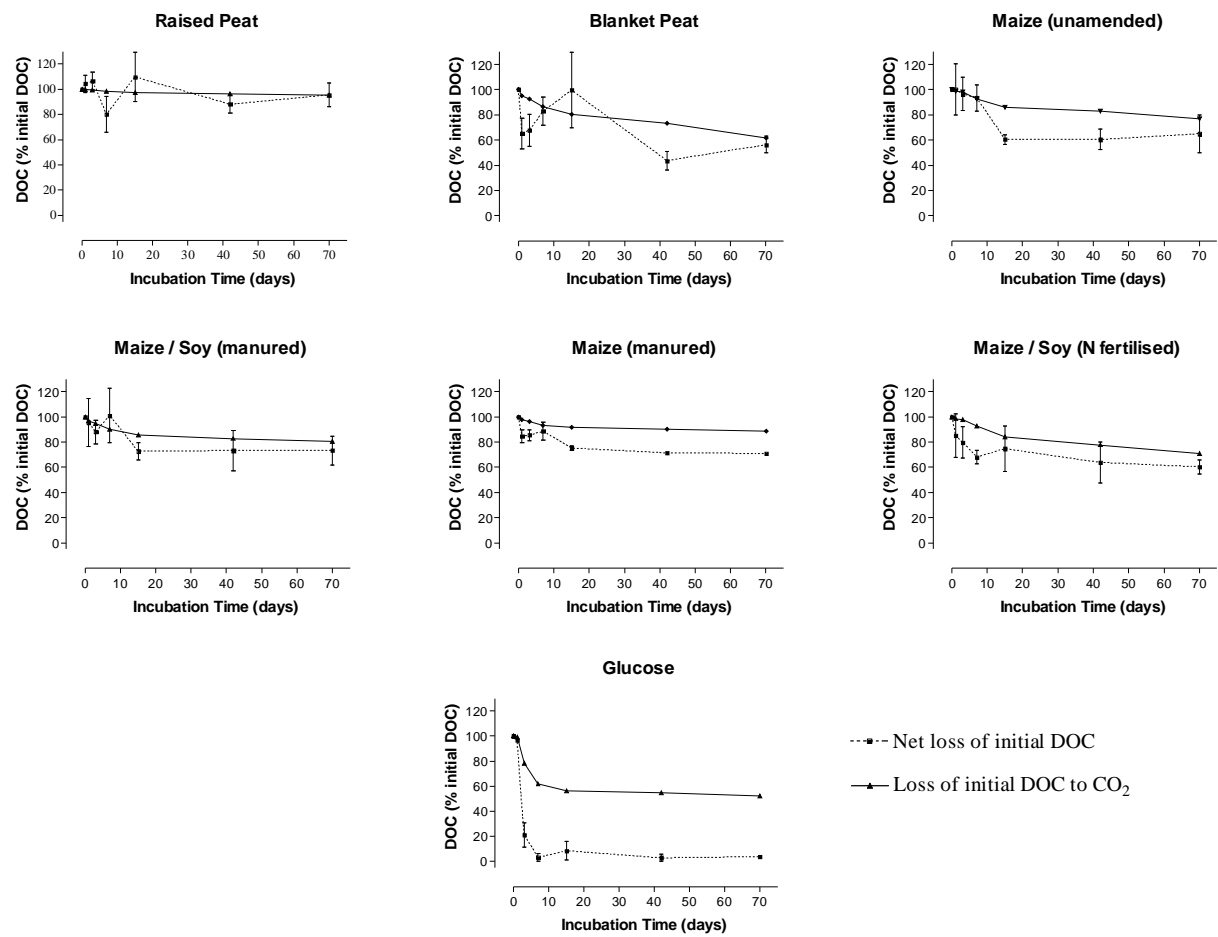
**Figure 26** Mean net loss of initial DOC from solution (% initial DOC) and loss of initial DOC due to measured $CO_2$ production (% initial DOC) (n = 3).
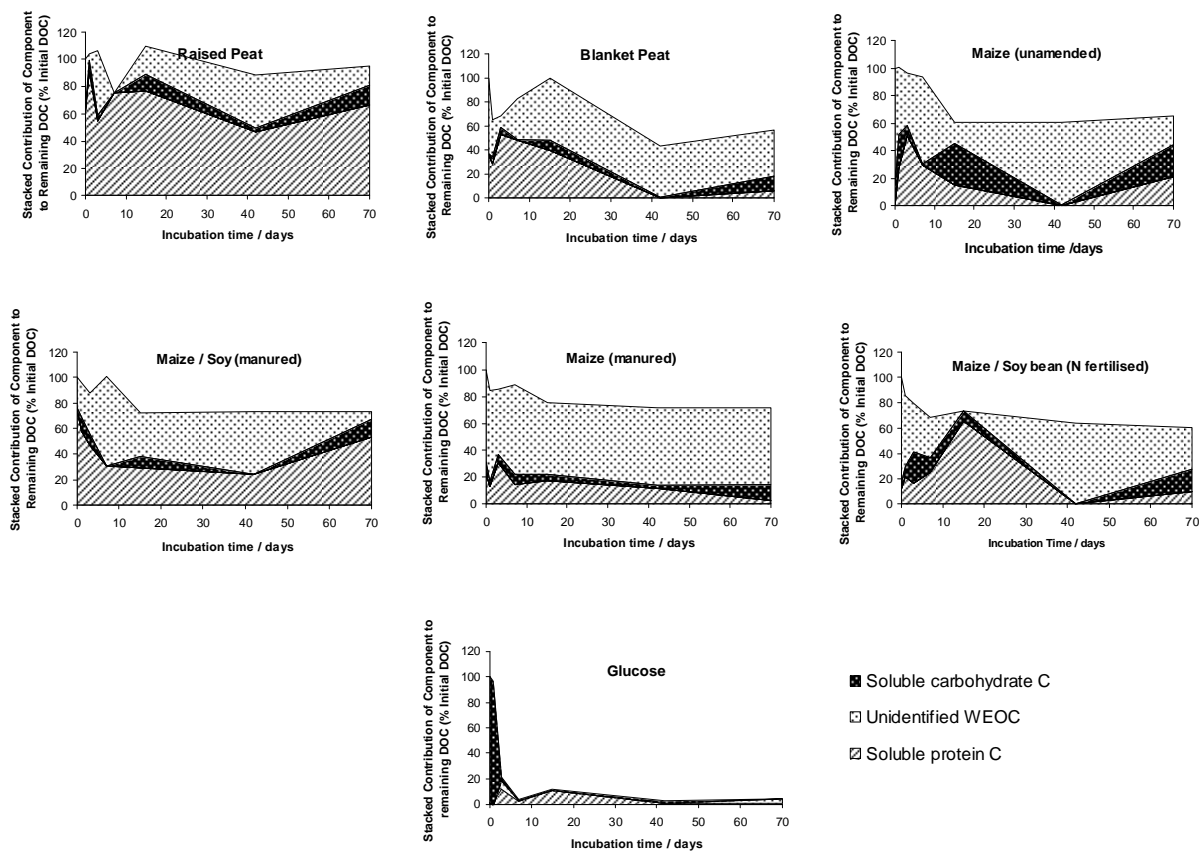
**Figure 27.** Mean stacked contribution of soluble carbohydrate carbon, soluble protein carbon and unidentified soluble carbon fractions (% initial DOC) to total DOC throughout the 70 day incubation period (n = 3). No amino acid carbon was detected.
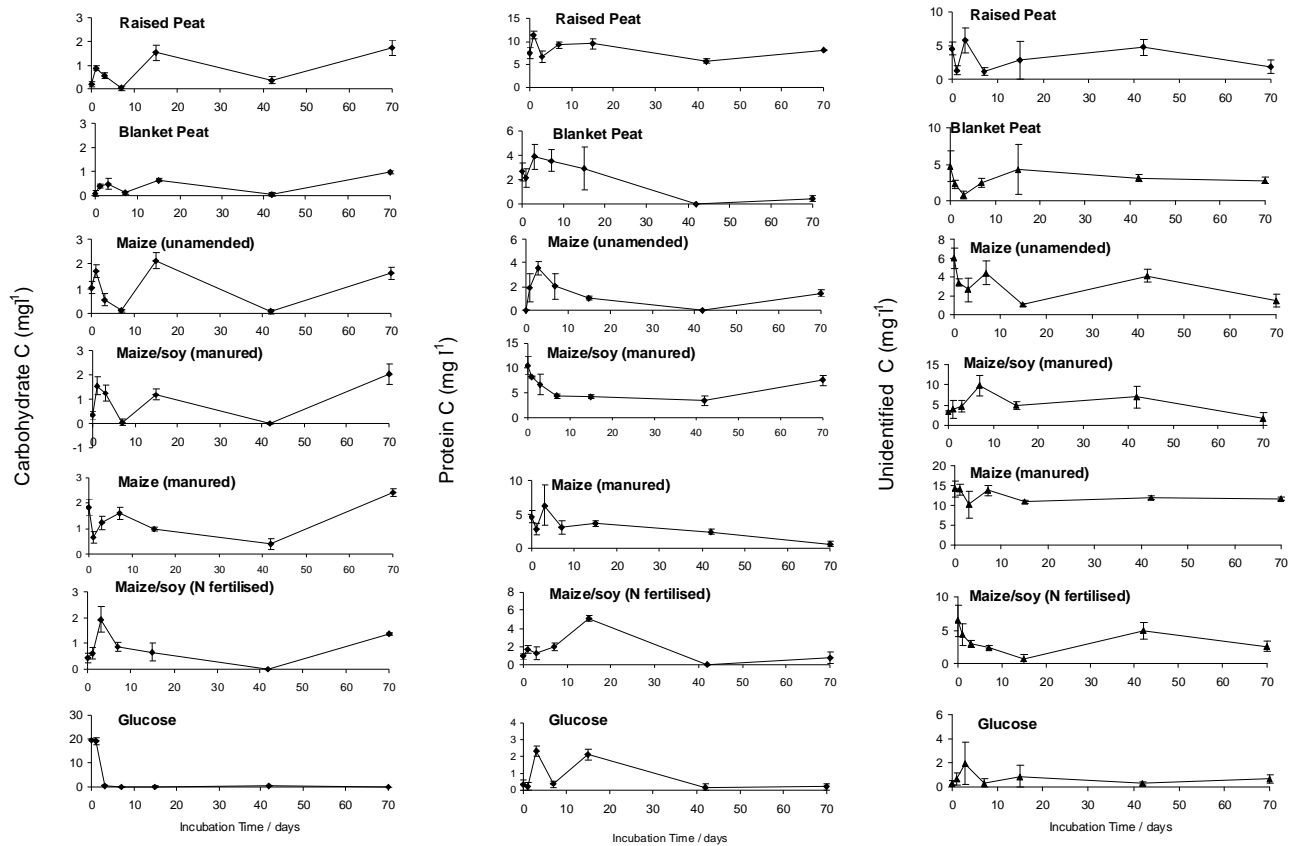
**Figure 28** Mean concentration (mg/l) of soluble carbohydrate C soluble protein C and unidentified soluble C for each soil type/treatment throughout the 70 day incubation period (n = 3). No amino acid carbon was detected. Error bars represent ± 1 S.E. due to spatial variation.

### 3.4.4   Microbial Population Dynamics

An increase in the number of microbial cells in the cultures containing DOC relative to the cultures containing inoculum only was clearly evident from day 3 (Figure 29). The magnitude of the microbial response and the appearance of the microbes were dependent on soil type/treatment (Figure 30). For all soil types/treatments there was a relatively rapid growth and subsequent decline in the size of the microbial biomass within the first 15 days (Figure 29). During this time numbers increased to 1 - 2 $x10^8$ bacterial / mg initial DOC, relative to day 0, for the blanket peat, maize/soy manured, maize manured and glucose treatments, and by 1 - 2 $x10^7$ bacterial / mg initial DOC for the raised peat and unamended maize.  By day 15 these numbers had fallen to 25 - 94% of their peak value Over the remaining days of the incubation period (days 15 - 70) numbers showed little change or slowly declined. A similar second peak in the microbial number data may have matched the second peak in microbial assimilation of DOC observed (see below) but was masked by lack of a direct count between days 15 and 70.

Calculations of net loss of DOC – loss of DOC to $CO_2$ to indicate net microbial assimilation/release of DOC, showed two periods of microbial assimilation (Figure 29). The first was a relatively rapid assimilation occurring within the first 7 days (6 – 30% initial DOC for soil samples; 59% for glucose), the second occurred by day 15 or later (6 – 30% for soil samples; 52% for glucose). The exception to this was the unamended maize DOC cultures where no net assimilation took place between days 0 and 7, however 23% initial DOC had been assimilated by day 15.

The magnitude of the response, in terms of both proliferation of microbial numbers and DOC assimilation, in the blanket peat DOC cultures was second only to the glucose only cultures.
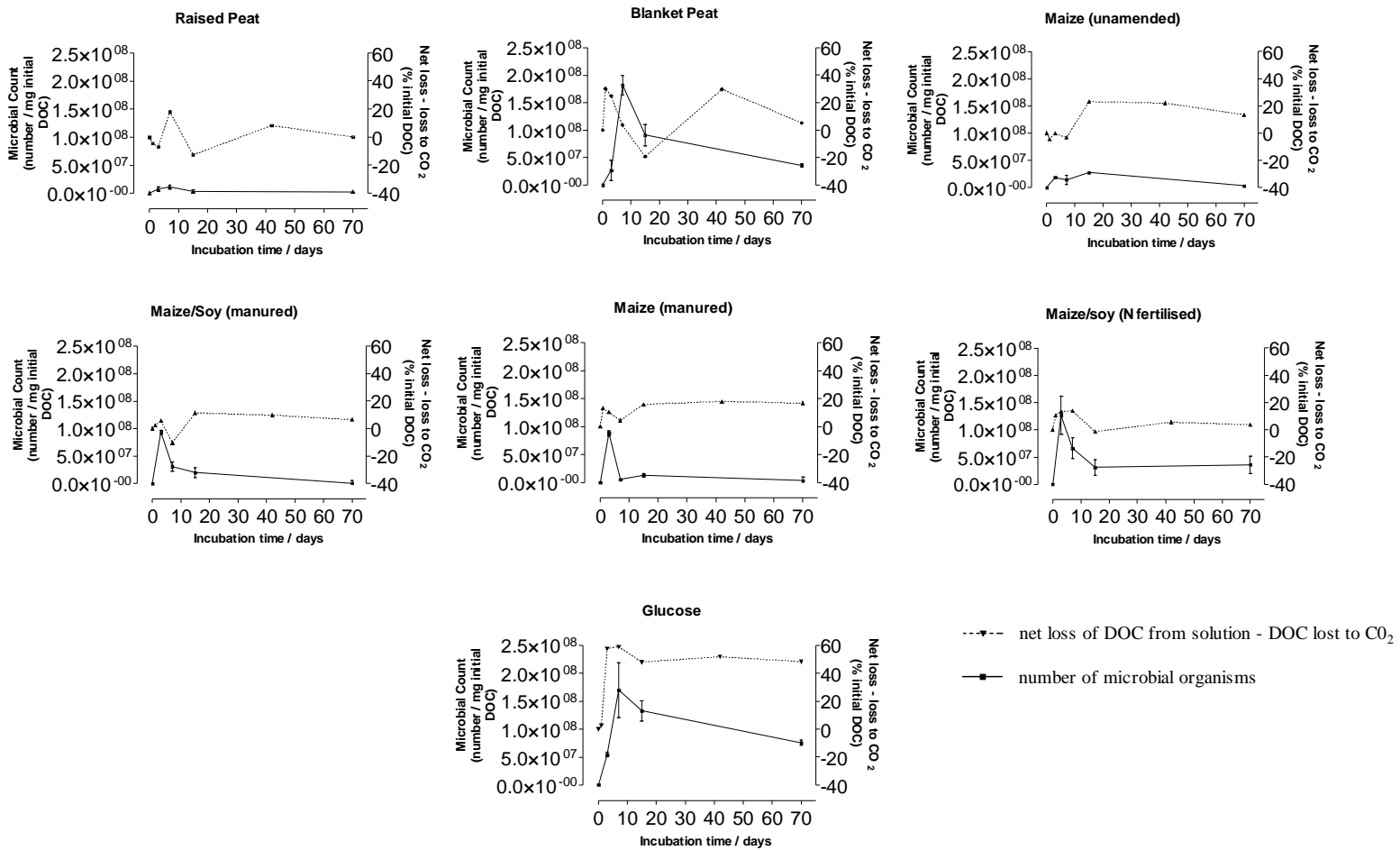
**Figure 29** Observed changes in microbial population size (mean number / mg initial DOC; n = 3) in response to the presence of DOC substrate and predicted changes in biomass carbon (mean % initial DOC; n = 3) based on calculations of net loss of DOC – DOC lost as $CO_2$.

Raised peat

Blanket peat

Maize (unamended)

Maize/soy (manured)

Maize (manured)

Maize/soy (N-fert)

Glucose

Blank (inoculum only)
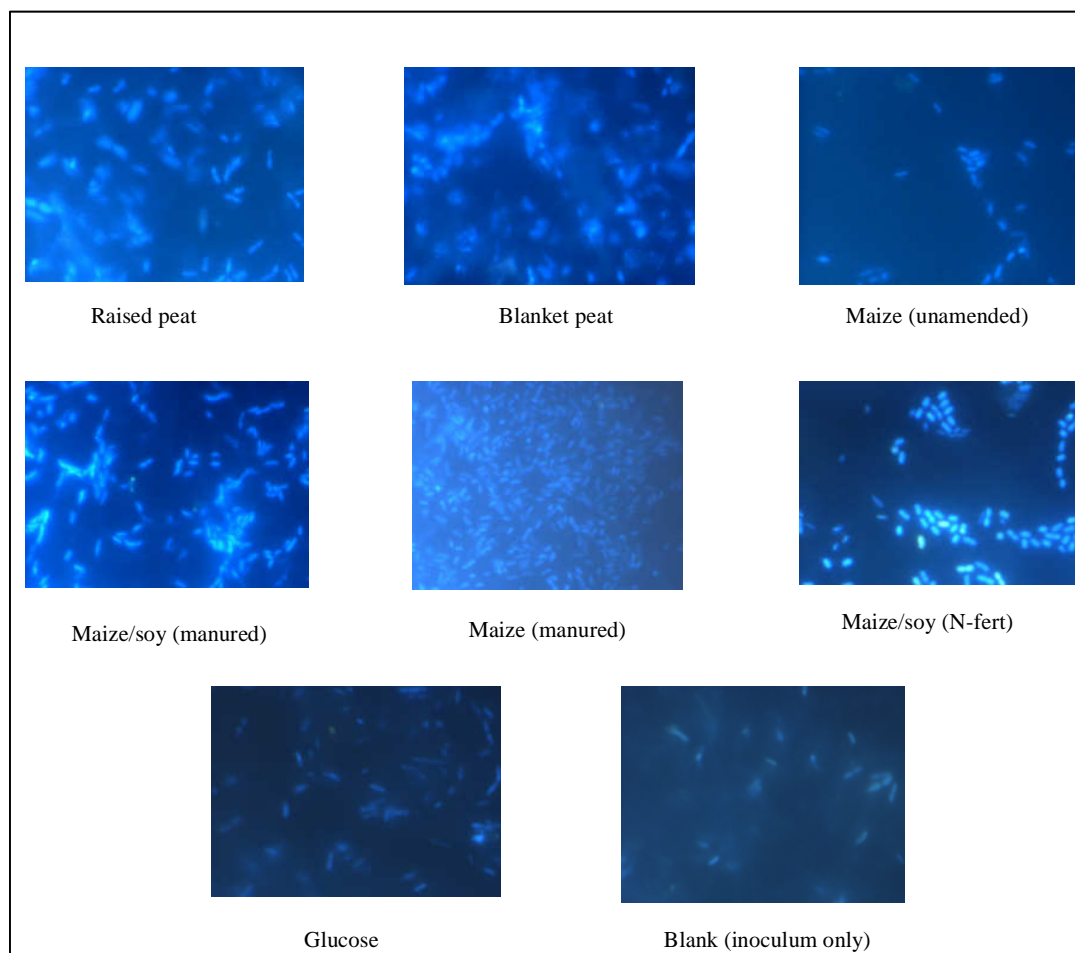
**Figure 30** Appearance of microbial populations following filtration on day 3 (DAPI stained, X 1000 magnification).

## 3.5     Discussion

Measuring biodegradation by measuring $CO_2$ production showed the DOC, for all soil types/treatments, to be comprised of two kinetically distinct pools and its decomposition described by a double exponential decay model. The rate constants for the biodegradation of both the labile and stable pools of the agricultural soils were of the same order as that found by Kalbitz et al. (2003a) using the A horizons of three different plots of a long-term agricultural field experiment (unfertilised; mineral fertilisation; and, manured (30 t ha$^{-1}$). The percentage of soluble carbon mineralised, during the 70 day incubation period for the agricultural soils (11.4 – 28.8%) is also similar to the range (17 – 32%) found by Kalbitz et al (2003a) for a 90 day incubation period carried out at the same temperature (20$^{o}$C). The only observed statistically significant difference in the extent of mineralization, between the two peat soils (blanket peat 38.5%, raised peat 4.7% of initial DOC), could be due to differences in vegetation cover and/or management practices. The blanket peat has a large amount of ground cover and relatively young trees growing on its surface releasing highly labile soluble carbon in the form of root exudates (Reid and Goss, 1983). This carbon may be accumulating within the peat due to reduced microbial activity (Küsel and Drake, 1999) and/or the inhibition of hydrolase enzymes (Kang and Freeman, 1999) by phenolic compounds, which build up in the absence of oxygen (Freeman *et al.*, 2001). This may also explain the relatively high mineralization constant for the labile pool of the blanket peat. The raised peat has fewer, much older trees and mainly bracken growing on its surface and within the past two hundred years has been drained. The combined effect of the older vegetation and the increasing aeration of the top layer, enabling the removal of phenolic compounds through increased phenol oxidase activity, may mean that highly labile root exudates produced by the ground cover are rapidly decomposed leaving only

the less labile to drain through and accumulate in the sampled layer of peat as soluble or potentially soluble organic matter. The extent of the mineralization of the dissolved glucose is typical of a glucose-induced respiratory response by soil microbes (Ladd *et al.*, 1992).

Measuring decomposition by measuring net loss of DOC from the culture mixture showed consistently lower concentrations of DOC than would be expected through loss to $CO_2$, and more complex dynamics than the mineralization dynamics with periods of increase as well as loss. This is in apparent contradiction with the results of Gregorich et al. (2003) who found that net loss of DOC of the same agricultural soils over a 42 day incubation period at $35^{\circ}C$ could be described by a double exponential decay model, with rate constants an order of magnitude higher for both the labile and stable pools. However, the results presented here reflect greater resolution resulting from a lower incubation temperature: sampling times in this study were the same as that used by Gregorich et al. (2003) however the incubation temperature was $15^{\circ}C$, effectively increasing the sampling frequency. Similarly, Gregorich et al found the extent of metabolisation of the mineral soils to be greater, 52 - 61 % despite a shorter incubation period (42 days), as compared to the 29 – 40 % observed here.

Discrepancies between the dynamics of decomposition were anticipated as only a portion of actual decomposition is accounted for when determining the decomposition rate by measuring $CO_2$ output (Paul and Clark, 1996). Some DOC will be used for biosynthesis within the microbial population i.e. the formation of new intracellular or extracellular material, therefore as the microbial population grows a net uptake of DOC would be expected. Likewise as the microbial biomass declines organic carbon would

be expected to be released as the cells lyse. Some of this released carbon will be soluble and that which is insoluble may itself undergo microbial decomposition to soluble material. Thus the differences between DOC mineralised and actual DOC lost from solution should give an indication of the microbial population dynamics, assuming formation of colloidal, particulate and precipitated C during the incubation period is negligible. Comparison of this data with the changes in microbial population size obtained from the direct counting method, showed this to be the case at least for the first 15 days of the incubation period where a rapid growth and decline in the microbial population was observed. Fontaine et al (Fontaine *et al.*, 2003) proposed that 'r-strategist' microorganisms, adapted to rapidly proliferate in response to an abundance of suitable substrate (Killham, 1994), decompose the most energetic compounds of fresh organic matter (e.g. green manure or straw) whilst 'K-strategists', dominate only in the latter stages of decomposition when the highly energetic compounds have been exhausted and only polymerised compounds remain. This theory could to some extent be applied to the pattern of microbial decomposition of DOC observed here: the r-strategists rapidly proliferate in response to the availability of the readily-decomposable labile pool present in the DOC, assimilating soluble organic carbon as they do so, and then, once this pool is exhausted, the population declines releasing soluble organic material. The predicted microbial dynamics based on 'net loss of DOC minus loss to $CO_2$' showed a second more gradual rise and decline supporting the theory that the K-strategist population subsequently, in response to reduced competition, grows slowly and begins to dominate the degradation of the more recalcitrant molecules that constitute the stable pool. However, insufficient direct counting data in the latter stages made this difficult to verify and it is possible that precipiation/dissolution processes taking place during the incubation may be confusing the picture. Although the patterns

of change observed for both changes in the microbial population size and changes in the predicted assimilation/release of DOC suggest both are a consequence of the two kinetically distinct pools of DOC, the relative times at which these changes take place is different for each soil type/treatment, making a direct link between the two difficult to confirm. Initial increases in DOC, observed between day 0 to 1 or 3 depending on soil type/treatment, may be the consequence of lysis of some of the original inoculum as the community adjust to the conditions in each specific culture.  The responsiveness of the microbial community to the composition of DOC is evident from the differences in the appearance of the microbial cells according to soil type evident from as early as day 3 (

Figure 30). Changes in appearance of microbes from any one culture were also evident as the incubation progressed (Figure 31). Although outside the scope of this study, it would be useful to determine whether these observed differences actually represent structural and functional differences in the microbial communities.


Examination of the protein and carbohydrate fractions did not show rapid decomposition/assimilation in the first few days as may be expected of such 'labile' molecules but instead revealed a complex pattern of both degradation/assimilation and release throughout the incubation period. Any link between the dynamics of each of these fractions and the microbial population was impossible to ascertain as the relationship between the two varied according to soil type/treatment. This variation however does not preclude the existence of a direct relationship as the differing substrate material and chemical conditions in each culture would be expected to illicit a different biochemical response by the microbial community. The observed net increase in the carbohydrate component in this study, except in the case of the glucose control,
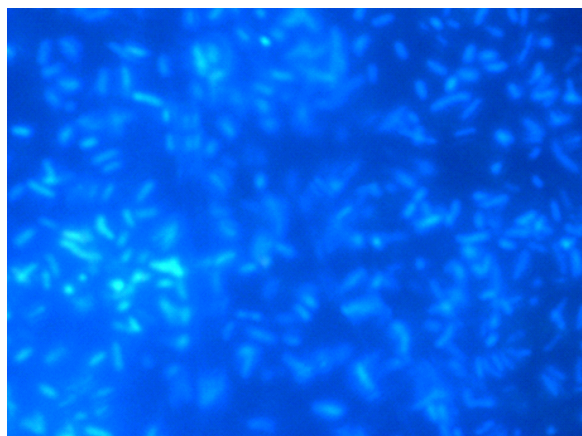
Day 3
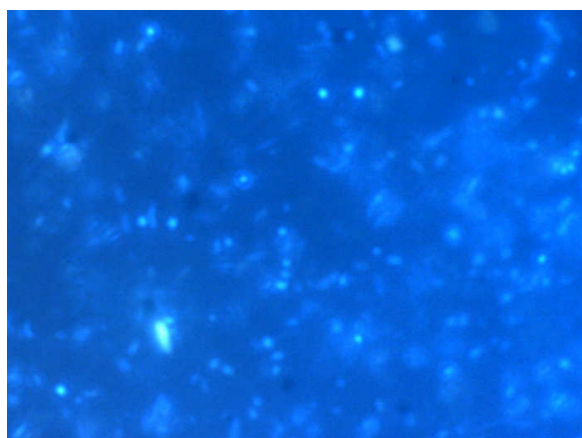


Day 70

**Figure 31** Examples of appearance of microbial populations on days 3 and 70 in maize/soy N-fertilised DOC culture (DAPI stained, X 1000 magnification).

was also observed by Kalbitz et al (2003b) using Oi and Oa spruce forest DOC samples in a 90 day incubation. This net production of carbohydrate could be due to synthesis of carbohydrate molecules by the microbial population, using internal carbon sources which is then released into solution as relatively recalcitrant products, or decomposition products, of extracellular secretions. Observed changes in viscosity of the culture solutions and the cloudy appearance of some samples when viewed under the microscope (Figure 32) suggest either cellular material undergoing lysis or the presence

of biofilm material. Biofilms are complex communities of microorganisms attached to surfaces or associated with interfaces (Davey and O'Toole, 2000) and are thought to be the normal environment for most microbial cells in many natural and artificial habitats (Christensen, 1989). The main 'cement' for all these cells and products is the mixture of polysaccharides secreted by cells established within the biofilm (Sutherland, 2001). Many of these polysaccharides are soluble and, because of their large molecular mass yield highly viscous aqueous solutions (Sutherland, 2001). However, the absence of a comparable production of carbohydrate by the glucose only culture, despite sustaining a relatively high biomass, suggests that the majority of carbohydrates released by the microbial population are not inherently stable but are stabilised by some external factor present in the soil DOC cultures only, perhaps through complexation with aromatic decomposition products of lignin or tannins. Similarly, this could explain the observed increase in protein concentration between days 42 to 70 for all the soil DOC cultures, except maize manured, at the same time that the pool of DOC is believed to be kinetically relatively stable: protein molecules released by microbes in the latter stages of the incubation may be binding to accumulating lignin degradation products, reducing their biodegradability. No amino acid C was detected during the incubation period suggesting that its turnover was so rapid that the concentration remained below the levels of detection (0.01 mg C $l^{-1}$).
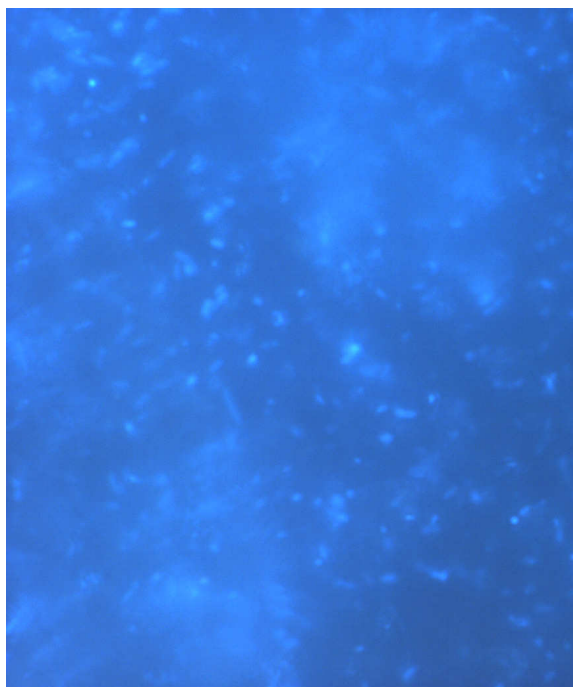
**Figure 32** Image showing 'cloudy' appearance of typical of many of the samples following filtration (maize manured sample day 70, stained with DAPI , X 1000 magnification).

All aspects of the decomposition process (mineralization kinetics, mass loss of DOC dynamics; fluctuations in carbohydrates and proteins; and, changes in microbial population size) were similar for both the peatland and mineral agricultural soils. This suggests that the microbial response to the native DOC is similar in the two ecosystems, irrespective of the differences in timing and chemical composition of organic inputs, local hydrological and temperature conditions and geochemical properties of the soil.

## 3.6    Conclusions

- the mineralisation dynamics previously observed for agricultural and forest ecosystems, described by a double exponential decay model, also hold true for peatlands

- the pattern of mass loss of DOC from solution during decomposition is more complex than the double exponential decay model observed by previous studies [Qualls and Haines, 199][Gregorich et al., 200][Kalbitz et al, 2003a)

- the changes in the size of the microbial community during the decomposition process show that during the initial stages of decomposition (days 0 to 15) one sub-group of the community proliferates in response to the presence of the labile pool of DOC and subsequently declines as the labile pool declines.

- the protein and carbohydrate fractions of DOC do not show rapid decomposition/assimilation as may be expected of such 'labile' molecules but instead a more complex pattern of both degradation/assimilation and release throughout the a 70 day incubation period. No amino acid C was detected at any stage during the incubation process.

- All aspects of the decomposition process (mineralization kinetics, mass loss of DOC dynamics; fluctuations in carbohydrates amino acids and proteins; and, changes in

microbial population size) are similar in both peatland (raised and blanket peat) and mineral agricultural soils, irrespective of crop and fertilisation management strategies.

# Chapter 4: Effects of Repeated Applications of Trace Amounts of Litter DOM on Activity of Peat Soil Microbial Biomass

## 4.1 Introduction

The results of many field studies have shown that DOC concentrations show seasonal variation (Cronan and Aiken, 1985; McDowell and Likens, 1988; Tipping *et al.*, 1999). These variations have been attributed to various environmental factors, including temperature, precipitation intensity and freeze-thaw cycles (reviewed by Kalbitz et al., 2000). No studies have, however, looked at the effect of rainfall frequency. Rainfall contains both natural DOM, initially released as dust from the canopy and subsequently washed out in the rain (Guggenberger and Zech, 1994), and anthropogenic DOM such as herbicides, pesticides and petroleum products. In temperate forest ecosystems, as rainfall passes through the canopy, DOM is mobilised by the washing of dry-deposited organic carbon, leaf leachate and the metabolic products of microbial activity (Zech and Guggenberger, 1996). As the rainwater passes through undecomposed partially decomposed litter horizons more DOM is solubilised. Thus each time it rains 'fresh' DOC is delivered to the underlying soil. Does the frequency at which this fresh soluble organic matter is made available to the soil microbial biomass affect their activity and consequently the rate of mineralization of soil organic carbon?

O'Dowd and Hopkins (1998) observed that repeated additions (every 21 days) of D-glucose and L- and D-amino acids (2 mg g$^{-1}$ soil) to forest soil significantly increased the subsequent rate of $CO_2$ production. De Nobili et al. (2001) observed that even trace amounts (µg g$^{-1}$ soil) of solutions such as glucose and root extract cause a rapid, but relatively short-lived increase in the metabolic activity of soil microbial biomass which could be reactivated with further additions. De Nobili et al. (2001) hypothesised that this response of the microbial biomass is triggered by trace concentrations of low molecular weight compounds derived from a substrate, in anticipation of a more substantial 'food event' to come. Thus it is possible that in periods of frequent rainfall, frequent doses of 'trigger molecules', derived from throughfall and/or litter leachate, are delivered to the bulk of the soil microbial biomass increasing or maintaining the microbial biomass at a high level of activity and consequently increasing or maintaining a high rate of mineralization of available substrates.

## 4.2.    Hypothesis

The intervals between periods of rainfall will influence the extent of soil carbon mineralization through the action of trace amounts of litter DOM on the activity of the endogenous biomass. Frequent rainfall will lead to sustained 'alertness' of soil biomass because of the frequent supply of trace organic nutrients in the litter DOM acting as trigger molecules.  Frequent rainfall distributing fresh doses of trigger molecules should maintain the biomass in active state. Less frequent rainfall will reduce activity and therefore the extent of C mineralization.

## 4.3.    Materials and Methods

### 4.3.1  Soil Description

Peat soil was taken from the Of horizon of a drained, raised peat bog (NGR: NS747961) lying in an area of the Carse of Forth, known as Ochtertyre Moss Wood in Central Scotland (see section 2.3.1 for detailed description of site and soil characteristics). The samples were taken in June 2005 from a flat area under a silver birch canopy. Samples were sorted by hand to remove plant and animal material >2 mm in size. Prior to use the soil was bought to 50 % water-holding capacity (WHC) and equilibrated at 20 $^{\circ}$C for 7 days.

### 4.3.2  DOM Preparation

The DOM solution was prepared using litter (L layer) material removed from the same site as the peat soil samples. Sample material (200 g) was secured within a nylon netting bag suspended in 800 ml of distilled water, shaken for 2 hours and left overnight at 4$^{\circ}$C. The water extract was filtered through 0.22 μm cellulose nitrate membrane filter (Whatman) before use as the DOM solution. The organic carbon content of the DOM solution was determined using a Shimadzu TOC-V$_{CSN}$$^{®}$ Total Organic Carbon Analyser (see Appendix A for technical details) and its chemical composition adjusted to ensure a final C:N:P:S:K ratio $\geq$ 5:1:1:1:1 through the addition of $(NH_4)_2SO_4$ and $KH_2PO_4$. Control solutions contained identical amounts of $(NH_4)_2SO_4$ and $KH_2PO_4$ only.

### 4.3.3  Soil Incubation – Investigation 1

The respiration rate of the soil was determined using a multi-chamber respirometer (Respicond IV, Nordgren Innovations, Umea, Sweden). This allows near continuous monitoring of $CO_2$ production in the headspace above the soil sample by measuring decreases in conductance as $CO_2$ is absorbed in a KOH trap (Nordgren, 1988). 50 g moist soil (7 g dry soil equivalent) amended with 2 mg cellulose C/ g dry soil (equivalent to 4.448 mg cellulose/ g dry soil) in 0.5 g talc as an inert carrier (after O'Dowd and Hopkins,1998; Meli et al., 2002) was added to each chamber, and incubated at $20^oC$ for 24 days. 3 ml DOM solution containing 24.77 µg organic C $ml^{-1}$ were added to the experimental chambers (each 3 ml equivalent to an addition of 10.6 µg C $g^{-1}$ dry soil) and 3 ml nutrient only solution were added to the control chambers throughout the incubation period at varying frequencies: once at time 0 and at time 0 and every 3, 6 or 9 days thereafter. Such additions increased the moisture content of the soil during the incubation period from 50 to 54, 78, 64 and 61 % of its WHC respectively. No additions were made to a final set of chambers. At the time additions were made all chambers were exposed to the atmosphere for 20 seconds. $CO_2$ production was recorded every 6 hours. Each experimental set up was replicated three times.

### 4.3.4  Soil Incubation – Investigation 2

A lack of 3 day interval data and the apparent, but not statistically significant, divergence of the rates of respiration between DOM + nutrients and nutrients only additions for all the frequencies of application by day 24 for investigation 1 (see section 4.4) necessitated a second investigation. A second set of chambers were set up as before

(50 g moist soil, 7 g dry soil equivalent, amended with 2 mg cellulose C $g^{-1}$ dry soil in 0.5 g talc as an inert carrier), incubated at $20^{o}C$ and 3 ml additions of DOM solution containing 32.6 µg organic C ml (each 3 ml equivalent to an addition of 14.0 µg C $g^{-1}$ dry soil), and 3 ml nutrients only' were made every 3.5, 7 and 10.5 days. As a consequence of each addition soil moisture content increased from 50 to 85, 68 and 64 % of its WHC respectively during the incubation period. To determine the contribution of the inorganic nutrient content of the DOM extractant itself on respiration an additional set of chambers were included and 3 ml 'DOM (32.6 µg organic C $ml^{-1}$) only' additions were applied at the same frequencies as for the other amendments. Cumulative $CO_2$ was quantified every 3.5 days for 35 days by measuring the $CO_2$ concentration in the headspace of each incubation chamber using gas chromatography (Varian Aerograph 90-P). A hypodermic needle was used to pierce a resealing rubber bung in the lid of each chamber, remove 1 ml of air from the headspace and inject it in to the chromatograph.


### 4.3.5  Data Analysis

Raw data obtained using the respirometer was manipulated to compensate for the $CO_2$ absorbed from the atmosphere during removal of the chamber lids, by deducting the observed increase at that time from the subsequent data points. The 2-sample t-test was used to test for significant differences ($p \leq 0.05$) between sets of data (population from which data set collected was assumed to be normally distributed).

$CO_2$ carbon data collected using the gas chromatogram was cumulative and therefore differences between treatments were analysed using a repeated measures ANOVA technique.

## 4.4. Results – Investigation 1

The frequency of application (once on day 0, every 9 and every 6 days) of trace amounts of 'DOM + nutrients' had no statistically significant effect on the degree of C mineralization of the peat soil over a 24 day period relative to nutrient only additions (p = 0.271, 0.656, and 0.271 respectively) (Figure 33, Figure 34 and Figure 35). Although the difference between 'DOM + nutrient' amendment and 'no amendment' was not significant, a continuing divergence in rate of $CO_2$ production was evident, with 'DOM + nutrient' amendment consistently exceeding that of nutrient only amendment. Data for 3 day interval applications was not available due to technical difficulties with the respirometer. Nutrient addition significantly increased the rate of mineralization relative to no amendment, however, frequency of nutrient application had no significant effect.
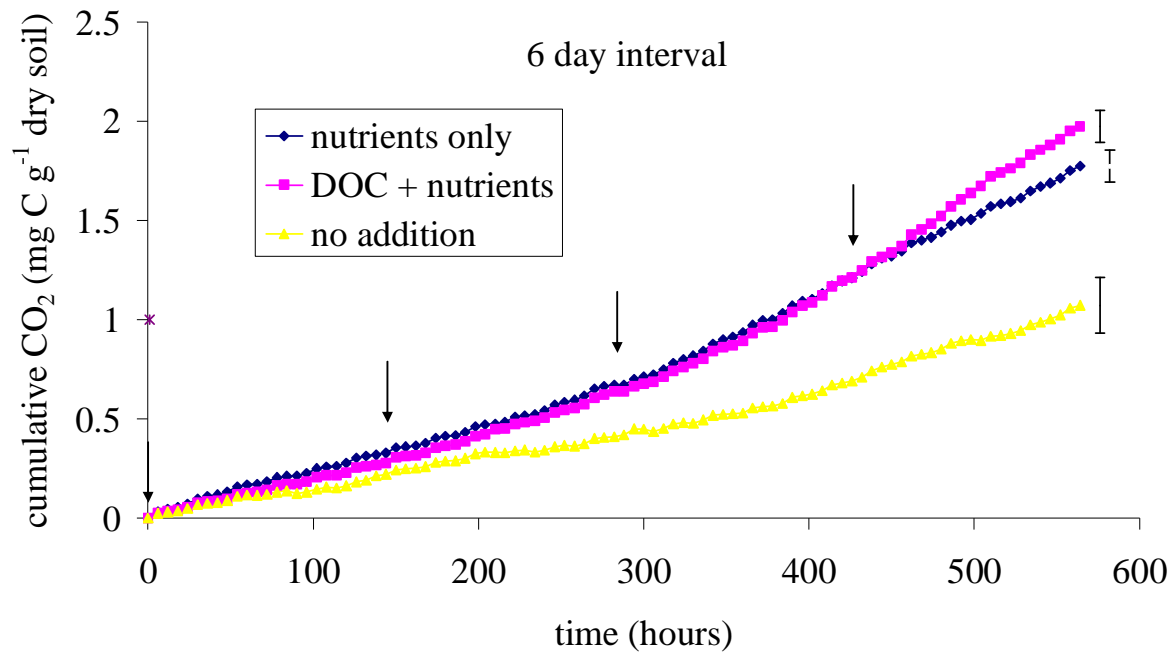
**Figure 33** The effects of repeated addition of trace amounts of leaf litter leachate DOM at 6 day intervals on C mineralization (cumulative $CO_2$ mg C $g^{-1}$ dry soil). Arrows indicate time of additions. Error bars represent $\pm$ 1 S.E. of the final mean (n = 3).
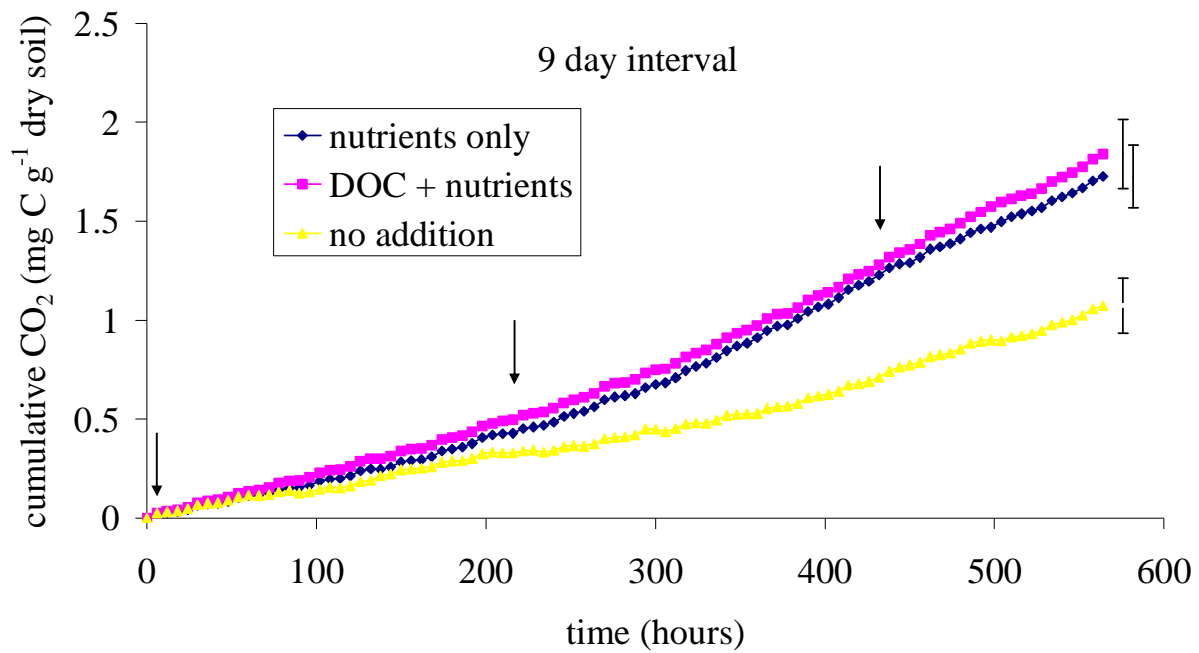
**Figure 34** The effects of repeated addition of trace amounts of leaf litter leachate DOM at 9 day intervals on C mineralization (cumulative $CO_2$ mg C $g^{-1}$ dry soil). Arrows indicate time of addition. Error bars represent $\pm$ 1 S.E. of the final mean (n = 3).
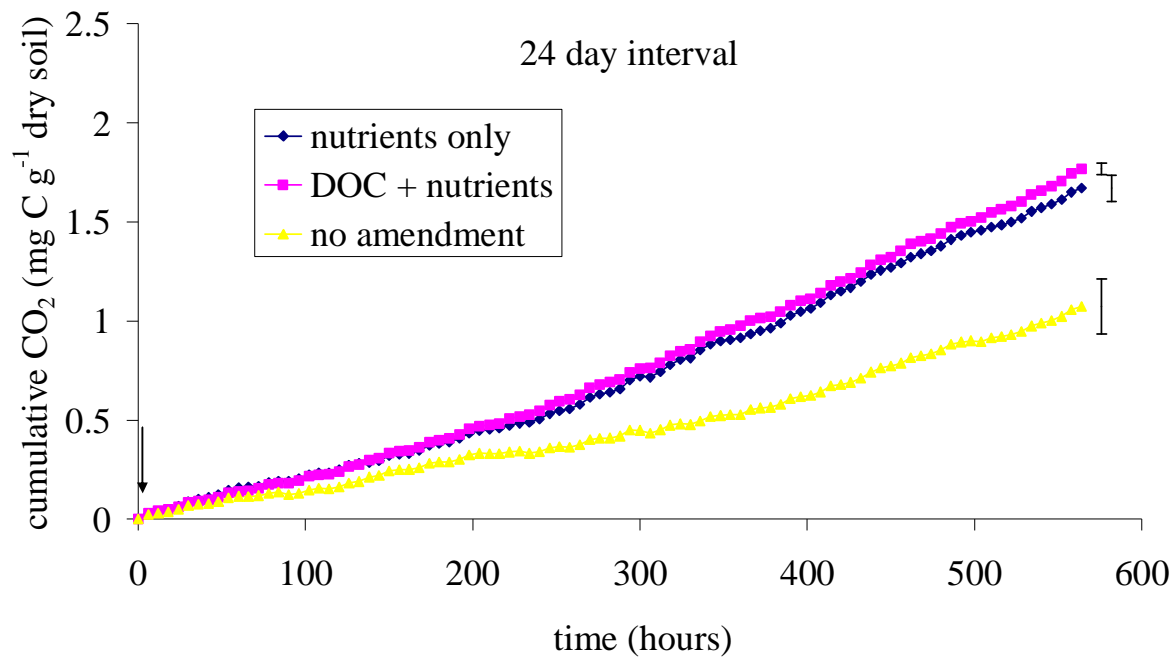
**Figure 35** The effects of repeated addition of trace amounts of leaf litter leachate DOM at 24 day intervals on C mineralization (cumulative $CO_2$ mg C g$^{-1}$ dry soil). Arrows indicate time of addition. Error bars represent $\pm$ 1 S.E. of the final mean (n = 3).

## 4.5.    Results – Investigation 2

After 35 days (840 h) of incubation, the application of trace amounts of 'DOM + nutrients' every 3.5 days had no statistically significant effect on the total C mineralised relative to 'nutrient only' additions, despite a total addition of 140 µg DOM carbon (Figure 36, 32 and Figure 38). Applications of 'DOM + nutrients' at the lower frequencies of 7 and 10.5 day intervals, however, did have a significant effect relative to 'nutrient only' additions (after 17 and 31 days of incubation), causing an additional 213 and 111 µg C $g^{-1}$ dry soil to be mineralised in total (Figure 39). These values exceeded the total DOC additions for these treatments of 70 and 56 µg C $g^{-1}$ dry soil by 3 and 2 fold respectively. For both 7 and 10.5 day applications, total C mineralised at the end of the incubation period in 'DOM + nutrient' treatments exceeded that of 'DOM only', but only in 10.5 day interval treatment did 'DOM only' significantly exceed 'no amendment'


A period of acceleration in the rate of C mineralised in the 'DOM + nutrient' relative to 'nutrient only' treatments was observed between 336-420 h for 7 day interval additions and, between 420-588 h (Figure 39) for 10 interval additions. The acceleration period was more sustained for the 10 day interval treatments. After this period of acceleration, the rate of 'extra' C mineralization declines to a relatively steady rate for both treatments. However, the rate of C mineralization for the remainder of the incubation period for the 10 day interval addition treatments exceeds that of 7.5 day interval treatments. No such acceleration was observed at the same time for 3.5 day interval applications.
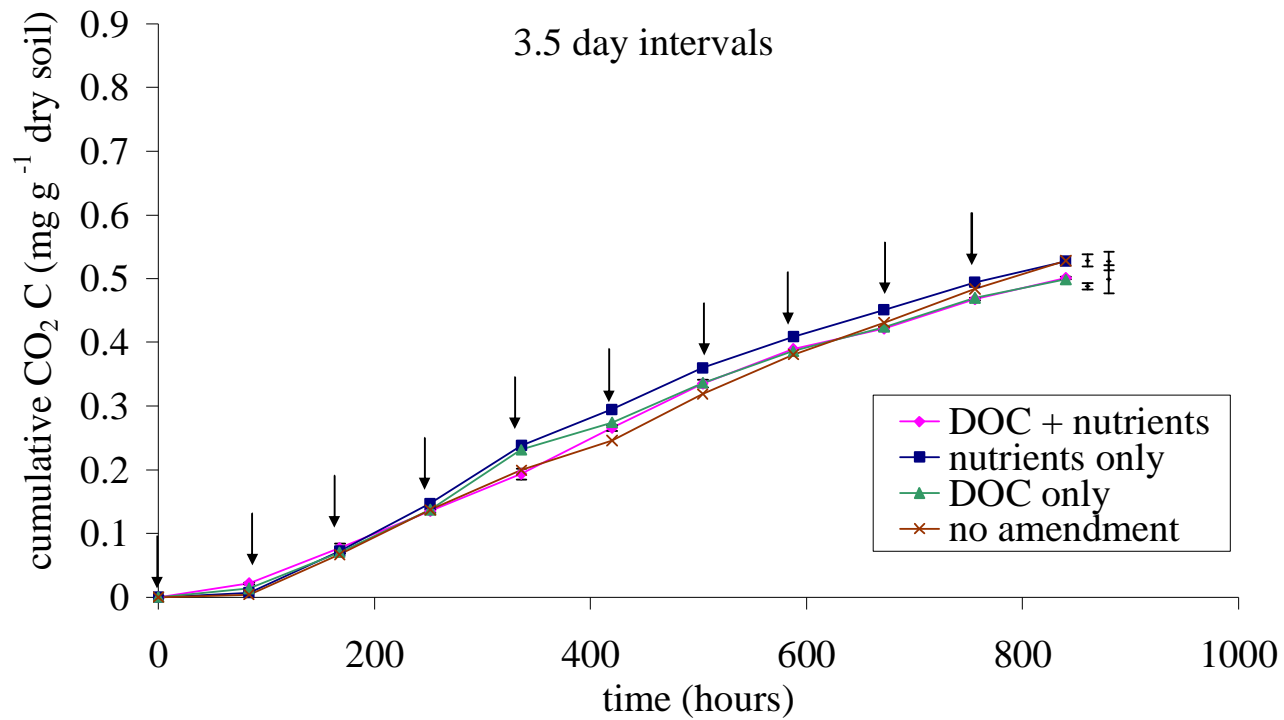
**Figure 36** The effects of repeated addition of trace amounts of leaf litter leachate DOM at different time intervals on C mineralization (cumulative $CO_2$ mg C $g^{-1}$ dry soil). Arrows indicate time of additions. Error bars represent $\pm$ 1 S.E. of the means (n = 3).
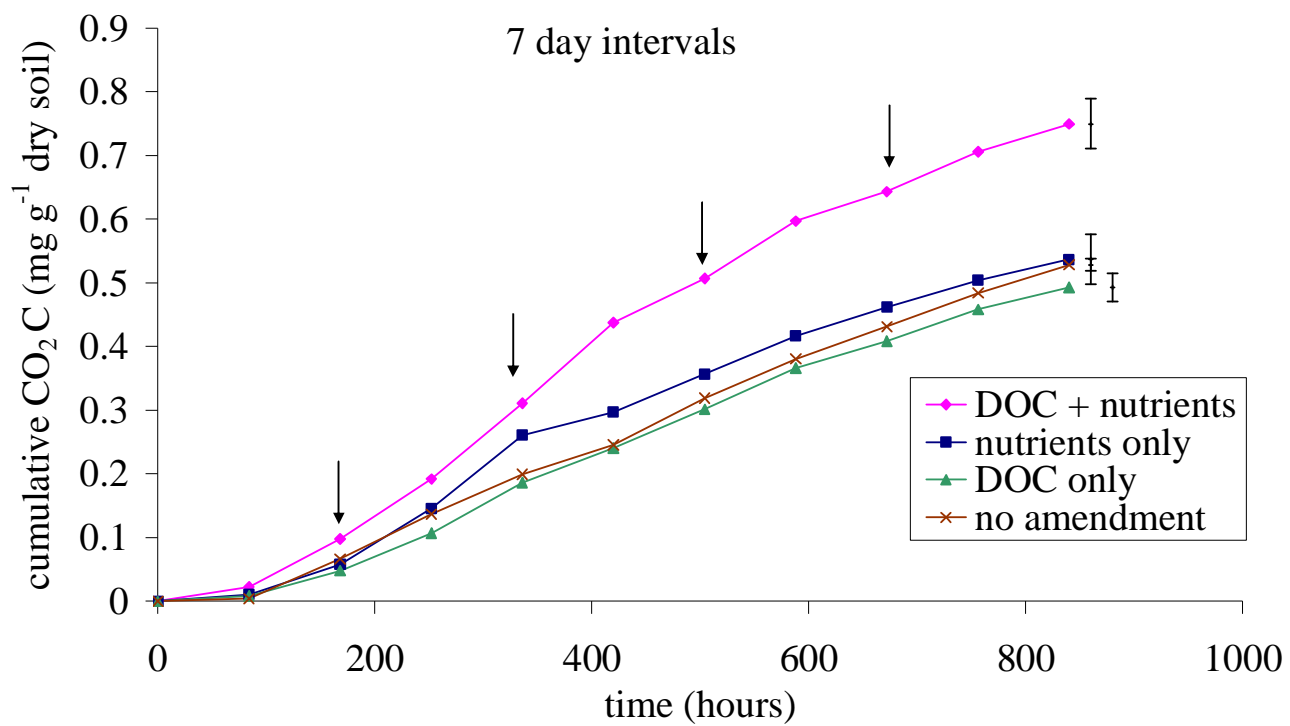
**Figure 37** The effects of repeated addition of trace amounts of leaf litter leachate DOM at different time intervals on C mineralization (cumulative $CO_2$ mg C $g^{-1}$ dry soil). Arrows indicate time of additions. Error bars represent $\pm$ 1 S.E. of the means (n = 3).
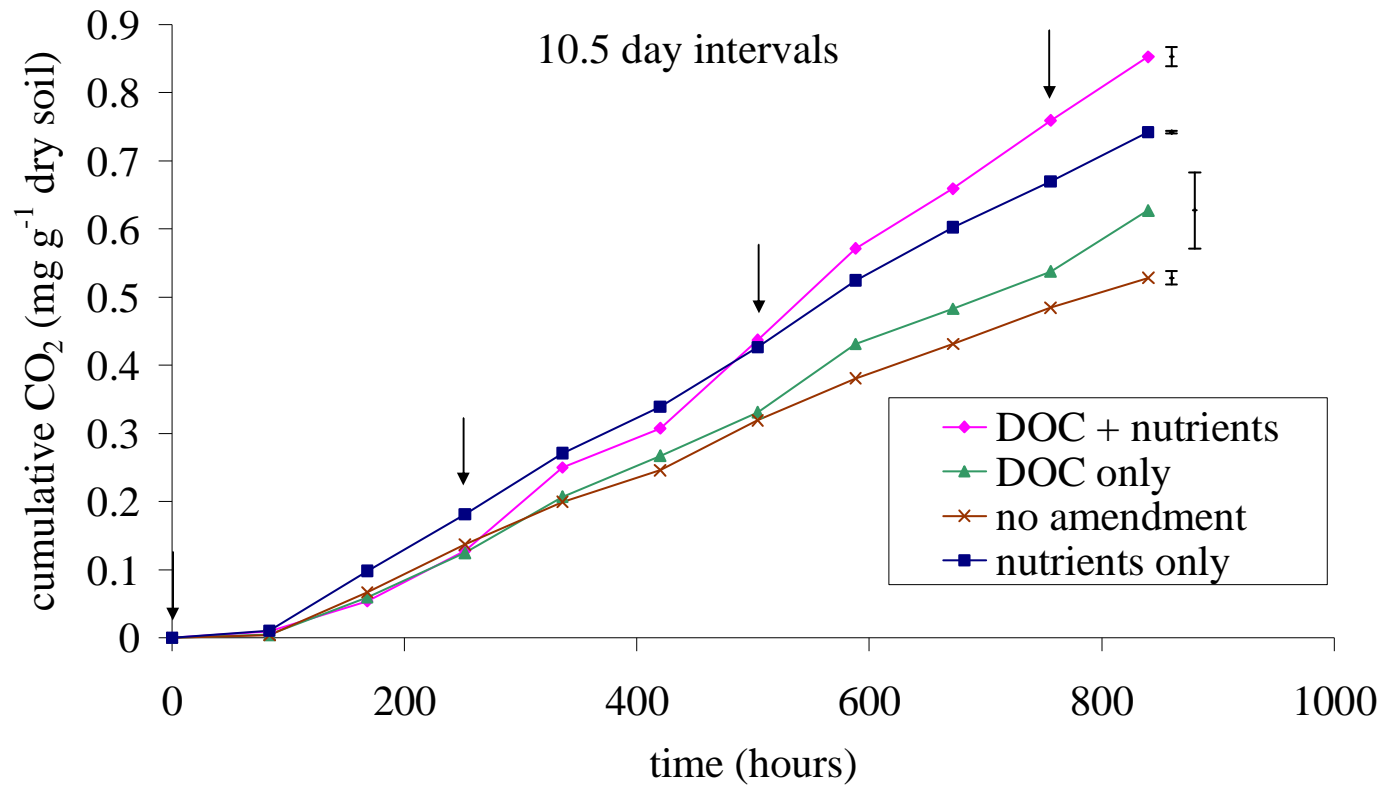
**Figure 38** The effects of repeated addition of trace amounts of leaf litter leachate DOM at different time intervals on C mineralization (cumulative $CO_2$ mg C $g^{-1}$ dry soil). Arrows indicate time of additions. Error bars represent $\pm$ 1 S.E. of the means (n = 3).
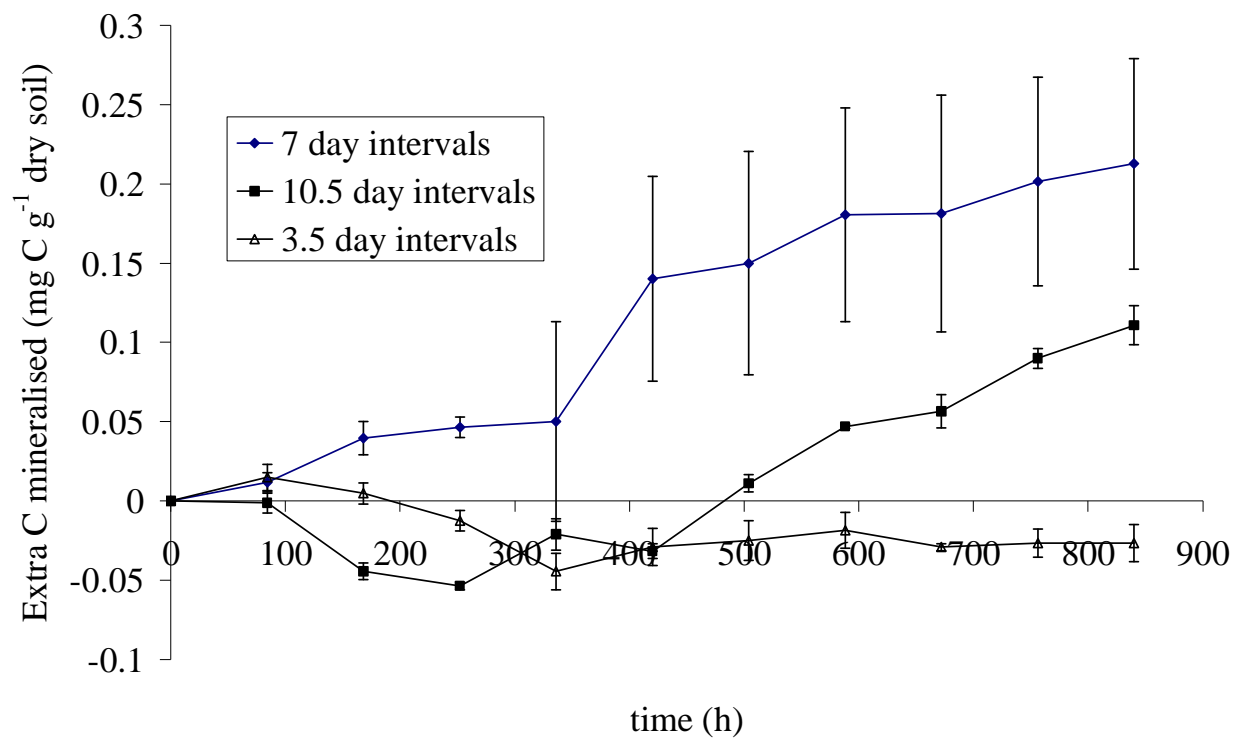
**Figure 39** Extra C mineralised (mg C g$^{-1}$ dry soil) as a result of repeated amendment with trace amounts of DOM ('DOM + nutrients' – 'nutrient only') at 3.5, 7 and 10.5 day intervals, during an 840 h incubation period. Data significantly differs from 0 after 420 h incubation time for 7 day interval applications and after 756 h for 10.5 day interval applications. No significant differences exist between 3.5 day interval data and 0 extra C mineralised. Error bars represent ± 1 S.E (n = 3).

## 4.6.   Discussion

The repeated addition of trace amounts of 'DOM + nutrients' to raised peatland soil over a 35 day incubation period caused an increase in $CO_2$ mineralisation, relative to nutrient only additions, when the time intervals between applications were 7 and 10.5 days. The extra C mineralised exceeded total DOC additions by more than 2 fold showing that components of the added DOM (in the presence of added inorganic nutrients) caused an increase in metabolic activity of the microbial biomass beyond that which could directly result from mineralization of all of the added carbon. As only a proportion of the added DOM would actually be sufficiently labile to be degraded within the 35 day incubation period, it is likely that the extra C mineralised actually exceeds added mineralisable C added by greater than 2 fold. The effect of 7 day interval applications was greater than for 10.5 day applications suggesting that the heightened activity of the biomass declined between 7 and 10.5 days after each addition of 'trigger molecules' within the DOM.

The period of acceleration of 'extra' C mineralised for 7 day interval additions begins earlier than that observed 420-504 h for the 10.5 day interval treatment but was not so sustained. Both responses, however, occur 7 days after the second addition was made. This suggests that (i) the microbial production of at least some of the enzymes responsible for biodegradation of the native and/or added carbon, or (ii) an increase in microbial biomass, are only initiated once a critical concentration of a specific substrate or substrates has been achieved. The microbes may be responding directly to the molecules added and hence the time lag of 7 days may be the time taken to elicit an appropriate response. Alternatively the microbes may increase their activity in response

to one or more breakdown products of the added carbon, in which case the time lag would be partly due to the time taken for these breakdown products to occur. The coincidental timing of a third addition during the period of acceleration for the 10.5 day interval treatment may have reinforced a response already taking place and therefore sustained the period for longer than for the 7 day interval treatment.

The timing of the point of acceleration in the rate of mineralization observed here for the 7 day interval additions is similar to that observed by De Nobili et al. (2001) (280-300 h) having made three repeated additions of root DOC extract (each addition = 11.3 µg C g$^{-1}$ dry soil) to cellulose-amended soils at time 0, 6 and 10.5 days in a 24 day incubation. In the case of De Nobili et al.'s work the period of greatest acceleration appeared to occur just over a day after the third addition, but the time lag between the second addition and the period of acceleration was 5.6 days, similar to the 7 day time lag observed here. Such comparisons between the two pieces of work, however, are difficult as the effectiveness and spectrum of potential 'trigger' molecules in root extract DOM may be very different to that of litter leachate, as may the sensitivity of the endogenous soil microbes to the presence of trigger molecules.

No period of acceleration of 'extra' C mineralization was observed for the 3.5 day interval treatments. In fact, the addition of 'DOM + nutrients' every 3.5 days had no significant effect on the total rate of mineralization relative to 'nutrient only' additions. This is difficult to explain considering the mineralization response to the less frequent additions. It is possible that additions made at this frequency enabled the accumulation of molecules in the culture which either inhibit extracellular enzyme activity or inhibit

microbial metabolism and/or reproduction to some degree. Such constituents may be present in the added DOC or result from partial degradation of the added DOC. With less frequent additions there may be sufficient time to enable such molecules to be broken down to a level where this effect is significantly reduced. Indeed, this may be the reason for the apparent time lag between additions and the heightened microbial response in the 7 and 10.5 day interval treatments discussed above. Alternatively, the high frequency of additions may be effectively water-logging the soil such that conditions are tending towards anaerobic conditions more rapidly than for the less frequent additions. A regimen of 3.5 day interval additions significantly increased the WHC with respect to the soils under a regimen of 7 and 10.5 day interval additions. Under anaerobic conditions biodegradation is known to be less efficient as the soils become so wet that the larger pores are water-filled and decomposition is limited by the rate at which oxygen can diffuse to the sites of microbial activity (Jenkinson, 1988). However, there is an absence of any negative effect of the relatively high WHC of the 3.5 day treatments on C mineralization for 'nutrient only' and 'DOM only' in comparison to the 7 and 10.5 day treatments. This suggests at the levels of WHC achieved here, the effects of increased pore saturation on C mineralization are minimal.

For both 7 and 10.5 day applications, total C mineralised in 'DOM + nutrient' treatments exceeded that of 'DOM only', and only at 35 days for 10.5 day interval treatment did 'DOM only' significantly exceeded 'no amendment' indicating that virtually all of the increases in total C mineralization seen here as a result of litter leachate DOM are inorganic nutrient limited. Thus enhanced mineralization of these particular soil samples in the field due to 'doses' of leaf litter leachate would be unlikely to be observed unless there was a source of nutrients over and above that

already present in the soil. Such sources may exist but be temporally and spatially dependent: influenced by rates of primary production, microbial activity and/or hydrological conditions. Additionally increases in anthropogenic sources of these nutrients could remove the nutrient limitation on enhanced mineralization.

The 'trigger' molecules present in the extracted peat litter DOM would be expected to be leached into the soil by periods of rainfall. From the evidence presented here the frequency at which periods of rainfall occur may therefore influence the degree of mineralization of native organic matter. Theoretically rainfall with return periods of 7 and 10.5 days could lead to an increase in the mineralization of organic carbon already present in the soil, with a 7 day return period having a greater effect than a 10.5 day return period. A higher frequency return period of 3.5 days would not be expected to affect the rate of mineralization of native organic carbon. The extent to which these effects would be negated by increased soil water content, leading to increased anaerobic conditions, would be dependent on the rate of rainfall and local hydrological conditions.

## 4.7    Conclusions

- Molecules present in water-extracted DOM from the litter layer of a raised peat bog appear to be able to trigger microbial mineralisation of carbon. The repeated addition of trace amounts of water-extracted 'DOM + nutrients' to raised peatland soil  over a 35 day incubation period in the laboratory caused an increase in $CO_2$ mineralisation, relative to nutrient only additions, when the time intervals between applications were 7 and 10.5 days. The extra C mineralised exceeded total DOC additions by more than 2 fold.

- A period of acceleration in the rate of extra C mineralised ('DOM + nutrients' – 'DOC only') was observed at 336-420 h for 7 day interval additions and at 420-504 h. In both cases, the increased rate occurred 7 days after the second addition, suggesting that the microbial production of enzymes responsible for biodegradation and/or an increase in microbial biomass, are initiated once a critical concentration of a specific substrate or substrates has been achieved. Alternatively, the time lag may a result of the time required to break down constituents of the added DOM that inhibit microbial activity and/or extracellular enzyme activity.

- The effect of 7 day interval applications was greater than for 10.5 day applications suggesting that the heightened activity of the biomass declined between 7 and 10.5 days after each addition of 'trigger molecules' within the DOM.

- The addition of 'DOM + nutrients' every 3.5 days had no effect on the total rate of mineralization   relative to 'nutrient only' additions. This may be due to an accumulation of inhibitors of microbial activity and/or extracellular enzyme activity, present in the added DOM.

- The supply of inorganic nutrients acts as a limiting factor on the enhancement of C mineralization in a raised peat soil resulting from the addition of DOM extracted from the litter layer of that soil.

- 'trigger' molecules present in the extracted peat litter DOM would be expected to be leached into the soil by periods of rainfall and, therefore rainfall return frequencies of 7 and 10.5 days may increase the degree of mineralization of native organic matter, assuming degree of soil saturation is not a limiting factor. A rainfall return frequency of 3.5 days, on the other hand, would be expected to have zero effect on the rate of C mineralization.

# Chapter 5: General Discussion

## 5.1    Summary of Findings

The broad aim of this research was to characterise soil DOM in such a way that would be useful for further studies on the biotic aspects of DOM production and loss. Within this context hypotheses were made in relation to several primary objectives (section 1.2.9). The findings of this research with respect to each objective and hypothesis are summarised below.

### 5.1.1  Objective 1:

**To evaluate the influence of method of isolation of DOM from peat soil on its biochemical composition.**

**Hypothesis:** *The proportion of proteins carbohydrate and amino acid carbon, as a fraction of the total organic carbon content in soil DOM, will vary according to the method used to isolate the DOM from the soil.*

**Findings:** In late summer/autumn and spring the size of the total protein, carbohydrate and amino acid fraction of peatland DOC varied with the method used to extract it, suggesting that different methods of extraction are effectively sampling fractions of DOC that are biochemically distinct at certain times of the year. For most of the year, where differences existed, DOC extracted using the water-extraction method contained

a greater proportion of proteins and/or carbohydrates than that obtained using the centrifugation or suction methods. Only in February was this situation altered with centrifuge-extracted DOC containing a larger proportion of carbohydrate than either water- or suction-extracted DOC.

## 5.1.2  Objective 2:

**To quantify changes in the biochemical composition and concentration of peat DOM over the period of a year.**

**Hypothesis:** *The total proportion of protein, carbohydrate and amino acid carbon as a fraction of DOC (% protein + carbohydrate + amino acid C), and total proportion of protein and amino acid nitrogen as a fraction of DON (% protein + amino acid N), extracted in the autumn/winter will be higher than in the spring/summer reflecting differences in degradability observed in previous studies (Kaiser et al., 2001; Marschner and Kalbitz, 2003; Nelson et al., 1994).*

**Findings:** The % carbohydrate + protein + amino acid C of DOC for water extracted DOC was higher in autumn than summer, suggesting that autumn DOC is more biodegradable than summer DOC. Autumn proportions of this fraction were also higher than winter levels contradicting other studies which have suggested higher biodegradabilities in winter/spring than summer/autumn (Kaiser *et al.*, 2001); Nelson et al., 1994). The levels of % carbohydrate + protein + amino acid C in suction-extracted DOC in October were significantly higher than in summer and spring. Centrifuge extracted DOC levels peaked in February but showed no significant change in %

carbohydrate + protein + amino acid with season. Highest levels of % protein + amino acid N as a fraction of DON were found in autumn (Oct) for all methods of extraction suggesting highest biodegradability of DON at this time of year. These levels were significantly higher than February for water-extracted DON and higher than April and August for centrifuge-extracted DON.

**Hypothesis:** *The concentration of DOC and DON extracted from a raised peat bog will show seasonal variability with higher amounts in summer than winter.*

**Findings:** DOC concentrations were highest in June for all methods of extraction: significantly higher than December for water-extracted DOC, higher than August, October and February for centrifuge-extracted DOC, and higher than all months sampled apart from April for suction-extracted DOC. DON concentrations for water- and centrifuge-extracted DON were highest in August: significantly higher than October and December for water-extracted DON and significantly higher than at any other time for centrifuge-extracted DON. No significant seasonal changes in suction-extracted DON concentration were observed.

**Hypothesis:** *DON concentration is independent of DOC concentration*

**Findings:** In water extracted DOC, concentrations of DOC and DON were significantly correlated but the correlation was weak. There was no significant correlation between DOC and DON in centrifuge and suction extracted DOC. DOC/DON ratios varied with

respect to both method of extraction and time of sampling. Whilst the average DOC/DON ratios for water-, centrifuge- and suction-extracted DOC were 35:1, 22:1 and 41:1 respectively individual values ranged from as little as 7:1 to as high as 61:1. Highest DOC/DON ratios for the year were observed in October and the lowest in August for both water- and centrifuge-extracted DOC. For DOC extracted using a suction-lysimeter, DOC/DON ratios were highest in October, April and June and lowest in December.

### 5.1.3  Objective 3:

**To study the dynamics of total DOC, key biological molecules and the microbial population during decomposition of soil DOC in both peat and agricultural ecosystems.**

**Hypothesis:** *The mineralization dynamics of peatland DOC will fit a double exponential decay model as observed in previous studies on forest and agricultural soil DOC (Qualls and Haines, 1992; Gregorich et al., 2003; Kalbitz et al., 2003).*

**Findings:** Measuring biodegradation by measuring $CO_2$ production over a 70 day incubation period showed DOC, for all soil types/treatments to be comprised of two kinetically distinct pools with its decomposition described by a double exponential decay model. The labile pool had a half-life ranging from 3 – 8 days and the stable pool from 0.4 to 6 years, depending on soil type and management.

**Hypothesis:** *The mass loss of total residual DOC for both peatland and agricultural soils will be greater than that predicted by the rate of mineralization due to assimilation of DOC by the microbial population and the pattern of loss will reflect changes in the size of the microbial population.*

**Findings:** Mass changes in DOC concentrations over a 70 day incubation period were different to that expected as a result of mineralization, both in terms of extent and dynamics. For all soil types/treatments, except for raised peat where expected matched mass loss, the total loss of DOC was greater than could be accounted for by the process of respiration/mineralization. In contrast to the expected double exponential decay pattern of decline based on mineralization rate, there was at least one increase in DOC observed within the culture medium for each soil types and treatments within the first 15 days. For all soil types/treatments there was a rapid growth and decline in the microbial population within the first 15 days, based on a direct counting method. Over the subsequent days of the incubation period the populations remained relatively steady or slowly declined.

**Hypothesis:** *Analyses of the composition of the DOC during the decomposition process will show a rapid decline in carbohydrate, protein and amino acid content within the first few days, in accordance with the rapid degradation of the labile pool observed in previous studies (Qualls and Haines, 1992; Gregorich et al., 2003; Kalbitz et al., 2003).*

**Findings:** Examination of the protein and carbohydrate fractions did not show rapid decomposition/assimilation in the first few days as may be expected of such 'labile' molecules but instead revealed a complex pattern of both degradation/assimilation and release throughout the incubation period. Release and degradation/assimilation of unidentified C tended to oppose that of protein and carbohydrate. Any link between the dynamics of each of these fractions and the microbial population was impossible to ascertain as the relationship between the two varied according to soil type/treatment.

### 5.1.4  Objective 4:

**To investigate the effects of repeated applications of trace amounts of litter DOM on the activity of peat soil microbial biomass.**

**Hypothesis***: The intervals between periods of rainfall will influence the extent of soil carbon mineralization through the action of trace amounts of litter DOM on the activity of the endogenous biomass. Frequent rainfall will lead to sustained 'alertness' of soil biomass because of the frequent supply of trace organic nutrients in the litter DOM acting as trigger molecules.  Frequent rainfall distributing fresh doses of trigger molecules should maintain the biomass in active state. Less frequent rainfall will reduce activity and therefore the extent of C mineralization.*

**Findings:** The repeated addition of trace amounts of litter-extracted DOM to raised peatland soil  did trigger an increase in C mineralization, over a 35 day incubation

period, when the time intervals between applications were 7 and 10.5 days. The increase in C mineralization exceeded DOC additions by more than 2 fold. The observed increases were inorganic-nutrient supply limited. A period of acceleration in the rate of extra C mineralised 7 days was seen after the second addition for both treatments, suggesting that the microbial production of enzymes responsible for biodegradation and/or an increase in microbial biomass, are initiated once a critical concentration of a specific substrate/s or inhibitory factor has been achieved. The effect of 7 day interval applications was greater than for 10.5 day applications suggesting that the heightened activity of the biomass declined between 7 and 10.5 days after each addition of 'trigger molecules' within the DOM.

## 5.2     Implications of Findings

Three different methods of extraction isolated three biochemically distinct pools of DOM from peatland soil: 'water-extracted DOM', 'centrifuge-extracted DOM' and 'suction-extracted DOM'. Each pool differed in its composition with respect to the relative proportions of carbohydrates, proteins and amino acids, and C:N ratios, and responded differently to seasonal influences. The source of these pools was likely to be the larger macropores (Figure 41: 'DOM 3') for suction-extracted DOM, and intermediate pores ('DOM 2') plus macropores ('DOM 1') for centrifuge-extracted DOM. The exact source of water-extracted DOM was less clear as the differences that exist between centrifuge and water-extracted DOM may have been a consequence of the inclusion of DOM from micropores ('DOM 1') and/or additional dissolution of molecules from intermediate and macropores (Figure 41: arrows 'a' and 'b'). The biochemical differences and responses of these pools to the seasons highlighted the functional distinctiveness of the different soil pore sizes i.e at least partial compartmentalisation of the biological, chemical and/or physical processes involving organic carbon within the soil. No one method of extraction is likely to accurately represent whole soil DOM but a consideration of the source of the DOM when using specific methods should help us to understand the processes taking place within the different soil compartments and the potential influence of factors such as hydrological conditions and microbial accessibility on its properties.

The seasonal changes in the proportion of 'labile' material, i.e carbohydrate, protein and amino acid, observed in this study were different for each pool of extracted DOM and

the specific times of the year at which absolute concentrations of DOC and DON peaked and troughed were different to some previous studies. Determining which processes, inputs and/or losses are causing the observed changes in DOM composition and concentration of each of the fractions with the time of year would be complex. It is highly likely several factors that change with the seasons e.g. temperature, hydrological conditions, leaf litter input etc. may be exerting an influence on DOM production and loss, and these factors are likely to be very site specific both on the macro and micro scale. A conceptual model of the influences, sources and examples of the differing characteristics of DOM extracted using each of the three techniques is illustrated in Figure 41. Carrying out a detailed study correlating seasonal changes in DOC and DON concentrations and composition with seasonal changes in the field, such as time and type of falls in leaf litter, first indications of plant activity in the spring, temperature, precipitation patterns etc. over a period of several years, may give some insight into which seasonal factors may be having the dominant effect.

DOC and DON concentrations throughout the sampled year were found to be independent of one another. DOC and DON concentrations will depend on the interaction of many factors including the relative concentrations present in throughfall and litter leachate, and the relative rates of production and loss through microbial decomposition/assimilation, plant uptake, sorption/desorption and hydrologic flushing. It would seem highly unlikely that the net effect of these factors throughout the seasons would influence DOC and DON concentrations to the same degree. For instance, in N-poor soils, such as peat, microbial growth will not occur unless important nutrients such

as N are present. At times of the year when there is a more than sufficient supply of labile DOC to sustain the population e.g. inputs of labile DOC from fresh litter in the autumn, then N-containing compounds may be preferentially hydrolysed by microbes reducing DON concentrations relative to DOC. At other times of the year, when decomposition rates are high, N-rich decomposition products may accumulate because of their relative recalcitrance, causing an increase in DON concentrations relative to DOC.

The seasonal dependency of both the concentration and composition of DOM indicates its vulnerability to climate change. If there is a long-term change in one or more external environmental driving forces then this could have far reaching consequences. As highlighted in the introduction, terrestrial soils contain some $1500 \times 10^3$ Mt of organic carbon, roughly double that of plants or in the atmosphere. Changes in the concentration and composition of DOM may represent significant changes in the rate at which carbon is cycled back into the atmosphere. It will also have far reaching consequences for all the other factors influenced by the presence of DOM such as the annual carbon budget of headwater streams, soil formation, the fate and transport of inorganic and organic contaminants and water quality.

One potential seasonal factor, which has received little attention to date, is that of rainfall frequency. For reasons discussed in Chapter 4 (section 4.1), each time rain falls, at the very least, trace amounts of fresh, soluble organic matter will be leached into the soil. The frequency at which trace amounts of DOM are added to peatland soil has been

shown, in the work presented here, to influence the rate of mineralization of soil organic carbon. Additions made every 7 and 10.5 days appeared to trigger the activity of the microbial biomass such that the rate of mineralization increased beyond that which could be accounted for by the added carbon. Additions made more frequently than this (every 3.5 days), however, appeared to have no effect on carbon mineralization. Whilst there has been little seasonal variation in the frequency of periods of rainfall in the vicinity of the extraction site of the soil used for this study over the past 10 years (Figure 40), this may not be the case at other locations. Indeed even at this locality climate change may, at some point in the future, cause the frequency pattern to change such that seasonal differences become apparent, or, more importantly in terms of carbon cycling, the annual frequency pattern changes altogether. If intervals between rainfall began to show a trend towards 7 days, the interval observed to cause the greatest increase in mineralization (relative to 3.5 and 10 day intervals), then the annual rate of loss of soil carbon may increase. Whether this increase would be significant, relative to all the other effects that other factors associated with climate change may induce in carbon mineralization, remains to be seen. Additionally, it should be remembered that the enhancement of C mineralization in the peat soil was inorganic nutrient limited, and therefore any rainfall frequency effects would only become apparent if the nutrient input increased beyond the rate-limiting threshold. Such inputs may exist in the field but be temporally and spatially dependent e.g. influenced by rates of primary production, microbial activity, anthropogenic sources, and/or hydrological conditions. Once again, even if there is insufficient nutrient availability to enable enhanced C mineralisation at the present time, environmental conditions may change and sufficient inputs may become available in the future.
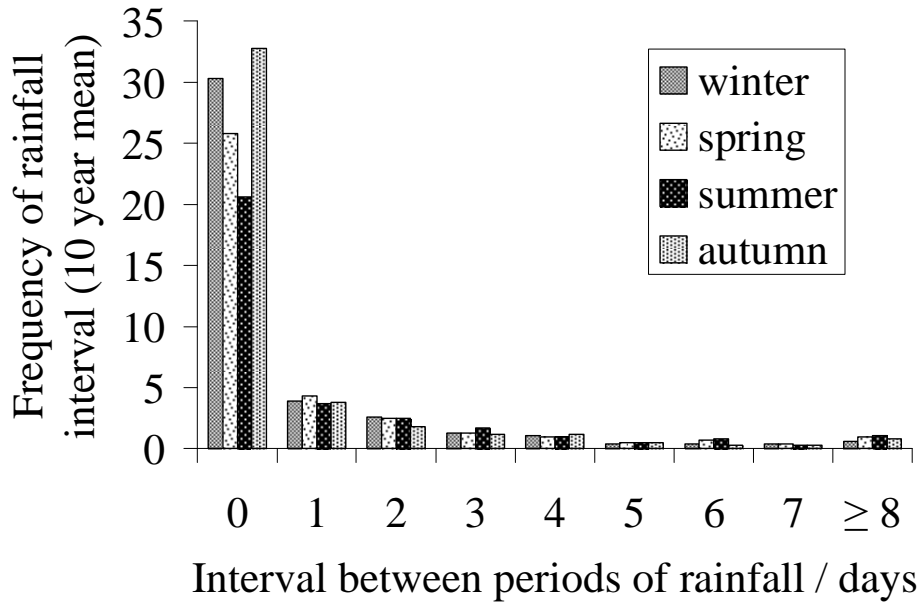
**Figure 40** Mean frequency of rainfall interval (days) for the period 1995-2004, recorded at the University of Stirling, Scotland.

Analysis of the mineralization kinetics of the water-extractable fraction of peatland DOC showed the presence of a labile pool with a half-life of less than 10 days and a stable pool with a half-life of the order of months or years (<10), for blanket peat and raised peat respectively. Analysis of the mineralisation kinetics of the water-extractable fractions of a mineral agricultural soil also showed the presence of a labile and a stable pool, with half-lives of a similar magnitude, regardless of fertilisation and crop management strategy. Other aspects of the decomposition process (mass loss of DOC dynamics; fluctuations in carbohydrates amino acids and proteins; and, changes in microbial population size) were also similar for both the peatland and mineral agricultural soils. Thus it seems, despite the variety in timing and chemical composition

of organic inputs, local hydrological and temperature conditions and geochemical properties of the soil, the degradability of DOC is universal across peatland and agricultural ecosystems. This may be a function of the capabilities of microbial communities generally, rather than the specific chemical composition of the DOC. Each soil will have a community highly adapted to the specific environmental conditions of that soil, and therefore able to utilise the available DOC substrate material to maximum effect. The half lives of each of the labile and stable pools may be a consequence of the time taken for adapted soil microbial communities to respond to any mixture of simple, easily degradable material plus more complex macromolecules, regardless of differences in specific chemical composition of that mixture. Alternatively, because the majority of organic inputs to all the soils will be plant material, dominated by molecules common to all plants, any differences between types of plant input, and the addition of other organic sources such as manure, will have only a relatively small effect. However before accepting or rejecting any theory, it must be remembered that, for reasons discussed above, the mineralization kinetics of this particular fraction of DOC may not accurately represent whole soil DOC nor represent the mineralization dynamics of DOC extracted at different times of the year.

Measuring decomposition by measuring net loss of DOC showed more complex dynamics than previously observed, with consistently lower concentrations of DOC than would be expected through loss to $CO_2$ and periods of increase as well as loss. In biological terms this makes sense as only the oxidation of DOC to $CO_2$ in the process of respiration is accounted for when determining the rate of mineralization i.e respiration: loss of DOC from solution also takes place as the microbial biomass assimilates DOC for growth. Measuring both the rates of mineralization and loss of DOC from solution is

useful in gaining an insight into the actual dynamics of the microbial community. The rate of mineralization is, after all, determined by both the chemical nature of the substrate and how the microbial community responds to that substrate. Evidence from this study seems to suggest that a sub-group of the microbial community rapidly proliferate in response to the availability of readily-decomposable, labile substrate. The proliferation of a second sub-group in response to the more recalcitrant substrate as proposed by Fontaine et al (Fontaine *et al.*, 2003) seems likely but requires further investigation. The activity of the microbial community during the decomposition process will itself influence the composition of the DOC. Thus the protein and carbohydrate fractions of soil DOC do not show a straightforward rapid decline in the first few days of decomposition, as may be expected of such highly labile molecules, but show periods of release as well as loss. If the metabolism of the microbes is taken into account then this is not particularly surprising. For instance, active microbes will release proteins into solution in the form of extracellular enzymes in response to the presence of suitable substrate material and maybe releasing carbohydrates in the form of biofilm polysaccharides. At the end of their lives microbes lyse, and proteins, carbohydrates and amino acids will all be released into solution.
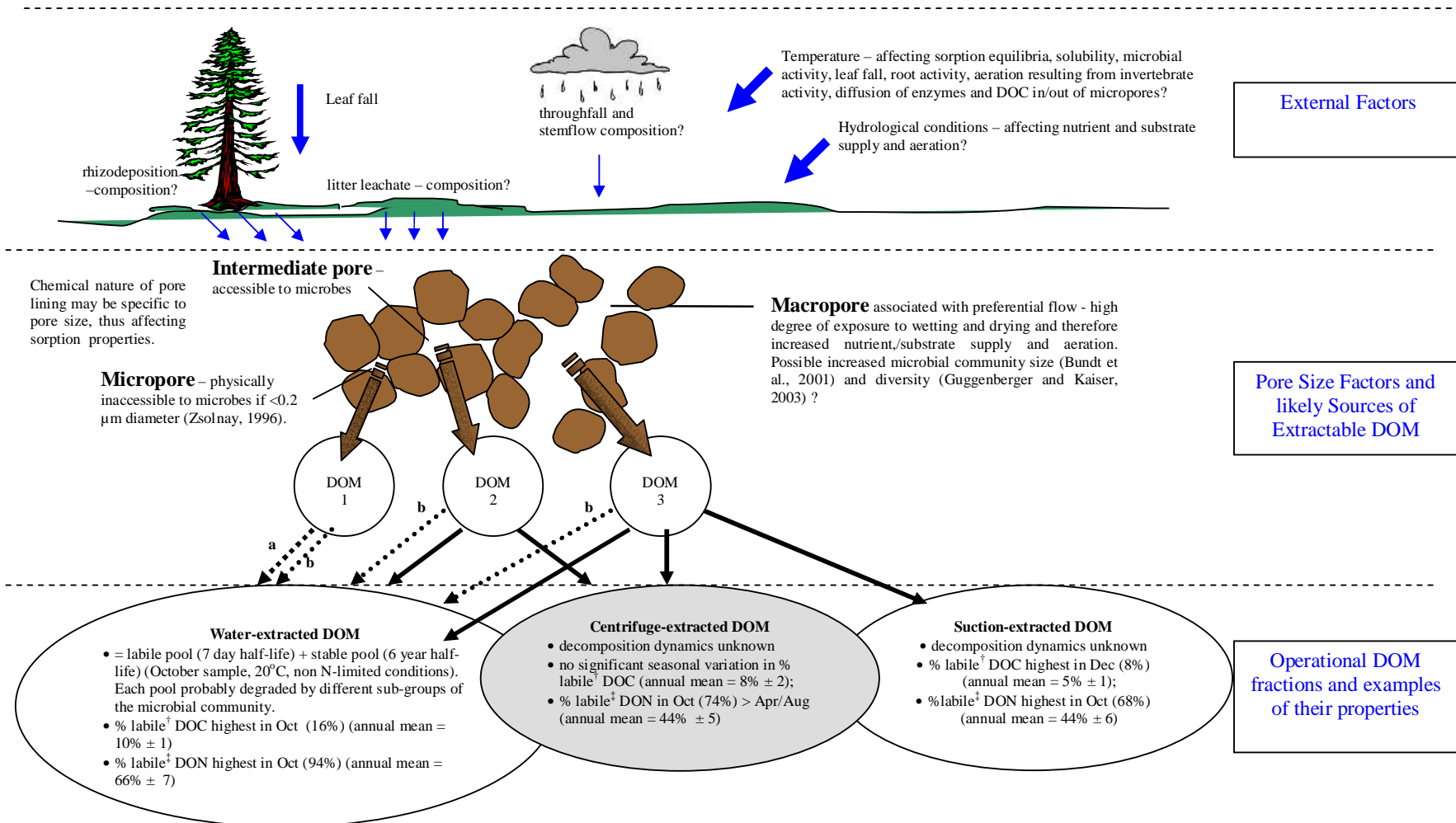
**Figure 41** Conceptual model illustrating the possible interaction of seasonal factors, pore size and method of extraction on observed biochemical properties of DOM from a raised peat bog in Central Scotland ( ——▶ = possible seasonal influences on DOM composition; [†]labile DOC = carbohydrate + protein + amino acid; [‡]labile DON = protein + amino acid; a = possible direct extraction of DOM; b = possible dissolution of DOM insoluble in situ).

**External Factors**

Leaf fall

throughfall and stemflow composition?

Temperature – affecting sorption equilibria, solubility, microbial activity, leaf fall, root activity, aeration resulting from invertebrate activity, diffusion of enzymes and DOC in/out of micropores?

Hydrological conditions – affecting nutrient and substrate supply and aeration?

rhizodeposition –composition?

litter leachate – composition?

**Pore Size Factors and likely Sources of Extractable DOM**

Chemical nature of pore lining may be specific to pore size, thus affecting sorption properties.

**Intermediate pore** – accessible to microbes

**Macropore** associated with preferential flow - high degree of exposure to wetting and drying and therefore increased nutrient,/substrate supply and aeration. Possible increased microbial community size (Bundt et al., 2001) and diversity (Guggenberger and Kaiser, 2003) ?

**Micropore** – physically inaccessible to microbes if <0.2 µm diameter (Zsolnay, 1996).

DOM 1    DOM 2    DOM 3

a    b    b

b

**Operational DOM fractions and examples of their properties**

**Water-extracted DOM**
- = labile pool (7 day half-life) + stable pool (6 year half-life) (October sample, 20°C, non N-limited conditions). Each pool probably degraded by different sub-groups of the microbial community.
- % labile[†] DOC highest in Oct (16%) (annual mean = 10% ± 1)
- % labile[‡] DON highest in Oct (94%) (annual mean = 66% ± 7)

**Centrifuge-extracted DOM**
- decomposition dynamics unknown
- no significant seasonal variation in % labile[†] DOC (annual mean = 8% ± 2);
- % labile[‡] DON in Oct (74%) > Apr/Aug (annual mean = 44% ± 5)

**Suction-extracted DOM**
- decomposition dynamics unknown
- % labile[†] DOC highest in Dec (8%) (annual mean = 5% ± 1);
- %labile[‡] DON highest in Oct (68%) (annual mean = 44% ± 6)

## 5.3    Concluding Remarks

There has been increasing interest in the dynamics of soil DOM over recent years as its influence on terrestrial and aquatic ecosystems and C cycling has become better understood. Whilst current research is making headway into gaining a fuller understanding of the production, loss and fluxes of DOM, the overall picture appears to become increasingly complex. Many biological and geochemical factors appear to be influencing DOM dynamics and each of these factors in turn may be affected by changes in environmental conditions. Added to this is the increasing awareness of the complexity of soil structure such that the influence of each of these factors may be different in different compartments of the soil. This research has attempted to investigate some of the biological properties of soil DOM. To date most research has operationally characterised soil DOM, making it difficult to make any deductions as to its involvement in biological processes. Whilst this work has gone some way to elucidating the biologically-relevant characteristics of soil DOM, it represents only a very small fraction of the knowledge needed to be able to predict the effects of environmental change on the production, loss and mineralization of soil DOM.

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# Appendix A: Analytical Methods

## A.1 Total Dissolved Organic Carbon Assay

Total dissolved organic carbon (TOC) was determined using a Shimadzu TOC-VCSN® Total Organic Carbon Analyzer. The difference between the total carbon (TC) and inorganic carbon (IC) analysis values determined TOC.

### A.1.1   TC Analysis

The sample is injected into a combustion tube filled with an oxidation catalyst (platinum), and heated to $680^{o}$C. The TC of the sample is oxidised in the combustion tube to form carbon dioxide and carried by a carrier gas (high purity oxygen), to a dehumidifier, where it is cooled and dehydrated. It is then passed through a halogen scrubber before reaching the cell of a non-dispersive infrared (NDIR) gas analyser, where the gas is detected. The analogue detection signal of the NDIR forms a peak, and the area of this peak is measured by a data processor.

Three sample injections are oxidised to $CO_2$ and the mean, standard deviation (SD) and coefficient of variance (CV%) of the peak area output of the NDIR are calculated. The limits for SD and CV were set at 0.1 and 2% respectively. If both were exceeded further sample injections are analysed until one of the set limits was achieved, using data from three of the injections. The resulting mean peak area was taken to be proportional to the TC concentration of the sample.

### A.1.2  IC Analysis

The sample is acidified with a small amount of hydrochloric acid to obtain a pH of less than 3. Any carbonates present are presumed to produce carbon dioxide according to the following reactions:

$$CO_3^{\,2-} + 2HCl \Longrightarrow CO_2 + 2Cl^- + H_2O$$

$$HCO_3^- + HCl \Longrightarrow CO_2 + Cl^- + H_2O$$
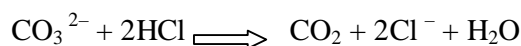
The dissolved carbon dioxide is volatised by bubbling carrier gas through the sample and detected by the NDIR. Peak area is taken to be proportional to IC concentration.

### A.1.3  Calibration

Standards of TOC were made up in double-distilled de-ionised water, using potassium hydrogen phthalate ($KC_8H_5O_4$) for the TC standard and sodium hydrogen carbonate ($NaHCO_3$) for the IC standard. 4 point calibration curves were generated and shifted to the origin to correct for TC and IC concentrations present in the water of the standard solutions.

## A.2 Total Dissolved Organic Nitrogen (TON) Assay

Total dissolved organic nitrogen was determined using a Shimadzu TOC-VCSN® Total Nitrogen Analyzer (TNM-1). Total organic nitrogen (TON) was calculated as the difference between total nitrogen (TN) concentration and inorganic nitrogen (ammonium + nitrate) concentrations determined using the methods outlined below (see A.4.2 and A.6 respectively).

### A.2.1  <u>TN analysis</u>

The sample is injected into the combustion tube at $720^{o}C$ where the TN in the sample decomposes to form nitrogen monoxide. The nitrogen monoxide is then cooled and dehumidified before entering a chemiluminescence gas analyser where it is detected. The detection signal from the chemiluminescence gas analyser generates a peak and the TN concentration in the sample can be measured.

### A.2.2  <u>Calibration</u>

Standards of TN were made up in double-distilled de-ionised water, using potassium nitrate ($KNO_3$). 4 point calibration curves were generated and shifted to the origin to correct for any TN present in the water of the standard solutions.

## A.3 Soluble Carbohydrate Assay

Soluble carbohydrate concentration determination was based on a colorimetric test using anthrone reagent. Anthrone in $H_2SO_4$ reacts with furan derivatives of monosaccharides, polysaccharides and glycosides to form a blue/green colour (Allen, 1989). 10 $cm^3$ ice-cold anthrone reagent (0.5g anthrone, 380 ml conc. $H_2SO_4$, and 120 ml distilled water) is pipetted onto the surface of 2 ml of each sample. The tubes are shaken for 15 s and kept cool prior to heating in an ice bath to prevent formation of colour as a result of the heat of dilution. The mixture is then held at $90^{o}C$ for 16 minutes, cooled in ice water and kept for 15 minutes at room temperature before measuring absorbance (Doutre *et al.*, 1978). Blanks were set up to compensate for the natural brown colouration of the soil water. For each blank both the sample and the anthrone reagent underwent the same processes as described above but were not added to each other until just prior to absorbance measurement. Concentrations of glucose

equivalents were calculated by subtracting blank absorbencies from sample absorbencies at 620 nm and comparing to those of glucose standards. In order to express all measures of C in common units, glucose equivalents were converted to mg C $l^{-1}$ of soil water by assuming that all reactants were hexoses, after Davidson et al. (Davidson *et al.*, 1987). It is important to note that there may be a bias associated with the use of anthrone: anthrone is considerably more sensitive to glucose than any other sugar present (Doutre et al., 1978), therefore when glucose is used to establish the standard curve, determination of the sum concentration of a mixture of sugars would be expected to be an under-estimate of the true amount.

## A.4 Amino Acid Assay

Amino acid concentrations were determined using a ninhydrin-N assay (see A.4.1). Ninhydrin reacts with amino-acids in the presence of a reducing agent at a pH of about 5 to form a purple complex (Allen, 1989). Other nitrogenous compounds, principally ammonium, interfere and therefore the ammonium-N concentration will also be determined in a separate assay.

### A.4.1   Ninhydrin-N Assay

1 ml citric acid buffer and 1 ml ninhydrin reagent are added slowly to 1 ml of each sample, mixed thoroughly and placed in a boiling water bath for 25 minutes. After heating the reaction mixture is cooled quickly to room temperature and absorbance at 570 nm is measured using a spectrophotometer (Moore and Stein, 1951). In order to compensate for the natural brown colour of the samples 'blanks', in which the sample is not added to the reaction mixture until after the heating and cooling process, are also created. Concentrations of glycine equivalents were calculated by subtracting the

absorbance of the blanks, at 570 nm, from the sample absorbencies and comparing to those of glycine standards. Glycine equivalents were converted to mg N $l^{-1}$ of soil water based on the assumption that plant proteins contain on average 16 % N (Allen, 1989) and a N-to-C ratio of 0.36(Meli *et al.*, 2003). Values for ammonium N (mg N $l^{-1}$) were deducted from results of ninhydrin-N assay to determine final amino acid concentration.

### A.4.2  **Ammonium Assay**

Ammonium concentration was determined colorimetrically using the indophenol-blue method. In the reaction ammonium nitrogen is oxidised by sodium hypochlorite and then coupled with a phenolic compound (sodium salicylate) to produce the indo-phenol blue colour (Allen, 1989).

5 ml of reagent 1 (10 g sodium salicylate, 8 g sodium citrate, 8 g sodium tartrate, 250 ml distilled water) are added to 0.1 ml of each sample, thoroughly mixed and left to stand for 15 minutes. 5 ml of reagent 2 (10 g sodium hydroxide, 750 ml distilled water) are added and mixed to each tube and left for 1 hour to achieve full colour development. Absorbance of colour is measured at 655 nm. To compensate for the natural brown of the samples, 'blank' tubes underwent the same process but the sample was not added to reagents 1 and 2 until just before absorbance is measured. Concentrations of ammonium were calculated by subtracting the absorbance of the blanks from sample absorbancies and comparing to those of ammonium standards. Ammonium concentration was converted to mg N / l soil water.

## A.5 Soluble Protein Assay

Protein concentration were assayed using the micro Lowry, Ohnshi and Barr modification method (Ohnishi and Barr, 1978) procedure (see below). Under alkaline conditions divalent copper ions form a complex with peptide bonds in which they are reduced to monovalent ions. Monovalent copper ions and the R-groups of tyrosine, tryptophan, and cysteine react with Folin reagent to produce an unstable product that becomes reduced to a purple/blue colour.

2.2 ml Biuret Reagent (Sigma Diagnostics, catalog. No. 690-1) is added to 0.5 ml of sample solution and allowed to stand at room temperature for 10 minutes. ). 1 ml Folin and Ciocaiteu's Reagent (Sigma Diagnostics, catalog. No. 690-2) is then added and each tube immediately mixed thoroughly. The tubes are left to stand at room temperature for 30 minutes before absorbance is measured at 725 nm. A 'blank' was created for each sample by carrying out the same procedure as above except that the sample was not added to the reagents until just prior to absorbance measurement. Concentrations of protein were calculated by subtracting the blank absorbance values from the sample values and comparing to albumin standards. Protein concentrations (mg protein/ml) were converted to mg C or mg N was based on the assumption that plant proteins contain on average 16 % N (Allen, 1989) and a N-to-C ratio of 0.36 (Meli *et al.*, 2003).

## A.6 Nitrate Assay

Nitrate concentrations were determined direct colorimetric method based on the nitration of salicylic acid in alkaline conditions (Keeney and Nelson, 1982). 1 ml of 5% salicylic acid (in conc. $H_2SO_4$) is added to 0.5 ml of sample and allowed to stand for 30 mins., before adding 10 ml 4 M NaOH. After 1 h to allow full colour development,

absorbance is measured at 410 nm on a spectrophotometer. Standards were made using

potassium nitrate in water. To compensate for the brown coloration of each sample, a

'blank' containing 0.5 ml of sample, 1 ml of conc. $H_2SO_4$ and 10 ml 4 M NaOH. The

absorbance of the blank was deducted from the sample absorbance, before comparing to

potassium nitrate standards.

# Appendix B: Results Data

## B.1 Influence of Method and Time of Extraction on Biochemical Composition

### B.1.1 Water-Extracted DOC

**Table 7.** Results of the biochemical analysis of the composition of water-extracted DOC, sampled at two month intervals during the year August 3003 to June 2004.

| Sample Number | TOC (mg/l) | TN (mg/l) | $NH_4^+$ (mg/l) | $NO_3^-$ (mg/l) | TON (mg/l) | Carbohydrate C (mg/l) | Protein C (mg/l) | ninhydrin-N (mg/l) | pH |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 34.18 | 3.14 | 0.00 | 1.07 | 2.07 | 0.55 | 3.45 | 0.00 | 3.7 |
| 2 | 29.55 | 3.13 | 0.01 | 0.83 | 2.29 | 0.55 | 4.56 | 0.03 | 3.6 |
| 3 | 16.66 | 2.28 | 0.00 | 1.02 | 1.26 | 0.69 | 1.76 | 0.03 | 3.5 |
| 1 | 32.85 | 0.75 | 0.04 | 0.30 | 0.41 | 3.23 | 0.98 | 0.06 | 3.6 |
| 2 | 19.85 | 1.20 | 0.03 | 0.65 | 0.52 | 2.53 | 1.23 | 0.05 | 3.6 |
| 3 | 30.40 | 0.85 | 0.00 | 0.42 | 0.43 | 3.51 | 1.01 | 0.07 | 3.3 |
| 1 | 29.70 | 1.93 | 0.00 | 0.86 | 1.07 | 0.27 | 0.64 | 0.06 | 3.5 |
| 2 | 14.50 | 1.07 | 0.02 | 0.64 | 0.41 | 0.27 | 0.73 | 0.06 | 3.4 |
| 3 | 12.56 | 0.91 | 0.03 | 0.65 | 0.23 | 0.48 | 0.56 | 0.09 | 3.6 |
| 1 | 54.66 | 2.36 | 0.03 | 1.32 | 1.01 | 0.27 | 0.64 | 0.04 | 3.5 |
| 2 | 25.01 | 2.71 | 0.01 | 1.69 | 1.01 | 0.20 | 0.74 | 0.04 | 3.3 |
| 3 | 19.34 | 1.72 | 0.05 | 1.06 | 0.61 | 0.41 | 0.69 | 0.03 | 3.4 |
| 1 | 39.96 | 4.06 | 0.01 | 2.34 | 1.71 | 2.03 | 0.98 | 0.04 | 3.4 |
| 2 | 32.42 | 2.08 | 0.02 | 1.07 | 0.99 | 1.40 | 2.34 | 0.06 | 3.3 |
| 3 | 42.68 | 2.68 | 0.02 | 1.39 | 1.27 | 1.68 | 2.21 | 0.04 | 3.3 |
| 1 | 55.39 | 3.28 | 0.01 | 1.43 | 1.84 | 1.04 | 3.90 | 0.07 | 3.4 |
| 2 | 46.33 | 2.49 | 0.03 | 1.29 | 1.17 | 1.82 | 2.65 | 0.05 | 3.4 |
| 3 | 47.18 | 2.18 | 0.04 | 1.01 | 1.13 | 1.54 | 2.29 | 0.05 | 3.4 |

## B.1.2 Centrifuge-Extracted DOC

**Table 8.** Results of the biochemical analysis of the composition of centrifuge-extracted DOC, sampled at two month intervals during the year August 3003 to June 2004.

| Sample Number | TOC (mg/l) | TN (mg/l) | NH$_4^+$ (mg/l) | NO$_3^-$ (mg/l) | TON (mg/l) | Carbohydrate C (mg/l) | Protein C (mg/l) | ninhydrin-N (mg/l) | pH |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 76.96 | 18.63 | 0.00 | 3.54 | 15.09 | 2.31 | 3.32 | 0.09 | 2.9 |
| 2 | 114.38 | 16.50 | 0.00 | 3.22 | 13.28 | 2.39 | 5.54 | 0.04 | 2.8 |
| 3 | 112.54 | 17.38 | 0.03 | 3.03 | 14.32 | 2.39 | 4.34 | 0.06 | 3.1 |
| 1 | 91.94 | 2.77 | 0.02 | 1.46 | 1.29 | 5.77 | 2.45 | 0.10 | 2.8 |
| 2 | 56.00 | 5.99 | 0.00 | 3.23 | 2.76 | 5.07 | 4.98 | 0.07 | 3.0 |
| 3 | 134.69 | 3.72 | 0.04 | 2.12 | 1.56 | 5.77 | 3.23 | 0.13 | 2.8 |
| 1 | 100.04 | 7.22 | 0.04 | 3.73 | 3.45 | 1.54 | 4.30 | 0.07 | 2.9 |
| 2 | 115.54 | 6.03 | 0.02 | 3.02 | 2.99 | 1.68 | 3.98 | 0.04 | 2.9 |
| 3 | 127.14 | 5.39 | 0.04 | 3.42 | 1.93 | 0.27 | 5.02 | 0.09 | 3.0 |
| 1 | 88.87 | 9.51 | 0.05 | 4.56 | 4.90 | 3.58 | 5.64 | 0.09 | 2.8 |
| 2 | 48.57 | 7.00 | 0.03 | 3.89 | 3.08 | 2.46 | 4.35 | 0.06 | 2.8 |
| 3 | 51.65 | 8.83 | 0.03 | 4.24 | 4.56 | 2.95 | 2.65 | 0.11 | 2.8 |
| 1 | 132.36 | 13.41 | 0.00 | 5.94 | 7.47 | 2.03 | 4.97 | 0.10 | 2.8 |
| 2 | 104.66 | 11.02 | 0.01 | 4.70 | 6.31 | 2.53 | 4.98 | 0.15 | 2.9 |
| 3 | 93.68 | 10.11 | 0.02 | 2.90 | 7.19 | 1.96 | 4.02 | 0.14 | 2.8 |
| 1 | 163.96 | 9.98 | 0.00 | 3.48 | 6.50 | 6.05 | 6.48 | 0.17 | 3.0 |
| 2 | 154.86 | 9.83 | 0.01 | 4.81 | 5.01 | 3.73 | 7.34 | 0.19 | 3.0 |
| 3 | 179.36 | 11.18 | 0.01 | 5.89 | 5.28 | 5.00 | 8.67 | 0.16 | 3.0 |

## B.1.3 Suction-Extracted DOC

**Table 9.** Results of the biochemical analysis of the composition of suction-extracted DOC, sampled at two month intervals during the year August 3003 to June 2004.

| Sample Number | TOC (mg/l) | TN (mg/l) | $NH_4^+$ (mg/l) | $NO_3^-$ (mg/l) | TON (mg/l) | Carbohydrate C (mg/l) | Protein C (mg/l) | ninhydrin-N (mg/l) | pH |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 51.26 | 1.32 | 0.07 | 0.23 | 1.02 | 0.62 | 1.10 | 0.10 | 3.8 |
| 2 | 46.69 | 1.33 | 0.07 | 0.31 | 0.95 | 0.62 | 0.98 | 0.08 | 3.6 |
| 3 | 74.38 | 1.90 | 0.06 | 0.29 | 1.55 | 0.62 | 1.02 | 0.12 | 4.2 |
| 1 | 51.11 | 1.32 | 0.01 | 0.32 | 0.99 | 1.19 | 1.98 | 0.03 | 2.8 |
| 2 | 46.54 | 1.33 | 0.05 | 0.45 | 0.83 | 2.10 | 1.34 | 0.09 | 2.8 |
| 3 | 74.23 | 1.90 | 0.00 | 0.56 | 1.34 | 1.26 | 2.23 | 0.08 | 2.8 |
| 1 | 60.92 | 14.48 | 0.07 | 3.35 | 11.06 | 1.40 | 4.50 | 0.11 | 3.2 |
| 2 | 56.53 | 6.61 | 0.04 | 3.56 | 3.01 | 1.04 | 3.99 | 0.04 | 3.3 |
| 3 | 55.60 | 6.72 | 0.00 | 4.23 | 2.49 | 1.26 | 2.01 | 0.05 | 3.4 |
| 1 | 63.24 | 7.45 | 0.00 | 3.64 | 3.81 | 1.04 | 3.22 | 0.03 | 2.8 |
| 2 | 79.47 | 7.07 | 0.03 | 3.23 | 3.81 | 1.11 | 4.40 | 0.00 | 2.8 |
| 3 | 72.99 | 4.40 | 0.01 | 2.99 | 1.40 | 0.34 | 1.02 | 0.00 | 3.0 |
| 1 | 72.78 | 6.04 | 0.00 | 3.00 | 3.04 | 0.62 | 1.05 | 0.07 | 2.6 |
| 2 | 95.13 | 2.85 | 0.03 | 1.93 | 0.89 | 0.55 | 1.65 | 0.02 | 3.0 |
| 3 | 117.86 | 2.85 | 0.06 | 1.48 | 1.31 | 0.76 | 2.10 | 0.08 | 2.8 |
| 1 | 119.46 | 4.89 | 0.08 | 1.32 | 3.49 | 1.82 | 3.01 | 0.20 | 3.0 |
| 2 | 138.96 | 3.22 | 0.02 | 1.96 | 1.24 | 2.10 | 2.23 | 0.15 | 3.0 |
| 3 | 117.96 | 4.31 | 0.09 | 2.10 | 2.12 | 1.40 | 2.21 | 0.11 | 3.1 |

## B.2 Decomposition Dynamics of DOC

### B.2.1 $CO_2$ Production in Headspace

**Table 10.** $CO_2$ content of headspace (mg C) of incubation flasks during 70 day incubation period. Data determined using a gas chromatograph (Varian Aerograph 90-P).

| Soil Type / Treatment | Sample Number | Headspace $CO_2$ content (mg C) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 3 | Day 7 | Day 15 | Day 36 | Day 42 | Day 70 |
| raised peat | 1 | 0.41 | 0.46 | 0.58 | 0.57 | 0.86 | 0.64 | 0.74 |
| | 2 | 0.42 | 0.48 | 0.56 | 0.55 | 0.79 | 0.59 | 0.65 |
| | 3 | 0.35 | 0.50 | 0.53 | 0.55 | 0.75 | 0.57 | 0.59 |
| blanket peat | 1 | 0.58 | 0.60 | 0.67 | 0.67 | 0.79 | 0.66 | 1.01 |
| | 2 | 0.46 | 0.48 | 0.56 | 0.58 | 0.71 | 0.60 | 0.62 |
| | 3 | 0.48 | 0.52 | 0.72 | 0.72 | 0.95 | 0.77 | 1.05 |
| maize (unamended) | 1 | 0.44 | 0.45 | 0.54 | 0.56 | 0.73 | 0.63 | 0.75 |
| | 2 | 0.39 | 0.47 | 0.71 | 0.80 | 0.80 | 0.72 | 0.79 |
| | 3 | 0.41 | 0.56 | 0.64 | 0.60 | 0.75 | 0.64 | 0.65 |
| maize / soy (manured) | 1 | 0.52 | 0.50 | 0.50 | 0.57 | 0.64 | 0.69 | 0.73 |
| | 2 | 0.55 | 0.56 | 0.84 | 0.77 | 0.76 | 0.66 | 0.69 |
| | 3 | 0.46 | 0.65 | 0.91 | 0.87 | 0.97 | 0.78 | 0.70 |
| maize (manured) | 1 | 0.48 | 0.51 | 0.58 | 0.54 | 0.66 | 0.64 | 0.67 |
| | 2 | 0.47 | 0.59 | 0.74 | 0.65 | 0.85 | 0.69 | 0.73 |
| | 3 | 0.62 | 0.68 | 0.79 | 0.67 | 0.85 | 0.70 | 0.76 |
| maize / soy ( N-fertilised) | 1 | 0.49 | 0.54 | 0.67 | 0.77 | 1.02 | 0.82 | 0.96 |
| | 2 | 0.38 | 0.44 | 0.56 | 0.66 | 0.69 | 0.61 | 0.66 |
| | 3 | 0.37 | 0.51 | 0.66 | 0.69 | 0.69 | 0.57 | 0.61 |
| glucose | 1 | 0.44 | 1.72 | 1.46 | 0.84 | 0.71 | 0.72 | 0.79 |
| | 2 | 0.40 | 1.52 | 1.73 | 0.90 | 0.86 | 0.67 | 0.83 |
| | 3 | 0.43 | 1.94 | 1.34 | 0.83 | 0.73 | 0.67 | 0.70 |
| distilled/deionised water | 1 | 0.39 | 0.46 | 0.54 | 0.50 | 0.64 | 0.59 | 0.57 |
| | 2 | 0.36 | 0.47 | 0.49 | 0.53 | 0.77 | 0.69 | 0.57 |
| | 3 | 0.42 | 0.48 | 0.50 | 0.54 | 0.73 | 0.64 | 0.73 |

## B.2.2 DOC Concentration of Culture Solutions

**Table 11.** DOC concentration of culture solution (mg C $l^{-1}$) during 70 day incubation period. Data determined using a Shimadzu TOC-VCSN® Total Organic Carbon Analyser, with correction for inorganic-C.

| Soil Type / Treatment | Sample Number | DOC Concentration (mg C $l^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 3 | Day 7 | Day 15 | Day 42 | Day 70 |
| raised peat | 1 | 13.99 | 17.55 | 16.63 | 14.62 | 14.91 | 16.08 | 17.70 |
| | 2 | 12.11 | 14.62 | 14.73 | 14.96 | 13.29 | 13.43 | 14.50 |
| | 3 | 12.85 | 15.86 | 13.69 | 9.52 | 21.24 | 13.42 | 13.94 |
| blanket peat | 1 | 11.02 | 9.82 | 8.87 | 7.70 | 8.39 | 7.76 | 8.77 |
| | 2 | 6.75 | 7.31 | 5.78 | 10.31 | 7.95 | 6.37 | 7.08 |
| | 3 | 6.69 | 6.93 | 6.29 | 10.08 | 14.86 | 6.01 | 7.66 |
| maize (unamended) | 1 | 6.62 | 12.24 | 6.86 | 8.34 | 7.69 | 6.69 | 6.96 |
| | 2 | 6.92 | 7.44 | 9.89 | 10.84 | 6.78 | 7.83 | 7.34 |
| | 3 | 9.62 | 10.95 | 9.36 | 10.09 | 7.23 | 8.63 | 10.31 |
| maize / soy (manured) | 1 | 10.91 | 12.16 | 11.79 | 13.09 | 11.69 | 10.84 | 11.80 |
| | 2 | 16.88 | 16.58 | 15.73 | 23.36 | 13.28 | 12.46 | 13.19 |
| | 3 | 16.92 | 21.53 | 15.83 | 16.22 | 14.99 | 18.37 | 17.24 |
| maize (manured) | 1 | 18.52 | 18.49 | 17.89 | 19.41 | 17.99 | 18.28 | 18.37 |
| | 2 | 22.60 | 21.97 | 20.86 | 24.35 | 18.46 | 18.28 | 18.20 |
| | 3 | 22.89 | 21.46 | 20.03 | 20.75 | 19.13 | 18.08 | 18.37 |
| maize / soy ( N-fertilised) | 1 | 7.01 | 9.86 | 7.80 | 7.85 | 11.55 | 10.88 | 9.04 |
| | 2 | 5.74 | 7.42 | 6.62 | 9.26 | 7.09 | 6.67 | 7.56 |
| | 3 | 12.60 | 12.05 | 9.93 | 8.30 | 7.70 | 7.73 | 8.34 |
| glucose | 1 | 20.31 | 22.24 | 5.96 | 5.08 | 3.59 | 4.23 | 4.75 |
| | 2 | 20.40 | 21.79 | 9.34 | 3.49 | 7.48 | 4.93 | 4.64 |
| | 3 | 19.86 | 22.10 | 2.89 | 2.94 | 2.95 | 3.07 | 3.91 |
| distilled/deionised water | 1 | 0.78 | 3.31 | 2.84 | 2.41 | 2.54 | 2.84 | 3.59 |
| | 2 | 0.73 | 2.93 | 0.57 | 4.23 | 2.40 | 3.61 | 3.50 |
| | 3 | 0.59 | 3.28 | 2.38 | 2.93 | 3.99 | 3.94 | 3.83 |

## B.2.3 Carbohydrate C Concentration

**Table 12.** Concentration of carbohydrate C in culture solution (mg C $l^{-1}$) during 70 day incubation period.

| Soil Type / Treatment | Sample Number | Carbohydrate Concentration (mg C $l^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 3 | Day 7 | Day 15 | Day 42 | Day 70 |
| raised peat | 1 | 0.96 | 1.21 | 1.04 | 0.31 | 1.28 | 0.83 | 2.10 |
| | 2 | 0.64 | 0.89 | 1.24 | 0.19 | 2.44 | 0.38 | 2.25 |
| | 3 | 0.58 | 0.96 | 1.44 | 0.64 | 1.86 | 0.51 | 1.25 |
| blanket peat | 1 | 0.45 | 0.39 | 1.51 | 0.57 | 1.02 | 0.25 | 1.09 |
| | 2 | 0.83 | 0.64 | 0.77 | 0.25 | 0.83 | 0.44 | 0.94 |
| | 3 | 0.52 | 0.58 | 1.17 | 0.51 | 1.09 | 0.06 | 1.25 |
| maize (unamended) | 1 | 2.03 | 1.44 | 1.24 | 0.31 | 2.63 | 0.53 | 1.25 |
| | 2 | 1.21 | 1.71 | 0.77 | 0.57 | 1.86 | 0.25 | 2.02 |
| | 3 | 1.40 | 2.38 | 1.64 | 0.44 | 2.95 | 0.02 | 1.94 |
| maize / soy (manured) | 1 | 0.58 | 1.04 | 1.31 | 0.06 | 1.86 | 0.00 | 3.02 |
| | 2 | 1.02 | 1.64 | 2.45 | 0.31 | 1.02 | 0.17 | 1.71 |
| | 3 | 0.96 | 2.38 | 2.05 | 0.76 | 1.66 | 0.32 | 1.79 |
| maize (manured) | 1 | 2.22 | 0.64 | 1.78 | 2.38 | 1.41 | 1.02 | 2.48 |
| | 2 | 1.90 | 1.21 | 1.51 | 1.78 | 1.15 | 0.48 | 2.33 |
| | 3 | 2.97 | 0.52 | 2.45 | 1.64 | 1.41 | 0.32 | 2.79 |
| maize / soy ( N-fertilised) | 1 | 1.27 | 0.70 | 3.59 | 0.97 | 1.09 | 0.09 | 1.48 |
| | 2 | 0.96 | 1.15 | 2.11 | 1.58 | 1.54 | 0.17 | 1.56 |
| | 3 | 0.64 | 0.39 | 2.11 | 1.10 | 0.38 | 0.17 | 1.40 |
| glucose | 1 | 19.84 | 21.92 | 1.51 | 0.57 | 0.57 | 0.55 | 0.09 |
| | 2 | 19.46 | 17.13 | 0.90 | 0.50 | 0.57 | 0.55 | 0.02 |
| | 3 | 20.47 | 18.71 | 1.17 | 0.16 | 0.44 | 0.71 | 0.17 |
| distilled/deionised water | 1 | 0.52 | 0.00 | 0.77 | 0.36 | 0.38 | 0.17 | 0.17 |
| | 2 | 0.45 | 0.80 | 0.50 | 0.43 | 0.00 | 0.25 | 0.02 |
| | 3 | 0.58 | 0.40 | 0.77 | 0.23 | 0.70 | 0.17 | 0.17 |

## B.2.4 Protein C Concentration

**Table 13.** Concentration of protein C in culture solution (mg C $l^{-1}$) during 70 day incubation period.

| Soil Type / Treatment | Sample Number | Protein Concentration (mg C $l^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 3 | Day 7 | Day 15 | Day 42 | Day 70 |
| raised peat | 1 | 10.76 | 12.71 | 8.09 | 12.18 | 11.82 | 5.60 | 8.62 |
| | 2 | 7.02 | 14.31 | 6.31 | 12.89 | 8.62 | 7.38 | 8.27 |
| | 3 | 7.02 | 11.64 | 10.40 | 10.58 | 8.62 | 5.96 | 8.27 |
| blanket peat | 1 | 2.22 | 4.89 | 7.20 | 5.07 | 6.31 | 0.44 | 0.80 |
| | 2 | 4.71 | 4.00 | 5.60 | 7.91 | 2.93 | 0.00 | 0.98 |
| | 3 | 3.29 | 2.22 | 3.64 | 5.60 | 0.07 | 0.27 | 0.09 |
| maize (unamended) | 1 | 0.80 | 5.60 | 5.78 | 5.24 | 1.33 | 0.00 | 1.51 |
| | 2 | 0.44 | 1.51 | 5.60 | 6.13 | 1.51 | 0.09 | 1.33 |
| | 3 | 0.62 | 3.29 | 4.00 | 1.51 | 0.98 | 0.09 | 2.22 |
| maize / soy (manured) | 1 | 7.56 | 9.87 | 4.18 | 6.13 | 4.00 | 3.29 | 6.13 |
| | 2 | 13.07 | 9.33 | 11.64 | 7.73 | 5.07 | 5.78 | 9.69 |
| | 3 | 13.07 | 10.04 | 8.80 | 7.02 | 4.18 | 2.93 | 7.56 |
| maize (manured) | 1 | 6.49 | 4.53 | 12.71 | 3.82 | 3.64 | 2.93 | 0.80 |
| | 2 | 5.96 | 2.93 | 8.44 | 5.96 | 3.29 | 2.40 | 0.27 |
| | 3 | 3.64 | 5.60 | 2.40 | 7.20 | 4.71 | 3.64 | 1.51 |
| maize / soy ( N-fertilised) | 1 | 2.04 | 4.00 | 0.27 | 4.00 | 5.96 | 0.27 | 0.00 |
| | 2 | 1.33 | 2.93 | 2.76 | 5.42 | 5.07 | 0.00 | 0.44 |
| | 3 | 1.51 | 2.76 | 4.18 | 4.36 | 4.89 | 0.44 | 2.22 |
| glucose | 1 | 0.62 | 2.22 | 4.36 | 3.29 | 2.93 | 0.00 | 0.27 |
| | 2 | 0.09 | 0.62 | 3.29 | 3.11 | 1.87 | 1.16 | 0.00 |
| | 3 | 1.69 | 1.51 | 4.00 | 1.87 | 2.22 | 0.44 | 0.80 |
| distilled/deionised water | 1 | 0.27 | 2.93 | 2.04 | 2.58 | 0.44 | 0.00 | 0.44 |
| | 2 | 1.16 | 1.69 | 1.69 | 3.29 | 0.27 | 0.62 | 0.27 |
| | 3 | 0.80 | 0.09 | 0.98 | 2.04 | 0.00 | 1.16 | 0.00 |

## B.2.5 Amino Acid C Concentration

**Table 14.** Concentration of amino acid C in culture solution (mg C l$^{-1}$) during 70 day incubation period.

| Soil Type / Treatment | Sample Number | Amino Acid Concentration (mg C l$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 3 | Day 7 | Day 15 | Day 42 | Day 70 |
| raised peat | 1 | 5.60 | 5.11 | 4.58 | 5.02 | 5.55 | 3.93 | 5.47 |
| | 2 | 6.90 | 5.17 | 4.74 | 4.68 | 4.56 | 3.31 | 4.66 |
| | 3 | 5.47 | 5.08 | 4.65 | 4.52 | 5.31 | 3.90 | 5.07 |
| blanket peat | 1 | 5.20 | 5.90 | 4.44 | 4.68 | 4.28 | 3.70 | 4.79 |
| | 2 | 5.02 | 7.16 | 4.67 | 4.80 | 5.53 | 4.11 | 4.06 |
| | 3 | 5.10 | 6.45 | 4.55 | 5.07 | 4.41 | 3.13 | 4.06 |
| maize (unamended) | 1 | 5.11 | 5.20 | 6.91 | 4.32 | 5.14 | 3.24 | 3.76 |
| | 2 | 5.13 | 4.80 | 6.62 | 4.30 | 4.21 | 5.33 | 4.09 |
| | 3 | 7.05 | 4.90 | 5.66 | 4.33 | 4.52 | 3.68 | 5.19 |
| maize / soy (manured) | 1 | 5.17 | 6.54 | 5.68 | 4.72 | 4.81 | 3.59 | 3.49 |
| | 2 | 4.91 | 5.64 | 5.07 | 4.70 | 4.63 | 4.48 | 5.34 |
| | 3 | 5.36 | 5.07 | 5.31 | 4.55 | 4.32 | 3.62 | 3.36 |
| maize (manured) | 1 | 6.60 | 5.48 | 6.17 | 4.33 | 4.47 | 4.15 | 4.79 |
| | 2 | 5.68 | 6.77 | 4.35 | 4.14 | 3.92 | 2.98 | 3.68 |
| | 3 | 5.26 | 5.20 | 5.83 | 5.15 | 4.49 | 3.57 | 3.62 |
| maize / soy ( N-fertilised) | 1 | 4.76 | 5.78 | 5.33 | 6.36 | 4.16 | 3.63 | 3.72 |
| | 2 | 4.55 | 5.72 | 5.58 | 5.42 | 3.68 | 3.70 | 3.65 |
| | 3 | 4.54 | 5.19 | 4.51 | 5.97 | 4.59 | 3.58 | 4.13 |
| glucose | 1 | 5.27 | 6.02 | 3.63 | 3.03 | 2.69 | 3.85 | 3.35 |
| | 2 | 5.07 | 5.25 | 3.60 | 3.09 | 2.74 | 3.14 | 3.07 |
| | 3 | 5.67 | 6.33 | 3.68 | 3.01 | 2.66 | 2.43 | 3.20 |
| distilled/deionised water | 1 | 5.09 | 5.17 | 4.21 | 3.68 | 3.74 | 3.68 | 3.84 |
| | 2 | 4.85 | 4.99 | 4.43 | 3.87 | 3.81 | 3.50 | 4.11 |
| | 3 | 4.95 | 4.73 | 4.49 | 4.05 | 3.96 | 3.59 | 3.97 |

# B.3 Effect of Repeated Applications of Trace Amounts of DOC on

# Microbial Activity

## B.3.1 <u>Investigation 1</u>

**Table 15.** Cumulative $CO_2$ production (mg C) resulting from 6 day interval additions of trace amounts of 'DOC + nutrients' and 'nutrients only' to 7.3 g dry soil equivalent peat soil. Data determined using a multi-chambered respirometer.

| Time / h | Cumulative $CO_2$ production (mg C) following 6 day interval additions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DOC + nutrients | | | | nutrients only | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6 | 0.204 | 0.202 | 0.147 | 0.225 | 0.316 | 0.193 | 0.237 | 0.164 |
| 12 | 0.261 | 0.250 | 0.196 | 0.324 | 0.476 | 0.239 | 0.369 | 0.224 |
| 18 | 0.298 | 0.289 | 0.233 | 0.388 | 0.586 | 0.271 | 0.493 | 0.274 |
| 24 | 0.387 | 0.377 | 0.310 | 0.486 | 0.728 | 0.354 | 0.655 | 0.364 |
| 30 | 0.561 | 0.561 | 0.446 | 0.640 | 0.929 | 0.529 | 0.883 | 0.525 |
| 36 | 0.611 | 0.600 | 0.496 | 0.704 | 1.010 | 0.575 | 1.016 | 0.589 |
| 42 | 0.635 | 0.628 | 0.522 | 0.745 | 1.058 | 0.601 | 1.131 | 0.636 |
| 48 | 0.714 | 0.706 | 0.597 | 0.834 | 1.148 | 0.688 | 1.290 | 0.731 |
| 54 | 0.882 | 0.886 | 0.731 | 0.986 | 1.310 | 0.869 | 1.512 | 0.889 |
| 60 | 0.947 | 0.925 | 0.781 | 1.044 | 1.372 | 0.919 | 1.642 | 0.962 |
| 66 | 0.984 | 1.026 | 0.784 | 1.026 | 1.415 | 0.955 | 1.683 | 0.955 |
| 72 | 1.067 | 1.116 | 0.846 | 1.105 | 1.543 | 1.021 | 1.764 | 1.008 |
| 78 | 1.243 | 1.300 | 0.972 | 1.258 | 1.739 | 1.177 | 1.910 | 1.138 |
| 84 | 1.298 | 1.341 | 1.006 | 1.309 | 1.829 | 1.199 | 1.956 | 1.170 |
| 90 | 1.314 | 1.356 | 1.018 | 1.336 | 1.882 | 1.202 | 1.981 | 1.182 |
| 96 | 1.409 | 1.445 | 1.094 | 1.431 | 2.008 | 1.275 | 2.068 | 1.263 |
| 102 | 1.587 | 1.633 | 1.225 | 1.587 | 2.214 | 1.447 | 2.213 | 1.413 |
| 108 | 1.654 | 1.682 | 1.284 | 1.654 | 2.325 | 1.490 | 2.267 | 1.475 |
| 114 | 1.669 | 1.700 | 1.292 | 1.673 | 2.379 | 1.505 | 2.280 | 1.498 |
| 120 | 1.775 | 1.796 | 1.375 | 1.776 | 2.515 | 1.600 | 2.366 | 1.604 |
| 126 | 1.963 | 1.991 | 1.529 | 1.944 | 2.728 | 1.792 | 2.520 | 1.786 |
| 132 | 2.028 | 2.036 | 1.589 | 2.010 | 2.836 | 1.852 | 2.573 | 1.870 |
| 138 | 2.089 | 2.021 | 1.787 | 1.950 | 2.770 | 1.965 | 2.526 | 2.017 |
| 144 | 2.167 | 2.094 | 1.868 | 2.013 | 2.852 | 2.059 | 2.553 | 2.107 |
| 150 | 2.389 | 2.302 | 2.040 | 2.190 | 3.058 | 2.279 | 2.685 | 2.314 |
| 156 | 2.449 | 2.339 | 2.086 | 2.227 | 3.117 | 2.340 | 2.680 | 2.386 |
| 162 | 2.483 | 2.365 | 2.110 | 2.235 | 3.144 | 2.388 | 2.659 | 2.439 |
| 168 | 2.596 | 2.462 | 2.200 | 2.323 | 3.245 | 2.507 | 2.704 | 2.570 |
| 174 | 2.815 | 2.684 | 2.369 | 2.495 | 3.443 | 2.738 | 2.829 | 2.786 |
| 180 | 2.889 | 2.735 | 2.426 | 2.544 | 3.509 | 2.821 | 2.837 | 2.881 |
| 186 | 2.956 | 2.786 | 2.475 | 2.590 | 3.561 | 2.902 | 2.708 | 2.979 |
| 192 | 3.093 | 2.902 | 2.574 | 2.695 | 3.681 | 3.043 | 2.767 | 3.127 |
| 198 | 3.324 | 3.131 | 2.747 | 2.883 | 3.885 | 3.298 | 2.911 | 3.361 |
| 204 | 3.419 | 3.191 | 2.816 | 2.954 | 3.963 | 3.397 | 2.936 | 3.478 |
| 210 | 3.825 | 3.208 | 3.037 | 2.972 | 3.986 | 3.440 | 2.935 | 3.534 |
| 216 | 3.846 | 3.247 | 3.080 | 3.000 | 4.053 | 3.547 | 2.925 | 3.569 |
| 222 | 4.071 | 3.427 | 3.230 | 3.167 | 4.261 | 3.786 | 3.040 | 3.765 |
| 228 | 4.156 | 3.444 | 3.268 | 3.214 | 4.342 | 3.876 | 3.029 | 3.829 |
| 234 | 4.244 | 3.468 | 3.300 | 3.259 | 4.405 | 3.972 | 3.024 | 3.902 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 240 | 4.418 | 3.568 | 3.395 | 3.372 | 4.542 | 4.142 | 3.077 | 4.055 |
| 246 | 4.663 | 3.768 | 3.562 | 3.553 | 4.754 | 4.407 | 3.197 | 4.300 |
| 252 | 4.791 | 3.834 | 3.638 | 3.639 | 4.859 | 4.551 | 3.213 | 4.440 |
| 258 | 4.899 | 3.904 | 3.688 | 3.709 | 4.938 | 4.672 | 3.213 | 4.561 |
| 264 | 5.086 | 4.048 | 3.814 | 3.847 | 5.095 | 4.877 | 3.282 | 4.759 |
| 270 | 5.350 | 4.289 | 4.035 | 4.071 | 5.338 | 5.175 | 3.447 | 5.060 |
| 276 | 5.491 | 4.390 | 4.122 | 4.168 | 5.447 | 5.326 | 3.469 | 5.211 |
| 282 | 5.548 | 4.437 | 4.432 | 4.196 | 5.492 | 5.404 | 3.460 | 5.280 |
| 288 | 5.518 | 4.492 | 4.433 | 4.171 | 5.539 | 5.520 | 3.450 | 5.061 |
| 294 | 5.749 | 4.734 | 4.597 | 4.350 | 5.758 | 5.801 | 3.610 | 5.247 |
| 300 | 5.855 | 4.821 | 4.667 | 4.425 | 5.871 | 5.950 | 3.641 | 5.331 |
| 306 | 5.936 | 4.911 | 4.746 | 4.491 | 5.951 | 6.080 | 3.651 | 5.405 |
| 312 | 6.140 | 5.096 | 4.892 | 4.675 | 6.152 | 6.310 | 3.767 | 5.631 |
| 318 | 6.406 | 5.378 | 5.081 | 4.821 | 6.399 | 6.623 | 3.908 | 5.853 |
| 324 | 6.573 | 5.511 | 5.206 | 4.941 | 6.549 | 6.801 | 3.964 | 6.019 |
| 330 | 6.731 | 5.644 | 5.302 | 5.086 | 6.681 | 6.960 | 4.025 | 6.206 |
| 336 | 6.916 | 5.830 | 5.467 | 5.197 | 6.858 | 7.182 | 4.120 | 6.388 |
| 342 | 7.223 | 6.139 | 5.723 | 5.448 | 7.128 | 7.499 | 4.324 | 6.691 |
| 348 | 7.399 | 6.290 | 5.868 | 5.588 | 7.282 | 7.675 | 4.422 | 6.903 |
| 354 | 7.402 | 6.327 | 5.952 | 5.689 | 7.379 | 7.780 | 4.479 | 6.993 |
| 360 | 7.640 | 6.463 | 6.125 | 5.849 | 7.539 | 7.945 | 4.633 | 7.215 |
| 366 | 7.989 | 6.735 | 6.406 | 6.128 | 7.818 | 8.229 | 4.841 | 7.518 |
| 372 | 8.242 | 6.886 | 6.563 | 6.323 | 8.040 | 8.424 | 4.928 | 7.685 |
| 378 | 8.392 | 6.982 | 6.510 | 6.303 | 8.081 | 8.499 | 4.922 | 7.717 |
| 384 | 8.647 | 7.180 | 6.758 | 6.538 | 8.288 | 8.717 | 5.113 | 7.997 |
| 390 | 8.990 | 7.510 | 7.042 | 6.827 | 8.570 | 9.037 | 5.336 | 8.283 |
| 396 | 9.237 | 7.700 | 7.244 | 7.058 | 8.777 | 9.241 | 5.458 | 8.471 |
| 402 | 9.385 | 7.838 | 7.370 | 7.147 | 8.842 | 9.343 | 5.519 | 8.538 |
| 408 | 9.626 | 8.059 | 7.596 | 7.460 | 9.037 | 9.562 | 5.697 | 8.762 |
| 414 | 9.957 | 8.395 | 7.919 | 7.784 | 9.323 | 9.876 | 5.963 | 9.068 |
| 420 | 10.180 | 8.581 | 8.115 | 8.004 | 9.515 | 10.057 | 6.086 | 9.247 |
| 426 | 10.325 | 8.739 | 8.261 | 8.121 | 9.624 | 10.186 | 6.185 | 9.375 |
| 432 | 10.578 | 8.994 | 8.520 | 8.385 | 9.850 | 10.416 | 6.394 | 9.617 |
| 438 | 10.894 | 9.332 | 8.813 | 8.693 | 10.137 | 10.719 | 6.659 | 9.898 |
| 444 | 11.064 | 9.508 | 8.978 | 8.895 | 10.395 | 10.878 | 6.802 | 10.191 |
| 450 | 11.176 | 9.633 | 9.209 | 9.008 | 10.448 | 10.968 | 6.867 | 10.301 |
| 456 | 11.457 | 9.830 | 9.491 | 9.236 | 10.661 | 11.107 | 7.050 | 10.525 |
| 462 | 11.889 | 10.155 | 10.001 | 9.680 | 10.872 | 11.393 | 7.355 | 10.897 |
| 468 | 12.155 | 10.317 | 10.251 | 9.791 | 10.990 | 11.478 | 7.429 | 10.988 |
| 474 | 12.404 | 10.480 | 10.515 | 9.943 | 11.142 | 11.579 | 7.522 | 11.088 |
| 480 | 12.722 | 10.713 | 10.870 | 10.192 | 11.366 | 11.759 | 7.718 | 11.277 |
| 486 | 13.101 | 11.019 | 11.278 | 10.500 | 11.640 | 12.011 | 7.967 | 11.494 |
| 492 | 13.399 | 11.210 | 11.610 | 10.705 | 11.810 | 12.143 | 8.126 | 11.630 |
| 498 | 13.692 | 11.371 | 11.963 | 10.771 | 11.870 | 12.191 | 8.204 | 11.683 |
| 504 | 13.927 | 11.563 | 12.263 | 11.139 | 12.218 | 12.394 | 8.359 | 11.875 |
| 510 | 14.269 | 11.888 | 12.581 | 11.566 | 12.630 | 12.697 | 8.525 | 12.033 |
| 516 | 14.451 | 11.991 | 12.740 | 11.696 | 12.763 | 12.770 | 8.609 | 12.100 |
| 522 | 14.598 | 12.156 | 12.886 | 11.843 | 12.893 | 12.859 | 8.697 | 12.145 |
| 528 | 14.808 | 12.365 | 13.108 | 12.030 | 13.045 | 13.009 | 8.851 | 12.237 |
| 534 | 15.083 | 12.664 | 13.409 | 12.329 | 13.324 | 13.255 | 9.115 | 12.452 |
| 540 | 15.255 | 12.774 | 13.616 | 12.551 | 13.519 | 13.380 | 9.287 | 12.584 |
| 546 | 15.387 | 12.946 | 13.805 | 12.734 | 13.671 | 13.488 | 9.455 | 12.692 |
| 552 | 15.581 | 13.158 | 14.045 | 12.972 | 13.880 | 13.653 | 9.674 | 12.852 |
| 558 | 15.865 | 13.476 | 14.363 | 13.294 | 14.173 | 13.920 | 9.975 | 13.098 |
| 564 | 16.026 | 13.566 | 14.577 | 13.505 | 14.353 | 14.040 | 10.162 | 13.243 |

**Table 16.** Cumulative $CO_2$ production resulting from 9 day interval additions of trace amounts of 'DOC + nutrients' and 'nutrients only' to 7.3 g dry soil equivalent peat soil. Data determined using a multi-chambered respirometer.

| Time / h | Cumulative $CO_2$ production (mg C) following 9 day interval additions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DOC + nutrients | | | | nutrients only | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6 | 0.147 | 0.214 | 0.170 | 0.189 | 0.154 | 0.140 | 0.165 | 0.176 |
| 12 | 0.207 | 0.307 | 0.235 | 0.253 | 0.209 | 0.161 | 0.207 | 0.239 |
| 18 | 0.238 | 0.379 | 0.287 | 0.313 | 0.255 | 0.163 | 0.237 | 0.287 |
| 24 | 0.310 | 0.500 | 0.381 | 0.414 | 0.342 | 0.220 | 0.318 | 0.379 |
| 30 | 0.434 | 0.686 | 0.535 | 0.582 | 0.487 | 0.356 | 0.479 | 0.537 |
| 36 | 0.488 | 0.778 | 0.603 | 0.643 | 0.539 | 0.382 | 0.529 | 0.607 |
| 42 | 0.506 | 0.836 | 0.644 | 0.688 | 0.571 | 0.380 | 0.554 | 0.648 |
| 48 | 0.573 | 0.956 | 0.733 | 0.787 | 0.643 | 0.439 | 0.641 | 0.741 |
| 54 | 0.693 | 1.140 | 0.883 | 0.950 | 0.772 | 0.577 | 0.805 | 0.904 |
| 60 | 0.752 | 1.238 | 0.949 | 1.014 | 0.819 | 0.613 | 0.864 | 0.978 |
| 66 | 0.795 | 1.269 | 0.974 | 1.034 | 0.814 | 0.595 | 1.003 | 0.987 |
| 72 | 0.866 | 1.397 | 1.080 | 1.133 | 0.859 | 0.662 | 1.094 | 1.052 |
| 78 | 0.984 | 1.594 | 1.246 | 1.304 | 0.962 | 0.813 | 1.270 | 1.187 |
| 84 | 1.034 | 1.697 | 1.312 | 1.368 | 0.995 | 0.850 | 1.332 | 1.232 |
| 90 | 1.047 | 1.763 | 1.345 | 1.412 | 1.016 | 0.854 | 1.363 | 1.243 |
| 96 | 1.119 | 1.910 | 1.447 | 1.530 | 1.094 | 0.936 | 1.472 | 1.331 |
| 102 | 1.239 | 2.114 | 1.610 | 1.713 | 1.229 | 1.094 | 1.658 | 1.483 |
| 108 | 1.301 | 2.239 | 1.681 | 1.797 | 1.291 | 1.146 | 1.738 | 1.558 |
| 114 | 1.306 | 2.303 | 1.703 | 1.831 | 1.318 | 1.142 | 1.769 | 1.583 |
| 120 | 1.385 | 2.465 | 1.807 | 1.959 | 1.411 | 1.232 | 1.892 | 1.704 |
| 126 | 1.516 | 2.706 | 1.979 | 2.164 | 1.564 | 1.410 | 2.097 | 1.899 |
| 132 | 1.577 | 2.839 | 2.043 | 2.257 | 1.632 | 1.463 | 2.182 | 2.001 |
| 138 | 1.579 | 2.938 | 1.981 | 2.250 | 1.610 | 1.397 | 2.160 | 2.046 |
| 144 | 1.638 | 3.089 | 2.035 | 2.327 | 1.661 | 1.436 | 2.230 | 2.170 |
| 150 | 1.781 | 3.362 | 2.202 | 2.524 | 1.818 | 1.599 | 2.434 | 2.408 |
| 156 | 1.825 | 3.495 | 2.234 | 2.570 | 1.871 | 1.605 | 2.488 | 2.517 |
| 162 | 1.834 | 3.594 | 2.238 | 2.609 | 1.913 | 1.581 | 2.519 | 2.594 |
| 168 | 1.908 | 3.782 | 2.317 | 2.734 | 2.022 | 1.645 | 2.634 | 2.755 |
| 174 | 2.051 | 4.056 | 2.476 | 2.950 | 2.195 | 1.812 | 2.850 | 2.999 |
| 180 | 2.106 | 4.205 | 2.518 | 3.042 | 2.281 | 1.840 | 2.928 | 3.126 |
| 186 | 2.135 | 4.343 | 2.551 | 3.141 | 2.360 | 1.862 | 3.001 | 3.240 |
| 192 | 2.220 | 4.552 | 2.644 | 3.299 | 2.477 | 1.959 | 3.136 | 3.423 |
| 198 | 2.371 | 4.838 | 2.812 | 3.541 | 2.655 | 2.156 | 3.372 | 3.680 |
| 204 | 2.437 | 5.010 | 2.866 | 3.657 | 2.750 | 2.219 | 3.468 | 3.828 |
| 210 | 2.439 | 5.085 | 3.091 | 3.712 | 2.799 | 2.228 | 3.565 | 3.890 |
| 216 | 2.474 | 5.119 | 3.109 | 3.780 | 2.809 | 2.197 | 3.591 | 3.916 |
| 222 | 2.592 | 5.318 | 3.279 | 4.002 | 2.941 | 2.342 | 3.792 | 4.129 |
| 228 | 2.617 | 5.410 | 3.329 | 4.095 | 2.976 | 2.352 | 3.866 | 4.237 |
| 234 | 2.615 | 5.489 | 3.365 | 4.197 | 3.036 | 2.354 | 3.938 | 4.329 |
| 240 | 2.675 | 5.664 | 3.470 | 4.378 | 3.153 | 2.435 | 4.090 | 4.503 |
| 246 | 2.801 | 5.932 | 3.639 | 4.640 | 3.321 | 2.596 | 4.332 | 4.750 |
| 252 | 2.856 | 6.109 | 3.707 | 4.784 | 3.418 | 2.650 | 4.446 | 4.910 |
| 258 | 2.869 | 6.258 | 3.746 | 4.903 | 3.501 | 2.682 | 4.530 | 5.044 |
| 264 | 2.964 | 6.492 | 3.856 | 5.108 | 3.637 | 2.806 | 4.698 | 5.261 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 270 | 3.131 | 6.818 | 4.052 | 5.409 | 3.843 | 3.038 | 4.968 | 5.562 |
| 276 | 3.203 | 7.004 | 4.114 | 5.550 | 3.941 | 3.131 | 5.080 | 5.733 |
| 282 | 3.202 | 7.084 | 4.117 | 5.609 | 3.985 | 3.155 | 5.114 | 5.806 |
| 288 | 3.259 | 7.160 | 4.277 | 5.737 | 4.045 | 3.215 | 5.226 | 5.862 |
| 294 | 3.394 | 7.450 | 4.504 | 6.025 | 4.229 | 3.450 | 5.474 | 6.102 |
| 300 | 3.439 | 7.630 | 4.599 | 6.176 | 4.328 | 3.546 | 5.592 | 6.239 |
| 306 | 3.439 | 7.699 | 4.654 | 6.239 | 4.400 | 3.584 | 5.657 | 6.316 |
| 312 | 3.541 | 7.945 | 4.843 | 6.461 | 4.573 | 3.755 | 5.844 | 6.540 |
| 318 | 3.709 | 8.255 | 5.034 | 6.759 | 4.796 | 4.018 | 6.103 | 6.832 |
| 324 | 3.799 | 8.457 | 5.168 | 6.933 | 4.933 | 4.151 | 6.238 | 7.021 |
| 330 | 3.851 | 8.585 | 5.312 | 7.054 | 5.061 | 4.253 | 6.344 | 7.178 |
| 336 | 3.973 | 8.894 | 5.443 | 7.335 | 5.251 | 4.457 | 6.534 | 7.422 |
| 342 | 4.164 | 9.165 | 5.674 | 7.608 | 5.472 | 4.726 | 6.784 | 7.718 |
| 348 | 4.274 | 9.398 | 5.823 | 7.776 | 5.627 | 4.895 | 6.940 | 7.944 |
| 354 | 4.352 | 9.453 | 6.047 | 7.858 | 5.704 | 4.980 | 7.020 | 8.114 |
| 360 | 4.477 | 9.632 | 6.266 | 8.011 | 5.785 | 5.176 | 7.197 | 8.423 |
| 366 | 4.717 | 9.843 | 6.575 | 8.286 | 6.003 | 5.474 | 7.432 | 8.773 |
| 372 | 4.875 | 9.991 | 6.751 | 8.440 | 6.148 | 5.616 | 7.545 | 8.995 |
| 378 | 4.851 | 10.031 | 6.745 | 8.551 | 6.240 | 5.612 | 7.538 | 9.083 |
| 384 | 5.027 | 10.279 | 6.996 | 8.780 | 6.459 | 5.861 | 7.736 | 9.395 |
| 390 | 5.261 | 10.551 | 7.246 | 9.083 | 6.695 | 6.133 | 7.975 | 9.709 |
| 396 | 5.444 | 10.732 | 7.429 | 9.256 | 6.853 | 6.301 | 8.099 | 9.941 |
| 402 | 5.547 | 10.841 | 7.492 | 9.402 | 6.988 | 6.357 | 8.143 | 10.067 |
| 408 | 5.722 | 11.063 | 7.698 | 9.635 | 7.219 | 6.560 | 8.415 | 10.349 |
| 414 | 5.986 | 11.366 | 7.987 | 9.949 | 7.475 | 6.860 | 8.693 | 10.673 |
| 420 | 6.128 | 11.536 | 8.163 | 10.114 | 7.649 | 7.010 | 8.821 | 10.884 |
| 426 | 6.218 | 11.673 | 8.294 | 10.273 | 7.835 | 7.117 | 8.916 | 11.047 |
| 432 | 6.425 | 11.912 | 8.544 | 10.515 | 8.058 | 7.341 | 9.118 | 11.311 |
| 438 | 6.682 | 12.203 | 8.835 | 10.820 | 8.316 | 7.624 | 9.378 | 11.597 |
| 444 | 6.820 | 12.359 | 9.001 | 10.980 | 8.460 | 7.772 | 9.512 | 11.764 |
| 450 | 6.898 | 12.480 | 9.116 | 11.109 | 8.390 | 7.774 | 9.537 | 11.983 |
| 456 | 7.020 | 12.726 | 9.335 | 11.345 | 8.530 | 7.957 | 9.713 | 12.285 |
| 462 | 7.309 | 12.989 | 9.767 | 11.632 | 8.713 | 8.304 | 9.993 | 12.682 |
| 468 | 7.395 | 13.120 | 9.885 | 11.769 | 8.805 | 8.349 | 10.066 | 12.829 |
| 474 | 7.495 | 13.234 | 10.040 | 11.893 | 8.912 | 8.431 | 10.146 | 12.992 |
| 480 | 7.676 | 13.424 | 10.288 | 12.092 | 9.084 | 8.602 | 10.305 | 13.216 |
| 486 | 7.909 | 13.669 | 10.562 | 12.359 | 9.289 | 8.817 | 10.514 | 13.467 |
| 492 | 8.052 | 13.829 | 10.760 | 12.519 | 9.425 | 8.943 | 10.630 | 13.647 |
| 498 | 8.113 | 14.388 | 10.859 | 12.606 | 9.493 | 8.991 | 10.708 | 13.733 |
| 504 | 8.321 | 14.498 | 11.119 | 12.672 | 9.564 | 9.272 | 10.935 | 13.980 |
| 510 | 8.546 | 14.647 | 11.206 | 12.721 | 9.694 | 9.447 | 11.130 | 14.182 |
| 516 | 8.620 | 14.768 | 11.364 | 12.844 | 9.787 | 9.533 | 11.243 | 14.336 |
| 522 | 8.682 | 14.746 | 11.507 | 12.962 | 9.889 | 9.606 | 11.336 | 14.474 |
| 528 | 8.838 | 14.913 | 11.699 | 13.119 | 10.034 | 9.733 | 11.454 | 14.625 |
| 534 | 9.093 | 15.157 | 11.993 | 13.374 | 10.268 | 9.965 | 11.691 | 14.869 |
| 540 | 9.262 | 15.318 | 12.221 | 13.526 | 10.411 | 10.114 | 11.844 | 15.050 |
| 546 | 9.400 | 15.465 | 12.424 | 13.680 | 10.551 | 10.239 | 11.986 | 15.212 |
| 552 | 9.597 | 15.660 | 12.672 | 13.875 | 10.721 | 10.409 | 12.181 | 15.416 |
| 558 | 9.885 | 15.930 | 12.993 | 14.151 | 10.960 | 10.661 | 12.463 | 15.689 |
| 564 | 10.050 | 16.106 | 13.225 | 14.310 | 11.110 | 10.811 | 12.639 | 15.876 |

**Table 17.** Cumulative $CO_2$ production (mg C) resulting from 24 day interval additions of trace amounts of 'DOC + nutrients' and 'nutrients only' to 7.3 g dry soil equivalent peat soil. Data determined using a multi-chambered respirometer.

| Time / h | Cumulative $CO_2$ production (mg C) following 24 day interval additions | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DOC + nutrients | | | | nutrients only | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6 | 0.225 | 0.212 | 0.250 | 0.190 | 0.213 | 0.242 | 0.137 | 0.187 |
| 12 | 0.332 | 0.298 | 0.352 | 0.224 | 0.324 | 0.371 | 0.175 | 0.241 |
| 18 | 0.399 | 0.353 | 0.428 | 0.255 | 0.444 | 0.480 | 0.206 | 0.286 |
| 24 | 0.495 | 0.453 | 0.543 | 0.329 | 0.603 | 0.613 | 0.288 | 0.375 |
| 30 | 0.651 | 0.610 | 0.722 | 0.491 | 0.831 | 0.798 | 0.432 | 0.532 |
| 36 | 0.714 | 0.674 | 0.793 | 0.524 | 0.952 | 0.900 | 0.491 | 0.582 |
| 42 | 0.751 | 0.710 | 0.849 | 0.547 | 1.056 | 0.973 | 0.518 | 0.621 |
| 48 | 0.841 | 0.802 | 0.950 | 0.622 | 1.215 | 1.089 | 0.602 | 0.709 |
| 54 | 0.993 | 0.952 | 1.121 | 0.788 | 1.432 | 1.254 | 0.747 | 0.864 |
| 60 | 1.056 | 1.015 | 1.193 | 0.830 | 1.563 | 1.344 | 0.803 | 0.919 |
| 66 | 1.051 | 1.001 | 1.204 | 0.966 | 1.583 | 1.312 | 0.798 | 1.068 |
| 72 | 1.138 | 1.061 | 1.259 | 1.055 | 1.633 | 1.380 | 0.691 | 1.142 |
| 78 | 1.294 | 1.191 | 1.387 | 1.235 | 1.726 | 1.528 | 0.840 | 1.290 |
| 84 | 1.359 | 1.235 | 1.413 | 1.272 | 1.746 | 1.616 | 0.877 | 1.338 |
| 90 | 1.386 | 1.241 | 1.408 | 1.295 | 1.731 | 1.649 | 0.880 | 1.353 |
| 96 | 1.494 | 1.327 | 1.481 | 1.385 | 1.804 | 1.767 | 0.961 | 1.446 |
| 102 | 1.654 | 1.473 | 1.625 | 1.563 | 1.919 | 1.920 | 1.095 | 1.602 |
| 108 | 1.732 | 1.541 | 1.676 | 1.615 | 1.969 | 2.025 | 1.160 | 1.667 |
| 114 | 1.749 | 1.546 | 1.684 | 1.635 | 1.943 | 2.054 | 1.164 | 1.681 |
| 120 | 1.860 | 1.652 | 1.783 | 1.733 | 2.020 | 2.177 | 1.268 | 1.784 |
| 126 | 2.043 | 1.829 | 1.968 | 1.924 | 2.173 | 2.358 | 1.448 | 1.966 |
| 132 | 2.120 | 1.903 | 2.042 | 1.977 | 2.225 | 2.456 | 1.523 | 2.038 |
| 138 | 2.609 | 1.933 | 2.158 | 1.978 | 2.270 | 2.369 | 1.664 | 2.010 |
| 144 | 2.672 | 2.022 | 2.262 | 2.039 | 2.345 | 2.437 | 1.758 | 2.062 |
| 150 | 2.856 | 2.227 | 2.483 | 2.218 | 2.537 | 2.642 | 1.960 | 2.229 |
| 156 | 2.899 | 2.293 | 2.560 | 2.247 | 2.574 | 2.733 | 2.027 | 2.253 |
| 162 | 2.908 | 2.323 | 2.608 | 2.271 | 2.592 | 2.784 | 2.066 | 2.252 |
| 168 | 3.008 | 2.439 | 2.735 | 2.367 | 2.690 | 2.906 | 2.189 | 2.331 |
| 174 | 3.194 | 2.634 | 2.954 | 2.569 | 2.867 | 3.098 | 2.393 | 2.504 |
| 180 | 3.267 | 2.713 | 3.042 | 2.626 | 2.922 | 3.197 | 2.480 | 2.550 |
| 186 | 3.332 | 2.780 | 3.125 | 2.693 | 2.967 | 3.264 | 2.559 | 2.601 |
| 192 | 3.466 | 2.913 | 3.270 | 2.822 | 3.074 | 3.390 | 2.706 | 2.721 |
| 198 | 3.672 | 3.118 | 3.502 | 3.048 | 3.262 | 3.588 | 2.928 | 2.930 |
| 204 | 3.766 | 3.216 | 3.606 | 3.126 | 3.331 | 3.685 | 3.040 | 3.021 |
| 210 | 3.796 | 3.244 | 3.646 | 3.161 | 3.343 | 3.716 | 3.088 | 3.055 |
| 216 | 3.852 | 3.303 | 3.704 | 3.198 | 3.351 | 3.919 | 3.103 | 3.098 |
| 222 | 4.056 | 3.511 | 3.908 | 3.371 | 3.482 | 3.807 | 3.263 | 3.274 |
| 228 | 4.144 | 3.606 | 3.985 | 3.388 | 3.581 | 3.901 | 3.322 | 3.333 |
| 234 | 4.216 | 3.681 | 4.058 | 3.425 | 3.590 | 3.943 | 3.367 | 3.383 |
| 240 | 4.369 | 3.842 | 4.205 | 3.531 | 3.693 | 4.055 | 3.499 | 3.513 |
| 246 | 4.593 | 4.066 | 4.437 | 3.729 | 3.865 | 4.233 | 3.709 | 3.734 |
| 252 | 4.708 | 4.188 | 4.634 | 3.808 | 3.938 | 4.334 | 3.833 | 3.853 |
| 258 | 4.783 | 4.273 | 4.736 | 3.877 | 3.975 | 4.384 | 3.933 | 3.950 |
| 264 | 4.950 | 4.447 | 4.912 | 4.024 | 4.109 | 4.537 | 4.128 | 4.140 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 270 | 5.208 | 4.712 | 5.182 | 4.278 | 4.339 | 4.776 | 4.417 | 4.427 |
| 276 | 5.320 | 4.836 | 5.300 | 4.379 | 4.418 | 4.894 | 4.566 | 4.569 |
| 282 | 5.346 | 5.110 | 5.340 | 4.428 | 4.424 | 4.919 | 4.620 | 4.719 |
| 288 | 5.405 | 5.182 | 5.423 | 4.555 | 4.611 | 4.995 | 4.760 | 4.867 |
| 294 | 5.654 | 5.457 | 5.682 | 4.824 | 4.914 | 5.283 | 5.096 | 5.224 |
| 300 | 5.773 | 5.619 | 5.851 | 4.943 | 4.986 | 5.400 | 5.261 | 5.398 |
| 306 | 5.754 | 5.642 | 5.893 | 5.014 | 4.941 | 5.329 | 5.240 | 5.422 |
| 312 | 5.921 | 5.843 | 6.090 | 5.198 | 5.106 | 5.512 | 5.474 | 5.655 |
| 318 | 6.164 | 6.115 | 6.365 | 5.477 | 5.339 | 5.743 | 5.768 | 5.956 |
| 324 | 6.303 | 6.282 | 6.514 | 5.607 | 5.456 | 5.912 | 5.989 | 6.163 |
| 330 | 6.363 | 6.365 | 6.605 | 5.708 | 5.501 | 5.930 | 6.077 | 6.254 |
| 336 | 6.654 | 6.670 | 6.845 | 5.930 | 5.707 | 6.231 | 6.439 | 6.549 |
| 342 | 6.842 | 6.889 | 7.094 | 6.193 | 5.927 | 6.379 | 6.656 | 6.805 |
| 348 | 6.976 | 7.069 | 7.268 | 6.333 | 6.016 | 6.472 | 6.811 | 6.976 |
| 354 | 7.062 | 7.127 | 7.346 | 6.400 | 6.049 | 6.520 | 6.867 | 7.031 |
| 360 | 7.183 | 7.257 | 7.525 | 6.530 | 6.163 | 6.560 | 6.824 | 7.159 |
| 366 | 7.394 | 7.419 | 7.697 | 6.741 | 6.377 | 6.497 | 7.025 | 7.378 |
| 372 | 7.500 | 7.530 | 7.809 | 6.828 | 6.465 | 6.598 | 7.177 | 7.532 |
| 378 | 7.535 | 7.563 | 7.831 | 6.868 | 6.541 | 6.653 | 7.290 | 7.636 |
| 384 | 7.728 | 7.773 | 8.046 | 7.052 | 6.705 | 6.831 | 7.515 | 7.859 |
| 390 | 7.975 | 8.025 | 8.308 | 7.240 | 6.965 | 7.079 | 7.810 | 8.162 |
| 396 | 8.115 | 8.182 | 8.464 | 7.380 | 7.079 | 7.214 | 7.985 | 8.336 |
| 402 | 8.196 | 8.274 | 8.552 | 7.476 | 7.183 | 7.309 | 8.131 | 8.477 |
| 408 | 8.385 | 8.482 | 8.754 | 7.677 | 7.360 | 7.489 | 8.353 | 8.706 |
| 414 | 8.651 | 8.762 | 9.042 | 7.978 | 7.625 | 7.740 | 8.659 | 9.021 |
| 420 | 8.774 | 8.906 | 9.189 | 8.115 | 7.729 | 7.860 | 8.822 | 9.183 |
| 426 | 8.880 | 9.026 | 9.311 | 8.244 | 7.853 | 7.974 | 8.984 | 9.340 |
| 432 | 9.084 | 9.241 | 9.536 | 8.460 | 8.046 | 8.155 | 9.224 | 9.580 |
| 438 | 9.346 | 9.516 | 9.816 | 8.750 | 8.308 | 8.400 | 9.510 | 9.887 |
| 444 | 9.534 | 9.784 | 9.967 | 8.903 | 8.439 | 8.525 | 9.665 | 10.049 |
| 450 | 9.598 | 9.898 | 10.093 | 9.022 | 8.592 | 8.613 | 9.755 | 10.142 |
| 456 | 9.830 | 10.152 | 10.333 | 9.179 | 8.735 | 8.768 | 9.964 | 10.315 |
| 462 | 10.080 | 10.407 | 10.626 | 9.439 | 8.970 | 8.961 | 10.171 | 10.530 |
| 468 | 10.191 | 10.522 | 10.734 | 9.524 | 9.076 | 9.078 | 10.303 | 10.648 |
| 474 | 10.277 | 10.619 | 10.837 | 9.622 | 9.173 | 9.171 | 10.425 | 10.765 |
| 480 | 10.438 | 10.792 | 11.021 | 9.804 | 9.340 | 9.324 | 10.621 | 10.958 |
| 486 | 10.670 | 11.024 | 11.268 | 10.059 | 9.579 | 9.544 | 10.875 | 11.227 |
| 492 | 10.800 | 11.167 | 11.427 | 10.202 | 9.705 | 9.675 | 11.036 | 11.388 |
| 498 | 10.858 | 11.235 | 11.498 | 10.274 | 9.953 | 9.735 | 11.116 | 11.472 |
| 504 | 10.967 | 11.391 | 11.609 | 10.468 | 10.050 | 9.839 | 11.148 | 11.557 |
| 510 | 11.165 | 11.559 | 11.778 | 10.696 | 10.251 | 9.925 | 11.097 | 11.726 |
| 516 | 11.303 | 11.700 | 11.878 | 10.827 | 10.361 | 9.983 | 11.174 | 11.838 |
| 522 | 11.424 | 11.810 | 11.971 | 10.959 | 10.473 | 10.052 | 11.276 | 11.965 |
| 528 | 11.578 | 11.939 | 12.123 | 11.134 | 10.629 | 10.168 | 11.446 | 12.144 |
| 534 | 11.848 | 12.179 | 12.369 | 11.407 | 10.868 | 10.371 | 11.693 | 12.410 |
| 540 | 12.029 | 12.326 | 12.524 | 11.557 | 10.990 | 10.487 | 11.844 | 12.566 |
| 546 | 12.194 | 12.463 | 12.662 | 11.708 | 11.124 | 10.593 | 11.989 | 12.713 |
| 552 | 12.410 | 12.645 | 12.853 | 11.906 | 11.299 | 10.745 | 12.173 | 12.909 |
| 558 | 12.714 | 12.909 | 13.126 | 12.192 | 11.559 | 10.977 | 12.440 | 13.196 |
| 564 | 12.919 | 13.071 | 13.291 | 12.349 | 11.693 | 11.106 | 12.597 | 13.356 |

**Table 18.** Cumulative $CO_2$ production (mg C) resulting from no additions of trace amounts of 'DOC + nutrients' or 'nutrients only' to 7.3 g dry soil equivalent peat soil. Data determined using a multi-chambered respirometer.

| Time / h | Cumulative $CO_2$ production (mg C) following no additions | | | |
| | no amendment | | | |
| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 0 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6 | 0.211 | 0.162 | 0.149 | 0.180 |
| 12 | 0.269 | 0.207 | 0.193 | 0.243 |
| 18 | 0.317 | 0.243 | 0.223 | 0.292 |
| 24 | 0.403 | 0.322 | 0.300 | 0.387 |
| 30 | 0.564 | 0.468 | 0.430 | 0.543 |
| 36 | 0.605 | 0.513 | 0.477 | 0.598 |
| 42 | 0.633 | 0.531 | 0.494 | 0.626 |
| 48 | 0.708 | 0.612 | 0.566 | 0.714 |
| 54 | 0.859 | 0.752 | 0.690 | 0.864 |
| 60 | 0.899 | 0.797 | 0.745 | 0.925 |
| 66 | 0.922 | 0.775 | 0.718 | 0.921 |
| 72 | 0.956 | 0.797 | 0.744 | 0.978 |
| 78 | 1.068 | 0.867 | 0.791 | 1.090 |
| 84 | 1.068 | 0.918 | 0.862 | 1.163 |
| 90 | 1.041 | 0.639 | 0.793 | 1.143 |
| 96 | 1.086 | 0.682 | 0.830 | 1.231 |
| 102 | 1.210 | 0.795 | 0.899 | 1.341 |
| 108 | 1.227 | 0.834 | 0.950 | 1.554 |
| 114 | 1.200 | 0.779 | 0.879 | 1.571 |
| 120 | 1.258 | 0.857 | 0.947 | 1.682 |
| 126 | 1.402 | 1.017 | 1.073 | 1.855 |
| 132 | 1.426 | 1.068 | 1.122 | 1.947 |
| 138 | 1.711 | 1.251 | 1.204 | 2.035 |
| 144 | 1.755 | 1.308 | 1.261 | 2.132 |
| 150 | 1.909 | 1.461 | 1.407 | 2.319 |
| 156 | 1.914 | 1.498 | 1.454 | 2.389 |
| 162 | 1.898 | 1.467 | 1.564 | 2.400 |
| 168 | 1.953 | 1.516 | 1.630 | 2.493 |
| 174 | 2.108 | 1.674 | 1.781 | 2.662 |
| 180 | 2.124 | 1.711 | 1.840 | 2.737 |
| 186 | 2.142 | 1.708 | 1.840 | 2.756 |
| 192 | 2.217 | 1.793 | 1.928 | 2.860 |
| 198 | 2.390 | 1.962 | 2.082 | 3.036 |
| 204 | 2.422 | 2.019 | 2.154 | 3.118 |
| 210 | 2.420 | 1.994 | 2.129 | 3.112 |
| 216 | 2.434 | 1.983 | 2.079 | 3.139 |
| 222 | 2.564 | 2.101 | 2.162 | 3.086 |
| 228 | 2.559 | 2.121 | 2.199 | 3.143 |
| 234 | 2.547 | 2.082 | 1.948 | 3.120 |
| 240 | 2.603 | 2.154 | 2.010 | 3.206 |
| 246 | 2.746 | 2.293 | 2.123 | 3.356 |
| 252 | 2.769 | 2.332 | 2.162 | 3.426 |
| 258 | 2.768 | 2.298 | 2.114 | 3.419 |
| 264 | 2.848 | 2.390 | 2.187 | 3.533 |

| | | | | |
|------|-------|-------|-------|-------|
| 270 | 3.028 | 2.594 | 2.351 | 3.740 |
| 276 | 3.056 | 2.635 | 2.384 | 3.818 |
| 282 | 3.039 | 2.817 | 2.316 | 3.791 |
| 288 | 3.141 | 2.903 | 2.362 | 3.845 |
| 294 | 3.375 | 3.115 | 2.586 | 4.063 |
| 300 | 3.432 | 3.065 | 2.529 | 4.057 |
| 306 | 3.392 | 2.978 | 2.391 | 3.952 |
| 312 | 3.511 | 3.087 | 2.486 | 4.079 |
| 318 | 3.715 | 3.267 | 2.627 | 4.243 |
| 324 | 3.792 | 3.290 | 2.645 | 4.305 |
| 330 | 3.783 | 3.280 | 2.605 | 4.296 |
| 336 | 3.939 | 3.391 | 2.691 | 4.428 |
| 342 | 4.115 | 3.582 | 2.840 | 4.611 |
| 348 | 4.162 | 3.602 | 2.846 | 4.672 |
| 354 | 4.323 | 3.574 | 2.801 | 4.686 |
| 360 | 4.516 | 3.581 | 2.605 | 4.738 |
| 366 | 4.763 | 3.748 | 2.760 | 4.912 |
| 372 | 4.878 | 3.754 | 2.776 | 4.970 |
| 378 | 4.959 | 3.745 | 2.766 | 5.010 |
| 384 | 5.134 | 3.812 | 2.830 | 5.133 |
| 390 | 5.396 | 4.000 | 2.999 | 5.360 |
| 396 | 5.511 | 4.018 | 3.027 | 5.464 |
| 402 | 5.597 | 4.043 | 3.051 | 5.557 |
| 408 | 5.759 | 4.131 | 3.139 | 5.716 |
| 414 | 6.013 | 4.330 | 3.335 | 5.956 |
| 420 | 6.098 | 4.337 | 3.363 | 6.049 |
| 426 | 6.198 | 4.387 | 3.420 | 6.160 |
| 432 | 6.366 | 4.505 | 3.544 | 6.336 |
| 438 | 6.610 | 4.719 | 3.750 | 6.580 |
| 444 | 6.760 | 4.808 | 3.996 | 6.705 |
| 450 | 6.898 | 4.844 | 4.013 | 6.858 |
| 456 | 7.055 | 4.874 | 4.056 | 6.985 |
| 462 | 7.280 | 5.061 | 4.252 | 7.218 |
| 468 | 7.362 | 5.096 | 4.312 | 7.333 |
| 474 | 7.445 | 5.128 | 4.353 | 7.436 |
| 480 | 7.596 | 5.239 | 4.465 | 7.604 |
| 486 | 7.823 | 5.434 | 4.654 | 7.837 |
| 492 | 7.931 | 5.489 | 4.718 | 7.968 |
| 498 | 7.987 | 5.514 | 4.747 | 8.039 |
| 504 | 8.090 | 5.484 | 4.554 | 8.033 |
| 510 | 8.266 | 5.613 | 4.679 | 8.170 |
| 516 | 8.345 | 5.622 | 4.700 | 8.233 |
| 522 | 8.439 | 5.681 | 4.748 | 8.310 |
| 528 | 8.578 | 5.777 | 4.841 | 8.443 |
| 534 | 8.809 | 5.976 | 5.027 | 8.667 |
| 540 | 8.923 | 6.039 | 5.101 | 8.796 |
| 546 | 9.051 | 6.127 | 5.181 | 8.932 |
| 552 | 9.223 | 6.258 | 5.306 | 9.115 |
| 558 | 9.481 | 6.492 | 5.528 | 9.381 |
| 564 | 9.608 | 6.577 | 5.620 | 9.535 |

## B.3.5 <u>Investigation 2</u>

**Table 19.** Cumulative $CO_2$ production (mg C) resulting from 3.5 day interval additions of trace amounts of 'DOC + nutrients', 'nutrients only' and 'DOC only' to 7.3 g dry soil equivalent peat soil. Data determined using a gas chromatograph(Varian Aerograph 90-P).

| Time / h | Cumulative $CO_2$ production (mg C) following 3.5 day interval additions | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | DOC + nutrients | | | nutrients only | | | DOC only | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 84 | 0.10 | 0.20 | 0.17 | 0.09 | -0.01 | 0.06 | 0.25 | 0.03 | 0.02 |
| 168 | 0.61 | 0.58 | 0.49 | 0.57 | 0.47 | 0.54 | 0.69 | 0.44 | 0.41 |
| 252 | 1.03 | 0.98 | 0.95 | 1.11 | 0.99 | 1.12 | 1.17 | 0.93 | 0.91 |
| 336 | 1.38 | 1.53 | 1.32 | 1.74 | 1.70 | 1.78 | 1.90 | 1.66 | 1.51 |
| 420 | 1.97 | 2.05 | 1.80 | 2.08 | 2.19 | 2.18 | 2.31 | 1.92 | 1.77 |
| 504 | 2.56 | 2.48 | 2.30 | 2.58 | 2.67 | 2.63 | 2.70 | 2.45 | 2.23 |
| 588 | 2.97 | 2.88 | 2.70 | 2.94 | 3.07 | 2.94 | 3.05 | 2.94 | 2.46 |
| 672 | 3.16 | 3.10 | 2.98 | 3.39 | 3.32 | 3.16 | 3.40 | 3.25 | 2.64 |
| 756 | 3.46 | 3.44 | 3.34 | 3.68 | 3.73 | 3.41 | 3.60 | 3.59 | 3.09 |
| 840 | 3.72 | 3.65 | 3.59 | 3.92 | 3.99 | 3.64 | 3.83 | 3.77 | 3.32 |

**Table 20.** Cumulative $CO_2$ production (mg C) resulting from 7 day interval additions of trace amounts of 'DOC + nutrients', 'nutrients only' and 'DOC only' to 7.3 g dry soil equivalent peat soil. Data determined using a gas chromatograph (Varian Aerograph 90-P).

| Time / h | Cumulative $CO_2$ production (mg C) following 7 day interval additions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DOC + nutrients | | | nutrients only | | | DOC only | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 84 | 0.10 | 0.22 | 0.18 | 0.10 | 0.07 | 0.08 | 0.06 | 0.06 | 0.06 |
| 168 | 0.68 | 0.77 | 0.69 | 0.49 | 0.33 | 0.46 | 0.32 | 0.38 | 0.35 |
| 252 | 1.40 | 1.44 | 1.36 | 1.13 | 1.01 | 1.05 | 0.74 | 0.81 | 0.79 |
| 336 | 2.06 | 2.89 | 1.86 | 1.95 | 1.63 | 2.12 | 1.36 | 1.35 | 1.37 |
| 420 | 2.95 | 3.83 | 2.80 | 2.24 | 1.88 | 2.39 | 1.75 | 1.74 | 1.78 |
| 504 | 3.43 | 4.29 | 3.38 | 2.63 | 2.19 | 2.99 | 2.16 | 2.25 | 2.19 |
| 588 | 4.02 | 4.96 | 4.10 | 3.14 | 2.66 | 3.33 | 2.52 | 2.84 | 2.65 |
| 672 | 4.34 | 5.31 | 4.43 | 3.31 | 2.93 | 3.87 | 2.73 | 3.17 | 3.03 |
| 756 | 4.81 | 5.65 | 5.00 | 3.60 | 3.25 | 4.19 | 3.10 | 3.54 | 3.40 |
| 840 | 5.05 | 6.02 | 5.34 | 3.75 | 3.53 | 4.47 | 3.30 | 3.87 | 3.62 |

**Table 21.** Cumulative CO2 production (mg C) resulting from 10.5 day interval additions of trace amounts of 'DOC + nutrients', 'nutrients only' and 'DOC only' to 7.3 g dry soil equivalent peat soil. Data determined using a gas chromatogram (Varian Aerograph 90-P).

| Time / h | Cumulative $CO_2$ production (mg C) following 10.5 day interval additions | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DOC + nutrients | | | nutrients only | | | DOC only | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 84 | 0.11 | 0.06 | 0.03 | 0.03 | 0.14 | 0.06 | -0.04 | 0.06 | 0.07 |
| 168 | 0.40 | 0.42 | 0.35 | 0.65 | 0.79 | 0.71 | 0.42 | 0.39 | 0.49 |
| 252 | 0.90 | 0.99 | 0.90 | 1.26 | 1.38 | 1.32 | 0.93 | 0.82 | 0.98 |
| 336 | 1.82 | 1.96 | 1.69 | 1.89 | 2.05 | 1.99 | 1.47 | 1.32 | 1.74 |
| 420 | 2.23 | 2.33 | 2.16 | 2.43 | 2.53 | 2.46 | 1.69 | 1.77 | 2.38 |
| 504 | 3.25 | 3.27 | 3.06 | 3.17 | 3.12 | 3.04 | 2.31 | 2.20 | 2.73 |
| 588 | 4.24 | 4.18 | 4.09 | 3.91 | 3.82 | 3.75 | 3.01 | 2.90 | 3.52 |
| 672 | 4.97 | 4.76 | 4.70 | 4.41 | 4.45 | 4.32 | 3.28 | 3.38 | 3.91 |
| 756 | 5.61 | 5.49 | 5.53 | 4.88 | 4.92 | 4.86 | 3.64 | 3.71 | 4.41 |
| 840 | 6.43 | 6.09 | 6.15 | 5.45 | 5.40 | 5.40 | 3.96 | 4.42 | 5.35 |