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The effects of acidity on recent changes in carbon cycling in organic soils

Thesis submitted for the degree of Doctor of Philosophy

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Declaration:

I confirm that this my own work and the use of all material from other sources has been properly and fully acknowledged.

Signed: Catharine M Pschenyckyj

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Abstract

There has been an observed increase in dissolved organic carbon (DOC) concentrations in soil solutions and surface water bodies in acid sensitive areas of Europe and North America over the past four decades. This has been linked to changes in atmospheric chemistry and associated acid deposition, due to increased solubility of DOC in response to recovery from acidification. However, as DOC production (and consumption) is under biological control through the decomposition (and mineralisation) of organic material, there is uncertainty as to whether this increasing DOC trend is solely a chemical (solubility) response or whether there is a biological element also. In addition, there have been inconsistencies in DOC release from catchments receiving similar acid deposition loads, which suggests that differences in catchment characteristics may result in variations in the magnitude of response to acidification and recovery. Despite this and that fact that many catchments consist of peat and organo-mineral soil, much research has focused solely on peat.

In order to investigate both the chemical and biological responses to changing acidity behind these DOC trends, an acidity manipulation field experiment was run over two National Parks with contrasting historical pollution levels, which included both peat and peaty podzol soil. The chemistry of pore water, as well as soil and surface litter extracts, were monitored alongside a decomposition experiment to separate out the changing solubility of DOC from the biological production through decomposition. Bacterial and fungal communities were also sequenced to assess how microbial communities were affected by changes in acidity.

There was a clear chemically mediated DOC response to acidity in pore water, supporting previous findings and building on evidence of the pH-DOC hypothesis that recovery from acidification is increasing DOC solubility in organic soils. The DOC in the upper organic layer of peat and organo-mineral soil was found to be acid sensitive, but the surface litter DOC was not. However, overall there were limited responses of litter decomposition, Tea Bag Index (TBI) parameters and microbial diversity to acidity manipulations, and so there is little evidence that short-term changes in acidity effect microbial communities and biologically mediated processes (decomposition and associated DOC production).

Regardless of experimental insignificance, bacterial community diversity was found to be positively and significantly related to both soil pH and extract DOC, which suggests that there may be a functional response to changing acidity as well as changes in community structure. Further work is needed to assess the mechanistic functional response of bacteria in terms of DOC production and consumption in response to changing acidity.

Glossary of Terminology

ANOVA – Analysis of variance

ATP – Adenosine triphosphate

Au – Absorbance units

AWMN – Acid Waters Monitoring Network

C – Carbon

CEC – Cation exchange capacity

CO_2 – Carbon dioxide

CH_4 – Methane

dNTP – Deoxynucleotide

DOC – Dissolved organic carbon

DOM – Dissolved organic matter

DON – Dissolved organic nitrogen

DOP – Dissolved organic phosphorus

EC – Electrical conductivity

ER- Ecosystem respiration

GPP – Gross primary production

H_2O – Water

HMW – High molecular weight

Internal Transcribe Spacer – ITS

K – Decomposition rate

Kt – Kilotonnes

L – Litre

LMW – Low molecular weight

N – Nitrogen

NECB – Net ecosystem carbon balance

NEE – Net ecosystem exchange

NPP – Net primary production

rpm – reps per minute

S – Sulphur

S – Stabilisation factor

SD – Standard deviation

SO_2 – Sulphur dioxide

SO_4 – Sulphate

SOM – Soil organic matter

SUVA – Specific ultra-violet absorbance

PCR – Polymerase Chain Reaction

Pg - Petagram

POC – Particulate organic carbon

TBI – Tea bag index

TEA – Terminal electron acceptor

TED – Terminal electron donor

UK – United Kingdom

UKEAP – Eutrophying and Acidifying Atmospheric Pollutants

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Chapter 1: Introduction

1.1 Research context

Upland moorland catchments dominated by organic soils are important stores of carbon, providing mitigation against climate change; hydrological services such as water quality and flood risk regulation; tourism, recreation and cultural heritage; and other important ecosystem services. These organic upland catchments usually consist of a mix of peat on shallow slopes and organo-mineral soil on sloped areas. Milne and Brown (1997) estimate raw peat soils to cover an area of 3568 km^2 in England and Wales, and contain an estimated 400,166 kt of carbon. Peaty podzols are a common organo-mineral soil present in upland bogs, and are estimated to cover 1,313 km^2 and contain 25,400 kt of carbon in England and Wales. Peat and organo-mineral soils differ in their properties including hydrology, texture, and vegetation communities, all of which have been shown to correspond with dissolved organic carbon (DOC) concentrations in pore water (Dalva and Moore, 1991, Neff and Asner, 2001, van den Berg et al., 2012). The key differences between peat and organo-mineral soils relates to hydrological properties. Peats form in areas with impeded drainage and so tend to have slow hydraulic conductivity and form in areas with high water tables, resulting in permanently saturated conditions. In contrast, podzols form on slopes and hills over mineral material which is more free-draining, allowing for aeration of the soil. In addition, unlike organic-rich peat, podzols have a mineral content, with the potential for sorption of DOC (Stutter et al., 2012).

DOC also represents a major natural carbon export from peatlands and other organic rich peaty soils (Billett et al., 2004, Clark et al., 2007, Dinsmore et al., 2010, Hope et al., 1994). DOC is typically defined as organic carbon less than 0.45 µm in size (Thurman, 1985). DOC production is a biological process, either via the release of exudates from plants, or through microbial decomposition of polymeric particulate organic material. Therefore, waters draining catchments comprised of organic rich soils such as peat and peaty podzols are associated with high concentrations of DOC released during the decomposition of organic material (Aitkenhead et al., 1999, Evans et al., 2006a). However, much of the literature focuses on DOC exported from peatland areas, with little investment into the role of organo-mineral soils on DOC dynamics in upland non-forested catchments.

There has been a considerable increase in DOC concentrations in terrestrial waters draining catchments dominated by organic soils in much of the Northern Hemisphere since the 1980's (Couture et al., 2012, Evans et al., 2005, Monteith et al., 2007, Oulehle and Hruška, 2009, SanClements et al., 2012a). For instance, the Acid Waters Monitoring Network recorded a 91 % increase in DOC concentrations in surface waters between 1988 and 2003 in acid sensitive areas of the UK (Evans et al., 2006a). There has also been an increase in the amount of high molecular weight, coloured DOC of an aromatic and refractory nature leaching from peatlands, contributing to the 'brownification' of many terrestrial waters (Watts et al., 2001, Worrall et al., 2003a).

This increase in DOC export from peatlands and other organic soils not only affects carbon budgets (Dinsmore et al., 2010), but also creates expensive implications for water companies due to the removal of DOC through drinking water treatment processes (Ritson et al., 2014). In addition, greater DOC concentrations in terrestrial waters can also affect the functioning of aquatic ecosystems by influencing acidity (Eshleman and Hemond, 1985), bioaccumulation of organic chemicals (Haitzer et al., 1998), transport of trace metals (Lawlor and Tipping, 2003), nutrient (Stewart and Wetzel, 1981) and energy supply (Wetzel, 1992) and light absorbance (Schindler, 1971) and photochemistry (Scully et al., 2003). Therefore there is a dire need to understand these changing carbon dynamics.

Several explanations for this increasing DOC trend have been proposed in the literature and include changes in land management (Clutterbuck and Yallop, 2010), hydrology (Hejzlar et al., 2003, Tranvik and Jansson, 2002), increasing temperature (Freeman et al., 2001a), and nitrogen deposition (Aitkenhead and McDowell, 2000). However, there is now a large evidence base for the reduced deposition of sulphur as a major driver. From 1970 to 2013 there has been a 94 % decline SO_2 emissions in the UK (Defra, 2013). The UK Environmental Change Network highlights recovery from acidification as one of the three most significant long-term trends in the physical environment within the UK (Morecroft et al., 2009). This recovery from acidification as a result of reductions in atmospheric sulphur deposition, increases DOC solubility as soil pH recovers, releasing previously insoluble DOC from soils. This is widely supported by field (Ekström et al., 2011, Evans et al., 2008a, Evans et al., 2012, Moldan et al., 2012, Oulehle et al., 2013) and laboratory experiments (Clark et al., 2011, Palmer et al., 2013) as well as modelling (Evans et al., 2008a, Monteith et al., 2007, Rowe et

al., 2014, Sawicka et al., 2016) and field observations (Evans et al., 2006a, Oulehle et al., 2011, Oulehle et al., 2017, Oulehle and Hruška, 2009).

However, there have been discrepancies in rates of DOC release in areas receiving similar acidifying deposition loads, suggesting catchment specific properties may be a controlling factor in DOC production and release (Clark et al., 2010a). While organic catchments experiencing this increasing DOC release are comprised of peat and organo-mineral soils, the majority of research in this area has focused on peats only. Laboratory experiments have suggested that it is possible that the magnitude of response of peat and organo-mineral soil to acidity may differ, and this may account for some of the discrepancies in DOC release between catchments (Clark et al., 2011). In addition, as soil pH is crucial to enzyme functioning (Fog, 1988), and is highly correlated with microbial community structure (Griffiths et al., 2011), acidification could alter mechanisms involved in microbial decomposition of organic material and the subsequent DOC released. Therefore, it is possible there may be an additional biological mechanism behind these changing trends in response to changing sulphur deposition and recovery from acidification. We need to disentangle the role of biological processes controlling DOC production from chemical processes controlling DOC mobility.

These significant increases in carbon fluxes have raised concerns over the future of terrestrial carbon stocks (Freeman et al., 2001a) as well as contributing to accelerated climate change (Moody et al., 2013). Therefore it is vital we have a complete understanding of the mechanisms behind DOC dynamics in upland organic catchments in order to accurately predict future carbon release and responses to environmental change.

1.2 Aims and objectives

Aim: To understand how changes in acidity have contributed to recent changes in carbon cycling in organic soils in terms of chemical vs biological controls.

Objective 1: To assess how acidity effects DOC quantity and quality released from organic soils and surface litters across different sites representing a pollution deposition gradient.

Objective 2: To assess how litter type and quality effects decomposition and subsequent DOC production over a pollution deposition gradient in peat and organo-mineral soils.

Objective 3: To assess how acidity impacts litter decomposition and the associated DOC produced in peat and organo-mineral soil.

Objective 4: To evaluate whether microbial communities in peat and organo-mineral soil respond to acidification.

1.3 Explanation of objectives

Objective 1: To assess how acidity effects DOC quantity and quality released from organic soils and surface litters across different sites representing a pollution deposition gradient.

Recent studies have suggested that, in intact systems, the age of DOC in surface waters is less than 40 years old (Evans et al., 2007, Palmer et al., 2001, Tipping et al., 2010), and hydrological studies have shown that the surface layers are better connected with stream water DOC concentrations than deeper soil pore water (Billett et al., 2006, Clark et al., 2008). Radiocarbon ^{14}C dating within peatland catchments have demonstrated that between 96 - 100 % of DOC in surface water was recently produced and derived from the peat surface layer (Tipping et al., 2010).

While such field evidence shows that DOC flushed into water systems is recently formed and derives from the surface layer, it is not yet clear how DOC production is partitioned in this dynamic upper segment between decomposing surface litter and recently formed peat organic matter, and whether there is any difference in DOC properties and their sensitivity to environmental change such as acidification. In addition, much research to date has focused on peatlands, with less attention given to processes in freely-draining organo-mineral soils in non-forested environments. By conducting an acidity manipulation field experiment and monitoring the sensitivity of pore water, peat and surface litter samples, the production and release of DOC from different components of the upper organic layer of peat and podzol in response to changing acidity were investigated.

Objective 2: To assess how litter type and quality effects decomposition and subsequent DOC production over a pollution deposition gradient in peat and organo-mineral soils.

Litter decomposition is a major biological source of DOC through the biodegradation of polymeric particulate organic material by extracellular enzymes. The decomposition and subsequent quantity and quality of DOC produced can differ between different vegetation

species (Moore et al., 2007, Pinsonneault et al., 2016). Vascular plants, such as *Calluna*, have been shown to produce more DOC than *Sphagnum* in peatland ecosystems (Armstrong et al., 2012, Ritson et al., 2014). In addition, the release of *Calluna* flower capsules provides a seasonal input of labile material, with a potential to decompose quickly and produce aromatic DOC which is persistent in waters (Ritson et al., 2016).

Environmental factors can also influence the quality of litter and therefore decomposition and DOC production. Nitrogen accumulation in plant tissues has been observed in areas of high deposition (Berg and Matzner, 1997, Caporn et al., 2014, Van Vuuren and Van Der Eerden, 1992). For instance, Pitcairn et al. (1995) found *Calluna* to have a linear increase in tissue nitrogen of 0.045 mg g^{-1} kg^{-1} ha^{-1} $year^{-1}$ of increased atmospheric nitrogen deposition. There is also evidence that vascular plants are more efficient at utilising nitrogen, and so providing decomposers with a greater nutrient availability and enhancing DOC production (Ritson et al., 2016).

Here we investigate the decomposition and subsequent DOC produced from two litter types typical to upland organic catchments (*Eriophorum* and *Calluna*). As there are a lack of published studies presenting decomposition data for organo-mineral soil in a non-forested environment, the role of podzol soil and associated DOC production could be assessed here and compared to peat. A litter bag experiment was used to investigate decomposition over a 12 month period over a two sites with contrasting historical pollution deposition, and how this varied for each soil type. Therefore, the effect of pollution deposition on litter quality, and they subsequent effect this had on decomposition and DOC production for both soil types could also be investigated.

Objective 3: To assess how acidity impacts litter decomposition and the associated DOC produced in peat and organo-mineral soil.

To date, most attention has been given to understanding the chemical mobility of DOC within soils and surface waters. However, as recovery from acidification changes soil pH to more favourable conditions for biological activity, it is unclear to what extent increased DOC concentrations could have been driven by increased decomposition and, therefore, DOC production. Recent modelling analysis has indicated that increased nitrogen deposition could increase litter production, which in turn would stimulate DOC production (Sawicka et

al., 2017). The direct effects of acidity itself on DOC production from litter decomposition are poorly understood, with many studies focusing on nitrogen deposition effects (Berg and Matzner, 1997, Bragazza et al., 2012, Knorr et al., 2005, Lovett and Goodale, 2011, Manning et al., 2008) rather than acidification (Evans et al., 2008a). However, nitrogen deposition causes simultaneous acidification and this may be contributing to changing carbon dynamics (Oulehle et al., 2018), and yet there has been little attempt to separate out the individual responses of nitrogen enrichment and acidification.

Of the little research there is on the effects of acidity on litter decomposition, results are conflicting. There is some evidence of shifts in microbial community structure and suppressed decomposition with acidity but these focus on forested ecosystems (Adams and Angradi, 1996, Baath et al., 1980, Dangles et al., 2004, Oulehle et al., 2018, Prescott and Parkinson, 1985). Suppression of decomposition of *Calluna* and *Eriophorum* litter has been observed in a peat monolith acidification experiment (Sanger et al., 1993), whilst no effects have been shown for *Sphagnum* in a poor fen environment (Rochefort et al., 1990). Other studies show acidity as having a minimal effect on decomposition with other abiotic factors being more influential such as soil moisture content (Donnelly et al., 1990, Rochefort et al., 1990).

Therefore to tackle these knowledge gaps, a decomposition study using litter bags and the Tea Bag Index (TBI) was incorporated into an acidity manipulation field experiment to investigate how acidity impacts the decomposition of five litter types common to upland organic catchments, and the resulting effect on the DOC produced, across two sites representing a pollution deposition gradient, for both peat and organo-mineral soil.

Objective 4: To evaluate whether microbial communities in peat and organo-mineral soil respond to acidification.

Despite there being evidence for the important role that microorganisms play in moorland functioning, there is little research into microbial community composition and their associated functioning (Littlewood et al., 2010), or how environmental change may influence these communities and functions (Thormann, 2006). There is further little research comparing communities between peat and organo-mineral soil, despite the fact that upland moorland catchments are typically comprised of both soil types.

As soil pH is crucial to enzyme functioning (Fog, 1988), and is highly correlated with microbial community structure (Griffiths et al., 2011), it is possible that acidification could alter mechanisms involved in microbial decomposition of organic material. Using a mixed-effects model, Dawson et al. (2009) concluded that increased solubility of DOC alongside enhanced heterotrophic decomposition are behind increasing DOC trends for upland organic catchments in Scotland. Microbial community structure has been shown to be affected by acidity, with decreased bacteria and increased fungi in soils (Blagodatskaya and Anderson, 1998, Oulehle et al., 2018, Rousk et al., 2009), with bacterial growth rates being more sensitive (Walse et al., 1998). Reduced litter decomposition rates have been demonstrated under acidic conditions (Adams and Angradi, 1996, Baath et al., 1980, Dangles et al., 2004, Killham and Wainwright, 1981, Oulehle et al., 2017), including in peat (Sanger et al., 1993) and podzol soil (Brown, 1985), demonstrating reduced DOC production with acidity. In addition, particular inhibition of microbial decomposition has been noted at sites with high sulphur pollution (Brown, 1985, Prescott and Parkinson, 1985).

Therefore, it is possible that changes in acidity may alter microbial communities and their functioning in terms of DOC production and consumption in organic catchments. In order to investigate further whether the increasing DOC trend may be due to a biological response to changing acidity, fungal and bacterial communities were sequenced from soils receiving acid and alkaline treatments, and correlations between changes in communities and soil extract DOC could be explored.

1.4 Thesis structure

This thesis is comprised of eight chapters. Chapter 2 is a review of the literature relevant to this research, and provides context and detailed discussion of relevant topics to-date. Chapter 3 is a detailed description of the field experiment underpinning each results chapters, including treatment applications, sample collections and site locations. Chapters 4-7 are the results chapters, presented in journal paper form, that tackle each objective separately. As a result, they are intended to be stand-alone chapters to be submitted for publication, and so some repetition is apparent in the introduction and methods sections. Chapter 4 addresses Objective 1 by presenting the results of one year monitoring data from the field experiment for pore water, peat and surface litter samples. Both Chapters 5 and 6 present data from a year-long litter bag experiment run as part of the field experiment. By

analysing litter decomposition and DOC production data of different litter types over a twelve month period, and presenting data from a litter bag translocation experiment, Objective 2 could be addressed within Chapter 5. The effects of acidity on litter decomposition and DOC production were then assessed in Chapter 6, and included both litter bag data and the utilisation of the Tea Bag Index to assess decomposition parameters in order to meet Objective 3. Finally, Objective 4 was addressed in Chapter 7 by presenting bacterial and fungal community data for soils collected from the field experiment. In Chapter 8, the key findings from each results chapter are drawn together and discussed, and conclusions and suggestions for future work are presented.

Chapter 2: The effect of acid deposition on dissolved organic carbon cycling in upland organic soils: Current understanding and key research gaps

2.1 Upland moorland environments and environmental change

2.1.1 Overview

Areas of uplands are located across the UK, including vast areas of Wales, Scotland and Northern Ireland, and areas in England including the Pennines, Dartmoor and the North York Moors, but also across much of the Northern Hemisphere. These areas are dominated by moorland environments, defined as “open areas with acid or strongly base-deficient soils” (Holden et al., 2007), which are characterised by organic soils including saturated peats and freely draining organo-mineral soils, such as peaty podzols (see Section 2.2).

In the UK, moorlands are predominantly used for low intensity grazing, but are also managed for grouse shooting or as wildlife reserves. Upland Moorlands provide a vast array of ecosystem services, from tourism, recreation and cultural heritage, to hydrological services such as flood risk regulation and water purification, to biodiversity protection, fire protection, and carbon regulation and climate change mitigation (Bonn et al., 2010).

Despite the international importance of upland moorlands, particularly for carbon storage (Section 2.3.1), there are a number of pressures on these systems, including climate change, land management, nitrogen saturation, heavy metal pollution and acidification (Section 2.5.2). Such pressures are causing severe damage, including soil erosion, flooding, poor water quality, biodiversity loss, nitrogen leaching and carbon destabilisation (Section 2.5.1) (Holden et al., 2007, Clark et al., 2010c, House et al., 2010, Caporn et al., 2011, Pilkington et al., 2005).

In particular, there has been increases in concentrations of dissolved organic carbon (DOC) in surface waters draining organic catchments (Evans et al., 2005). There has also been an increase in the amount of high molecular weight, coloured DOC of an aromatic and refractory nature leaching from peatlands, contributing to the ‘brownification’ of many terrestrial waters (Watts et al., 2001, Worrall et al., 2003a) and creating problems for water treatment companies (Ritson et al., 2016).

Despite the fact that many upland moorland catchments consist of both peat and organo-mineral soil (*Figure 2.2*), there has been a focus on peat and little work has been done on organo-mineral soils. As a result, there are few studies which assess and compare the impacts of such pressures for both soil types. Therefore, it is more important than ever that these pressures and their effects on carbon cycling and storage in these systems are fully understood, with scope for a fuller review of both soil types in these environments.

2.2 Upland moorland soil types

2.2.1 Peatland

Peatlands are areas where peat naturally forms and accumulates. The process of paludification, or rather the geological accumulation of organic material, causes peat to extend across the landscape by the addition of organic material under saturated conditions (Laamrani et al., 2014, Wheeler and Proctor, 2000). In the UK this is mainly due to the build-up of mosses, most commonly *Sphagnum*.

Peatlands cover just 3 % of the global land surface area (Yu et al., 2010), with 80 % classed as northern peatlands situated in North America, Northern Europe and Russia (Gorham, 1991) with an estimated store of 270 to 547 Pg of carbon (Gorham, 1991, Yu et al., 2010, Turunen et al., 2002). The UK holds between 9-15 % of Europe's peatland area, which includes 13 % of the world's blanket bog (Bain et al., 2011).

There are various different types of peatlands, and the basis of classification can vary from topography, surface vegetation, chemical properties, physical characteristics and so on depending on the region (Andriesse, 1988). Within the UK alone there are discrepancies in peat classification based on a minimum depth threshold of surface organic matter (*Figure 2.1*).

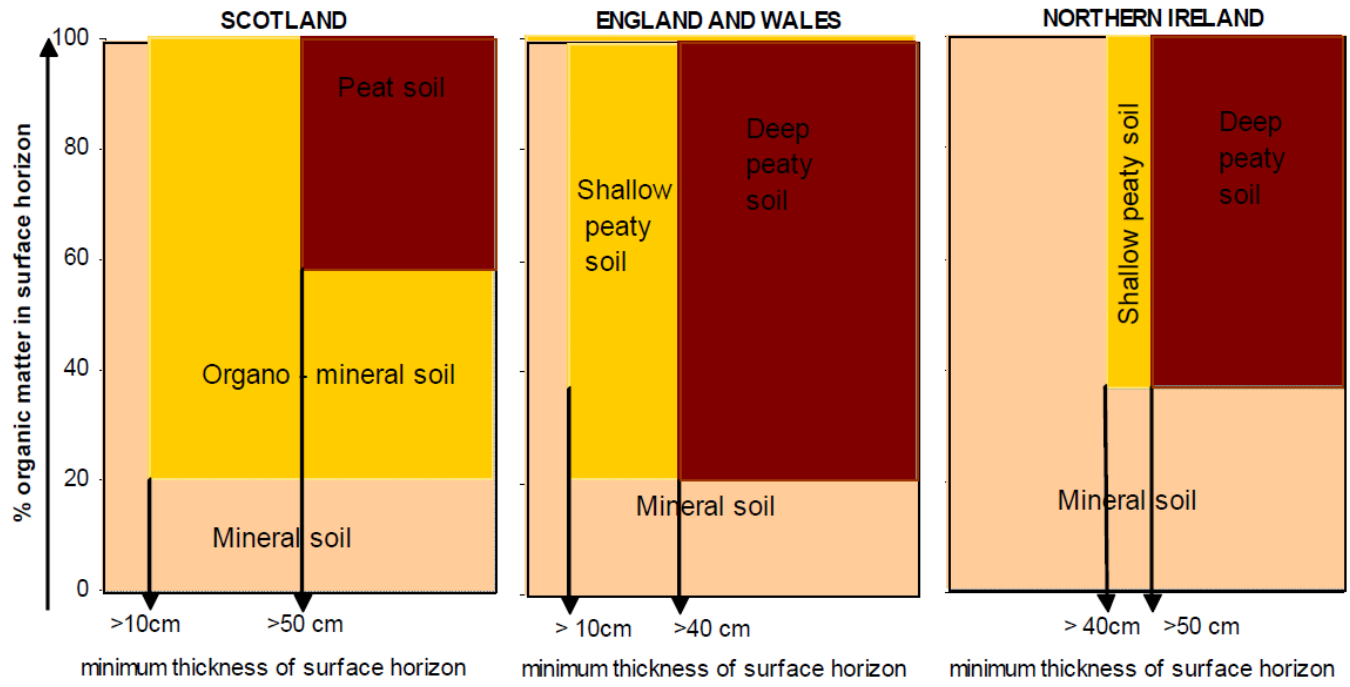


Figure 2.1: Diagram comparing the minimum depth and percentage organic matter content threshold used to define mineral, organo-mineral and peat soil in the Scottish, English and Welsh, and Northern Irish classification schemes (Joint Nature Conservation JNCC, 2011).

In the UK hydrology and nutrient source play a big part in peatland type classification, with a traditional distinction between ombrotrophic and minerotrophic groups (Wheeler and Proctor, 2000). Minerotrophic peatlands are areas of peat which are highly influenced by ground and/or surface water supply, also known as 'fens'. Alternatively, where the main influence of water supply stems from precipitation, peatlands are referred to as ombrotrophic, or 'bogs'. Bog peatlands represent the most common UK peatland type (Joint Nature Conservation JNCC, 2011), and are generally more acidic (pH < 5.0) than fens (pH > 6.0) (Wheeler and Proctor, 2000).

The hydromorphological system is another form of classification based on the climatic conditions influencing the formation of that particular peatland type (Charman, 2002). For instance, bogs can be further subcategorised into 'blanket bogs' over a hilly landscape, and lowland 'raised bogs' on wet floodplains, in basins and sometimes on existing fen peats making them slightly raised compared to the surrounding landscape. The UK falls into the bioclimate zone for these types of bogs, with warmer temperatures (> 0 °C) and high precipitation (> 900 mm/yr), than peat plateaus for instance, occurring in subarctic regions encompassing low temperatures (< -5 ℃) and low precipitation (< 400 mm/yr) (Wieder and

Vitt, 2006). A number of bioclimatic envelope models have been used to predict blanket peat cover, and changes in response to environmental change, based on a variety of thresholds for parameters such as precipitation and temperature in the UK (Gallego-Sala et al., 2010, Clark et al., 2010b) and globally (Gallego-Sala and Colin Prentice, 2012). As a result of this bioclimatic zone, blanket and raised bogs represent 95 % of peatland areas in the UK (Bain et al., 2011), and in upland areas blanket peats are the dominating peatland type.

The development of the World Reference Base (WRB) is facilitating a more harmonised international classification system. Many European institutions are now adopting this system, which is similar to the UK diagnosis for peat, but differs for other soil types (Joint Nature Conservation JNCC, 2011).

2.2.1.1 Peat

Peat is a classification within the taxonomic group histosols under the World Reference Base. More specifically, this is defined as a soil with a thick organic layer either greater than 10 cm depth and 20 % organic carbon content (directly overlying bedrock), or greater than 40 cm within the top 100 cm of the soil (FAO, 2006). Other definitions of peat include 'Organic material consisting largely of undecomposed or slightly decomposed plant remains' (Soil Survey Staff, 1999), or 'Dead and partially decomposed plant remains that have accumulated in situ under water logged conditions' under the Ramsar Convention (1971, 1971).

Peat does not have a mineral content, but is composed of organic matter and water (Charman, 2002). There are two distinctive layers within a peat profile; the acrotelm in the upper horizon comprising of roots and decomposing plant material, and the catotelm, consisting of dense peat (Holden and Burt, 2003a).

Peat properties are typically defined by the environmental conditions governing its formation, and in particular those controlling decay and humification, as well as the input of organic material from vegetation. Peats have slow decomposition rates due to waterlogged conditions, resulting in a thick organic layer at least 40 cm thick (Burnham et al., 1980). Saturation inhibits aerobic decomposition, and instead anaerobic decomposition occurs which is a much slower process (Swift et al., 1979). This leads to an accumulation of partially

decomposed organic material as the rate of decay is slower than the rate of input through net primary production.

Peat is heterogeneous with properties varying spatially between peatland sites to a scale of a few cm and throughout different depths. For instance, dry bulk density is greater at depth for some sites (Holden and Burt, 2003b) whilst the opposite has been observed at others (Frogbrook et al., 2009). Another example relates to preferential flow pathways, which can vary over a short distance creating hydraulic conductivity which differs both laterally and vertically (Holden and Burt, 2003b).

A key property of peatland hydrological systems is the high water table. This, alongside high porosity and slow hydraulic conductivity result in a significant store of water at more than 95 % (Charman, 2002). These factors create a positive feedback mechanism with permanent saturation and anoxic conditions impeding decomposition, further enhancing the organic matter content and water holding capacity of peat (Ise et al., 2008). Relationships between hydraulic conductivity and decomposition have been highlighted in models (Frolking et al., 2010, Morris et al., 2011), whilst the molecular composition and chemical structure of organic matter has been shown to correlate well to hydrological properties (Grover and Baldock, 2013).

Important components to the peatland hydrological system which may enhance flow include subsurface natural pipes, macropores, acrotelm stormflow and overland flow (Holden and Burt, 2003a). Hydrological properties can also vary with depth. Pore size and hydraulic conductivity has been observed to be much lower in deep and compact peats, whilst surface peats have a higher conductivity (Charman, 2002, Price, 2003), although others found no variation with depth (Chason and Siegel, 1986).

Peatlands have unique and extreme environments compared to other ecosystems, and as a result are generally considered to be species poor. Plant community composition is heavily influenced by hydrology, nutrient input, climate and land management. As a result, communities differ considerably between bogs and fens. For instance, bogs are dominated by ericaceous shrubs, such as heather (*Calluna vulgaris*), some graminoids including cotton grass (*Eriophorum vaginatum*) and a large proportion of bryophytes such as *Sphagnum* mosses. Fens have more graminoids and non-ericaceous shrubs (Weltzin et al., 2000). The

distinction between bogs and fens were originally drawn on the basis of plant species present as an indicator of groundwater inputs (Wheeler and Proctor, 2000).

Bryophytes dominate upland bog communities as they are well adapted to the wet, nutrient poor conditions (Hájek, 2014). *Sphagnum* mosses are not only well suited to this environment, but also contribute to creating and maintaining these conditions, and as a result actively out-compete other plant species (van Breemen, 1995). Acidification occurs when *Sphagnum* removes cations from solution, and replace them with hydrogen ions (Charman, 2002). Through their high water holding capacity and acidifying ability, *Sphagnum* mosses shape their habitat into an anoxic, acidic, nutrient poor environment, reducing its suitability for vascular plants (Rydin et al., 2006). In addition, the high C:N ratio of *Sphagnum* litter results in decomposers requiring more nitrogen and so decomposition and the release of carbon is reduced.

Peatland communities can be significantly affected by environmental change, including climate change and inputs of nutrients and acidity. Degraded areas have been associated with reduced *Sphagnum* species and increased presence of purple moor grass (*Molinia caerulea*) (Swindles et al., 2016, Ferguson et al., 1978). Areas receiving high levels of pollution deposition have seen significant *Sphagnum* loss, such as in the Pennines (Ferguson et al., 1978). Nitrogen deposition is thought to shift communities, with vascular plants outcompeting *Sphagnum* (Berendse et al., 2001, Heijmans et al., 2001). In addition, climate change related weather events can have adverse effects on *Sphagnum*, with irreversible desiccation and death with heat waves (Bragazza, 2008), and reduced production with lowered water tables (Weltzin et al., 2001).

2.2.2 Organo-mineral soils

Blanket peatlands are largely dominated by peats, but often found in mosaic of different soil types that include peaty podzols and peaty-gleys depending on topography and drainage (*Figure 2.2*) (Charman, 2002).These shallow peaty soils usually surround areas of peats on hilly slopes, and make up a large proportion of the moorland landscape. Using England as an example, peatlands are estimated to cover 11 % of total land area, with 48 % classed as deep peat and 37 % being shallow peaty soils including podzols. The remaining areas of

peatlands are classed as soils with peaty pockets, or “Areas of mostly non-peat soils, supporting smaller pockets of deep peat” and make up 15 % (Natural England, 2010).

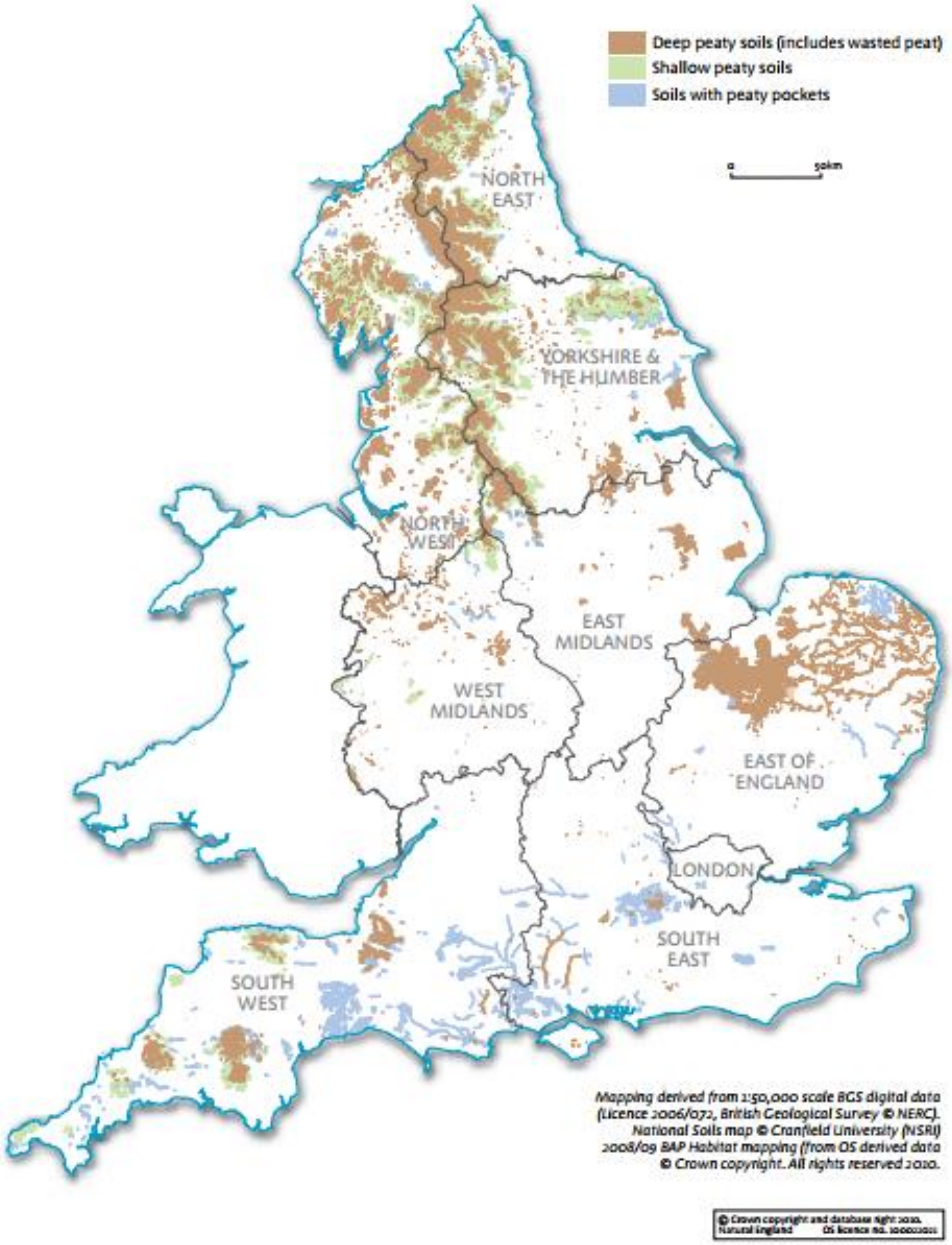


Figure 2.2: Map of England illustrating the distribution of different classifications of peat soils in peatland areas (Natural England, 2010).

Peaty podzols, or histic podzol termed under the World Reference Base taxonomic classification system (FAO, 2006), are classified by the subsurface B horizons where iron and aluminium sesquioxides, as well as other substances including organic matter and clay, have accumulated as a result of being leached through the soil (Burnham et al., 1980).

This process is podzolisation whereby soluble and suspended materials are leached down the profile through mechanical eluviation, resulting in acidic and nutrient deficient horizons higher up the profile (Burnham et al., 1980). The spodic B horizon develops below this due to illuvation of these suspended and soluble compounds leached from above, including DOC with sesquioxides (Dalsgaard et al., 2016). This illuvial B horizon ranges from red, darkish brown to black, and has a greater CEC (cation exchange capacity), clay and potentially the humic content than the above horizon (Wilding et al., 1983, Soil Survey Staff, 1999).

A resulting overlaying bleached horizon may be present (E, A2, Ae), which has been heavily leached of organic compounds, clay, iron and aluminium, the mobilisation of which is promoted by organic acids moving vertically from the overlying organic layers (Wilding et al., 1983, Little, 1997). This eluvial layer is easily distinguishable by its whitish-grey colouring, although this is no longer relied upon as a key diagnostic feature as it can easily be disturbed and be intermittent (Wilding et al., 1983).

A raw humus surface organic layer (Oi, Oe) may also be present as a peaty topsoil due to anoxic conditions which limit aerobic decomposition (Dalsgaard et al., 2016), and has the maximum organic matter content, CEC, total exchangeable bases and percentage base saturation values compared to other horizons present (Burnham et al., 1980, Little, 1997).

The podzolisation process is mediated by climatic variables, including the rainfall-evapotranspiration ratio, as well as vegetation, geography and topography which directly influence the hydrology and drainage capacity, and ultimately the acidity of the soil. The process may be accelerated by acid deposition and anthropogenic activities that increase soil exposure such as land clearance (Little, 1997).

The hydrology of podzol soil can be complex dependent on topography, but podzols are generally characterised as free draining. Flow pathways have been shown to switch from the lower mineral layer during times of low flow, to the upper organic layer during times of high flow (Clark et al., 2008). In terms of the vegetation communitites on moorland podzolic areas, bryophytes are less common, with calcifugous grassland communities dominating including *Festuca ovina* and *Juncus squarrosus*.

2.2.3 Key differences between peat and organo-mineral soils

A comparison in key characteristics between peat and podzol soil have been summarised below in *Table 2.1*.

Table 2.1: Comparison of key properties between peat and podzol soils.

Characteristic	Peat	Podzol
Depth of organic layer	Deep	Shallow
Mineral soil layers	None	Present
Hydrology	Impeded drainage Flow is always through organic layer.	Freely drained Flow shifts from mineral to organic layer when high.
Dominant vegetation	Bryophytes Ericaceous shrubs Graminoids	Calcifugous grassland communities
Redox status	Anoxic	Oxic

The key differences between peat and organo-mineral soils relates to hydrological properties. Peats form in areas with impeded drainage and so tend to have slow hydraulic conductivity and form in areas with high water tables, resulting in permanently saturated conditions. In contrast, podzols tend to form on slopes and hills over mineral material which is more free-draining, allowing for aeration of the soil. In addition, unlike organic-rich peat, podzols have a mineral content, with the potential for sorption of DOC, potentially increasing its residence time in the soil (Stutter et al., 2012).

Hydrologic flows and therefore transport of DOC differs between peat and organo-mineral soils. In podzols, high flow moves through the upper organic layer and low flow through the mineral layer. In peats, high saturation and organic matter content results in flow moving through a thick organic layer regardless of changes in flow severity (Clark et al., 2007).

The contrasting abiotic differences between peat and organo-mineral soils result in different vegetation communities developing, which ultimately influences the quality and quantity of organic material entering the decomposition process. For instance, species more tolerable of saturated conditions dominate areas of peat, such as *Sphagnum* mosses, whilst calcifugous grassland communities including *Festuca ovina* and *Juncus squarrosus* are more common to the podzol areas of moorlands.

In addition, different environmental conditions between peat and organo-mineral soils has a strong influence on the processing of organic material by microorganisms. Peatlands typically have a high water table, which alongside the ability to store large volumes of water due to large porosities in peat, creates anoxic conditions which impede aerobic decomposition (Ramchunder et al., 2009) resulting in anaerobic activity. In contrast, podzol soils are more freely draining allowing for more aerobic activity.

It is important to note these differences in properties between peat and organo-mineral soils, as they may result in variations in DOC dynamics within a single moorland catchment, including microbial DOC production and consumption, as well as sorption and leaching.

2.3 Carbon cycling in organic soils

2.3.1 Carbon store

Up to 80% of global peatlands are located in northern regions (Limpens et al., 2008), and are estimated to store between 270-547 Pg/C (Gorham, 1991, Turunen et al., 2002, Yu et al., 2010). In comparison, the remaining 20 % of global peatlands store 89 Pg/C in tropical peatlands (Page et al., 2011) and 18 Pg/C in southern peatlands (Yu et al., 2010). In the UK, peatlands cover 15 % of land area, and store between 1.7-2.3 Pg/C (Billett et al., 2010, Joosten, 2009), much of which is concentrated in Scotland. There are huge uncertainties in estimating peat carbon stores, with figures being refined frequently. Peat depth is an essential element for calculating carbon accumulation and storage as well as for developing maps of carbon distribution. However, peat depth is not homogenous, making it difficult to establish carbon storage across whole catchments (Parry et al., 2014). There is much less research on the carbon stocks of organo-mineral soils. Natural England (2010) estimate that shallow peaty soils cover an area of 5,272 km^2 in England and contain 58.5 megatonnes of carbon, this equating to 10 % of the total peatland carbon store.

2.3.2 Carbon balance

The difference between the net accumulation and loss of carbon will result in either ecosystem being a source or sink of atmospheric carbon, influencing its contribution to or mitigation against global climate change. A variety of gaseous and fluvial fluxes are used to estimate the net ecosystem carbon balance (NECB), the measurements approaches of which are summarised in *Figure 2.3*. Specifically:

$$NECB = -NEE + F_{CO} + F_{CH4} + F_{VOC} + F_{DIC} + F_{DOC} + F_{PC}$$

where NEE is net ecosystem exchange, F_{CO} is carbon monoxide flux, F_{CH4} is net methane flux, F_{VOC} is net volatile organic carbon flux, F_{DIC} is net dissolved inorganic carbon flux, F_{DOC} is net dissolved organic carbon flux, and F_{PC} is the net lateral transfer for particulate carbon (Chapin et al., 2006).

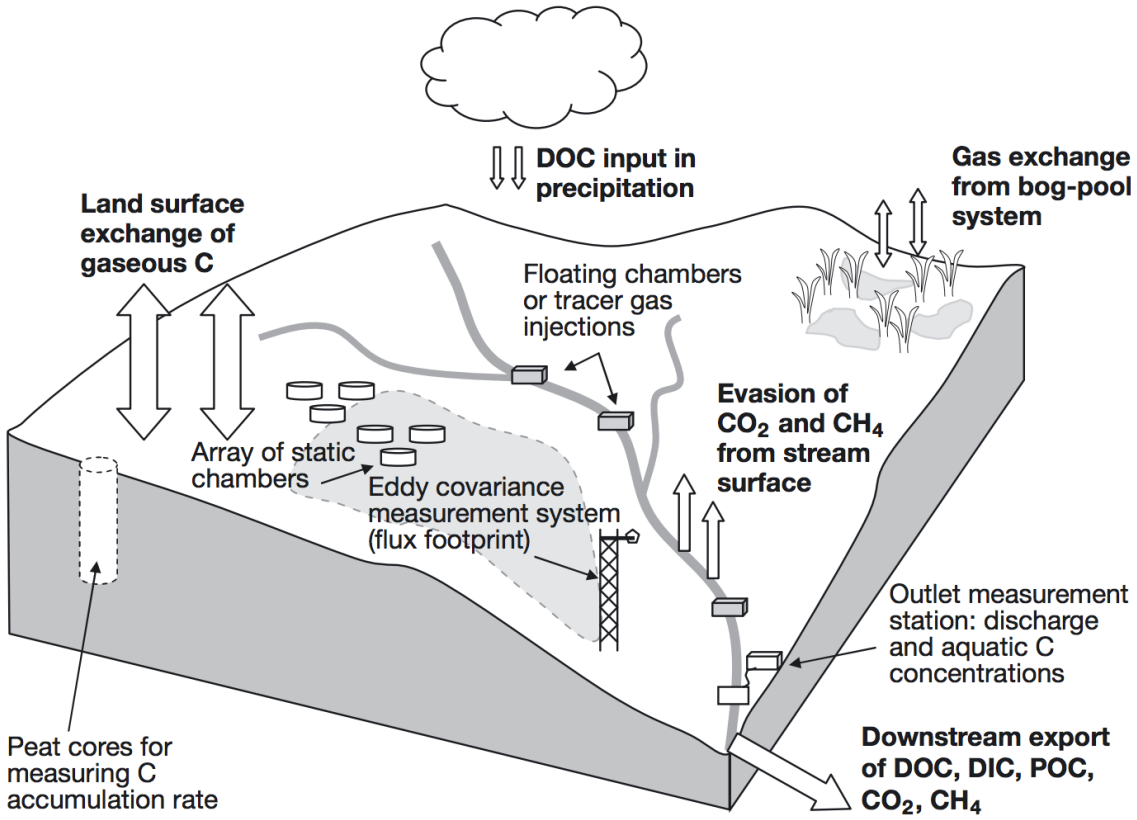


Figure 2.3: Key measurements of carbon inputs and outputs which are routinely used to establish net ecosystem carbon balance (NECB) (Billett et al., 2010).

Key carbon inputs in to moorlands are mainly from vegetation, which take in CO_2 during photosynthesis to incorporate carbon into plant tissues, which is then added to soil as exudates or through the decomposition of dead material. Soil microbial biomass is also another source of carbon, as well as DOC deposited in precipitation (although this is insignificant compared to other fluxes) (Rowson et al., 2010). Net primary production (NPP) is the autotrophic respiration subtracted from gross primary production (GPP) and represents the carbon added to the system through litter production and is assumed to be equal to this input (Yu et al., 2010). However, management including burning and grazing can result in an imbalance between the carbon fixed from the atmosphere and the carbon added to the soil once the plant has died.

Carbon outputs are through respiration from plants and soil microorganisms releasing CO_2 and CH_4, and the fluvial export of gaseous, particulate and dissolved carbon. Ecosystem respiration (ER), which comprises of above (plants) and below ground (plants and microbial heterotrophs) respiration is considered the largest loss of carbon from an undamaged moorland system (Billett et al., 2010). Again, estimating this flux can be problematic as heterotrophic respiration results from litter decomposition, and so is affected by litter evenness (Ward et al., 2010) and quality (Limpens and Berendse, 2003).

The exchange of CO_2 is often measured by calculating net ecosystem exchange (NEE) from the sum of ER (positive flux) and photosynthesis (negative flux) in daylight (Rowson et al., 2010). NEE has been shown to be the greatest flux of carbon in peatland catchments (Dinsmore et al., 2010). However, there are few studies which have measured NEE over a long term period spanning several years, and there is much variation annually as well as between sites, as well as minimal work on upland organo-mineral soils within the literature. For instance, a blanket bog in south west Ireland had an estimated NEE of 84.0 ± 4.8 g C m^{-2} yr^{-1} in 2005, which decreased to -12.0 ± 3.4 g C m^{-2} yr^{-1} in 2006. Based on six years of monitoring at this site, the estimated mean annual cumulative NEE was -47.8 ± 30.0 ($\pm$ 1 SD) g C m^{-2} yr^{-1} (Koehler et al., 2011).

The fluvial flux is another important outsource of carbon from moorland systems. DOC fluxes represent a major natural outsourcing of carbon from organic soils. Reported values are variable and range from 1 – 50 g DOC m^{-2} yr^{-1} in forested peatland catchments (Dillon and Molot, 1997), representing 10% of total carbon loss (Limpens et al., 2008, Gorham, 1995). However, this can be much higher for specific sites, particularly damaged peatlands. The south Pennines in the UK consists of a vast area of damaged and eroding peat which has a high fluvial flux of particulate and organic carbon draining into nearby reservoirs, with the potential to then become a gaseous export of carbon. The River Ashop peatland catchment in the South Pennines had an annual fluvial organic carbon loss of 29-106 Mg C km^{-2} between late 2005 and early 2007, with more eroded areas being dominated by POC loss over DOC (Pawson et al., 2012). The River Kinder reservoir in the South Pennines received 93 % more POC than DOC in 2012 and 2013 (Stimson et al., 2017). Complete carbon budgets for relatively undamaged peatlands also show the importance of the fluvial flux. DOC was shown to be the second largest exporter of carbon out of an ombrotrophic peatland in southern Scotland, equating to 24 % of carbon taken up through NEE based on two years of monitoring (Dinsmore et al., 2010). In addition, whilst much research focuses on monitoring the fluvial flux in peat, long term monitoring of DOC at three moorland catchments in the UK have shown that concentrations in podzol soil solution increased by 48 and 215 % for organic and mineral horizons between 1993-2007, whilst there were no changes in peat (Stutter et al., 2011). Such research highlights the importance of the fluvial flux in organo-mineral soil for carbon stability of an upland moorland catchment.

Carbon accumulation occurs due to slow decomposition rates which result in a build-up of organic material in saturated conditions. Accumulation rates have been estimated at an average of 18.6 g C m^{-2} yr^{-1} for northern peatlands, 22.0 g C m^{-2} yr^{-1} for southern peatlands and 12.8 g C m^{-2} yr^{-1} for tropical peatlands (Yu et al., 2010). However, there is much uncertainty in accumulation rate estimates, particularly as carbon balance calculations for individual peatlands are based on site specific monitoring and there is uncertainty surrounding the how representative these are for other peatlands. Also, there are few long term carbon budget monitoring studies, many spanning one or two years (Roulet et al., 2007), as well as little work on upland organo-mineral soils.

There are many environmental controls influencing the mechanisms involved in carbon inputs and outputs from a moorland system. Controls which have the greatest influence on organic soil carbon cycling are temperature, hydrology, plant community and chemistry of organic inputs (plant tissue and peat) (Moore et al., 1998). Such controls can fluctuate temporally and spatially, resulting in shifts in carbon source and accumulation rates, which ultimately affect the accuracy of annual carbon budgets. Koehler et al. (2011) found that over a six year monitoring period for an Atlantic blanket bog peatland in Ireland, the site was a source of carbon for two years. However, estimates of carbon accumulation rates do offer a useful basis for comparing sites and region as well as changes over time. Peat has been a consistent sink of carbon since the last glacial period (Charman et al., 2013), but climate change may destabilise these systems resulting in them becoming an net source of carbon. For instance, accumulation rates are thought to be decreasing gradually over time for sites in the UK. Billett et al. (2010) showed that recent accumulation rates for two UK sites (-56 to -72 g C m^{-2} yr^{-1}) are much lower than those seen over 150 years (-35 to -209 g C m^{-2} yr^{-1}) in peat cores. However, unlike peat, there has been little research invested into measuring long term carbon accumulation rates in peaty podzol soils in upland moorland environments.

2.3.3 Decomposition process

2.3.3.1 Biodegradation

Biodegradation is the decomposition of organic matter by microbial organisms, producing gases (CO_2, CH_4), DOC, humus and nutrients (both organic and inorganic) (Bragazza et al., 2009). Below is an introduction to the mechanisms involved in the decomposition of organic matter.

2.3.3.1.1 Biodegradation process

Metabolism is an essential process and a key driver of decomposition and biogeochemical cycles, where elements are released from the breakdown of organic matter to be used as an energy source, enabling the sustenance of cellular life functions. A range of metabolic strategies can be adopted depending on environmental conditions. The availability of a terminal electron acceptor and organic substrates associated with a microbe's ecological

niche will result in their ecological dominance and the type of metabolism which occurs (Hunter et al., 1998).

Organic matter is full of polymeric structures, where repeating monomers form a chemical compound with a large molecular mass. Microbes use a technique termed depolymerisation in order to break down long chains to produce monomer structures which are then small enough to pass through the cell membrane, working simultaneously with metabolism (Blankinship et al., 2014). Enzymes (a catalyst of biochemical reactions) are the key tool used to achieve this. Extracellular enzymes bind to the reactants at active sites to catalyse the degradation of a polymer, whilst intracellular enzymes catalyse biochemical reactions occurring within the cell.

For instance, cellulose is broken down exterior to the cell by the enzyme cellulase, to produce the oligomer cellobiose, which is further broken down to the monomer glucose by the enzyme β-glucosidase. Glucose is then small enough to cross the cell membrane, where it is used as the terminal electron donor (TED) and therefore oxidised during a redox reaction catalysed by intracellular enzymes for catabolic respiration. Oxygen is converted and H_2O is released as a waste product of aerobic metabolism. Some of the energy released during this process is stored in adenosine triphosphate (ATP) bonds as electrons, where it is used for the sustenance of cellular life functions, such as the biosynthesis of cellular components during anabolism.

2.3.3.2 Variation with soil depth

Early stage decomposition occurs at the surface where oxygen is most available. This process is largely led by detritivores, which break litter down into smaller pieces ready for microbial processing. This is followed by chemical transformation by bacteria and fungi as described above, producing inorganic molecules such as phosphate, ammonia, water and CO_2 (Swift et al., 1979, Aerts, 2006).

During this process, microbes use oxygen as the terminal electron acceptor (TEA), releasing CO_2 as a by-product. This process is the most efficient form of respiration and is more thermodynamically favoured by microorganisms (Keller et al., 2009). As organic material moves vertically into more saturated conditions, anaerobic activities take over, with the redox potential being reduced (Belyea, 1996). Microbes use alternative TEA's for reductions,

processes of which include (in order of decreasing thermodynamic yield) denitrification, iron reduction, manganese reduction and sulphate reduction. Once these TEA's are depleted, methanogenesis will take place (using carbon as TEA and releasing CH_4 as a by-product) (Keller et al., 2009). During this step-wise shift in microbial processing of organic material, there is a decrease in decomposition rate (Beer and Blodau, 2007).

2.3.3.3 Chemical transformation of organic matter

Microbes are capable of degrading both humic and non-humic substances in soils. Microbial communities are thought to preferentially decompose material which is more labile and easier to degrade. Such material usually has a relatively short residence time, being preferentially consumed by soil microorganisms, and include sugars, carbohydrates, proteins, amino acids, peptides, low molecular weight organic acids, fats, waxes and so on. DOC molecules are continuously being decomposed, altered and produced by a variety of microorganisms, resulting in a substance which is more stable and has a higher molecular weight than the original product (Malik and Gleixner, 2013). These stable, recalcitrant products of late decomposition stages are thought to be the largest fraction of stable dissolved organic matter (Kalbitz et al., 2003b). For instance, humic substances are more resistant to microbial degradation, and therefore stable (Schnitzer, 1978).

2.4 Dissolved organic carbon

2.4.1 Definition and characteristics

Dissolved organic matter (DOM), defined as organic compounds able to pass through a filter with a pore size of 0.45 µm (Thurman, 1985, Sleutel et al., 2009), makes up part of the SOM pool. DOM has various components which are not mutually exclusive of one another, including dissolved organic nitrogen (DON), phosphorous (DOP), and dissolved organic carbon (DOC). In summary, DOM derives from the exudation or decomposition of organisms, including microbial mass, plants and animals, which are further biodegraded to produce DOC (Evans et al., 2005).

The soil organic matter pool (SOM) consists of a mixture of plant, faunal and microbial debris, residues and exudates, as well as soil biomass which primarily comprises of microorganisms and humus (Tipping, 2002). SOM is a continuum comprising of fresh litter

and exudates, alongside material in various stages of decomposition (Stockmann et al., 2013). Plant biomass is the main source of SOM, supplying a complex mixture of many organic components, particularly lignin, polysaccharides, tannins and biopolymers (Kögel-Knabner, 2002). Therefore SOM receives a considerable amount of polymers from plant material with a variety of turnover times (Trinsoutrot et al., 2000).

The composition of DOC can be categorised into low and high molecular weight compounds, both of which differ in their lability. For instance, stable humic substances are complex, high molecular weight compounds including a mixture of aromatic and aliphatic hydrocarbon structures with attachments such as ketone, hydroxyl, carboxyl and other minor functional groups, which dominate DOC (Tipping, 2002, Evans et al., 2005, Leenheer and Croué, 2003). Whilst low molecular weight, non-humic compounds have simpler biochemical structures which are easier to identify, including carbohydrates, fats, waxes and amino acids (Tipping, 2002, Evans et al., 2005).

There are three main sources of DOC; root exudates, DOC leaching from fresh plant litter, and the resulting product from microbial DOM decomposition. This variety of sources means that DOC is not homogenous, indicated by the wide range of DOC concentrations conveyed within studies (Zsolnay, 1996, Kalbitz et al., 2003a). Correlations have been made between the flux of DOC in water systems, and the amount of organic matter in the catchment soil, with peat producing the greatest source of DOC (Hope et al., 1997). Soils generally have decreasing DOC concentrations with depth, as new material is continuously being added to the topsoil from plant litter and exudates (Michalzik et al., 2001, Kalbitz, 2001).

2.4.2 Pools of DOC

As plants photosynthesise and harvest CO_2 from the atmosphere, carbon is incorporated into plant material, which provides a key input of carbon into the SOM pool. Within peatlands, DOC is produced, modified and transported between different components of the soil system before it reaches terrestrial waters. These include living plant material, the aboveground decomposing plant material and belowground soil organic matter consisting of a solid phase, a solution phase and the biological biomass (*Figure 2.4*).

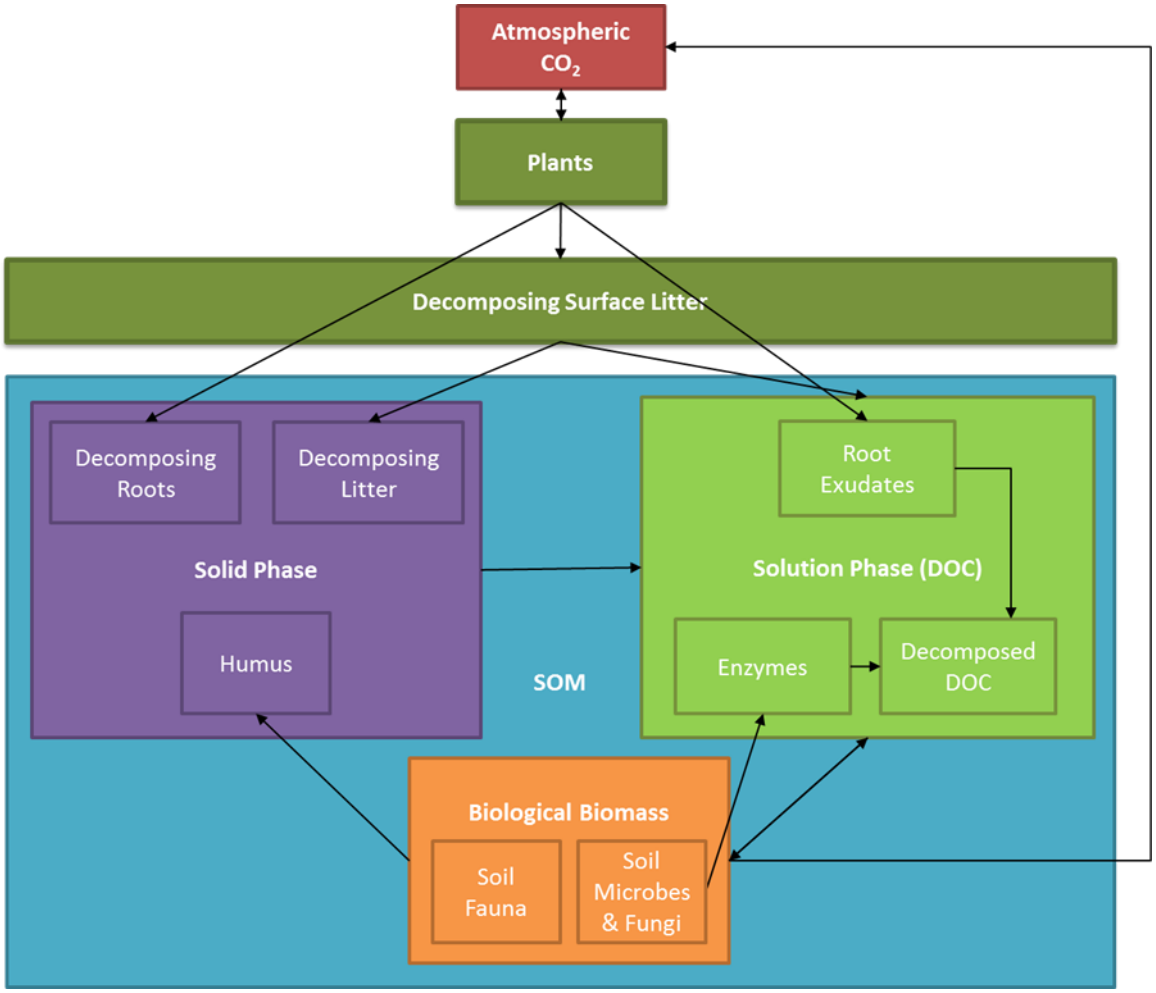


Figure 2.4: Illustration of the how carbon is transported around different components of a moorland environment.

Carbon input into the SOM compartment can occur both above and below ground. Plants release DOC belowground by either producing root exudates, which are readily dissolvable in the solution phase, or via the decomposition of dead root material in the solid phase (Blodau et al., 2007, Neff and Asner, 2001, Chanton et al., 1995). Aboveground inputs include the shedding of exudates, which then leach into the soil as DOC, or through the decomposition of freshly senesced litter above the soil surface in the decomposing surface litter zone (Neff and Asner, 2001).

In an undamaged peatland, much of the surface vegetation is not fully decomposed under the cool, wet climate, and anoxic and nutrient-deficient conditions, resulting in an accumulation of organic matter (Ise et al., 2008). Alternatively, podzol soils are more easily drained than peats, resulting in more aerated conditions and microbial activity (Cook and

Orchard, 2008, Howard and Howard, 1993), and so the material which enters this belowground solid phase is of a more processed nature (Berg, 2000).

The solid phase of SOM (including partly decomposed plant inputs from aboveground, decomposing roots and humus) will continue to degrade in soil, continuously releasing DOC into the solution phase, until the most recalcitrant, aromatic fraction remains. Within the solution phase, DOC is also continuously transformed by microbial activity and is eventually either respired back into the atmosphere during mineralisation (Marschner and Bredow, 2002), or reprocessed into more aromatic, recalcitrant DOC (Malik and Gleixner, 2013). However, microbial activity is limited under the anoxic conditions of peat compared to the more aerated podzol soil, resulting in slower processing of organic material belowground (Scanlon and Moore, 2000, Freeman et al., 2001b).

2.4.3 DOC production

2.4.3.1 Production and consumption

Biological processes produce DOC from the recycling of organic material, primarily from plant and animal biomass. Microbial decomposition releases DOC through the action of extracellular enzymes, which depolymerize higher molecular weight organic matter of low solubility, creating lower molecular weight DOC monomers and oligomers. DOC consumption is also a biological process: DOC compounds which are small enough (<~600 Da) can be actively transported through microbial cell walls and enter the anabolic and catabolic reactions of microbial metabolism with catabolism resulting in the ultimate oxidation and release of DOC as CO_2 (Blankinship et al., 2014).

Radiocarbon dating of DOC draining from peatland catchments suggests that DOC is recently formed, suggesting it is the product of microbial degradation of fresh litter or peat (Palmer et al., 2001, Tipping et al., 2010). Once small enough to become soluble, DOC is either transport from the catchment (Clark et al., 2008), or consumed and respired by microbes (Moore and Dalva, 2001). The aqueous phase is essential for the mechanisms by which soil microorganisms utilise organic matter (Marschner and Kalbitz, 2003), and so the dissolved fraction is fundamental to degradation of soil organic matter and DOC production (Kalbitz et al., 2003b). This is supported where a CO_2 efflux can be attributed to a decrease in water-

extractable organic carbon during selective degradation and modification of DOC by microorganisms (Marschner and Bredow, 2002).

DOC can also be produced from plants, where it is released as low molecular weight exudates. The priming effect of exudates is greater than with root mucilage or root residues (Mary et al., 1993). Such an input of fresh, labile material can stimulate microbial activity and therefore prime organic matter decomposition (Xiao et al., 2014, Blagodatskaya and Kuzyakov, 2008, Kuzyakov et al., 2000). Plants are thought to benefit from the decomposition of their exudates through the uptake of extra nutrients that have been released during mineralisation (Kuzyakov et al., 2000). For instance, during a controlled laboratory experiment, microbial activity was increased from the input of root exudates of *Lolium perenne*, which equated to additional decomposition of around 60 kg C ha^{-1} d^{-1} and the mineralisation of 6 kg N ha^{-1} d^{-1} (Kuzyakov et al., 2001).

2.4.4 Controls on production

2.4.4.1 Water table movement and aeration

DOC produced through organic matter decomposition is noted to increase following water table draw down in peat soils (Clark et al., 2009, Tipping et al., 1999, Ritson et al., 2017). DOC concentrations have also been noted to increase following drought in peat due to recovery from drought-induced acidification and associated effects on DOC solubility (Clark et al., 2012, Clark et al., 2006, Clark et al., 2005). A high water table and saturated conditions inhibits the decomposition of organic matter, and lowering the water tables increases aeration of organic matter, stimulating the production of DOC. By contrast, podzols are more freely drained and aerobic and water table fluctuations play less important roles in changes in DOC production.

The aqueous phase is essential for the mechanisms by which soil microorganisms utilise organic matter (Marschner and Kalbitz, 2003). DOC located within aggregates or air filled pores would be inaccessible for microbial processing. Soil microbes are aquatic, and organic carbon dissolved in water is the most bioavailable form of substrate (Marschner and Kalbitz, 2003).

The redox reactions utilised by soil microorganisms is dependent on environmental conditions, particularly pore air to water ratio and therefore aeration, gaseous volume and

ease of exchange. The local hydrology controls the supply rates of inorganic nutrients, redox species and dissolved carbon, which in turn influences microbial activity and metabolism (Hunter, et al., 1998). Anoxic environments will result in anaerobic decomposition which is less thermodynamically favoured compared to aerobic activities, and so the decomposition rate and therefore production of DOC is much slower (Keller et al., 2009).

An optimum soil moisture content of 60 % increases decomposition rates, particularly when coupled with warmer temperatures (e.g. 24 °C) (Donnelly et al., 1990). However, upland peat bogs are much colder and wetter. Howard and Howard (1993) demonstrated that increasing moisture content results in higher levels of respiration up to an optimum point, with organic soils experiencing the lowest rates of CO_2 evolution, such as raw peat.

2.4.4.2 Temperature

Temperature as a limiting environmental factor has remained controversial within the scientific community, with disagreement over the sensitivity of soil carbon decomposition (Stockmann et al., 2013, Giardina and Ryan, 2000). Many studies agree that temperature is a key factor controlling SOM decomposition (Knorr et al., 2005, Kirschbaum, 1995, Trumbore et al., 1996, Andersson and Nilsson, 2001b, Craine et al., 2010, Curtin et al., 2012, Yuste et al., 2007), whilst some dispute this, concluding that temperature has a minimal effect (Giardina and Ryan, 2000, Liski et al., 1999).

The range of organic compounds in soil are so vast, and therefore so are their physical properties, including their intrinsic temperature sensitivity to decompose, resulting in confusion in results between studies and arguments over the type of feedback that may be seen with temperature increases (Davidson and Janssens, 2006). Theoretically, the non-labile pools should be more stable and therefore less sensitive to higher temperatures compared to recalcitrant pools of SOM, which is supported across multiple scales and soil types (Craine et al., 2010). However, most decomposition models apply a single relationship to all pools, which may be unrepresentative and unreliable to the in situ situation (Stockmann et al., 2013). It is even argued that the resistant pool is more temperature sensitive than labile organic material (Knorr et al., 2005), although other studies challenge this (Fang et al., 2006).

DOC production is thought to be sensitive to changes in temperature in organic soils, but less is known about the temperature sensitivity of DOC compared to that of gaseous carbon losses from organic soils (Billett et al., 2004, Chapin et al., 2006). There is still much confusion on the effect of temperature on decomposition in organic soils (Davidson and Janssens, 2006). A controlled laboratory experiment on peat cores showed that temperature and water table draw-down result in changes to DOC production, with a higher Q_{10} (i.e. rise in the rate of net DOC production over a 10°C range) with a lower water table (Clark et al., 2009). However, modelling suggests that temperature increases only explain around 12 % of the 78 % increase in DOC production observed at a monitoring site in the North Pennines, with suggestions that other factors may be more important such as land management or changes in enzyme activity (Worrall et al., 2004a). Phenol oxidase activity has been shown to increase with higher temperatures in peat, which may increase the proportion of phenolic compounds in the peat and impair the metabolism of DOC, resulting in a greater proportion of DOC available to leached into surface waters (Freeman et al., 2001a).

Temperature may also indirectly increase microbial decomposition through increased plant respiration and photosynthesis, resulting in a greater volume of root exudates being shed, stimulating microbial activity (Tang et al., 2005). If inputs exceeded microbial decomposition rates, a negative feedback to the carbon cycle would be reached. Alternatively, if higher temperatures increase decomposition, transferring carbon to the atmosphere, a positive feedback would be achieved (Davidson and Janssens, 2006).

2.4.4.3 Organic matter substrate

Availability, chemical composition and amount of substrate will ultimately impact the quantity and quality of DOC produced during the biodegradation of organic material. A negative relationship exists between the biodegradation and lability of DOC (Kalbitz et al., 2003a). Biota consume more labile components of organic matter first (Boyer and Groffman, 1996), converting this to more refractory and aromatic compounds (Thurman, 1985), whilst leaving recalcitrant material behind to be degraded further (Boyer and Groffman, 1996). Therefore, microbes will follow a consistent and predictable path, performing chemical and molecular change on organic material, which involves a variety of organisms working at different stages of decomposition under a particular environment

(Grandy and Neff, 2008). For example, Kalbitz et al (2003a) compared DOM extractions from different samples and measured biodegradation through CO_2 efflux. Less humified organic material, such as straw, litter and fermentation layers of forest floors, had the greatest labile fraction (59-88 %), and the highest DOC mineralisation (61-93 %). In contrast, stable material such as DOM extracted from peats, had the smallest labile fraction (3-6 %), and the lowest measurement of DOC mineralisation (4-9%), whilst DOM obtained from agricultural soils had intermediate labile material (14-25 %) and mineralisation (17-32 %).

However, there is evidence that other biological and environmental factors can be more influential over SOM decomposition than the molecular structure of the substrate (Schmidt et al., 2011, Thiessen et al., 2013, Stockmann et al., 2013). Compound-specific isotopic analysis has shown that the more stable molecules which should theoretically persist in soil, such as lignin and plant lipids, can decompose as quickly as more labile compounds, some of which can persevere for decades, including sugars (Marschner et al., 2008, Schmidt et al., 2011). Acidity has been shown to suppress microbial activities. Rousk et al (2009) found microbial inhibition below a pH value of 4.5. The observed reduction in carbon mineralisation was attributed to the observed increase in fungal growth and decrease in bacterial grown with acidity.

Conversely, other studies have found soil moisture content and temperature to be more influential on microbial biodegradation than acidity (Donnelly et al., 1990). The type of respiration and therefore microbial taxa dominating at a particular decomposition stage will depend on the environmental conditions, which in turn can influence the rate of decomposition of particular carbon fractions, resulting in the variation in organic matter structure and function between ecosystems (Grandy and Neff, 2008, Stockmann et al., 2013). It is important to note that differences in sample preparation and method of analysis may contribute to the disparities between studies (Marschner and Kalbitz, 2003, Jones and Willett, 2006, Urbansky, 2001), and the controversy surrounding the key controls on biodegradation of different carbon fractions.

On another note, substrate quality and biodegradation can vary with depth. Leaching of DOC containing fresh plant material transports labile DOC from the organic topsoil to the subsoil horizons (Andersson and Nilsson, 2001b), which are then either leached out of the profile to nearby water courses, or are utilised by microorganisms. Both HMW and LMW

DOC have been attributed to microbial processing, formation and stabilisation of organic matter, as well as plant root exudates (Malik and Gleixner, 2013). Therefore evidence increasingly suggests that DOC found at depth is derived from the processing of organic matter (Froberg et al., 2006, Sanderman and Amundson, 2008).

2.4.5 Controls on DOC mobility

Once DOC is produced, physico-chemical processes affect its mobility in soils and partitioning between solid and dissolved phases. In organo-mineral soils, DOC can be sorbed onto the mineral components within the soil. The process of podzolisation, where materials are leached from upper E horizon, to accumulate in the lower B horizon, is central to controlling DOC movement. Co-precipitation of sequioxides and organic matter occurs in the B horizon where DOC is stored (McDowell and Wood, 1984).

Soil water chemistry is another key feature controlling DOC solubility and the sorption/desorption from solid phase. Acidity and ionic strength of a solution are key controls on DOC solubility. For instance, Clark et al. (2011) showed DOC released from organo-mineral O horizon and peats decreased by 21-60 % with sulphuric acid additions and associated changes in acidity and ionic strength. Acidity has been shown to reduce DOC solubility and mobility in peat and organo-mineral soils, particularly when exchangeable aluminium concentrations are greater and base saturation is lower (Clark et al., 2011). DOC release sensitivity to sulphur additions can be increased with aluminium in organo-mineral soils due to complexation and co-precipitation of DOC with aluminium (Jansen et al., 2003).

The chemical composition of DOC can also influence it's solubility. For instance, at pH <2 humic acids will precipitate but fulvic acids will remain in solution (Thurman, 1985). This has been demonstrated in laboratory sulphur addition experiments, where the concentration of acid-sensitive coloured aromatic humic acids have been observed to decrease with acidity in soil pore water (Clark et al., 2011). In addition, DOC itself can influence the pH of a solution through the dissociation or protonation of H^+ ions, meaning it is often referred to as a weak organic acid (Langmuir, 1997). The degree of dissociation depends on the solution pH; with a low pH and therefore high concentration of H^+ ions, protonation occurs which increases pH, and with a higher pH and therefore fewer H^+ ions, dissociation is higher, actively reducing pH (Thurman, 1985).

Biological and chemical processes result in changes to the pH of soils and waters in natural environments. For example, the removal of cations (such as through erosion) and the addition of anions (such as through precipitation) can also influencing the hydrogen balance in soils leading to acidification (De Vries and Breeuwsma, 1987, Edwards et al., 1985). In particular, the addition of sea-salt deposited on coastal areas results in the addition of Cl^- which can be acidifying. Another example is the reaction of CO_2 in soil gases with soil pore water to produce carbonic acid (H_2CO_3) (Reuss and Johnson, 1986). Soil acidification can also occur naturally through biogeochemical cycles which alter the ion balance. Ions can be produced or consumed as part of reactions performed by microorganisms (Helyar and Porter, 1989).

2.4.5.1 Controls on hydrological transport

DOC which is not bound by physio-chemical processes and has not been consumed during microbial metabolism may be controlled by hydrological processes. Key controls on hydrological transport relate to soil hydrology and associated flow paths. Hydraulic conductivity will dictate the movement and residence time of DOC in peat and organic soil. For instance, in peat, where the hydraulic conductivity is slow and saturation is high, DOC mobility is reduced and residence time is high. Both high and low flow move through the organic layer where DOC is mobile (Clark et al., 2008). Seasonal changes in the hydraulic head also result in patterns in DOC production and consumption and spatial redistribution of DOC throughout the peat profile (Waddington and Roulet, 1997).

Pockets of organo-mineral soils on peatlands have different profile characteristics and therefore hydrological behaviour. Such behaviour is complex and has been poorly studied. In general, the hill slope topography which allows podzols to form enhances drainage, whilst the mineral content of podzols also enhances pore volume and size and allows for easier throughflow. DOC which is not sorbed onto mineral surfaces is easily leached away due to greater drainage capacity and faster hydraulic conductivity. Flow pathways can shift during stormflow events from the main runoff pathway in the lower mineral layer, to the upper organic layer (McDowell and Likens, 1988, Clark et al., 2008).

2.5 Long term DOC trends and links to declining acid rain

2.5.1 DOC trends

DOC monitoring was limited to a few small sites prior to national monitoring programmes coming into force in the 1980's. However, even within these early site-specific datasets, increases in DOC concentrations were apparent both in North Wales (Reynolds et al., 1997, Robson and Neal, 1996) and Scotland (Harriman et al., 2001). In England, early data is limited to monitoring changes in water colour, with water notably becoming darker in colour from the 1960's in Yorkshire (Watts et al., 2001) and areas draining the Pennines (Worrall et al., 2003a).

The Acid Waters Monitoring Network has been monitoring DOC concentrations in lakes and streams across the UK since 1988. All 22 sites, located in acid sensitive areas of the UK, have experienced on average a 91 % increase in DOC concentrations (*Figure 2.5*), all of which were significant and consistent (Evans et al., 2006a). Further monitoring of another 198 sites across the UK showed that 77 % had a significant upward trend in DOC concentration, whilst 23 % did not have a significant trend, and no sites had a significant decrease in concentration (Worrall et al., 2004b).

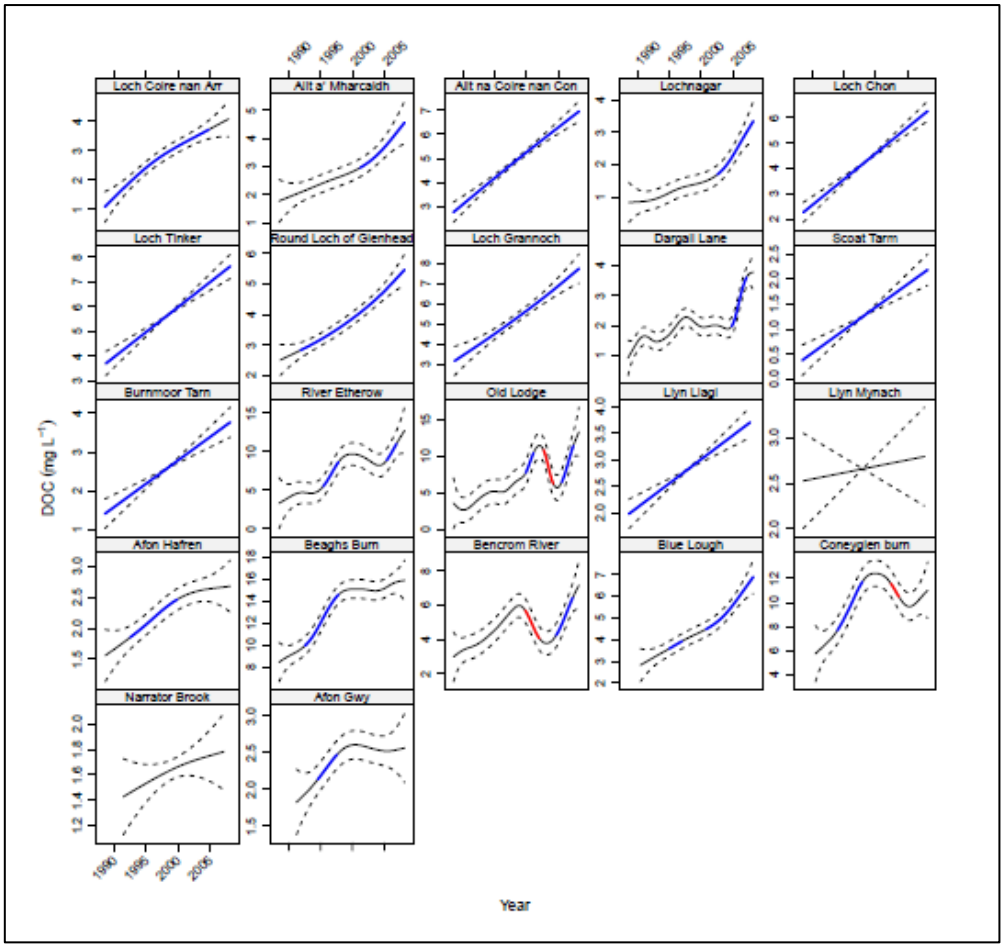


Figure 2.5: Fitted trends in dissolved organic carbon for the 22 AWMN sites. Coloured sections of the trend line indicates periods of significant change (determined by derivative analysis) (blue = increase; red = decrease) (M. Kernan, 2010).

In addition, there have been similar trends across Northern Europe and North America (Skjelkvåle et al., 2005, Driscoll et al., 2003, Hejzlar et al., 2003). The international UNECE programme (ICP Waters) monitored the impact of atmospheric deposition on surface waters, covering 12 geographical regions across Europe and North America. Data shows that DOC concentrations have increased across Nordic countries and the UK, but not across Central Europe (Skjelkvåle et al., 2005). Contrastingly, there is now much evidence of increased DOC concentrations in water bodies in the Czech Republic (Hejzlar et al., 2003, Oulehle and Hruška, 2009), as well as Sweden (Erlandsson et al., 2008) and Norway (de Wit et al., 2007).

In the US, a study covering the period 1990 to 2000 monitored DOC trends across northern and eastern US. Overall, there was an average 10% increase in DOC concentrations at four

out of five sites (Stoddard et al., 2003). Increases have also been observed at sites in New York (1982) (Driscoll et al., 2003) and Quebec (1985 – 1993) (Bouchard, 1997).

Monteith et al. (2007) summarised data on DOC trends across Europe and North America (*Figure 2.6*). It is clear from this review that there have been increases in DOC concentrations across much of the Northern Hemisphere between 1990 and 2004, with many of these increases being significant.

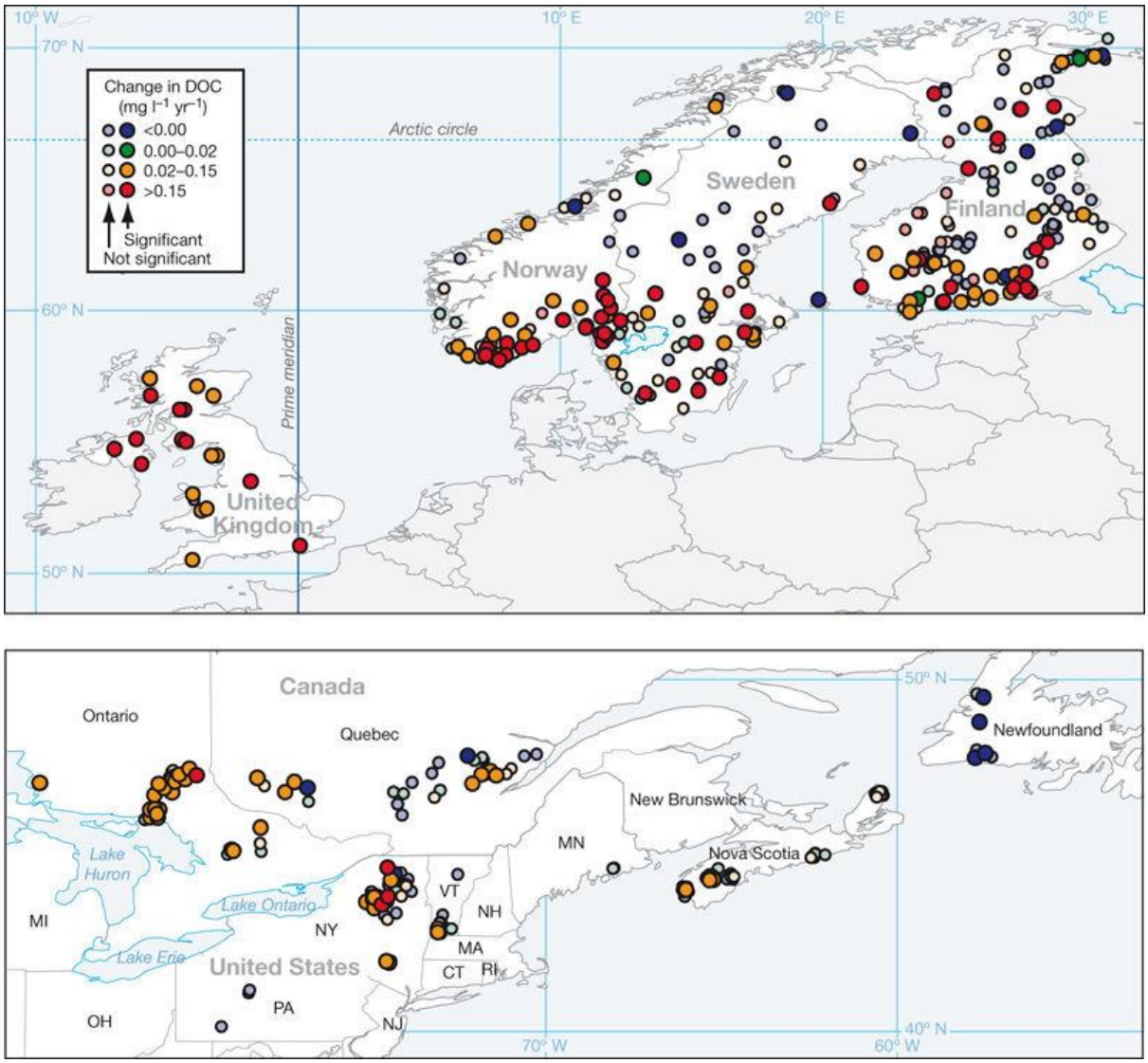


Figure 2.6: Map showing summary of datasets collected from monitoring sites across the Northern Hemisphere, for Europe (upper panel) and North America (lower panel) between 1990 and 2004 (Monteith et al., 2007).

2.5.2 Deposition of acidifying pollutants

Natural events and cycles can emit acidifying compounds into the atmosphere and onto terrestrial and aquatic environments, such as volcanic eruptions, sea sprays and the weathering of rocks. However, emissions of sulphur dioxide and oxides of nitrogen, as well as other pollutants, increased rapidly across developing areas of Western Europe and North America during the Industrial Revolution. Activities releasing these emissions include the combustion of fossil fuels, industrial processes, waste incineration, agricultural activities and even the use of explosives. This anthropogenic input of acidifying compounds into the atmosphere increased and quickly exceeded natural background levels (Badr and Probert, 1994).

Acidification of terrestrial and aquatic ecosystems has occurred largely as a results of NO and SO_2 emissions from fossil fuel combustion, which is transformed into nitric and sulphuric acid and then deposited on terrestrial and aquatic ecosystems (Galloway, 2001), increasing the cation concentration in solutions (Reuss and Johnson, 1986). The UK Environmental Change Network highlights recovery from acidification as one of the three most significant long-term trends in the physical environment within the UK (Morecroft et al., 2009).

There has been an awareness of precipitation contamination by atmospheric pollutants since the early Industrial Revolution (Smith, 1872). Analysis of peat cores have provided a useful environmental archive of pollution deposition rates over time, particularly for sulphur as natural and pollutant forms are isotopically distinct. Examination of cores from the Pennines show evidence of increases in sulphur deposition around 1400 AD, possibly reflecting inputs from small-scale lead smelting in local areas. Increases in lighter isotopic compositions towards the surface of the cores correlate with increased sulphur deposition loadings from 1750 onwards, corresponding with the beginning of the Industrial Revolution (Coulson et al., 2005).

The term ‘acid rain’ was first stated in scientific literature in March 1972 by Likens et al (1972), followed by discussion at the United Nations Conference on the Human Environment in June 1972; the first international conference focussing on environmental issues including air pollution and acid deposition. Action to tackle these issues were then outlined by the Geneva

Convention on Long-Range Transboundary Air Pollution in 1979, where a legally binding international agreement was established. This provided a basis for widespread action to monitor and reduce air pollution and acid deposition across Europe, including the 2008 EU Ambient Air Quality Directive, which set limits on concentrations of air pollutants, and was incorporated into UK law through legislation including the Air Quality Standards Regulations (2010) in England.

The scientific literature generally agree that global sulphur emissions peaked around 1980 followed by a decline (*Figure 2.7*), and that this decline was largely governed by environmental legislation, including fossil fuel use changes, controls on coal fired power plants such as the introduction of scrubbers, and the removal of sulphur from oil and non-ferrous metals (Smith et al., 2004).

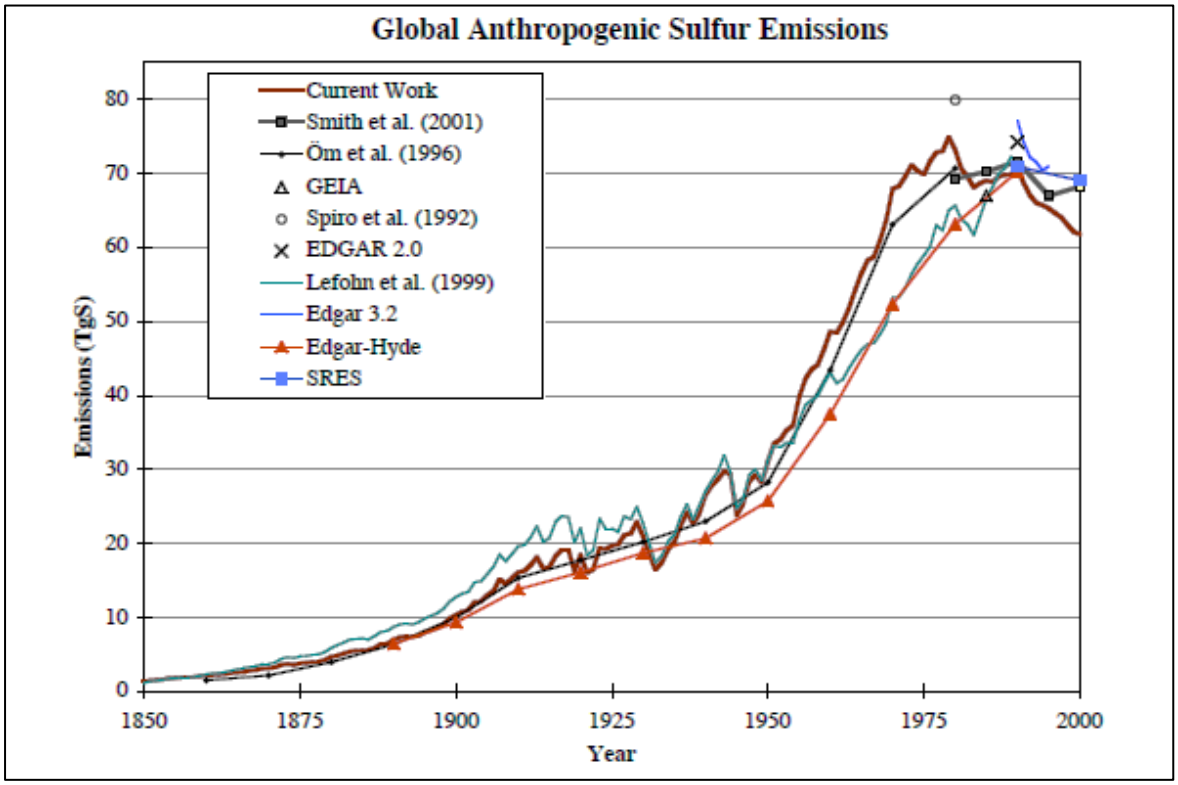


Figure 2.7: Global sulphur dioxide emissions between 1850 and 2000 based on estimates from within the literature (Smith et al., 2004).

When focusing on the sources of sulphur dioxide (*Figure 2.8*), it is clear that fossil fuel combustion emitted the most, with coal dominating followed by oil, and even with a reduction in all emissions from 1980 this still remained a dominant contributor in 2000. The third largest contributor is metal smelting, followed by ocean bunkers (fossil fuel used for

ocean shipping). Other sources include natural gas processing and combustion, land use and land use changes, traditional biomass combustion and other industrial processes.

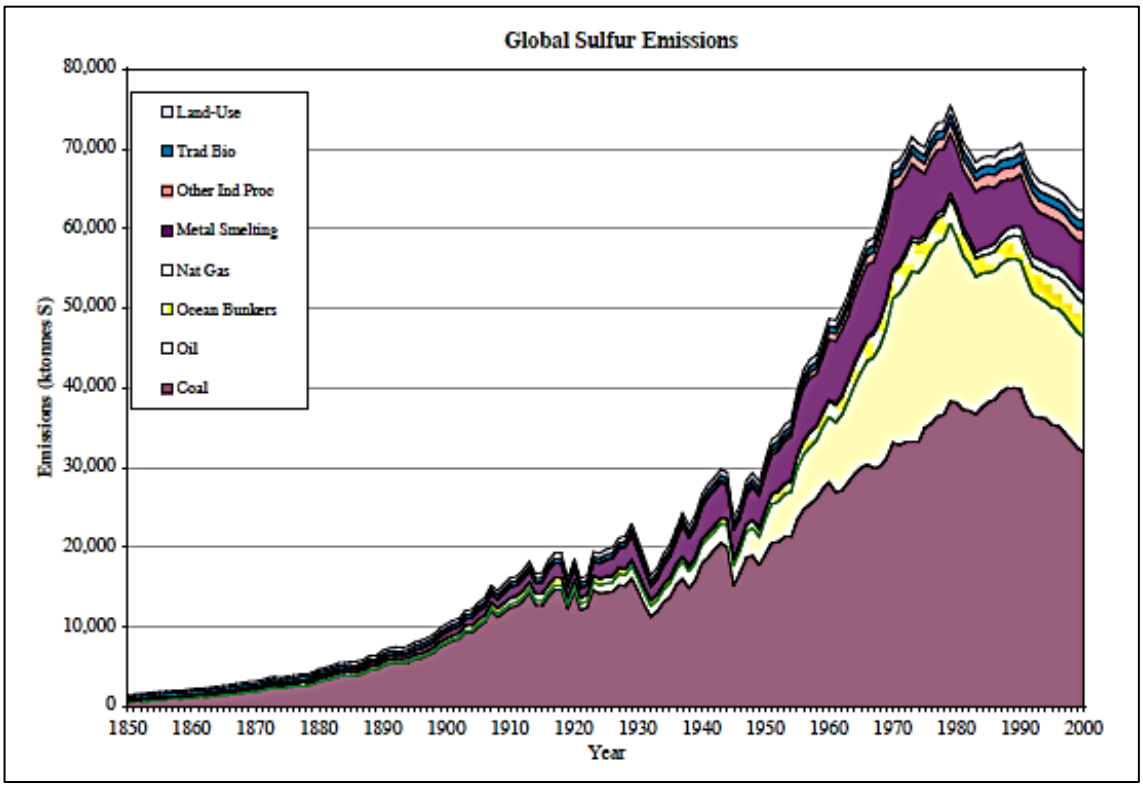


Figure 2.8: Global sulphur dioxide emissions estimates by source between 1850 and 2000 (Smith et al., 2004).

However, it is important to note that whilst sulphur dioxide emissions have peaked and are in decline in Europe and North America, emissions are still increasing in many other areas of the world, particularly in Asia (*Figure 2.9*). Therefore whilst some regions are seeing the effects of recovery from acidification, others are currently experiencing acidification. This highlights the continued relevance and importance of research on the effects of acidification and recovery on a variety of ecosystems internationally.

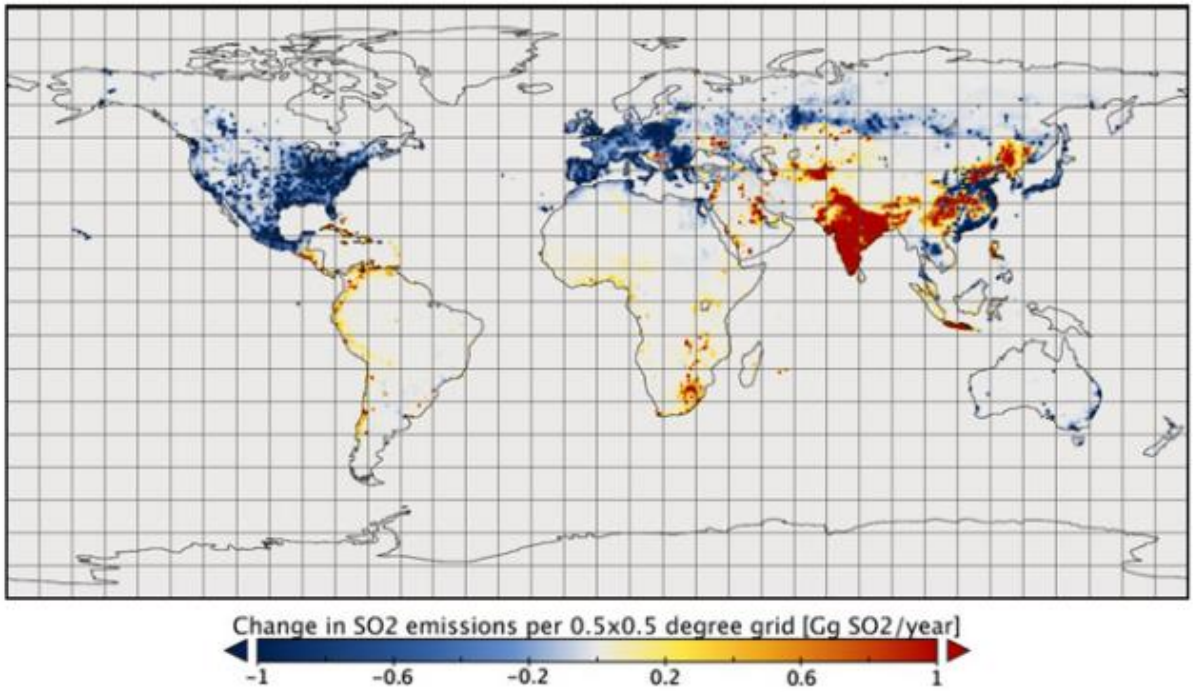


Figure 2.9: Change (as difference between 2010 and 2005 emissions) in regional distribution of anthropogenic sulphur dioxide emissions (Klimont et al., 2013).

Reductions in sulphur dioxide emissions in the UK can be seen from the 1970's (the earliest date at which emission rates were monitored), with a steep decline in the 1990's (*Figure 2.10*). In total from 1970 to 2013 there has been a 94 % decline SO_2 emissions in the UK. The largest source of emissions were and continue to be from electricity generation, followed by industrial combustion. Both have seen a dramatic decline in emissions over the last four decades. Emissions from residential and commercial combustion have also reduced significantly since 1970 (Defra, 2013).

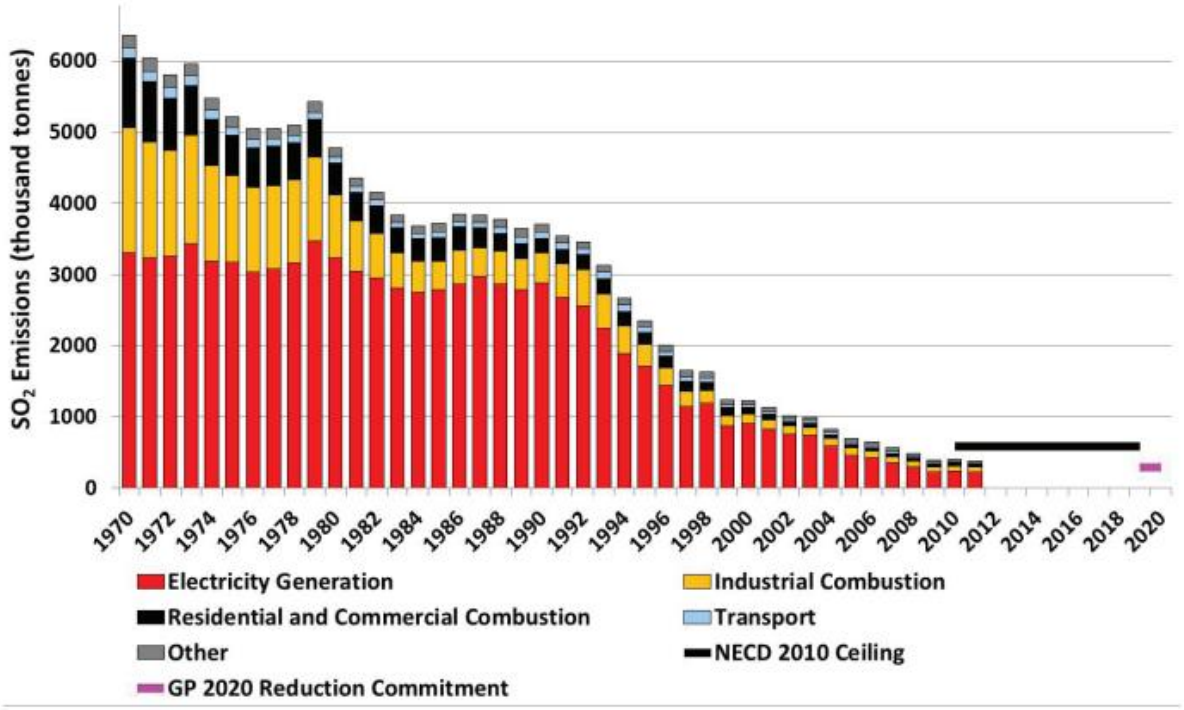


Figure 2.10: Sulphur dioxide emissions in the UK (1970 – 2011) alongside 2010 NECD target (black line) and 2020 Gothenburg Protocol emission reduction target (purple line) (Defra, 2013).

Contrastingly to SO_2 emissions, NOx emissions did not begin to decline in the UK until 1990 (*Figure 2.11*). This reduction was largely due to vehicle regulations as part of Euro Standards which brought in enforcement of the three-way catalytic converter, and a shift from coal to natural gas power stations (Defra, 2013).

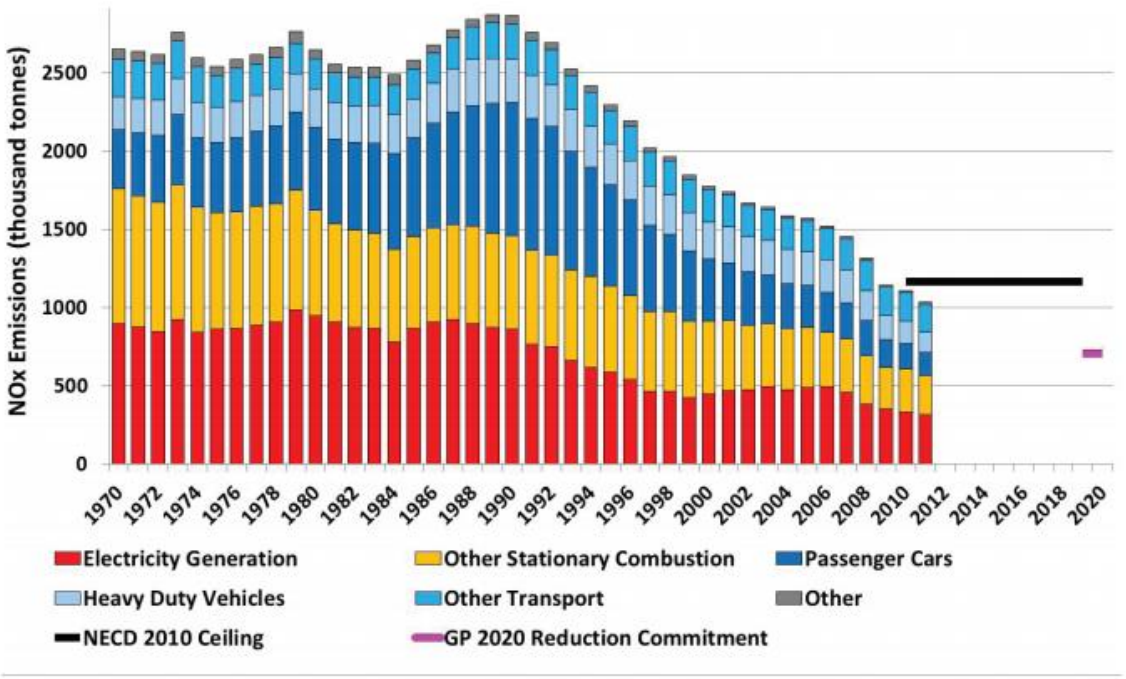


Figure 2.11: Oxides of nitrogen emissions in the UK (1970 – 2011) alongside 2010 NECD target and 2020 Gothenburg Protocol emission reduction target (Defra, 2013).

Data collected through the Acid Gas and Aerosol Network (AGANET) monitoring programme from sites across the UK in 2011 show SO_2 (*Figure 2.12*) and NOx (*Figure 2.13*) emissions are associated with the location of industrial areas, including London, the Midlands and lower North of England, Cardiff in Wales, Belfast in Northern Ireland and Glasgow and Edinburgh in Scotland. However, wet deposition of non-seasalt sulphate and nitrate is mainly in rural areas of the UK, highlighting the transboundary transportation potential of atmospheric pollutants.

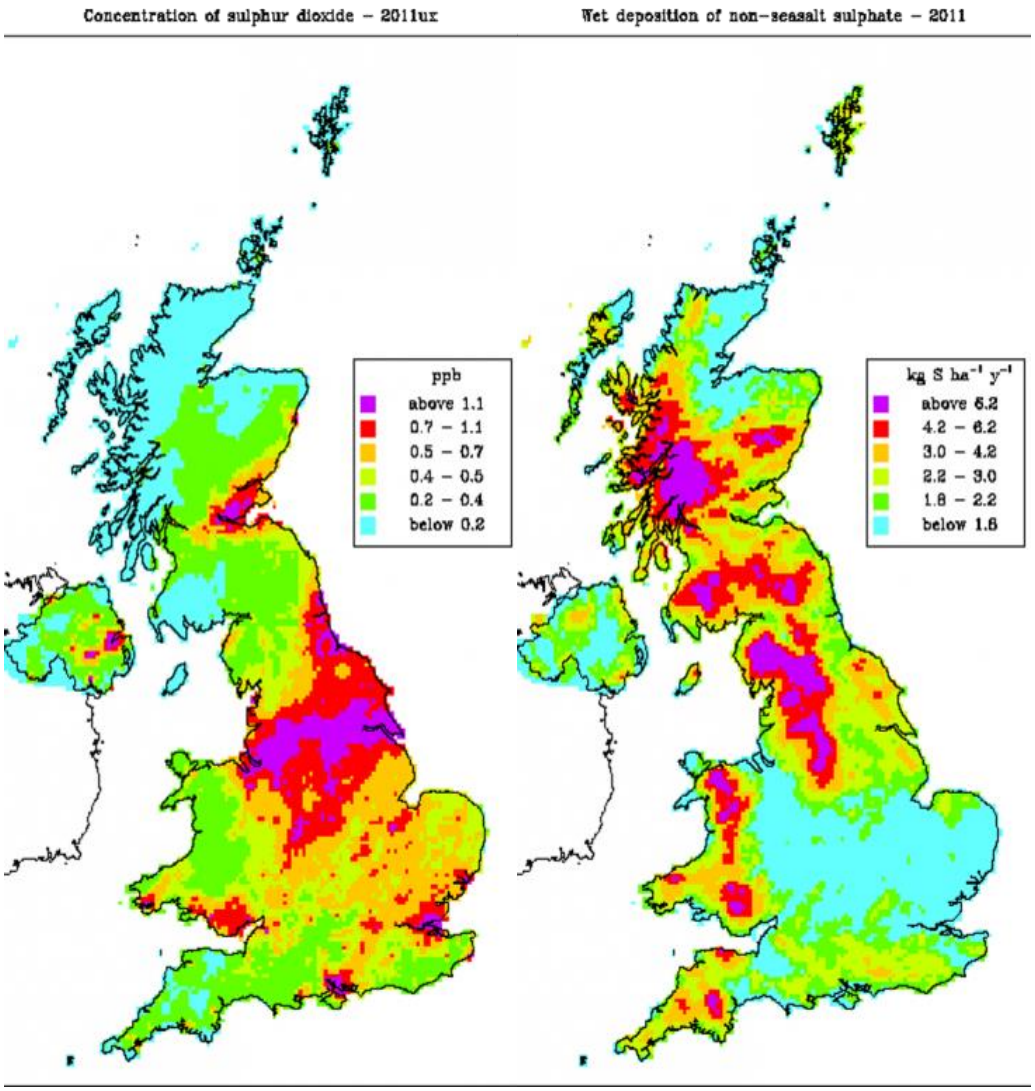


Figure 2.12: Data maps showing concentration of sulphur dioxide emissions (left) and wet deposition of non-seasalt sulphate (right) across the UK in 2011 (CEH, 2015b).

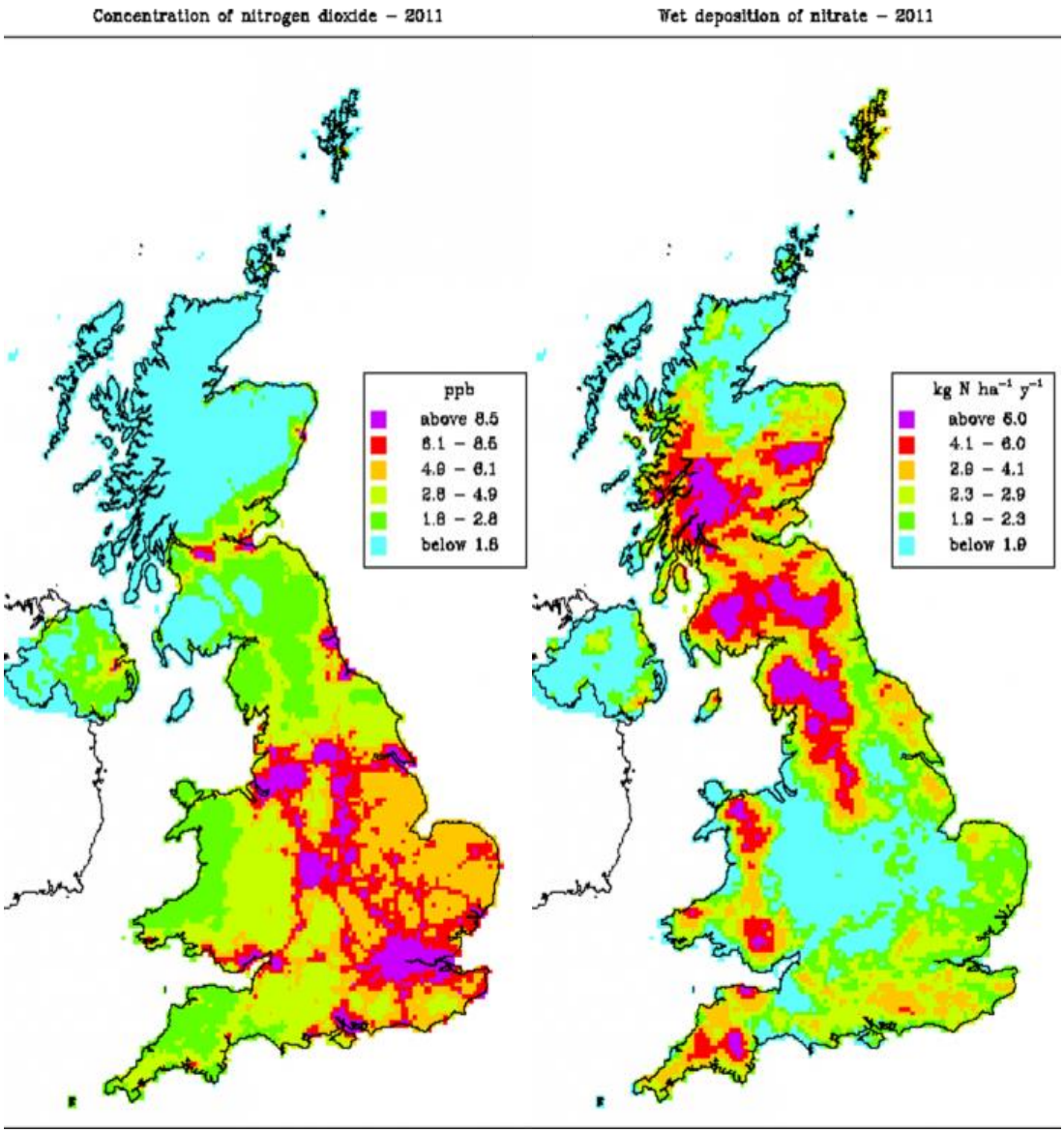


Figure 2.13: Data maps showing concentration of nitrogen dioxide emissions (left) and wet deposition of nitrate (right) across the UK in 2011 (CEH, 2015a).

Another monitoring programme, the Eutrophying and Acidifying Atmospheric Pollutants (UKEAP) network has been monitoring precipitation chemical composition at 41 sites across the UK since 1986. A clear relationship can be seen in the data between the reduction in emissions of sulphur dioxide and oxides of nitrogen, and reduced non-seasalt sulphate and nitrate concentrations in rainwater for the UK (Conolly et al., 2016). The deposition of sulphur across the UK has also been widely documented in freshwater systems as part of the long term monitoring of the Acid Waters Monitoring Network (AWMN) (*Figure 2.14*). Between 1988 and 2003, sulphate concentrations have been declining gradually and this has been linked to reduced sulphur deposition (Evans et al., 2006a).

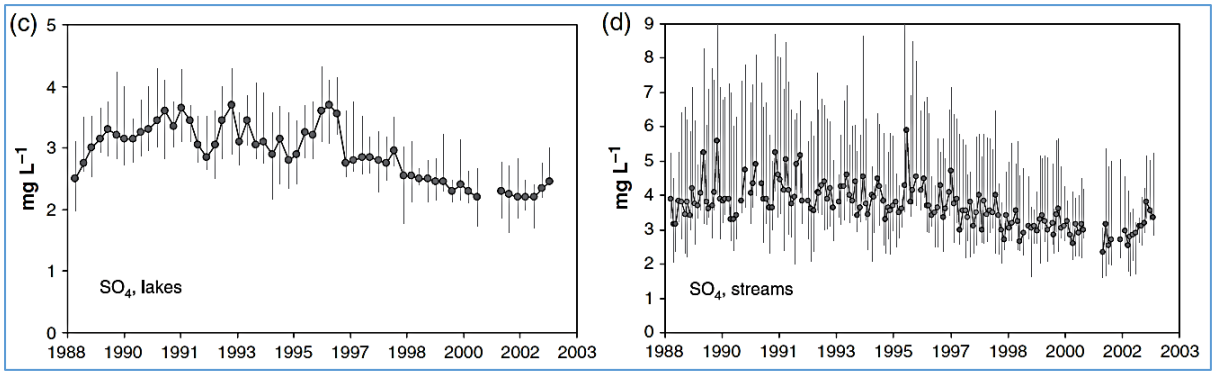


Figure 2.14: SO_4 concentrations for 10 lakes and 8 streams across the AWMN between 1988 and 2003 (Evans et al., 2006a).

2.5.3 DOC physio-chemical response mechanism

Marine and atmospheric sulphur have decreased over the last three decades, whilst DOC concentrations in surface waters draining organic catchments has been increasing (*Figure 2.15*). The key hypothesis behind this relationship is that declining sulphur deposition has led to decreased acidity and ionic strength as soils recover, which has increased DOC solubility (Evans et al., 2006a).

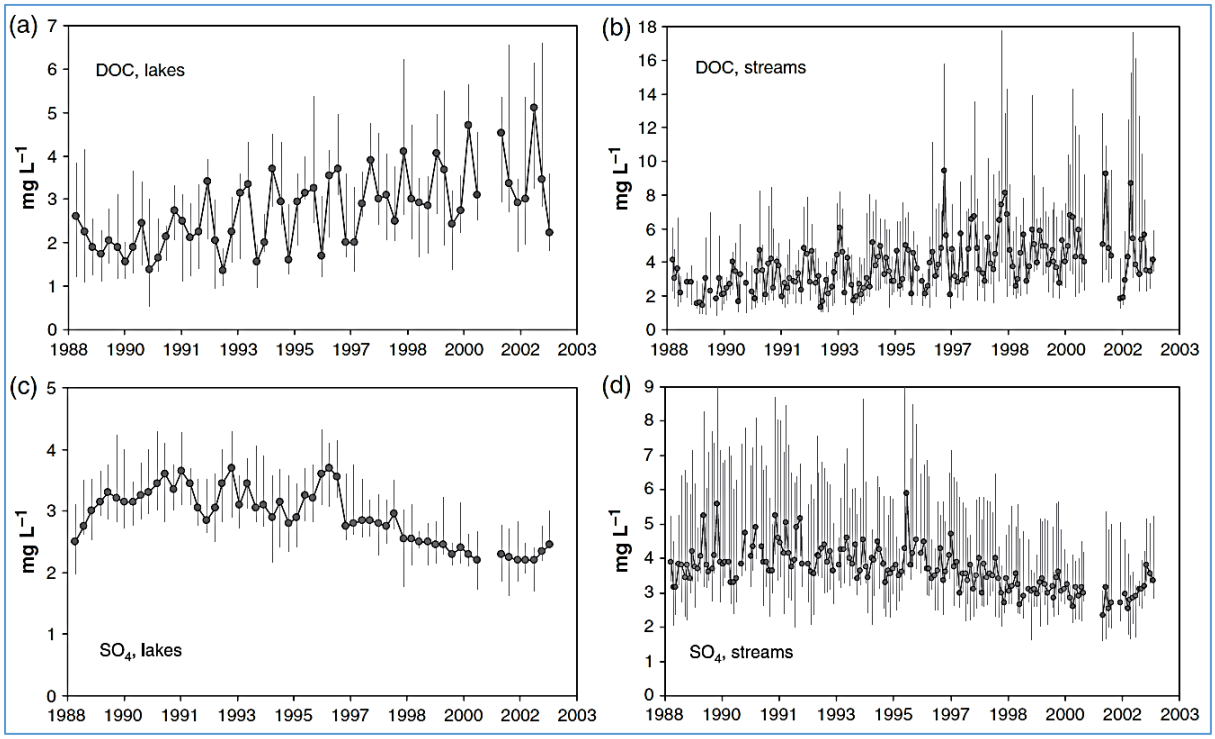


Figure 2.15: DOC and SO_4 concentrations for 10 lakes and 8 streams across the AWMN between 1988 and 2003 (Evans et al., 2006a).

During times of high acid deposition, the pH of soil solutions decreased, which lowered DOC solubility as organic compounds precipitated out of solution and coagulated together. As acid deposition decreased, pH of solutions increased as soils recovered towards pre-pollution levels and a high DOC production state. In addition, the solubility of the previously locked away DOC increased, resulting in a significant release of DOC into nearby water systems. However, there have been discrepancies in the amount of DOC released from catchments receiving similar acid deposition loads, raising the question of the importance of catchment specific characteristics such as the proportion of peat and organo-mineral soils (Clark et al., 2010a).

Electrical conductivity (measured as a proxy for ionic strength) is also increased with acidity, which may also retain DOC in peat. However, DOC solubility is more sensitive to changes in acidity than to conductivity (Clark et al., 2011). Variations in soil properties can also affect the extent to which this mechanism occurs and accounts for the differences in magnitude of DOC responses to acidity at different sites. This includes variations in soil acid/base status such as acidity buffering in peat, aluminium concentrations in organo-mineral soils resulting in co-precipitation with DOC and lower base saturation (Clark et al., 2011).

2.5.3.1 Evidence of driving mechanism

There are many theories which have been put forward to explain these increases in DOC concentrations. These include atmospheric carbon dioxide (Freeman et al., 2004), increased nitrogen deposition (Findlay, 2005), warmer temperatures (Freeman et al., 2001a), precipitation (Tranvik and Jansson, 2002), changes to land management (Clutterbuck and Yallop, 2010) and catchment hydrology (Erlandsson et al., 2008).

However, there is a strong evidence base in the literature to supporting the pH-DOC hypothesis. Long term monitoring of DOC concentrations in terrestrial water bodies has been conducted across sites in the Northern Hemisphere since the 1980's. The AWMN in the UK has shown a 91% increase in DOC concentrations in the water bodies monitored between 1988 and 2003, which correlate to a reduction in sulphur concentrations (41% reduction between 1988 and 1993 (Davies et al., 2005)) (Evans et al., 2006a). This relationship has also been observed across Europe and North America (Driscoll et al., 2003,

Skjelkvåle et al., 2005, Stoddard et al., 2003, Oulehle and Hruška, 2009, Oulehle et al., 2017, Oulehle et al., 2011).

Numerous laboratory and field experiments have provided strong evidence for this pH-DOC link. For instance, acidity manipulation field experiments in both peatlands and forested environments have shown strong relationships between acidity and DOC mobility in peat and organo-mineral soils (Evans et al., 2012, Oulehle et al., 2013, Evans et al., 2008a, Moldan et al., 2012, Ekström et al., 2011). Laboratory experiments have been conducted on samples collected from peatland sites across the UK, which provides further evidence for this hypothesis (Clark et al., 2011, Palmer et al., 2013). For instance, Clark et al. (2011) showed DOC released from organo-mineral O horizon and peats decreased by 21-60 % with sulphuric acid additions.

Studies involving models have also provided further support for this pH-DOC mechanism (Monteith et al., 2007, Rowe et al., 2014, Evans et al., 2008b, Sawicka et al., 2016). Sawicka et al. (2017) used the soil chemistry model MADOC and found that acidification from sulphur deposition was the dominant control on DOC trends, but suggests that the effects of nitrogen deposition on N-limited soils may have raised the 'acid recovery DOC baseline' to above pre-industrial levels. It is also suggested that changes in DOC leaching previously attributed to nitrogen deposition could be due to the simultaneous acidification of nitrogen pollutants (Evans et al., 2008a, Oulehle et al., 2018).

2.6 Gaps in knowledge

There has been inconsistencies in how different sites have responded to decreasing acidity and deposition, with some sites showing no significant increase in DOC. Whilst this may relate to variation in soil type and acid/base status between sites (Clark et al., 2011), there are still questions as to whether a biological mechanism may also be contributing to this disparity in magnitude of DOC changes at different sites.

There is evidence that biological activity in terms of DOC production and consumption may have increased with decreasing sulphur deposition. Dawson et al. (2009) used a statistical mixed-effects model to analyse the mechanisms behind the increased DOC concentration and hydrophobic fraction observed over two decades of monitoring at two upland moorland catchments in Scotland. Results suggest that increased solubility of DOC alongside enhanced

heterotrophic decomposition are behind these trends. Alternatively, high sulphur inputs may increase bacterial sulphate reduction, which may increase consumption of labile DOC (Bartlett et al., 2005).

It is important we fully understand the mechanisms behind increased DOC concentrations to further understand the implications for future carbon stores and accelerated climate change. There is a clear acceptance of the pH-DOC link within the scientific literature, and yet a knowledge gap exists in whether a biological mechanism may also be involved. In particular, how changing acidity might affect microbial communities and their functions, with a focus on DOC production and consumption.

2.7 Conclusion

Since the industrial revolution, fossil fuel combustion has resulted in an unintended field experiment spanning a momentum scale, resulting in acidification of terrestrial and aquatic ecosystems across much of the Northern Hemisphere. In recent decades, environmental legislation has led to a huge reduction in the emissions of acidifying pollutants, including a 94 % drop in SO_2 emissions (Defra, 2013). As a result, these environments are now in a state of recovery. The UK Environmental Change Network highlights recovery from acidification as one of the three most significant long-term trends in the physical environment within the UK (Morecroft et al., 2009).

Over the same time scale, DOC concentrations in terrestrial waters has increased by more than 90 % in acid sensitive areas across the UK (Evans et al., 2005). Much of this DOC is leaching from catchments containing carbon rich peat and organo-mineral soils, leading to concerns over the future of these internationally important carbon stocks and climate change implications. Further work is needed to better understand the mechanisms behind this increased DOC trend.

Chapter 3: Field sites and experiment

3.1 Description of study area

Two experimental sites were set up in contrasting areas of historic pollution deposition in two National Parks in the UK (*Figure 3.1*).

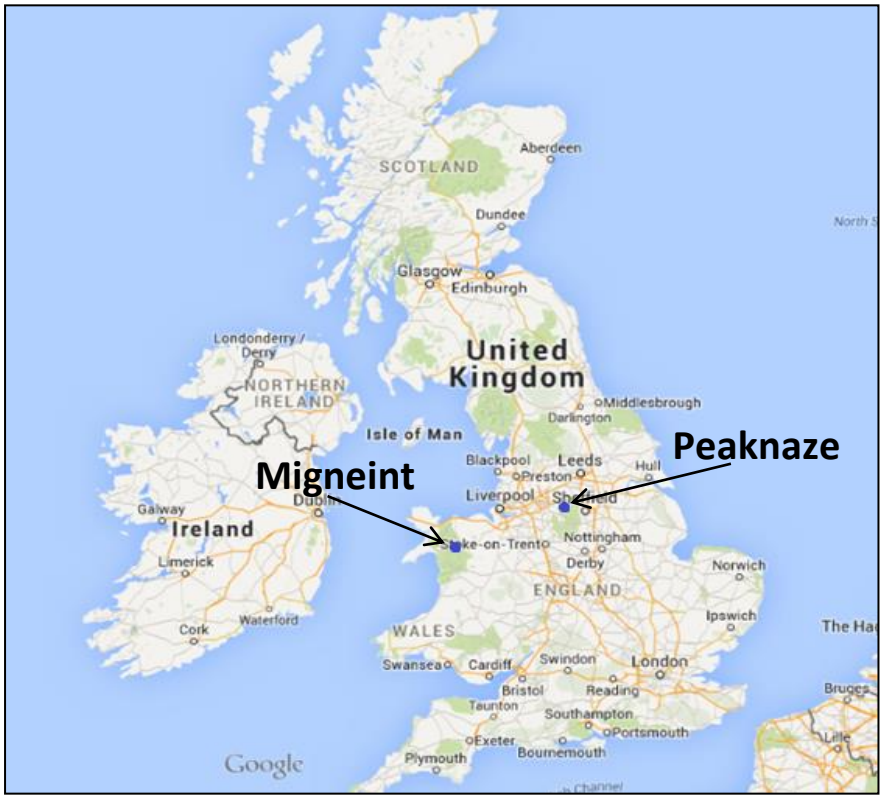


Figure 3.1: Map of UK illustrating the location of the two experimental sites (GoogleMaps, 2015).

Migneint (*Figure 3.2*) is a large area of upland moorland (experimental plots at ~460 m above sea level (a.s.l.)) located in the Snowdonia region of North Wales (3°48.8’ W, 52°59.6’ N), dominated by extensive blanket peat (*Table 3.1*). Although the Migneint has experienced low levels of pollution (Section 3.1.1) and is considered to be in relatively good condition (Evans et al., 2012), this still exceeds critical loads for a blanket bog and has resulted in significant acidification of the catchment (Evans et al., 2006b), with increased DOC concentrations in surface waters. This has been observed from nearby Upland Water Monitoring Network (UWMN) sites (M. Kernan, 2010, Monteith et al., 2014). Llyn Llagi, located 13 km west of the experiment site, has seen an 89 % increase in annual mean DOC concentrations between 1989 and 2009; and Afon Gwy, situated 50 km south, has had an increase of 51 % over the same time period (Evans et al., 2012).

Figure 3.2: A photograph of the surrounding landscape at the Migneint field site.

Peaknaze (*Figure 3.3*) (1°54.5’ W, 53°28.3’ N) is located in the upland moorland area (experimental plots at ~440 m a.s.l.) of the Peak District National Park, which encompasses the southern end of the Pennines located in the Midland / Northern area of England (*Table 3.1*). Contrastingly to the Migneint, high levels of sulphur and nitrogen deposition (Section 3.1.1) alongside intensive land management has led to peatland degradation, including *Sphagnum* loss and erosion (Oulehle et al., 2013, Tallis, 1987, Carroll et al., 2009). UWMN data indicates a rise in annual DOC concentration in nearby water courses, such as at the River Etherow (located 6 km from the study site) where a 194 % increase between 1989 and 2009 has been observed (Evans et al., 2012).

Figure 3.3: A photograph of the surrounding landscape at the Peaknaze field site.

	Migneint		Peak District	
	Peat	**Podzol**	**Peat**	**Podzol**
Condition	Good		Degraded	
Water Table (2008-2011)	9 cm below surface		13 cm below surface	
Height Above Sea Level	460 m	486 m	440 m	440 m
Annual Average Rainfall	2400 mm/yr		1000 mm/yr	
Increase in annual mean DOC Concentration 1989-2009	**Llyn Llagi**		**River Etherow**	
	89 %		194 %	

Table 3.1: A summary of the key characteristics at the two experimental sites. DOC concentrations are for two nearby Upland Waters Monitoring Network sites; River Etherow which is 6 km from Peaknaze, and Llyn Llagi located 13 km from Migneint (Evans et al., 2012).

3.1.1 Pollution deposition

Both sites have received very contrasting levels of pollution deposition (*Table 3.2*) which has strongly acidified soils and surface waters (Evans et al., 2000). The levels of pollution being experienced over these regions have significantly changed over the years. For instance,

between 1970 and 2007 there was an estimated 66 % reduction in sulphur deposition at the Migneint (as valued by the FRAME model), with 4.91 kg S ha^{-1} yr^{-1} of non-marine oxidised sulphur being deposited in 2014. Contrastingly, the Peak District represents an area also dominated by peat that has experienced much higher levels of sulphur deposition. Non-marine sulphur deposition was measured at 6.31 kg S ha^{-1} yr^{-1} in 2014, following a 69 % decline in sulphur deposition between 1970 and 2005 (Dore et al., 2007, Evans et al., 2012, CEH, 2014). Despite the decline in sulphur deposition, nitrogen deposition remains much higher, with 17.98 kg N ha^{-1} yr^{-1} being measured near the Migneint experimental plots in 2014, and 22.91 kg N ha^{-1} yr^{-1} near Peaknaze (CEH, 2014).

Waters draining from the catchments encompassing the experimental sites have shown a large decrease in sulphate and nitrate concentrations. Llyn Llagi, located 13 km from the Migneint experimental site, had a sulphate concentration of 62.9 kg S ha^{-1} yr^{-1} and a nitrate concentration of 10.4 kg N ha^{-1} yr^{-1} during UWMN baseline years (1988 – 1993), which has now fallen to 36.6 kg S ha^{-1} yr^{-1} and 6.4 kg N ha^{-1} yr^{-1} (2014 – 2015 data). Much higher concentrations were measured at the River Etherow, located 6 km from Peaknaze. During baseline years, the sulphate concentration was 295.3 kg S ha^{-1} yr^{-1} and the nitrate concentration was 44.8 kg N ha^{-1} yr^{-1}. Although these have reduced considerably to 139.6 kg S ha^{-1} yr^{-1} and 29.4 kg N ha^{-1} yr^{-1} (2014 - 2015), these are still high values depicting pollution deposition still remains high in this region (Shilland et al., 2016). In addition, when assessing the recent UKEAP (UK Eutrophying and Acidifying Network) dataset for wet and dry deposition as well as acid gas concentrations for nearby waters, we can see that the Peak District still has higher concentrations of pollutants compared to the Migneint area of Snowdonia (*Table 3.2*).

Regardless of the lower levels deposited over the North Wales region in comparison to the Peak District, considerable acidification is suspected to have occurred (Evans et al., 2006a). This can be seen in the pH of precipitation collected in 2016, which was 0.32 units lower at Llyn Llagi than at the River Etherow (Defra, 2016b). Also, recent (2014) deposition data shows total acidity deposition was much higher near the Peaknaze experimental site at 1.83 keq ha^{-1} yr^{-1} compared to 1.47 keq ha^{-1} yr^{-1} near the Migneint site (CEH, 2014).

Data	Year	Migneint	Peaknaze
Non-marine Sulphur Deposition (kg S ha^{-1} yr^{-1})[1]	Historical (1970 – 2007)	Decreased by 66%	Decreased by 69%
Total None-marine Oxidised Sulphur Deposition (kg S ha^{-1} yr^{-1})[2]	2014	4.91	6.31
Non-marine Wet Sulphate Deposition (kg S ha^{-1} yr^{-1})[2]	2014	4.41	5.41
Sulphur Dioxide Dry Deposition (kg S ha^{-1} yr^{-1})[2]	2014	1.32	2.55
Total Nitrogen Deposition (kg N ha^{-1} yr^{-1})[2]	2014	17.98	22.91
Total Acidity Deposition (keq ha^{-1} yr^{-1})[2]	2014	1.47	1.83
UWMN River Concentrations[3]		**Llyn Llagi**	**River Etherow**
Sulphate Concentrations ($\mu eq\ l^{-1}$)	Baseline Years	62.9	295.3
	2014 – 2015	36.6	139.6
Nitrate Concentrations ($\mu g\ l^{-1}$)	Baseline Years	10.4	44.8
	2014 – 2015	6.4	29.3
UKEAP Wet Deposition[4]		**Llyn Llagi**	**River Etherow**
Non-marine Sulphate as S in Precipitation (mg/L)	2016	0.13	0.24
Nitrate as N in Precipitation (mg/L)	2016	0.17	0.37
pH of Precipitation	2016	5.38	5.70
UKEAP Acid Gases Concentrations[5]		**Plas Y Brenith**	**Lady Bower**
Sulphur Dioxide ($\mu g/m^3$)	2016	0.31	0.42
Nitric Acid ($\mu g/m^3$)	2016	0.18	0.23
Nitrous Acid ($\mu g/m^3$)	2016	<0.002	<0.03
UKEAP Particulate Deposition[5]		**Plas Y Brenith**	**Lady Bower**
Particulate Sulphate ($\mu g/m^3$)	2016	0.90	0.82
Particulate Nitrite ($\mu g/m^3$)	2016	<0.05	<0.05
Particulate Nitrate ($\mu g/m^3$)	2016	1.19	1.62

Table 3.2: A summary of present and historical pollution deposition collected near experimental sites. Sources are as follows: 1) CEH moorland deposition data and estimates from the FRAME model (Evans et al., 2012); 2) UK deposition data downloaded from CEH website (CEH, 2014); 3) UWMN (Shilland et al., 2016). The mean between 1988 and 1993 is deemed the baseline; 4) UKEAP data of wet deposition at the UWMN sites (Defra, 2016b) and 5) concentration of acid gases and particulate deposition at nearby Plas Y Brentih (29 km from Migneint) and Lady Bower (23 km from Peaknaze) (Defra, 2016a).

3.1.2 Soil type and vegetation

The field experiment encompasses two different soil types at each site location. The first is blanket peat, or histosols under the FAO classification system (FAO, 2006). These dominate areas where rainfall is high with minimum relief. The second soil type is peaty podzols (histic podzol (FAO, 2006)) which is an organo-mineral soil typical to areas with high rainfall and moderate drainage (see Section 2.2). These represent two common soils types typical to UK upland organic catchments and which represent two slightly different moorland environments. At the Migneint, the peat is approximately 460 m a.s.l. with a mean water table depth of 9 cm (as measured between 2008-2011) and an average peat depth of 2.0 m across the study area (*Figure 3.6*). The NVC (National Vegetation Classification) code for this site is M19 (*Calluna vulgaris – Eriophorum vaginatum* blanket mire) (Rodwell, 1998b) with vegetation including *Calluna vulgaris* and *Eriophorum vaginatum* with some *Cladonia spp.* above a deep *Sphagnum* layer (*Figure 3.4*). A large diversity exists in *Sphagnum* species across the Migneint area. Alternatively, humic podzol soils are present on hilly areas (*Figure 3.5*), the experimental plots being approximately 486 m a.s.l., emerging from a blanket bog. The peaty organic horizon is 5-18 cm deep, with a shallow stony E and B horizon underlying, followed by bedrock (*Figure 3.6*). Vegetation includes *Festuca ovina* and *Juncus squarrosus* with some *Galium saxatile, Eriophorum vaginatum* and *Calluna vulgaris*, as well as mosses such as *Polytrichum commune* and *Pleurozium shreberi* (Evans et al., 2012), with the NVC community U6 (*Juncus squarrosus – Festuca ovina* grassland (Rodwell, 1998a)).

Figure 3.4: A photograph of the peat plots located at Migneint.

Figure 3.5: A photograph of the podzol plots located at Migneint.

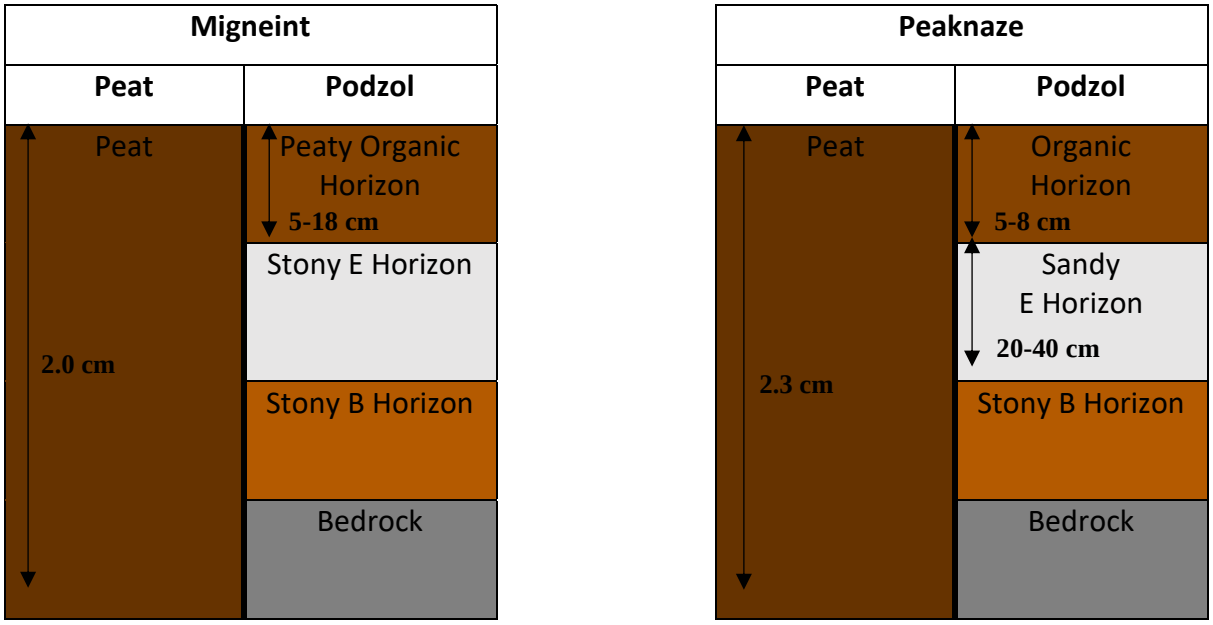


Figure 3.6: Diagram of the horizon structures of two soil types at the two experimental locations (no scale applied).

The experimental area of Peaknaze is mainly dominated by peat, with an average depth over the experimental area of 2.3 m. Amongst bare patches of exposed peat (*Figure 3.7*), degraded vegetation includes *Eriophorum vaginatum*, *Calluna vulgaris* and *Vaccinium myrtillus* with widespread mosses largely consisting of *Pleurozium shreberi* and some *Caldonia spp.* (*Figure 3.8*) (NVC M20b *Calluna vulgaris – Cladonia spp*. sub-community (Rodwell, 1998b)). The podzol soil has a 5-8 cm organic horizon overlying a 20-40 cm sandy E horizon and a stony B horizon (*Figures 3.6* and 3.*9*). Vegetation community has been categorised as NVC U6 with *Vaccinium myrtillus* sub-community (Rodwell, 1998a), which largely consists of *Festuca Ovina, Calluna vulgaris* and extensive *Vaccinium myrtillus,* with some *Eriophorum vaginatum* and *Juncus squarrosu*. Mean water table depth is 13 cm (as measured between 2008-2011) (Evans et al., 2012).

Figure 3.7: A photograph taken near Peaknaze showing an exposed area of peat where extensive erosion has occurred.

Figure 3.8: A photograph of the peat plots based at Peaknaze.

Figure 3.9: A photograph of the podzol plots based at Peaknaze.

3.2 Field experiment

3.2.1 Experiment design

This research builds on an existing long-term pH manipulation field experiment established in 2007 (Evans et al., 2012). Plots consist of twelve 9 m^2 plots at each of the four sites, with a randomised blocked design compromising of four replicates of control, acid and alkaline treatment plots at each location (*Figure 3.10* and 3.*11*). Therefore for statistical analysis, there are two factors; sites (four levels), and treatments (three levels), the design being applicable for a Two-way ANOVA test to investigate significant differences in data. Two treatments (acid and alkaline) were applied to two soil types (peat as a sulphur reducing system; and podzol as a sulphur oxidising system) at each location (Migneint and Peaknaze) on a monthly basis with additional control plots receiving ambient rainfall only.

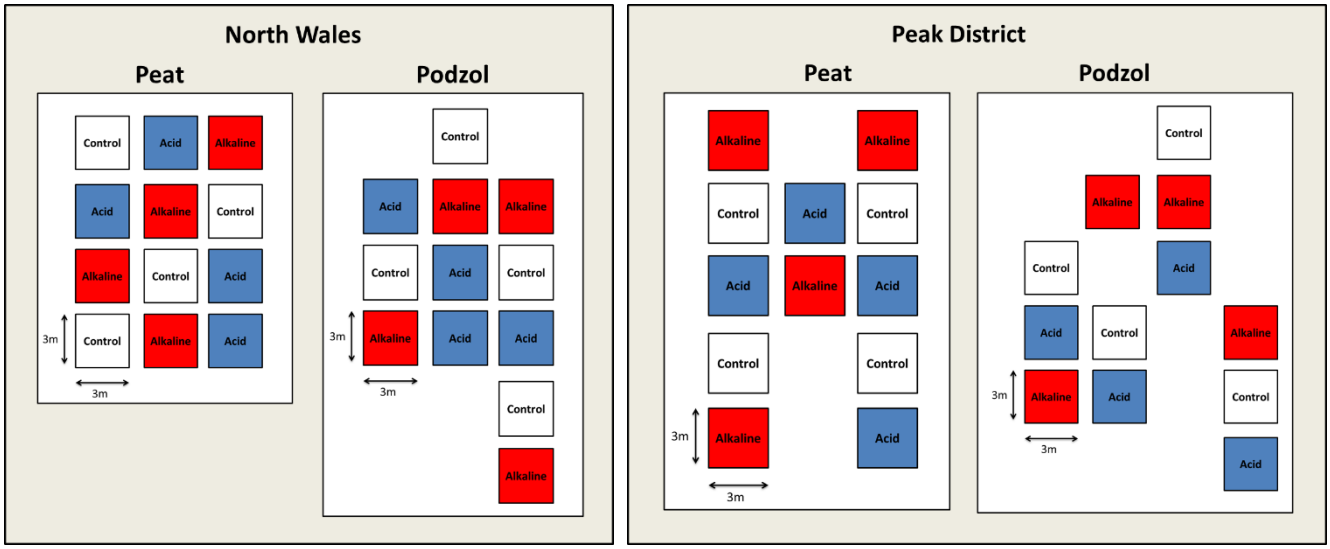


Figure 3.10: Illustration of field experiment layout, with three treatments organised in a randomised block design at two locations for two soil types.

Figure 3.11: Photograph of the 3x3 m plots located at Migneint on peat soil. The corners of the plots are marked out with posts, colour coded in reference to treatment type (white is control, red is acid and blue is alkaline).

3.2.2 Treatment applications

Treatments were applied initially from October 2008 until December 2012 (Evans et al., 2012), and then re-established (using the same methods, treatments and plot allocations) from January 2015 until October 2016. Acid plots received a monthly dose of sulphuric acid (H_2SO_4) mixed with rainwater collected at the site with 20 L of rainwater using a watering can (*Figures 3.12* and *3.13*). The concentration applied was 50 kg S ha^{-1} yr^{-1} at the podzol sites and 100 kg S ha^{-1} yr^{-1} at the peat sites, the latter concentration being similar to the ambient sulphur deposition in the Peak District in the 1970's. A higher dose was applied to peat plots to take account of the buffering effects of sulphur reduction. It should be noted that the aim of this experiment was to measure the effect in soil pH rather than simulate exact deposition loads. The original dose was 25 kg S ha^{-1} yr^{-1} in the early experimental years, which was increased to 50 kg S ha^{-1} yr^{-1} in January 2009, and again to 100 kg S ha^{-1} yr^{-1} at the peat plots in September 2009 to represent an acceleration in the experiment and ensure measureable pH change could be captured against the sulphur reduction mechanism of peat. A 10 L rinse of rainwater followed application to ensure the treatment infiltrated into the soil and to minimise and direct toxicity effects on plant foliage.

The same procedure was used for the alkaline plots with sodium hydroxide (NaOH) and potassium hydroxide (KOH). This was followed by a rinse containing magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) to maintain base cation ratios similar to those observed in rainfall, as well as maintaining a Na:Mg ratio similar to sea-salt. The molar OH^- concentration in the alkaline treatments was intended to be comparable to the H^+ concentration in the acid treatments. In 2009 treatments dosages were increased in parallel to those at the acid plots. Control plots received 20 L of rainwater only. See Appendix in Chapter 4 for protocol on treatment solution preparation.

Figure 3.12: A photograph of treatment solutions for a site prior to being diluted with rainwater.

Figure 3.13: A photograph showing alkaline treatment being applied to a plot at the Migneint peat site.

3.2.3 Sample collections

3.2.3.1 Sample collection timing

Prior to treatments recommencing in 2015, soil samples were collected from all plots in January and a chemical analysis was undertaken on soil extracts. In September 2015, monthly soil pore water collection began, and continued until October 2016 (Chapter 4). Soil samples were taken quarterly from January 2016 until October 2016 (Chapter 4). Litter was collected from a variety of vegetation species in September 2015 (Chapter 5 and 6) and three collections of decomposing surface litter were made in April, July and October 2016 (Chapter 4).

3.2.3.2 Soil sample collection

A square 'flap' of approximately 10cm^2 was cut through the vegetation using a serrated edge knife, including the top productivity layer, and the dead undecomposed layer below for peat. Using the knife and a trowel, the required quantity of soil or peat was removed (~30 g) from a depth of 10-20 cm (*Figure 3.14*), and placed into a plastic re-sealable bag. The flap was then put back in place and lightly pressed down, in order to restore the plot to its original condition and minimise disturbance as much as possible. Four soil samples were taken from each plot, 10-15 cm in from the edge to avoid impacts from compaction, and avoiding 15 cm around the gas chamber cylinders to avoid disturbance which may affect future gas flux readings.

Figure 3.14: A photograph of a peat sample being collected.

3.2.3.3 Soil pore water sample collection

Rhizon suction samplers (19.21.35, www.rhizosphere.com) were installed into plots to a depth of 10 cm below the surface at a slight angle during July 2015, with four rhizons per plot approximately 30-60 cm from each corner. For sample collection, a 20 ml syringe was inserted into the end of the rhizon (*Figure 3.15*), and a vacuum was created inside the syringe by pulling out the plunger and using a retainer to keep this in place. These were then left overnight and collected the following morning. Syringes were covered in silver tape to minimise direct sunlight and the potential for sample degradation. Samples were bulked by dispensing all four syringes into the relevant bottle for each plot. If less than 10 ml was collected in a syringe, the total volume was recorded on the field sheet.

Figure 3.15: A photograph of soil pore water being collected using a rhizon and syringe.

3.2.3.4 Decomposing surface litter collection

Prior to collecting a soil sample, the decomposing litter (*Figure 3.16*) directly on the soil surface was scraped away and placed inside a re-sealable plastic bag. A soil sample was then taken from this position. A total of four samples were taken per plot and bulked.

Figure 3.16: A photograph of some decomposing surface litter being collected.

3.2.3.5 Litter collection

Freshly senesced litter samples were collected at the end of the growing season during September 2015 when DOC production was at its highest. Vascular plants including *Eriophorum vaginatum* and *Festuca ovina* were collected as standing biomass using scissors to cut through the stem directly above the soil surface, whilst branches of *Calluna vulgaris* were also removed with scissors. *Sphagnum* was collected as whole blocks whilst *Pleurozium schreberi* was cut from the ground using scissors. All samples were placed into large re-sealable plastic bags.

3.2.3.6 Storage and transport of samples

Samples were stored in a cool box with ice blocks during transportation from the sites to CEH Bangor. Once at CEH, samples were stored at 4°C in a refrigerator prior to analysis, whilst soil subsamples were stored at -20°C destined for Next Generation Sequencing (NGS).

3.2.4 Sample analysis overview

The type of analysis performed on each sample type, and where this is presented in this thesis is summarised below in *Table 3.3.*

Sample Type	**Type of Analysis**	**Chapter**
Soil pore water	Chemistry	4
Soils	Chemistry of extracts Microbial communities	4 7
Decomposing surface litter	Chemistry of extracts	4
Litter	Decomposition Chemistry of extracts	5, 6 6

Table 3.3: Summary of analysis on each sample type, with reference to the chapters where this is detailed.

Chapter 4: How do acidity manipulations effect DOC quantity and quality in peatland soil extracts, pore water and surface litters across different sites?

Abstract

There has been an observed increase in dissolved organic carbon (DOC) concentration in soil solutions and surface water bodies over the past 30 years in acid sensitive areas of Europe and North America, which has been linked to recovery from acidification of soils in response to decreasing levels of atmospheric pollution. In addition, there is evidence from radiocarbon ^{14}C dating that DOC in surface waters is from recently formed DOC in the upper organic layer of peat. The key aim of this study was to improve understanding of whether increases in DOC observed in surface waters in response to changing acid deposition are the result of increased export from surface litter or near surface peat. This research was built upon an existing long-term pH manipulation field experiment in contrasting areas of historical pollution; North Wales and the Peak District, UK. Here, we present analysis of one year monitoring data of peat pore waters and DOC extracts from surface litter and peat.

When comparing DOC production in peat and litter within the upper organic layer, litter was the largest source with nearly 3 times more DOC, whilst peat had more aromatic DOC (as indicated by $SUVA_{254}$) during July and April which significantly reduced during October. Organo-mineral soil was found to contain more aromatic DOC in the upper peaty layer than peat itself, which suggests that podzol areas of an organic catchment may release this coloured humic acid fraction of DOC during times of high flow. A theoretical pathway model has also been proposed for the production and movement of aromatic DOC through the different components of the surface layer. Finally, results show that pore water pH and DOC concentration are strongly correlated, and the reproducibility of results from a previous acidification experiment at these sites provide further support for the hypothesis that increasing DOC concentrations in surface waters is due to increasing solubility of DOC with recovery from acidification. This pH-DOC relationship was also seen in peat samples, but not in surface litter.

Chapter 4

4.1 Introduction

Dissolved organic carbon (DOC), which is typically defined as organic carbon less than 0.45 µm in size (Thurman, 1985), represents a major natural carbon export from peatlands and other organic rich peaty soils (Clark et al., 2007, Dinsmore et al., 2010, Billett et al., 2004, Hope et al., 1994). Therefore, waters draining catchments comprised of organic rich soils such as peat and peaty podzols are associated with high concentrations of dissolved organic matter (DOM) released during the decomposition of organic material (Evans et al., 2006a, Aitkenhead et al., 1999). However, much of the literature focuses on DOC exported from peatland areas, with little investment into the role of organo-mineral soils on DOC dynamics in organic catchments.

There has been a considerable increase in DOC concentrations in terrestrial waters draining catchments dominated by organic soils in much of the Northern Hemisphere since the 1980's (Evans et al., 2005, Monteith et al., 2007, Oulehle and Hruška, 2009, SanClements et al., 2012b, Couture et al., 2012). This has been largely attributed to recovery from acidification in many regions, with increased solubility of DOC with increasing pH, releasing previously suppressed DOC from soils. This is widely supported by field (Evans et al., 2012, Oulehle et al., 2013, Evans et al., 2008a, Moldan et al., 2012, Ekström et al., 2011) and laboratory experiments (Clark et al., 2011, Palmer et al., 2013) as well as modelling (Monteith et al., 2007, Rowe et al., 2014, Evans et al., 2008b, Sawicka et al., 2016) and field observations (Oulehle et al., 2017, Oulehle and Hruška, 2009, Evans et al., 2006a, Oulehle et al., 2011). With peatlands being a major store of carbon (Gorham, 1991), and with drastic changes in DOC export from these ecosystems, concerns have been raised over the future of carbon balances from these organic catchments (Freeman et al., 2001a).

This increase in DOC export from peatlands and other organic soils not only affects carbon budgets (Dinsmore et al., 2010), but also creates expensive implications for water companies due to the removal of DOC through drinking water treatment processes (Ritson et al., 2014). In addition, greater DOC concentrations in terrestrial waters can also affect the functioning of aquatic ecosystems by influencing acidity (Eshleman and Hemond, 1985), bioaccumulation of organic chemicals (Haitzer et al., 1998), transport of trace metals (Lawlor and Tipping, 2003), nutrient (Stewart and Wetzel, 1981) and energy supply (Wetzel,

1992), and light absorbance (Schindler, 1971) and photochemistry (Scully et al., 2003). Therefore there is a dire need to understand these changing carbon dynamics.

DOC is produced through the decomposition of both above and below ground plant material, as well as organic matter decomposition and the release of plant exudates. As a result of these diverse sources, DOC consists of a range of molecules with a variety of molecular weights and properties (Leenheer and Croué, 2003). It is likely that the dominant vegetation community of upland organic catchments will influence the quantity and quality of DOC produced and exported into water bodies. For example, *Sphagnum* has been shown to produce highly aromatic DOC during decomposition, as has peat, whilst vascular plants result in a greater quantity of DOC which is more labile in nature (Ritson et al., 2016, Armstrong et al., 2012). The source of DOC and its chemistry, specifically the proportion of aromatic compounds will affect the sensitivity to changes in acidity (Clark et al., 2011, SanClements et al., 2012a).

Recent studies have suggested that in intact systems the DOC in surface waters is less than 40 years old (Palmer et al., 2001, Tipping et al., 2010, Evans et al., 2007), and that surface layers are better connected with stream water DOC concentrations than deeper soil pore water (Clark et al., 2008, Billett et al., 2006). Radiocarbon ^{14}C dating within peatland catchments have demonstrated that between 96 - 100 % of DOC in surface water was recently produced and derived from the peat surface layer (Tipping et al., 2010). However, it is difficult to determine the relative role of litter compared to near surface peat as a source of DOC from these studies alone.

There are uncertainties as to why increases in DOC concentrations have not been uniform across sites receiving similar deposition levels, possibly due to site specific catchment characteristics. Field evidence shows that DOC flushed into water systems is recently formed and derives from the upper layer of peat. However, there has been little effort invested into partitioning this dynamic upper segment to understand in detail the roles of decomposing litter and peat organic matter. Much research to date in the UK has focused on peatlands, with less attention given to processes in freely-draining organo-mineral soils. It is unclear to what extent the riverine DOC derives from surface litter or newly formed peat, and whether there is any difference in DOC properties and their sensitivity to environmental change such as acidification. Therefore, the key aim of this study is to

improve understanding of where in the surface layer DOC increases are likely to be originating by assessing the quantity and quality of DOC in surface litter and peat and their response to acidification and recovery.

Specific objectives are:

- To quantify, characterise and compare the direct measures of DOC in soil pore water with laboratory water extracts of decomposing surface litter and peat and organo-mineral soil O horizon.
- To assess how DOC quantity and quality differs among peat and organo-mineral soil, and across different sites representing a damaged and pristine moorland as well as a 'natural' acidity gradient.
- To assess the effect of acidity manipulations on DOC derived from soil pore water, litter extracts and soil extracts in terms of mobility and aromaticity.

4.2 Methods

4.2.1 Site description and experimental design

This work is built upon an existing long-term acidity manipulation field experiment set up in 2007, situated across two moorland locations with contrasting historic rates of acid deposition, and therefore present-day soil acidity (Evans et al., 2012). At each site, replicated acidity manipulations were established within two soil types; blanket peat and peaty podzol, which are among the commonest soils present in the UK uplands, and which also occur extensively in other cool, humid temperate regions.

The first study site, the Migneint (3°48.8' W, 52°59.6' N, 460 m a.s.l.), is a relatively undisturbed moorland area with historically low levels of pollution, based in North Wales. Peaknaze (1°54.5' W, 53°28.3' N, 440 m a.s.l.), Northern England, is a more disturbed region affected by relatively intensive land management and historically high levels of atmospheric pollution, which has led to degradation of the ecosystem including *Sphagnum* loss and erosion. More details for both sites can be found in Chapter 3 and (Evans et al., 2012).

4.2.2 Field experimental operation

The experimental sites were established in August 2007 and consist of twelve 9 m^2 plots at each of the four sites, with a randomised blocked design compromising four replicates of control, acid and alkaline treatments at each location. Treatments were applied initially from October 2008 until December 2012 (Evans et al., 2012), and then re-established for the purposes of this study (using the same methods, treatments and plot allocations) from January 2015 until October 2016. Acid plots received a monthly dose of sulphuric acid (H_2SO_4) mixed with rainwater (20L) collected at the site. The concentration applied was 50 kg S ha^{-1} yr^{-1} at the podzol sites and 100 kg S ha^{-1} yr^{-1} at the peat sites, this concentration being similar to the ambient sulphur deposition in the Peak District in the 1970's (a higher dose was applied to peat plots to take account of the buffering effects of sulphur reduction (Evans et al., 2012)). A 10 L rinse of rainwater followed to ensure the treatment infiltrated into the soil and to minimise any direct toxicity effects on plant foliage.

The same procedure was followed for the alkaline plots with sodium hydroxide (NaOH) and potassium hydroxide (KOH), followed by a rinse containing Magnesium Chloride ($MgCl_2$) and Calcium Chloride ($CaCl_2$) to maintain base cation ratios similar to those observed in rainfall. The molar OH^- concentration in the alkaline treatments was intended to be comparable to the H^+ concentration in the acid treatments. Control plots received 20 L of rainwater only. All treatments and rinses were applied using a watering can.

4.2.3 Sampling and analysis

4.2.3.1 Pore water sampling

Pore water samples were collected monthly from September 2015 until October 2016, approximately one week after treatments were applied. Samples were collected from a depth of 10 cm below the surface using syringes and rhizon suction samplers (part number 19.21.35, www.rhizosphere.com), from four locations within the plot. These were then bulked into one sample per plot following the protocol from previous monitoring by Evans et al. (2012).

4.2.3.2 Peat and decomposing surface litter sampling

Decomposing litter directly on the peat surface was scraped away and placed inside a re-sealable plastic bag. A peat sample was then taken from this position as follows. A square 'flap' of approximately 10cm^2 was then cut through the vegetation using a serrated edge knife, including the top productivity layer, and the dead undecomposed layer below for peat. Using the knife and a trowel, the required quantity of soil or peat was removed (~30 g) from a depth of 10-20 cm, and placed into a plastic re-sealable bag. The flap was then put back in place and lightly pressed down, in order to restore the plot to its original condition and minimise disturbance as much as possible. Four decomposing litter and peat samples were taken from each plot, 10-15cm in from the edge to avoid areas impacted by compaction. Samples were collected during April, July and October 2016.

4.2.4 Laboratory analysis

Peat and litter samples were processed in the lab by cutting and/or chopping into 1 – 2 cm pieces and homogenising. Unwanted material such as stones, insects, thick roots and living plant material was removed. Using 4 g of sample and ultrapure water, samples underwent a cold water extraction on a horizontal shaker (30 rpm) at room temperature for 3 hours for peat (1:10 mass to volume ratio) and 24 hours for litter (1:20 mass to volume ratio). Samples were then centrifuged (3500 rpm for 20 minutes) and vacuum filtered through 0.45 µm cellulose membrane filter paper. This extraction method was adapted from Ghani et al. (2003).

Extracts and pore water samples underwent a chemical analysis which included pH, electrical conductivity, total organic carbon, and ultra-violet absorbance. A Thermalox TC-TN analyser (Analytical Sciences, Ltd., UK) was used to measure the concentration of DOC (by subtracting the amount of total inorganic carbon (TIC) from the amount of total carbon (TC)).

DOC concentration in peat and litter extracts were expressed in terms of mg DOC extracted per g of dry material, as is standard practice for this measure. Pore water DOC concentrations were expressed in mg DOC/L as these samples were direct measures of DOC concentrations in situ. To correct the extract data to mg DOC/g, the mean moisture content of *Calluna vulgaris* and *Eriophorum vaginatum* following three months of decomposition at

each site was used in the calculation for decomposing surface litter, and the moisture content of peat and organo-mineral soil was used to correct DOC concentrations in surface peat (*Table 4.1*).

Table 4.1: Percentage moisture content used in the calculations for peat and surface litter, at each site.

Site	Peat	Litter
Migneint Peat	1298.23	913.45
Migneint Podzol	604.85	531.74
Peaknaze Peat	311.89	685.61
Peaknaze Podzol	287.12	521.81

Optical measures were used to define spectroscopic properties as a proxy measure of DOC quality. Samples were diluted to less than 1 au, as determined by measuring absorbance at 240 nm. UV visible absorbance spectra were determined using UV transparent 96 well plates on a Spectromax M2e Microplate Reader (Molecular Devices, San Jose, CA) set to scan at wavelengths between 240 and 600 nm with a 1 nm increment. As absorbance data obtained by the microplate method is slightly lower than the cuvette method (due to the difference in absorbance between plastic and quartz), data was multiplied by correction factors (Tim Jones, pers comm). Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) has been identified as being a useful proxy for measuring the aromatic fraction (Weishaar et al., 2003) and molecular weight (Chowdhury, 2013) of DOC, as it is strongly linked to the hydrophobic organic acid fraction of DOM (Spencer et al., 2012). Therefore the $SUVA_{254}$ value was used as a measure of aromaticity and calculated by dividing the absorbance value at 254 nm by the DOC concentration (mg l^{-1}) (Weishaar et al., 2003).

4.2.5 Data Analysis

Data was statistically analysed using R statistical package (RDevelopment CORE TEAM, 2008). Data was assessed as to whether it met the assumptions of Analysis of Variance (ANOVA), including normality and equal variance, and transformations were applied where necessary. ANOVA was used to examine the effect of various factors and their interactions on sample chemical properties of pH, DOC concentration and $SUVA_{254}$. When significance was apparent, a post hoc test was run using the 'Tukey HSD' function in R to confirm where

significant differences occurred between groups. In addition, Spearman's Rank Correlation Coefficient was used to assess the significance, direction and strength of relationships between pH and DOC concentration.

4.3 Results

4.3.1 Characterising and comparing DOC in different samples

4.3.1.1 Peat and litter comparison

Significantly more DOC was extracted from litter than from peat during April (P = <0.001), July (P = <0.001) and October (P = <0.001) (*Figure 4.1*). Extract DOC increased from 2.4 mg/g during spring to 3.2 mg/g during autumn for litter (P = 0.039). However, extract DOC in peat remained similar across all months at ~1.0 mg/g.

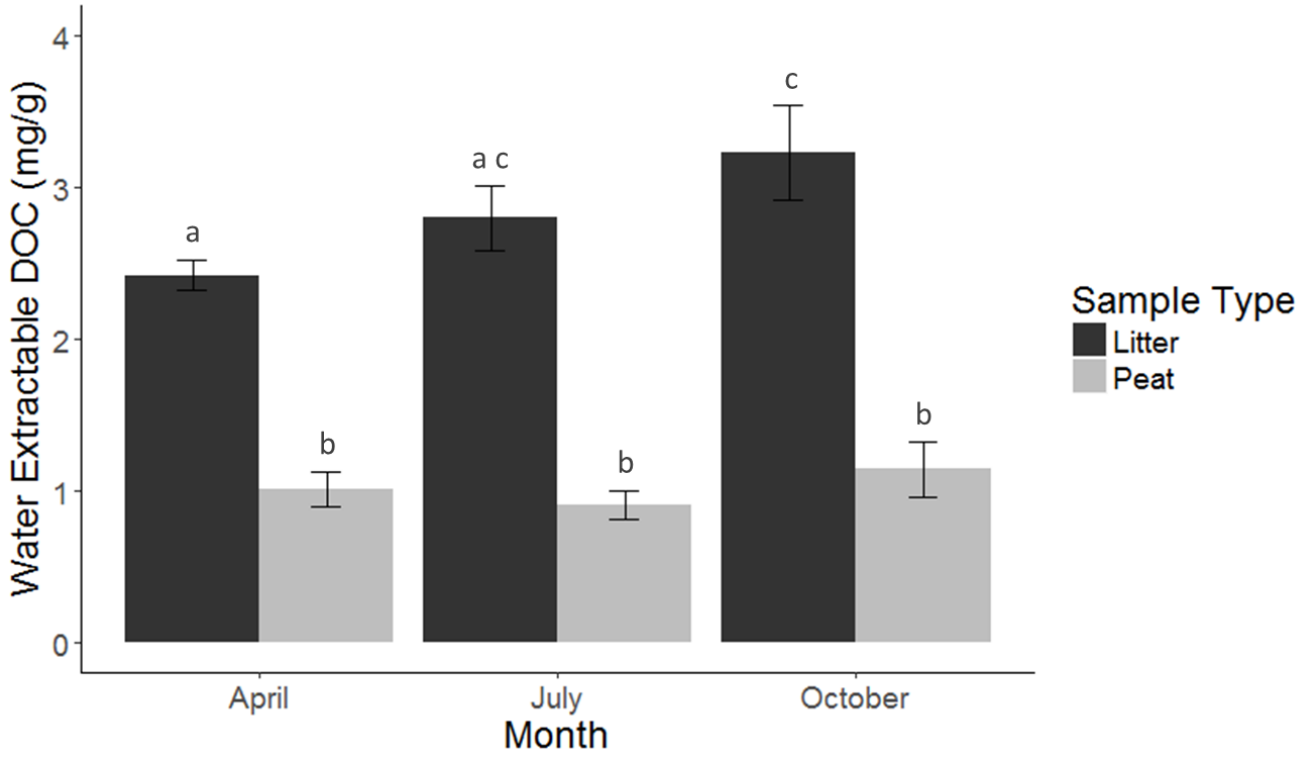


Figure 4.1: DOC extracted from peat and surface litter samples, collected from control plots only during April, July and October 2016. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), sample (two levels) and month (three levels). Data was log transformed.

$SUVA_{254}$ was used as a proxy for measuring the aromatic fraction of DOC. DOC was significantly more aromatic when extracted from peat samples than from litter samples, by 0.67 L/mg C^{-1}/m^{-1} during April (P = 0.017) and 1.2 L/mg C^{-1}/m^{-1} during July (P = <0.001) (*Figure 4.2*). However, in October the $SUVA_{254}$ value of peat is similar to that of litter. This is because of an apparent seasonal effect on DOC quality in peat samples, with a significantly lower $SUVA_{254}$ in October than in April (by 0.82 L/mg C^{-1}/m^{-1}) (P = 0.033) and July (by 1.41 L/mg C^{-1}/m^{-1}) (P = <0.001). The mean $SUVA_{254}$ value remains similar for surface litter across all months at 2.3 - 2.6 L/mg C^{-1}/m^{-1}.

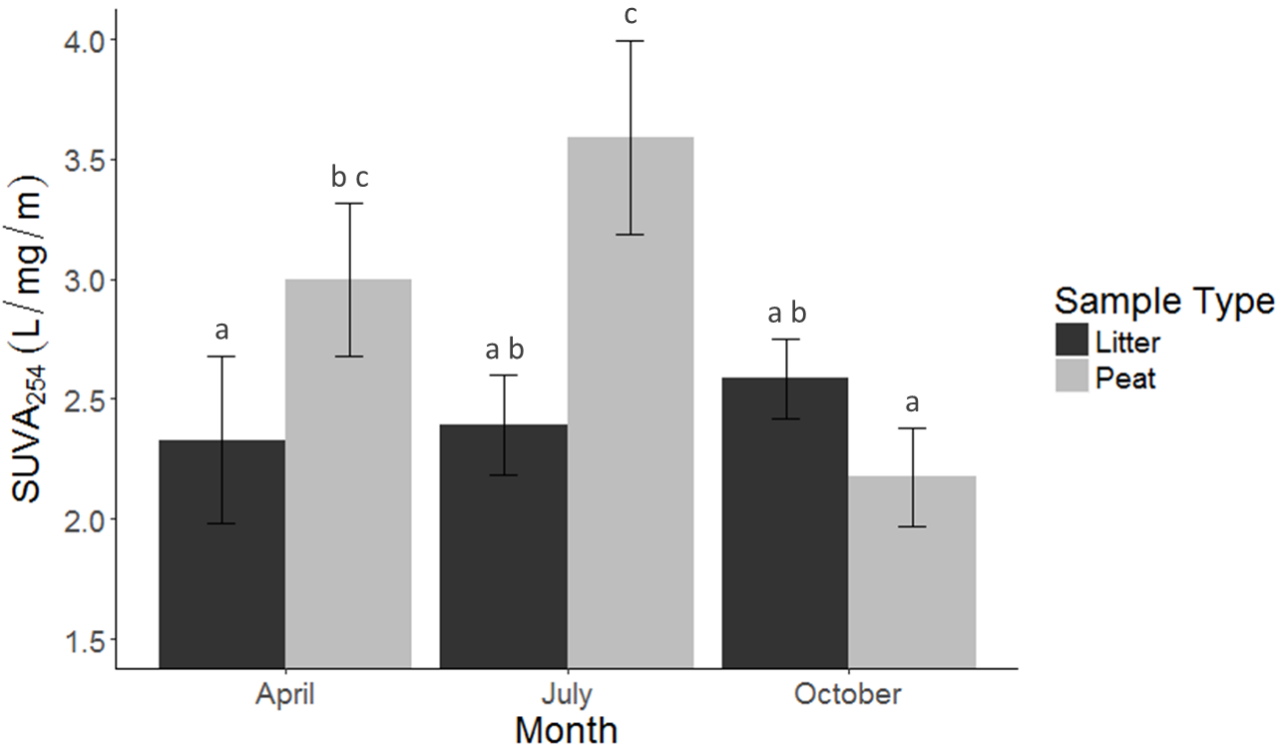


Figure 4.2: $SUVA_{254}$ of DOC in extracts from peat and surface litter samples, collected from control plots only during April, July and October 2016. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), sample (two levels) and month (three levels). Data was log transformed.

4.3.1.2 Relationships in DOC released between different sources and methods

There was no relationship between litter and peat DOC extracts (P = 0.114). There were also no significant relationships between the DOC concentration in pore water, and that in litter extracts (P = 0.376) and peat extracts (P = 0.442).

There was a significant positive relationship between the $SUVA_{254}$ in pore water with the amount extracted from peat (P = 0.002, Rho = 0.446) (*Figure 4.3*). However, the $SUVA_{254}$ in surface litter was found not to correlate with that in pore water (P = 0.106) (*Figure 3*), but did correlate with that in peat (P = 0.005, Rho = 0.407) (*Figure 4.4*).

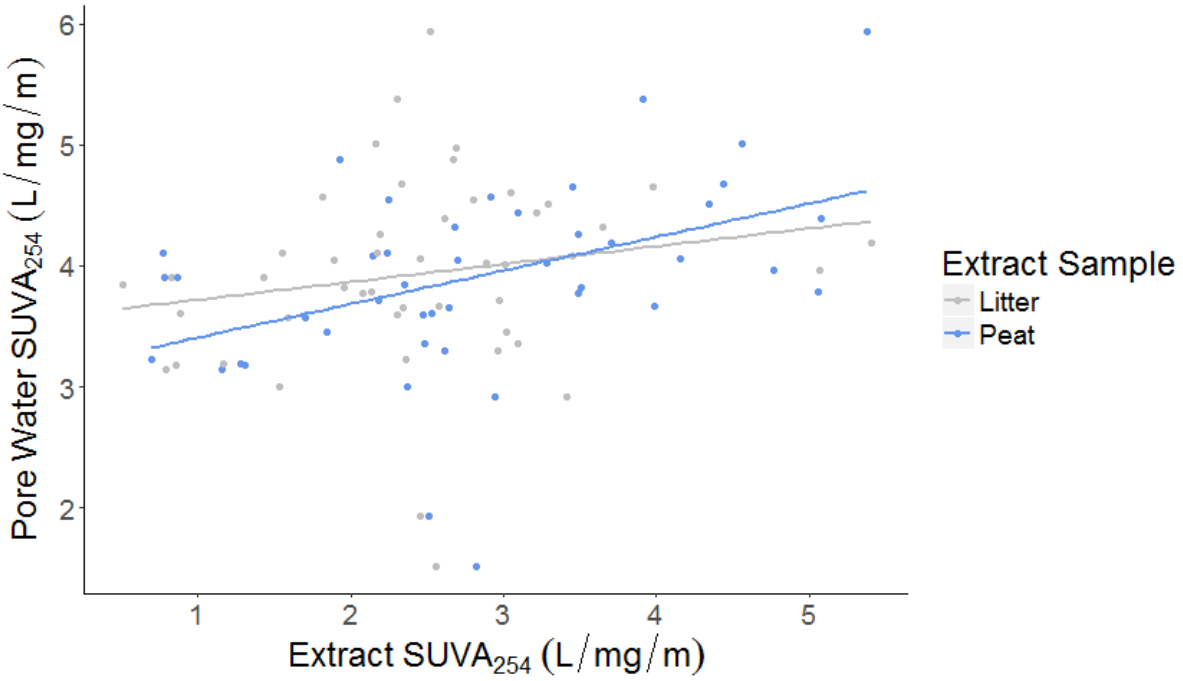


Figure 4.3: $SUVA_{254}$ of DOC in extracts from peat and surface litter samples plotted against direct measurements from pore water. Samples were collected from control plots only during April, July and October 2016. Spearman's Rank tests were used to assess the strength and significance of relationships.

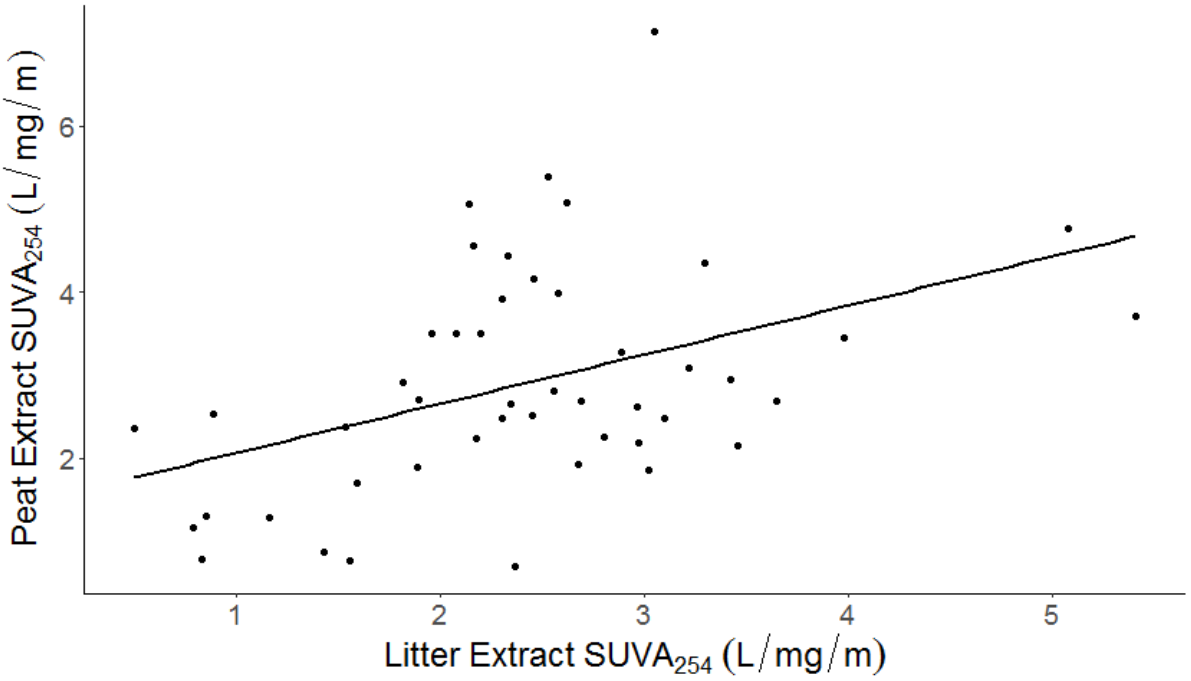


Figure 4.4: $SUVA_{254}$ of DOC in extracts from peat and surface litter samples. Samples were collected from control plots only during April, July and October 2016. Spearman's Rank tests were used to assess the strength and significance of relationships.

4.3.2 Influence of site and soil type on DOC in extracts and direct measurements in pore water

Significantly more DOC was measured in the organic surface layer of peat than in organo-mineral soil at both Migneint (1.65 and 0.94 mg/g for Migneint Peat and Migneint Podzol respectively) (P = <0.001) and Peaknaze (0.97 and 0.52 mg/L for Peaknaze Peat and Peaknaze Podzol respectively) (P = <0.001) (*Figure 4.5*). However, Migneint Podzol had a significantly greater concentration of DOC in surface litter (3.41 mg/g) than at Migneint Peat (2.46 mg/g) (P = <0.001), which reflects the higher net primary production (NPP) which occurs with organo-mineral soils compared to peat. Surface peat also contained more DOC at the Migneint compared to Peaknaze, when comparing both peat (difference in mean of 0.68 mg/L) (P = <0.001) and podzol (difference in mean of 0.42 mg/g) (P = <0.001) sites. For surface litter, this site difference was only apparent at podzol sites, with Migneint Podzol having 0.77 mg/g more DOC than Peaknaze Podzol (P = 0.014). When assessing direct measurements of DOC concentration in pore water, it is apparent there is considerably more DOC mobile in pore water at Peaknaze Peat at 80 mg/L, compared to just 24 - 34 mg/L at other sites.

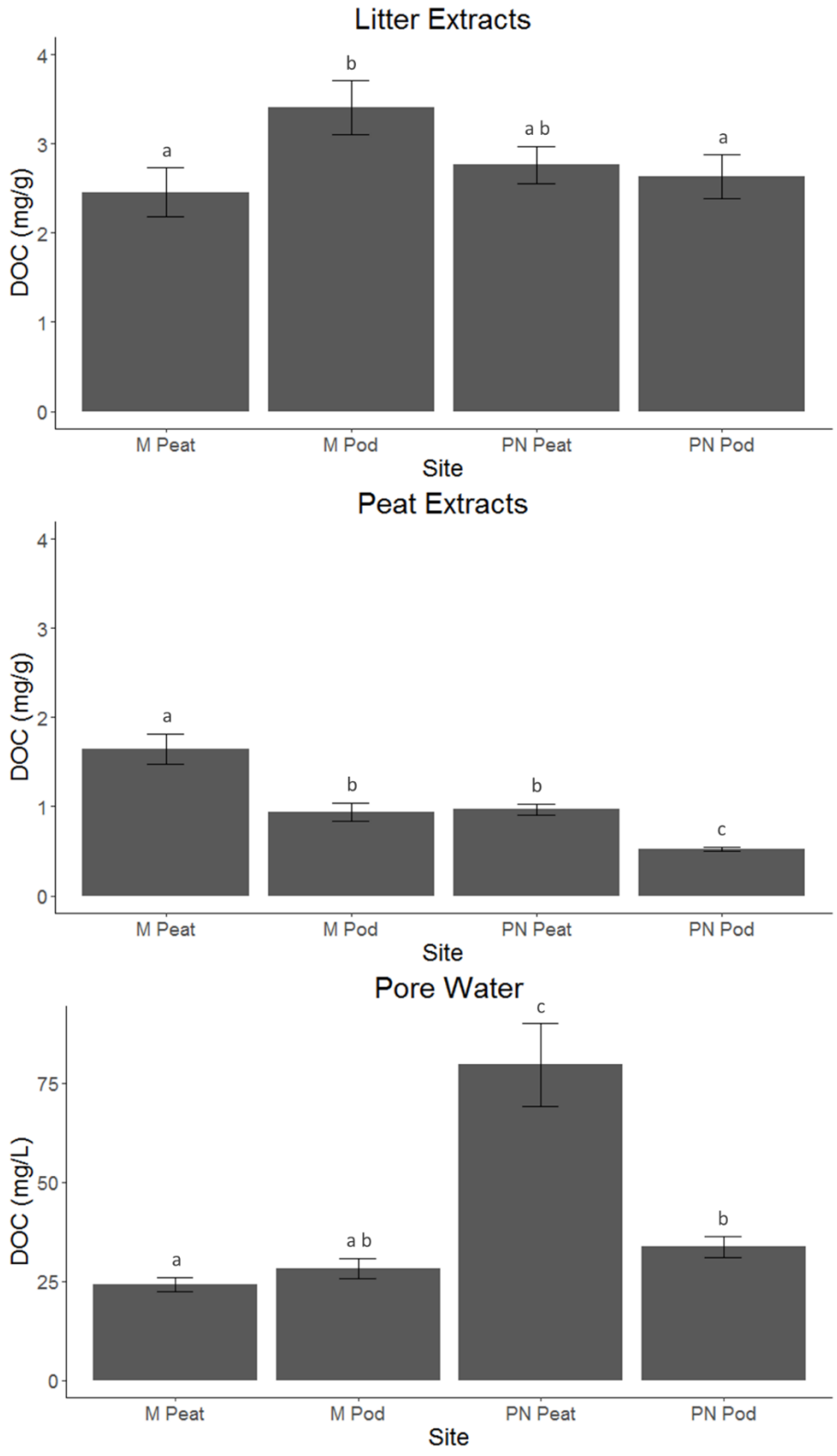


Figure 4.5: DOC in extracts of peat and surface litter, and direct measurements of DOC concentration in pore water. Samples were collected from control plots only during April, July and October 2016. Letters signify where significant differences occurred, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels) and month (three levels). Litter and peat data were log transformed, whilst pore water data was transformed using the square root.

$SUVA_{254}$ was significantly higher at Peaknaze Peat than at Migneint Peat, for all sample types (soil P = <0.001; litter P = <0.001; pore water P = 0.016) (*Figure 4.6*). However, there was no difference in $SUVA_{254}$ between podzol sites. Furthermore, $SUVA_{254}$ was higher at Migneint Podzol than at Migneint Peat in both litter (P = <0.001) and soil (P = <0.001) extracts, but not pore water samples (P = 0.814). $SUVA_{254}$ was also greater at Peaknaze Podzol compared to Peaknaze Peat for surface peat samples (P = 0.013), but not for litter extracts (P = 0.896) and pore water (P = 0.387) samples.

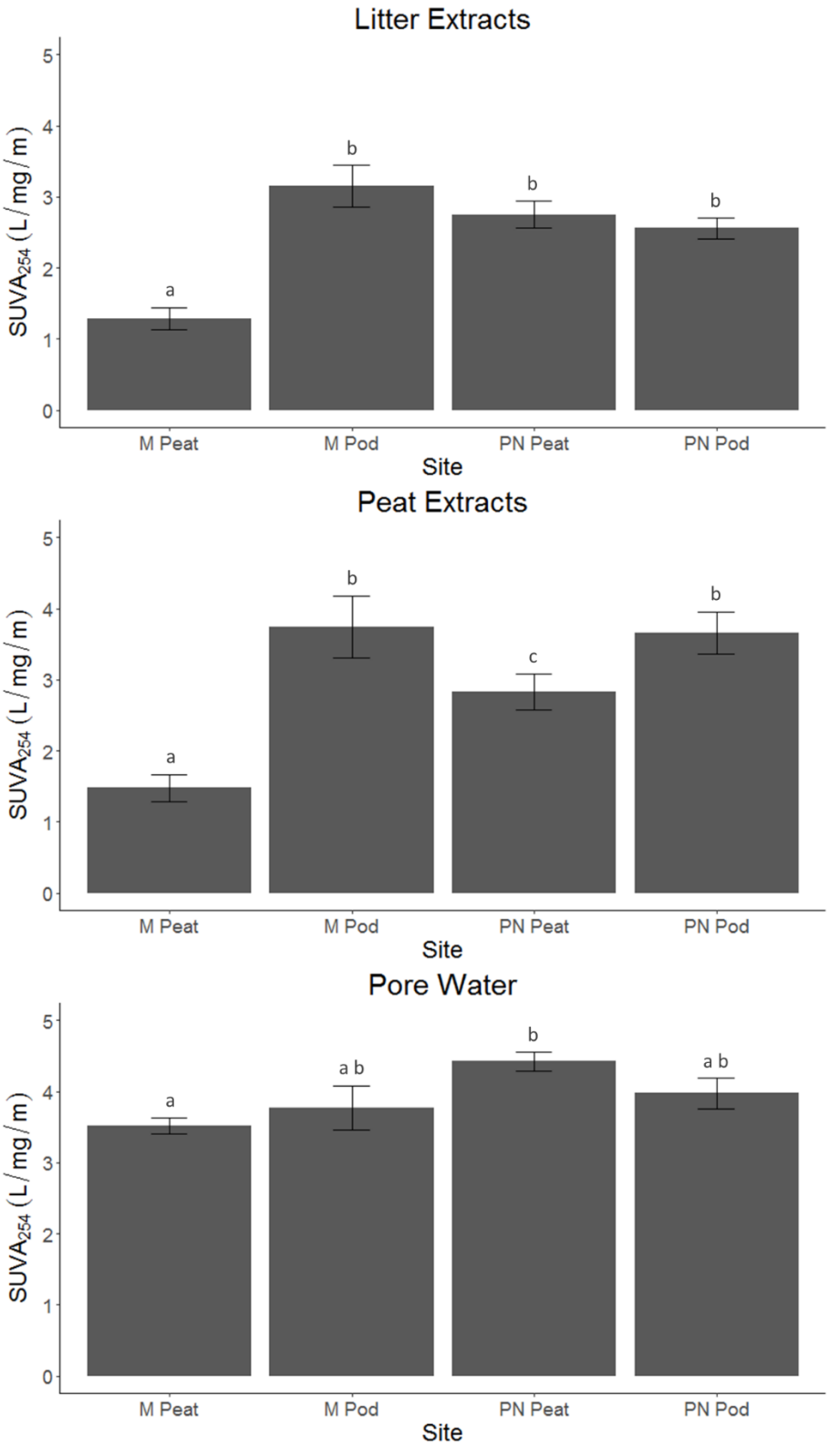


Figure 4.6: $SUVA_{254}$ of DOC in pore water samples, and extracts of peat and surface litter. Samples were collected from control plots only during April, July and October 2016. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels) and month (three levels). Litter and peat data was transformed using the square root.

4.3.3 Effect of acidity on DOC

4.3.3.1 Pore water thirteen month dataset

Treatment applications have successfully altered the pH of pore water at all sites excluding Migneint Peat (*Figure 4.7*). The pH of pore water was reduced by 0.18 – 0.28 pH units with the application of acid treatments, and was increased by 0.23 – 0.68 pH units with alkaline treatments. The treatments have had the greatest effect at the Migneint Podzol site, with a pH range of 4.06 – 5.02 (based on mean of treatment plots). The most acidic site was Peaknaze Peat, which had a pH at control plots of 3.98 units, followed by Peaknaze Podzol with a pH of 4.11. Both Migneint sites had a similar pH at control plots of ~4.30 units.

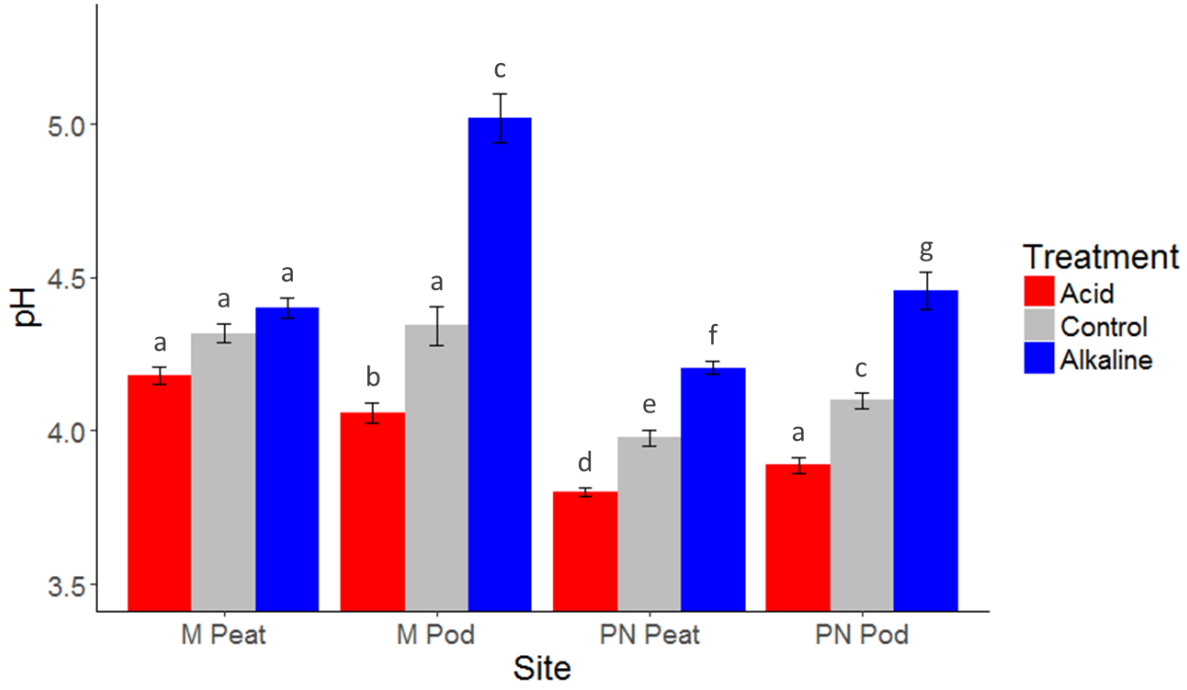


Figure 4.7: Mean pH of pore water samples with acid, alkaline and control treatments, collected monthly over a 13 month period. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), treatment (three levels) and month (thirteen levels).

There are some clear seasonal dynamics apparent with DOC mobility in pore water (*Figure 4.8*). DOC concentration is lower during winter months (November through to February) at all sites except Peaknaze Peat. This site has the highest amount of DOC which is mobile in pore water for most of the experimental period. In addition, whilst other sites have a gradual increase or decrease in DOC concentration spanning several months, at Peaknaze Peat DOC mobility changes rapidly from one month to the next. For instance, in April the

concentration is 82 mg/L (mean of control plots), which then falls to 33 mg/L in June, and then rapidly increases again to 119 mg/L in July.

In terms of acidity manipulations, the DOC concentration has responded to the acidity treatments at all sites excluding Migneint Peat, and this is consistent throughout the thirteen month experimental period despite the seasonal dynamics. In general, pore water DOC concentration has been lowered with the acidity treatments, and is greater with alkaline treatments.

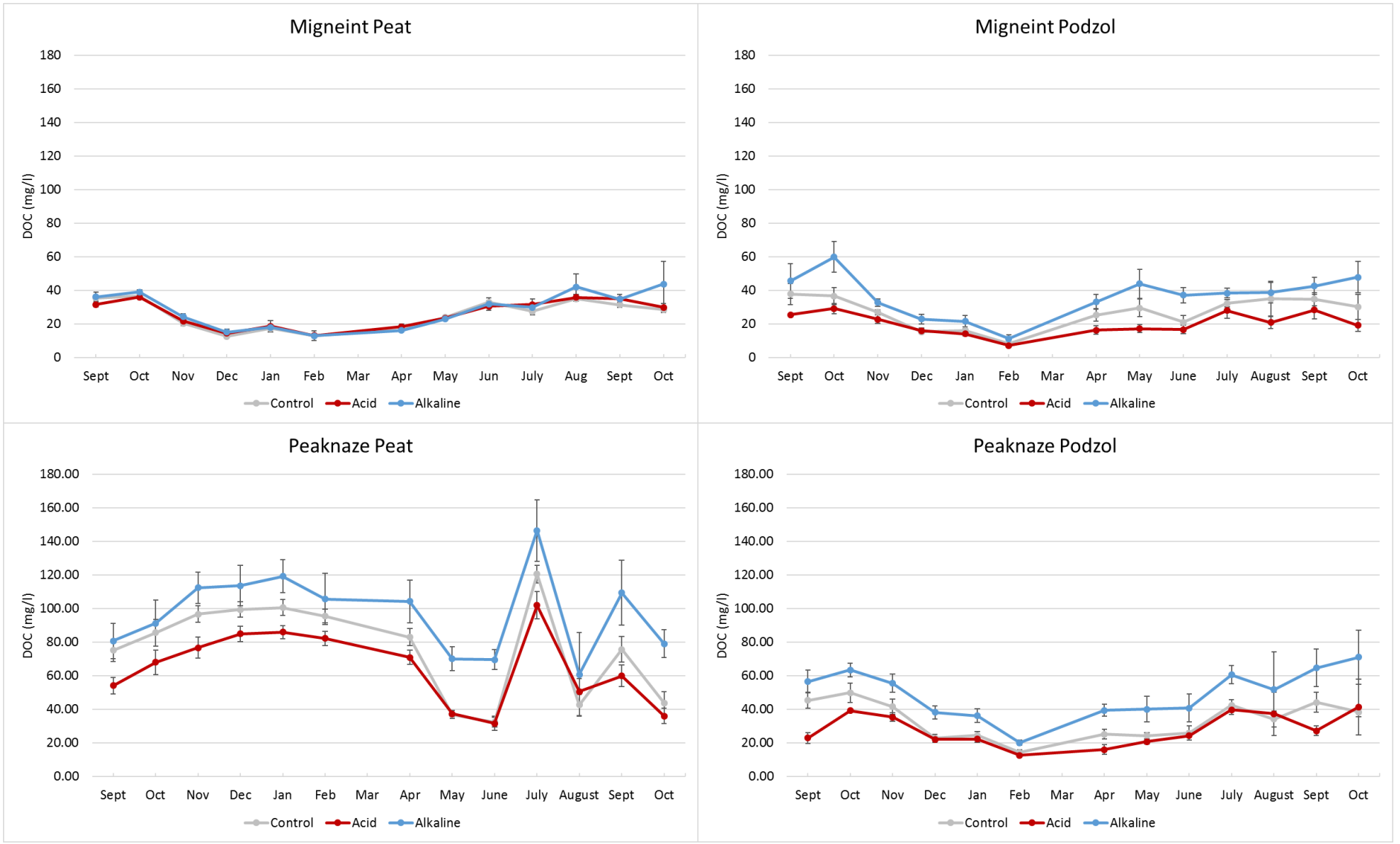


Figure 4.8: DOC concentration in pore water samples, collected monthly from September 2015 until October 2016.

There are many fluctuations in the concentration of the aromatic fraction ($SUVA_{254}$) of pore water DOC over the thirteen month experimental period at all sites (*Figure 4.9*). Overall, there is no clear seasonal trend in DOC aromaticity other than a decrease in $SUVA_{254}$ between July and October at all sites except Migneint Peat. This mirrors the difference in the aromatic fraction of DOC indicated by a higher $SUVA_{254}$ also seen in peat samples between July and October, confirming that this is a seasonal response rather than an artefact of sampling those individual months.

The response of $SUVA_{254}$ and so the aromatic fraction of DOC to acidity treatments is dependent on month ($P = 0.014$). In some months, such as August, September and October 2016, $SUVA_{254}$ appears to respond to acidity manipulations, with less aromatic DOC being mobile with acid treatments, and more with alkaline treatments. However, such a response is not apparent during many other months at all sites.

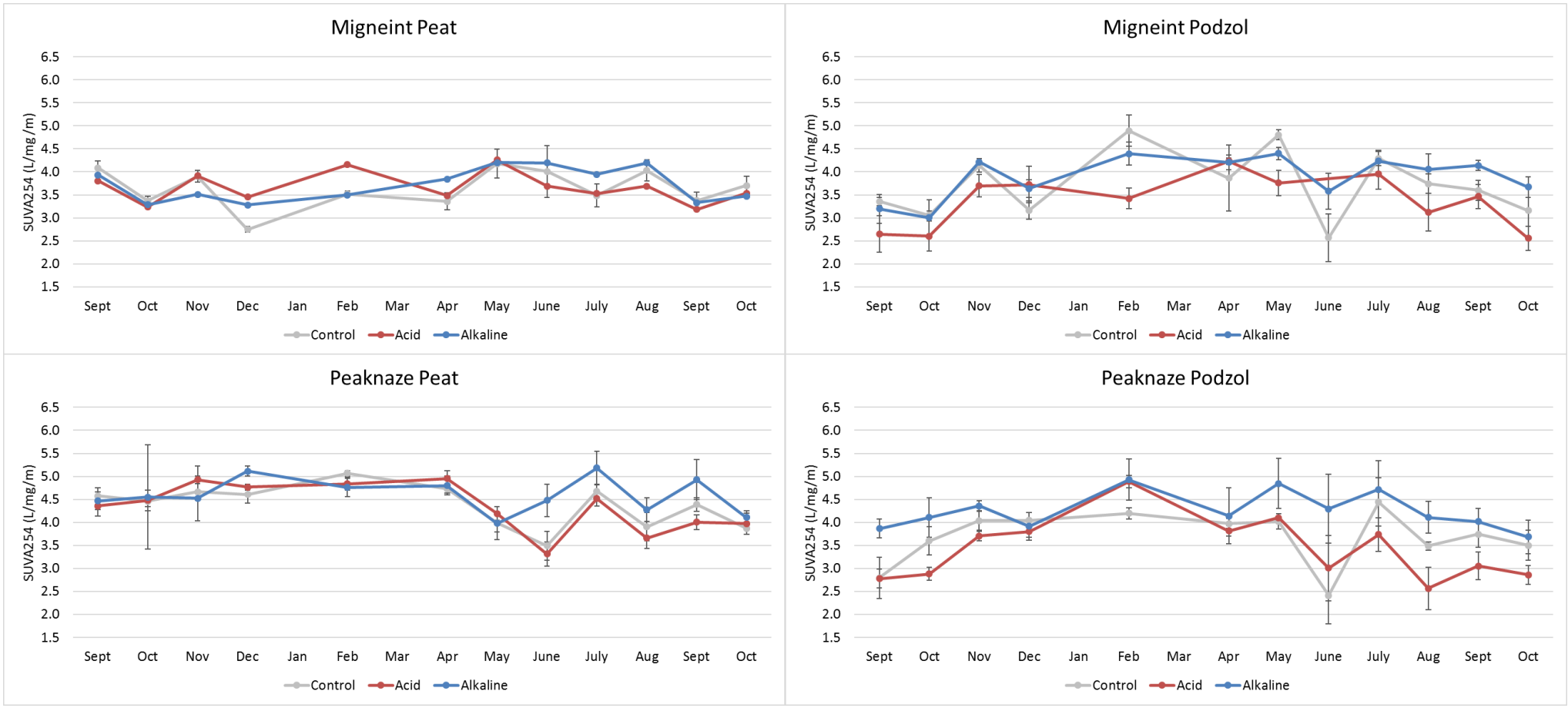


Figure 4.9: $SUVA_{254}$ of DOC in pore water samples, collected monthly from September 2015 until October 2016.

The DOC concentration in pore water have responded to acidity manipulations at all sites except Migneint Peat (*Figure 4.10*). There was a significantly lower DOC concentration in pore water with acidity treatments, with a reduction in the range of 7-12 mg/L (mean of thirteen month dataset, per site and treatment, excluding Migneint Peat), and an increased DOC concentration in the range of 9-21 mg/L with alkaline treatments.

Peaknaze Peat had a significantly higher DOC concentration compared to other sites. Peaknaze Podzol also had significantly more DOC mobile in pore water than Migneint Podzol ($P = <0.001$). Finally, at Peaknaze there is a difference in the DOC concentration in pore water between soil types, with much more DOC mobile in peat compared to podzol ($P = <0.001$). Unlike at Peaknaze, there was no significant difference in the concentration of DOC in the pore water in peat and podzol at Migneint ($P = 0.973$).

At podzol sites, alkaline treatments resulted in a greater $SUVA_{254}$, and so a higher proportion of aromatic DOC was mobile in pore water, whilst acidity resulted in less aromatic DOC (*Figure 4.10*). However, statistical significance only occurred between control and acid plots at Migneint Podzol, and control and alkaline plots at Peaknaze Podzol. There were no significant differences in $SUVA_{254}$ between treatments at peat sites. As with DOC concentration, pore water from Peaknaze Peat had a significantly greater concentration of the aromatic fraction of DOC at 4.4 L/mg C^{-1}/m^{-1} at control plots compared to other sites which had a mean of ~3.7 L/mg C^{-1}/m^{-1} at control plots.

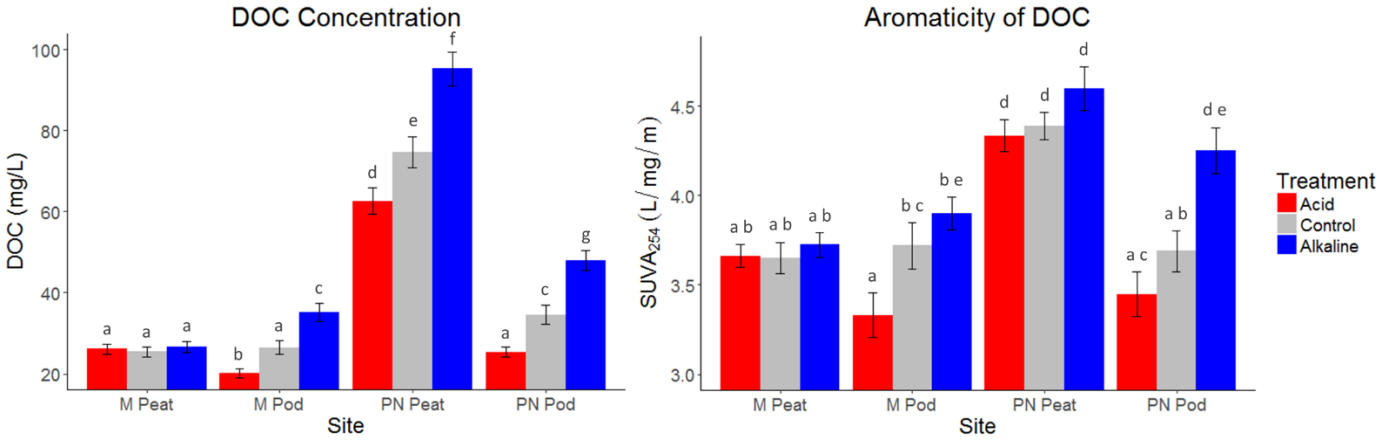


Figure 4.10: Mean DOC concentration and $SUVA_{254}$ of DOC in pore water samples, collected monthly over a 13 month period. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), treatment (three levels) and month (thirteen levels).

4.3.3.1.1 Relationship between pH and DOC change

When assessing pore water data collected from all sites over a 13 month period, we see a strong relationship between pH and DOC concentration (*Figure 4.11*). As pH increases, so does the DOC concentration, with a significant correlation coefficient of 0.82 signifying a strong positive relationship (P = <0.001).

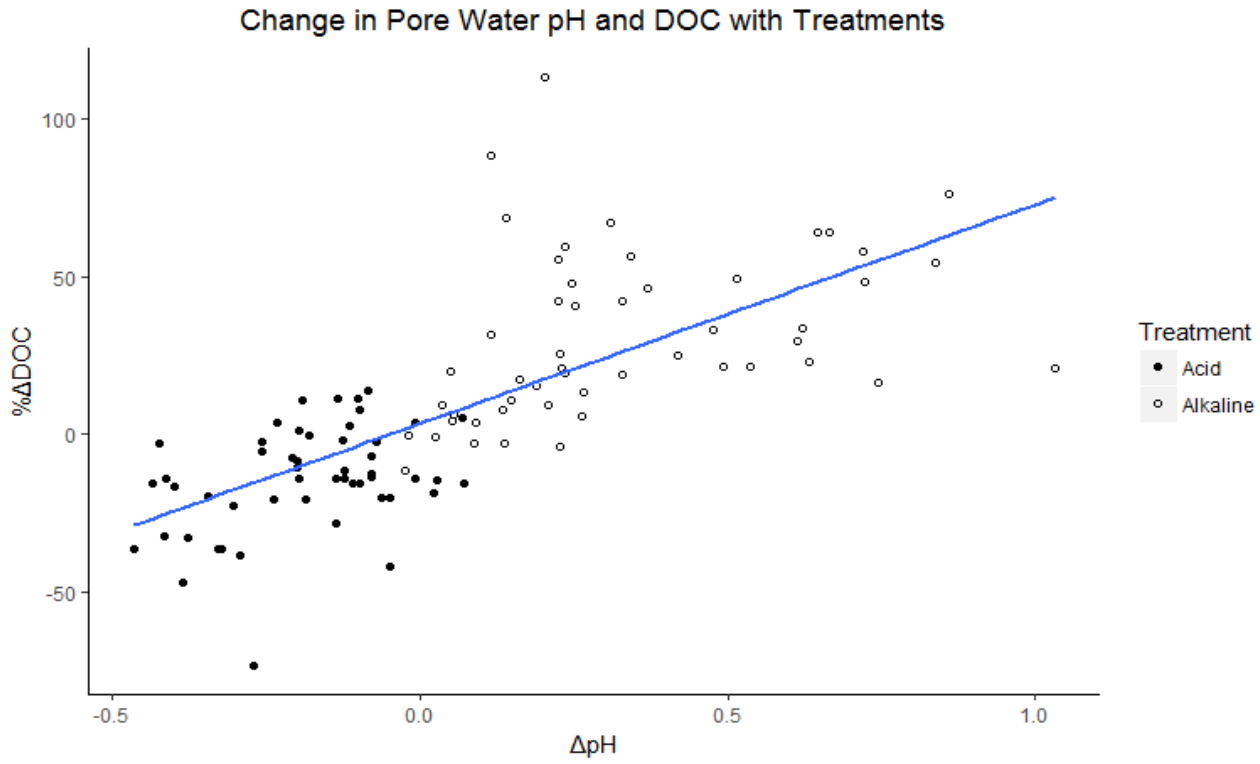


Figure 4.11: Scatterplot comparing change in pH and percentage DOC for each treatment compared to the control, based on pore water data collected monthly over 13 months.

4.3.3.2 Pore water, peat and litter (3 month dataset)

When focusing on the three month dataset for comparison with soil and litter data, the treatment effect on pore water pH is still apparently, and is largely significant at all sites except Migneint Peat (*Figure 4.12*). For soil and litter extracts, there is little significance in terms of the pH of each treatment with respect to the control, suggesting that the treatments were not as successful in altering the pH of these samples. For surface litters, both acid and alkaline treatments were significantly different to the control at Peaknaze Peat, and there was a significant increase in pH with alkaline treatments at the podzol sites (which is also apparent in peat samples), but there were no significance differences at Migneint Peat.

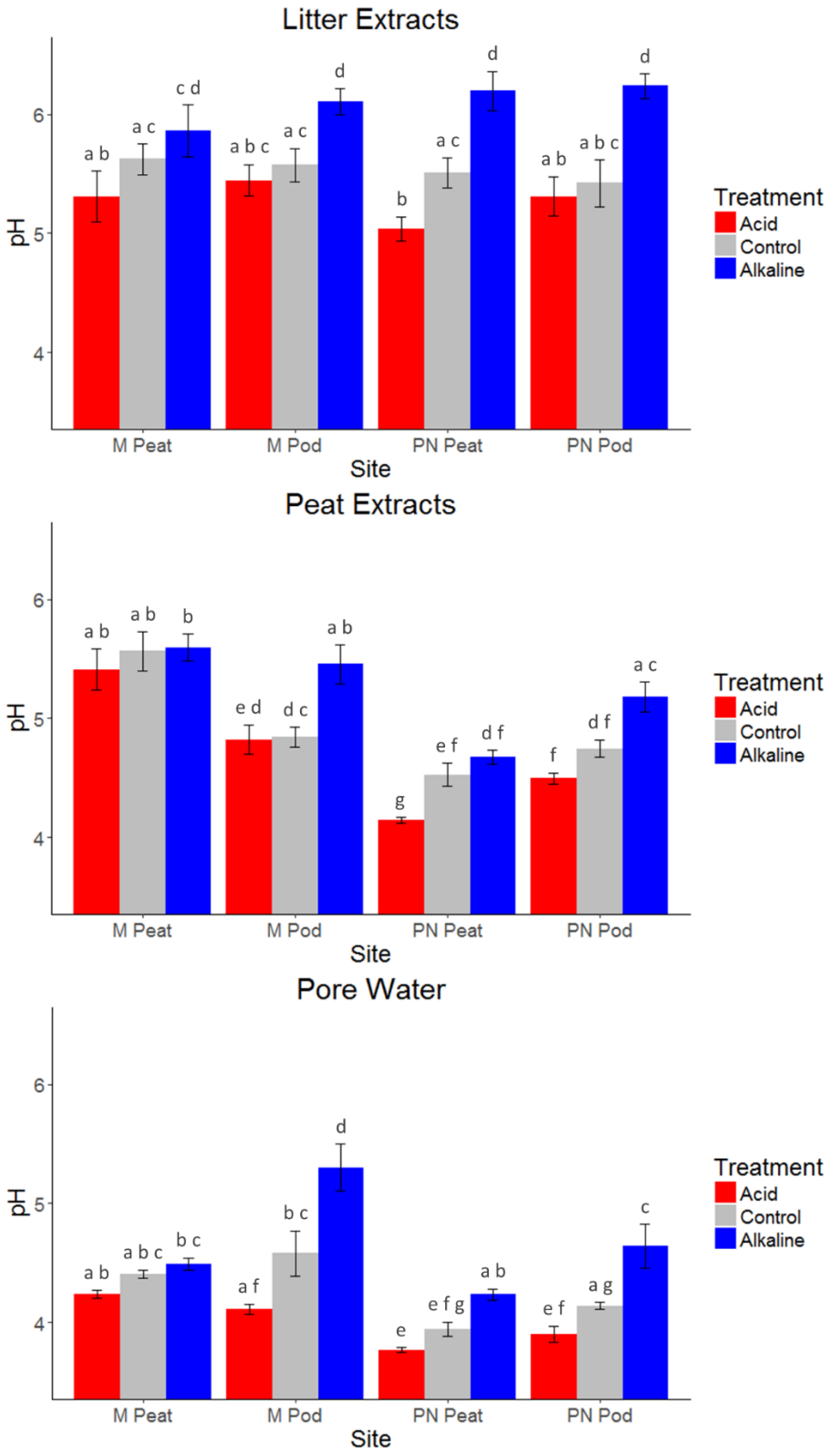


Figure 4.12: Mean pH of DOC in extracts of peat and surface litter samples, and pore water samples. Samples were collected during April, July and October 2016. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), treatment (three levels) and month (three levels). Peat and pore water data were transformed using boxcox transformation.

When assessing the effect of treatments on DOC extracted from peat and litters, the interaction between sites (four levels) and treatments (three levels) was found not to be significant for peat samples (P = 0.296) (*Figure 4.13*). However, this was significant for litter extracts (P = 0.045), but when investigated further with a Posthoc test extract DOC between treatments at each site were found not be significant. For pore water samples, DOC concentrations were only significantly different between acid and alkaline plots at all sites except Migneint Peat, where DOC concentrations remained the same regardless of treatment. There is however considerably more DOC mobile in Peaknaze Peat pore water at 68 – 102 mg/L (range in mean of treatment plots), compared to just 21 – 35 mg/L at Migneint sites and 27 – 50 mg/L at Peaknaze Podzol.

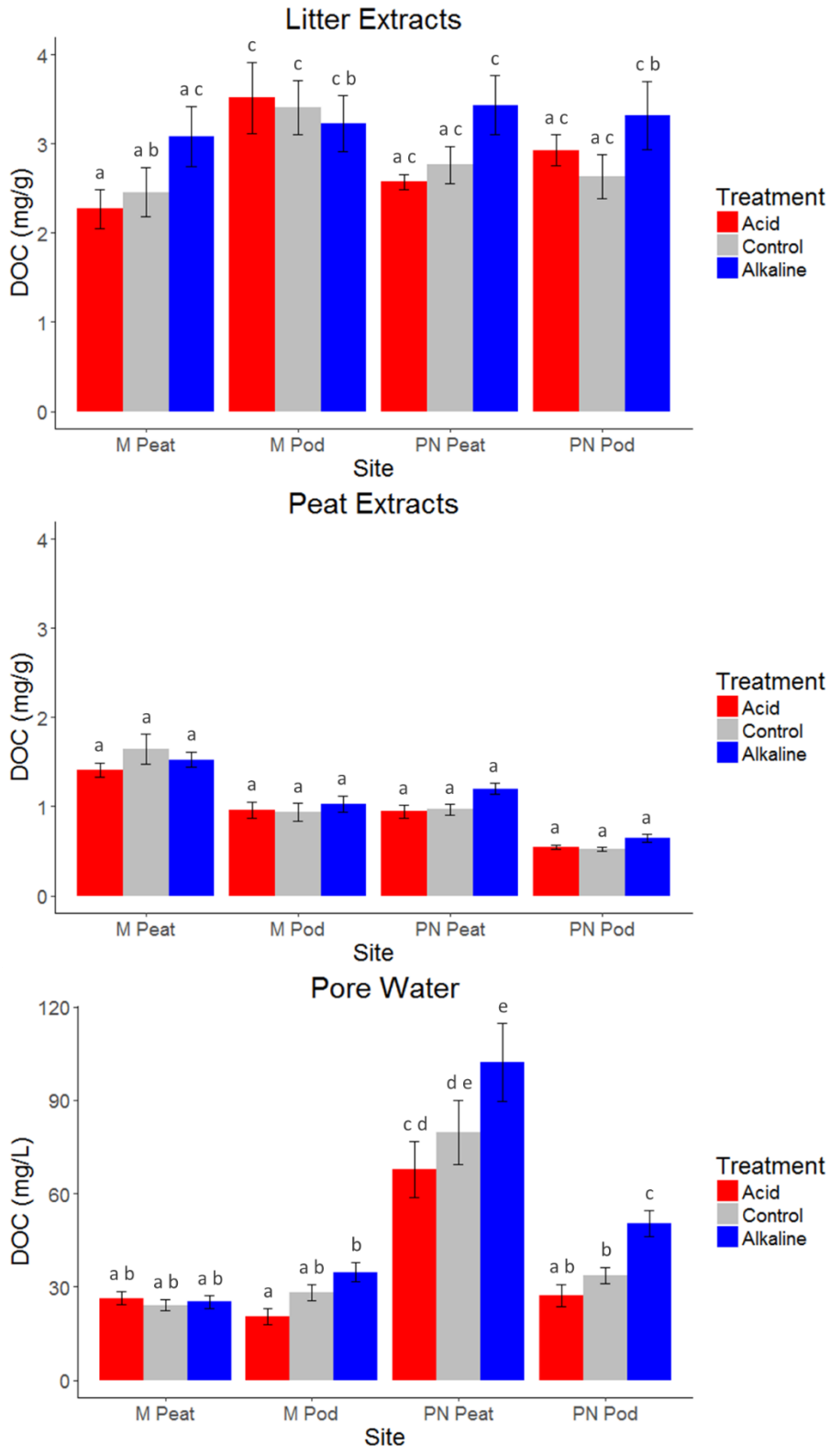


Figure 4.13: Mean DOC concentration of peat and surface litter samples, and pore water samples. Samples were collected during April, July and October 2016. Letters signify where significant differences occur, obtained using a Posthoc analysis on an ANOVA test comparing site (four levels), treatment (three levels) and month (three levels). All data was log transformed.

The aromatic fraction of DOC as indicated by $SUVA_{254}$ was not effected by treatments for either litter extracts (P = 0.073), or soil extracts (P = 0. 591) during the monitoring period of April, July and October 2016. However, an ANOVA on pore water data showed a significant P value for treatments (0.006), but when investigated further with a Posthoc analysis, there were no significant differences of interest.

4.3.3.2.1 Relationship between pH and DOC change

The effect of treatments on pH were apparent for pore water and peat extracts, with a reduction in pH with the acid treatment, whilst alkaline treatments increased pH (*Figure 4.14*). Both soil extracts and pore water show a strong and significant positive relationship with increasing DOC with increasing pH (*Figure 4.14*). However, there was no such relationship with surface litter (P = 0.885).

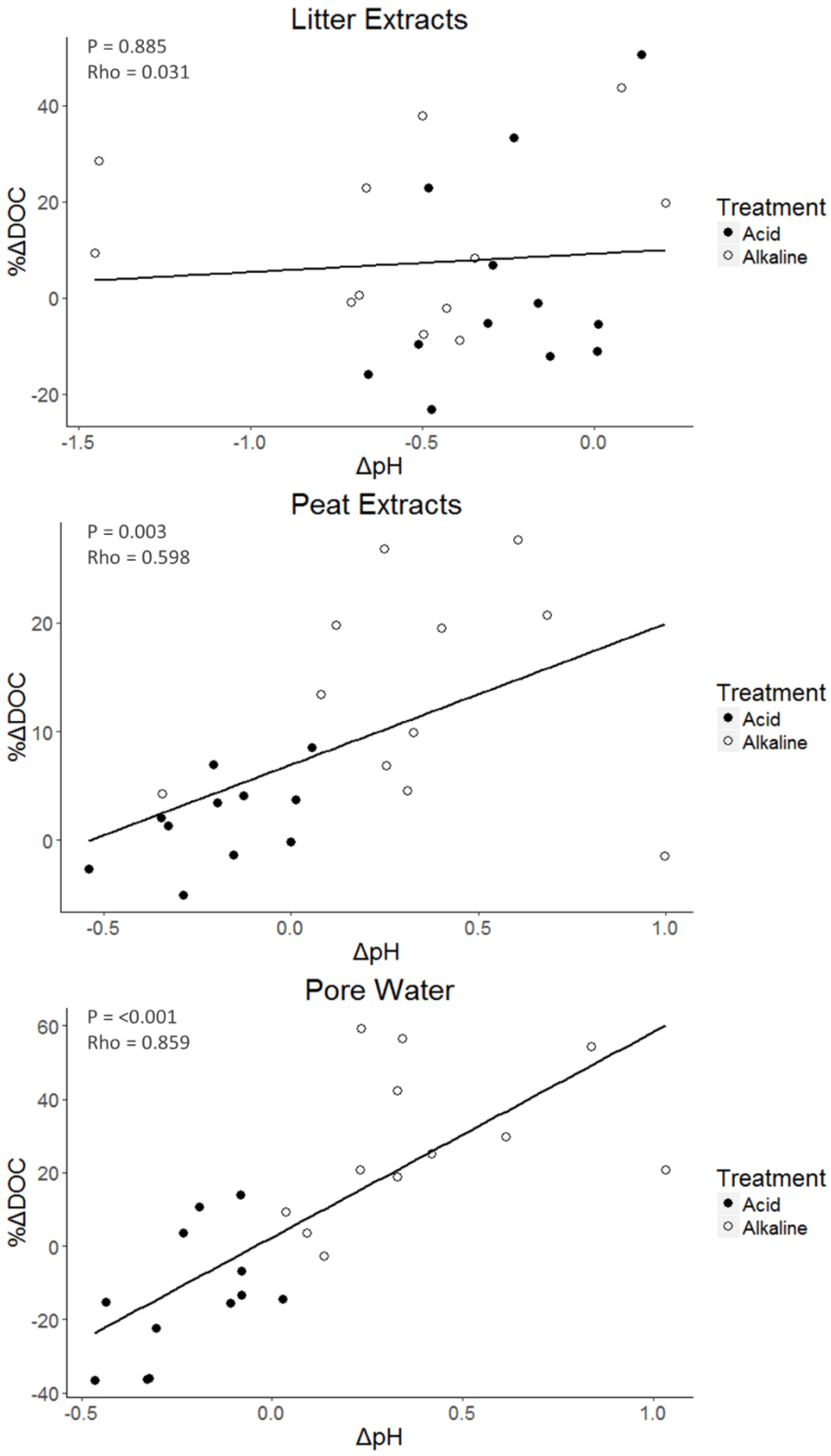


Figure 4.14: Scatterplots comparing change in pH and percentage DOC for each treatment compared to the control, for litter and peat extracts, and pore water samples. Samples were collected during April, July and October 2016. Significance (P value) and strength of relationship (Rho value) were obtained using Spearman's Rank.

4.4 Discussion

4.4.1 Peat and litter DOC production

Surface litter produced more DOC relative to the dry mass of substrate than peat, regardless of sampling month. This highlights the importance of substrate for DOC production and potential transport. DOC released from litter increased from 2.4 mg/g to 3.2 mg/g between April and October. This reflects the seasonal cycle in the build-up of the quality of the litter pool over the year, from increased NPP through the summer months and production of organic material, to the seasonal senescence of plant material during autumn that adds additional decomposing litter on the peat surface. This seasonal change in the quality of litter driven by the seasonal cycle of NPP increased the quantity of DOC produced in this surface layer per unit mass of substrate. Similar values of DOC released from litters through water extracts are reported in the literature for peat at ~1 mg/g, and various litters including *Calluna* (<6 mg/g), *Eriophorum* (<5 mg/g) and *Sphagnum* (<3 mg/g) (Ritson et al., 2016, Mastný et al., 2018). Few studies are available that compare the effect of seasonality, so further work on this is needed to better understand the relationship between seasonal litter quality, NPP and DOC release.

The lack of correlation in DOC concentration between sample types may be an artefact of the experiment. It may be particularly difficult to detect signals with the limited sampling months used in this study (April, July and October). It is also possible there are more influential mechanisms on the concentration of DOC in pore water, peat and surface litter, and so it is not possible to detect relationships between each sample type. For instance, DOC concentration in pore water is controlled by acidity and ionic strength (Evans et al., 2012, Thurman, 1985), whilst aluminium can influence the solubility of DOC to acidity in organo-mineral soils through complexation and co-precipitation with aluminium (Jansen et al., 2003).

DOC released from peat had a greater proportion of aromatic DOC as measured by $SUVA_{254}$ during April and July of >3 L/mg C^{-1}/m^{-1} compared to ~2.3 L/mg C^{-1}/m^{-1} in litter. Ritson et al. (2017) also observed a higher $SUVA_{254}$ value for peat compared to *Calluna* litter based on samples collected in May. This is likely due to the build-up of more microbially degraded products in peat from the biodegradation of organic material in litter and peat. Soil microorganisms are thought to preferentially decompose labile material, and much of this

fraction of the freshly senesced litter at the surface is decomposed and released as CO_2, leaving the more recalcitrant aromatic material (Kalbitz et al., 2003a, Saadi et al., 2006, McDowell et al., 2006) which may enter the peat layer below. This is further supported by the significant and positive relationship in $SUVA_{254}$ and so the concentration of aromatic DOC between the surface litter and peat below. This material is continuously decomposed and altered into a substance which is more stable and has a higher molecular weight than the original product (Malik and Gleixner, 2013), as well as an increased aromaticity as indicated by the SUVA value (Hur et al., 2009). These stable, recalcitrant products of late decomposition stages in peat, such as humic acids, are more resistant to microbial degradation and are thought to be the largest fraction of stable dissolved organic matter (Kalbitz et al., 2003b, Schnitzer, 1978), meaning they are an essential component of the carbon pool in these systems.

The aromatic humic and fulvic acid fraction of DOC, which is estimated to make up 50-75 % of DOC in water and is strongly related to a brown colouring in water (Worrall et al., 2003a, Tipping et al., 1988, Hongve et al., 2004, Grieve, 1990), has been shown to be sensitive to acidity (Clark et al., 2011, SanClements et al., 2012a). Therefore this coloured and persistent DOC is also likely to increase with recovery from acidification. This problem of 'brownification' of waters draining peatland areas in the Pennines is well documented (Chapman et al., 2010, Worrall et al., 2003b, Watts et al., 2001). Therefore based on this $SUVA_{254}$ data, peat derived DOC may be more sensitive to changes in acidity than litter derived DOC during April and July.

During October, there is a decline in the $SUVA_{254}$ by 1.4 L/mg C^{-1}/m^{-1} in peat making values similar to that in litter. This reduction also occurs in pore water samples at all sites except Migneint Peat, suggesting that this change in the aromatic DOC pool in peat is a response to seasonal variation rather than a random artefact of sampling surface peat during those specific months. In addition, when looking at pore water DOC concentrations, there is a reduction in winter months with concentrations ranging from 8-24 mg/L from November till February, to 21-43 mg/L in summer months (mean of control plots across different sites excluding Peaknaze Peat). Clark et al. (2005) reported similar seasonal values for pore water from blanket peat over a 10 year period, at <20 mg/L during winter months, and ~25 – 40 mg/L during the summer. These seasonal variations in DOC concentrations have also been

observed in rivers draining organic catchments in the Pennines, with values reported as 5 mg/L in winter months, increasing to 20 mg/L in late summer/early autumn (Scott et al., 1998).

DOC concentration is usually higher during summer months due to greater NPP, and the influence of temperature driving biological activity and therefore DOC production compared to the winter months (Dawson et al., 2008). There is also less rainfall volume and frequency, and higher evapotranspiration rates. The water table is typically lower during the summer, which is apparent for sites when looking at the pH of pore water, which is more acidic during July, and increases by 0.1-0.52 units depending on site between July and October. A lower water table results in reduced hydraulic conductivity and therefore removal of DOC. The reduced flux and lower volume of water increases concentrations in summer, and once more frequent rain events occur, this summer store is depleted in the winter months, reducing DOC concentration and increasing the proportion of hydrophobic DOC (Scott et al., 1998). This Autumnal release of DOC from peatlands into water systems creates seasonal implications for water companies (Ritson et al., 2016).

There are some interesting relationships in DOC quality between the different elements of the upper peat layer, which provide some insights into the production and movement of aromatic DOC within peat systems. There is a positive and significant relationship in $SUVA_{254}$ between surface litter and peat, and between peat and pore water. The latter may be an artefact of measuring the $SUVA_{254}$ on peat extract, which is also measuring $SUVA_{254}$ on the pore water within the field moist peat sample. However, if this were the case, we would also expect to see a similar magnitude of pH and DOC concentration response to treatments in peat as with pore water, which we do not.

These relationships suggest there is a process by which aromatic DOC is produced and moves through the upper peat system into pore water, where it then becomes mobile and is able to leach from the peatland system into water bodies. Here a theoretical pathway model on the production and movement of aromatic DOC is proposed below based on the evidence of this research (*Figure 4.15*).

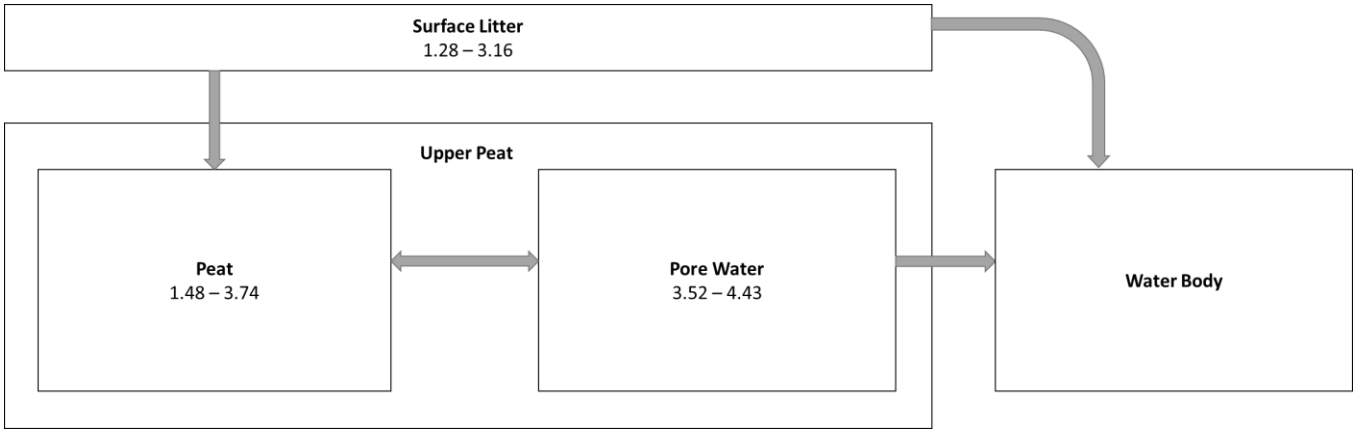


Figure 4.15: Theoretical pathway model showing the movement of aromatic DOC through the different components of the upper surface layer of peat into terrestrial waters. The $SUVA_{254}$ value (L/mg C^{-1}/m^{-1}), which is a proxy for measuring the aromatic fraction of DOC, is given for pore water, and litter and peat extracts (mean of control plots at each site) as an indication of quality.

To begin with, aboveground exudates and freshly senesced litter is added to the peat surface, where it begins decomposition by macro, meso and micro organisms. This litter layer contributes to the input of aromatic DOC into the peat. However, it's important to note that the decomposability of litter, and therefore the release of decomposition products such as CO_2 and DOC is dependent on vegetation type (Neff and Hooper, 2002). Other studies have shown vegetation type to be an important factor for the release of aromatic DOC in peatlands (Ritson et al., 2016, Pinsonneault et al., 2016).

DOC produced in this layer can be transported directly into water systems through overland flow (Clark et al., 2007). Alternatively, the partly decomposed organic material and aromatic DOC may enter the peat system below as more litter is added above. In both peat and pore water, organic material is continuously decomposed, altered and produced by a variety of microorganisms, resulting in a substance which is more stable and has a higher molecular weight than the original product (Malik and Gleixner, 2013). This is supported by the higher concentration of aromatic DOC in peat and pore water compared to litter.

The material which has become small enough to be dissolvable can move into pore water, where it can potentially be transported through hydrological processes into terrestrial water bodies (Clark et al., 2008). This is supported by the simultaneous changes in concentration between both sample types, such as the reduction in the concentration of aromatic DOC during October in both peat and pore water samples. Dissolvable material can also move

out of solution, again becoming part of the peat complex, such as with precipitation and coagulation with acidity (Thurman, 1985).

4.4.2 Variations across sites and soil types

The degraded peatland site, Peaknaze, had a much greater mean concentration of total DOC in peat pore water at 80 mg/L. This is higher than the mean pore water value measured at this site during the previous monitoring period of 60 mg/L, but it is not surprising that such high DOC concentrations are recorded at this site. A survey of surface waters in the South Pennines in 1998 produced DOC concentrations in the range of 117 – 1296 mg/L in waters (Evans et al., 2000). This site also had the highest $SUVA_{254}$ value in pore water, and whilst this was not significant, it is consistent with the problem of 'brownification' of waters draining peatland areas in the Pennines (Chapman et al., 2010, Worrall et al., 2003b, Watts et al., 2001).

This area has experienced significant damage, including previously high sulphur deposition which has seen a 69 % reduction between 1970 and 2005 (Dore et al., 2007, Evans et al., 2012), with a significant store of sulphur still being present in the South Pennine peats (Daniels et al., 2008), and significant acidification (Evans et al., 2000). In addition, this areas has experienced previous and current high levels of nitrogen deposition and saturation (Helliwell et al., 2007, Curtis et al., 2005, Evans et al., 2000), intensive land management (Clutterbuck and Yallop, 2010), all of which have contributed to extensive *Sphagnum* loss, a lowering of the water table and peat erosion (Oulehle et al., 2013, Tallis, 1987, Carroll et al., 2009, Noble et al., 2018). It is possible that the drier conditions in peat have resulted in more rapid decomposition and therefore DOC production (Mitchell and McDonald, 1992). However, there is also evidence that aerobic conditions can stimulate CO_2 production, and therefore microbial consumption of DOC, which would reduce concentrations (Freeman et al., 2004, Pastor et al., 2003).

An alternative explanation for high DOC concentrations in the pore water at Peaknaze Peat is the response to recovery from acidification. This area is currently in a state of recovery following some of the highest sulphur deposition levels in Europe, and previously suppressed DOC is being released as solubility increases with increasing pH (Evans et al., 2012, Ekström et al., 2011, Oulehle et al., 2017, Clark et al., 2011, Evans et al., 2006a).

Neither peat nor surface litter samples at Peaknaze Peat have significantly higher DOC or aromatic DOC concentration compared to other sites, which suggests there's no difference in substrate. This implies that the high DOC concentrations in pore water is therefore not the result of recently produced DOC, but from the release of previously retained DOC at this site. On the other hand, higher density and greater DOC concentrations compared to other sites have been recorded nearby at the River Etherow, and so it is possible that more peat may simply result in more DOC (Clark et al., 2012).

In the upper organic peaty layer, there is consistently more DOC in peat samples than in podzol. However, barring the Peaknaze Peat site, pore water samples show no difference in the concentration of total or aromatic DOC (as indicated by $SUVA_{254}$) between peat and organo-mineral soil. There are few studies which have compared DOC concentrations in peat and podzol soils in a non-forested area. van den Berg et al. (2012) reported pore water DOC values as 46 mg/L for histosols (average of 8 sites) and 27 mg/L for podzols (average of 15 sites, of which one podzol site occurred in a moorland environment). Alternatively, a survey of the literature reporting DOC values show there is more DOC in podzol pore water (39 mg/L) than in peat (22 mg/l) (van den Berg et al., 2012), which suggests that podzols are as important as a source of DOC as peat, yet more research has been done on peat. This discrepancy in DOC concentration in podzol, with a greater amount in the upper organic layer but a similar concentration in pore water compared to peat, is likely due to the influence of the mineral component of podzol. In organo-mineral soil, the mineral horizon has been shown to control the concentration of DOC in pore water through sorption onto mineral surfaces (Kennedy et al., 1996).

Despite there being more DOC concentration in the surface organic layer of peat compared to podzol, there is more aromatic DOC, as indicated by $SUVA_{254}$, in the surface organic layer of podzol. This is consistent with findings from the Pennines which have shown waters to be browner in colour when a greater contribution of flow originates from the mineral soil horizons rather than peat dominated run off (Chapman et al., 2010). Furthermore, the podzol surface litter layer at the undamaged site (Migneint) contains both more DOC and more aromatic DOC compared to the surface litter at the peat site. This suggests that the greater aromatic fraction of DOC found in the podzol organic layer originates from the

decomposition of the litter above, which is further supported by the significant and positive relationship in aromatic DOC between peat and litter.

This holds important implications for the store of carbon in these soils and transport into terrestrial waters. Some studies based in Canadian catchments have shown a positive relationship between discharge and DOC concentrations in streams draining podzol soils, whilst there is no such significant relationship with waters draining peats (Hinton et al., 1998). Other studies have also found a correlation between DOC concentrations in organo-mineral catchments and stream flow (McDowell and Likens, 1988, Hope et al., 1994, Dawson et al., 2002, Boyer et al., 1997, Hornberger et al., 1994).

Water typically travels through the mineral layer in podzol soil where DOC is retained by mineral sorption during times of low flow. However, around 36 % to more than 50 % of annual DOC flux have been shown to occur during peak discharge over short periods of time (Clark et al., 2007, Inamdar et al., 2006, Buffam et al., 2001, Hinton et al., 1997). During times of high flow, the hydrological pathway switches to the organic layer, which, according to the results of this study, contain significantly more aromatic DOC in the organic peaty material than in peat. Overland flow may well increase also, transporting again more aromatic DOC but also a greater proportion of total DOC from the above surface litter compared to peat.

As a result of the potential increase in frequency and magnitude of rainfall events predicted with climate change (Tebaldi et al., 2006, Meehl et al., 2005), there may be an increase in flux and concentration of DOC and aromatic DOC from organo-mineral soils (Clark et al., 2008). This provides insights into the temporal and spatial transport of DOC out of these catchment systems comprising of peat and organo-mineral soils. It also provides implications for modelling and predicting the production and movement of persistent aromatic DOC into water systems.

4.4.3 How does DOC respond to acidity treatments?

Firstly, it is apparent that treatments were successful in altering the pH of pore water over the 13 month experimental period. Treatments generated a pH range of at least 0.2 pH units to a maximum range of 0.9 units (when comparing means for acid and alkaline plots at each site). This is comparable to the change in pH observed in the Countryside Survey broad habitats between 1978 and 2007 (Evans et al., 2012).

There was a strong and significant relationship between pore water pH and change in DOC concentration, suggesting that pore water DOC concentrations are consistently sensitive to changes in acidity. In addition, the results from this experiment are similar to that of a previous acidity manipulation experiment at these sites (Evans et al., 2012), showing reproducibility and providing a strong support base for the hypothesis that increased DOC concentrations in terrestrial waters are due to increased solubility with recovery from acidification in organic soils. This change in solubility of DOC in soil solution is related to the degree of dissociation of organic acids (Oulehle et al., 2013) and is simultaneously related to changes in ionic strength (Clark et al., 2005).

DOC in both peat and podzol soil pore water responded to acid and alkaline treatments at Peaknaze, whilst only the podzol soil respond at the Migneint site. The lack of response of pore water pH and DOC concentration at the Migneint Peat site has been observed before in a prior acidity manipulation experiment at this site (Evans et al., 2012) and has been attributed to the wetter conditions which a) dilutes any treatment solutions applied, and b) enhances sulphate reduction which buffers against acidity change and therefore response in soil solution chemistry (Oulehle et al., 2013). Sulphate reduction occurs at wetter sites and this explains why the DOC trends of waters draining drier peat sites are greater, such as at the Peak District, or streams draining better drained organo-mineral soils such as in the Pennines (Chapman et al., 2010).

However, at the Migneint Podzol site, which achieved the greatest pH increase with the alkaline treatment of 0.68 pH units, only an increase of 9 mg/L of DOC was achieved in pore water. Migneint had a higher baseline pH of pH 4.3 compared to ~pH 4 at Peaknaze, and yet despite the high pH increase at this site there was only a marginal increase in DOC suggesting that there is a pH threshold at which solubility controls DOC concentration, a

trend which was also observed by Evans et al. (2012). They suggested that this shift from 'solubility control' to 'supply control' on DOC leaching could have implications on the future of DOC release from peatlands as sensitivity to processes influencing DOC production increase, such as climate change and land management. For instance, DOC release from organo-mineral soils (Christ and David, 1996) and peat have been shown to increase with increased temperature. Increases in temperature may also increase enzyme activity and consequently DOC release from organic soils (Freeman et al., 2001a), whilst the sensitivity of different vegetation species to DOC production may increase with changes in climate, particularly for *Calluna* (Ritson et al., 2014).

At Peaknaze, which is situated in the strongly acidified area of the Peak District, the increase in DOC in response to the alkaline treatment was particularly large (13 – 22 mg/L for peat and podzol sites respectively). There was a smaller response to the acid treatment (decrease of 9 – 12 mg/L), likely due to the highly acidified baseline state of peat (3.98 units) and podzol (4.11 units) and so further additions had a limited effect on DOC solubility.

When assessing the response of pore water, peat and surface litter over April, July and October, there is less of a response of pH and DOC concentration to treatments. For instance, whilst a significant change in pH was achieved in pore water at podzol sites, it was not possible to achieve a significant decrease in pH with acid plots at Peaknaze Peat, whilst no change in pH was achieved at Migneint Peat. This is to be expected due to the low baseline pH at Peaknaze Peat, making further reductions undetectable when taking the mean over three months spanning three seasons. Also, the lack of response was seen at Migneint Peat when taking a mean of the 13 month dataset, for reasons mentioned above. The change in DOC concentration and strength of relationship with pH was not as strong as when taking the mean of the 13 month dataset, which again is to be expected due to increased error and seasonal variability when taking the mean of three months spanning three different seasons. There was however a decrease in DOC concentration with acid treatments, and an increase with alkaline treatments, although this change was not significant to the control.

There was no significant change in peat DOC production in response to acidity treatments, whilst the extracts showed there was some change in pH at all sites except Migneint Peat. For litter, as the treatment applications were being applied directly to the litter surface, it

was to be expected that they would achieve a greater magnitude of pH change than with the peat below, despite the effort of rinses. However, there were no responses of litter and peat DOC released in water extracts to treatments, which, considering the lack of pH response, is to be expected. This suggests that either a) this is an artefact of the minimum sampling period of three months, which is supported by the significant relationship between pH and DOC change in peat, or b) changes in deposition chemistry has a minimal effect on the hydrogen ion concentrations of these samples, and thus does not influence the production of DOC for either peat or litter in the surface layer of peat and organo-mineral soil, and instead the pH effect on DOC solubility is mediated within the upper peat horizons through in situ soil processes and buffering.

The sensitivity of $SUVA_{254}$ to acidity has been demonstrated in the literature (Clark et al., 2011, SanClements et al., 2012a), yet peat and litter extract $SUVA_{254}$ did not respond to treatments, and the response of pore water $SUVA_{254}$ was dependent on month and not consistently apparent throughout the 13 month monitoring period. Clark et al. (2011) found that a change in pH of more than half a unit resulted in a response in $SUVA_{254}$. Peat and podzol O-horizon extracts experienced a change in pH of 0.73-1.08 units with acid treatments and 0.59-0.92 with alkaline treatments. However, in this study samples experienced a lower magnitude of change in pH. Acid treatments reduced pH by 0.11-0.47 units (mean of all sampling months at individual sites) in litter extracts, 0.16-0.70 units in peat and podzol O-horizon extracts, and 0.17-0.47 units in pore water, whereas alkaline treatments increased pH by 0.25-0.82 units in litter extracts, 0.03-0.62 units in peat and podzol O-horizon extracts and 0.09-0.72 units in pore water. Therefore it is likely that the limited response of $SUVA_{254}$ is due to the lower magnitude of change in pH. In addition, unlike Clark et al. (2011) which was based on a controlled laboratory experiment, it is likely that there is environmental variation which may limit the response of $SUVA_{254}$ to treatments. A longer acidity manipulation field experiment with more replication is needed to see the effects of long term acidification and recovery, and therefore a greater magnitude of change in pH, on the quality of DOC released from organic catchments.

In summary, litter is a greater source of DOC, which is less aromatic (as indicated by $SUVA_{254}$), whilst peat produced less DOC which is more aromatic. Whilst there was no significance of treatments on $SUVA_{254}$ in this study, the sensitivity of the aromatic fraction

of DOC has been demonstrated in the literature (Clark et al., 2011, SanClements et al., 2012a), suggesting that this acid sensitive fraction in peat is likely to become mobile during recovery from acidification. The relationship between peat and pore water supports this movement and suggests that the acid sensitive fraction of DOC is transported through leaching in the upper peat layer. In addition, the poor correlation in $SUVA_{254}$ between pore water and litter suggests that aromatic DOC produced in the surface litter layer is transported to waters directly through overland flow.

4.5 Conclusion

This research provides insights into the seasonal dynamics of both DOC quantity and quality within the different components of the upper surface layer of typical organic catchment soils, and therefore the temporal and spatial transport of DOC out of catchment systems comprising of both peat and organo-mineral soils. It also creates implications for modelling and predicting the production and movement into water systems. A theoretical pathway model has also been proposed for the production and movement of acid sensitive aromatic DOC through the different components of the surface layer, identifying sources and potential pathways. Finally, it is highly likely that increased solubility of DOC in pore waters due to recovery from acidification has contributed to the increase in DOC concentration in surface waters. Furthermore, there is limited evidence from this study that DOC production from litter and peat decomposition is sensitive to acidity, and so further work is needed to assess the impact of DOC production with recovery from acidification on DOC release from these sources.

Appendix

Protocol of treatment solution preparation

Equipment

- 6x small glass beaker
- 4 x medium glass beaker
- 4 x 1 L beaker
- 1 x 1 L measuring cylinder
- 1 x small measuring cylinder (50-100ml)
- 1 x glass rod
- 1 x plastic pipette
- 1 x spatula

1. Acid solution

1a. Peat

- Half fill the 16 red acid bottles with distilled water
- Add 11g of concentrated H_2SO_4 to each bottle
- Add more distilled water to the bottle so it is reasonably full

1b. Podzol

- Half fill the 16 red acid bottles with distilled water
- Add 5.5g of concentrated H_2SO_4 to each bottle
- Add more distilled water to the bottle so it is reasonably full

2. Alkaline solution

2a. Peat

- Add 400 ml of distilled water to a 1 L beaker
- Weigh out 135.58 g of NaOH and 3.76 g of KOH and add to the 1L beaker
- Add another 400 ml of distilled water to the beaker and mix with a glass rod until compound has dissolved
- Divide this solution into the 16 blue Alkaline 1 bottles, each containing 50 ml

2b. Podzol

- Add 400 ml of distilled water to a 1 L beaker
- Weigh out 135.58 g of NaOH and 3.76 g of KOH and add to the 1 L beaker
- Add another 400 ml of distilled water to the beaker and mix with a glass rod until compound has dissolved
- Divide this solution into the 16 blue Alkaline 1 bottles, each containing 50 ml

3. Alkaline rinse solution

3a. Peat

- Weigh out 39.37 g of $MgCl_2$ and 8.74 g of $CaCl_2$ and add to a dry 1 L beaker
- Slowly add 400 ml of distilled water to the beaker (compounds will fizz and react rapidly) and mix with a glass rod until all compound has dissolved
- Divide this solution into the 8 blue Alkaline 2 bottles, each containing 50 ml

3b. Podzol

- Weigh out 22.15 g of $MgCl_2$ and 4.92 g of $CaCl_2$ and add to a dry 1 L beaker
- Slowly add 400 ml of distilled water to the beaker (compounds will fizz and react rapidly) and mix with a glass rod until all compound has dissolved
- Divide this solution into the 8 blue Alkaline 2 bottles, each containing 50 ml

Chapter 5: Assessing the impacts of litter type and quality on decomposition in peat and organo-mineral soils during a one-year, multi-scale incubation experiment.

Abstract

With evidence that carbon sequestration in peatlands is slowing, and with the observed increase in DOC concentrations in waters draining organic catchments over the past 30 years, it is vital the mechanisms behind carbon storage and release, and the responses to environmental change in these sensitive ecosystems are fully understood. In addition, despite their presence in many organic catchments and importance for carbon storage, there is relatively little research on upland organo-mineral soils and even few studies which compare them directly to peat. Therefore the main aims of this research were to investigate moorland litter decomposition and how this varies with respect to underlying soil type (peat and humic podzol), and different acid deposition loads between two sites over a nitrogen (N) deposition gradient. A litter bag experiment was run using two common moorland species; *Calluna vulgaris* and *Eriophorum vaginatum*, to investigate decomposition over a 12 month period. In addition, the extent to which changes in N deposition have altered litter quality and associated decomposition of *Calluna* were investigated through a translocation experiment.

Results show that decomposition is faster in podzols than in peat, and is suppressed where N and acid deposition is high. N content of *Calluna* from the polluted site was significantly higher, suggesting that deposited nitrogen has accumulated in plant tissue. Furthermore, litter quality in terms of N content and C:N ratio did not influence the decomposition of *Calluna* at most sites. However, the *Calluna* which had significantly more N accumulated in tissue decomposed significantly less at the most polluted peat site. This may be an artefact of the experiment, or due to the interactions between high N and lignin content, the mechanisms of which are discussed in this chapter. More work is needed assessing the potential interacting effects of high N in plant tissues and organic material, and lignin content on the decomposition of *Calluna* in a moorland environment. This research provides insights into the spatial and temporal variations on the decomposition of common litters in an upland organic catchment comprising of peat and organo-mineral soil over a 12 month period.

Chapter 5

5.1 Introduction

5.1.1 Why is litter decomposition important for DOC dynamics?

Peatlands are estimated to store 20-30 % of the total global carbon (C) stock (Gorham, 1991). In England and Wales this has been estimated at 400,166 kt of C for an area of 3568 km^2 (Milne and Brown, 1997). Organo-mineral soils are also present on the sloped areas of these organic upland catchments, and are also an important component of the catchment C store, with an estimated 25,400 kt of carbon for an area of 1,313 km^2 in England and Wales. Dissolved organic carbon (DOC) represents a major natural C export from upland organic soils (Clark et al., 2007, Billett et al., 2004, Hope et al., 1994, Dinsmore et al., 2010, Roulet et al., 2007, Nilsson et al., 2008).

Litter decomposition is a major source of DOC and so plays a vital role in C dynamics, stabilisation and transport in soils (Don and Kalbitz, 2005, Kalbitz et al., 2000). Radiocarbon studies, for instance, show that the vast majority of DOC in surface waters is recent, typically less than 40 years old in undisturbed systems (Tipping et al., 2010, Evans et al., 2007, Palmer et al., 2001). Other studies also show a correlation between DOC in surface layers of peat and DOC in stream water (Clark et al., 2008, Billett et al., 2006). Such evidence suggests that DOC in stream water likely derives from litter and soil decomposition.

There is a huge diversity in the quantity and quality of DOC produced by different vegetation species in a peatland ecosystem (Pinsonneault et al., 2016). Moore et al (2007) conducted a long-term litterbag experiment and found litter type to be the most dominant control on litter decomposition over site differences. Vascular plants, such as *Calluna*, have been shown to produce more DOC than *Sphagnum* in peatland ecosystems (Ritson et al., 2014, Armstrong et al., 2012). In addition, the release of *Calluna* flowers capsules provides a seasonal input of labile material, with the potential to decompose quickly and produce aromatic DOC which is persistent in waters (Ritson et al., 2016). There is also evidence that vascular plants are more efficient at utilising N, and so providing decomposers with a greater nutrient availability and enhancing DOC production (Ritson et al., 2016).

Sphagnum has been consistently shown to produce a lower quantity of DOC compared to vascular plants (Pinsonneault et al., 2016, Armstrong et al., 2012), which has been attributed to the initial labile property of *Sphagnum*, resulting in less DOC being produced

which is of a more aromatic nature once processed by microorganisms (Ritson et al., 2016). *Sphagnum* is a key peat building bryophyte with a high phenolic content (Rasmussen et al., 1995), which suppresses biodegradation, contributing to a slow decomposition rate (Verhoeven and Toth, 1995, Fenner et al., 2004). Therefore a peatland ecosystem with a higher proportion of vascular plants will produce more DOC of a more labile nature leading to a greater loss of carbon, whilst a *Sphagnum* dominated peatland will produce less DOC of an aromatic nature and increase the soil C pool (Ritson et al., 2016).

Peatlands represent one of the largest stores of C in the UK soil C pool, containing an estimated 3.2 billion tonnes (Bain et al., 2011). Changes in the sink/source dynamics can result from the destabilisation of the C pool which may have major impacts on climate change feedback systems, both directly (through the atmospheric pathway) or indirectly (by means of the aquatic pathway) (Billett et al., 2007). There has been an observed increase in DOC concentrations in surface waters draining from catchments dominated by organic soils since the 1980's, across large areas of the Northern Hemisphere (Evans et al., 2005, Monteith et al., 2007, Oulehle and Hruška, 2009, SanClements et al., 2012b, Couture et al., 2012). Such significant changes in C fluxes have raised concerns over the future of terrestrial C stocks (Freeman et al., 2001a).

Most research on this issue to date has focused on examining soil processes, such as the physico-chemical response of DOC to acidification, with few studies looking at the potentially changing role of litter and DOC production. Also, despite the proximity of peaty podzols to areas of reported increases in DOC concentrations in surface waters, organo-mineral soils are much less understood compared to peatlands (Stutter et al, 2011). Additionally, whilst acid deposition progresses towards background levels, future climate change scenarios depict changes in temperature and hydrological processes with unclear consequences on DOC quality and quantity (Dieleman et al., 2016). In addition, there are suggestions that the DOC baseline is being shifted to above pre-industrial levels as a result of nitrogen fertilisation (Sawicka et al., 2017). All of this presents much uncertainty in the response of DOC production to environmental change, creating implications for predicting future climate change scenarios, as well as creating possible future impacts on the drinking water industry (Ritson et al., 2016). In this study we aim to improve knowledge of the

sources of DOC and factors influencing production rates in order to better understand there changes in C fluxes.

5.1.2 Variation in DOC across different soils and pollution gradients

5.1.2.1 Peat and organo-mineral soil

Catchments vary in their characteristics and therefore may respond differently to environmental change. For instance, there have been discrepancies in rates of DOC release in areas receiving similar acidifying deposition loads, suggesting catchment specific properties may be a controlling factor in DOC production and release (Clark et al., 2010a). Upland organic catchments consist of a combination of both peat and organo-mineral soils. These shallow organic soils, usually peaty podzols, surround areas of peats on hilly slopes, and make up a large proportion of upland carbon-rich landscapes. Therefore the topography of the catchment is a determining factor in the proportion of peat and peaty podzol present within the catchment. Peat and organo-mineral soils differ in their properties including hydrology, texture, and vegetation communities, all of which have been shown to correspond with DOC concentrations in pore water (Dalva and Moore, 1991, Neff and Asner, 2001, van den Berg et al., 2012).

Peatland UK BAP Priority Habitats are estimated to cover a total area of 2,287,665 ha in the UK, of which 2,208,553 ha are classed as blanket peat (JNCC, 2011). Milne and Brown (1997) estimated an area of 356,800 ha of peat to contain 400,166 kt of C in England and Wales. Peat typically has slow decomposition rates due to waterlogged conditions, resulting in the accumulation of a thick organic layer of partially decomposed material at least 40 cm thick (Burnham et al., 1980). The high water table results in a slow hydraulic conductivity and therefore a significant store of water at more than 95 % (Charman, 2002). Such properties allow peat to hold a large C store, which has been correlated with concentrations and fluxes of DOC in surface waters (Aitkenhead et al., 1999, Hope et al., 1997).

Peaty podzols are a common organo-mineral soil present in upland bogs, estimated to cover 131,300 ha and contain 25,400 kt of carbon in England and Wales (Milne and Brown, 1997). Pockets of peaty podzol soils on upland organic catchments have different profile characteristics, hydrological behaviour, plant communities and therefore DOC dynamics to peat. Such behaviour is complex and has been poorly studied in non-forested environments.

In general, the hill slope topography which allows podzols to form enhances drainage, whilst the mineral content of podzols improves pore volume and size and allows for easier throughflow as well as sorption of DOC onto mineral particles in the mineral horizons (Stutter et al., 2012). In addition, flow pathways can shift during stormflow events from the main runoff pathway in the lower mineral layer, to the upper organic layer where DOC is mobile (McDowell and Likens, 1988, Clark et al., 2007).

Peat and organo-mineral soil have different plant communities. For instance, peatland areas are typically dominated by ericaceous shrubs, such as heather (*Calluna vulgaris*), some graminoids including cotton grass (*Eriophorum vaginatum*) and a large proportion of bryophytes such as *Sphagnum* mosses over peat areas. In contrast, podzol areas consist of some Ericaceous shrub and graminoids, but are dominated by grasses including *Festuca ovina* and rushes such as *Juncus squarrosus*. As DOC characteristics are related to vegetation type (van den Berg et al., 2012, Pinsonneault et al., 2016), it is likely that these different plant communities will produce DOC with different properties.

5.1.2.2 Environmental factors

Environmental factors can also influence decomposition and therefore the quantity and quality of DOC produced. Litter chemistry may be a product of the concentration of nutrients in the ecosystem, which may ultimately affect its degradability. In areas with higher N deposition, greater foliar N concentrations have been measured in dwarf shrub and bryophyte species (Caporn et al., 2014). For instance, in areas receiving an historic increase in atmospheric N deposition, *Sphagnum* has been found to contain higher N concentrations, resulting in a higher decay rate (Aerts et al., 2001, Limpens and Berendse, 2003). Pitcairn et al. (1995) found *Calluna* to have a linear increase in tissue N of 0.045 mg g^{-1} kg^{-1} ha^{-1} $year^{-1}$ of increased atmospheric N deposition.

Alternatively, the plant community structure may itself be impacted by the atmospheric chemistry and pollution deposited on the ecosystem, such as reduced species richness (Caporn et al., 2014, Payne et al., 2017, Caporn et al., 2011). Vascular plants, which release more labile DOC (Del Giudice and Lindo, 2017), outcompete *Sphagnum* in areas with increased N deposition (Berendse et al., 2001). Therefore the change in deposition chemistry can result in a shift in plant community composition and, thereby the potential to affect the quantity and quality of DOC produced.

Other environmental conditions likely to influence litter decomposition and the resulting DOC quantity and quality is changes in abiotic conditions in peat and organo-mineral soil. For instance, soil chemical properties can affect how bioavailable DOC is, such as pH and ionic strength (Clark et al., 2010a). Also, the hydrological properties of different soils will affect DOC residence time and decomposition rates. Temperate and boreal peatlands generally have low rates of net primary production (Moore et al., 2002) alongside slow decomposition (Moore et al., 2007) which could increase with increased incidence of drought with climate change (Fenner and Freeman, 2011).

5.1.3 Objectives of this study

This research assesses litter decomposition in both common soil types across a pollution gradient, and therefore improves knowledge on the spatial difference in DOC production across a peatland catchment in response to environmental change. The main aims of this study were to investigate litter decomposition and how this varies with respect to underlying soil type, and different acid deposition loads between two sites over a nitrogen deposition gradient. The specific objectives were: (1) to determine the impacts of site and soil type on the decomposition of *Calluna vulgaris* and *Eriophorum vaginatum* using litter native to each field location; and (2) Determine to extent to which changes in N deposition have affected litter quality and associated decomposition of *Calluna* through a translocation experiment. Work will build on an established acidity manipulation field experiment (Evans et al., 2012).

5.2 Methods

5.2.1 Site description and experimental design

This work was built upon an existing long-term acidity manipulation field experiment set up in 2007, comprising of four replicated sites situated across two moorland locations with contrasting historic rates of acid deposition (see Chapter 3 for further detail) (Evans et al., 2012). The first study site, the Migneint (3°48.8’ W, 52°59.6’ N, 460 m a.s.l.), is a relatively undisturbed blanket bog area in North Wales with historically low levels of pollution. Peaknaze (1°54.5’ W, 53°28.3’ N, 440 m a.s.l.), Northern England, is a more disturbed region affected by relatively intensive land management and historically high levels of atmospheric

pollution, which has led to degradation of the ecosystem including extensive *Sphagnum* loss and erosion (Carroll et al., 2009, Noble et al., 2018, Tallis, 1987) (*Table 5.1*).

Table 5.1: A summary of present and historical pollution deposition data collected near experimental sites. Sources are as follows: 1) CEH moorland deposition data and estimates from the FRAME model (Evans et al., 2012); 2) UK deposition data downloaded from CEH website (CEH, 2014).

Data	Year	Migneint	Peaknaze
Non-marine Sulphur Deposition ($kg\ S\ ha^{-1}\ yr^{-1}$)[1]	Historical (1970 – 2007)	Decreased by 66%	Decreased by 69%
Total None-marine Oxidised Sulphur Deposition ($kg\ S\ ha^{-1}\ yr^{-1}$)[2]	2014	4.91	6.31
Non-marine Wet Sulphate Deposition ($kg\ S\ ha^{-1}\ yr^{-1}$)[2]	2014	4.41	5.41
Total Nitrogen Deposition ($kg\ N\ ha^{-1}\ yr^{-1}$)[2]	2014	17.98	22.91
Total Acidity Deposition ($keq\ ha^{-1}\ yr^{-1}$)[2]	2014	1.47	1.83

At each site, two soil types were investigated (blanket peat and humic podzol) which are among the commonest soils present in the UK uplands, and which also occur extensively in other cool, humid temperate regions. This study focuses on ambient deposition treatments within the control plots of this field experiment only, which received 20 L monthly applications of local rainwater without pH adjustment.

5.2.2 Litter bag experiment

The litter bag technique was used to measure the decomposition of litter in the field (Beyaert and Fox, 2007). *Calluna vulgaris* and *Eriophorum vaginatum* litter samples were harvested at the end of the growing season during autumn when DOC production from litter is typically at its greatest. *Eriophorum* and *Calluna* were chosen because they were common vegetation types to both sites and soils types.

The vascular plants were collected as dead standing biomass, were sorted to remove other material and processed by cutting into 2 cm long pieces and homogenised. A commonly used pre-treatment procedure was applied to the samples (followed by several studies including (Bragazza et al. (2007), Moore et al. (2007))). This involved air drying to a constant

mass at ambient temperature. The dry mass equivalent of 3 g were added to the litter bags, all of which were buried at a depth of 5 cm in October 2015. Pre-sown 10 x 10 cm litter bags made of polyamide monofil were used (Filtrations Technik, Germany). Subsamples of litter were ball milled and analysed for carbon and nitrogen content using a Thermo Flash 2000 Carbon Nitrogen analyser (Thermo Fisher Scientific, Massachusetts, USA).

Mesh size has been found to significantly influence what community of decomposers are exposed to the litter based on faunal size classes (Bradford et al., 2002). A mesh size as small as 74 µm has been used for sphagnum studies, and whilst this prevents the loss of litter pieces, it excludes many meso and macro-faunal decomposers (Limpens and Berendse, 2003). Alternatively much larger mesh sizes have been used such as 1-2 mm to encourage macrofaunal decomposition (Moore et al., 2007, Latter et al., 1997), but there is a risk that litter may be lost, particularly *Sphagnum* which becomes very crumbly when dry. Therefore a mesh size of 0.5 mm was chosen to allow decomposers into the bag, whilst minimising loss of fine litter particles (Bragazza et al., 2007). Litters were then sorted in to two groups to reflect different experiments (1) native litter to site and (2) litter translocation from other site (*Figure 5.1*). Litter bags were buried in the field at a depth of 5-10 cm in October 2015.

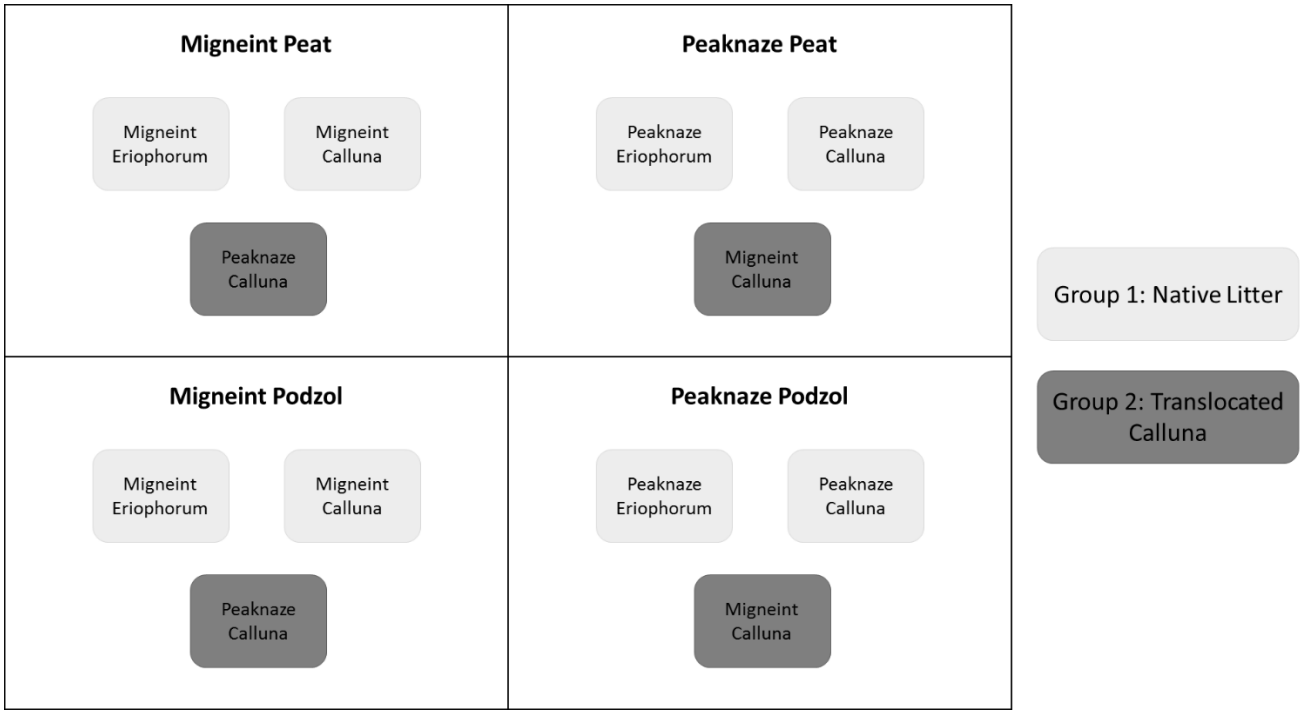


Figure 5.1: Illustration of which litters from each group were buried at each site. Group 1 represents two litter types (*Eriophorum* and *Calluna*) which are common at all sites, to investigate how decomposition is influenced by different sites and soils over a 12 month period. All were harvested from the site where they were buried. Group 2 contains *Calluna* from the opposing site to test the hypothesis of whether site, soil or litter quality is more influential on decomposition after 12 months of incubation. For instance, *Calluna* harvested from Peaknaze was buried at Migneint, and vice versa.

Collections occurred quarterly (January, April, July and October 2016) to assess how decomposition rate changed over time. Once retrieved, litters were processed by removing all ingrown material and soil invertebrates, and litter was weighed. To determine DOC production potential for another study, a cold water extraction was then performed with a 1:20 litter to water ratio using ultrapure water with a horizontal shaker for 24 hours. Extracts were centrifuged (3500 rpm, 10 minutes) and filtered through 0.45 µm cellulose membrane filter paper. The litter was then reweighed to establish a post-extraction weight and oven dried at 70 °C for 48 hours and weighed again for an oven dry mass.

5.2.3 Data analysis

A simple negative exponential decay model was used to express the mass loss over time (Olson, 1963, Pandey et al., 2007):

$$In\left(\frac{X_t}{X_0}\right) = -Kt$$

where t is time (year), k is the decomposition rate (year^{-1}), X_0 is original litter mass (g) and X_t is the mass of litter remaining at time t (g).

Data were statistically analysed using R statistical package (RDevelopment CORE TEAM, 2008). Data were assessed as to whether it met the assumptions of Analysis of Variance (ANOVA), including normality and equal variance, and transformations were applied where necessary. When significance was apparent, a post hoc test was run using the 'Tukey HSD' function in R to confirm where significant differences occurred between groups.

5.3 Results

5.3.1 Group 1: Native litters

The decomposition rates of litters at different sites after 12 months of decomposition are presented below in *Table 5.2*.

Table 5.2: Mean (± SE) litter decomposition rates (*k*) of *Calluna* and *Eriophorum* at each site based on 12 months of decomposition.

Litter	Site	*k*
Calluna	Migneint Peat	1.080 ± 0.063
	Migneint Podzol	0.861 ± 0.047
	Peaknaze Peat	1.400 ± 0.106
	Peaknaze Podzol	1.012 ± 0.068
Eriophorum	Migneint Peat	0.771 ± 0.029
	Migneint Podzol	0.684 ± 0.046
	Peaknaze Peat	1.367 ± 0.162
	Peaknaze Podzol	0.800 ± 0.041

There was a clear and significant effect of soil type on the decomposition of *Eriophorum*. Mass loss was lower in peat compared to podzols and this was consistent for both sites (Migneint P = 0.001; Peaknaze P = <0.001) and throughout the 12 month experimental

period (*Figure 5.2*). Decomposition was also higher at Migneint than at Peaknaze, this site difference being significant between peat sites (P = <0.001) but not for podzol sites (P = 0.956).

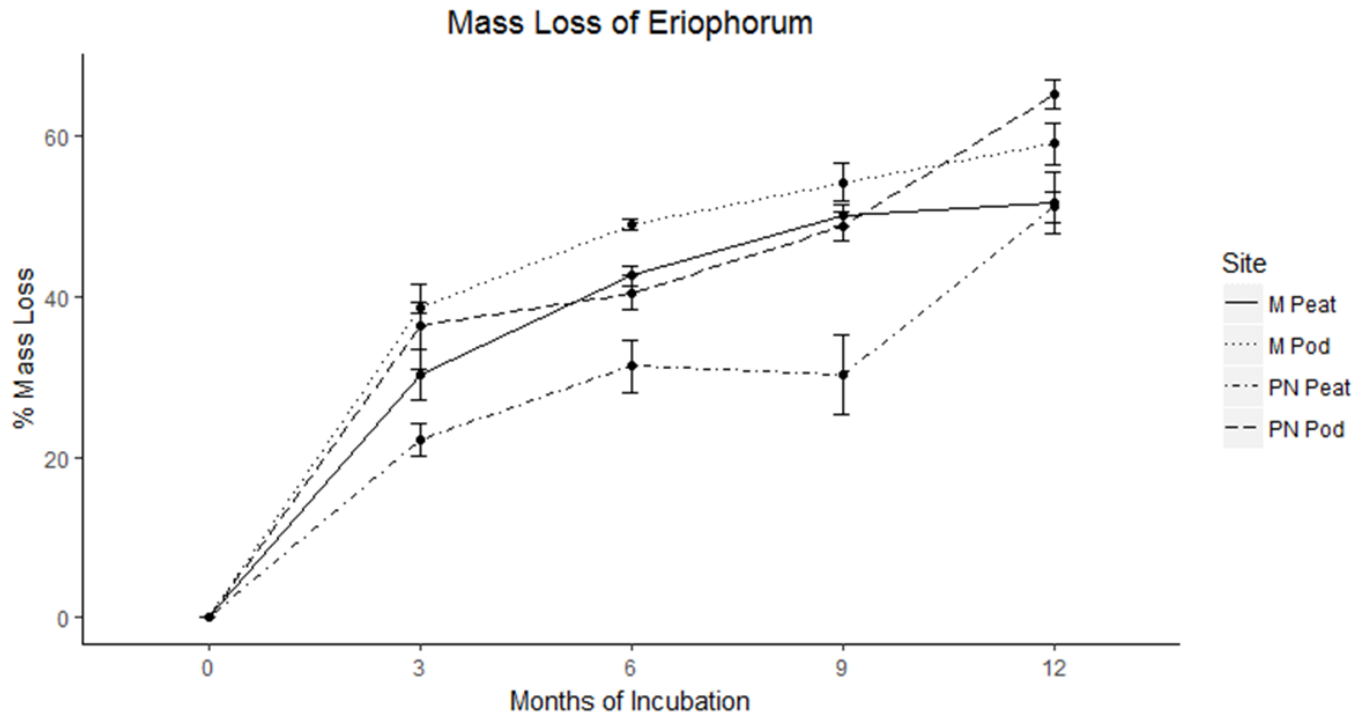


Figure 5.2: Percentage mass loss of *Eriophorum* from litter bags collected at quarterly intervals over a 12 month period. An ANOVA test was run on this data comparing sites (4 levels) and month (4 levels).

The decomposition of *Calluna* litter responded in a similar way to that of *Eriophorum* litter at different sites and soil types (*Figure 5.3*). Soil is significantly influential on mass loss for both sites (Migneint P = 0.004; Peaknaze P = <0.001) although there was less variation between treatment groups than with *Eriophorum*. Litter generally decomposed more when incubated in podzol soil than in peat at both sites. As with *Eriophorum*, decomposition was greater at the Migneint when comparing peat sites (P = <0.001), yet after 12 months of decomposition in podzol soils decomposition was greatest at Peaknaze (P = <0.001).

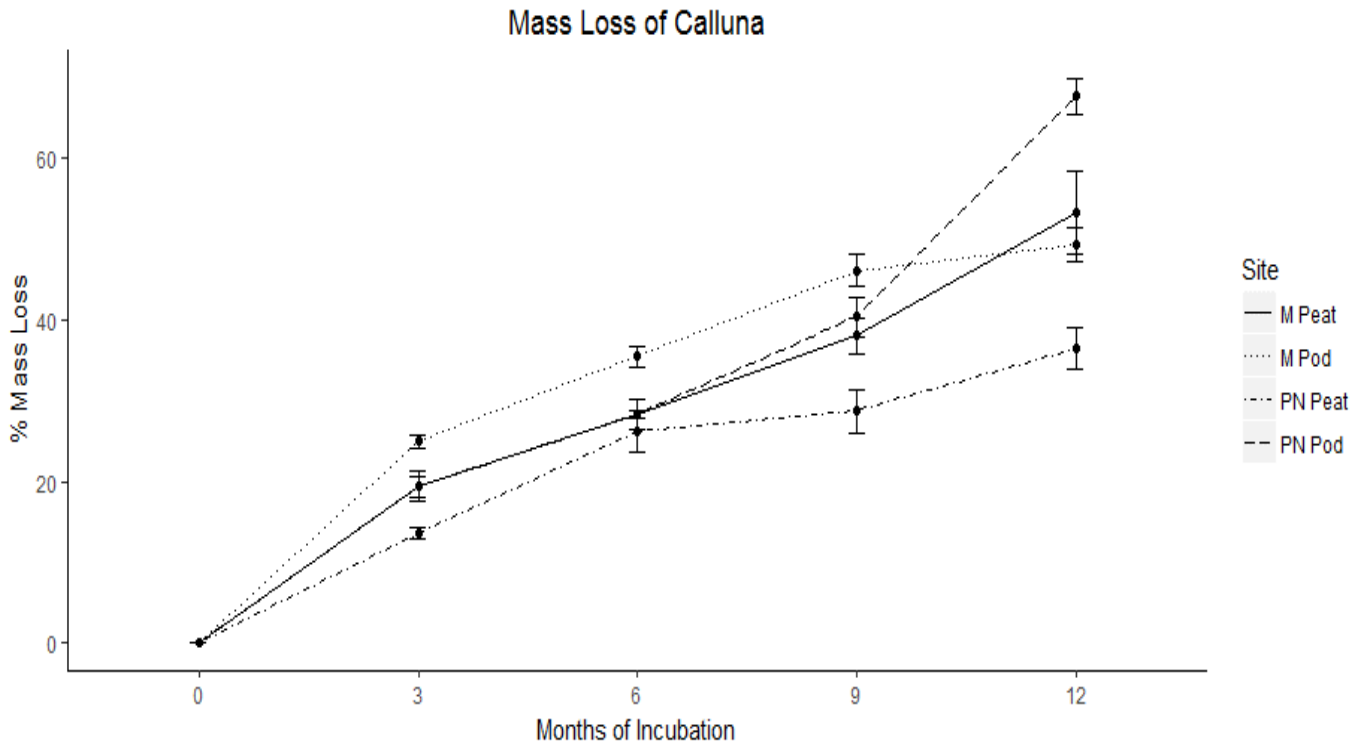


Figure 5.3: Percentage mass loss of *Calluna* from litter bags collected at quarterly intervals over a 12 month period. An ANOVA test was run on this data comparing sites (4 levels) and month (4 levels).

For both litter types, decomposition rates occurred in the following order at sites from 0-9 months of incubation: Migneint Podzol > Migneint Peat ≈ Peaknaze Podzol > Peaknaze Peat. By 12 months of incubation, for *Eriophorum* this changed to Peaknaze Podzol > Migneint Podzol > both peat sites, and for *Calluna* this changed to Peaknaze Podzol > Migneint sites > Peaknaze Peat. Therefore, by 12 months both litter types decomposed the most at Peaknaze Podzol due to an increase in decomposition rates at this site between July and October. During this time there was a change in mass loss of 16 % for *Eriophorum* and 27 % for *Calluna* at Peaknaze Podzol, compared to just 2-5 % at Migneint sites for *Eriophorum* and 3-15 % at all other sites for *Calluna*. Decomposition also increased rapidly between July and October at Peaknaze Peat for *Eriophorum*, with a change in mass loss of 21 %. Regardless, Peaknaze Peat had the lowest rate of decomposition across the experimental period for both litters, with a mass loss of 51 % for *Eriophorum* and 37 % for *Calluna* after 12 months of decomposition.

Soil type appears to be the dominating factor on decomposition, with consistently more decomposition in podzol soil than in peat. By 12 months, *Eriophorum* litter had a 7-14 % greater mass loss at podzol sites than at peat sites, and *Calluna* had a 31 % greater mass loss

in Peaknaze Podzol than in Peaknaze Peat, with an insignificant difference between Migneint sites. Also, for both litter types decomposition is greatest at the undamaged site (Migneint) compared to the degraded site (Peaknaze) for most of the experimental period.

5.3.2 Litter quality

For both litter types decomposition is greatest at the relatively undisturbed site (Migneint) compared to the degraded site (Peaknaze), suggesting a possible impact of the pollution deposition gradient between the two sites. The C and N content of litter were analysed in order to assess the role of litter quality as a factor affecting site variation. Significant differences in quality of litter between sites was assessed with a paired 2-sample t-test, or a Wilcoxon matched-pairs test when distributions were not normal (see *Table A5.1* in appendix).

There was no significant difference in the quality of *Eriophorum* between the sites (*Table 5.3*). For N and C:N ratio, this is possibly due to an error in the lab, as one replication had to be excluded from analysis due to a malfunction with the C:N Analyser during the analysis of N content, and so only two replications were available for N. However, for *Calluna*, there was significantly more N in litter harvested from Peaknaze than from Migneint (P = 0.027), as well as a lower C content (P = 0.046). This resulted in a significantly lower C:N ratio in litter harvested from Peaknaze (P = 0.011).

Table 5.3: Table illustrating the mean (± standard error) carbon and nitrogen content, and C:N ratio of *Calluna* and *Eriophorum* collected from each of the two sites. Due to an instrumental error during the analysis of *Eriophorum* N content for one replication, the mean of N is based on two replications only.

Litter	Site	N %	C %	C:N
Eriophorum	**Migneint**	2.71 ± 1.29	47.15 ± 0.14	22.56 ± 10.78
	Peaknaze	1.47 ± 0.01	47.45 ± 0.22	32.19 ± 0.38
Calluna	**Migneint**	0.82 ± 0.02	51.57 ± 0.50	63.26 ± 0.64
	Peaknaze	0.93 ± 0.02	50.15 ± 0.16	54.02 ± 1.31

5.3.3 Group 2: Translocated *Calluna*

Calluna which was harvested from Peaknaze (*Calluna* PN) was more N enriched than *Calluna* harvested from the Migneint (*Calluna* M). However, when comparing how native and translocated *Calluna* decomposed at each site, there was only a significant difference at Peaknaze Peat (P = 0.002) (*Figure 5.4*). Here, 34 % of *Calluna* M had decomposed after 12 months of incubation, whilst 27 % of *Calluna* PN had decomposed. Therefore at the most acidic site, the *Calluna* with highest N content which was native to that site decomposed the least.

When comparing how each litter decomposed across the different sites and between peat and organo-mineral soil, there was no significant difference for *Calluna* M between any of the four sites. There was also no significant difference in *Calluna* PN decomposition between sites (peat sites P = 0.068; podzol sites P = 0.496). However, *Calluna* PN decomposed significantly more in podzol soil than in peat at both locations (Migneint P = <0.001; Peaknaze P = <0.001). Therefore soil type is a dominating factor in litter decomposition when N content is greater and C:N ratio in lower.

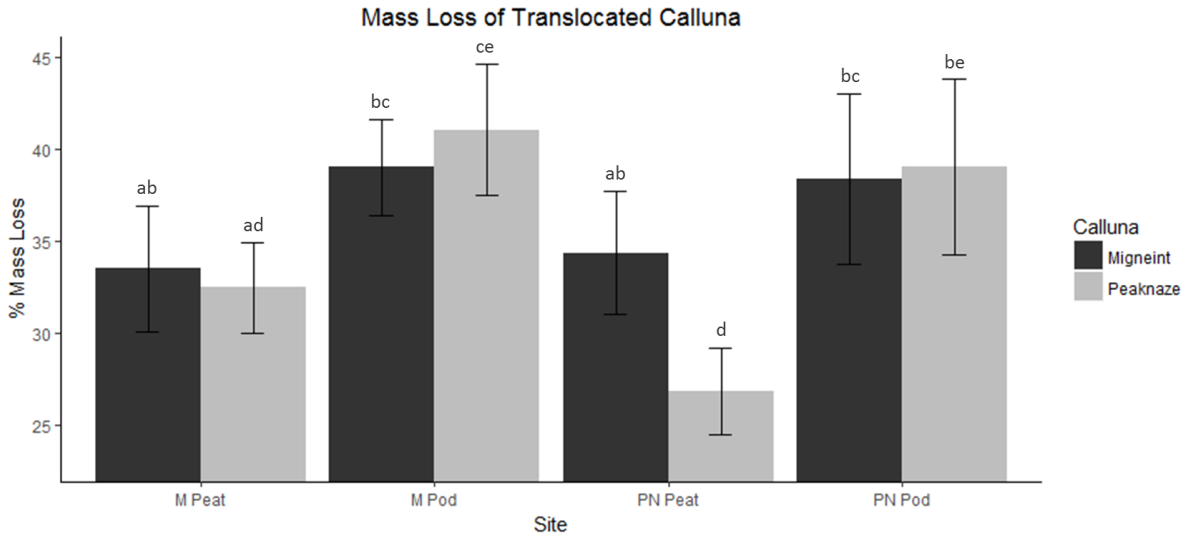


Figure 5.4: Percentage mass loss of *Calluna* from litter bags collected from different sites after a 12 month incubation period. An ANOVA test was run on this data comparing litter (2 levels), sites (4 levels) and month (4 levels). Letters signify where significant differences occur, obtained using a Posthoc analysis.

5.4 Discussion

5.4.1 How do litters decompose in peat and organo-mineral soil?

Litters decomposed more when incubated in podzol soil than in peat, with 5-9 % more mass loss for *Calluna* and 7-13 % for *Eriophorum* (mean difference for all months). This suggests that soil type is a dominating factor on litter decomposition in an organic catchment. The organic horizon of podzol has been shown to contain a large amount of DOC which has been attributed to microbial decomposition (Stutter et al., 2012), as well as more aromatic DOC (Stutter et al., 2012) (Chapter 4), which is also associated with microbial transformation (Hur et al., 2009). This suggests that the organic upper peaty layer of podzol may be an important source of DOC when flow shifts from the mineral to the organic horizon where DOC is mobile during times of high flow (Clark et al., 2007).

This difference in decomposition between peat and podzol is likely due to the moisture content differences. Undrained peats almost are permanently saturated which creates anoxic conditions and inhibits aerobic decomposition (Ramchunder et al., 2009) resulting in anaerobic activity which is a much slower process (Swift et al., 1979, Keller et al., 2009). In contrast, podzol soils are more freely draining allowing for more aerobic activity. Furthermore, NPP is greater at podzol areas compared to peat areas, and so there is a greater input of organic material from litter above, which arguably could result in a more biologically active and primed microbial community, resulting in faster decomposition (Xiao et al., 2014, Kuzyakov et al., 2000).

Much of the literature has focused on either decomposition in peat, or on podzols in forested environments. However, FTIR analysis indicates that less decomposition occurs in the upper organic layer of peat and peaty podzols in a moorland environments than in forested podzols, likely due to the drier conditions under the forest canopy (Chapman et al., 2001). Such evidence suggests that podzols behave differently in forested and moorland environments. Coulson and Butterfield (1978) compared decomposition in peat and mineral soils at Moor House (a moorland area in North Pennines, UK) and generally found little difference for most plants, and slightly more decomposition of young *Calluna* shoots in peat than in podzol, this being the opposite to the findings of this study. However, no other such studies have been found in the literature, which highlights the lack of studies on moorland organo-mineral soils and comparisons with peats.

5.4.2 Decomposition at different sites over a pollution deposition gradient

Between 0-9 months of incubation between October and July, litters decomposed the most at the less disturbed site (Migneint) for both peat and podzol soil, suggesting that decomposition is being suppressed at Peaknaze. This area has experienced high sulphur deposition which has seen a 69 % reduction between 1970 and 2005 (Dore et al., 2007, Evans et al., 2012), with a substantial store of sulphur still being present in the South Pennine peats (Daniels et al., 2008), and significant acidification of soils and waters (Evans et al., 2000). In addition, this areas has received previous and current high levels of N deposition and consequently has a high level of ecosystem N saturation (Helliwell et al., 2007, Curtis et al., 2005, Evans et al., 2000).

Acidification could alter mechanisms involved in microbial decomposition of organic material, particularly as soil pH is crucial to enzyme functioning (Fog, 1988), and is highly correlated with microbial community structure (Griffiths et al., 2011). Suppression of decomposition of *Calluna* and *Eriophorum* litter has also been observed in a peat monolith acidification experiment (Sanger et al., 1993), whilst no effects have been shown for *Sphagnum* in a poor fen environment (Rochefort et al., 1990). Acidity has been shown to suppress microbial activities. Rousk et al (2009) found microbial inhibition below a pH value of 4.5 units. The observed reduction in C mineralisation was attributed to the observed increase in fungal growth and decrease in bacterial grown with acidity. In a wooded podzolic soil environment, acid treatments have reduced microbial activity and the decomposition of bracken litter (Brown, 1985). In a spruce forested system, sulphur additions have been shown to reduce microbial biomass and soil respiration, as well as alter fungal:bacterial ratios and enzyme activities (Oulehle et al., 2018). However, soil respiration has been shown to be unaffected by acidity in other forested environments (Cronan, 1985, Oulehle et al., 2018). Such evidence suggests that acidification may be suppressing decomposition, and if so with recovery there will be an increase in decomposition and therefore release of C from such sites.

The high level of N deposition and saturation may also be affecting mechanisms involved in microbial decomposition at the Peaknaze site. Microbial community structure is thought to be disturbed with high N additions, and biodegradation of cellulose material is enhanced leading to an accumulation of more recalcitrant lignocellulose, all of which suppress

decomposition (Fog, 1988). Reduced phenol oxidase activity and therefore the decomposition of phenolic compounds has been demonstrated in moorland sites with high N deposition (Caporn et al., 2014). There is also an argument for the abiotic stabilisation of organic material with N inputs, such as phenolic compounds being polymerised by nitrogen bridges (Nommik and Vahtras, 1982) which are resistant to biodegradation (Janssens et al., 2010), or from the condensing of amino compounds with polyphenols and some decomposition products, leading up a build-up of inhibitory products which suppress decomposition (Fog, 1988).

In addition, the "microbial nitrogen mining" theory may explain lower decomposition rates under high N conditions. Under low N conditions, microbes maintain the functionality to mineralise carbon through shifts in enzyme synthesis and activity towards preferential decomposition of labile sources in order to acquire N from more recalcitrant sources. With increasing N supply, mineral N is more readily available and so decomposition is reduced (Craine et al., 2007, Janssens et al., 2010, Carreiro et al., 2000).

Such effects have been shown to be dependent on decomposition stage. In the early stage, decomposition of cellulose and soluble compounds in fresh litter is stimulated, particularly where N has accumulated in plant tissues. In later stages, such as in humus, decomposition is regulated by the lignin degradation rates, and additional N further suppresses decomposition (Berg and Matzner, 1997). This is supported by an inverse relationship between N concentration and microbial respiration in the late decomposition stage of humus (Berg and Matzner, 1997, Michel and Matzner, 2002).

As acidification also occurs in areas where N deposition is high, it is difficult to determine whether suppressed decomposition is a response to either or both forms of environmental change. However, in a nitrogen and sulphur addition experiment in a forested environment in the Czech Republic, sulphur additions were found to alter microbial functioning the most, suggesting that such responses previously attributed to nitrogen deposition could also be due to the simultaneous acidification effect (Oulehle et al., 2018). Furthermore, decomposition of litters in a sub-artic bog in response to N additions has been found to be species-specific, with no effect on the decomposition of *Eriophorum* (Aerts et al., 2006), suggesting that slow decomposition of *Eriophorum* at Peaknaze Peat may be due acidification from previous sulphur deposition.

Much of the previously published research focuses on forested environments, and so once again there is a need for research separating the effects of acidity and N deposition on litter decomposition in upland organic soils. In addition, as organic upland catchments often contain both peat and organo-mineral soil, and with very little research on peaty podzol soils, there is a need for further research into such effects on both soil types.

5.4.3 Decomposition stages and seasonal dynamics

Limiting environmental factors on decomposition of litter include soil moisture content, soil aeration and temperature (Marschner and Bredow, 2002) all of which change over the year and influence biotic elements of the ecosystem. The effect of time on decomposition is highly complex with abiotic and biotic variables and their interconnectivity changing over the year which ultimately influences how organic material is processed (Kalbitz et al., 2000, Solinger et al., 2001).

The decomposition for both litters was fastest between 0-3 months of incubation despite this occurring during late autumn/early winter when seasonal influences mean decomposition rates are at their lowest rate, with a mass loss of 22-39 % for *Eriophorum* and 19-25 % for *Calluna* across all sites. This is likely to be due to preferential decomposition of in the early decomposition stage. Freshly senesced litter contains compounds which are readily degradable and of a labile and soluble nature. Soil microorganisms are thought to preferentially decompose labile material, and much of this fraction of the freshly senesced litter at the surface is decomposed and released as CO_2, leaving the more recalcitrant aromatic material which is less labile, such as lignin (Kalbitz et al., 2003a, Saadi et al., 2006, McDowell et al., 2006, Don and Kalbitz, 2005, Nordén and Berg, 1990). Between 3-9 months of incubation, through winter and spring, decomposition of *Calluna* and *Eriophorum* slowed, reflecting the potential decrease in proportion of labile material from earlier decomposition, and a greater proportion of more recalcitrant material which is more difficult to decompose (Moore et al., 2007).

During 9-12 months of incubation (July to October) decomposition rates increased at Peaknaze by 8-27 % for *Calluna*, and 16-21 % for *Eriophorum*, and by 3-15 % and 2-5 % respectively at Migneint. During late summer conditions are typically warmer which may increase biological activity. Early autumn is also when peak litterfall occurs, including a

seasonal flux of *Calluna* flower capsules (Ritson et al., 2016, Cormack and Gimingham, 1964). This seasonal input of labile material, some of which may be in dissolved forms, combined with more favourable abiotic conditions of higher temperatures are likely to stimulate biological activity and decomposition (Mitchell and McDonald, 1992). However, the discrepancy in increases between sites during this period is unclear and reflects the differences in responses to seasonality. For instance, Migneint is much more saturated and so may be more able to buffer against increasing temperatures, whilst the drier more degraded site of Peaknaze may experience more severe evapotranspiration and so greater aeration of peat and podzol soil, increasing decomposition rates during this period.

The large decomposition rate experienced by both litters during late autumn/early winter, and the lower decomposition rate despite rising temperatures from late winter until summer, suggests that litter quality in terms of the proportion of labile and recalcitrant compounds associated with the decomposition stage is more influential than seasonal controls. However, from late summer into autumn decomposition increases at some sites suggesting that the interaction of climatic variables on the biology is boosting decomposition (such as high temperature, litterfall including dissolved forms which stimulate microbial activity, and reduced soil moisture content), despite the fact that litter is now in a much later decomposition stage with the lowest amount of labile material. Overall, this suggests that litter quality is the main control on decomposition, but this is dependent on the time of year, with a shift to other controls associated with seasonality being dominant during late summer and early autumn. However, this appears to be site dependent, with a particular increase at the degraded site of Peaknaze during this period.

5.4.4 Control of litter quality on decomposition

Calluna harvested from Peaknaze had a greater N content and lower C:N ratio, than at the Migneint. N deposition over the Peak District is currently exceptionally high and has been for decades, with evidence of N saturation in the soil (Helliwell et al., 2007, Curtis et al., 2005, Evans et al., 2000). N accumulation in plant tissues has been noted in the literature (Berg and Matzner, 1997, Van Vuuren and Van Der Eerden, 1992, Caporn et al., 2014). Pitcairn et al. (1995) found *Calluna* to have a linear increase in tissue N of 0.045 mg g^{-1} kg^{-1} ha^{-1} $year^{-1}$ of increased atmospheric N deposition. Such evidence suggests that *Calluna* at

Peaknaze has accumulated N in the plant tissue in response to high and long term deposition.

Despite having different quantities of N in plant tissue, there was no difference in how the two *Calluna* litters decomposed at three of the sites (Migneint Peat, Migneint Podzol and Peaknaze Podzol). This suggests that N content is not a controlling factor on decomposition. This was also seen with Van Vuuren and Van Der Eerden (1992) who enriched *Calluna* with ^{15}N and found *Calluna* took in the added N, and the litter retained and released N simultaneously, but the rate of decomposition of *Calluna* leaves was unaffected.

Migneint *Calluna* decomposed the same amount regardless of site or soil type. This does not support the 'home-field advantage' theory in this case, whereby plants promote communities of decomposers which create optimum decomposition of their litter, meaning litter will decompose more in their native soil (Austin et al., 2014, Ayres et al., 2009).

At Peaknaze Peat, *Calluna* which was more N enriched decomposed the least compared to *Calluna* from the Migneint. It is possible that the more favourable microbial community from the low pollution site may have been translocated with Migneint *Calluna*, but we would also expect to see a greater decomposition of Migneint *Calluna* at Peaknaze Podzol also if this were the case. Alternatively, as this is the most acidic site, it is possible that acidity is suppressing decomposition (Oulehle et al., 2018). However, we would expect the decomposition of both *Calluna* litters to be suppressed if this were the case. Alternatively, it may be possible that the excessive N at this site may be suppressing decomposition of N enriched litter in some way. This is discussed below in more detail.

Litter quality, particularly the composition of labile and aromatic compounds, affects how easily it can be broken down by the microbial community. A litter containing a high amount of aromatic material such as lignin may have a slower decomposition rate than a highly labile litter material (Moore et al., 2007). Alternatively, studies have found that C:N ratio is strongly correlated with decomposition (Szumigalski and Bayley, 1996) and DOC (Ritson et al., 2016, Soong et al., 2014, Aitkenhead and McDowell, 2000), although Moore et al (2007) found lignin content to be more influential. There is evidence in the literature that plants produce more lignin when under stress (Moura et al., 2010), and so it is possible that *Calluna* from this site also had a higher proportion of lignin in plant tissue, which inhibits

decomposition (Bragazza et al., 2007). If this is the case, then lignin may have accumulated in the peat, which may be another factor influencing suppressed decomposition at Peaknaze Peat. Furthermore, there is evidence that interactions between N and lignin content may create decay resistant compounds which actually slows decomposition (Fog, 1988, Berg and Matzner, 1997) and in fact causes stabilisation into humus (Prescott, 2010), which would explain why Peaknaze *Calluna* had suppressed decomposition at Peaknaze Peat only.

Nitrogen enrichment has been shown to slow the loss of lignin from decomposing litters, which further suppresses decomposition (Xia et al., 2017). Also, N has been found to reduce activity of lignin degrading extracellular enzymes of litters with a high lignin content (Waldrop and Zak, 2006, Sinsabaugh et al., 2002). Whether N or lignin controls decomposition is dependent on litter quality. Litters with a high lignin content are controlled by lignin:N ratio, but when lignin content is lower, C:N ratio is more influential on decomposition (Taylor Barry et al., 1989). For instance, litter decomposition in litter with a high lignin content can be suppressed with exogenous N availability through the N condensation with phenolic compounds (Waldrop Mark et al., 2004, Bridgham and Richardson, 2003).

This mechanism may be particularly important when considering peat (which naturally contains more phenolic compounds), and may explain why there is not a difference in decomposition of the *Calluna* litters at Peaknaze Podzol, but such a vast difference at Peaknaze Peat. Bragazza et al. (2007) also found the decomposition of *Calluna* litter to be negatively related to the high lignin content of its litter in a peat environment. Therefore it is possible that the differences in litter decomposition at Peaknaze Peat are due to the interaction between high nitrogen deposition and accumulation of N in litter, and the high lignin content associated with the woody parts of *Calluna*, which may have been increased as a plant response mechanism to stress. Such evidence suggests suppression of Peaknaze *Calluna* litter decomposition in a high deposition area, and preferential decomposition of Migneint *Calluna* with a lower N content where fewer decay resistant compounds are created from the interaction with high litter N and lignin.

There are many mechanisms in the literature assessing decomposition in response to nitrogen deposition, many of which have contradictory results (Knorr et al., 2005). Despite the importance of decomposition in organic catchments for carbon storage and release, and

with N deposition remaining high, there are a lack of studies looking at interactions between N deposition, plant N accumulation and lignin accumulation on decomposition of litter in both peat and podzol soil in an organic environment. Therefore more work is needed to fill this gap in the literature.

Overall, both *Calluna* and *Eriophorum* litters decompose more in podzol soils than in peat, which suggests that soil type is a major control on decomposition, and that there is spatial heterogeneity in litter decomposition within a moorland catchment. There is variation in how litters decompose over the course of 12 months which is associated with different controls dominating. Litter decomposition rates are initially high, reflecting the preferential biodegradation of labile material, and then slows as bioavailable material is depleted. During this period (0-9 months), decomposition was significantly suppressed at the Peaknaze site, reflecting the influence of chronic acidification and N saturation at this site. However, between late summer and early autumn decomposition increased at Peaknaze, suggesting there is potentially a switch from the controls associated with decomposition stage progression and pollution deposition, to seasonality such as increased temperatures and the priming effect of increased litter fall. Finally, N content and C:N ratio were found not to influence decomposition of *Calluna* litter except at the most polluted peat site. At this site, the litter with the higher N content decomposed the least, possibly due to the interacting effects of high N content and exogenous available N, and high lignin content associated with the woody parts of *Calluna* litter and accumulation as a plant response mechanism to stress.

5.5 Conclusion

This research provides insights into the spatial and temporal variations in the decomposition of two key upland organic catchment litters; *Calluna* and *Eriophorum*, over a 12 month period. Results show that, as expected, decomposition is faster in podzols than in peat, and is suppressed in areas of chronic N deposition and acidification. Furthermore, litter quality in terms of N content and C:N ratio does not influence the decomposition of *Calluna* at most sites. However, the *Calluna* which had significantly more N accumulated in tissue decomposed significantly less at the most polluted peat site. More work is needed assessing the potential interacting effects of high N deposition and accumulation in plant tissue and

peat, and lignin content and accumulation in plant tissue, on the decomposition of *Calluna* in organic soils.

Appendix

Table A5.1: P values results from comparison of N, C and C:N ratio content of *Eriophorum* and *Calluna* litter between different sites, using either [1]Paired t-test (when data was normal), or [2] Wilcoxon matched-pairs test (when data was not normal).

Litter	N	C	C:N
Eriophorum	1.000[2]	0.353[1]	1.000[2]
Calluna	**0.027**[1]	**0.046**[1]	**0.011**[1]

Table A5.2: P values results from Anova comparing *Calluna* litter (2 levels), sites (4 levels) and month (4 levels).

Factors	P Value
Litter	**0.043**
Site	**<0.001**
Month	**<0.001**
Litter:Site	**<0.001**
Litter:Month	0.659
Site:Month	**<0.001**
Litter:Site:Month	**0.032**

Table A5.3: P value results from Posthoc performed on Anova test comparing *Calluna* litter (2 levels), sites (4 levels) and month (4 levels) (*Table A5.2*).

		Calluna M				**Calluna PN**		
		M Peat	**M Pod**	**PN Peat**	**PN Pod**	**M Peat**	**M Pod**	**PN Peat**
Calluna M	**M Pod**	0.235						
	PN Peat	1.000	0.315					
	PN Pod	0.100	1.000	0.142				
Calluna PN	**M Peat**	0.962	**0.013**	0.923	**0.004**			
	M Pod	**0.001**	0.404	**0.001**	0.751	**<0.001**		
	PN Peat	**0.003**	**<0.001**	**0.002**	**<0.001**	0.068	**<0.001**	
	PN Pod	0.175	1.000	0.241	1.000	**0.008**	0.496	**<0.001**

Chapter 6: Acid deposition impacts on litter decomposition and DOC production within peat and organo-mineral soils.

Abstract

There has been an observed increase in dissolved organic carbon (DOC) concentrations in soil solutions and surface water bodies over the past 30 years in acid sensitive areas of the Northern Hemisphere, which has been linked to recovery from acidification of soils in response to decreasing levels of atmospheric pollution. However, it is unclear to what extent increased DOC concentrations could have been driven by increased decomposition and therefore DOC production, as recovery from acidification changes soil pH to more favourable conditions for biological activity. A decomposition study using litter bags and the Tea Bag Index (TBI) was incorporated into an acidity manipulation field experiment across two sites representing a pollution deposition gradient, for both peat and organo-mineral soils, to investigate how acidity impacts the decomposition of five litter types common to upland moorland catchments, and the resulting effect on the DOC produced.

Only *Eriophorum* litter decomposition responded to acidity treatments, which may be related to the higher nitrogen content within plant tissue compared to other litters. Acidity had no effect on the TBI. This suggests that controls on litter decomposition could be limited by nitrogen availability, such that acidity effects were only seen where litter had sufficient nitrogen availability. Soil type was found to be highly influential on litter decomposition and DOC production. Decomposition rates were greater on podzol soil than peat. Greater decomposition on podzols was associated with more DOC release (with *Eriophorum*) and a higher $SUVA_{254}$ value indicating more aromatic DOC (with *Eriophorum* and *Calluna*) being extracted, compared to the same litter decomposing on peat. This suggests that litter has the potential to decompose faster in podzol soil than in peat, and after one year of decomposition this results in more DOC being produced which is of a more aromatic nature due to the later decomposition stage reached compared to peat. Such research provides insights into the spatial variable and limiting factors on litter decomposition and DOC production in an upland organic catchment encompassing both organo-mineral soil and peat. Further research is needed to unpick the interactions between nitrogen limitation and acidity on decomposition processes.

Chapter 6

6.1 Introduction

6.1.1 Why is litter decomposition important for DOC dynamics?

Upland soils hold a significant fraction of the UK's soil carbon pool (Billett et al., 2010), consisting primarily of peats, but also organo-mineral soils including gleys, podzols and leptosols (Howard et al., 1995). DOC fluxes represent a major natural outsourcing of carbon from moorland catchments dominated by organic soils. Dinsmore et al. (2010) monitored a peatland catchment in southern Scotland over two years, and found the amount of carbon lost through DOC export (25 g DOC m^{-2} yr^{-1}) equated to nearly a quarter of the carbon taken in through net ecosystem exchange. Alternatively, some catchments can act as either carbon sources or are carbon neutral when loss of total organic carbon in waters is equal to or greater than net carbon uptake on land (Billett et al., 2004). Blodau et al. (2007) used an in-situ incubation experiment to further estimate production, storage and export of carbon, and found turnover to be dominated by pore water DOC, which was produced and consumed at greater rates than CH_4 or CO_2 in an ombrotrophic temperate peatland. Studies have shown differences in DOC quality between peat and organo-mineral soils, with organo-mineral soils containing more aromatic DOC compared to peat (Chapman et al., 2010). Differences in quality can be attributed to changes in desorption in the mineral horizons with changes in acid sulphur deposition (Chapman et al., 2010), particularly as flow shifts from the mineral to the organic horizon during times of high flow (Clark et al., 2007). Such studies highlight the importance of DOC fluxes for carbon storage in both peat and organo-mineral soil, and the different processes that operate in organo-mineral and peat soils within an upland moorland catchments dominated by organic soils.

DOC originates from different sources, including ancient groundwater, mineral soils and newly formed DOM with an average age of 5 years within the peat surface (Tipping et al., 2010). The newly formed DOC derives from peat in the surface layer, and litter. This newly formed DOC in the surface peat layer is transported to streams through a combination of weak sorptive retention being exerted by the peat solids, and with precipitation largely passing through the upper surface (Clark et al., 2008, Holden and Burt, 2003b). This is supported by correlations between DOC in surface layers of peat and DOC in stream water (Clark et al., 2008, Billett et al., 2006). Further studies investigating radiocarbon (^{14}C) levels of DOC suggests that fresh DOC may derive more from litter than the surface peat (Evans et

al., 2014, Evans et al., 2007). Therefore litter decomposition is a major source of DOC and plays a vital role in C dynamics, stabilisation and transport in organic soils (Don and Kalbitz, 2005, Kalbitz et al., 2000). Litter decomposition is commonly measured using litter bags, whereby the mass loss of litter can be measured and compared between litter types under different experimental conditions (Graça et al., 2005). Alternatively, the Tea Bag Index (TBI) is a standardised method of measuring decomposition of established litter types which is comparable to other studies and experiments (Keuskamp et al., 2013).

There has been an observed increase in DOC concentrations in surface waters draining catchments dominated by organic soils since the 1980's, across large areas of the Northern Hemisphere (Evans et al., 2005, Monteith et al., 2007, Oulehle and Hruška, 2009, SanClements et al., 2012b, Couture et al., 2012). Such significant increases in carbon fluxes have raised concerns over the future of terrestrial carbon stocks (Freeman et al., 2001a) as well as contributing to accelerated climate change (Moody et al., 2013). This highlights the need for a greater understanding of the mechanisms behind carbon dynamics in upland catchments, and in particular their response to environmental change (Tipping et al., 2007).

The UK Environmental Change Network highlights recovery from acidification as one of the three most significant long-term trends in the physical environment within the UK (Morecroft et al., 2009). There is now much evidence that this increasing trend in DOC concentrations is due to recovery from acidification as a result of air pollution controls and reduced deposition of acidifying pollutants. A physio-chemical mechanism controlling DOC movement with high acidity is supported by field (Evans et al., 2012, Oulehle et al., 2013, Evans et al., 2008a, Moldan et al., 2012, Ekström et al., 2011) and laboratory experiments (Clark et al., 2011, Palmer et al., 2013) as well as modelling (Monteith et al., 2007, Rowe et al., 2014, Evans et al., 2008b, Sawicka et al., 2017) and field observations (Oulehle et al., 2017, Oulehle and Hruška, 2009, Evans et al., 2006a, Oulehle et al., 2011).

6.1.2 What are the known effects of acidity on DOC production through litter decomposition?

To date, most attention has been given to understanding the chemical mobility of DOC within soils and surface waters. The direct effects of acidity itself on DOC production from litter decomposition are poorly understood, with many studies focusing on nitrogen deposition effects (Manning et al., 2008, Berg and Matzner, 1997, Knorr et al., 2005, Bragazza et al., 2012, Lovett and Goodale, 2011) rather than acidification (Evans et al., 2008a). Nitrogen fertilization may also be changing the soil acid-base status (Evans et al., 2008a, Chen et al., 2015), and yet there has been little attempt to separate out the individual responses to acidification and nitrogen deposition. Oulehle et al. (2018) concluded that carbon accumulation which has previously been attributed to nitrogen deposition may also be a response to the simultaneous acidification, based on results of a nitrogen and sulphur addition field experiment in a forest stand. Recent modelling studies have indicated that nitrogen deposition may have contributed to increased DOC production in nitrogen limited systems by stimulating primary productivity and the production of litter (Sawicka et al., 2017).

Changes in plant community structure in response to acidification may indirectly influence DOC release from organic catchments. A combination of acidification, nitrogen deposition and drainage has already resulted in widespread loss of bryophyte species in UK moorlands, such as the severe *Sphagnum* loss seen in the Peak District which has been largely attributed to high levels of sulphur dioxide and associated acid deposition (Carroll et al., 2009). Loss of *Sphagnum* species alongside increasing acidification has been recorded in other UK bogs (Hogg et al., 1995, Lee, 1998). Lichens have also experienced a widespread loss in species richness in many habitats where acid deposition has been high (NEGTAP, 2001). Acidification has also been attributed to a loss of other species in UK bogs, including a decline in birch in forested areas (Hogg et al., 1995). In addition, Molina has expanded in areas of high acidification (Hogg et al., 1995).

Moorland vegetation varies in quality, decomposability and production of DOC. *Sphagnum* in particular has a high phenolic content (Rasmussen et al., 1995). Phenolic compounds are from plant metabolites including phenolic acids, lignin, tannins and so on, which have more than one aromatic ring with at least one hydroxyl functional group, which suppress

decomposition (Verhoeven and Toth, 1995, Fenner et al., 2004). *Sphagnum* mosses are consistently shown to have the slowest decomposition rates, produce the least amount of DOC which is most resistant to biodegradation compared to other vegetation types (Pinsonneault et al., 2016, Armstrong et al., 2012, Moore et al., 2007, Ritson et al., 2016, Ritson et al., 2014). Vascular plants, which release more labile DOC (Del Giudice and Lindo, 2017), have been reported to outcompete *Sphagnum* in areas with increased N deposition (Berendse et al., 2001). In general, vascular plants are found to decompose faster, produce more DOC, and more labile DOC than mosses in peatland ecosystems, such as *Calluna* (Armstrong et al., 2012, Ritson et al., 2016). Therefore a peatland ecosystem dominated by *Sphagnum* will likely produce less DOC of an aromatic nature, whilst a higher proportion of vascular plants will produce more DOC of a more labile nature leading to a reduced soil C pool and greater loss of C (Ritson et al., 2016).

There is little research on decomposition of vegetation in organo-mineral soils in moorland environments. In peaty podzol soil, the typical vegetation consists of grasses and dwarf shrubs with few bryophytes, and so a dominance of vascular plants may result more labile and therefore less stable DOC being produced compared to bryophyte dominated peat. Also, there are more suitable aerobic conditions for microbial activity, which may result in faster and more complete decomposition (Kalbitz and Kaiser, 2008).

It is unclear to what extent increased DOC concentrations could have been driven by increased decomposition and, therefore, DOC production, as recovery from acidification changes soil pH to more favourable conditions for biological activity (Andersson and Nilsson, 2001a). Therefore, a decomposition study using litter bags was incorporated into an acidity manipulation field experiment to investigate how acidity impacts the decomposition of litters common to upland organic catchments, and the resulting effect on the DOC produced. As well as in situ treatment applications being applied, two locations with contrasting historical pollution deposition levels and therefore baseline pH were used to investigate the effect of a 'natural' acidity gradient. Two organic soil types typical to UK moorland ecosystems were also used, building upon the experimental framework presented by Evans, et al., (2012). As such, a study of this kind that focuses solely on the effects of acidification on litter decomposition, with an attempt to dissect biological production from

the solubility effects of DOC in an upland organic catchment in the UK, has not been published before to our knowledge.

6.1.3 Objectives of this study

The specific objectives of this work were:

- To investigate the effect of acidity on the decomposition of different moorland litters, and the quantity and quality of DOC produced in peat and organo-mineral soils and across two sites representing a 'natural' acidity gradient.
- To assess acidity effects on decomposition processes generally using standard litters from the Tea Bag Index across different soils at different sites.

6.2 Methods

6.2.1 Site description and experimental design

This work built upon an existing long-term acidity manipulation field experiment set up in 2007, comprising four replicated plot-scale experiments situated across two peatland locations with contrasting historic rates of acid deposition, and therefore present-day soil acidity (Evans et al., 2012). At each site, replicated acidity manipulations were established within two soil types; blanket peat and peaty podzol, which are among the commonest soils present in the UK uplands, and which also occur extensively in other cool, humid temperate regions.

The first study site, the Migneint (3°48.8' W, 52°59.6' N, 460 m a.s.l.), is a relatively undisturbed peatland area with historically low levels of pollution in North Wales. Peaknaze (1°54.5' W, 53°28.3' N, 440 m a.s.l.), Northern England, is a more disturbed region affected by relatively intensive land management and historically high levels of atmospheric pollution, which has led to degradation of the ecosystem including *Sphagnum* loss and erosion. More details for both sites can be found in Chapter 3 and (Evans et al., 2012).

6.2.2 Field experimental operation

The experimental sites were established in August 2007 and consist of twelve 9 m^2 plots at each of the four sites, with a randomised blocked design compromising four replicates of control, acid and alkaline treatments at each location. Treatments were applied initially from October 2008 until December 2012 (Evans et al., 2012), and then re-established (using the same methods, treatments and plot allocations) from January 2015 until October 2016.

Acid plots received a monthly dose of sulphuric acid (H_2SO_4) mixed with rainwater collected at the site with 20 L of rainwater using a watering can. The concentration applied was 50 kg S ha^{-1} yr^{-1} at the podzol sites and 100 kg S ha^{-1} yr^{-1} at the peat sites, this concentration being similar to the ambient sulphur deposition in the Peak District in the 1970's (a higher dose was applied to peat plots to take account of the buffering effects of sulphur reduction (Evans et al., 2012)). A 10 L rinse of rainwater followed to ensure the treatment infiltrated into the soil and to minimise any direct toxicity effects on plant foliage.

The same procedure was followed for the alkaline plots with sodium hydroxide (NaOH) and potassium hydroxide (KOH), followed by a rinse containing magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) to maintain base cation ratios similar to those observed in rainfall. The molar OH^- concentration in the alkaline treatments was intended to be comparable to the H^+ concentration in the acid treatments. Control plots received 20 L of rainwater only.

6.2.3 Sampling and analysis

6.2.3.1 Litter bag experiment

The litter bag technique was used to measure the decomposition of litter in the field (Beyaert and Fox, 2007). Litter samples were harvested at the end of the growing season during autumn when DOC production from litter was at its greatest and included *Calluna vulgaris, Eriophorum vaginatum, Festuca ovina, Pleurozium schreberi* and *Sphagnum spp.*. These were categorised into two groups (*Table 6.1*). *Calluna* and *Eriophorum* litters are common in upland organic catchments and are categorised as Group 1. Group 2 captures dominant vegetation species specific to each of the four sites. For instance *Festuca* is common at the podzols sites whilst mosses characterise peat sites, with a distinction between the *Sphagnum* dominated Migneint site and the *Pleurozium* dominated degraded Peaknaze site. This captured the dominant vegetation community specific to each site (for more details see Chapter 3). The quality of litter in terms of percentage carbon and nitrogen content, and C:N ratio for each litter harvested from each site is presented below in *Table 6.2*.

Table 6.1: Summary of litters buried at each site. All were harvested from the site where they were buried.

Site	Group 1	Group 2
Migneint Peat	*Eriophorum, Calluna*	*Sphagnum*
Migneint Podzol	*Eriophorum, Calluna*	*Festuca*
Peaknaze Peat	*Eriophorum, Calluna*	*Pleurozium*
Peaknaze Podzol	*Eriophorum, Calluna*	*Festuca*

Table 6.2: Mean (± standard error) carbon and nitrogen content, and C:N ratio of litters collected from the Migneint and Peaknaze. Due to a problem during the analysis of nitrogen content of *Eriophorum* from Migneint, results from one replication have been excluded and so the mean of this sample is based on two replications.

Litter	Site	N %	C %	C:N
Eriophorum	**Migneint**	2.71 ± 1.29	47.15 ± 0.14	22.56 ± 10.78
	Peaknaze	1.47 ± 0.01	47.45 ± 0.22	32.19 ± 0.38
Calluna	**Migneint**	0.82 ± 0.02	51.57 ± 0.50	63.26 ± 0.64
	Peaknaze	0.93 ± 0.02	50.15 ± 0.16	54.02 ± 1.31
Festuca	**Migneint**	0.42 ± 0.01	46.29 ± 1.18	109.53 ± 1.79
	Peaknaze	0.49 ± 0.01	46.99 ± 0.63	96.53 ± 1.79
Sphagnum	**Migneint**	0.77 ± 0.02	42.89 ± 0.82	55.76 ± 0.23
Pleurozium	**Peaknaze**	1.15 ± 0.05	43.65 ± 0.20	37.99 ± 1.66

Vascular plants were collected as standing biomass, whilst whole blocks of *Sphagnum* were collected. Samples were then sorted to remove other material and processed by cutting into 2 cm long pieces and homogenised. It is not possible to establish the starting point of decomposition for Sphagnum as this is a continuous process, so following protocols adopted by other studies (Aerts et al., 2001, Moore et al., 2007, Bragazza et al., 2007) the capitulum (2-4 cm below the tip) of each individual was removed, as this is likely to be living and photosynthesising. Therefore only freshly senesced litter was used.

A commonly used pre-treatment procedure was applied to the samples (Moore et al., 2007, Bragazza et al., 2007). This involved air drying the samples to a constant mass at ambient

temperature. The dry mass equivalent of 3 g of *Calluna, Eriophorum* and *Festuca,* and 1 g of *Sphagnum* and *Pleurozium* (a lower amount was used due to the low density of these species) were added to the litter bags, all of which were buried at a depth of 5 cm in October 2015. Pre-sown 10 x 10 cm litter bags made of polyamide monofil were used (Filtrations Technik, Germany). Subsamples of litter were ball milled and analysed for carbon and nitrogen content using a Thermo Flash 2000 Carbon Nitrogen analyser (Thermo Fisher Scientific, Massachusetts, USA).

Mesh size has been found to significantly influence what community of decomposers are exposed to the litter based on faunal size classes (Bradford et al., 2002). A mesh size as small as 74 µm has been used for sphagnum studies, and whilst this prevents the loss of litter pieces, it excludes many meso and macro-faunal decomposers (Limpens and Berendse, 2003). Alternatively much larger mesh sizes have been used such as 1-2 mm to encourage macrofaunal decomposition (Moore et al., 2007, Latter et al., 1997), but there is a risk that litter may be lost, particularly *Sphagnum* which becomes very crumbly when dry. Therefore a mesh size of 0.5 mm was chosen to allow decomposers into the bag, whilst minimising loss of fine litter particles (Bragazza et al., 2007).

Bags were collected after 12 months of incubation in October 2016. Once retrieved, litters were processed by removing all ingrown material and soil invertebrates, and litter was weighed. A cold water extraction was then performed with a 1:20 litter: water ratio using ultrapure water with a horizontal shaker for 24 hours, followed by centrifuging (15 minutes at 3500 rpm) and filtering through 0.45 µm cellulose membrane filter paper. The litter was then reweighed to establish a post-extraction weight and oven dried at 70 °C for 48 hours and weighed again for an oven dry mass.

The Tea Bag Index (TBI) is a standardised method to establish a decomposition rate (*k)* and stabilisation factor (*S*) of soil which is comparable to other studies. Two types of tea were buried (Green and Rooibos, both Lipton Tea Bags), which have contrasting decomposition rates. Green tea is more labile whilst Rooibos tea is more recalcitrant, meaning once the labile fraction of Green tea has been consumed by decomposers, the decomposition of the labile fraction of the more recalcitrant Rooibos tea still continues. Therefore, by comparing the mass loss, it is possible to estimate the decomposable fraction of Green tea and decomposition rate constant of Rooibos tea. From this the stabilisation factor of soil can be

calculated, which is the inhibiting effect of environmental conditions on the decomposition of the labile fraction. Three sets of bags were incubated in each plot for 90 days and received three treatment applications. The bags were then retrieved and the mass loss was established, allowing the decomposition rate (*k*) and stabilisation factor (*S*) to be calculated following the method outlined by Keuskamp et al. (2013).

6.2.4 Laboratory analysis

Litter extracts were analysed for pH, electrical conductivity, DOC, and absorbance. The extracts indicate the quantity and quality of DOC produced at the stage of decomposition reached after twelve months, whilst the mass loss of litters were used to indicate decomposition rate.

The Thermalox TC-TN analyser (Analystical Sciences, Ltd., UK) was used to measure the concentration of dissolved organic carbon (DOC) (by subtracting the amount of total inorganic carbon (TIC) from the amount of total carbon (TC)). Optical measures were used to define spectroscopic properties of filtrate DOC. Samples were diluted to less than 1 au, as determined by measuring absorbance at 240 nm. Ultraviolet (UV) visible absorbance spectra were determined using UV transparent 96 well plates on a Spectromax M2e Microplate Reader (Molecular Devices, San Jose, CA) set to scan at wavelengths between 240 and 600 nm with a 1 nm increment. As absorbance data obtained by the microplate method is slightly lower than the cuvette method (due to the difference in absorbance between plastic and quartz), data was multiplied by correction factors (Tim Jones, pers comm). The specific ultraviolet absorbance (SUVA) value has been identified as being a useful proxy for measuring the aromatic fraction (Weishaar et al., 2003) and molecular weight (Chowdhury, 2013) of DOC, as it is strongly linked to the hydrophobic organic acid fraction of DOM (Spencer et al., 2012). Therefore the $SUVA_{254}$ value was used as a measure of aromaticity and calculated by dividing the absorbance value at 254 nm by the DOC concentration (mg l^{-1}) (Weishaar et al., 2003).

6.2.5 Data analysis

Data were statistically analysed using R statistical package (RDevelopment CORE TEAM, 2008). Data were assessed as to whether it met the assumptions of Analysis of Variance (ANOVA), including normality and equal variance, and transformations were applied where necessary. When significance was apparent, a post hoc test was run using the 'Tukey HSD' function in R to confirm where significant differences occurred between groups.

Different Anova tests were run on the data for different litters based on the number of factors. For instance, *Eriophorum*, *Calluna* and Teabags were buried at all plots, and so a two-way Anova was used to compare how decomposition and extract chemistry varied over different sites, soils (combined to create four levels) and treatments (three levels). However, *Pleurozium* and *Sphagnum* were buried in peat at one site only, and so it was only possible to investigate how these litters responded to treatments (three levels) (one-way Anova). *Festuca* were incubated in podzol soil only, but at both sites and so a two-way Anova was used to compare treatments (three levels) and sites (two levels). Significant differences in the percentage carbon and nitrogen content, and C:N ratio between litter were assessed using a Two-Way Anova comparing sites (two levels) and litter (five levels).

6.3 Results

6.3.1 How effective were treatments at changing sample pH?

The pH of most of the litter extracts were more acidic when litters received acid treatments, and had a higher pH when they received the alkaline treatment (*Figure 6.1*), ranging from pH 4.93-5.10 for acid to 5.59-6.11 units for alkaline and 5.06-5.34 units for control (based on mean of plots for each litter type, excluding *Sphagnum*). The exception was *Sphagnum*, where the pH of extracts were similar regardless of treatments at ~5.60 units ($P = 0.755$). The extractant pH of litters from alkaline plots differed significantly from acid and control treatments for all litters (*Table 6.3*). However only *Calluna* extracts had a significantly lower pH for acid treatments when compared to the control ($P = 0.032$). Overall, this shows the persistence of the pH effect at the depth the bags were buried (~5 cm), as with soil extracts and pore water samples taken at a depth of 5-10 cm (Chapter 4).

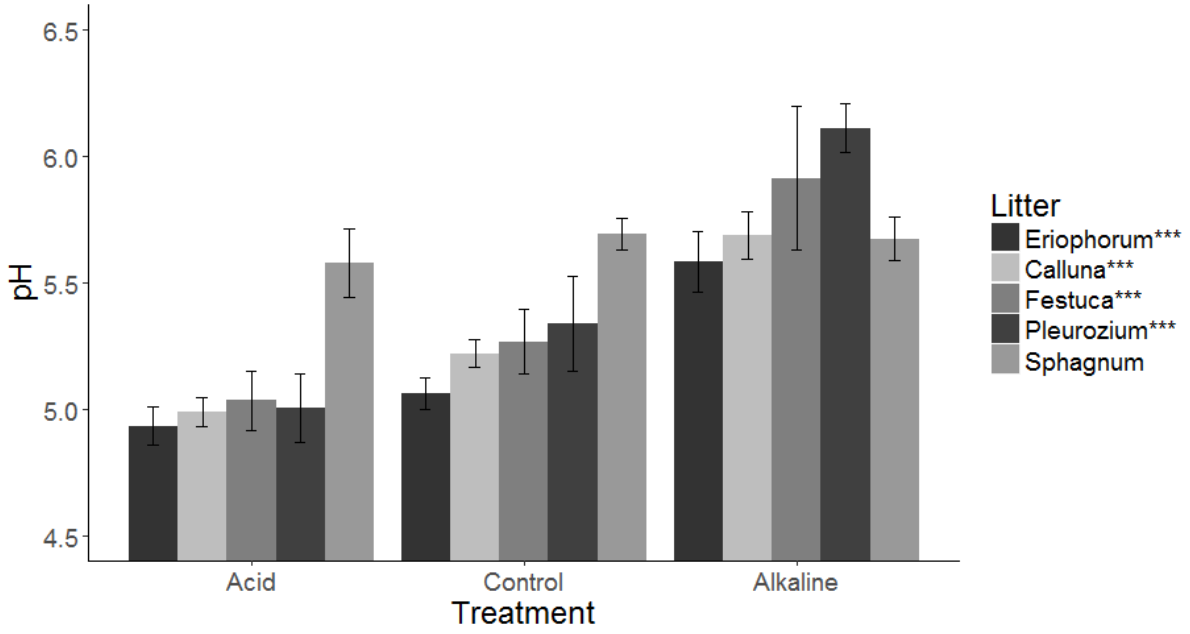


Figure 6.1: Mean pH (pooled for all sites) of extracts from different litters which received acid, alkaline and control treatments. Anova results of significance are represented in the legend (***P = <0.001, **P = 0.001 – 0.010, *P = 0.010 – 0.050). For full Anova results, see Table A6.1 in Appendix. Post hoc results are below in Table 6.3.

Table 6.3: P value results of Post hoc test, comparing treatments for the pH of litter extracts. For full Anova results, see Table A6.1 in Appendix. Data transformations to meet assumptions of Anova are stated in table.

	Eriophorum Boxcox	***Calluna*** log	***Festuca*** log	***Pleurozium*** ^4
Con & Acid	0.192	**0.032**	0.314	0.348
Con & Alk	**<0.001**	**<0.001**	**0.011**	**0.005**
Acid & Alk	**<0.001**	**<0.001**	**<0.001**	**<0.001**

6.3.2 Does acidity influence the decomposition of different litters, and ultimately to quantity and quality of DOC produced?

6.3.2.1 Group 1: Common Litters

The mass loss of *Eriophorum* litter was found to be significantly affected by treatments (P = 0.035) (*Figure 6.2*). Litters decomposed more when incubated in alkaline plots compared to acid plots (P = 0.029). However, neither treatments were significantly different to the control (acid P = 0.355; alkaline P = 0.406). Decomposition of *Calluna* was similar across all treatments (P = 0.877). There was also no significant interaction between treatments and

sites (*Eriophorum* P = 0.379; *Calluna* P = 0.148), suggesting that the effect of treatment did not depend on site or soil type.

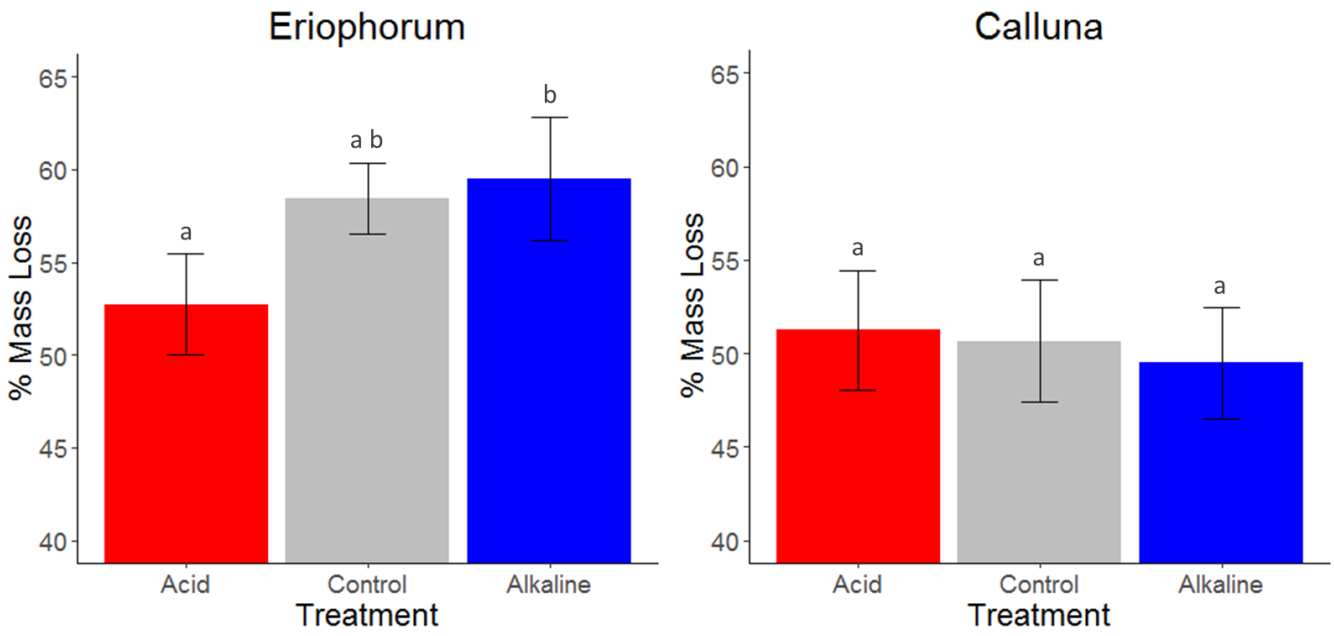


Figure 6.2: Mean percentage mass loss (pooled for all sites) of *Eriophorum* and *Calluna* litter after 12 months of incubation with different treatments. Error bars represent standard error of the mean, whilst letters indicate where significant differences occur based on results of a Posthoc test on an Anova comparing sites (four levels) and treatments (three levels).

Litters decomposed more in podzol soil than in peat, with 62 % (mean of control plots at both sites) mass loss of *Eriophorum* (P = 0.024 for Migneint and P = 0.006 for Peaknaze) and 59 % mass loss of *Calluna* (P = <0.001) when incubated in podzol soil after 12 months of decomposition, compared to 51 % and 43 % mass loss respectively when incubated in peat (*Table 6.4*). However, there was no significant difference in decomposition for *Calluna* between soil types at Migneint (P = 0.557) with a mass loss of ~50 %. The only site difference in mass loss of litter was for *Calluna*, which decomposed the most at Peaknaze Podzol when compared to Migneint Podzol (P = <0.001), whilst there was no difference in how *Eriophorum* decomposed between sites.

Significantly more DOC was extracted from *Eriophorum* litter which was incubated in podzol than in peat. A total of 3.86 mg/g (mean of 12 plots) of DOC was extracted from *Eriophorum* when incubated at Migneint Podzol compared to 2.06 mg/g at Migneint Peat (P = 0.010), and 3.90 mg/g when incubated at Peaknaze Podzol compared to 2.57 mg/g at Peaknaze Peat (P = 0.028). Conversely, for *Calluna*, more DOC was extracted from litter incubated in

peat (2.93 mg/g) than in podzol (1.90 mg/g) at Migneint (P = 0.026), whilst there was no difference between soils at Peaknaze (P = 0.623). The only difference in DOC extracted from litter incubated at different sites was between podzol soils (1.90 mg/g at Migneint Podzol and 3.28 mg/g at Peaknaze Podzol) for *Calluna* (P = 0.005). Treatments had no effect on the extracted DOC relative to the dry mass of litter for both *Eriophorum* (P = 0.338) and *Calluna* (P = 0.722). Treatment effect was also found not to be dependent on site (*Eriophorum* P = 0.369; *Calluna* P = 0.767).

There was a significantly higher extractant $SUVA_{254}$ value for litters which were incubated in podzol soil compared to peat at both Migneint (*Calluna* P = <0.001; *Eriophorum* P = 0.001) and Peaknaze (*Calluna* P = 0.002; *Eriophorum* P = <0.001), which is an indication of the quality of DOC in terms of the concentration of the aromatic fraction (Weishaar et al., 2003). For instance, more aromatic DOC was extracted from *Eriophorum*, with a $SUVA_{254}$ value of 2.77 L/mg C^{-1}/m^{-1} in podzol compared to 2.49 L/mg C^{-1}/m^{-1} in peat. *Calluna* extracts also had a higher $SUVA_{254}$ from litter incubated in podzol (1.80 L/mg C^{-1}/m^{-1}) compared to peat (1.26 L/mg C^{-1}/m^{-1}).

Overall, this suggests that litter decomposes faster in podzol, and this results in more DOC (in the case of *Eriophorum*) and more aromatic DOC being produced (for both *Calluna* and *Eriophorum*) from this litter earlier. However, in peat, decomposition and therefore DOC production is suppressed. However, $SUVA_{254}$ was unaffected by treatments for both *Calluna* (P = 0.692) and *Eriophorum* (P = 0.767), and this was not dependent on site or soil type (*Calluna* P = 0.522; *Eriophorum* P = 0.116).

Table 6.4: Mean and standard error (SE) of mass loss, water-extractable DOC concentration and $SUVA_{254}$ values for *Eriophorum* and *Calluna* litter extracts, incubated at different sites with different treatments. 'M' refers to Migneint, 'PN' refers to Peaknaze and 'pod' is podzol soil.

	Treatment	*Eriophorum*				*Calluna*			
		M Peat	**M Pod**	**PN Peat**	**PN Pod**	**M Peat**	**M Pod**	**PN Peat**	**PN Pod**
Mass Loss (%)	**Acid**	50.22	60.26	41.35	59.14	43.93	50.39	41.02	66.90
	SE	1.66	5.88	4.03	4.39	3.38	2.98	4.69	0.71
	Control	51.68	58.98	51.10	65.09	53.27	49.33	36.48	67.78
	SE	3.86	2.51	1.87	1.76	5.18	2.21	2.47	2.21
	Alkaline	54.91	72.74	54.84	58.17	42.38	49.70	47.82	66.64
	SE	3.44	6.71	5.74	0.27	5.16	2.79	5.93	1.26
DOC (mg/g)	**Acid**	2.13	3.51	2.40	3.54	2.50	2.19	2.66	3.40
	SE	0.22	0.88	0.25	0.59	0.20	0.19	0.30	0.90
	Control	2.01	4.72	2.14	4.58	3.51	2.09	2.69	3.12
	SE	0.04	0.85	0.18	0.78	1.06	0.32	0.40	0.35
	Alkaline	2.02	2.54	2.97	2.94	2.94	1.50	2.73	3.36
	SE	0.14	0.42	0.72	0.24	0.48	0.16	0.39	1.16
$SUVA_{254}$ ($L/mg\ C^{-1}/m^{-1}$)	**Acid**	2.06	2.79	1.55	3.05	1.06	2.35	1.21	1.88
	SE	0.19	0.19	0.45	0.10	0.14	0.05	0.17	0.30
	Control	1.87	2.75	2.32	2.78	1.45	1.83	1.15	1.76
	SE	0.45	0.12	0.28	0.30	0.10	0.19	0.13	0.12
	Alkaline	1.97	3.04	1.88	3.20	0.86	1.97	1.46	2.18
	SE	0.13	0.13	0.27	0.07	0.15	0.33	0.19	0.10

6.3.2.2 Group 2: Site specific litters

Acidity treatment had no significant effect on the decomposition of *Sphagnum* (P = 0.209), *Pleurozium* (P = 0.281) or *Festuca* (P = 0.085) after 12 months of incubation (*Figure 6.3*). Furthermore, *Festuca*, which unlike *Sphagnum* and *Pleurozium* was incubated at two sites, had a similar mean mass loss at Migneint and Peaknaze of 54 and 57 % (mean of 12 plots) (P = 0.162), and the effect of treatment was found not to be dependent on site (P = 0.793).

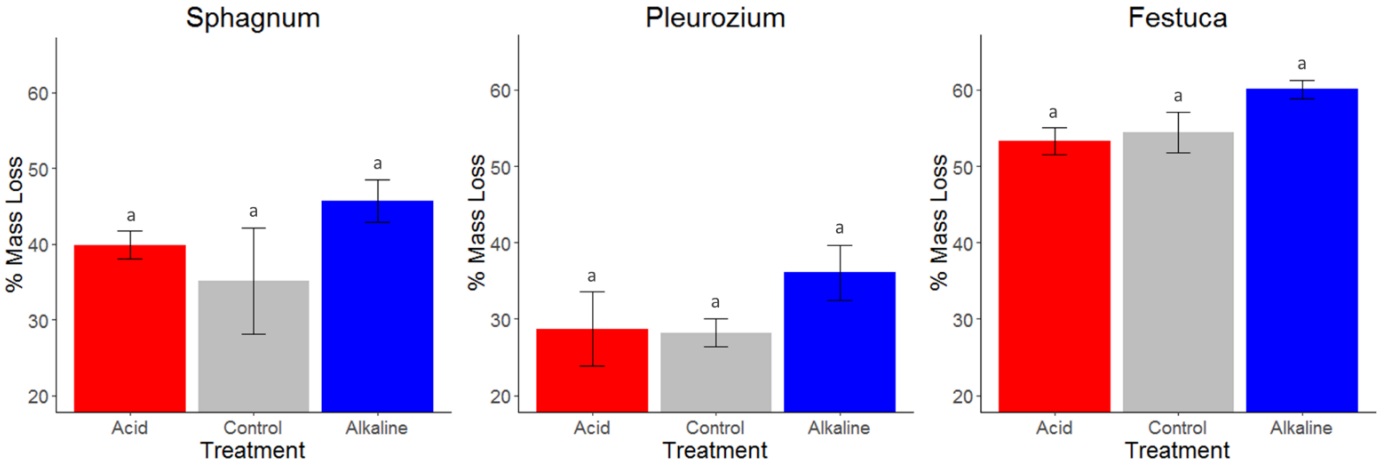


Figure 6.3: Mean percentage mass loss of *Sphagnum*, *Pleurozium* and *Festuca* (pooled mean of two sites) litter after 12 months of incubation with different treatments. Error bars represent standard error of the mean, whilst letters indicate where significant differences occur based on results of a Posthoc test on an Anova comparing treatments (three levels) for *Sphagnum* and *Pleurozium*, and site locations (two levels) and treatments (three levels) for *Festuca*.

More DOC was extracted from *Festuca* which was incubated at Peaknaze Podzol at 3.61 mg/g than when litter was incubated at Migneint Podzol at 2.21 mg/g (P = <0.001), whilst the $SUVA_{254}$ value of DOC did not differ between sites (P = 0.595). Treatments had no effect on the quantity of DOC extracted from *Sphagnum* (P = 0.566), *Pleurozium* (P = 0.121) and *Festuca* (P = 0.066), or on the quality of DOC in terms of the $SUVA_{254}$ value (*Sphagnum* P = 0.548; *Pleurozium* P = 0.784; *Festuca* P = 0.454) (*Table 6.5*). Furthermore, there was no significant interaction between site and treatment for both DOC (P = 0.620) and $SUVA_{254}$ (P = 0.412) on *Festuca* extracts.

Table 6.5: Mean and standard error (SE) of water-extractable DOC concentration and $SUVA_{254}$ values for *Sphagnum*, *Pleurozium* and *Festuca* litter extracts, incubated at different sites with different treatments. 'M' refers to Migneint, 'PN' refers to Peaknaze and 'pod' is podzol soil.

	Treatment	Sphagnum M Peat	Festuca M Pod	Pleurozium PN Peat	Festuca PN Pod
DOC (mg/g)	**Acid**	3.76	2.32	4.73	4.24
	SE	0.79	0.22	0.29	0.83
	Control	3.60	2.57	3.86	3.58
	SE	0.37	0.44	0.14	0.18
	Alkaline	4.57	1.60	4.42	3.01
	SE	0.99	0.43	0.25	0.08
$SUVA_{254}$ ($L/mg\ C^{-1}/m^{-1}$)	**Acid**	0.56	2.14	1.49	2.20
	SE	0.09	0.47	0.34	0.36
	Control	0.49	2.25	1.56	2.37
	SE	0.04	0.04	0.24	0.38
	Alkaline	0.46	2.23	1.28	1.58
	SE	0.07	0.13	0.13	0.11

6.3.2.3 Effect of treatments on the TBI at different sites and soils types

The effect of treatment on the *S* factor was found to be insignificant (P = 0.379) (*Figure 6.4*), as was the interaction with sites (at four levels) (P = 0.878). However, the *S* factor did differ significantly between sites (P = <0.001). This is due to the Peaknaze Peat site having the highest *S* factor of 0.24 (mean of control plots) compared to 0.17 at Migneint Peat (P = <0.001), 0.14 at Migneint Podzol (P = <0.001), and 0.11 at Peaknaze Podzol (P = <0.001).

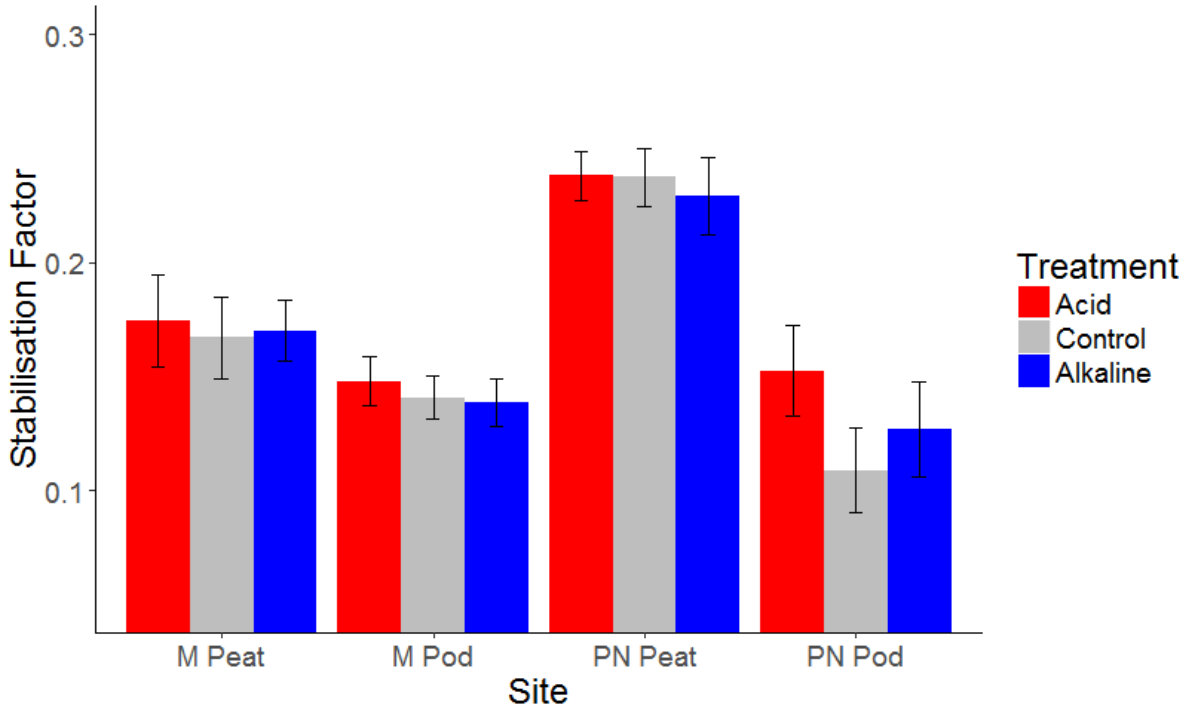


Figure 6.4: Stabilisation factor (*S*) calculated as part of the Tea Bag Index. Tea bags were incubated at different sites receiving acid, control and alkaline treatments.

Both *S* and *k* values are similar to comparable ecosystems based on values presented by Keuskamp et al. (2013), particularly for the peat sites (*Figure 6.5*). Mean Migneint Peat values were similar to those obtained from an undisturbed raised bog in central Ireland (Clara Bog), whilst the degraded Peaknaze Peat values were similar to a disturbed raised bog also in Ireland (also Clara Bog area). Podzol sites had a lower *S* value and a slightly greater *k* value compared to the peat sites. Migneint Podzol is most similar to the Netherlands pasture ecosystem, whilst Peaknaze Podzol *S* and *k* values are most comparable with the Netherlands wet forest.

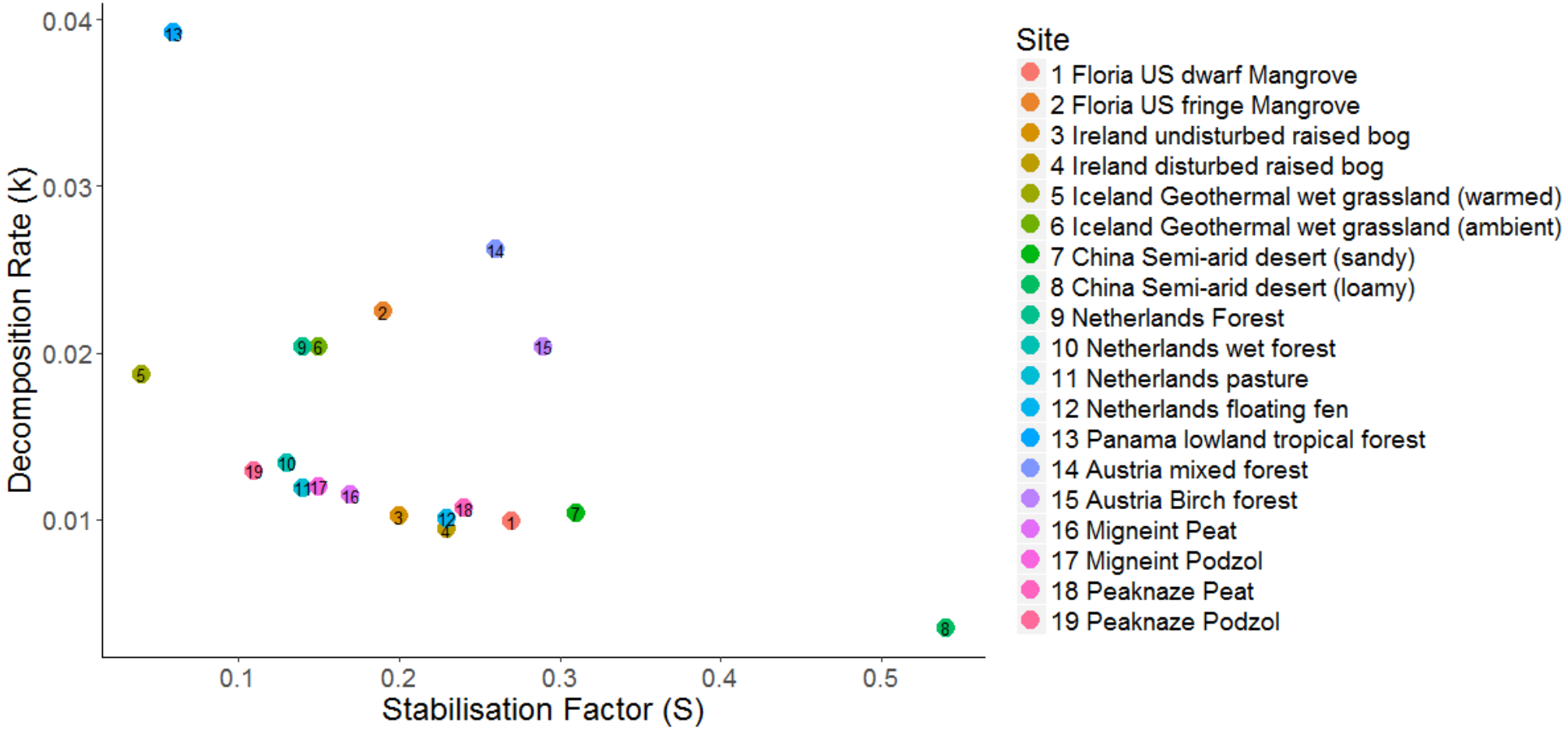


Figure 6.5: A comparison of the Tea Bag Index parameters *S* and *k* with data for other ecosystems as presented by Keuskamp et al. (2013). Data presented for the four experiment sites for this study are based on the mean from control plots only.

As with the *S* factor, treatments had no effect on the *k* value (P = 0.151) (*Figure 6.6*), and this was not dependent on site (P = 0.093). The *k* value was found to be significantly lower at Peaknaze Peat (0.011, mean of control plots) than at Migneint Podzol (0.012) (P = 0.030) and Peaknaze Podzol (0.013) (P = 0.028).

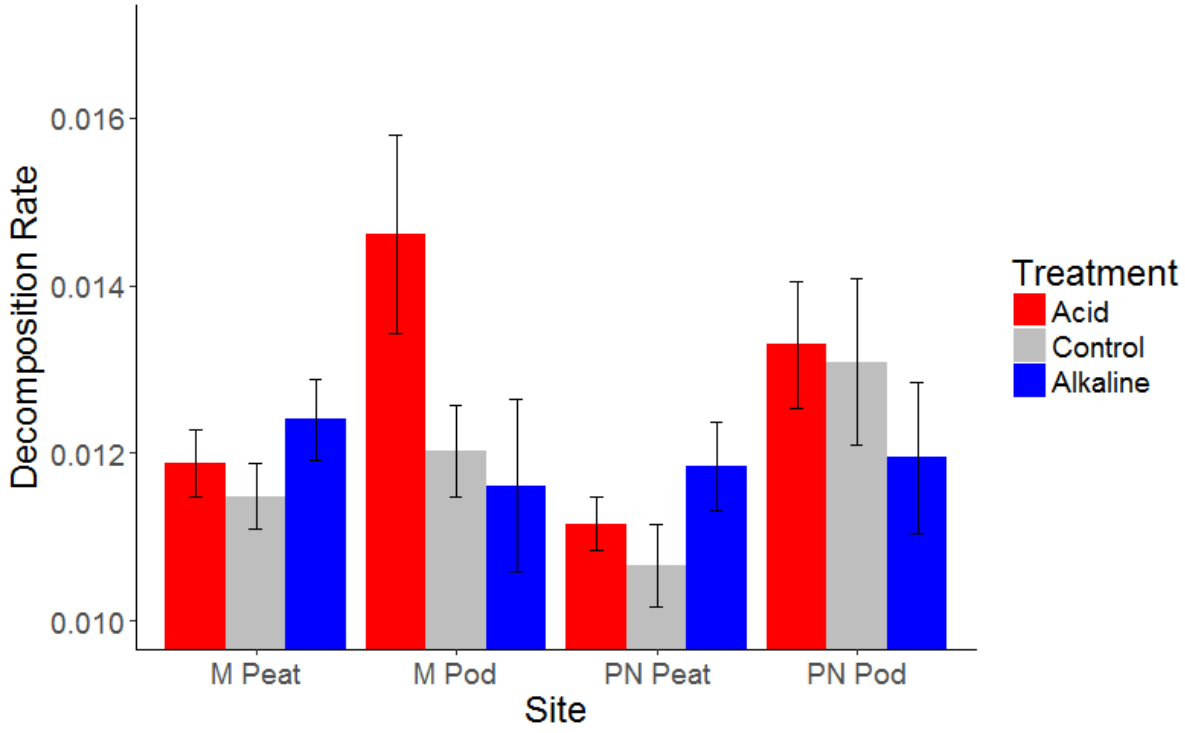


Figure 6.6: Decomposition rate (*k,* day^{-1}) calculated as part of the Tea Bag Index. Tea bags were incubated at different sites receiving acid, control and alkaline treatments.

6.4 Discussion

6.4.1 Decomposition and DOC production in peat and organo-mineral soil over a pollution deposition gradient

This difference in decomposition and DOC release between peat and podzol is likely due to the moisture content differences and their effect on soil aeration. Podzol soils are more freely draining allowing for more aerobic activity, whilst peats are permanently saturated which creates anoxic conditions and inhibits aerobic decomposition (Ramchunder et al., 2009) resulting in anaerobic activity which is a much slower process (Swift et al., 1979, Keller et al., 2009). Furthermore, net primary production is greater at podzol areas compared to peat, and so there is a greater input of organic material from litter above, which arguably could result in a more biologically activity and primed microbial community, resulting in faster decomposition (Xiao et al., 2014, Kuzyakov et al., 2000).

The decomposition and subsequent production of DOC from *Eriophorum* litter was similar between the two sites by 12 months of incubation in podzol soil and peat. This is also the case for *Calluna* decomposition and DOC production in peat. However, *Calluna* did decompose more at Peaknaze than at Migneint in podzol soil, and this resulted in slightly (yet significantly) more DOC being extracted. Interestingly, results from quarterly sampling of *Calluna* and *Eriophorum* litter bags from control plots at these sites over the same experimental period show that litter decomposed more at Migneint than at Peaknaze for both soil types between 0-9 months incubation (Chapter 5). This was followed by a rapid increase in *Calluna* litter decomposition at Peaknaze Podzol between July and October (9-12 months). This suggests that decomposition of *Calluna* was suppressed at Peaknaze and then increased in the more productive podzol soil in response to seasonal variables (see Chapter 5), releasing more DOC. However, when comparing the decomposition and DOC production of *Calluna* and *Eriophorum* litter across the experiment sites, overall soil type is more influential on decomposition than site differences (and therefore the influence of pollution deposition).

For the TBI, Peaknaze Peat had a significantly high *S* value and low *k* value, suggesting that there are environmental factors at this site which are inhibiting the decomposition of the labile fraction of organic material (Keuskamp et al., 2013). This is supported by results from Chapter 5 whereby decomposition of *Calluna* and *Eriophorum* litter was slowest at Peaknaze Peat between 0-9 months incubation. This area has experienced high sulphur deposition which has seen a 69 % reduction between 1970 and 2005 (Dore et al., 2007, Evans et al., 2012), with a substantial store of sulphur still being present in the South Pennine peats (Daniels et al., 2008). Soils and waters in this area have also experienced significant acidification (Evans et al., 2000). In addition, this area has received previous and current high levels of nitrogen (N) deposition and saturation (Helliwell et al., 2007, Curtis et al., 2005, Evans et al., 2000). Heavy metal pollution is also paramount, with concentrations of lead in near-surface peat recorded at 1000 mg kg^{-1} in the Peak District (Rothwell et al., 2005). N (Knorr et al., 2005) and sulphur deposition (Prescott and Parkinson, 1985), the associated acidification (Sanger et al., 1993), as well as heavy metal pollution (Cotrufo et al., 1995, Berg et al., 1991) have been shown to suppress litter decomposition. Such results from the TBI and Chapter 5 suggests that pollutants deposited on organic soils could be

contributing to suppression of decomposition in early stages during the biodegradation of labile material, and litter bag data from *Calluna*, *Eriophorum* and *Festuca* litters suggest that by 12 months there are other factors such as soil type which are more dominant. However, further work is needed to unpick these different drivers further.

6.4.2 The effect of changing acidity on litter decomposition and DOC production

Mass loss did not differ between treatments for any litters other than *Eriophorum*, which decomposed significantly less with acid treatments than with alkaline treatments, but neither treatments were significantly different to the control. Furthermore, the DOC extracted from litter, which represents the DOC produced during the decomposition stage reached by 12 months, did not differ between treatments. This is was also the case for the $SUVA_{254}$ value of extracted DOC, which is associated with the aromatic fraction of DOC (Weishaar et al., 2003), and so represents the aromatic fraction of DOC produced. In addition, the TBI *S* and *k* values were also unaffected by treatments. Interestingly, respiration and enzyme activity also did not respond to treatments during a previous acidification manipulation experiment at these sites (Oulehle et al., 2013).

There are few studies assessing the impact of acidification on litter decomposition. Donnelly et al. (1990) found microbial biomass, lignin and cellulose decomposition to be unaffected by acidity in a forested environment. Alternatively, suppression of decomposition of *Calluna* and *Eriophorum* litter has been observed in a peat monolith acidification experiment (Sanger et al., 1993), whilst no effects have been shown for *Sphagnum* in a poor fen environment (Rochefort et al., 1990), although a much lower annual treatment dose was applied of 18 kg S ha^{-1} yr^{-1}. In addition, the aerobic and anaerobic decomposition of glucose has been shown to be reduced in naturally acidic conditions (4.3 pH units) of surface peat dominated by *Sphagnum*, compared to experimentally increased pH (6.8 pH units) (Bergman et al., 1999). Such literature suggests that litter decomposition can be suppressed by acidity, which supports the results from this study for *Eriophorum*.

Acidity can affect decomposition by altering the quantity, community structure and functioning of decomposers. Little is known regarding soil faunal responses to acidity, but evidence suggests that community structure as well as the vertical distribution of faunal

groups may be impacted by acidity, which may affect functions such as decomposition (Wei et al., 2017). This may be more relevant in aerobic organo-mineral soils than in saturated peat. Furthermore, acidification could alter mechanisms involved in microbial decomposition of organic material, particularly as soil pH is crucial to enzyme functioning (Fog, 1988), and is highly correlated with microbial community structure (Griffiths et al., 2011). Such literature again supports the suppression of decomposition seen in this study for *Eriophorum* litter.

Although treatments were not significant to the control, *Eriophorum* decomposition did show signals of being affected by acidity. In contrast, the decomposition of other litters did not respond to treatments. It is possible that this disparity in response to treatments between *Eriophorum* and other litters is due to the quality of the litter. *Eriophorum* had significantly more nitrogen within plant tissue, with a difference of 0.82-1.51 % nitrogen content compared to other litters (based on mean of both sites), and a lower C:N ratio, with a difference of 10-75 C:N ratio compared to other litters. *Eriophorum* also decomposed the most after 12 months of incubation, with a mass loss of 58 % (mean of control plots from all sites) compared to 55 % for *Festuca*, 51 % for *Calluna*, 35 % for *Sphagnum* and 28 % for *Pleurozium*. Therefore it is possible that *Eriophorum* decomposition was not limited by N, and so instead the inhibition effect of acidity was controlling decomposition. However, with other litters, N content is lower and so decomposition may be limited by N, meaning that even when there is an optimum pH for microbial activity, decomposition is still suppressed.

However, if this were the case we would expect to see more decomposition of these low N litters at Peaknaze, where N deposition and saturation is high. In contrast, whilst *Calluna* decomposes more at Peaknaze Podzol than at Migneint Podzol, there is no difference between peat sites, and *Festuca* also had a similar mass loss between podzol sites. However, there are other environmental constraints at Peaknaze which may limit decomposition, and therefore decomposition of lower N content litters is not enhanced but in fact suppressed. For instance, heavy metal pollution have been shown to suppress litter decomposition (Cotrufo et al., 1995, Berg et al., 1991). Also, *Calluna* litter decomposition has been shown to be negatively related to the high lignin content of its litter in a peat environment (Bragazza et al., 2007). There is evidence that interactions between N and lignin content may create decay resistant compounds which actually slows decomposition (Fog, 1988, Berg

and Matzner, 1997). Therefore whilst we might expect higher N deposition at Peaknaze Peat to increase decomposition, actually the interaction with the lignin associated with the woody components of *Calluna* may suppress decomposition at this site.

Therefore whether decomposition is limited by acidity or nitrogen availability is complex and may depend on litter quality, whilst other environmental variables may become more dominant. Of the literature that is present, there are many contradictions on what the controlling factors are on litter decomposition. More work is needed to assess how recovery from acidification may be effecting the decomposition and the subsequent DOC produced from different litters in a controlled laboratory experiment where other variables such as litter tissue N content, heavy metal pollution and lignin content can be excluded, or interactions can be explored.

6.4.3 Limitations of study

It is possible that the minimal treatment response seen in this experiment could be a methodological problem. A possible reason may be due the pH of litter samples. Direct measurements from pore water as well as peat extracts of samples collected from 5-10 cm depth showed that treatments had successfully altered pH at the depth at which bags were buried (Chapter 4). The pH of extracts from litters also reflected the treatments they received, barring *Sphagnum* which had a similar pH between treatments. Extracts of litters retrieved from acid plots had a reduction in pH compared to the control by up to 0.33 pH units, whilst pH was higher when alkaline treatments had been applied, with an increase of up to 0.77 pH units. However, litters were also less acidic than peat, with the mean pH from control plots of peat extracts being 4.89-5.59 pH units for peat (the highest value being at Migneint Peat where treatment effect was difficult to establish in peat and pore water) and 4.74-4.89 pH units for podzol (Peaknaze and Migneint respectively) compared to 4.9-5.69 pH units for litter. Therefore the litter material itself had potentially not reached a pH low enough for acidity to influence microbial activity and therefore decomposition.

Another methodological problem may be the length of incubation time. Whilst there has not been a litter bag experiment conducted on a peatland specifically assessing the effects of sulphur on DOC production from litter decomposition, some peatland litter bag studies have been conducted over a period of two (Scheffer et al., 2001, Ward et al., 2015, Thormann et

al., 2001) and three (Bragazza et al., 2010, Moore et al., 2007) years. Mosses such as *Sphagnum* are consistently shown to have slow decomposition rates compared to other vegetation types (Pinsonneault et al., 2016, Ritson et al., 2014, Armstrong et al., 2012, Moore et al., 2007). *Sphagnum* also has a high phenolic content (Rasmussen et al., 1995), which suppresses biodegradation and contributes to its slow decomposition (Verhoeven and Toth, 1995, Fenner et al., 2004).

Oulehle et al. (2018) detected a suppression effect of sulphuric acid treatments on teabag decomposition after 6 months of incubation in a forested podzol soil. In this experiment, teabags were only buried for 3 months. Keuskamp et al. (2013) state that in environments where decomposition is slow, incubation time should be extended (> 90 days). Furthermore, the lack of differences in the TBI variables between soil types may be due to the differences in incubation time, with tea bags being buried for 3 months and litter bags for one year. It may be possible that the labile fraction of green tea had not completely decomposed and so *S* may have been overestimated in peat, making it difficult to detect differences in soil types. Overall, this may explain why treatment responses were not seen, and it may be possible that a longer incubation period was necessary to see any influences of acidity. Further work is needed to test this idea.

6.5 Conclusion

Only *Eriophorum* litter decomposition showed signals of responding to acidity treatments, which may be related to the higher nitrogen content within plant tissue compared to other litter types, meaning that nitrogen availability is not limiting decomposition and so acidity controls become apparent. This suggests that controls on litter decomposition can be limited by acidity or nitrogen availability and this is dependent on litter quality.

The lack of significant difference between treatments and the control with *Eriophorum* litter mass loss may be due to the short incubation time of 12 months. More work is needed to assess how recovery from acidification may be effecting the decomposition and the subsequent DOC produced from different litters with a) a longer term field experiment, and b) in a controlled laboratory experiment where other variables such as litter tissue N content, heavy metal pollution and lignin content can be excluded, or interactions can be explored.

The TBI suggests that decomposition at the most degraded and polluted site (Peaknaze Peat) has environmental factors that are limiting the decomposition of the labile fraction of organic material, supporting findings from Chapter 5. By 12 months of incubation of litter, soil type was more influential on decomposition, with more mass loss of litter when bags were incubated in podzol soil than in peat. This resulted in more DOC (*Eriophorum*) and a higher $SUVA_{254}$ value indicating more aromatic DOC (*Eriophorum* and *Calluna*) from extractions. This suggests that litter has the potential to decompose faster in podzol soil than in peat, and after one year of decomposition this results in more DOC being produced which is of a more aromatic nature due to the later decomposition stage reached compared to peat. *Eriophorum* was found to produce DOC which was of a more aromatic nature, as indicated by $SUVA_{254}$, compared to other litter types.

Chapter 7: How do microbial communities in peat and organo-mineral soil respond to acidification?

Abstract

There has been an observed increase in dissolved organic carbon (DOC) concentration in soil solutions and surface water bodies over the past 30 years in acid sensitive areas of Europe and North America, which has been linked to recovery from acidification of soils in response to decreasing levels of atmospheric pollution. It has been hypothesized that this increase in DOC is a result of increased solubility of DOC in response to recovering pH. However, as DOC production (and consumption) is under biological control through the decomposition (and mineralization) of organic material, there is uncertainty as to whether the increase in DOC concentrations is solely a chemical (solubility) response or whether there is a biological element also, with increased microbial DOC production, or reduced DOC consumption, with acidification recovery. Therefore the aim of this research was to investigate how microbial communities respond to soil acidification and recovery in peat and organo-mineral soils, as well as assessing relationships with soil pH and DOC. Fungal and bacterial communities from soil in an existing long-term pH manipulation field experiment in contrasting areas of historical pollution; North Wales and the Peak District, UK, were characterized by 16S rDNA- and ITS-based amplicon sequencing.

The alpha diversity of fungal communities was found to be significantly ($P = <0.05$) greater in podzol soils than in peat, whilst bacterial communities were strongly influenced by site differences, with less diversity at the polluted site in the Peak District. Acidity manipulations did not influence bacterial alpha diversity, but did increase the abundance of core Acidobacteria taxa. Therefore biological responses to experimental treatments were not detected using broad community metrics, which highlights the importance of focusing on indicator taxa. Finally, despite the insignificance with experimental treatments, bacterial community diversity was found to be positively and significantly related to both soil pH and soil extract DOC, which suggests that there may be a functional response to changing acidity as well as biological. Further work is needed to assess the mechanistic functional response of bacteria in terms of DOC production and consumption in response to changing acidity.

Chapter 8

7.1 Introduction

Peatlands are a valuable store of carbon, containing an estimated 20-30 % of the total global carbon stock (Gorham, 1991). However, there have been recent drastic changes in carbon release from these ecosystems, raising concerns over the future of peatland carbon balances (Freeman et al., 2001a). Considerable increases in dissolved organic carbon (DOC) concentrations have been observed in terrestrial waters draining catchments dominated by organic soils across acid sensitive areas of the Northern Hemisphere since the 1980's (Evans et al., 2005, Monteith et al., 2007, Oulehle and Hruška, 2009, SanClements et al., 2012b, Couture et al., 2012). This has been largely attributed to recovery from acidification as a result of reductions in atmospheric sulphur deposition in many regions, with DOC solubility increasing as soil pH recovers, releasing previously insoluble organic carbon as DOC from soils. This is widely supported by field (Evans et al., 2012, Oulehle et al., 2013, Evans et al., 2008a, Moldan et al., 2012, Ekström et al., 2011) and laboratory experiments (Clark et al., 2011, Palmer et al., 2013) as well as modelling (Monteith et al., 2007, Rowe et al., 2014, Evans et al., 2008b, Sawicka et al., 2016) and field observations (Oulehle et al., 2017, Oulehle and Hruška, 2009, Evans et al., 2006a, Oulehle et al., 2011). There has also been an increase in the amount of high molecular weight, coloured DOC of an aromatic and refractory nature leaching from peatlands, contributing to the 'brownification' of many terrestrial waters (Watts et al., 2001, Worrall et al., 2003a).

When the pH of soil is more acidic, the chemical solubility of DOC is reduced as organic material precipitates out of solution and coagulates with acidity, which leads to a lower concentration of DOC in pore water. Acid deposition has the greatest effect on DOC solubility when soil pH is 4-5 units (Thurman, 1985, Evans et al., 2006a). Clark et al. (2011) also showed a positive correlation between coloured aromatic humic acid concentrations and acidity, suggesting that the solubility of aromatic DOC is also acid sensitive and may be increasing with recovery. Reduced atmospheric pollution deposition can also lead to increasing ionic strength of soil-water and stream water which again can increase DOC solubility (Hruška et al., 2009).

However, there is evidence suggesting there may also be a biological mechanism behind these changing trends in response to reduced sulphur deposition and the subsequent recovery from acidification. Dawson et al. (2009) used a statistical mixed-effects model to

analyse the mechanisms behind the increased DOC concentration and aromatic fraction observed over two decades of monitoring at two upland organic catchments in Scotland. Results suggest that increased solubility of DOC alongside enhanced heterotrophic decomposition in responses to recovery from acidification are behind these trends. Alternatively, high sulphur inputs may increase bacterial sulphate reduction, which may increase consumption of labile DOC (Bartlett et al., 2005).

Soil microbes play a considerable role in the regulation, retention and release of carbon in peatland systems. DOC production is a biological process, either via the release of exudates from plants, or through microbial decomposition of polymeric particulate organic material. Microbial decomposition releases DOC through the action of extracellular enzymes, which depolymerize higher molecular weight organic matter of low solubility, creating lower molecular weight DOC monomers and oligomers. DOC consumption is also a biological process: DOC compounds which are small enough (<~600 Da) can be actively transported through microbial cell walls and enter the anabolic and catabolic reactions of microbial metabolism with catabolism resulting in the ultimate oxidation and release of DOC as CO_2 (Blankinship et al., 2014). As microbes preferentially decompose and consume labile DOC, the remaining by-product is the high molecular weight, aromatic and refractory DOC which is harder to break down (Kalbitz et al., 2003b, Malik and Gleixner, 2013).

It is possible that changes in acidity may alter DOC production in organic catchments. There is little research on the production of DOC from plants specific to peatlands and organic upland soils, let alone the effect of changing acidity on this DOC source (Fenner et al., 2004, Basiliko et al., 2012). However, as soil pH is crucial to enzyme functioning (Fog, 1988), and is highly correlated with microbial community structure (Griffiths et al., 2011), it is possible that acidification could alter mechanisms involved in microbial decomposition of organic material. There is evidence that acidity can slow biological processes involved in DOC production. Increases in soil pH with liming has been shown to stimulate microbial activity in terms of respiration, and mineralisation, alongside increases in DOC in peat and organo-mineral soils in both laboratory (Ivarson, 1977, Persson et al., 1989, Andersson et al., 2000, Andersson et al., 1994) and field experiments (Shah et al., 1990, Andersson and Nilsson, 2001a, Nilsson et al., 2001). This suggests that as soil pH changes to more favourable conditions for biological activity with recovery from anthropogenic acidification, DOC

production may increase as a result. Reduced litter decomposition rates have been demonstrated under acidic conditions (Killham and Wainwright, 1981, Adams and Angradi, 1996, Dangles et al., 2004, Baath et al., 1980, Oulehle et al., 2017), including in peat (Sanger et al., 1993) and podzol soil (Brown, 1985), demonstrating reduced DOC production with acidity. Particular inhibition of microbial decomposition has been noted at sites with high sulphur pollution (Prescott and Parkinson, 1985, Brown, 1985).

Changing acidity may influence microbial activities and taxa competitiveness, which may alter microbial communities and therefore functions such as DOC production and consumption. The soil microbial abundance and community structure is also an important limiting factor on DOC production. The decomposer community (microorganisms and larger decomposers) present has been shown to regulate changes in litter chemistry during decomposition, and ultimately the production of DOC including its quality and stability (Wickings et al., 2012). A relationship has been highlighted in a forest ecosystem between the bacterial community in particular and C-biochemistry, whilst fungi community is more influential on nutrient dynamics (Liu et al., 2016).

Soil microbial community structure has been shown to differ in soils with different pH (Frostegård et al., 1993, Griffiths et al., 2011, Hartman et al., 2008). Acidic conditions decrease bacteria and increase fungi in soils (Blagodatskaya and Anderson, 1998, Rousk et al., 2009, Oulehle et al., 2018), with bacterial growth rates showing a greater sensitivity (Walse et al., 1998). Rousk et al (2009) found microbial inhibition in terms of carbon mineralisation below a pH value of 4.5 units which was attributed to increased fungal growth and decreased bacterial growth with acidity.

Therefore it is possible that the observed trend of increasing DOC concentrations with acidification recovery could be partly due to changes in microbial communities and associated functions and DOC production, alongside the chemical mechanism which is demonstrated strongly in the literature. However, other studies show acidity as having a minimal effect on decomposition (and therefore DOC production) with other abiotic factors being more influential such as soil moisture content (Donnelly et al., 1990, Rochefort et al., 1990). For instance, Thormann et al. (2003) found that there are different fungal assemblages associated with decomposing different litter types in peatland ecosystems, and concluded that litter quality variables, such as total carbon and nitrogen, determined the

fungal community present more than pore water chemistry or physical variables. This raises uncertainty over the extent that a biological mechanism may be contributing to increased DOC concentrations in response to changing acidity, and highlights the need for research in this area.

Despite their critical role in the turnover of DOC, there is a lack of knowledge surrounding taxonomic composition and diversity of soil microbes in peatlands, and their community structural and functional responses to environmental change (Hartman et al., 2008). If community structure is shown to change in response to environmental change, this leads to the next debate as to whether the altered community has different functions with respect to DOC production and consumption. We need an improved understanding of ecological responses of soil biota to environmental change, such as acidification and recovery, to understand the potential relationships with peatland functions, critically the carbon cycle. There is uncertainty as to whether the increase in DOC concentrations in terrestrial waters is solely a chemical response due to increased solubility with recovering pH, or whether there is a biological element also, with increased microbial DOC production, or reduced DOC consumption, with acidification recovery.

The main aim of this chapter is to tackle part of this knowledge gap by assessing how microbial communities respond to soil acidification and recovery in peat and organo-mineral soils, building upon the experimental framework presented by Evans, et al., (2012).

Specific objectives are:

- To investigate how microbial diversity (in terms of alpha diversity indices incorporating eveness, and beta diversity assessing variation in species composition) differs between soils typical to UK upland organic catchments (peat and peaty podzol), and across different sites representing an acidity/pollution gradient.
- To establish whether microbial diversity is significantly affected by acidity treatments at these different sites for peat and organo-mineral soil.
- To assess whether the abundances of core microbiome taxa respond to acidity treatments within different soils and across different sites.
- To establish whether alpha diversity is significantly related to soil pH and extracted DOC.

7.2 Methods

7.2.1 Site description and experimental design

This work is built upon an existing long-term acidity manipulation field experiment set up in 2007, situated across two moorland locations with contrasting historic rates of acid deposition, and therefore present-day soil acidity (Evans et al., 2012). At each site, replicated acidity manipulations were established within two soil types; blanket peat and peaty podzol, which are among the commonest soils present in the UK uplands, and which also occur extensively in other cool, humid temperate regions. During the rest of this chapter, 'site' will refer to the four individual experimental sites (Migneint Peat, Migneint Podzol, Peaknaze Peat, Peaknaze Podzol), 'site location' will refer to either Peaknaze or Migneint, and 'soil' will refer to peat and podzol.

The first study site, the Migneint (3°48.8' W, 52°59.6' N, 460 m a.s.l.), is a relatively undisturbed moorland area with historically low levels of pollution, based in North Wales. Peaknaze (1°54.5' W, 53°28.3' N, 440 m a.s.l.), Northern England, is a more disturbed region affected by relatively intensive land management and historically high levels of atmospheric pollution, which has led to degradation of the ecosystem including *Sphagnum* loss and erosion. More details for both sites can be found in Chapter 3 and (Evans et al., 2012).

7.2.2 Field experimental operation

The experimental sites were established in August 2007 and consist of twelve 9 m^2 plots at each of the four sites, with a randomised block design comprising of four replicates of control, acid and alkaline treatments at each location. Treatments were applied initially from October 2008 until December 2012 (Evans et al., 2012), and then re-established (using the same methods, treatments and plot allocations) from January 2015 until October 2016. Acid plots received a monthly dose of sulphuric acid (H_2SO_4) mixed with rainwater collected at the site with 20 L of rainwater using a watering can. The concentration applied was 50 kg S ha^{-1} yr^{-1} at the podzol sites and 100 kg S ha^{-1} yr^{-1} at the peat sites, this concentration being similar to the ambient sulphur deposition in the Peak District in the 1970's (a higher dose was applied to peat plots to take account of the buffering effects of sulphur reduction (Evans et al., 2012)). A 10 L rinse of rainwater followed to ensure the treatment infiltrated into the soil and to minimise any direct toxicity effects on plant foliage.

The same procedure was followed for the alkaline plots with sodium hydroxide (NaOH) and potassium hydroxide (KOH), followed by a rinse containing magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) to maintain base cation ratios similar to those observed in rainfall. The molar OH^- concentration in the alkaline treatments was intended to be comparable to the H^+ concentration in the acid treatments. Control plots received 20 L of rainwater only.

Peat and organo-mineral soils samples were collected and processed as described in Chapter 4. Samples were stored at -20°C until extraction of DNA could be performed.

7.2.3 DNA Extraction

DNA was extracted from 0.25 g of soil samples (plus two blanks) based on the manufacturer's instructions with a PowerSoil™ DNA isolation kit (MO BIO Laboratories, Carlsbad, CA). In summary, samples were added to Powerbead Tubes containing a buffer solution of guanidine thiocyanate, allowing the dispersal of soil particles, protection of nucleic acids from degradation, and dissolving of humic acids. The detergent sodium lauryl sulphate was then added (60 µl) to aid cell lysis. Tubes were then vortexed using the TissueLyser II (QIAGEN, Germany), set at 30 Hz/S for 1 minute, enabling mechanical cell lysis and homogenisation. Following centrifugation (10,000 rcf for 30 seconds), 250 µl of Inhibitor Removal Technology ® (IRT) was added, tubes were centrifuged again (10,000 rcf for one minute) and the supernatant was removed from the resulting pellet. This allowed non-DNA organic and inorganic material that may reduce DNA purity to precipitate out of solution, including cell debris, proteins and humic substances. The extraction with IRT was repeated, and the supernatant was then mixed with a salt solution and passed through a silica membrane. Finally, the captured DNA was washed with an ethanol solution to remove contaminants such as humic acids, and then eluted using 10mM tris buffer for downstream analysis.

7.2.4 DNA amplification and sequencing

A two-step Polymerase Chain Reaction (PCR) procedure was used for amplification of the section of DNA of interest, including the rDNA 16S (bacteria and archaea) and fungal Internal Transcribe Spacer (ITS) region. The procedure is based around the use of DNA primers, also known as DNA olionucleotides, and Taq polymerase, a heat-stable enzyme which synthesises a new DNA strand from nucleotides. Using a multichannel pipette in a

sterile PCR cabinet, 1 µl of DNA was transferred into a 96 well PCR plate with a solution containing the Taq polymerase (Sigma Taq for 16S and Q5 Taq for ITS), deoxynucleotide (dNTP) and primers. Bacterial 16S rDNA were amplified with forward (515f, sequence GTG**Y**CAGCMGCCGCGGTAA) and reverse (806rB, sequence GGACTAC**N**VGGGTWTCTAAT) primers targeting the V4 region (Walters et al., 2016), and the fungal ITS region were amplified using forward (ITS7, sequence GTGARTCATCGAATCTTTG) and reverse (ITS4, sequence TCCTCCGCTTATTGATATGC) primers targeting the ITS2 region (Ihrmark et al., 2012). This created amplicon fragments of approximately 380 base pairs.

The first PCR step consisted of cycles of denaturing, annealing and extended DNA strands. The programme differed for ITS and 16S and is summarised below in *Table 7.1*. This step allows the primers to target the region of interest and create amplicons.

Table 7.1: Summary of the first PCR step programme used for 16S and ITS amplification, involving six steps with samples being held at different temperatures for different amounts of time. Steps 2-4 were repeated 25 times. Samples were stored at a low temperature in stage 6 until the plate was retrieved.

Analysis	**1**	**2**	**3**	**4**	**5**	**6**
		x25				
16S	95°*C*	95°*C*	55°*C*	72°*C*	72°*C*	4°*C*
	02:00	00:15	00:30	00:30	10:00	-
ITS	95°*C*	95°*C*	52°*C*	72°*C*	72°*C*	10°*C*
	03:00	00:15	00:30	00:30	10:00	-

Gel electrophoresis with agarose gel was used to check that DNA had amplified correctly during PCR. Post-cycle sequencing reaction contaminants including enzymes and dNTPs were removed from DNA samples using the ZR-96 DNA Sequencing Clean-up Kit™ (Zymo Research, USA). The cleaned PCR product (1 µl) was then added to a solution containing Sigma Taq polymerase, dNTP and primers. During the second PCR step (the programme of which is summarised in *Table 7.2*), the amplicons are given individual 'barcodes' to determine which sequence belongs to which sample during sequencing.

Table 7.2: Summary of the second PCR step programme used for 16S and ITS amplification, involving six steps with samples being held at different temperatures for different amounts of time. Steps 2-4 were repeated 8 times. Samples were stored at a low temperature in stage 6 until the plate was retrieved.

Analysis	1	2	3	4	5	6
		X8				
16S & ITS	95°C	95°C	55°C	72°C	72°C	4°C
	02:00	00:15	00:30	00:30	10:00	-

Gel electrophoresis was once again used to check for correct amplification of the second PCR product. Amplicon purification and normalisation of the product was then performed following instructions from the Applied Biosystems SequalPrep Normalization Plate (96) Kit (Thermo Fisher Scientific, USA). The quantity of DNA in the resulting pooled samples were then assessed using the Quibit 3.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, USA). Based on this assessment, DNA was diluted to 0.21 ng/ml using elution buffer from the normalisation kit to create a final volume of 400 µl. The resulting pooled diluted sample was then vacuum concentrated to 40 µl. For both 16S and ITS, 55 µl mixed with 10 µl of loading buffer were loaded onto a 2 % agarose gel containing 5 ml of SYBR™ Safe DNA gel stain (Invitrogen, Thermo Fisher Scientific, USA). Gel electrophoresis proceeded at 80 volts for 35 minutes, after which the DNA was cut out of the gel and weighed into an Eppendorf tube. Finally, the DNA was purified following the QIAquick Gel Extraction Kit (QIAGEN, Germany) instructions, and then analysed with Illumina MiSeq (Illumina, UK).

7.2.5 Bioinformatics

Bioinformatics were perform using R Statistical Software and the Dada2 package (Callahan et al., 2016) following the amplicon workflow: filtering; dereplication; sample inference; chimera identification; and merging of paired-end reads. During the first filtering step, forward and reverse reads were truncated based on the interpretation of quality profile plots. After truncation, readings with higher than expected errors were discarded. Identical sequences were then combined into ‘unique sequences’ during dereplication, which retains the quality information of each unique sequence to inform the error parameters. The sample inference step then applied the core sequence-variant inference algorithm to the data. Finally, forward and reverse reads which overlapped were merged. Chimeras were removed using the ‘removeBimeraDenovo’ function. The number of reads passing through each step in the workflow are summarised below in *Table 7.3* for a random

selection of samples. Microbial sequences were identified by comparing them to Greengenes (DeSantis et al., 2006) and UNITE (Kõljalg et al., 2013) sequence classifier databases.

Table 7.3: Number of reads obtained following each amplicon workflow step using DADA2 package.

Analysis	Sample	Input	Filtered	Merged	Chimera
16S	**PN Peat 1**	93978	82184	77761	77482
	M Peat 12	93072	86331	75629	75258
	PN Pod 12	110240	101540	95457	95346
	PN Pod 11	69518	63518	57897	57636
ITS	**PN Peat 1**	229	182	169	0
	M Peat 12	30950	23478	22724	22724
	PN Pod 12	102822	92193	88683	88674
	PN Pod 11	87282	78750	77206	77050

7.2.6 Data analysis

Data was analysed using Microbiome Analyst (http://www.microbiomeanalyst.ca) (Dhariwal et al., 2017) and R Statistical Software. Data was uploaded onto Microbiome Analyst and underwent a data filtering step. Samples with zero or low reads were removed manually prior to this. Unfortunately, this step removed a total of 15 samples for ITS, meaning many replications were removed and the effect of treatment could not be assessed. The optimum filtering parameters which removed data noise whilst retaining key taxa were identified. Variables with low abundance features were removed and so features remaining after the filtering step had a minimum count of two with 15% prevalence for 16S, and a minimum count of two with a 10% prevalence for ITS. This removed a total of 5147 low abundance features for 16S (873 remained) and 3384 for ITS (238 remained). In order to address the variability within data and allow for biologically meaningful comparisons so that communities between groups could be compared, data was normalised by applying cumulative sum scaling (CSS) (Paulson et al., 2013, Weiss et al., 2017).

Alpha diversity indices (Shannon, Simpson and Chao1) were calculated in Microbiome Analyst. A two-Way analysis of variance (ANOVA) was performed in R using site (four levels)

and treatments (three levels) as factors for 16S and a One-way Anova on ITS data comparing sites (four levels). Where ANOVA revealed a significant effect, further analysis was performed using 'Tukeys HSD' post hoc test.

Beta diversity was also calculated using the Bray-Curtis distance matrix to quantify the compositional dissimilarity between sites and treatments. Principle coordinates analysis (PCoA) was used to visually represent the distance matrix data over a two-dimensional space using Microbiome Analyst. The core microbiome was also obtained by setting abundance thresholds on relative abundance data in Microbiome Analyst (see section 7.3.3). The relative abundance of each member of the core microbiome was compared between sites (four levels) and treatments (three levels) using a Two-Way ANOVA in R for 16S data, and site locations (two levels) and soil type (two levels) for ITS data. Finally, relationships between alpha diversity indices (both 16S and ITS) with soil extract pH and DOC concentrations were assessed using scatterplots and Spearman's Rank statistical test.

Analysis of both 16S and ITS data was done at three taxonomic levels for comparison. For 16S these levels were phylum, order and genus, and for ITS these were order, genus and species as more taxa groups were identified at lower taxonomic levels.

7.3 Results

7.3.1 Community composition

7.3.1.1 Bacterial and archaeal composition

A total of 2,301,849 raw reads were obtained from 48 samples for 16S rRNA gene amplicons. After quality filtering, a total of 1,982,396 sequences were acquired which ranged from 13,519 to 90,679 sequences per sample, after two samples were removed from analysis due to low reads (Migneint Podzol Control plot 2, Peaknaze Podzol Acid plot 8).

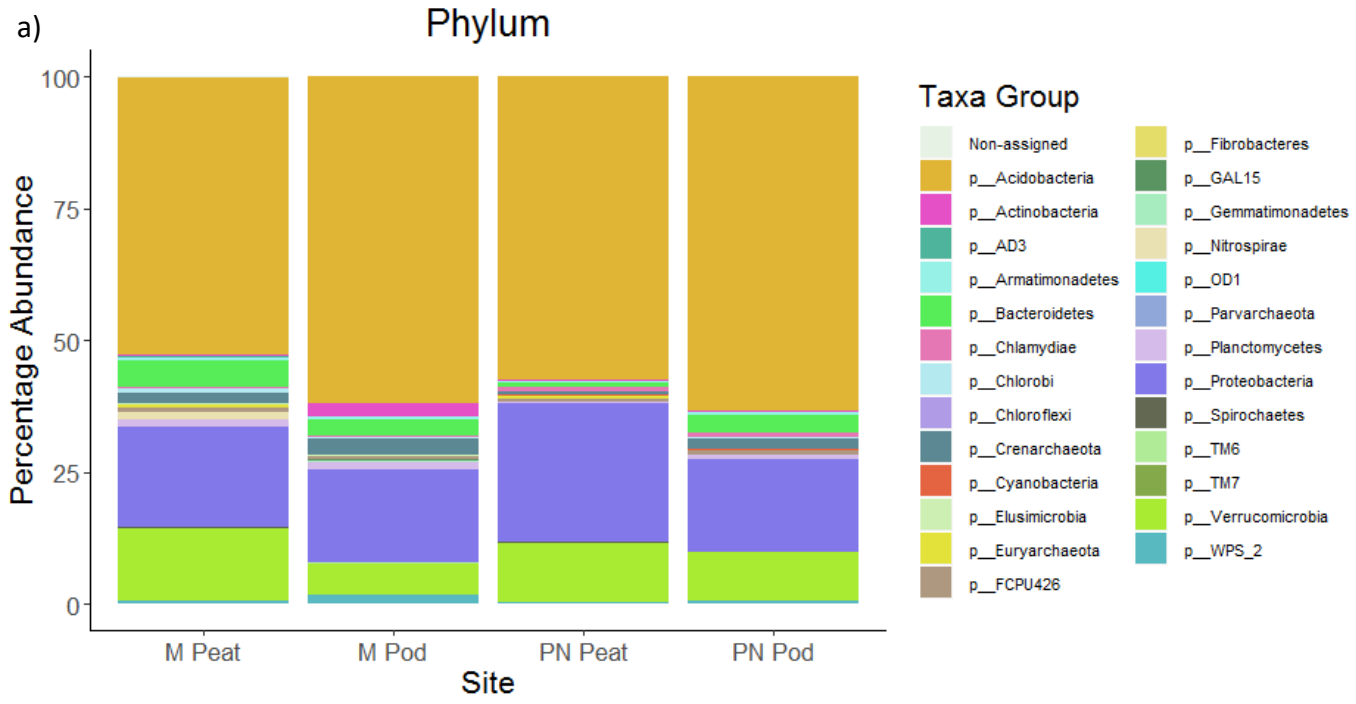
Composition is shown for four taxonomic levels below; phylum, order, genus and species (*Figure 7.1*). A total of 26 taxonomic groups were identified at phyla level (*Figure 7.1a*). Soil bacterial and archaeal taxonomic composition were similar between all sites. The most common phyla groups were Acidobacteria, Proteobacteria and Verrucomicrobia, accounting for 86-95% of abundance depending on site. Relative abundance of some bacterial groups varied slightly between soil types. For example, podzol sites had a higher abundance of Acidobacteria than peat sites, but a lower abundance of Verrucomocribia. Some taxonomic

groups also varied slightly between Peaknaze and Migneint. For instance, Nitrospirae were present at Migneint peat (~1 %), but not at other sites. Proteobacteria were also more abundant at Peaknaze peat (26 %), yet had a similar abundance across other sites (~ 17 %).

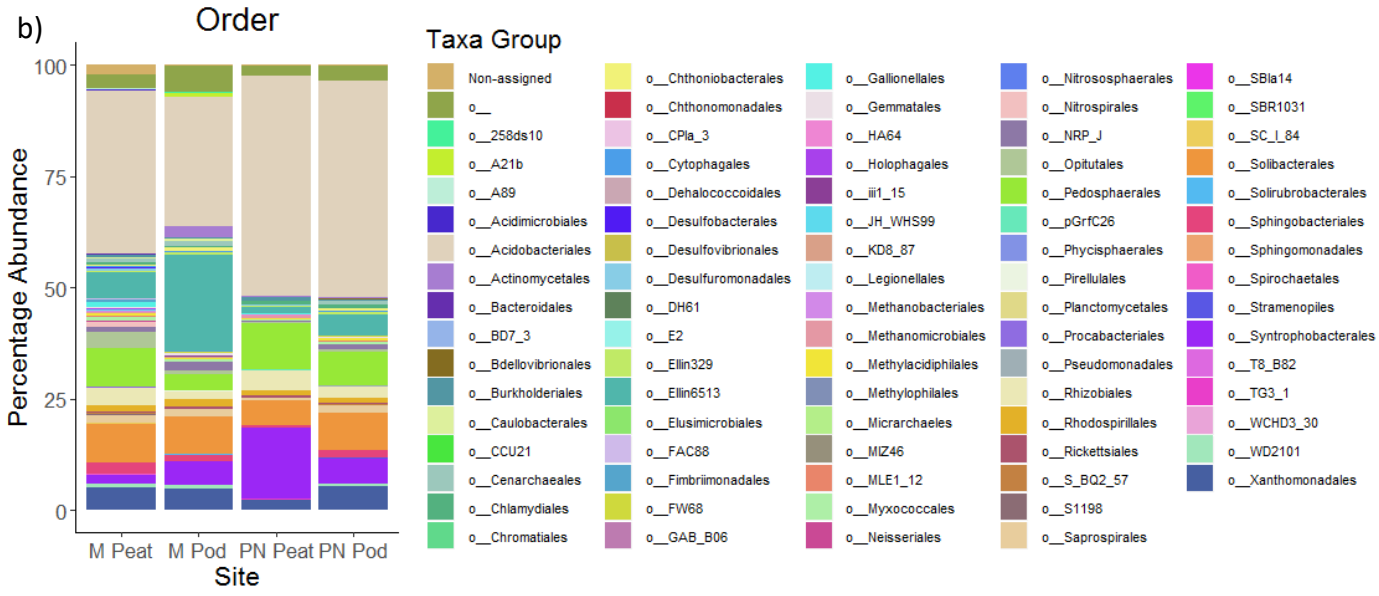
A total of 82 taxonomic groups were identified at order level (*Figure 7.1b*). Again, the most common taxa were Acidobacteriales, which were more prevalent at Peaknaze (49 % at both soil types) than at Migneint (29-36 % at podzol and peat sites respectively). Other common taxonomic groups included Solibacterales (6-9 %), Pedosphaerales (4-10 %) and Ellin6513, of which were more abundant at podzol sites (5 % at Peaknaze and 22% at Migneint) than at peat sites (1 % at Peaknaze and 6 % at Migneint)

A total of 48 taxonomic groups were identified at genus level (*Figure 7.1c*), the most abundant of which were unnamed groups (g_) and comprised 79-86 % relative abundance. Common taxa groups included Candidatus koribacter (2-6 %) and Candidatus solibacter (3-5 %). There were no apparent differences in community composition between different site locations and soils other than a slightly higher abundance of unnamed taxa (g_) at podzol sites than at peat sites. At species level (*Figure 7.1d*), unnamed taxa (s_) comprised 95-98 % relative abundance, and NA (unknown taxa) comprised <4 % relative abundance. All nine identified taxa at this level had a relative abundance of <1 % at each site. Due to the small amount of taxa identified, further analysis was not performed at this level.

a)


Phylum
Percentage Abundance
100
75
50
25
0
M Peat
M Pod
PN Peat
PN Pod
Site
Taxa Group
Non-assigned
p__Acidobacteria
p__Actinobacteria
p__AD3
p__Armatimonadetes
p__Bacteroidetes
p__Chlamydiae
p__Chlorobi
p__Chloroflexi
p__Crenarchaeota
p__Cyanobacteria
p__Elusimicrobia
p__Euryarchaeota
p__FCPU426
p__Fibrobacteres
p__GAL15
p__Gemmatimonadetes
p__Nitrospirae
p__OD1
p__Parvarchaeota
p__Planctomycetes
p__Proteobacteria
p__Spirochaetes
p__TM6
p__TM7
p__Verrucomicrobia
p__WPS_2

b)


Order
Percentage Abundance
100
75
50
25
0
M Peat
M Pod
PN Peat
PN Pod
Site
Taxa Group
Non-assigned
o__
o__258ds10
o__A21b
o__A89
o__Acidimicrobiales
o__Acidobacteriales
o__Actinomycetales
o__Bacteroidales
o__BD7_3
o__Bdellovibrionales
o__Burkholderiales
o__Caulobacterales
o__CCU21
o__Cenarchaeales
o__Chlamydiales
o__Chromatiales
o__Chthoniobacterales
o__Chthonomonadales
o__CPla_3
o__Cytophagales
o__Dehalococcoidales
o__Desulfobacterales
o__Desulfovibrionales
o__Desulfuromonadales
o__DH61
o__E2
o__Ellin329
o__Ellin6513
o__Elusimicrobiales
o__FAC88
o__Fimbriimonadales
o__FW68
o__GAB_B06
o__Gallionellales
o__Gemmatales
o__HA64
o__Holophagales
o__iii1_15
o__JH_WHS99
o__KD8_87
o__Legionellales
o__Methanobacteriales
o__Methanomicrobiales
o__Methylacidiphilales
o__Methylophilales
o__Micrarchaeles
o__MIZ46
o__MLE1_12
o__Myxococcales
o__Neisseriales
o__Nitrososphaerales
o__Nitrospirales
o__NRP_J
o__Opitutales
o__Pedosphaerales
o__pGrfC26
o__Phycisphaerales
o__Pirellulales
o__Planctomycetales
o__Procabacteriales
o__Pseudomonadales
o__Rhizobiales
o__Rhodospirillales
o__Rickettsiales
o__S_BQ2_57
o__S1198
o__Saprospirales
o__SBla14
o__SBR1031
o__SC_I_84
o__Solibacterales
o__Solirubrobacterales
o__Sphingobacteriales
o__Sphingomonadales
o__Spirochaetales
o__Stramenopiles
o__Syntrophobacterales
o__T8_B82
o__TG3_1
o__WCHD3_30
o__WD2101
o__Xanthomonadales

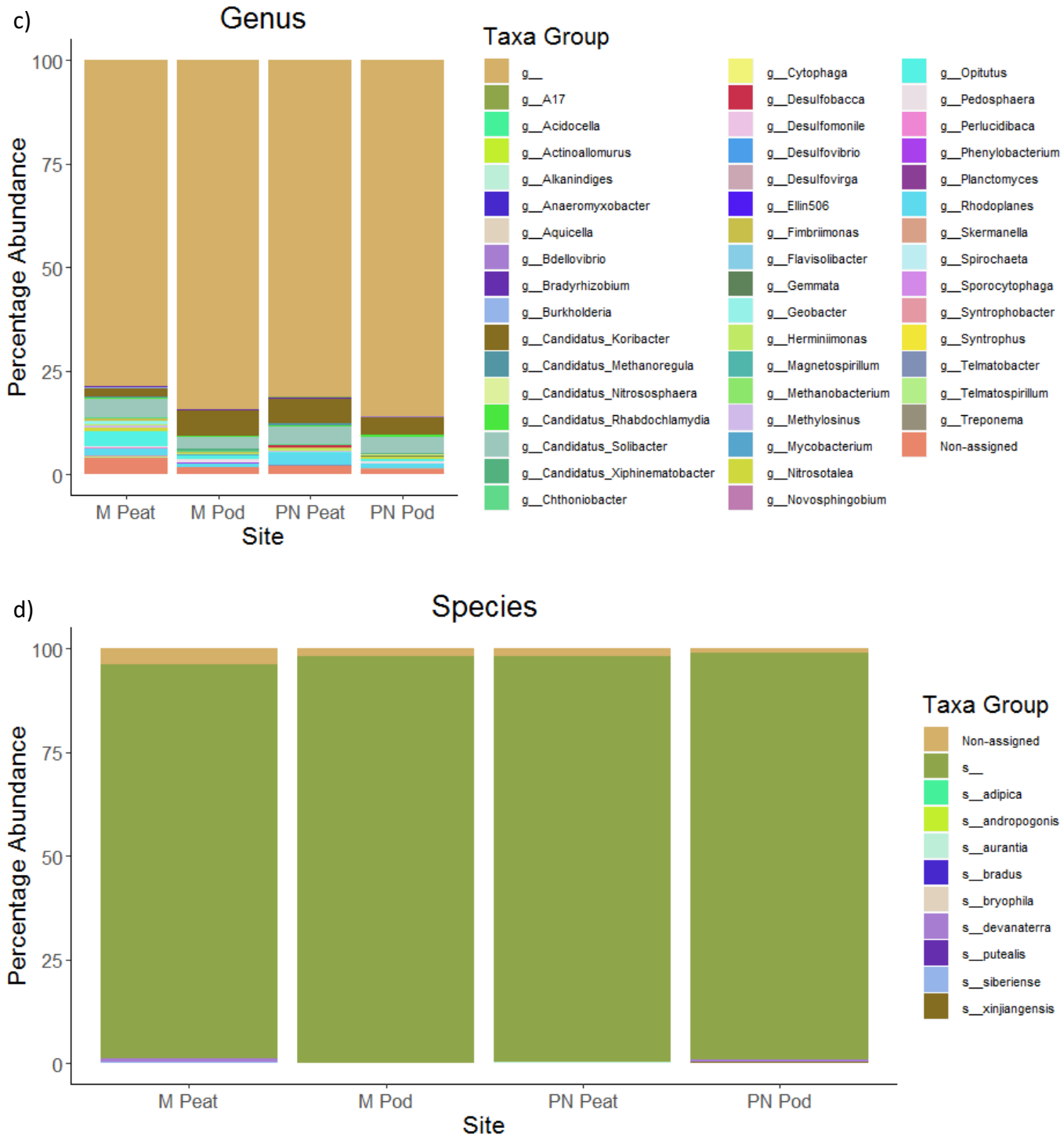


Figure 7.1: Percentage relative abundance of bacterial and archaeal taxa at phyla (a), order (b), genus (c) and species (c) taxonomic levels for each site. 'M' refers to Migneint, 'PN' refers to Peaknaze and 'Pod' refers to podzol soil.

7.3.1.2 Fungal composition

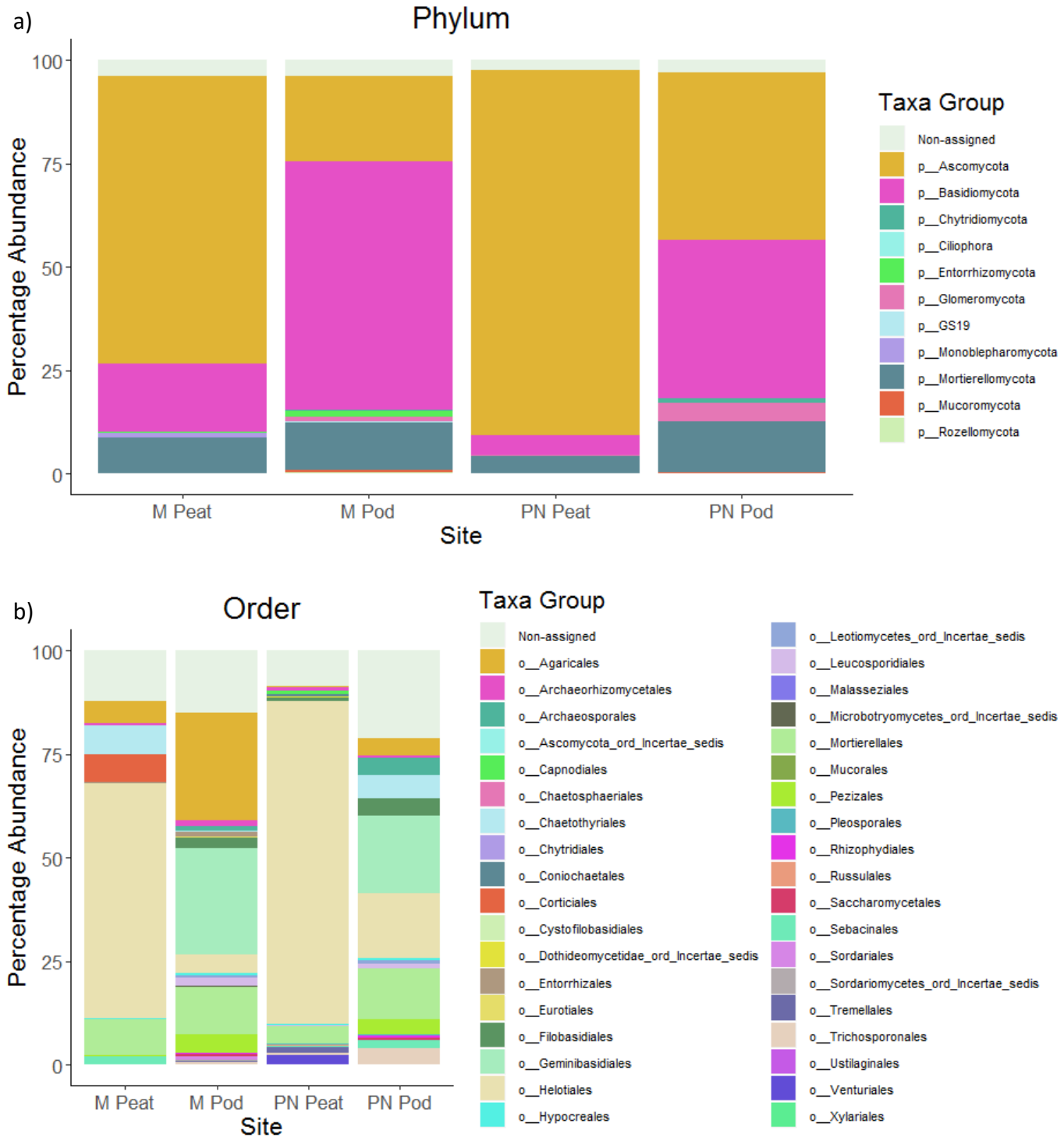
For ITS amplicons, a total of 1,768,795 raw reads were obtained from 48 samples. Fifteen samples were removed for having zero or low reads (Migneint Podzol plot 2 (control), 3 (control), 10 (alkaline); Peaknaze Peat plot 1 (control), 2 (control), 4 (control), 7 (acid), 8 (acid), 10 (alkaline); Peaknaze Podzol plot 2 (control), 6 (acid), 10 (alkaline)). After quality filtering, a total of 1,448,105 sequences were acquired which ranged from 302 to 82,104 sequences per sample.

At phyla level, 11 taxonomic groups were identified (*Figure 7.2a*). The main dominating taxa were Ascomycota, which were more abundant in peat (>70 %) than in podzols (<20 %), and Basidiomycota which were more abundant in podzols (40-60 %) than in peat (<15 %). A total of 37 taxonomic groups were identified at order taxonomic level (*Figure 7.2b*), the dominant of these being Helotiales which were more abundant in peat (57-78 %) than in podzols (<15 %), Geminibasidiales, which were more abundant at podzol sites (>20 %) than in peat (<1 %), and Mortierellales, which were slightly more abundant in podzols (~12 %) than in peat (<10 %). There were also some rarer taxa groups which were specific to podzol sites only including Filobasidiales (~3 %) and Pezizales (~3 %).

At genus level, a total of 68 taxonomic groups were identified (*Figure 7.2c*). The most common ITS taxa category was non-assigned taxa, which made up to 29-40 % of community composition depending on site. There were some key similarities between common taxa groups and soil type. For instance, taxa which were common in peat soils included Meliniomyces (28-36 %) and Pezoloma (~9 %), whilst taxa more common in podzol soils included Basidioascus (19-26 %) and Solicoccozyma (~3 %). Migneint Podzol also had a higher abundance of Hypochnicium (7 %) and Gliophorus (17 %).

At species level, there were 6 taxa identifications (*Figure 7.2d*). Non-assigned taxa made up a large amount of proportion of the relative abundance at each site (50-80 %). There were some clear differences in taxa community composition between peat and podzol soils. For instance, Undulatus was highly abundant at podzol sites (19-26%) but had an abundance of < 1 % at peat sites. Equally, Ericae was more abundant in peat sites (8 %), but less so at podzol sites (<4 %). There were some more site specific taxa also, including Xylopini and

Megaspora at Peaknaze Podzol (both at 3 % abundance), Cylichnium at Peaknaze Peat (12 %) and Albostramineum at Migneint Peat (7 %).



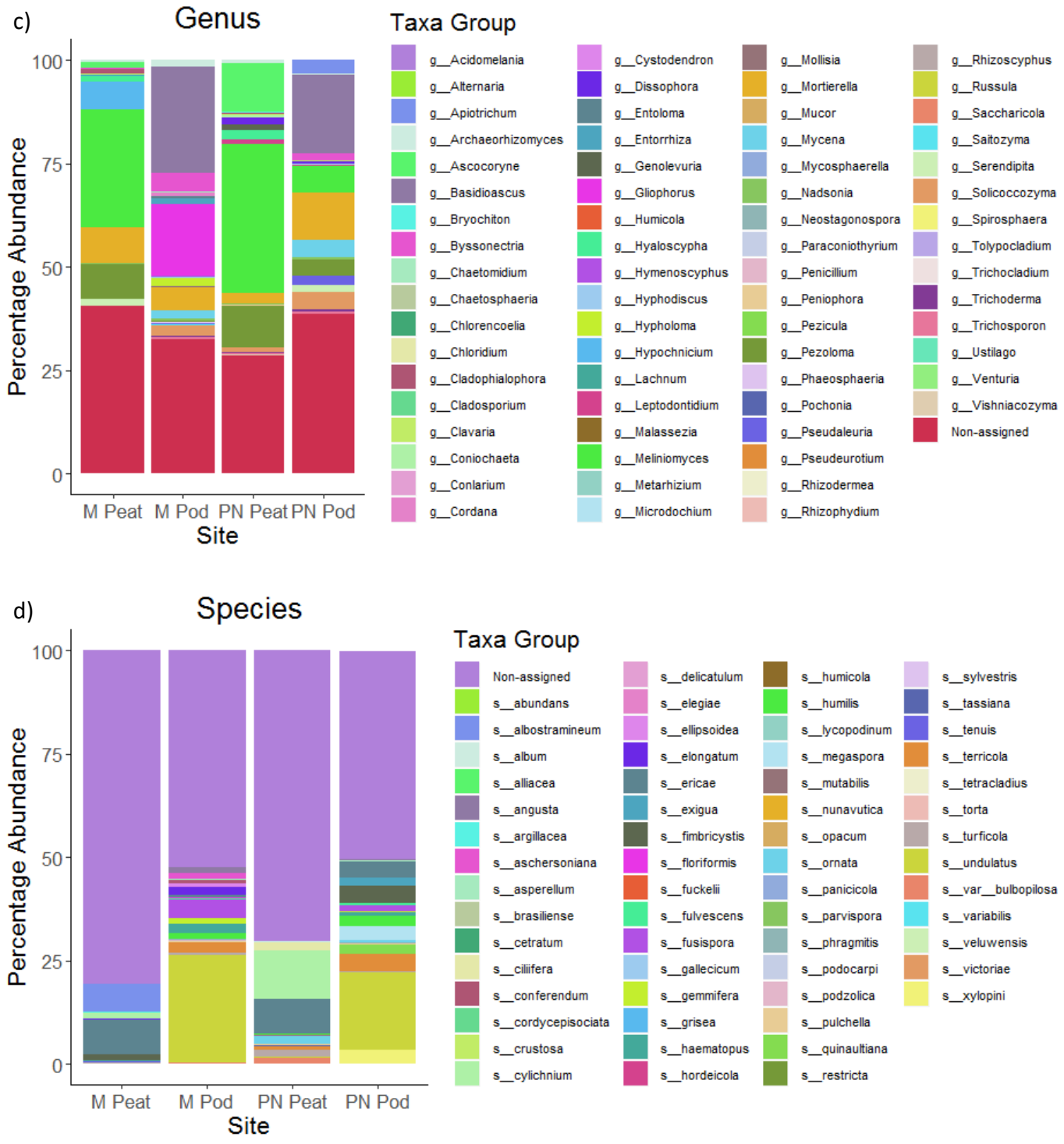


Figure 7.2: Percentage relative abundance of fungal taxa at phyla (a), order (b), genus (c) and species (c) taxonomic levels for each site. 'M' refers to Migneint, 'PN' refers to Peaknaze and 'Pod' refers to podzol soil.

7.3.2 Diversity

7.3.2.1 Alpha diversity

7.3.2.1.1 Bacterial and archaeal alpha diversity

Alpha diversity is a measure of taxa richness and abundance within a sample, and is a way of comparing total diversity in different communities (Lozupone and Knight, 2008). There are three indices which have been used to measure alpha diversity, each reflect a different element of alpha diversity. Chao1 measures richness (Chao, 1984), Shannon's index measures both richness and eveness of taxa (Ludwig and Reynolds, 1988), as does Simpson's, which reflects the probability of two individuals of the same taxa being randomly chosen (Simpson, 1949, Lemos et al., 2011). Data was assessed with a Two-way Anova comparing alpha diversity between sites (four levels) and treatments (three levels). As the effect of treatment on diversity was found to be insignificant, and so data is only presented below for sites across all treatments.

At phyla level, bacterial alpha diversity was found to be significantly different between sites for all diversity indices assessed using a Two-way ANOVA (Chao 1 P = <0.001; Shannon P = <0.001, Simpson's P = <0.001). Further analysis with Tukey's Posthoc showed a significant difference between peat sites for all indices (Chao1 P = <0.001, Shannon P = <0.001, Simpson P = 0.001) (*Figure 7.3a*), with a higher diversity at Migneint Peat than at Peaknaze Peat (*Figure 7.3*). Shannon's Index was also significantly different between podzol sites (P = 0.008).

However, diversity did not differ between soil types at each site location for any of the indices used. In addition, when comparing treatments, Simpson's and Shannon's index had a significant P value (P = 0.012; P = 0.016) but further investigation with a Tukey Posthoc showed no significant differences of interest between treatment plots at individual sites. Treatments were also shown to be insignificant in influencing diversity under Chao1 (P = 0.326). Finally, the treatment effect was found not to be dependent on site location or soil type (Chao1 P = 0.766; Shannon P = 0.386; Simpson's P = 0.555).

At order level, both Shannon and Simpson's diversity indices, which both account for eveness as well as richness, show there was a significant difference in diversity between site locations, with more diversity at Migneint both for peat (Shannon P = <0.001; Simpson's P =

<0.001) and podzol (Shannon P = <0.001; Simpson's P = <0.001) soils than at Peaknaze (*Figure 7.3b*). Chao1 shows that diversity was significantly greater at Migneint compared to Peaknaze for peat sites (P = 0.037), but not for podzol sites (P = 0.862).

There was a significant difference in diversity between soils types at Peaknaze (Chao1 P = 0.043; Shannon P = 0.002; Simpson's P = 0.034), with a greater diversity in podzol than in peat, but diversity was similar between soils at Migneint (Chao1 P = 0.953; Shannon P = 0.848; Simpson's P =0.784). Treatments were found to be significant for both Shannon (P = 0.042) and Simpson's (P = 0.001), but there were not significant differences between treatment plots at each site when investigated further with a Post hoc test.

At genus level, all indices show there was significantly more diversity at Migneint peat than at Peaknaze peat (Chao1 P = 0.005; Shannon P = <0.001; Simpson's P = <0.001) (*Figure 7.3c*). Only Shannon show a significant site location difference between podzol sites (P = 0.037). Finally, as with order level, there was significantly more diversity in podzol than in peat at Peaknaze only with Shannon (P = 0.009) and Simpson's (P = 0.041) indices. Treatment was found to be significant for Shannon (P = 0.045) but there were no significant differences between treatment plots at each site when investigated further with a Post hoc test.

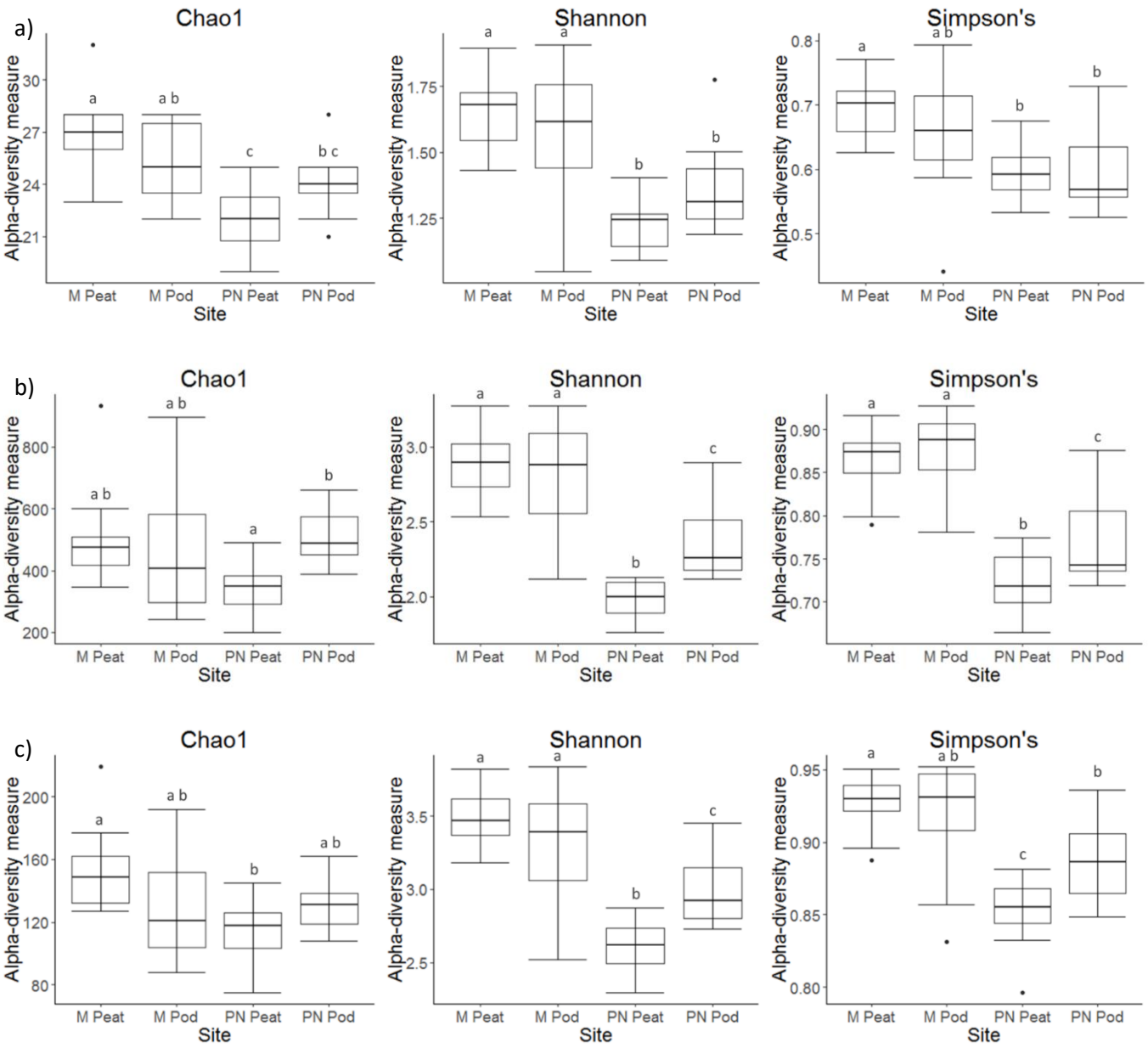


Figure 7.3: Boxplots of 16S alpha diversity indices (Chao1, Shannon and Simpson's) based on phyla (a), order (b) and genus (c) taxonomic levels, for each site. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way ANOVA (comparing sites (4 levels) and treatments (3 levels)).

7.3.2.1.2 Fungal alpha diversity

There was significantly more fungal diversity in podzol soils compared to peat at both Migneint (Chao1 P = <0.001; Shannon P = <0.001; Simpson's P = 0.007) and Peaknaze (Chao1 P = <0.001; Shannon P = <0.001; Simpson's P = <0.001) at order level (*Figure 7.4a*). At lower taxonomic levels (*Figures 7.4b & c*), this difference in diversity between peat and podzols becomes less apparent, with significance occurring between Migneint soils with Chao1

diversity index at genus (P = 0.006) and species (P = 0.031) level. There were no significant differences in fungal diversity between site locations for any of the taxonomic levels investigated or any of the diversity indices used.

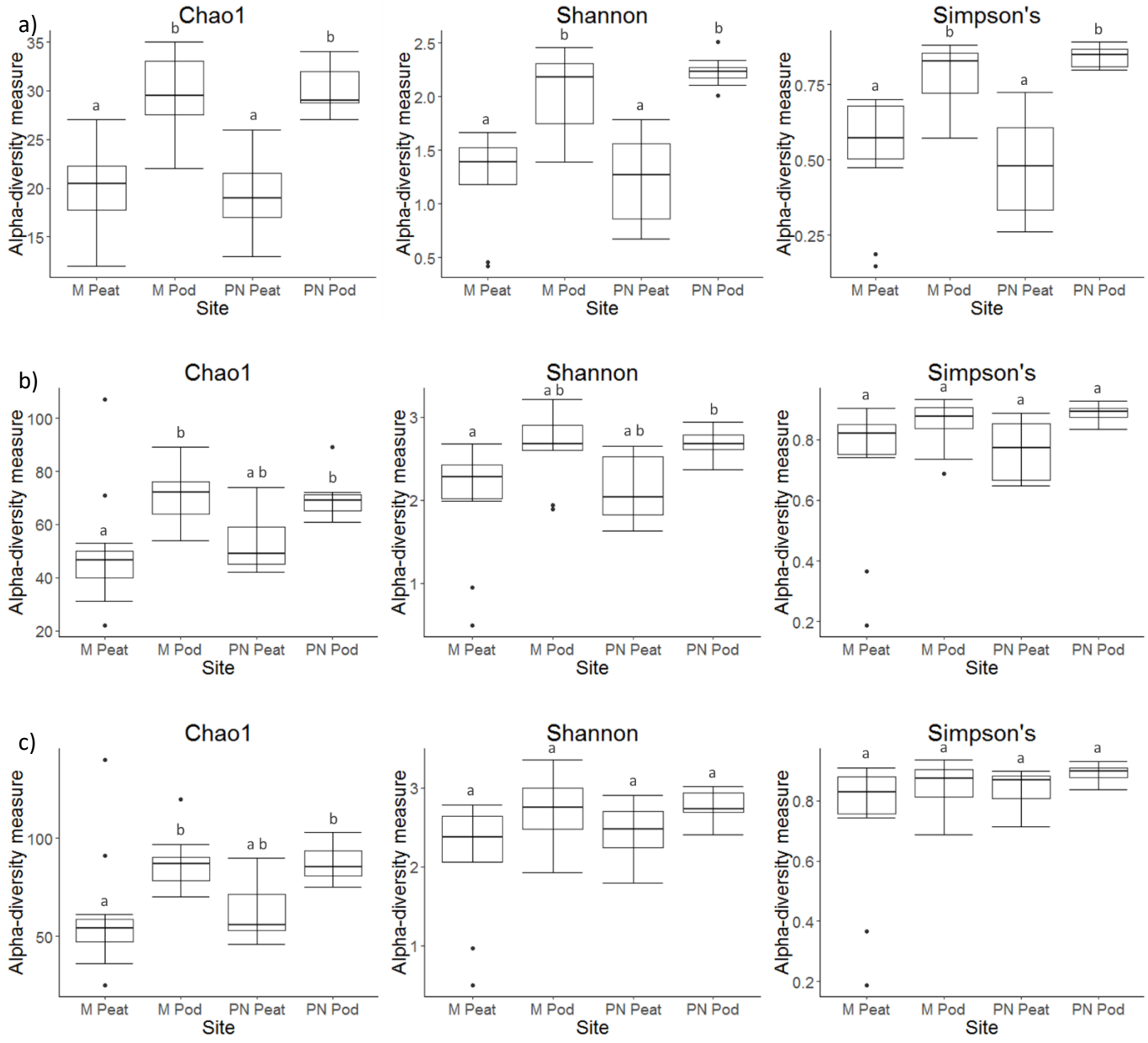


Figure 7.4: Boxplots of ITS alpha diversity indices (Chao1, Shannon and Simpson's) based on order (a), genus (b) and species (c) taxonomic levels, for each site. Letters show where statistically significant differences occurred, as determined using a post hoc test of a One-Way Anova (comparing sites (4 levels)).

The effect on treatment could not be tested as part of a Two-way ANOVA with sites, due to the amount of replications lost from the dataset due to low reads. However, all samples from Migneint Peat were retained, resulting in four replications per treatment at this site. A One-way ANOVA was performed on all alpha diversity indices and treatment was found to

be insignificant at Migneint Peat for order (Shannon P = 0.251; Simpson's P = 0.188; Chao1 P = 0.788), genus (Shannon P = 0.522; Simpson's P = 0.571; Chao1 P = 0.595), and species (Shannon P = 0.555; Simpson's P = 0.608; Chao1 P = 0.564) taxonomic levels.

7.3.2.1.3 Alpha diversity summary

There was more diversity at Migneint than at Peaknaze regardless of soil type for bacterial and archaeal data. There were also some soil differences, with more diversity in podzol soil than in peat. However, this was only apparent at Peaknaze and at higher taxonomic levels. This suggests that bacterial and archaeal diversity are most sensitive to site location differences, which in this case represents a 'natural' pollution deposition gradient. Alternatively, fungal diversity did not differ between site locations, but was more sensitive to soil differences, in this case diversity being greater in podzol soil than in peat. Neither fungal nor bacterial diversity responded to acidity treatments (see *Figures A7.1-A7.6* in Appendix).

7.3.2.2 Beta Diversity

7.3.2.2.1 Bacterial and archaeal beta diversity

Beta diversity is the diversity between habitats, or rather the ratio between local (alpha) and regional diversity. It is often used to measure a change in diversity along environmental gradients (Lozupone and Knight, 2008). Bray-Curtis distance matrix was used to quantify the compositional dissimilarity between sites and treatments. Principal coordinates analysis (PCoA) was used to visually represent the distance matrix data over a two-dimensional space, in an attempt to visualise shifts in bacterial community composition between sites and treatments. The significance and strength of sample groupings based on distance matrix was analysed statistically using ANOSIM (analysis of similarities).

When comparing sites using Bray-Curtis for 16S data results from ANOSIM statistical test were highly significant (P = <0.001), with an R value of 0.929, suggesting a significant difference in bacterial community composition between the four experimental sites. In addition, there were no significant dissimilarities in bacterial community diversity between treatments (P = 0.989). PCoA plots at phyla level (*Figure 7.5a*) show a clear clustering of samples from Peaknaze Peat which are distinct from Migneint sites, with a slight crossover with Peaknaze Podzol, suggesting that bacterial composition at Peaknaze Peat is dissimilar

to the other sites. In contrast, there is no clustering for treatments, suggesting there is no significant dissimilarity in bacterial community composition between treatments.

At both order and genus taxonomic levels (*Figures 7.5b & c*) there was a greater distance between site locations, with individual clusters for each site location being distant from each other with minimal overlap, and a clear separation between experimental sites at Peaknaze and experimental sites at Migneint. As with phyla level, Peaknaze Peat is the most dissimilar site.

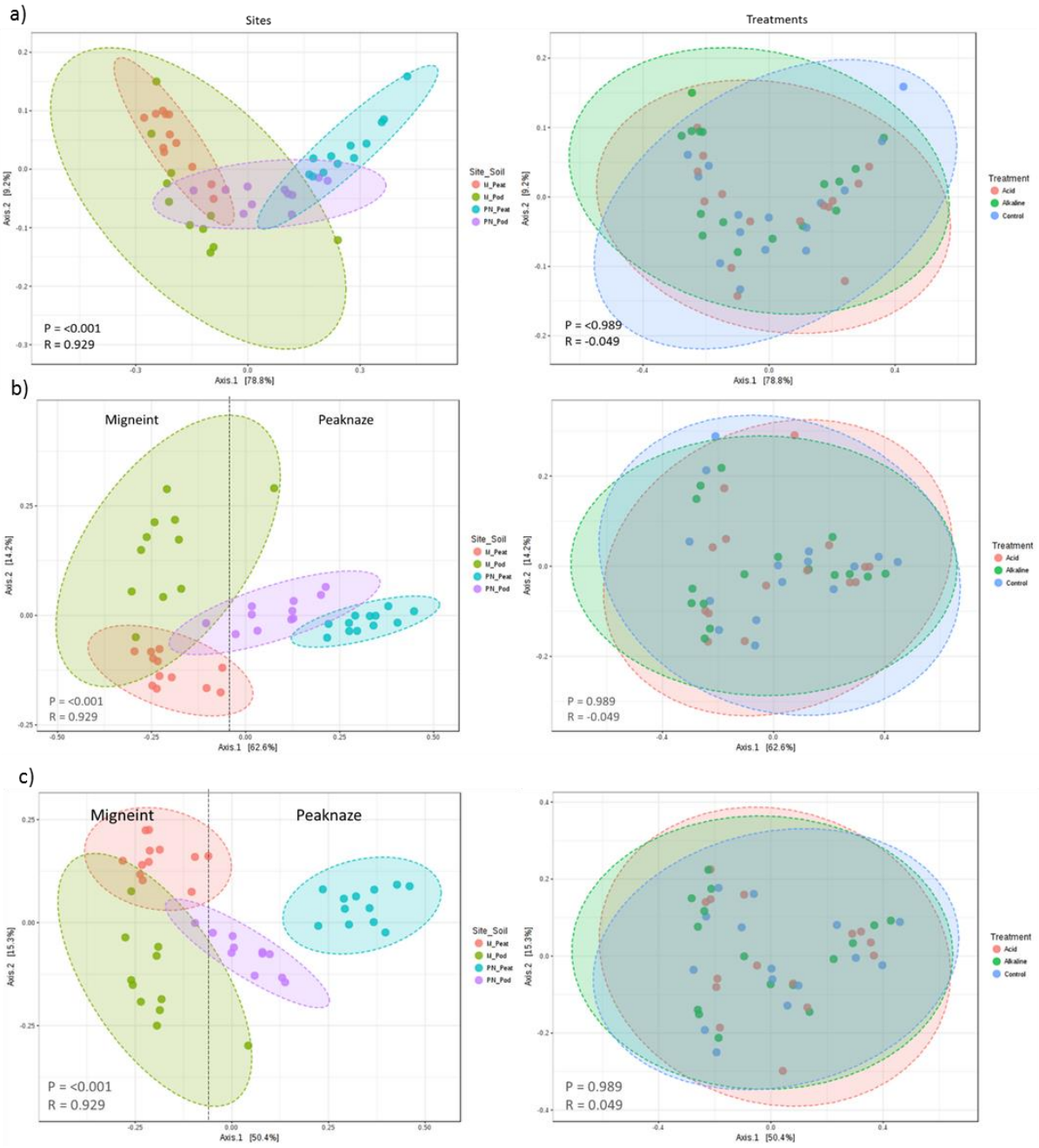


Figure 7.5: PCoA plot for 16S beta diversity at phyla (a), order (b) and genus (c) taxonomic levels using Bray-Curtis distance matrix. Results of ANOSIM test are stated (P and R values). Left panel is groupings for sites and right panel is groupings for treatments for all sites.

7.3.2.2.2 Fungal beta diversity

When comparing sites using Bray-Curtis for ITS data, results from ANOSIM statistical test were significant (P = <0.001), with an R value of 0.740, suggesting a significant difference in fungal community composition between experimental sites. PCoA plots show similar clustering between peat sites, which are separate from podzol sites, at all taxonomic levels (*Figure 7.6*). This suggests that fungal community composition is different between peat and podzol soils, but that there is little difference in fungal communities between Peaknaze and Migneint.

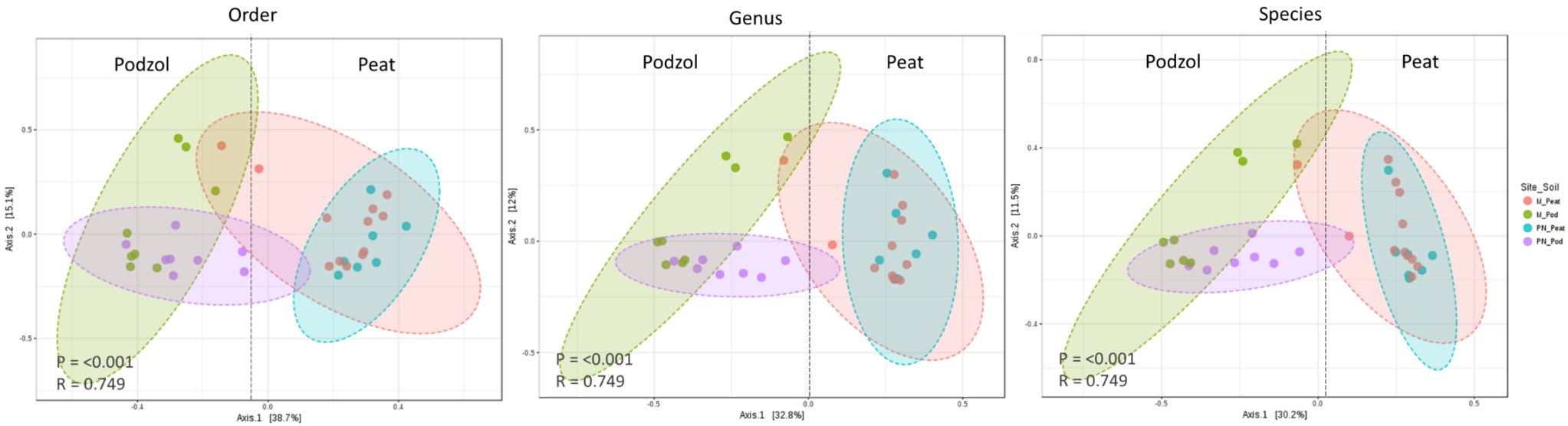


Figure 7.6: PCoA plots for ITS beta diversity at three taxonomic levels (order, genus and species) using Bray-Curtis distance matrix. Results of ANOSIM test are stated (P and R values). Plots have been annotated to show differences in groupings between soil types.

7.3.2.2.3 Beta diversity summary

The PCoA ordination plots show a clear separation in clustering of samples from different site locations for 16S data, whilst ITS data show separate clustering of samples for soil type. This suggests that community diversity for bacteria and archaea are different between Migneint and Peaknaze, whilst fungal diversity are different between peat and podzol soils regardless of site location.

7.3.3 Abundance of core microbiome and rare taxa

7.3.3.1 Bacterial and archaeal core microbiome

The core microbiome consists of the most abundant taxa across a high proportion of samples. A total of ten core taxa were identified at phyla level with detection thresholds set at 0.2 % relative abundance and a sample prevalence of 80 %. Therefore these taxa had a relative abundance of at least 0.2 % in 80 % of samples. As expected, the core consisted of the most common taxa, as presented in *Table 7.4*, with Acidobacteria, Proteobacteria and Verrucomicrobia having the greatest prevalence in samples. At genus level, the relative abundance threshold was reduced to 0.1 % with an 80 % sample prevalence, which resulted in seven taxa being identified as the core. The most prevalent of these included the unnamed group (g_), Candidatus solibacter and Candidatus koribacter.

A two-way ANOVA test was run on core microbiome taxa comparing their actual abundance between sites (four levels) and treatments (three levels) (*Table 7.4*) for each taxonomic group, at two taxonomic levels (phylum and genus). Treatments had no significant effect on abundance of any taxa at any taxonomic level, baring Candidatus Koribacter ($P = 0.010$). Interestingly, there was a significant interaction between site and treatment for Acidobacteria at phyla level, suggesting that abundance may be affected by treatments and this is dependent on site. This was also the case for an unnamed group and Candidatus Koribacter abundances at genus level. All taxa differed significant between sites at both taxonomic levels, suggesting that there are environmental variables associated with the different soils and site locations which are influencing abundance.

Table 7.4: P value results from a Two-way ANOVA, comparing sites (four levels) and treatments (three levels), performed on actual abundance data for 16S core microbiome taxa at phyla and genus taxonomic levels. Any transformations applied to data to meet assumptions of Anova are stated.

Taxonomic Group	Taxa	Site	Treatment	Site:Treatment
Phylum	Acidobacteria (log)	**<0.001**	0.249	**0.026**
	Actinobacteria (sqrt)	**<0.001**	0.405	0.542
	Bacteroidetes	**<0.001**	0.421	0.507
	Chlamydiae (log)	**<0.001**	0.559	0.515
	Crenarchaeota (log)	**0.024**	0.615	0.425
	FCPU426	**0.024**	0.425	0.425
	Planctomycetes (sqrt)	**<0.001**	0.479	0.127
	Proteobacteria	**0.003**	0.759	0.129
	Verrucomicrobia	**<0.001**	0.405	0.542
	WPS_2 (log)	**<0.001**	0.141	0.082
Genus	g_ (unamed)	**<0.001**	0.844	**0.039**
	Candidatus solibacter (log)	**<0.001**	0.516	**0.002**
	Candidatus koribacter (log)	**<0.001**	**0.010**	0.075
	Rhodoplanes	**<0.001**	0.771	0.987
	Opitutus	**<0.001**	0.133	0.181
	Candidatus Rhabdochlamydia	**<0.001**	0.459	0.671
	Herminiimonas	**<0.001**	0.176	0.229

7.3.3.1.1 Acidobacteria taxa

Acidobacteria abundance were greater at Peaknaze than at Migneint, although this was only significant for podzol sites (P = 0.016) and not for peat (P = 0.182) when comparing control plots (*Figure 7.7*). Abundance did not differ between treatment plots at any site other than Migneint Podzol, where abundance was significantly greater for acid plots compared to alkaline (P = 0.026), but this difference was barely significant when compared to the control (P = 0.076).

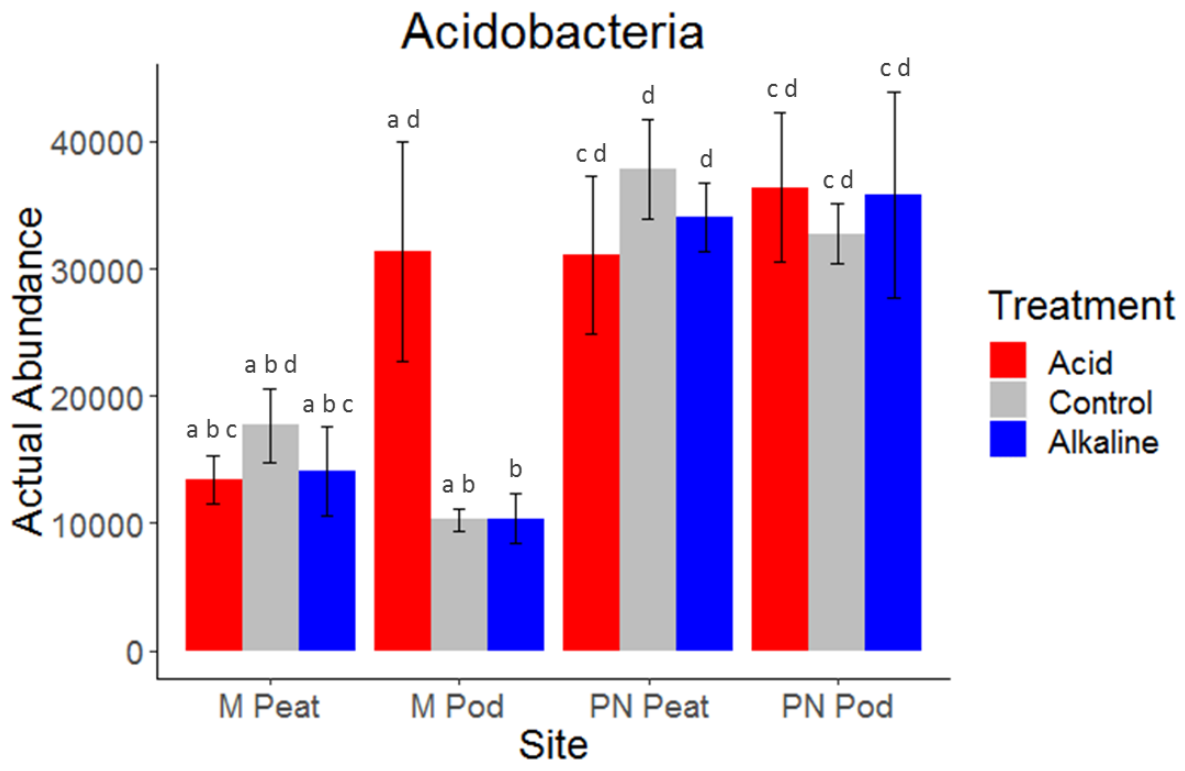


Figure 7.7: Mean of actual abundance of Acidobacteria at different sites and receiving different treatments. Data was log transformed to meet the assumptions of Anova. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing sites (four levels) and treatments (three levels)).

Within the Acidobacteria phyla taxonomic group, there were four taxa identified at genus level. Three of these were part of the core microbiome at genus level (unnamed group (g__), Candidatus Koribacter and Candidatus Solibacter), and Telmatobacter was a rare taxa with few data points and site specific to Peaknaze Peat (*Table 7.4*).

Table 7.4: Mean (± standard error) of actual abundance of Telmatobacter at different sites and receiving different treatments.

Site	Acid	Control	Alkaline
M Peat	0.00 ± 0.00	5.50 ± 5.50	0.00 ± 0.00
M Podzol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
PN Peat	18.00 ± 11.27	59.00 ± 28.62	41.00 ± 21.86
PN Podzol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Abundances of C. Koribacter and C. Solibacter were greater at Peaknaze than at Migneint, with a difference in actual abundance of 2324 (when comparing mean of control plots) and 1181 respectively for peat, and a difference of 1874 and 1811 for podzol soil (*Figure 7.8*). However, this difference between control plots was only significant when comparing abundances of C. Solibacter between Migneint Podzol and Peaknaze Podzol (P = 0.001). Acidity treatments had no effect on abundances of either taxa at the acidic site of Peaknaze, or at the Migneint Peat site where soil and pore water pH did not respond to treatments (Chapter 4). However, at the Migneint Podzol site, there is an increase in abundance of 2753 for C. Koribacter and 1193 for C. Solibacter with the acid treatment compared to the control, although this was only significant for C. Solibacter (P = 0.020), and barely significant for C. Koribacter (P = 0.079).

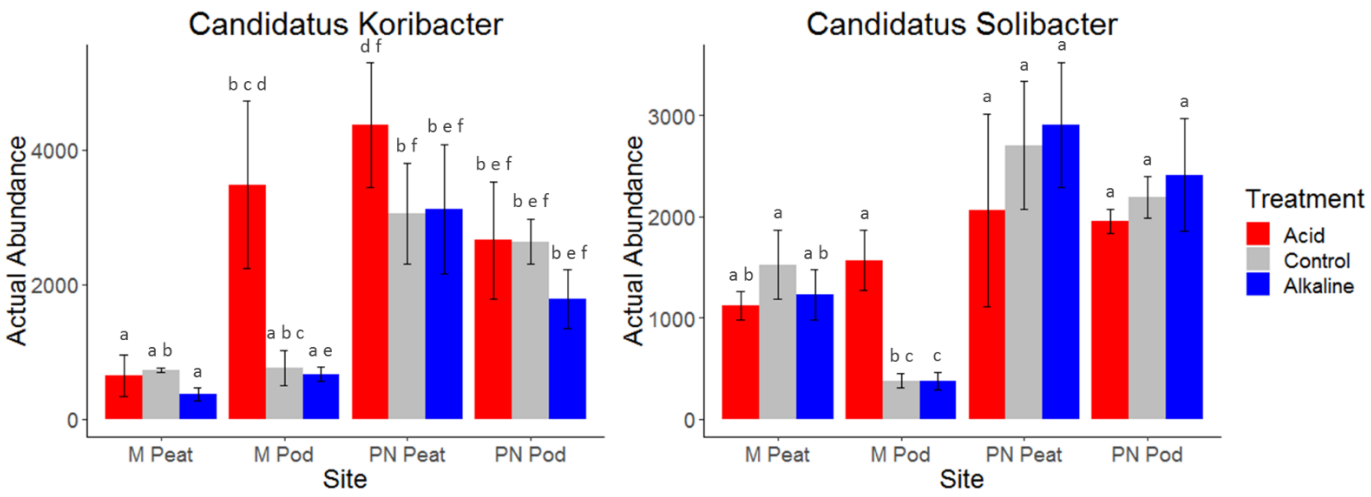


Figure 7.8: Mean of actual abundance of Candidatus Koribacter (left panel) and Candidatus Solibacter (right panel) at different sites and receiving different treatments. Both datasets were log transformed to meet the assumptions of Anova. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing sites (four levels) and treatments (three levels)).

7.3.3.1.2 Other 16S taxa groups

Other phylum taxonomic groups were also assessed as to whether they responded to treatments using a Two-way Anova comparing sites (4 levels) and treatments (3 levels) (*Table 7.5*). The only group which showed any statistical indication of responding to acidity treatments was TM6, which although there was an insignificant P value associated with treatments (P = 0.221), the interaction between sites and treatments was found to be significant (P = 0.005), suggesting that abundance may be responding to acidity at a particular site.

Table 7.5: Table of P value results from a Two-way Anova performed on 16S phyla taxonomic groups, comparing sites (four levels) and treatments (three levels).

Taxa	Site	Treatment	Site:Treatment
AD3	**<0.001**	0.910	0.742
Armatimonadetes	0.077	0.903	0.635
Chlorobi	**<0.001**	0.375	0.278
Chloroflexi	0.094	0.538	0.746
Cyanobacteria	**<0.001**	0.661	0.865
Eluximicrobia	**<0.001**	0.453	0.806
Euryarchaeota	**0.039**	0.232	0.478
Fibrobacteres	**<0.001**	0.989	0.663
GAL15	**<0.001**	0.257	0.276
Gemmatimonadetes	**<0.001**	0.516	0.241
Nitrospirae	**<0.001**	0.928	0.999
OD1	**0.026**	0.990	0.998
Parvarchaeota	**<0.001**	0.754	0.930
Spirochaetes	**0.004**	0.252	0.658
TM6	**0.046**	**0.046**	**0.026**
TM7	**<0.001**	0.320	0.631
Non-assigned	**<0.001**	0.550	0.468

When investigated further with a Poshoc test (*Figure 7.9*), abundance of TM6 in acid plots was significantly higher than in control (P = 0.045) and alkaline (P = 0.019) plots at the Migneint Podzol site. This suggests that abundance was significantly lower at the Migneint Podzol site (~1 compared to 55-134 at other sites), and then increased with acid treatments to 87 at this site, making abundance similar to other sites. At lower taxonomic levels, individual taxa have not been assigned to groups and so it was not possible to investigate which taxa were responding to treatments at this site further.

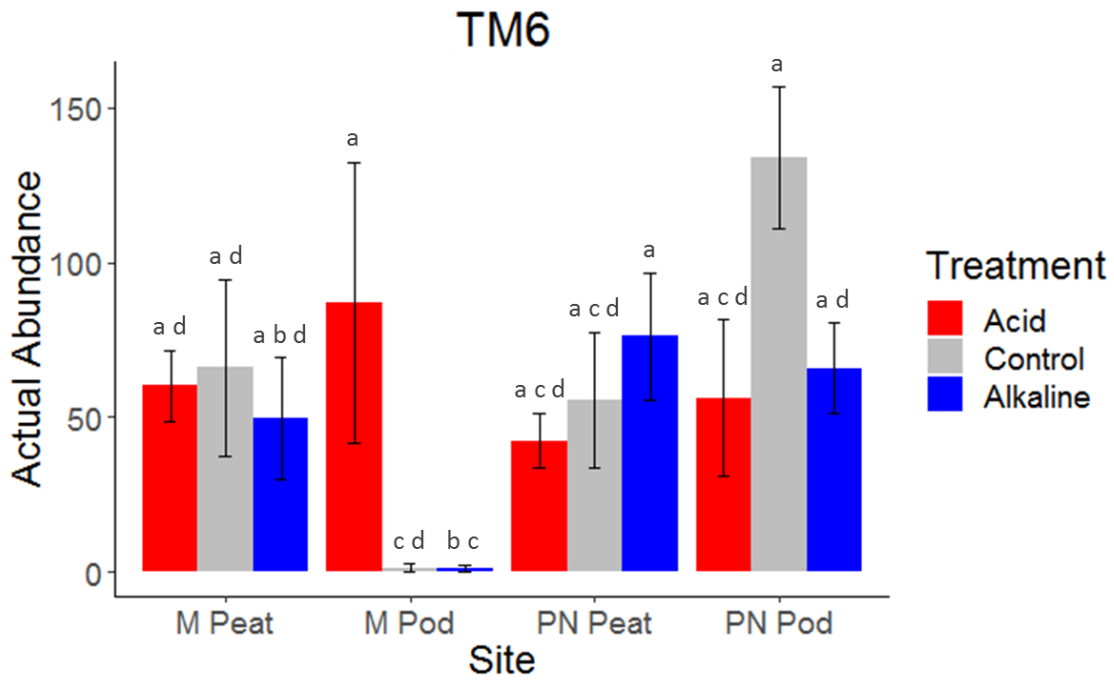


Figure 7.9: Mean of actual abundance of phyla taxa group TM6 at different sites and receiving different treatments. Data was transformed (sqrt) to meet the assumptions of Anova. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing sites (four levels) and treatments (three levels)).

7.3.3.2 Fungal core microbiome

The core microbiome was obtained for ITS data at family taxonomic level. A total of five core taxa were identified with detection thresholds set at 0.2 % relative abundance and a sample prevalence of 80 %, which included a group of non-assigned taxa (these being the most prevalent). The identified core taxa groups were assessed as to whether abundances differed between different site locations and soil types (*Table 7.6*). The abundance of three groups were found to differ significantly between peat and podzol soil (Leotiaceae P = 0.003; Helotiaceae P = <0.001; Helotiales fam Incertae sedis P = 0.002). For the abundance of Leotiaceae, this was found to depend on site location (P = 0.019). In addition, the abundance of Helotiales fam Incertae sedis was found to also differ between site locations (P = 0.028).

Table 7.6: P value results from a Two-way Anova, one comparing site locations (two levels) and soil type (two levels), performed on actual abundance data for ITS core microbiome taxa. Transformations that were applied to meet the assumptions of Anova are also stated in the table.

Family Group	Site	Soil	Site:Soil
Leotiaceae (sqrt)	0.190	**0.003**	**0.019**
Mortierellaceae	0.614	0.499	0.163
Helotiaceae (sqrt)	0.051	**<0.001**	0.096
Helotiales fam Incertae sedis (sqrt)	**0.028**	**0.002**	0.699
Hyaloscyphaceae (sqrt)	0.502	0.088	0.407

When investigated further with a Posthoc analysis (*Figure 7.10*), abundance of all taxa was found to be lower at Migneint Podzol compared to other sites, with an abundance of just 89 for Leotiaceae, 25 for Helotiaceae, and 375 for Helotiales fam Incertae sedis. This difference in abundance at Migneint Podzol was significantly different to all other sites for Leotiaceae and Helotiaceae, but only significantly different to Peaknaze Peat for Helotiales fam Incertae sedis. Abundance of all taxa groups was also lower at Peaknaze Podzol compared to Peaknaze Peat, although this was only significant for Helotiaceae (P = 0.013).

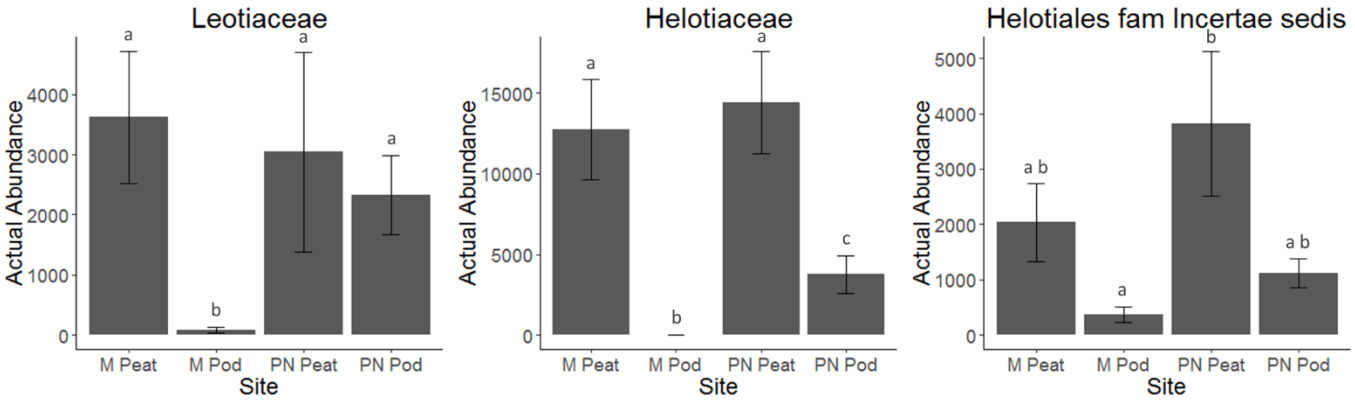


Figure 7.10: Mean (of all plots) of actual abundance of family taxa groups at different site locations and soil types. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing site locations (two levels) and soil type (two levels)).

7.3.3.2.1 Leotiaceae taxa

Two taxa groups were identified at species level within the taxa group of Leotiaceae. Ericae was the most abundant taxa with a maximum mean abundance of 3795 (measured at Migneint Peat) (*Figure 7.11*). This is similar to the maximum mean abundance of 3622 for the Leotiaceae taxa group at family level. Abundance of Ericae is significantly lower at Migneint Podzol, at 150 compared to an abundance of 1862-4373 at other sites. This was also the case for Leotiaceae, and so this suggests that Ericae accounts for the soil differences seen in the Leotiaceae family group. Ciliifera is site specific to Peaknaze, with a mean abundance of 557, whilst it was not detected at other sites.

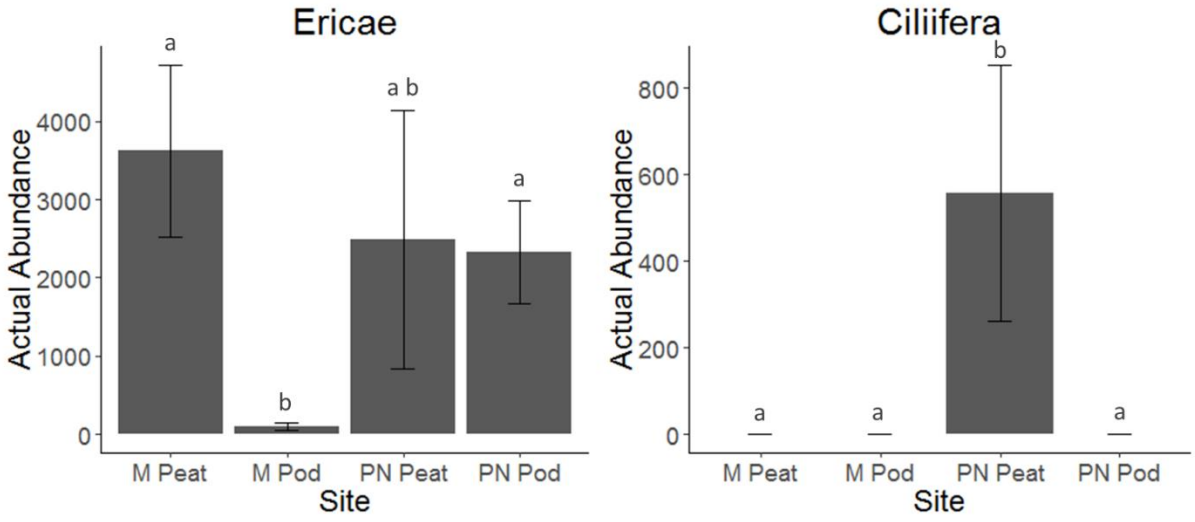


Figure 7.11: Mean (of all plots) of actual abundance of Ericae and Ciliifera taxa groups at species level, at different site locations and soil types. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing site locations (two levels) and soil type (two levels)).

7.3.3.2.2 Helotiaceae taxa

Within the Helotiaceae family, there were four taxa identified at species level (*Figure 7.12*). Cylichnium had the greatest abundance compared to other taxa within the Helotiaceae family, with a maximum mean abundance of 3529, whilst other groups were rare with very low mean maximum abundances of 31 for Tetracladius, 27 for Torta and 32 for Variabilis. As the maximum mean abundance of the Helotiaceae group at family taxonomic level was 14423, a large proportion of this group is non-assigned taxa at species level, suggesting that

there are other unidentified groups which are contributing to this difference in abundance between soil types.

Cylichnium was only detected at Peat sites and abundance was significantly higher at Peaknaze. Torta was only measured at Peaknaze, with higher abundances measured at the podzol site, whilst due to the high variability in the data and the low abundances measured, there were no significant differences between site locations. This was also the case for Variabilis. Finally, Tetracladius was site specific to Migneint Podzol.

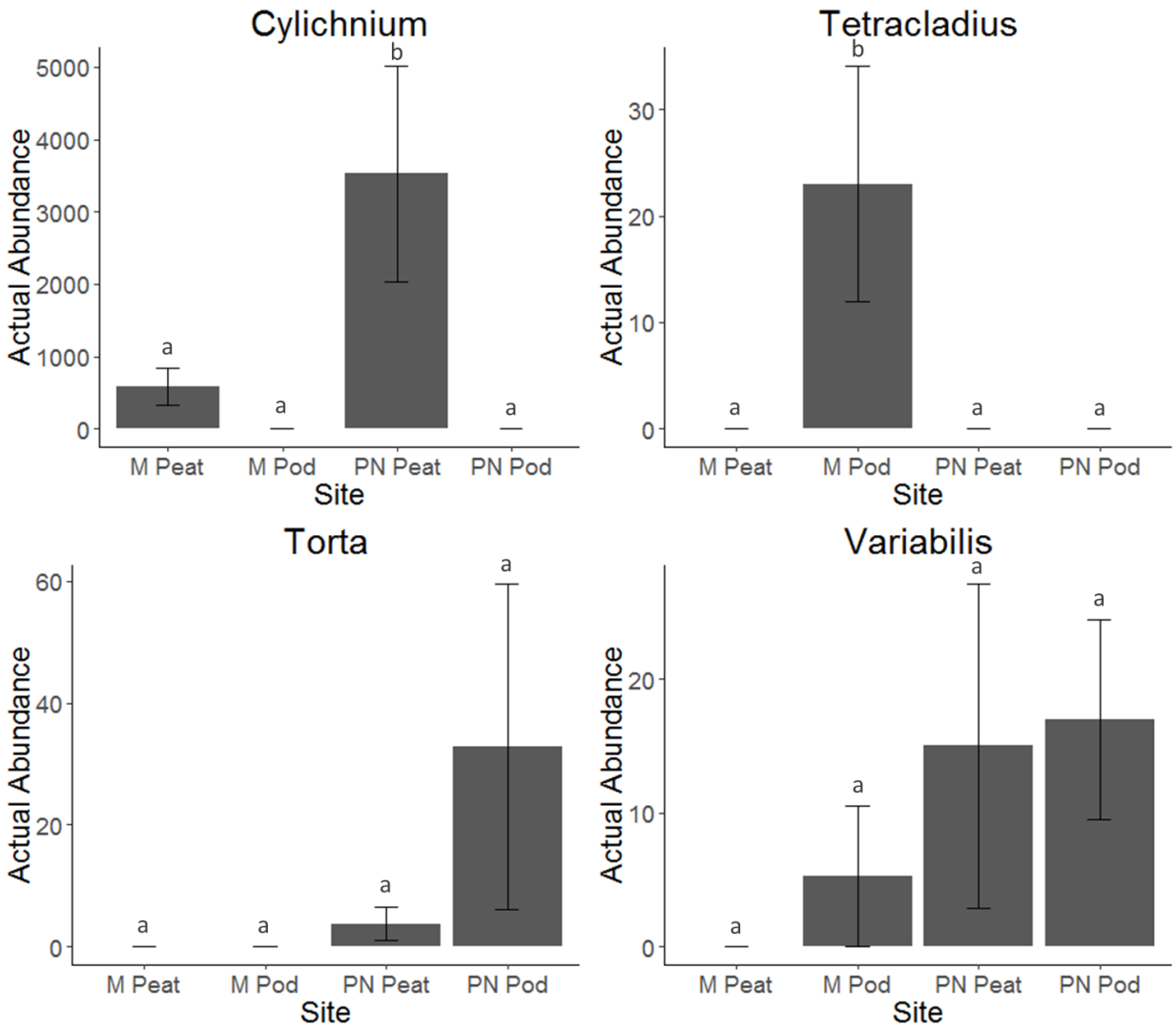


Figure 7.12: Mean (of all plots) of actual abundance of Cylichnium, Tetracladius, Torta and Variabilis taxa groups at species level, at different site locations and soil types. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing site locations (two levels) and soil type (two levels)).

7.3.3.2.3 Helotiales fam Incertae sedis taxa

For the Helotiales fam Incertae sedis taxonomic group, only three taxa were identified at species level, all of which had very low abundances of up to 61, compared to a maximum mean abundance of 3817 for Helotiales fam Incertae sedis which suggests there are other unidentified taxa at species level which are contributing to differences in abundance between soil types (*Figure 7.13*). Panicicola had significantly higher abundance at Peaknaze Podzol compared to other sites, whilst Floriformis was only detected at Migneint Podzol. Humicola was only measured at Peaknaze, with a significantly higher abundance at the podzol site.

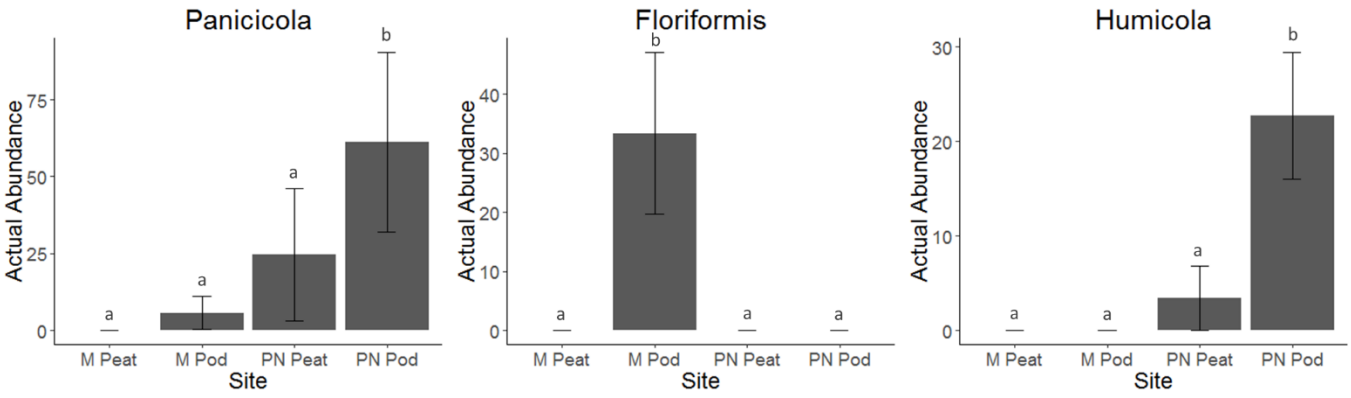


Figure 7.13: Mean (of all plots) of actual abundance of Panicicola, Floriformis and Humicola taxa groups at species level, at different site locations and soil types. Error bars represent standard error. Letters show where statistically significant differences occurred, as determined using a post hoc test of a Two-Way Anova (comparing site location (two levels) and soil type (two levels)).

7.3.3.2.4 Fungal core microbiome taxa summary

The core microbiome which occupy most sites was identified at family level, and differences in abundance across site locations and soil types was assessed. Within the core, there were three taxa family groups which had significant soil differences (Leotiaceae, Helotiaceae, and Helotiales fam Incertae sedis). There was generally a lower abundance in podzol soil than in peat, with abundances being lowest at Migneint Podzol for all taxa. The taxa within these family groups was identified at species level, and these were assessed to see whether individual taxa contributing to these soil differences could be identified. Within the Leotiaceae family, Ericae accounted for much of the soil differences seen, whilst a rarer taxa which was specific to Peaknaze Peat was identified (Ciliifera). Mostly rare taxa with low

abundances were identified for the Helotiaceae and Helotiales fam Incertae sedis family groups, and so there were unidentified taxa which were contributing to the soil differences seen within these taxa groups.

7.3.4 Microbial community relationship with soil parameters

This section investigates whether there is a statistically significant relationship between microbial communities and soil parameters, with a focus on pH and DOC concentration. To do this scatterplots have been used to visualise relationship and Spearman's Rank to assess significance at 95 % certainty and strength of relationship.

7.3.4.1 Bacterial and archaeal alpha diversity

At genus taxonomic level, all diversity indices for bacterial and archaeal alpha diversity were found to be positively and significantly related to both soil extract pH and DOC (*Figure 7.14*). In addition, relationships were also found to be significant at phylum and order taxonomic levels, barring Chao1 at order level for pH (P = 0.085) and DOC (P = 0.629) (see *Figures A7.7 & 9*, and *Tables A7.3 & 4* in Appendix). Relationships with pH were also found to have high Rho values with a range of 0.416-0.735, suggesting relationships were strong. Relationships with soil DOC were weaker with a range in Rho values of 0.259-0.443.

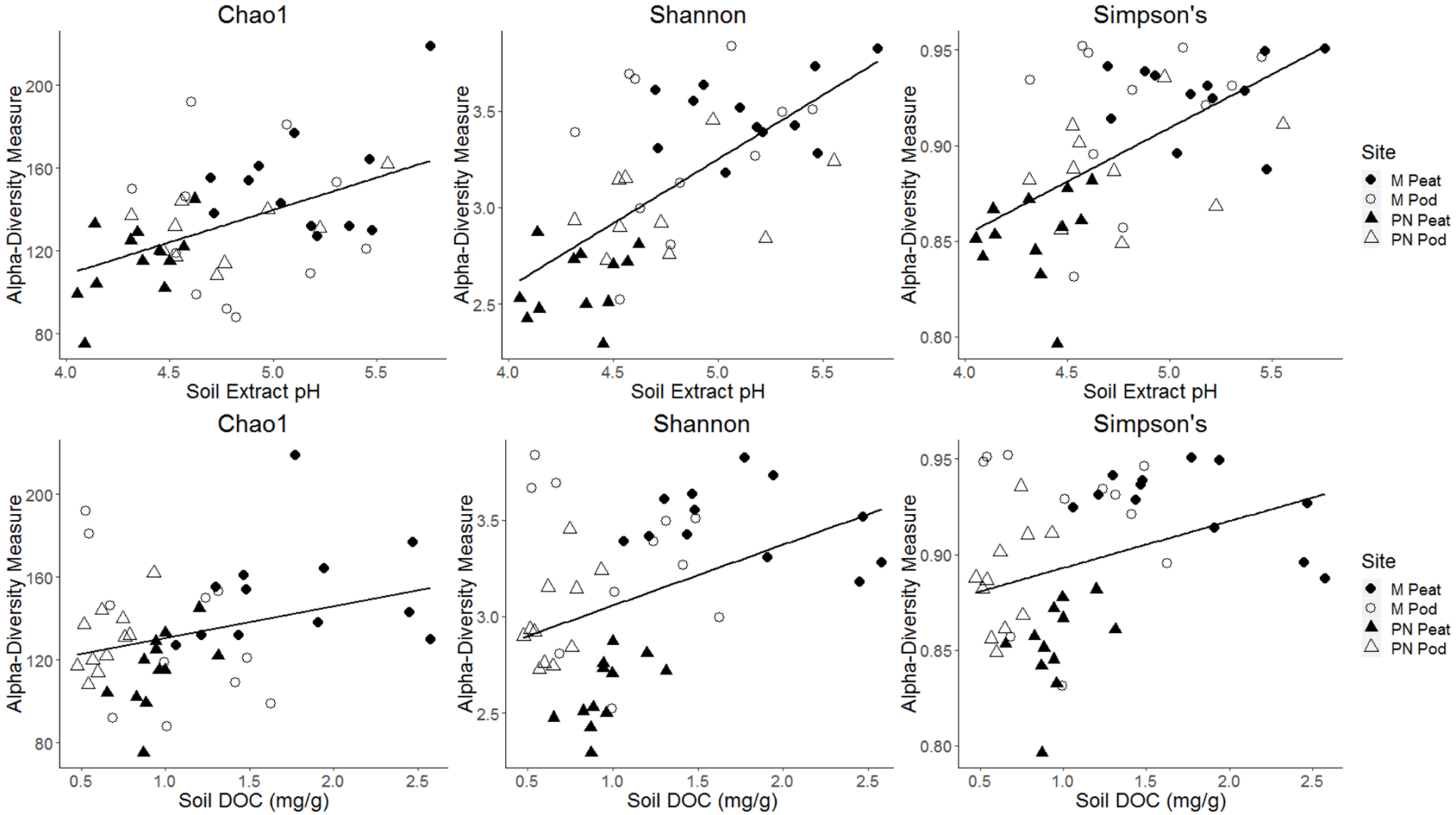


Figure 7.14: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH (top panel), and extracted DOC (lower panel), for 16S data at genus taxonomic level.

7.3.4.2 Fungal alpha diversity

In contrast to bacterial and archaeal data, fungal diversity was found not to be significantly related to soil extract pH (see *Table A7.5* in Appendix). Chao1 diversity index was significantly related to soil DOC at all taxonomic levels, although Rho values suggest relationship were weak, particularly at lower taxonomic levels (see *Table A7.6* in Appendix). Shannon and Simpson's indices were only significantly related to soil DOC at order taxonomic level. All relationships were negative, suggesting that fungal diversity is lower when soil DOC is greater (*Figure 7.15*). However, when studying this relationship further with, there is a clear divide in DOC concentration between soil types, with more DOC and lower diversity in peat, and less DOC but more diversity in podzol.

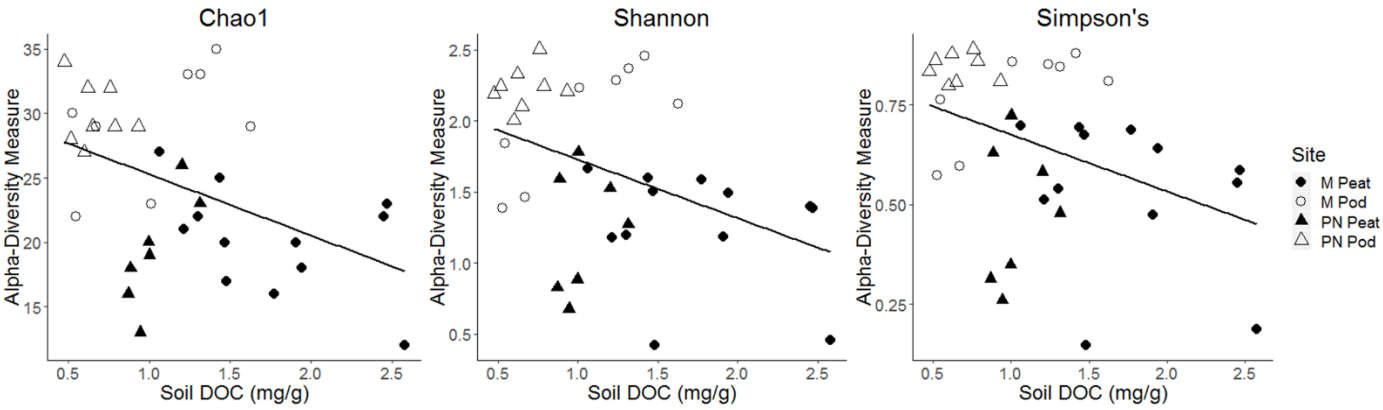


Figure 7.15: Relationships between diversity indices (Chao1, Shannon and Simpson's) with extracted DOC, for ITS data at order taxonomic level.

7.4 Discussion

7.4.1 How does microbial community diversity vary between peat and organo-mineral soil?

For bacterial and archaeal communities (16S rRNA gene amplicons), differences in alpha diversity metrics between peat and organo-mineral (podzol) soils within a particular site were only apparent at lower taxonomic levels at Peaknaze, where diversity (according to Chao, Shannon and Simpson's at order level and Shannon and Simpson's at genus level) was greater in podzol soil. For beta diversity, when assessing the PCoA plots, which allows for a visual comparison of communities between groups, it was only possible to see distinct differences in communities between soil types at genus level, whilst communities from

different soil types were similarly grouped at higher taxonomic levels. Overall, this suggests that bacterial and archaeal community diversity is similar between soil types.

The difference in community diversity between soil types was most apparent for fungi. Alpha diversity was greatest in podzol soil compared to peat, this being consistent at both site locations at order level for all indices, but was only significant for Chao1 at Peaknaze at lower taxonomic levels. This suggests that Chao1 is a better indicator of fungal alpha diversity in organic soils. In addition, PCoA plots of beta diversity show a clear separation between communities in peat and podzol soil at all taxonomic levels examined. Overall, this suggests that fungal community diversity is inherently different between peat and organo-mineral soils. Heterotrophic fungi are a main decomposer of litter in acidic soils, with decomposer and mycorrhizal fungi synthesising extracellular enzymes which degrade organic matter, and therefore play a key role in the cycling of carbon and nutrients (Killham, 1994, Thormann, 2006).

The core microbiome at family level was examined to assess how these differences in diversity between soil types may be affecting abundances of key taxa. Three taxa groups were found to differ significantly between soil types; Leotiaceae, Helotiaceae and Helotiales fam Incertae sedis. These taxa typically had a higher abundance in peat, and a lower abundance in podzol soil, which was lowest at Migneint Podzol. Unfortunately, mostly rare taxa have been assigned to these groups at species level, with a large proportion of abundance of these groups being unidentified taxa. (Artz et al., 2007, Thormann, 2006) also found a lack of identification of OTU's (operational taxonomic units) from peat to at least order level, and argued that the lack of representation of fungal taxa from peat in public databases reflects the uniqueness of fungal communities in peatlands compared to other ecosystems. The results of this study suggests that this argument may also be applied to peaty podzols from upland organic catchments as well as peats. Of the taxa from these core groups which had abundances of >1000, Clyichnium (from Helotiaceae family group), was found only in peat soils, and was particularly abundant at Peaknaze Peat, and Ericae (Leotiaceae family) was significantly less abundant at Migneint Podzol compared to other sites, suggesting there are other taxa which are outcompeting this taxa at this site.

Alpha diversity is higher in podzol, for instance by 31-38 % at Migneint (based on mean of each diversity index for each soil type at order level) and 36-45 % at Peaknaze, whilst the

abundance of the three core taxa examined at family level is lowest in podzol, for instance by 82 % to nearly 100 % at Migneint. A key environmental variable which makes peat distinct from podzols is moisture content. Peats are permanently saturated which creates anoxic conditions and inhibits aerobic decomposition (Ramchunder et al., 2009) resulting in anaerobic activity which is a much slower process (Swift et al., 1979, Keller et al., 2009). In contrast, podzol soils have mineral components and are more freely draining allowing for more aerobic activity, which arguably could result in a lack of hydrological connectivity and therefore spatial isolation and reduced physical opportunities for species to interact. This in turn may reduce competition and therefore allow a greater diversity of fungal taxa in podzol soils to co-exist without being competitively excluded (Torsvik and Øvreås, 2002).

Alternatively, this soil difference in fungal community diversity and abundance of core taxa may be due to the differences in vegetation communities between peat and organo-mineral soil. Both peat sites are dominated by *Eriophorum vaginatum* and *Calluna vulgaris,* but differ in bryophyte cover with *Sphagnum* dominating Migneint Peat (National Vegetation Classification (NVC) code M19) whilst *Pleurozium shreberi* dominates Peaknaze Peat (NVC M20b). Alternatively, the plant communities at podzol sites are classified as subcategories within the NVC U6, and are characterised by *Festuca ovina* and some *Juncus squarrosu*, but differ in that Migneint Podzol largely consists of grasses, whilst Peaknaze Podzol has a large proportion of *Vaccinium myrtillus*.

There is evidence of a linkage between peatland vegetation composition and fungal communities. For instance, Artz et al. (2007) sequenced the ITS region along a gradient of regenerating peatland vegetation, and found fungal community composition structure was related to the composition of vegetation at each site. Fungal abundance in soil has been shown to be strongly influenced by plant diversity (Zak et al., 2003). Thormann et al. (2003) found that there are different fungal assemblages associated with decomposing different litter types in peatland ecosystems, and concluded that litter quality variables, such as total carbon and nitrogen, determined the fungal community present more than pore water chemistry or physical variables. There is also an argument for plant selection of belowground microbial communities through rhizodeposits, which promote beneficial microbial populations, such as symbiotic fungi (Hartman et al., 2008). For instance, changes

in below-ground carbon relating to host specificity have been noted as potential drivers of fungal diversity (Artz et al., 2007, Thormann et al., 2004, De Boer et al., 2006).

The greater abundance of ericoid mycorrhizal fungi at peat sites is likely due to the presence of *Calluna* host plants. *Calluna* also contribute lignin compounds into the peat, and ericoid mycorrhizal fungi within the Helotiaceae family have been shown to produce lignin-degrading enzymes such as phenol oxidases (Burke and Cairney, 2002). Futhermore, some Helotiaceae fungal species have been identified as bryophilous pathogens and are associated with the *Sphagnum* microbiome, where they exploit its dispersal mechanisms for releasing sexual propagules by replacing spores with the fungal anamorph (Redhead and Spicer, 1981, Davey and Currah, 2006, Kostka et al., 2016). Again, this may explain why Helotiaceae had a greater abundance in peat sites where bryophytes are dominant.

Overall, this research suggests that soil type is resulting in a selection of fungal taxa, which may be important for understanding differences in soil processes and biogeochemical cycles between peat and organo-mineral soils in an organic upland catchment.

7.4.2 How does microbial community diversity differ between different sites representing a 'natural' acidity/pollution deposition gradient?

For alpha and beta analysis, when comparing within soil type, there were no apparent differences in fungal diversity between site locations for either soil type. However, both alpha and beta diversity showed a clear difference in bacteria and archaeal community diversity between site locations. Diversity was significantly greater at Migneint compared to Peaknaze for both soil types, as shown with at least one alpha diversity index at all taxonomic levels. Site location differences in diversity were significant for Shannon diversity index at all taxonomic levels studied and for both soil types, suggesting that Shannon is the most effective diversity index for detecting differences in diversity in bacterial and archaeal communities.

The Pennines where Peaknaze is situated has experienced high sulphur deposition which has seen a 69 % reduction between 1970 and 2005 (Dore et al., 2007, Evans et al., 2012), with a substantial store of sulphur still being present in the South Pennine peats (Daniels et al., 2008). Soils and waters in this area have also experienced significant acidification (Evans et al., 2000). Recent pore water measurements showed mean pH to be 3.98 units (mean

from control plots over 13 months) at the peat site and 4.10 units at the podzol site, compared to ~4.3 units respectively at Migneint (Chapter 4). In addition, this area has received previous and current high levels of nitrogen deposition and saturation (Helliwell et al., 2007, Curtis et al., 2005, Evans et al., 2000). Heavy metal pollution is also paramount, with concentrations of lead in near-surface peat recorded at 1000 mg kg^{-1} in the Peak District (Rothwell et al., 2005). Intensive land management in the form of grazing and management for grouse shooting has also contributed to degradation of the Pennines (Ward et al., 2007, Clutterbuck and Yallop, 2010). Such pollution deposition, acidification and degradation of this area may have contributed to a reduction in soil biodiversity in this area.

Microbial activity and community composition in soil has been shown to be negatively affected by heavy metal pollution (Müller et al., 2001, Wang et al., 2007). Sites in the South Pennines which have high levels of bioavailable metals have been shown to contain a greater proportion of bacteria taxa able to live in extreme environments, such as acidophilic and sulphur utilising bacteria (Linton et al., 2007). In polluted podzol soils in forested environments, heavy metal concentrations have been shown to negatively affect Chao1 diversity, although soil pH was found to be more influential (Chodak et al., 2013).

It may be possible that diversity is lower at Peaknaze as taxa which are suited to the harsher conditions have thrived and outcompeted other taxa which are less suited. The core taxa analysed in more detail in this study was Acidobacteria, which is known to be highly responsive to pH (Sait et al., 2006), and has been suggested as a potential indicator taxa for measuring restoration of wetlands and trophic status (Hartman et al., 2008). Acidobacteria was significantly more abundant at Peaknaze than at Migneint for both soil types. (Elliott et al., 2015) compared microbial communities between sites which were degraded or in a state of restoration in the South Pennines, and also found the degraded site to have higher levels of Acidobacteria. Interestingly, Fierer et al. (2012) found Acidobacteria abundance to decrease in response to nitrogen fertilisation, which suggests that the increased abundance at Peaknaze is due to chronic historical acidification rather than nitrogen deposition. In addition, although not always significant, Candidatus Koribacter and Candidatus Solibacter, which are members of the Acidobacteria taxa group and contribute to the core microbiome at genus level, were more abundant at Peaknaze than at Migneint. In addition,

Telmatobacter, a rare taxa also part of the Acidobacteria taxa group, was only present at Peaknaze Peat.

7.4.3 Are microbial communities impacted by acidification?

Fungal diversity was not affected by the acidity treatments, and there was also no relationship between fungal diversity and soil pH. Bacterial and archaeal diversity was also not effected by acidity manipulations, with no significant difference apparent between treatments at any site. However, when investigating individual core taxa, Acidobacteria was found to be more abundant with acidity treatments at the Migneint Podzol site. Hartman et al. (2008) found Acidobacteria abundance to be strongly correlated with soil pH in peat. Contrastingly, there was no difference in abundance between treatment plots at Migneint Peat. This was unsurprising as there was little success in changing soil and pore water pH at this site with treatment applications, likely due to the high water table and associated dilution, and sulphur reduction potential at this site (Chapter 4). There was also little response at Peaknaze for both soil types, and yet this site is already highly acidified so it was unlikely that communities would respond to further acidification when the baseline pH was already so low. Bardhan et al. (2012) also found soil microbial diversity to be unaffected by acidity along an acid deposition gradient of a spruce-fir forest. This area had also received heavy levels of acid deposition and pH of soil samples were very acid (< 4.0 pH units).

When taxa within the Acidobacteria group were investigated at genus level, two genera were also found to have a higher abundance with acidity at the Migneint Podzol site, with an increase in abundance of 78 % for Candidatus Koribacter and 76 % for Candidatus Solibacter when compared to the control. Such results suggest that the abundance of members of the Acidobacteria can be positively influenced by acidification. Furthermore, a rare taxa (abundance < 200) was found to also respond to acidity at Migneint Podzol (TM6, phyla level), with an increase in abundance of 76 % with the acid treatment.

Even though it was not possible to detect treatment effects on alpha diversity, it was possible to see a relationship between pH and diversity when data from all locations and soils was combined; when comparing alpha diversity indices to soil extract pH, there was a positive and significant relationship. This shows that in more acidic conditions, the diversity of bacterial and archaeal communities reduces in peat and peaty podzol soil, and that with

acidification recovery, diversity is also likely to increase. Other studies have found pH to be an important factor in influencing microbial community structure (Rousk et al., 2009) and diversity (Chodak et al., 2013, Hartman et al., 2008, Griffiths et al., 2011). Soil pH has been shown to be amongst the most important soil variables for controlling soil microbial communities in peat (Hartman et al., 2008) and podzol (Chodak et al., 2013) soils.

7.4.4 Correlations between microbial communities and DOC

Fungal communities were negatively correlated with soil extract DOC, although these relationships corresponded more with soil differences in DOC concentration. For instance, podzol soils had less extracted DOC and also a higher fungal diversity, whilst more DOC was extracted from peat which also had a lower diversity for fungal communities. Therefore both fungal diversity and soil extract DOC are likely both a function of plant communities and soil type, and so not necessarily directly correlated.

Interestingly, the diversity of bacterial and archaeal communities was positively and significantly related to soil extract DOC. There is a lack of knowledge surrounding the functions of the well-studied microbial taxa in organic soils, and the relationships between functions with microbial community structure, and so it is difficult to determine the cause and effect relating to microbial diversity and DOC production and consumption. Three potential scenarios behind this observed relationship have been suggested below.

The first scenario is that as pH increases to more favourable conditions, microbial diversity increases. The positive link between pH and microbial diversity has been demonstrated many times before by previous studies (Chodak et al., 2013, Hartman et al., 2008, Griffiths et al., 2011). However, net DOC production does not change in response to the increase in microbial diversity. This might be because additional taxa that are gained as pH increases are functionally redundant, such that the net DOC production function is already maximised by the lower diversity present in the more acidic soils. Simultaneously, DOC solubility also increases with increasing pH, and so there is an indirect relationship between microbial diversity and soil DOC, which favours the chemical control hypothesis behind increasing DOC trends.

The second scenario is that as recovery from acidification changes soil pH to more favourable conditions for biological activity (Andersson and Nilsson, 2001a), diversity is

increased and this results in a more biologically active community, and so decomposition of organic matter and therefore DOC production is enhanced. Therefore this relationship between pH and diversity may reflect a diversity control signal superimposed on the chemical control. However, there is a lack of knowledge linking changes in microbial community structure with functional responses (Torsvik and Øvreås, 2002, Nannipieri et al., 2003). Many studies demonstrate a change in microbial communities with acidity (Blagodatskaya and Anderson, 1998, Rousk et al., 2009, Oulehle et al., 2018) with bacterial growth rates being more sensitive than fungal growth rates (Walse et al., 1998). Rousk et al (2009) found microbial inhibition below a pH value of 4.5 units due to decreased bacterial grown with acidity. Acidity has also been shown to reduce microbial activity, including litter decomposition (Brown, 1985, Oulehle et al., 2018), as soil pH is crucial to enzyme functioning (Fog, 1988). Such evidence suggests that acidity suppresses decomposition of organic material and slows growth rates of bacteria, and if so with recovery and associated increase in pH there will be an increase in bacterial diversity, and activities such as decomposition and DOC production.

The final scenario is that DOC solubility and therefore bioavailability is increasing in response to increasing pH (Evans et al., 2012, Marschner and Kalbitz, 2003), and this increased substrate primes microbial communities, increasing growth and diversity, and decomposition of organic material (Fontaine et al., 2003), and so DOC production is also increased. This suggests that diversity is responding to elevated DOC concentrations, but are not controlling it.

7.4.5 Limitations of study

Despite the literature suggesting that microbial communities respond to acidity, we found a lack of significant relationships with treatments. Acidic conditions have been shown to decrease bacteria and increase fungi in soils (Blagodatskaya and Anderson, 1998, Rousk et al., 2009, Oulehle et al., 2018), with bacterial growth rates showing a greater sensitivity (Walse et al., 1998). This sensitivity of bacterial communities to soil pH may explain why relationships, when examined across the different locations and soils, were seen for bacteria and archaeal communities, but not for fungi. Furthermore, the effect of acidity on bacterial and archaeal diversity was not found to be significant with treatments, whilst a significant relationship was apparent between diversity and pH. This could be due to the

environmental variation associated with the field experiment, and perhaps there were not enough replications to overcome environmental heterogeneity and detect biological responses. Alternatively, the magnitude of change in pH achieved with treatment applications was less than the range of pH observed between plots across all experiment sites. For instance, the difference between maximum and minimum pH of soil extracts between treatment plots at each site ranged from 0.20-0.64 units (based on mean of four sampling months), whilst the difference in pH across all 48 plots was 1.9 units. It would be useful if a controlled acidity manipulation laboratory experiment was run to assess the response of bacterial and archaeal community diversity, as well as the response of individual taxa, to acidity in peat and organo-mineral soils. The magnitude of change in pH at which diversity is affected could then also be investigated.

The amount of taxa identified at lower taxonomic levels for both ITS and 16S datasets was limited at lower taxonomic levels. This suggests that taxa from upland organic catchment soils are underrepresented in public databases, and this reflects the uniqueness of these microbial communities compared to well-studied ecosystems. This has previously been concluded by (Artz et al., 2007, Thormann, 2006) for peat, and the results of this study suggests that this argument may also be applied to peaty podzols from upland organic catchments.

A final problem with this study was the amount of samples excluded from analysis of fungal communities due to having low or zero reads. This resulted in many treatment replications being lost and so the effect of acidity manipulations on fungal communities could not be examined in detail. This could be due to the large amount of PCR inhibitors such as humic acids in peat and organo-mineral soil, and although attempts were made to remove these from samples, it is possible that some remained and disrupted amplification of DNA using the ITS primers (Schrader et al., 2012). Another explanation is that fungal communities were virtually absent in those samples.

7.5 Conclusion

Despite there being evidence for the important role that microorganisms play in peatland functioning, there is little research into microbial community composition and their associated functioning (Littlewood et al., 2010), or how environmental change may

influence these communities and functions (Thormann, 2006). There is further little research comparing communities between peat and organo-mineral soil, despite the fact that organic catchments are comprised of both soil types. This research provides some insight into microbial community composition and diversity, as well as relationships with soil extract DOC, at different taxonomic levels for both peat and peaty podzol from an upland organic catchment.

The diversity of fungal communities was found to be significantly greater in podzol soils than in peat, whilst bacterial and archaeal communities were strongly influenced by site differences, with more diversity at Migneint compared to the degraded site of Peaknaze. Acidity manipulations did not influence the diversity of bacterial and archaeal communities. However, Acidobacteria abundance was found to be greater at the acidified site of Peaknaze, as well as with acid treatments at the Migneint Podzol site. When investigated further at lower taxonomic levels, two core Acidobacteria taxa, Candidatus Solibacter and Candidatus Koribacter, were found to also have a higher abundance at Peaknaze, and at acidified plots at Migneint Podzol. This suggests that assessing broad community metrics is not enough to detect biological responses to experimental treatments in the field, but by focusing on specific indicator or core taxa it is possible to see biological responses.

Finally, despite the insignificance with experimental treatments, bacterial and archaeal community diversity was found to be positively and significantly related to both soil pH and soil extract DOC when relationships were examined across all locations, soils and treatments. The relationship between biological response to pH and functional response in terms of DOC production, as well as the implications for recovery of acid sensitive areas from acidification and the associated DOC release, have been discussed.

Appendix

Table A7.1: P value results of Two-way Anova performed on each 16S alpha diversity index measure at each taxonomic level, comparing sites (4 levels) and treatments (3 levels).

Taxonomic Level	Diversity Index	Site	Treatment	Site:Treatment
Phyla	Chao1	**<0.001**	0.326	0.766
	Shannon	**<0.001**	**0.016**	0.386
	Simpson's	**<0.001**	**0.012**	0.555
Order	Chao1	**0.036**	0.499	0.520
	Shannon	**<0.001**	**0.004**	0.703
	Simpson's	**<0.001**	**0.001**	0.791
Genus	Chao1	**0.009**	0.109	0.800
	Shannon	**<0.001**	**0.045**	0.939
	Simpson's	**<0.001**	0.066	0.981

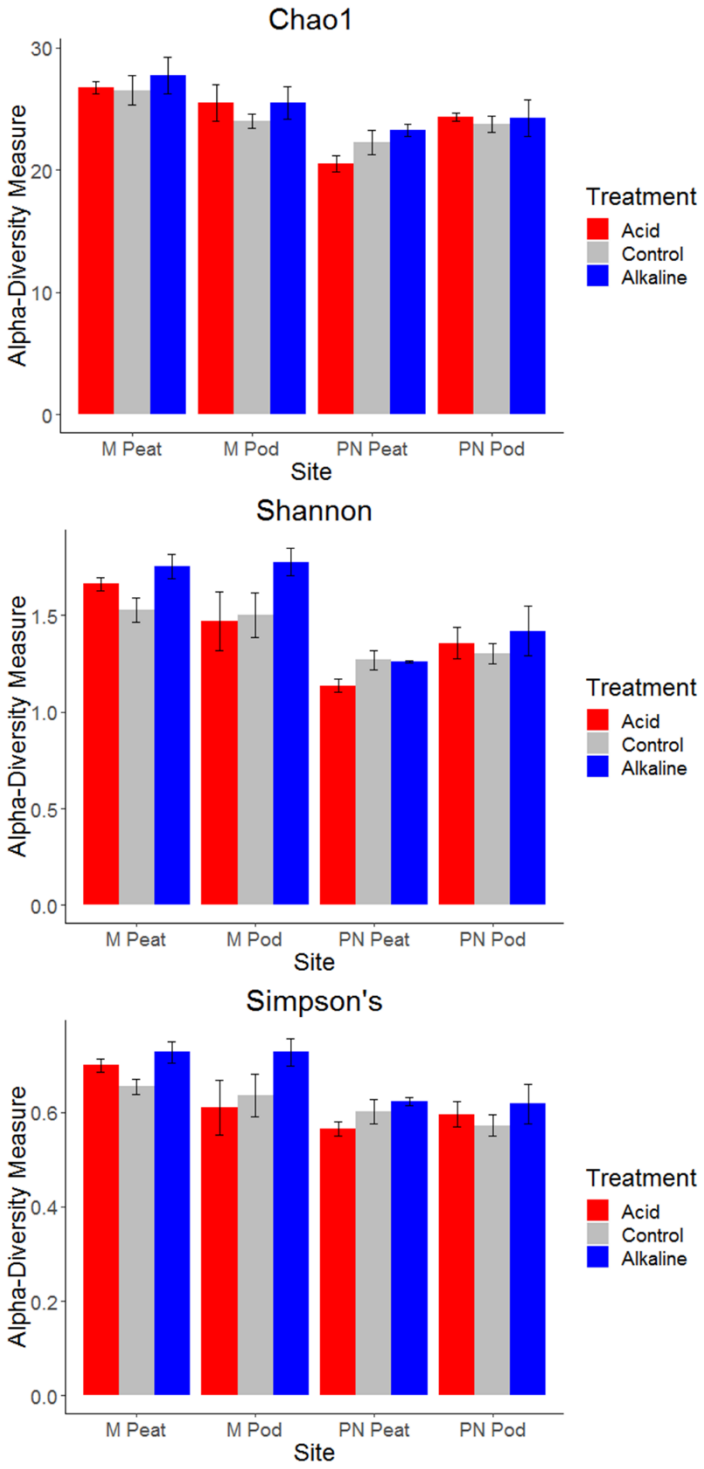


Figure A7.1: Diversity indices of 16S data at phylum level, of samples from different sites receiving treatments. Error bars represent standard error around the mean.

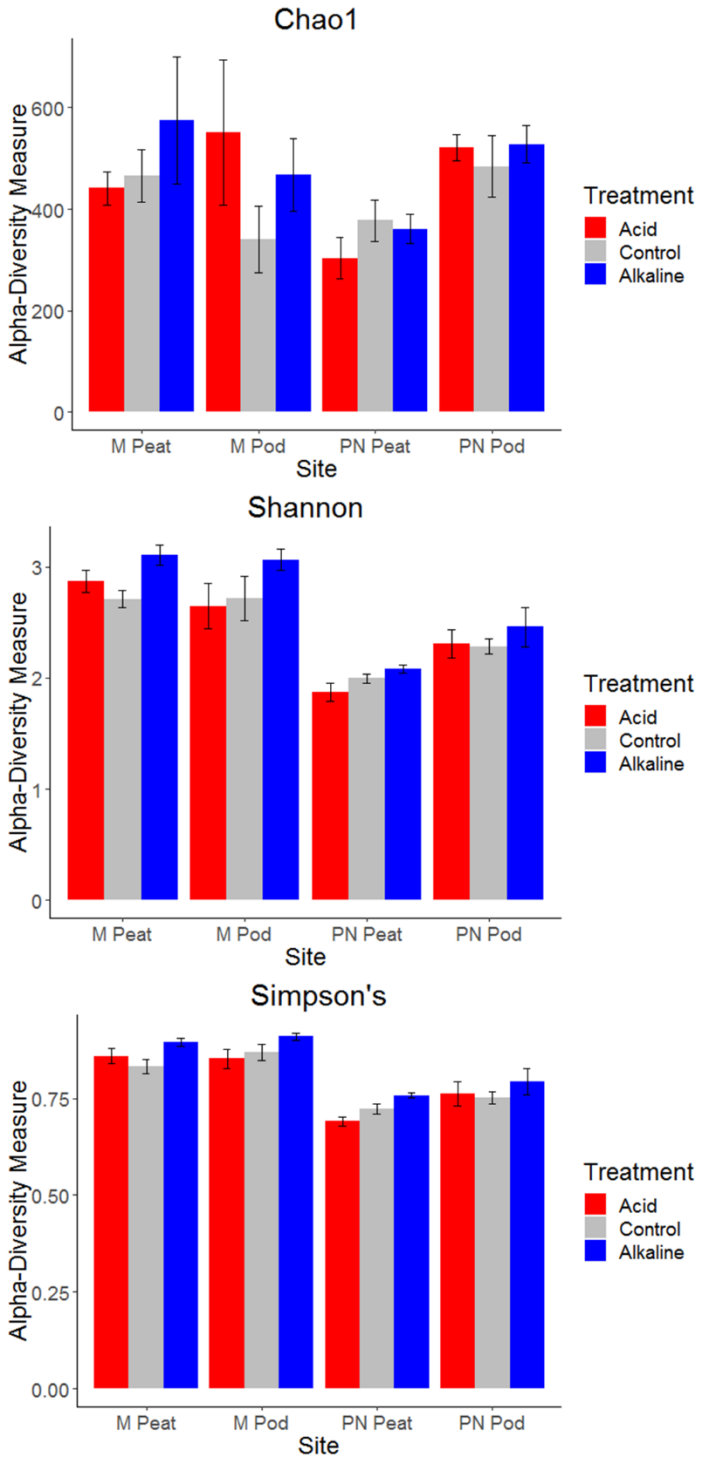


Figure A7.2: Diversity indices of 16S data at order level, of samples from different sites receiving treatments. Error bars represent standard error around the mean.

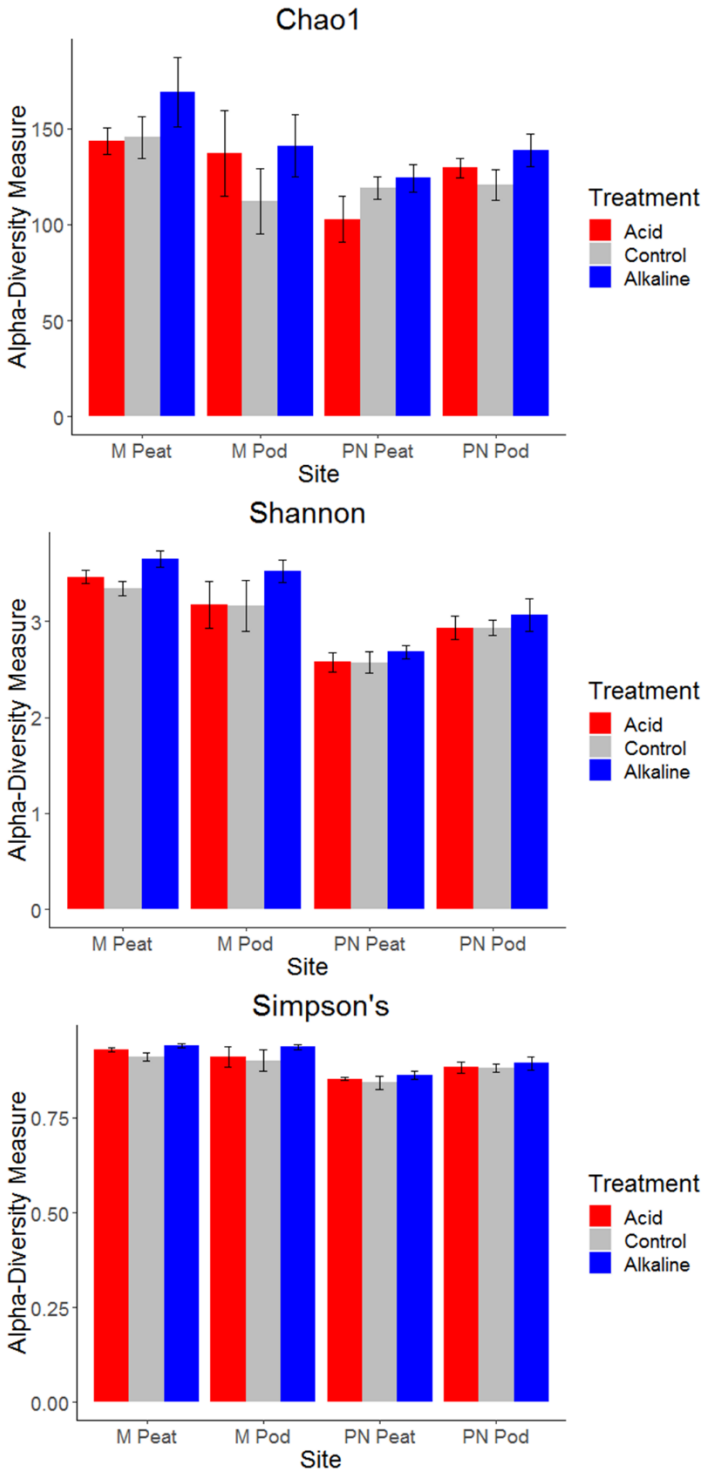


Figure A7.3: Diversity indices of 16S data at genus level, of samples from different sites receiving treatments. Error bars represent standard error around the mean.

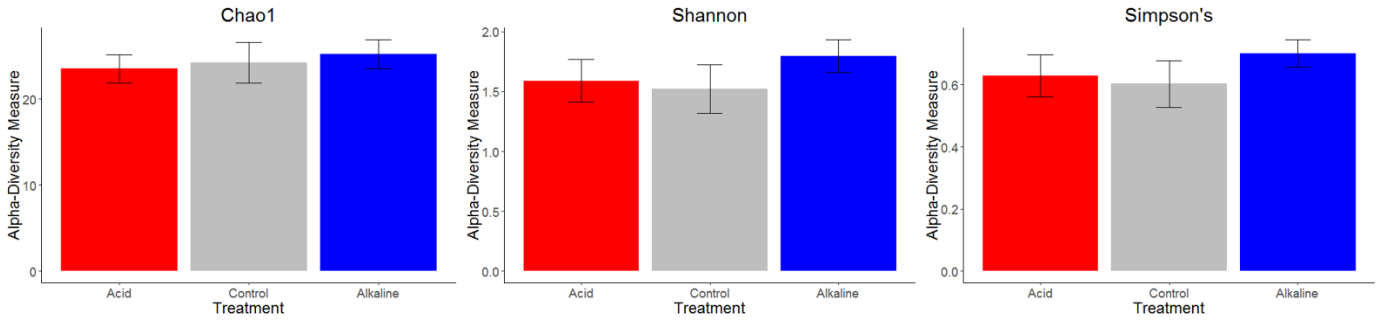


Figure A7.4: Bulked mean of treatment plots from all sites of diversity indices of ITS data at order level. Error bars represent standard error around the mean.

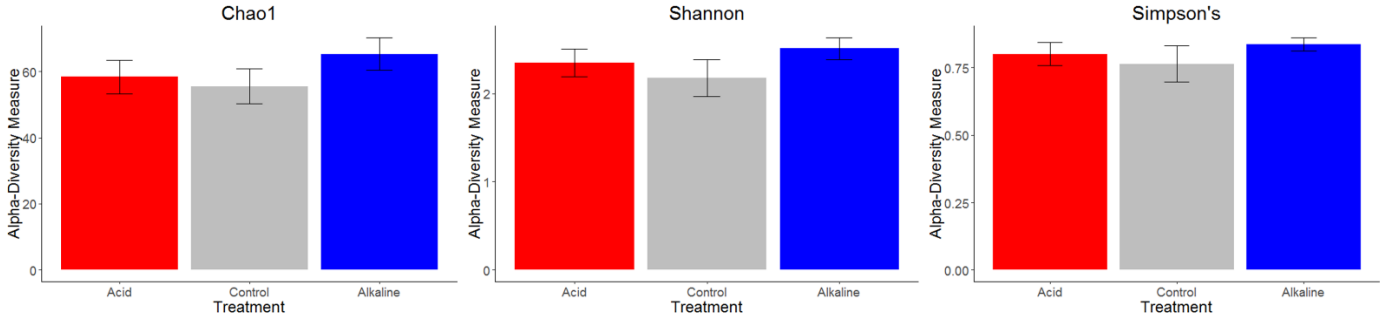


Figure A7.5: Bulked mean of treatment plots from all sites of diversity indices of ITS data at genus level. Error bars represent standard error around the mean.

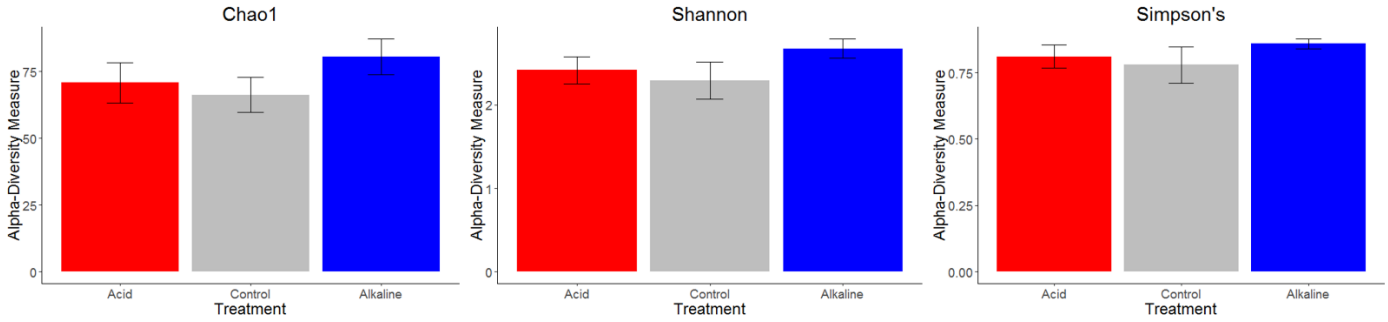


Figure A7.6: Bulked mean of treatment plots from all sites of diversity indices of ITS data at species level. Error bars represent standard error around the mean.

Table A7.2: P value results of One-way Anova performed on each ITS alpha diversity index measure at each taxonomic level, comparing sites (4 levels).

Taxonomic Level	Diversity Index	Site
Order	Chao1	**<0.001**
	Shannon	**<0.001**
	Simpson's	**<0.001**
Genus	Chao1 (log)	**0.002**
	Shannon	**0.016**
	Simpson's	0.111
Species	Chao1	**0.008**
	Shannon	0.072
	Simpson's	0.187

Table A7.3: Results (P and Rho values) of Spearman's Rank test comparing soil extract pH with 16S diversity index values, at three taxonomic levels.

Taxonomic Level	**Diversity Index**	**P Value**	**Rho Value**
Phylum	**Chao1**	**<0.001**	0.504
	Shannon	**<0.001**	0.650
	Simpson's	**<0.001**	0.539
Order	**Chao1**	0.085	0.260
	Shannon	**<0.001**	0.735
	Simpson's	**<0.001**	0.672
Genus	**Chao1**	**0.005**	0.416
	Shannon	**<0.001**	0.678
	Simpson's	**<0.001**	0.609

Table A7.4: Results (P and Rho values) of Spearman's Rank test comparing soil DOC (mg/g) with 16S diversity index values, at three taxonomic levels.

Taxonomic Level	Diversity Index	P Value	Rho Value
Phylum	**Chao1**	**0.035**	0.312
	Shannon	**0.011**	0.371
	Simpson's	**0.002**	0.443
Order	**Chao1**	0.629	-0.073
	Shannon	**0.013**	0.365
	Simpson's	**0.004**	0.419
Genus	**Chao1**	0.083	0.259
	Shannon	**0.013**	0.364
	Simpson's	**0.026**	0.328

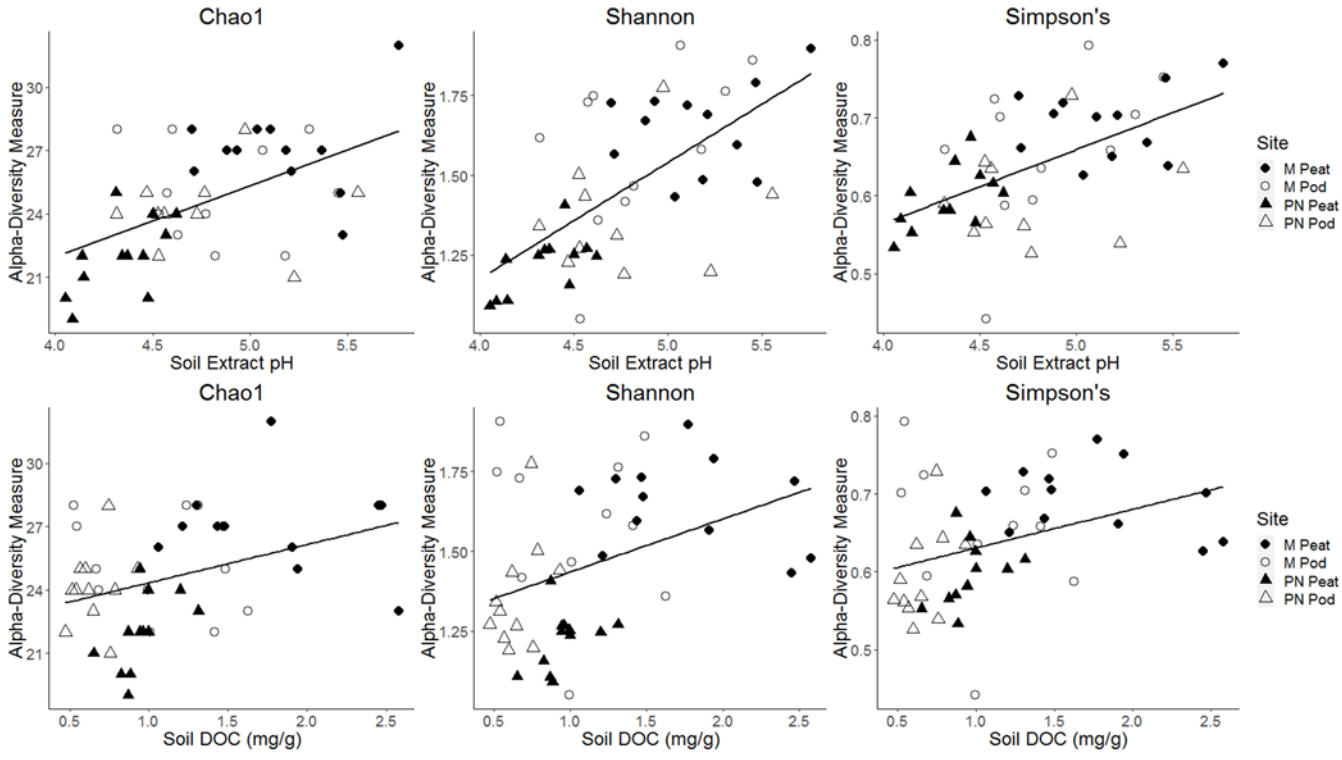


Figure A7.7: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH (top panel), and extracted DOC (lower panel), for 16S data at phylum taxonomic level.

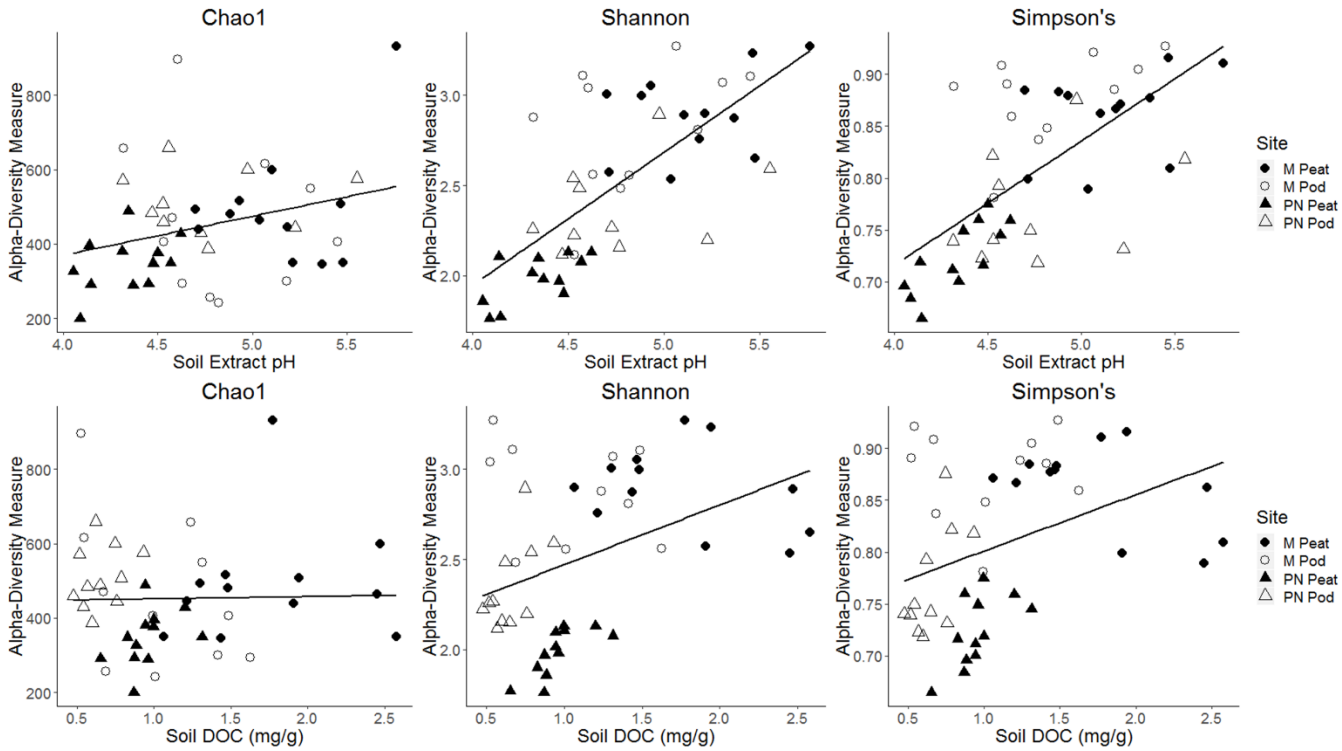


Figure A7.8: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH (top panel), and extracted DOC (lower panel), for 16S data at order taxonomic level.

Table A7.5: Results (P and Rho values) of Spearman's Rank test comparing soil extract pH with ITS diversity index values, at three taxonomic levels.

Taxonomic Level	Diversity Index	P Value	Rho Value
Order	**Chao1**	0.972	0.006
	Shannon	0.710	0.067
	Simpson's	0.662	0.078
Genus	**Chao1**	0.700	0.068
	Shannon	0.867	0.029
	Simpson's	0.937	-0.014
Species	**Chao1**	0.517	0.115
	Shannon	0.780	0.050
	Simpson's	0.811	-0.043

Table A7.6: Results (P and Rho values) of Spearman's Rank test comparing soil DOC (mg/g) with ITS diversity index values, at three taxonomic levels.

Taxonomic Level	Diversity Index	P Value	Rho Value
Order	**Chao1**	**0.015**	-0.408
	Shannon	**0.030**	-0.366
	Simpson's	**0.024**	-0.381
Genus	**Chao1**	**0.020**	-0.385
	Shannon	0.079	-0.297
	Simpson's	0.061	-0.316
Species	**Chao1**	**0.016**	-0.225
	Shannon	0.194	-0.225
	Simpson's	0.110	-0.275

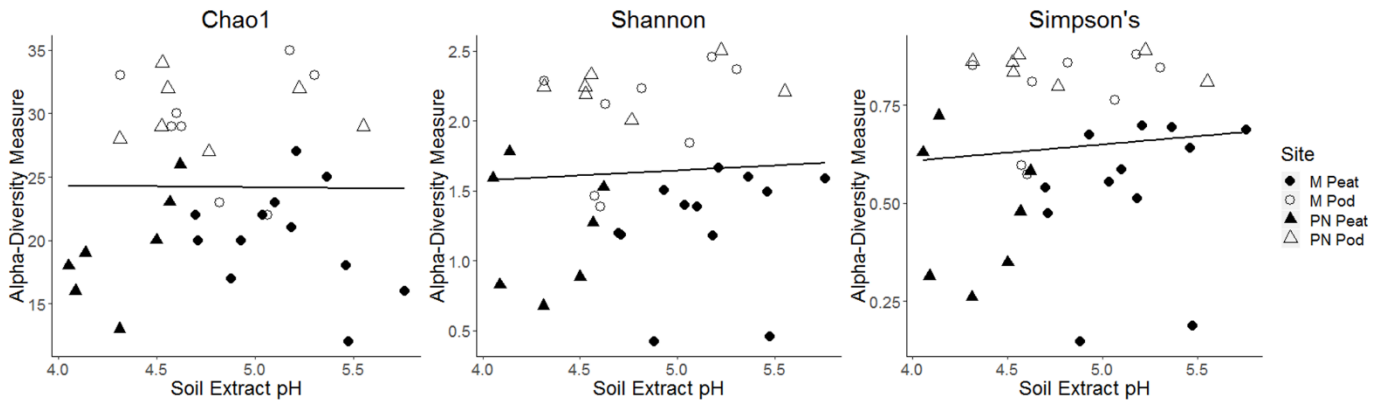


Figure A7.9: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH, for ITS data at order taxonomic level.

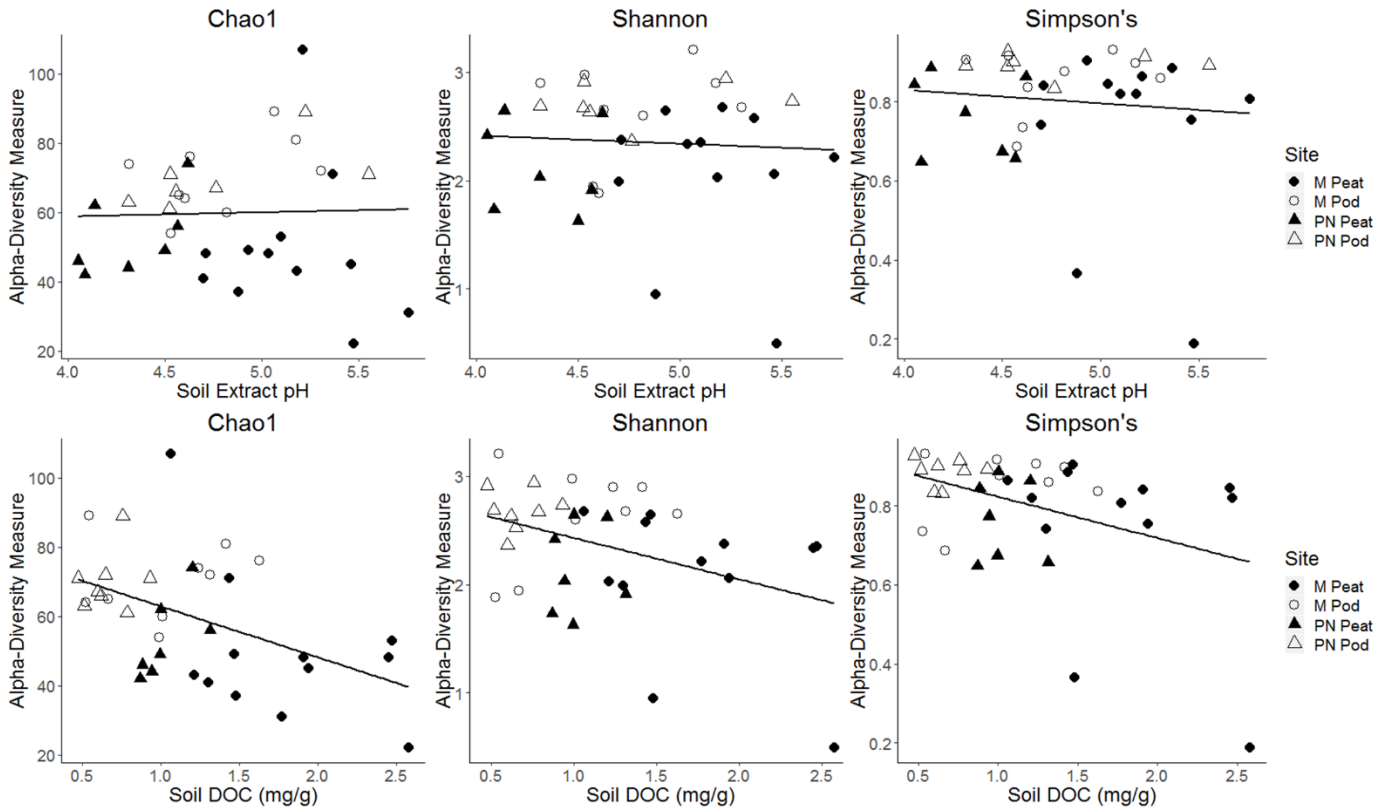


Figure A7.10: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH (top panel), and extracted DOC (lower panel), for ITS data at genus taxonomic level.

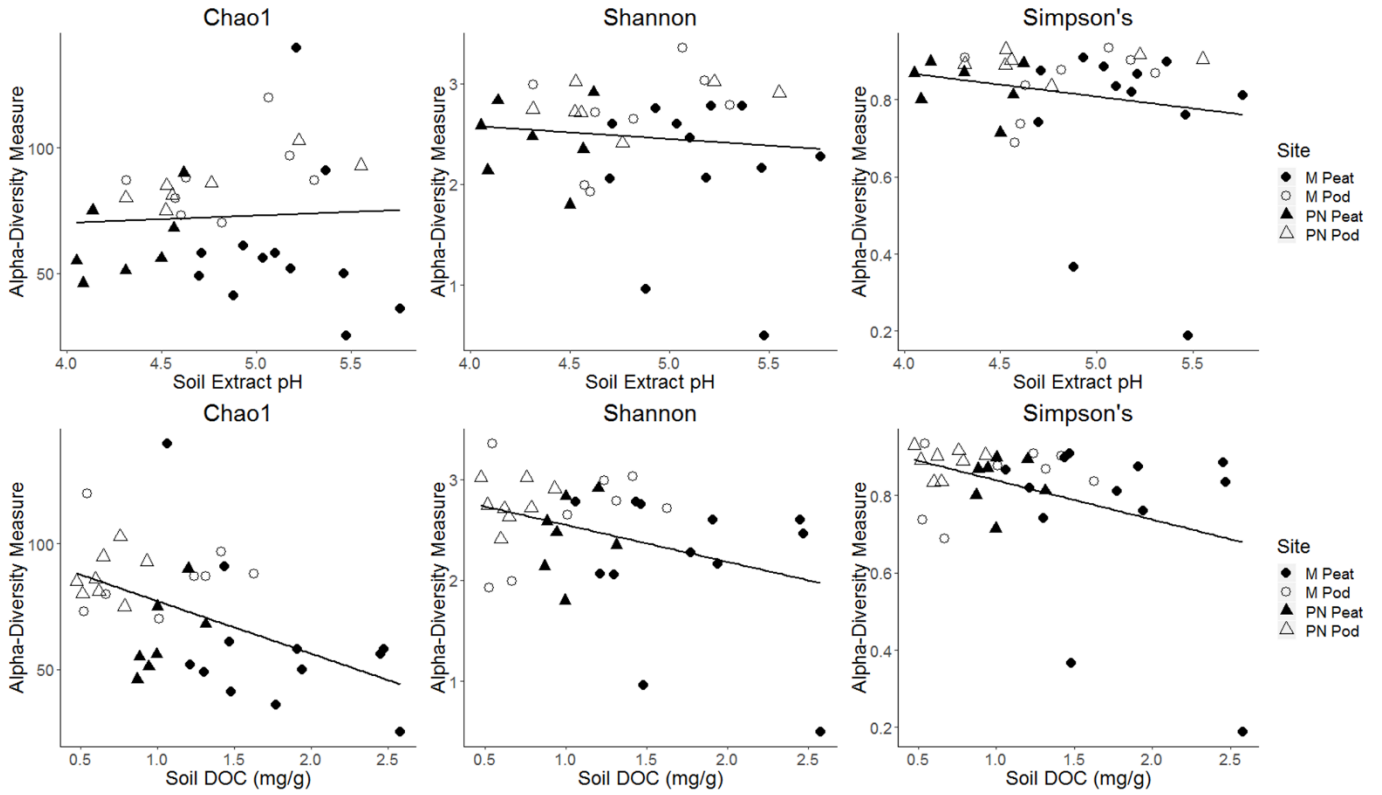


Figure A7.11: Relationships between diversity indices (Chao1, Shannon and Simpson's) with soil extract pH (top panel), and extracted DOC (lower panel), for ITS data at species taxonomic level.

Chapter 8: Summary

8.1 Thesis summary with respect to aim and objectives

Upland organic catchments comprising of both peat and organo-mineral soil are an important global store of carbon. However, many of these catchments in acid sensitive areas of the Northern Hemisphere have experienced increasing carbon losses in the form of dissolved organic carbon (DOC), concentrations of which have been increasing in the surface waters over the past four decades (Evans et al., 2005; Monteith et al., 2014). Whilst there is much debate surrounding the cause of this increased DOC trend, there is empirical evidence linking increasing DOC concentrations to changing atmospheric chemistry and associated acid deposition (Clark et al., 2011; Evans et al., 2012; Monteith et al., 2007; Oulehle et al., 2011; Palmer et al., 2013). Although there is strong evidence of a chemical control on DOC solubility (Ouhlehle et al., 2013), there is uncertainty as to whether there is also a biological mechanism behind this trend. Litter and peat decomposition is a major biological source of DOC, and yet it is unclear how this responds to changing acidity.

In addition, there have been inconsistencies in DOC release from moorland catchments receiving similar acid deposition loads, which suggests that differences in catchment characteristics may result in inconsistencies in the magnitude of response to acidification and recovery. Upland moorland catchments typically consist of both peat and organo-mineral soil, the development of which is dependent on site topography, and yet much of the literature has focused on either peatland areas, or podzols in forested environments. Therefore, we do not fully understand how both chemical and biological responses to changing deposition chemistry can vary in magnitude across an organic non-forested catchment consisting of both peat and organo-mineral soil.

It is important we understand in full the mechanisms behind this increasing DOC trend in response to changing acid deposition, and how these responses vary for different soils typical to organic catchments, in order to understand some of the variability and future response of specific catchments. With global climate change progressing at an accelerated rate due to anthropogenic activities, it is critical such information is available to allow models to accurately predict the storage and loss of carbon from these sensitive carbon rich

catchments, particularly in relation to changing quality of raw drinking water sources from many upland areas (Ritson et al., 2014).

In this thesis, a number of experiments were carried out as part of a long-term acidity manipulation field experiment, encompassing both peat and organo-mineral soil at two sites representing a pollution deposition gradient. The aim was to investigate both chemical and biological responses to changing acidity behind the increasing DOC trend in these different soil types. By using a field experiment, realistic responses to acidity that might be experienced under true acid deposition could be examined for two upland catchment areas. Overall, the key findings of this work were:

- A clear chemically mediated DOC response to acidity in soil pore water. This supports previous findings and builds on evidence of the pH-DOC hypothesis that recovery from acidification is increasing DOC solubility in organic soils.
- The DOC in the upper organic layer of peat and organo-mineral soil was acid sensitive, but the decomposing surface litter DOC was not.
- Responses to treatments were limited for litter decomposition, Tea Bag Index (TBI) parameters and microbial diversity, and therefore there is little evidence that short-term changes in acidity effect microbial communities and biologically mediated processes (decomposition and associated DOC production).

An extended summary of the findings addressing this aim in relation to each objective is given below.

8.1.1 Objective 1: To assess how acidity effects DOC quantity and quality in peatland soil extracts, surface litters and pore water at different sites representing a pollution deposition gradient.

The results of one year of experimental manipulation and measurement, building upon an existing long-term pH manipulation field experiment, were presented in Chapter 4 to investigate different sources of DOC in the upper organic layer of peat and organo-mineral soil. This enabled the sensitivity of different sources to acidity to be assessed in realistic field conditions, for different soils typical to upland organic catchments and across a pollution deposition gradient.

Organo-mineral soil was found to contain more aromatic DOC in the upper peaty layer than peat itself. This suggests that podzol areas of an organic catchment may release this coloured humic acid fraction of DOC during times of high flow when flow shifts from the mineral layer, where DOC is sorbed onto mineral surfaces, to the organic layer where DOC is mobile. Litter produced more DOC than peat, but this was less sensitive to acidity (as indicated by a lower $SUVA_{254}$ and associated aromaticity). By contrast, peat generated larger peaks of aromatic DOC during the peak of the growing season which is more sensitive to acidity. Pore water DOC responded strongly to acidity treatments, with lower concentrations observed at higher levels of acidity. This was further supported by pH-DOC relationships, also seen in peat samples, but not in surface litter. This provides supporting evidence for the hypothesis that increasing DOC concentrations in surface waters is due to increasing solubility of DOC with recovery from acidification, and that DOC solubility in pore water and peat is acid sensitive whereas DOC production in surface litter is not.

Therefore there is strong evidence for the chemical solubility control on DOC with acidity, which supports previous findings, and this is consistent within the soil for both peat and organo-mineral soil, but not for surface litter. Such information is important for modelling how carbon budgets of organic catchments may be affected by environmental change such as acidification and recovery.

8.1.2 Objective 2: To assess how litter type and quality effects decomposition and subsequent DOC production over a pollution deposition gradient in peat and organo-mineral soil.

In response to Chapter 4 which concluded that DOC production from mixed surface litter was not acid sensitive, the decomposition and subsequent DOC production of two individual litter types typical to organic catchments (*Eriophorum* and *Calluna*) were assessed to see if this differed over a pollution deposition gradient for different soils. As part of this, a translocation experiment involving *Calluna* litter was used to investigate the effect of litter quality. This involved a litter bag experiment in Chapter 5, with quarterly sampling over a 12 month period to assess the temporal decomposition and subsequent production of DOC.

Decomposition was faster in podzols than in peat, and was suppressed at the most polluted site where nitrogen deposition, heavy metal pollution and acidification are greatest.

Nitrogen content of *Calluna* from the polluted site was significantly higher, suggesting that deposited nitrogen has accumulated in plant tissue. However, litter quality in terms of nitrogen content and C:N ratio did not influence the decomposition of *Calluna* at most sites.

However, the *Calluna* which had significantly more nitrogen accumulated in plant tissue decomposed significantly less at the most polluted peat site. The reasons behind this are unclear, but may be due to the interactions between high exogenous and tissue content of nitrogen, and tissue lignin content of *Calluna*.

As well as raising important questions on the controls of decomposition at polluted peatland sites, this research also provides insights into the temporal variations on the decomposition of common litters, and how this varies across an organic catchment comprising of both peat and organo-mineral soil, comparisons of which are lacking in the literature.

8.1.3 Objective 3: To assess how acidity impacts litter decomposition and the associated DOC produced in peat and organo-mineral soil.

It is unclear to what extent increased DOC concentrations could have been driven by increased decomposition and therefore DOC production, as recovery from acidification changes soil pH to more favourable conditions for biological activity (Andersson and Nilsson, 2001)(Andersson and Nilsson, 2001a)(Andersson and Nilsson, 2001a). As Chapter 4 concluded that DOC release from mixed surface litter was not acid sensitive, an acidity manipulation litter bag experiment was assessed in Chapter 6 to further understand if the decomposition and subsequent production of DOC from individual plant species was effected by acidity. The TBI was also incorporated in order to assess the decomposition rate (*k*) and stabilisation factor (*S*) of different soils and sites, and whether effects of acidity were consistent across these. This also meant that TBI parameters could be compared with other studies and ecosystems.

Soil type was found to be highly influential on litter decomposition and DOC production. There was significantly more mass loss of litter when bags were incubated in podzol soil than in peat. This resulted in more DOC (as with *Eriophorum*) and a higher $SUVA_{254}$ value indicating more aromatic DOC (as with *Eriophorum* and *Calluna*) being extracted. This suggests that litter has the potential to decompose faster in podzol soil than in peat, which is to be expected, and that after one year of decomposition this resulted in more DOC being

produced compared to peat. Also, the same litter produced more aromatic DOC when decomposing in a more aerated environment (podzol soil), possibly due to the later decomposition stage reached resulting in a product which is more stable due to microbial processing (Malik and Gleixner, 2013).

Only decomposition of *Eriophorum* litter showed signals of responding to acidity treatments, which may be related to the higher nitrogen content within plant tissue compared to other litters. In short, the decomposition of this litter may not be limited by nitrogen availability as others may be, and so acidity becomes a dominant control. This suggests that controls on litter decomposition can be controlled by nitrogen availability or suppression with acidity, and this is dependent on litter quality. We would therefore expect other litter which had a lower tissue nitrogen content to have decomposed more at the site with highest nitrogen deposition, which was not observed in Chapter 5. However, as the TBI *S* factor suggests that there are environmental factors at this site which are suppressing the decomposition of the labile fraction of organic material, it is possible there are other environmental variables at this site which are acting as a further dominant control on decomposition. However, further investigation is needed.

This research provides insights into how different litters decompose and release DOC in peat and organo-mineral soil, and therefore the spatial variation across an upland organic catchment comprising of both soil types, information of which is important for modelling. It has also highlighted some of the limiting factors on litter decomposition and DOC production that need to be explored. Further work is needed to understand under what conditions acidity may become a dominant control on decomposition, and which environmental variables may outweigh this as the main control (see Section 8.2).

8.1.4 Objective 4: To evaluate whether microbial communities in peat and organo-mineral soil respond to acidification.

As DOC production through the decomposition of organic material is a biological process, there is uncertainty as to whether the increase in DOC concentrations is solely a chemical response due to increased solubility with recovering pH, or whether there is a biological element also, with increased microbial DOC production, or reduced DOC consumption, with acidification recovery. The soil microbial community response to acidity was investigated in

Chapter 7, with attempts to link these to functional responses in terms of DOC production and consumption. Fungal and bacterial communities were sequenced from peat and organo-mineral soil in an existing long-term pH manipulation field experiment in contrasting areas of historical pollution. From this, diversity of communities as well as abundances of core taxa were assessed, and potential relationships with soil extract DOC were investigated.

The diversity of fungal communities was found to be significantly greater in podzols than in peat. Bacterial communities were strongly influenced by site differences, with less diversity at the polluted site in the Peak District. Acidity manipulations did not influence the diversity of bacterial communities, but acidity did increase the abundance of core Acidobacteria taxa. Bacterial community diversity was found to be positively and significantly related to both soil pH and soil extract DOC. Such a correlation suggests that there could be a functional response to changing acidity as well as a response in community structure.

Therefore bacterial communities were more sensitive to acidity than fungi, whilst soil type, and potentially the associated plant community, were more influential on fungal communities. In addition, as biological responses to experimental treatments were not detected using broad community metrics, the importance of focusing on indicator taxa when detecting microbial responses to environmental change has been highlighted.

This research provides one of the first insights into microbial community composition and diversity at different taxonomic levels, for both peat and peaty podzol soil at different sites representing a pollution deposition gradient. However, a controlled laboratory experiment with more replications is needed to detect community response to the treatments. As it is difficult to unpick whether a) communities are responding to changing pH and this is causing a biological functional increase in DOC, or whether b) both diversity and DOC are simultaneously responding to changing pH, further work is also needed to assess the mechanistic functional response of bacteria in terms of DOC production and consumption in response to changing acidity in organic soils.

8.2 Wider implications and further research

This research set out to investigate the effect of acidity on the chemical and biological controls on DOC production in upland organic catchments. There was clear and consistent evidence that changing soil pH alters DOC solubility in organic soils, which supports previous findings, but that this effect does not extend to fresh litter. In contrast, there was little clear evidence that short-term changes in pH lead to changes in biologically mediated processes (decomposition and associated DOC production), or microbial community composition.

Finally, there is good evidence that a range of intrinsic (e.g. soil type, moisture content) and historic (e.g. N, S and metal pollution, management) environmental factors affect decomposition processes and DOC production either directly (by facilitating or inhibiting processes) or indirectly (by determining plant and microbial community composition). Acidity plays a key part in this, but probably as one of a number of interlinked variables.

As a result, a number of key areas where further research is needed have been raised. These are:

1. Linking responses of microbial communities to acidity to functional responses in terms of DOC production and consumption.
2. The effect of acidity on litter decomposition in a controlled laboratory experiment.
3. Establish under what conditions does acidity become a dominating control on decomposition.

These are discussed in more detail below, and a series of experiments have been suggested in order to fill the gaps raised during this thesis.

1. It was difficult to detect a response in microbial communities to acidity using broad community metrics for this field experiment, although responses for particular indicator taxa (Acidobacteria) were found to respond in terms of their abundance. However, bacterial and archaeal diversity was significantly and positively correlated with soil extract pH, suggesting that diversity does respond to changes in pH, but that this was not detected under field experimental conditions. There are a number of possible reasons for why communities were not significantly different between treatment plots. In particular, it is

possible that plant and microbial communities are adapted to long-term changes in acidity but don't adjust to short-term pH changes, and so the length of field experiment may have contributed to the lack of significant results. Other possible reasons include treatment frequency, sampling time, and other environmental variables which may also influence microbial communities. Furthermore, bacterial and archaeal diversity was also significantly and positively correlated with soil extract DOC, but it is difficult to establish whether this was due to a functional response of microbes to changing pH, particularly as a treatment response for litter decomposition was also not clear. Therefore a controlled laboratory experiment investigating acidity responses in both community structure and also function, whilst excluding other environmental confounding variables, would be valuable.

A possible experiment which could be used to answer these questions could be based on a dilution to extinction approach similar to (Wertz et al., 2006). This is based around the idea that although soils are very diverse environments, there are high levels of functional redundancy, and that functions are performed by specialised microbial groups. Therefore a reduction in diversity may not actually influence function, but function is affected when the diversity of specialised microbial groups are altered. Two experiments based around these ideas are suggested below:

1a) A controlled laboratory experiment similar to the field experiment run during this research, in order to exclude environmental heterogeneity and detect treatment responses using broad community metrics as well as identifying which taxa groups are responding. This could involve microcosms with treatments to manipulate pH of peat and organo-mineral soil, and sequencing of the communities.

1b) Using the removal approach described by (Wertz et al., 2006), sterile peat and organo-mineral soil are inoculated with communities and species which were not found to be acid sensitive under 1a are removed preferentially, whilst function is measured including respiration and DOC production.

2. The effect of acidity on litter decomposition was only detected for one litter type (*Eriophorum*) out of five during this research, whilst TBI parameters were also unaffected. It

is possible that only *Eriophorum* decomposition was acid sensitive, or that this was the result of statistical coincidence. However, there are a number of issues with the experiment itself which may have resulted in a lack of response to treatments for other litter types. These include frequency of treatment applications, length of field experiment and incubation of litter, and general environmental factors and variation which may have also effected decomposition rates and made treatment responses difficult to detect.

Therefore it is suggested that a controlled laboratory experiment is used to investigate the effect of acidity on litter decomposition further. A possible approach could follow on from experiments 1a and 1b, whereby taxa which are shown to respond to acidity and which show a functional response in terms of carbon cycling, could be inoculated in ^{14}C labelled sterile litter samples. By excluding soil, any interactions with soil minerals or older organic material can be eliminated, and so the effects of decomposition on fresh litter only can be assessed. Samples then receive acidity manipulation treatments, and the decomposition of litter types by particular microbial groups under different pH environments can be investigated by tracking ^{14}C isotopes, measuring final mass loss of litter, DOC production and respiration.

3. The effect of acidity on litter decomposition was found not to be simple during the course of this research, and it is suggested that there may be other variables which influence the magnitude of effect that acidity may have on litter decomposition and therefore DOC production, such as plant species and litter quality, as well as other forms of pollution including nitrogen and heavy metals. A summary of the suggested scenarios at which particular variables control decomposition has been presented below in *Table 8.1*, based on research for Objectives 2 and 3 and evidence in the literature.

Table 8.1: Theoretical scenarios at which variables dominate control on litter decomposition are suggested and explained.

Scenario	Dominating control on decomposition
High-lignin litter from polluted site (*Calluna*)	*Calluna* produces more lignin as a stress response to pollution. *Calluna* also incorporates more nitrogen (N) into plant tissue. The interaction of high exogenous N, high litter tissue N and high lignin content results in inhibition of decomposition.
Low-lignin litter with lower N content	Microbial decomposition is limited by N availability, and this overrides acidity effect (low N in litter and soil causes reduced decomposition, regardless of pH). However, increased N deposition does not increase decomposition of these litters where other pollutants (possibly heavy metals) are present, as these become the dominant control on decomposition.
Low-lignin litter with higher N content (*Eriophorum*)	Microbes have enough N from litter, and so decomposition is not limited by N availability, but instead is suppressed by acidity.

Whilst the experiment discussed under 2. would establish whether acidity effects the decomposition and DOC production of particular litters, this may not necessarily occur in situ, and so it is important to fully understand how acidity controls may be influenced when interacting with other variables that might be occurring in the field. By establishing the magnitude of response of litter decomposition and DOC production that might occur under acidification and recovery under different field conditions, this would provide vital information for modelling for particular catchments, and may explain some of the disparity in DOC release between different catchments receiving similar acid deposition loads. Again, multi-factor controlled laboratory experiments are needed to explore these interactions further.

8.3 Final thoughts

Organic soils are diverse and a valuable store of carbon. In an era of anthropogenically induced global climate change and its associated economic, social and environmental risks, it is vital we understand in full the earth's carbon dynamics and responses to environmental change. This thesis has demonstrated how recovery from acidification results in an increase in DOC solubility and release, and highlights the importance of considering organo-mineral soils as well as peats when assessing carbon dynamics of upland organic catchments. In addition, the complexity surrounding the biological mechanisms behind this increasing DOC trend with recovery have also been highlighted, and a number of research areas where future work could focus to further explore the role of microorganisms in DOC dynamics in organic soils experiencing acidification and recovery have been raised.

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