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Abiotic Controls on Soil Enzyme Activity and Community Composition of Micro-eukaryotes: A Comparison of Arctic, Temperate and Tropical Peatlands

Alajmi, Fatemah Enad M

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Abiotic Controls on Soil Enzyme Activity and Community Composition of Micro-eukaryotes: A Comparison of Arctic, Temperate and Tropical Peatlands.

A thesis submitted to Bangor University by:

Fatemah Alajmi

In candidature for the degree of:

Philosophiae Doctor

October 2020

School of Natural Sciences

Bangor University

Gwynedd

Abstract

A knowledge of the conditions that influence the activities of enzymes that mediate the key processes occurring within peat soils is a prerequisite for predicting potential ecosystem responses to climate change, human utilization and management policies.

In contrast to previous studies focussed on single biomes, the following study included three peatlands along a climatic gradient: Arctic (Svalbard, Norway), temperate (Wales, United Kingdom) and tropical (Andean mountains, Colombia). The study also encompasses a deliberately broad range of biotic and abiotic parameters, which were investigated along the peat profile.

Contrasting relationships were found between measured parameters across the three peatlands. Generally, the highest biogeochemical processing rates occurred in the superficial layer indicating it to be the most biologically active layer. However, in the deeper mineral layer of Svalbard, phenol oxidase activity was as high as in the superficial layer, with higher pH and nutrient concentrations. Also, nitrous oxide flux from the deeper layers did not significantly differ from that of the upper layers, further highlighting the potential for the deeper layers of peats to be more active than is commonly assumed. The dominant controlling factor for phenol oxidase activity in Svalbard and Colombia was pH, while in Wales only hydrolases were positively correlated with pH. The response of hydrolase activities and gas fluxes to phenolics concentration also varied between the three regions, indicating that the three climatic peatlands differed in parent plant chemistry that controls peat chemical composition across the latitudinal transect from the Arctic to the tropics.

A negative correlation was expected between enzyme activity and nutrient availability. However, contradictory results were found concerning this perspective, possibly because soil samples obtained from the three contrasting peatlands differed in nutrient concentrations, changing the dependency of *in situ* microbial communities on edaphic nutrients.

Significant differences in measured parameters were identified between different regions. Relatively high hydrolase activities were observed in Colombia, possibly due to the higher demand for nutrients and/or higher energy supply. Phenol oxidase was relatively higher in the Arctic, associated with significantly higher pH and concentrations of most inorganic nutrients. Conversely, the Welsh peatland exhibited the lowest phenol oxidase activity, consistent with lowest pH and consequently highest phenolics concentration. The higher dissolved organic carbon (DOC) concentration towards the tropics can be attributed to the

higher primary productivity towards the equator where there is a warmer climate and longer growing season. In addition, decreased DOC concentration has been linked to increased sea salt, which were high in Svalbard possibly due to the proximity of the sampling sites to the sea.

The effect of soil variables on community composition of micro-eukaryotes in peatlands along a climatic gradient is useful for predicting how peatland structure and function may respond to future climate change. In the present study, the diversity and relative abundance of micro-eukaryotes were determined. Also, attempts were made to relate micro-eukaryote community composition to abiotic and metabolic variables. The general pattern of peat soil micro-eukaryote abundance was consistent with other studies of peatlands, where the supergroups Alveolata, Opisthokonta, Rhizaria and Stramenopiles were dominant. Redundancy analysis revealed that the community composition of micro-eukaryotes differed significantly between the three regions in response to the selected variables; high pH, nutrients, organic matter content and oxygen concentration, and low temperature structured micro-eukaryotes community. The results showed that phagotrophs, phototrophs and mixotrophs dominated in the Arctic, while with increasing temperature and phenolics concentration, community composition showed an increasing dominance of parasites in the temperate peatland. Micro-eukaryotic community in the tropical peatland was dominated by osmotrophs and distinguished by the high enzyme activities, decreased phenolics concentration, and with relatively high pH and DOC concentration.

Acknowledgements

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Special thanks to my family, for all their patience and encouragement during the past four years.

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Chapter 1

Introduction

1.1. Wetland ecosystems

Wetlands are reported to occupy more than 6.4% of the earth's terrestrial surface (Maltby and Turner 1983), and to contain the highest carbon density (Kayranli *et al*. 2010). They occur in a range of climatic zones and differ in size, dominant plant species, water table depth and extent of human impact. As a result, the term "wetland" is difficult to clearly define (Mitsch and Gosselink 2000).

Amongst the many definitions of wetland that have been published, the most commonly used one is that proposed in 1971 at the Ramsar Convention: ″ Areas of marsh, fen, peatland, or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed six meters. ″ (Groombridge 1992). This definition integrates all wetland variable aspects, but the predominant feature of all wetland ecosystems is the persistently high-water level and its effect on biogeochemical processes.

Wetlands provide a range of valuable services such as climate regulation through carbon sequestration, water storage and consequent flood risk reduction, filtration of pollutants, food and freshwater supply (Parish *et al*. 2008). Furthermore, their unique ecological position straddling aquatic and terrestrial status tends to support high levels of biodiversity (Mitsch and Gosselink 2000). Wetlands, therefore, are an important resource for scientific research in a variety of fields (Moore 2002).

1.1.1. Peatlands

Wetlands are commonly associated with the accumulation of deep layers (at least 30 cm depth) of partly decomposed remains of plants known as peat. This process results from the suppression of decomposition rates brought about by waterlogged and anaerobic conditions (Mitsch and Gosselink 2000, Moore 2002, Holden 2005).

Peatlands are freshwater wetlands occupying extensive areas in northern hemisphere, as a consequence of low temperatures, humidity and high precipitation rates (Figure 1.01). Peatlands can also be found at high latitudes in the southern hemisphere as long as low temperatures and anaerobic conditions exist (Hodgkins *et al*. 2018). Their distribution and growth rate (peat formation) are mainly determined by climate, hydrological conditions, vegetation type, and the imbalanced between rates of organic matter production (inputs) and decomposition (outputs) (Parish *et al.* 2008). Within the terrestrial biosphere, peatlands

represent the most significant long-term reservoirs of carbon, which has accumulated over thousands of years (Holden 2005, Parish *et al.* 2008). Peatlands occur in more than 180 countries (Parish *et al.* 2008). They hold over a third of all organic carbon found in soils, which is equivalent to around two-thirds of the atmospheric carbon pool and double the quantity of carbon stock in the earth's forest biomass (IPCC 2007). Peatlands are therefore critical for global carbon capture and long-term storage, having exceptional preservative properties. This is attested to by their ability to preserve ancient human remains (bog bodies) (Freeman *et al.* 2012).

According to Holden (2005), peatland soils are usually associated with high water content, sometimes up to 98%, received from either precipitation or ground water influx. Long-term waterlogged conditions tend to result in low oxygen concentrations, creating anaerobic conditions where decomposition rates fail to keep up with primary productivity. Peatland soils accordingly are characterised by a high content of organic matter (more than 65% organic matter on a dry weight basis), dark colour and fibrous texture (Clymo 1983).

Peatlands can be divided into bogs (oligotrophic rain-fed) and fens (minerotrophic flow-fed) (Moore 2002). Their soils may be classified as mineral or organic, all of which contain organic matter to some degree. However, mineral soil is considered to contain only 20% to 35% organic matter and differ in many physicochemical properties from organic soil (Dunn 2013).

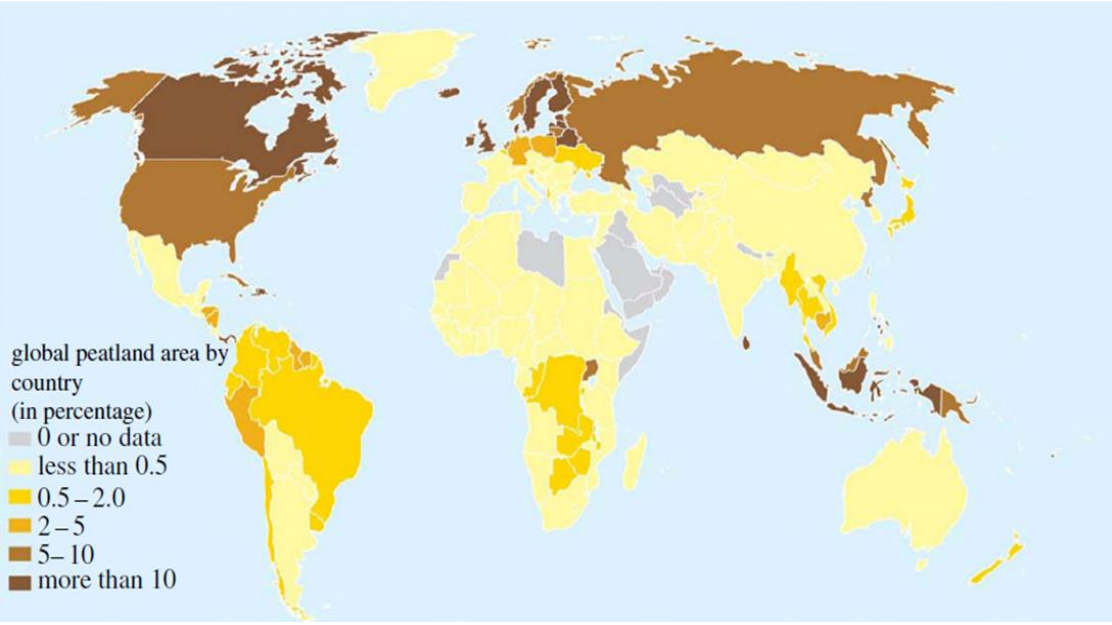

Figure 1.01. The global distribution of peatlands (from http://maps.grida.no/go/graphic/peat-distribution-in-the-world).

1.1.1.1. Arctic peatlands

Peat soils are mostly found in the Arctic and subarctic regions because of lower temperature and the permanently submerged conditions (Grønlund *et al.* 2008, Nakatsubo *et al.* 2015). The short growing season in the Arctic latitude limits primary production. The tendency for peat occurrence is enhanced by slowing decomposition processes due to cold Arctic temperatures and oxygen deficiency conditions which creating by the presence of permafrost, and melt-water in the summer season (Rozema *et al.* 2006, Hodgkins *et al*. 2018).

In Norway, where soil samples were collected during this project, peatlands are estimated to cover 24,000 km^2 (7%) of the land area and release up to 2 million tons of CO_2 per year (Grønlund *et al.* 2008). In Svalbard in the Norwegian high Arctic, the annual rainfall is 400mm, with continuous permafrost and relatively short growing season (40-70 days) (Rozema *et al.* 2006). Annual mean temperature is between -4 and -7 °C (Tveit 2014). According to Moore (2002), in very oceanic regions such as western Norway, blanket bog develops even at sea level. Elvebakk (1994) observed that the peat-forming moss tundra in the High Arctic tends to develop in wet conditions under bird nesting cliffs where bird manure provides a source of nutrients, stimulating primary productivity and carbon accumulation.

Arctic peatlands are estimated to hold a substantial amount (approximately 50%) of the world's soil carbon pool and are considered important potential sources for increased emissions of the GHG in a warming climate (Tarnocai *et al.* 2009).

Arctic regions, including permafrost peatlands have already been exposed to a substantial temperature increase and are expected to be exposed to a further 5.6-12.4°C by the end of this century (IPCC 2013). Resultant prolonged frost-free periods are likely to have a significant impact on vegetation and an increase in the depth of “active” layers (the active layer is the brown moss and peat layers above the permafrost subjected to seasonal thawing; 0- ~65cm) risks exposing vast carbon reservoirs to decomposition (Tarnocai *et al.* 2009, Voigt *et al.* 2017b). A growing number of studies have confirmed that Arctic peatlands also have enormous nitrogen stocks, with more than 67 billion tons accumulated over thousands of years, making them substantial potential sources of nitrous oxide (N_2O) if the Arctic permafrost thaws and its nitrogen stocks are mobilised (Voigt *et al.* 2017a, b).

Because of concerns about the consequences of climate change in such regions, researchers have carried out relatively detailed investigations regarding GHGs exchange in Arctic peatlands. Nevertheless, a limited number of studies consider microbial activities which regulate the production of GHG and are influenced by environmental factors especially in Norwegian peatlands.

1.1.1.2. Temperate peatlands

Peat-accumulating wetlands are mostly found in the temperate zone. Moore (2002) estimated that carbon uptake in temperate peatlands represented about 12% of anthropogenic emissions.

In the UK, where temperate climate conditions exist, peatlands cover around 24,640 km^2 of the land area (15%) and store 2,302 Megatons (Mt) of carbon (Billett *et al.* 2010), representing over 10% of the global peat store (IUCN 2014). In Wales, where samples were collected during this project, 732 km^2 of land, is occupied by peatland, which are reported to store 119 Mt of total carbon (Table 1.01). The temperate peatland site used in this study is located in the Migneint Valley (Snowdonia National Park), North Wales, UK. This area is one of the largest areas of blanket bog in Wales. The area is 460m above sea level and the annual rainfall is 2,400 mm (Dunn 2013).

Although peatlands in the UK form a relatively small portion of the peatlands of the northern temperate regions, they are of local and global importance (Billett *et al.* 2010). Besides their role in climate change mitigation, through holding enormous amounts of carbon that could otherwise be released into the atmosphere, they also provide multiple land uses, they supply freshwater to most major UK catchments, support livestock farming and a tourist destination to attract millions of visitors per year.

Since the last ice age, UK peatlands have accumulated millions of tonnes of atmospheric carbon (Billett *et al*. 2010). However, over 80% of the UK's peatlands have been the focus of protection and restoration efforts, following damage resulting from drainage practices, peat extraction for fertilizers and fuel, intensive grazing and fires (Holden *et al.* 2007).
UK peatlands have also been impacted by climate variability in the temperature and precipitation rates over the last years (Billett *et al.* 2010). According to Natural England (2010), damaged peatlands in the UK release 350,000 tonnes of CO_2 each year, mostly due to burning the vegetation on upland blanket bog areas. A report by the Committee on Climate

Change (2013) went as far as to say that only 4% of peatlands in the UK are considered in good conditions and actively developing peat (Committee on Climate Change 2013). Moore (2002) cited the future impacts of human practices and a consequent climate change on temperate peatlands in which more details can be found.

A number of studies, including field observations and manipulation experiments, have been conducted on the UK peatlands in order to relate specific microbial processes to a narrow range of physicochemical variables (e.g., Freeman *et al.* 1992, 1994, 1995, 1996, 2004, Pind *et al.* 1994, Kang *et al*. 1998, Kang and Freeman 1999, 2002, Fenner and Freeman 2011).

Table 1.01. Peatland areas and carbon stores in the UK from Billett *et al*. (2010).

		Soil depth		
Country	Area (km^2)	0-100 cm (Mt Carbon)	>100 cm (Mt Carbon)	Total (Mt Carbon)
Scotland	17,789	1104	516	1,620
England	4,246	296	123	419
Wales	732	67	52	119
Northern Ireland	1,873	90	54	144
UK	24,640	1,557	745	2,302

1.1.1.3. Tropical peatlands

Peatlands are distributed along a latitudinal transect from the Arctic to the tropics as long as low temperatures and anaerobic conditions exist. However, the chemical composition, especially carbohydrates and aromatics content, of parent vegetation and peat differs between climatic zones, with more aromatics and less carbohydrate content toward the tropics which could explain the very low decomposition and GHG production rates reported in these low latitude peatlands (Hodgkins *et al.* 2018).

It has been estimated that nearly 11% (0.44 Mkm^2 (M = million)) of peatlands occur in tropical regions, spread across Central and South America, Africa, the Caribbean and Southeast Asia. Tropical peatlands are estimated to hold around 18 to 25% of the total global peat volume (1758 Gm^3 ($G = 10^9$)) and around 15 to 19% of the global peat carbon pool. Southeast Asia is estimated to have the largest area of tropical peatland (247 778 km^2, 56%) and peat volume (1359 Gm^3, 77%) followed by South America (107 486 km^2, 24.4% and 192

Gm^3, 11% peat volume), Africa (55 860 km^2, 13% and 138 Gm^3, 8% peat volume) and the Caribbean and Central America (23 374 km^2, 5.3% and 60 Gm^3, 3% peat volume) (Page *et al.* 2011). There is a growing recognition of the global importance of tropical peatlands for carbon storage and carbon release and their critical role in climate change processes, especially in degraded Asian peatlands (Page *et al.* 2011). Interest in Southeast Asian peatlands has increased following extensive land use change for agricultural purposes, which has resulted in the degradation of soil carbon stores, and significant greenhouse gas (GHG) release (Page *et al.* 2002, Koh *et al.* 2009).

Recently, Gumbricht *et al.* (2017) published a new estimate of peatland extent and volume in the tropics. They estimated that tropical peatlands cover 1.7 Mkm^2 and contain 7 268 Gm^3 of peat, of which 750 000 km^2 (44%) and 3 117 Gm^3 (43%) are in South America. These latest figures suggest that Southeast Asian peatlands are surpassed in extent and volume by South American peatlands and highlight the importance of focussing studies of tropical peatlands in this region (Gumbricht *et al.* 2017). Specifically, more research is required to adequately understand tropical peatland enzyme dynamics and the factors affecting these processes, which will help in developing strategies for the restoration or reconstruction of drained peatlands and for the maintenance of healthy peatlands (Könönen *et al.* 2018). Within South America, Colombia is estimated to have 74 950 km^2 of the total tropical peatlands (Gumbricht *et al.* 2017). Like all tropical peatlands, Colombian peatlands at high elevations, where peat samples were collected during this project, are susceptible to the impacts of climate change including global warming (Benavides et al. 2013). The tropical peatland site used in this study is located at high altitude within the Sumapaz National Park in the Eastern mountain range of the Andes, Colombia. The area extension is about 154000 ha. The annual rainfall is between 1300 and 2400mm, with continuous and high humidity. The Park is a significant hydrographic center, containing river basins and lakes (Sesana 2006).

In the Andes Mountains, temperatures have already increased over the last two decades by 0.7°C for each decade with a further temperature rise of a total of about 5°C predicted over the next nine decades (Bradley *et al.* 2006, Ruiz *et al.* 2008). The resulting droughts and more oxygenated conditions pose a significant threat to the stability of peatlands, with carbon release also likely to cause positive climate feedbacks (Wieder 2001, Turetsky *et al*. 2002). Despite their global importance, South American peatlands have received less attention than other tropical peatlands. This is due partly to methodological and logistical difficulties (Gumbricht *et al*. 2017).

1.1.1.4. Why peatlands matter

Besides their unique biodiversity, peatlands play an important role in climatic processes and impact on water quality.

As previously stated, peatland plants absorb carbon dioxide (CO_2) from the atmosphere during photosynthesis. Much of this carbon becomes stored within peat soil as plants die back during the fall season, thereby locking it underground in waterlogged conditions that reduce decay. This combined with the decay-resistant nature of some plants forming peatlands, such as *Sphagnum* species, creating an unbalanced situation in which the production rates of organic matter exceed those of decomposition (Holden 2005). The process of accumulation of organic material over several millennia is known as carbon sequestration. In this way, peatlands can act as long-term atmospheric carbon sinks (Moore 2002, Kayranli *et al.* 2010). It has been estimated that, were it not for carbon storage in peat, global temperatures would have been 1.5-2.0 °C higher during the past 10,000 years (Holden 2005).

Comparing all oceanic and terrestrial ecosystems, peatlands have the highest carbon storage (Freeman *et al.* 2012), sequestering at least 550 Gtonnes of carbon within their peat, despite accounting for only 3% of the terrestrial ecosystems by area (Parish *et al.* 2008). Their existing carbon storage, combined with the potential for additional storage with certain management techniques, means that peatlands represent a vital resource for combating climate change (Holden 2005). Disturbed peatlands can, however, act as carbon sources. The released carbon may take different forms depending on the dominant decomposition reactions occurring in different layers within soil. For example, microbial respiration and methane oxidation primarily occur in the upper aerobic layers (acrotelm) and yield carbon mainly in the form of CO_2. In contrast, methane production (methanogenesis) as an anaerobic process and thus primarily arises in deeper, more anaerobic layers (catotelm) (Moore 2002).

Carbon is also released from peatlands in the form of dissolved organic carbon (DOC), which accumulates in pore waters and is leached into surface waters during rainfall events, with concentrations often highest following warm and dry periods, during which DOC has accumulated (Holden 2005, Fenner and Freeman 2011). Indeed, many UK peatlands have been drained over the last few decades with the consequent water table drawdown has led to the destabilisation of their carbon stores due to increased decomposition, resulting in increased DOC accumulation and export to surface waters (Holden *et al.* 2004). Evans *et al.* (2005) found that in the UK, DOC concentrations have increased by 91% in 22 different

upland sites since 1988 to 2003. This is likely to be due to an enzymatic response to rising temperatures as well as increased frequency and severity of droughts and more aeration, leading to higher peat decomposition rates (Freeman *et al.* 2001b).

The concentration of DOC in surface waters has important implications for drinking water providers since DOC reduce the aesthetic quality of the water and acts as a reaction precursor to potentially harmful disinfection by-products (Jones 2006). For all these reasons, peatlands have gained in recognition of their importance over recent decades (Dunn *et al.* 2014).

1.2. The role of organic matter decomposition in peatlands

Organic matter decomposition within peatlands is a complex process as it includes aerobic (respiration and methane oxidation) and anaerobic (fermentation, methanogenesis and iron, nitrate and sulfate reduction) processes (Kayranli *et al.* 2010).

Dunn and Freeman (2011) stated that the decomposition of soil organic matter leads to the release of carbon in soluble form as dissolved organic carbon (DOC), solid form as particulate organic carbon (POC) and gaseous form as methane (CH_4) and carbon dioxide (CO_2). Along with nitrous oxide (N_2O), these are known as greenhouse gases (GHGs) which are formed and emitted from peat soils and provide an indication of microbial respiration.

Natural organic matter (NOM), which includes particulate organic carbon (POC) and dissolved organic carbon (DOC), is a constituent of freshwaters and is composed of a mixture of microbial, animal and plant materials in different stages of disintegration. Carbon is the basic element of NOM and is mostly present in freshwaters in the dissolved form, passing through a 0.45 µm filter membrane (Thurman 2012). DOC in drinking water supplies is a very important water quality parameter affecting colour, safety and aesthetic value (Holden, 2005). DOC as an energy source promote microbial activities and processes, supporting the aquatic food chain. Within a drinking water distribution system, DOC therefore promotes bacterial regrowth. However, high concentrations of DOC can attenuate light penetration within the waterbodies (Kayranli *et al.* 2010) due to the presence of chromophoric functional groups (Leenheer and Croue 2003), reducing the growth rates of phototropic microorganisms.

Increasing atmospheric concentrations of GHG cause global warming by increasing the atmospheric sorption of infrared radiation (Kayranli *et al.* 2010). Among all the GHGs, CO_2 is produced largely as a result of the respiration of microorganisms in the upper layers of

peat, where the presence of oxygen and input of fresh litter allow for the continuous degradation of organic matter (Billett *et al.* 2010). CH_4 is produced by methanogens, prokaryotic microorganisms that belong to the domain of *Archaea*, and occur in the deeper layers of soil where conditions are anoxic and they use acetate and/or hydrogen as essential substrates (Segers 1998). Simultaneously, methanotrophs can consume and oxidize some of the atmospheric CH_4 to form CO_2, in the presence of O_2 in the uppermost layers (Freeman *et al.* 2002). Therefore, the degree of oxygenation, which is determined by the water table level, controls the methanogenic (methane production) and methanotrophic (methane oxidation) processes within wetlands (Kelley *et al.* 1995).

N_2O is formed in the soil by microbial communities during nitrification and denitrification processes (Kayranli *et al*. 2010), using mineral N forms, ammonium and nitrate, respectively. N_2O is a powerful greenhouse gas, nearly 300 times stronger than CO_2 in terms of resultant temperature increase. Therefore, nitrogen storage in peatlands and nitrogen cycling are receiving growing attention especially in Arctic peatlands where vast amounts of nitrogen (above 67 billion tons) accumulated over thousands of years (Voigt *et al.* 2017a, b).

Microbial decomposition and consequently GHG production occur due to the activity of extracellular enzymes, especially hydrolysing enzymes (β-D-xylosidase, β-D-glucosidase, phosphatase, arylsulfatase and N-acetyl-β-D-glucosaminidase (chitinase)) and oxidizing enzymes (phenol oxidases) (Dunn *et al.* 2014).

Hydrolase enzymes are a group of hydrolytic enzymes capable of hydrolysis of complex biomolecules, such as lipids, proteins, nucleic acids and starch. This process includes the breaking of substrate bonds with water. During the hydrolysis, the hydrogen ion (H^+) from the water molecule combines with one fragment, and the remaining hydroxyl ion (OH^-) combines with the other fragment (Figure 1.02) (Dunn 2013).

Soil hydrolases, which are produced normally by soil microbes and plant cells, are the main agents of carbon and nutrient cycling, controlling the rate at which organic compounds are degraded to become readily utilizable by microbial and plant communities (Marx *et al*. 2001).

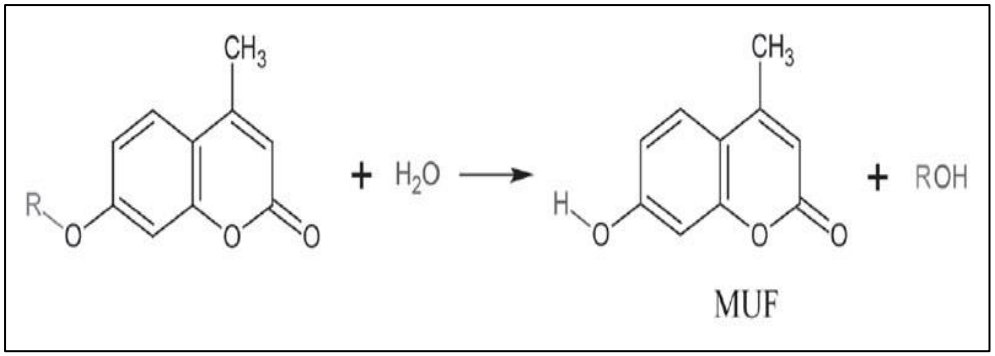

Figure 1.02. The fluorogenic assay used to determine the activity of the five hydrolytic enzymes based on monitoring the fluorescence of the model compound methylumbelliferone (MUF) liberated by enzymatic hydrolysis of the chemical bond between the MUF and the substrate (R) (From Dunn 2013).

Phenol oxidases are a group of oxidative enzymes responsible for promoting oxidation of aromatic or phenolic compounds using molecular oxygen (O_2), with complete degradation producing non-phenolic compounds, such as CO_2 (Figure 1.03) (Durán *et al.* 2002). Soil phenol oxidases originate naturally from microbial biomass and in small amounts from certain plant roots (Pelaez *et al.* 1995, Gramss *et al.* 1999, Hullo *et al.* 2001).

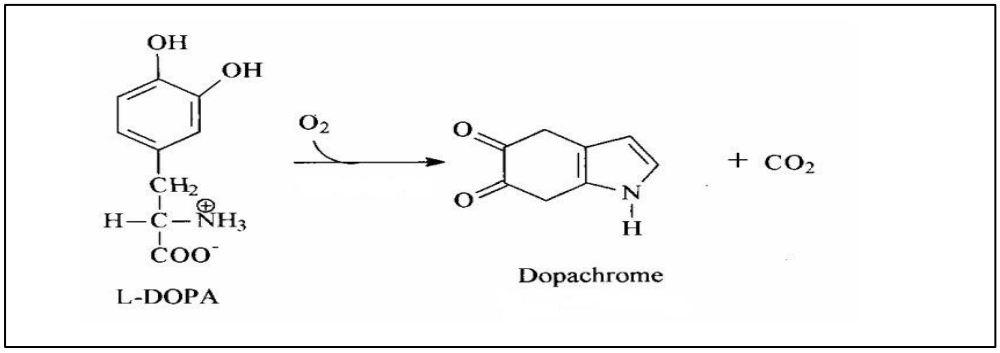

Figure 1.03. The colorimetric phenol oxidase assay in which the oxidation reaction of L-dihydroxy phenylalanine (L-DOPA) by phenol oxidase produces readily measured dopachrome (From Dunn 2013).

1.3. Soil variables affecting decomposition

As mentioned previously, peatlands sequester vast amounts of carbon, which enables them to play a significant role in minimizing the anthropogenic impact on the global climate (Freeman *et al.* 2012). However, human exploitation and on-going climate change induce decomposition processes and carbon loss, shifting the status of peatlands from an atmospheric carbon sink to a carbon source, thus compounding the greenhouse effect (Moore

2002). Wild (1993) suggested that the total annual amount of CO_2 produced globally from peatlands during the decomposition could be up to 0.5 Pg ($P = 10^{15}$), which poses a threat to the global climate stability. In soils, the rates of decomposition and the resultant release of biogenic GHGs are controlled by extracellular enzyme activities (Dunn *et al*. 2014). Measurement of these enzymes in relation to the biogeochemical properties of peat soils and their role in the carbon cycle and hence climate change has therefore been the focus of a significant amount of research in recent years.

Phenol oxidases are a suite of copper-containing enzymes with the ability to oxidise phenolic-based substances such as lignin, and convert them into smaller substances, in the presence of oxygen (Durán *et al.* 2002). Unlike phenol oxidases, hydrolase enzymes are not directly affected by the presence or absence of O_2 (Freeman *et al*. 2004). Evidence of the lack of a direct link between hydrolase activities and molecular oxygen was found by Lee *et al.* (1999) in the rumen, where completely anaerobic conditions persist, and hydrolase are still active.

Soil hydrolases are the proximate mediators of carbon and nutrient cycling (Marx *et al.* 2001). However, phenolic compounds can suppress the activity of hydrolase enzymes, limiting the degradation of organic matter, a mechanism known as the "enzymic latch" (Freeman *et al.* 2001b). Phenolics are a group of recalcitrant organic compounds, characterised by the presence of phenol units, which include an aromatic ring bonded with one or more hydroxyl groups. Phenolics are synthesized naturally by plants to support several physiological functions (Zak *et al.* 2019). They are classified into simple phenols and polyphenols depending on the number of phenolic units (Dunn and Freeman 2011). They persist in peat soils as a result of the extreme suppression of oxygen-dependent phenol oxidase activity and thus influence carbon and nutrient cycling by inhibiting organic matter-degrading enzymes (Freeman *et al.* 2001b).

Researchers have studied soil enzymology for more than a hundred years, and over the subsequent decades, researchers have developed their understanding of the role of extracellular enzymes activity in soil fertility, plant growth, carbon cycling and the associated influence on climate change. In addition, studies have been performed to investigate the relationship between enzymic activities and a range of environmental variables in peatlands and in turn inform management strategies for protecting their globally important carbon stores (Dunn *et al.* 2014).

Several physicochemical variables are known to influence microbial processes in peatlands (Kang *et al.* 1998). The key factors are discussed in turn below.

1.3.1. Oxygen concentration

Water table decline as a consequence of climate change or human intervention is an important factor affecting decomposition processes by controlling oxygen availability within soils (Moore 2002). Oxygen-dependent phenol oxidase is the key enzyme for carbon sequestration, when it remains inactive (Freeman *et al*. 2001b, 2004). A number of researchers have attempted to relate phenol oxidase activity to the concentrations of O_2 and phenolic compounds across the peat profile. For example, Pind *et al.* (1994) found that phenol oxidase activity falls gradually with depth due to decreased O_2 concentration, which allowed phenolics to accumulate in depth. Similarly, McLatchey and Reddy (1998) observed that higher activity of phenol oxidase was detectable only under oxygen-saturated conditions, in a Florida wetland.

Hydrological changes are the most studied subject concerning soil enzymes, drought-associated water level drawdown introduces O_2 into the system, stimulating phenol oxidase activity, resulting in a reduction in phenolics concentrations. By the removal of phenolics, hydrolase enzymes can accordingly resume their normal activity, leading to greater peat decomposition rates and carbon losses to the atmosphere and waterbodies (Fenner and Freeman 2011). Similarly, experimental lowering of the water level in peatlands resulted in an increase in calcium and magnesium concentrations and a substantial fall in the dominant inhibitors (iron and phenolics), which enhanced β-glucosidase, phosphatase and arylsulfatase activities (Freeman *et al.* 1996).

By influencing microbial processes, warming and more oxygenated conditions mainly accelerate the emission of CO_2 (Billett *et al.* 2010, Kayranli *et al*. 2010). By contrast, when the water table level is raised during flooding periods, CO_2 emission is reduced and the methanogenesis process is triggered, resulting in the release of CH_4 into the atmosphere in considerable amounts. Similarly, N_2O emission through denitrification process is associated also with anaerobic conditions (Kang *et al.* 1998). On the other hand, with a lowering of the water table level, methanogenesis decreases due to increased methanotrophy (Kayranli *et al.* 2010).

1.3.2. Phenolic compound concentrations

Phenolic compounds have inhibitory effects on hydrolase enzymes and undoubtedly on biodegradation and respiration rates because they can combine with the reactive sites of organic and inorganic substrates, making them resistant to further microbial attack. They can also inhibit litter decomposition by lowering pH (Min *et al*. 2015) and by themselves being strongly resistant to biodegradation (Zak *et al.* 2019). Generally, variations in the form, composition and the source of phenolic compounds, may be driving variations in enzyme activity and the resultant metabolic end products (Hoostal and Bouzat 2008, Min *et al.* 2015).

In peat soils, phenolic compounds accumulate due to the absence of oxygen which drastically inhibits phenol oxidase. The low hydrolase enzyme activities can be therefore indirectly attributed to O_2 constraints upon phenol oxidase activity. Indeed, in a series of experiments Freeman *et al*. (2004) found that the greatest impact of the addition of O_2 was seen on phenol oxidase, causing a sharp decline in phenolics abundance (27% fall) in less than 18 hours, which stimulated hydrolase enzyme activities (xylosidase 16%, glucosidase 26%, phosphatase 18%, arylsulfatase 47% and chitinase 22%).

So, through the reduction of the concentrations of phenolic compounds, phenol oxidase represents a key regulator for enzymic decomposition in peatlands (Freeman *et al*. 2001b, 2004). However, the correlation between phenol oxidase activity and phenolics concentration is not clear as contradictory results are currently present, while some studies demonstrated a negative relationship (Pind *et al.* 1994, Waldrop and Zak 2006, Stursova and Sinsabaugh 2008, Waldrop and Harden 2008, White *et al.* 2011), still others reported a positive (Freeman *et al.* 2001a, Fenner *et al.* 2005, Yao *et al.* 2009, Theuerl *et al.* 2010, Kang *et al.* 2018) or no relationship (Bending and Read 1997, Tian *et al.* 2010). Such conflicting evidence could be due to the dual functions of phenolic compounds that enabling them to serve as an enzyme substrate and/or as a product of enzyme action (Min *et al.* 2015).

As explained by Fenner *et al.* (2005), a rise in phenol oxidase activity simultaneously with increased concentration of phenolic compounds, may be due to the latter being cleaved as product of enzymatic action from the peat matrix faster than being degraded by the microbial biomass.

1.3.3. Hydrogen ions concentration (pH)

Alongside oxygen, a variety of factors have been appeared recently to weaken the enzymic latch mechanism, including pH value (Kang *et al*. 2018, Zak *et al.* 2019). Indeed, a simulation of moisture loss (increased oxygen ingress) during drought in a peat soil by Williams *et al.* (2000) did not result in increased phenol oxidase activity as would be expected. They attributed this to the low pH that characterized peatland ecosystems, the cation exchange capacity of carpet-forming *Sphagnum* mosses and the partial decay of plant remains introduce organic acids into the soil, all of which produce the characteristic acidity of peatlands (Gorham *et al.* 1987). Acidification of peat soils will indirectly limit hydrolase enzyme activities by suppressing phenol oxidase activity, which allowing the accumulation of inhibitory phenolics (Freeman *et al.* 2012).

According to Clymo (1983), low pH is an important factor in reducing decomposition rates in wetlands. The concentration of hydrogen ions is one of the primary factors controlling decomposition processes through its impact on phenol oxidase activity (Pind *et al.* 1994, Williams *et al.* 2000, Sinsabaugh *et al.* 2008). The pH value may affect the enzyme reaction site, as well as the interaction between immobilised enzymes and their associated matrix, thereby modifying enzyme activity in soil systems (Kang and Freeman 1999).

Across a survey of a wide range of peatlands and through field and laboratory experiments, pH emerged as the primary agent driving phenol oxidase activity and subsequently dissolved organic carbon (DOC) release (Kang *et al.* 2018). Furthermore, Kang and Freeman (1999) found that pH value was the dominant controlling factor for phosphatase activities in bog, fen and swamp of a Welsh peatland.

1.3.4. Organic and inorganic nutrient concentration

Organic carbon supplies are required by heterotrophic microorganisms as energy sources promoting microbial processes (Kang *et al.* 1998). DOC is reported to have a priming effect on organic matter decomposition and therefore may be expected to exhibit a positive correlation with extracellular enzyme activities (Song *et al.* 2019). DOC is also thought to stimulate the activity of enzymes involved in the mineralisation of other nutrients (e.g., phosphatase and arylsulfatase) (Kim and Kang 2008). However, even under a high concentration of DOC, microbial processes could be limited (Kang *et al*. 1998) due to the presence of phenolic compounds which tend to constitute a large proportion (50-75%) of the

peatland-derived DOC pool, dissolved organic carbon (DOC) in aquatic environments can originate from internal or photosynthetic sources (autochthonous inputs) and/or from external or terrestrial sources (allochthonous inputs) (Hope *et al*. 1994). In general, DOC from autochthonous sources is relatively uncoloured, and has low aromaticity and molecular weight and is easily broken down by soil microbes and therefore exhibits high flux rates in waters. Allochthonous DOC on the other hand has high molecular weight and is rich in aromatic compounds, highly coloured and relatively recalcitrant to decomposition (Gergel *et al.* 1999).

In addition to water table heights, inorganic nitrate supply and carbon supply have been reported as the main controlling factors for N_2O emission (Groffman 1991). Indeed, Kang *et al.* (1998) found that the N_2O emission increased with increasing both nitrate concentration (used as electron acceptor during denitrification) and β-glucosidase activity (used as an indicator of labile carbon availability) in a Welsh peatland. In the same study, they found a significant negative relationship between inorganic sulfate and CH_4 emission as sulfate addition decreased the production of CH_4 by 91%, a finding backed up by Freeman *et al*. (1994), who attributed the inhibitory effects of sulfate ions on CH_4 emission to the competition between methanogens and the most abundant sulfate-reducing bacteria for organic substrates.

Measurement of enzymes activity can reflect microbial nutrient demand as many microorganisms adjust their enzyme production in response to the demand for nutrients (Chròst 1991). Therefore, it is believed that a negative correlation would occur between soil enzyme activity and nutrient availability depending on the economic model of microbial metabolism indicating that if nutrients are limited, enzymes production increase and vice versa (Sinsabaugh *et al.* 1993, 2008). Indeed, a statistically significant negative relationship was detected between phosphatase activity and phosphate concentration, indicating that phosphatase production was induced in response to the low phosphate availability in wetland soils (Kang and Freeman 1999). However, there is conflicting evidence regarding the relationship between inorganic nutrients content and the enzymes activity, for example between phosphatase activity and inorganic P availability, while some studies observed a negative relationship (e.g. Kang and Freeman 1999, when compared on a spatial basis, Treseder and Vitousek 2001), still others reported a positive relationship (e.g. Weintraub and Schimel 2005) or no relationship (e.g. Kang and Freeman 1999, when compared on a temporal basis). The latter could be due to the measurement of stabilised enzymes which are

already present in large amounts in the soil and are independent of microbial control and thus do not respond to nutrient availability (Burns *et al.* 2013).

Increased nitrogen deposition has been linked with increased enzymes activity and carbon release from peatlands due to the lower release of polyphenols by *Sphagnum* mosses when N content is high, which in turn limit enzyme inhibition and promote soil metabolism (Bragazza *et al*. 2006).

1.4. Microbial processes along layers of peat soil

Soil microorganisms occur throughout the soil profile and even in deeper layers, where during their activity and function, they contribute to the processes involving organic matter decomposition, nutrient cycling and GHG emissions (Jackson *et al.* 2009, Senga *et al.* 2015).

The key processes in the anaerobic deeper layers are fermentation, methanogenesis, and iron, nitrate and sulfate reduction. Subsurface soil processes such as methanogenesis and denitrification are dependent on the oxygen status of the soil and hence the water table level (Kayranli *et al*. 2010). N_2O and CH_4 are potent greenhouse gases, having a much higher global warming potential (up to 298 and 34 times stronger, respectively) than CO_2 (IPCC 2013).

Several studies have shown that methanogenic *Archaea* increase as peat depth increases (Kotsyurbenko *et al.* 2004, Dedysh *et al*. 2006, Jackson *et al*. 2009). Likewise with N_2O flux, anaerobic conditions promote denitrification which produces more N_2O than during the nitrification process (which is more prominent under aerobic conditions). Moreover, deep soil layers may exhibit the same as or higher enzymes activity than surface layers (Senga *et al.* 2015).

Fenner and Freeman (2011) and Gough *et al.* (2016) suggest that under anoxic conditions, anaerobic metabolism becomes dominant, shifting metabolic end products towards CH_4, DOC and CO_2 rather than principally CO_2. The concentration of DOC in surface waters has important implications for drinking water providers since DOC reduce the aesthetic quality of the water and acts as a reaction precursor to potentially harmful disinfection by-products (Jones 2006).

Despite the importance of the microbial processes that occur at depth, lower layers of peat have received less attention than the upper layers (Senga *et al*. 2015). This is because the upper depths of the peat profile often represent the most enzymatically active layer, more

likely due to the higher temperatures, aeration, labile organic matter content and the fact that this layer corresponds with the rhizosphere and hence highest density of microbes (Moore 2002).

1.5. Micro-eukaryotes in peatlands

Peatlands are water-saturated environments thanks to the spongy layer of *Sphagnum* moss on their surface. These conditions allow planktonic organisms to be mobile and dynamic. These organisms include prokaryotes, microbial eukaryotes and small Metazoa that together form the microbial food web where nutrients and energy are transferred through the trophic interactions (Jassey *et al*. 2015). Unlike prokaryotes, research into eukaryotic microorganisms has been limited in many ecosystems including peatlands. These are globally significant environments since they represent huge carbon reservoirs, sequestering over one third of the global soil carbon in the form of peat (Kang *et al*. 2018) and are also distinguished by their rich biodiversity (Gilbert and Mitchell 2006, Mieczan 2012). The structure (biological communities) and function (storing carbon) of peatlands are threatened by ongoing global warming which has been attributed to high emissions of greenhouse gas into the atmosphere (IPCC 2013).

In the microbiomes found in soils, eukaryotic microbial communities are the most species rich (Geisen *et al*. 2015a). These organisms including fungi and protists (heterotrophic (protozoa) and autotrophic protists), which are both functionally diverse (Araujo *et al.* 2018), allowing them to play a number of essential roles within the ecosystem's food webs (Adl and Gupta 2006, Gilbert and Mitchell 2006), and can directly and/or indirectly affect the carbon balance of peatlands and hence possibly contribute to climate change (Dedysh *et al.* 2006, Jassey *et al*. 2015).

In soils, phototrophs containing photosynthetic pigments are the primary producers fixing carbon during photosynthesis (Seppey *et al.* 2017). Phagotrophs feed mainly on bacterial and fungal communities in addition to other eukaryotic microorganisms and thus they contribute to elemental cycling, nutrient release and soil fertility, which in turn stimulates plant growth (Geisen 2016, Geisen *et al.* 2018). Through predation, phagotrophs also control their microbial prey populations thus influencing indirectly the functioning of their prey in the ecosystems (Geisen *et al.* 2018). Indeed, Murase *et al.* (2006) found that without heterotrophic protists, total DNA increased in ɤ-radiation-sterilized soil that had been reinoculated with a bacterial assemblage, while in the soil that had been reinoculated with

both bacteria and protists after sterilization, there was a decrease in bacterial biomass due to protistan grazing effects. This demonstrates the significant role of phagotrophic protists in the regulation of bacterial populations (Gilbert and Mitchell 2006). Protozoan predation may also help to reduce diseases through the consumption of disease agents (e.g., *Cryptosporidium* oocysts) (Stott *et al.* 2001). Mixotrophic organisms are those that benefit from both trophic behaviours (phototrophy and phagotrophy), which enable them to significant contribution to carbon cycling. As primary producers they directly contribute to carbon fixation through photosynthesis, while as heterotrophs they indirectly affect organic matter decomposition by feeding on the decomposers (Jassey *et al.* 2015). Osmotrophs absorb soluble nutrients through the cell membrane and are mostly found within the fungal group (Adl and Gupta 2006). These have been identified as the main decomposers of organic substrates in soils (Gilbert and Mitchell 2006), converting dead organic matter into biomass and hence building the link between basic resources and higher trophic levels (Ferreira and Voronina 2016) mainly thanks to their extracellular enzymes' activities (Min *et al.* 2015).

Recently, high throughput sequencing-dependent approaches revealed that parasitic protists are abundantly present in soils (Geisen *et al.* 2015b, Mahé *et al*. 2017). Therefore, soil can be an important source of pathogenic infection for livestock, agricultural crops and even for humans. In soils, parasites are often found encysted in a cell wall (cysts, oocysts and spores). Prominent examples are: the cysts of *Giardia duodenalis*, *Balantidium coli* and *Entamoeba histolytica,* the oocysts of *Cryptosporidium* spp., *Cystoisospora belli* and *Toxoplasma gondii* and the spores of microsporidia. Parasites can also be found in soils as trophozoites, for example the facultative amoeba *Acanthamoeba* spp. and the excavate *Naegleria fowleri*. According to Thomas *et al.* (1997), parasites could act as key species in wetlands through their direct and indirect effects on their host species. These effects include survival, predation, castration, sexual selection and ecological distribution. In addition to the pathogenic effects, parasitic free-living dispersal stages can serve as prey for other organisms, contributing greatly to ecosystem biomass and energy transfer (Thieltges *et al.* 2013).

By controlling host communities, parasites can alter food web structures (Geisen *et al.* 2015b). Generally, micro-eukaryotes can directly and/or indirectly affect the carbon balance of the ecosystem through their diverse functions.

Micro-eukaryotic organisms are known to be vulnerable to changes in environmental conditions including water content, oxygen availability, temperature, pH, light intensity, nutrient availability, vegetation type and anthropogenic perturbations (Geisen *et al*. 2018).

Soil water availability is a major factor shaping the community composition of soil micro-eukaryotes along a transect from the Arctic to the tropics (Tsyganov *et al.* 2013). They permanently need water for their mobility and functions. Consequently, their activities are constrained by lack of water (Geisen *et al.* 2014). On the other hand, growth rates of micro-eukaryotes in anaerobic conditions created by excess water are lower than those in aerobic conditions (Fenchel and Finlay 1990). Temperature affects micro-eukaryotic communities both directly and indirectly. For example, relatively high temperature has a direct effect on the phototrophic ability of testate amoebae (Jassey *et al.* 2015) and has an indirect effect by regulating soil moisture content (Bamforth 1973). A wide range of tolerance to soil temperature is observed depending on the species present. However, eukaryotic life is severely limited at temperatures above 60°C (Clarke 2014). Micro-eukaryotes also show a wide range of pH optima. Acidic soils of *Sphagnum* peatlands host a broad diversity of protist communities (Lara *et al.* 2011). Light intensity primarily affects autotrophic microorganisms and hence affects their predators by regulating food availability (Seppey *et al.* 2017). Vegetation type can also affect micro-eukaryotic communities in soil through root exudates and/or litter composition (Bragazza *et al*. 2015, Geisen *et al.* 2018). Therefore, lengthened growing seasons and changes in vegetation cover due to climate warming could result in substantial microbial community composition changes (Shi *et al.* 2015).

Moreover, soil micro-eukaryotes have been found to be vulnerable to anthropogenic disturbances such as ploughing, pollution, elevated atmospheric CO_2 and the application of fertilizers and pesticides (Geisen *et al*. 2018). Soil parasite abundance can be an indicator of hostile environments, for example where pollution and the addition of pesticides may have weakened soil invertebrates and created a high susceptibility of hosts to infection by apicomplexan parasites (Foissner 1999). Application of fertilizers to naturally nutrient-depleted peatlands significantly increase the abundance of autotrophic microorganisms (Gilbert *et al.* 1998). Amblard (1991) reported that the development ability of many phototrophic diatoms has not impeded by dark conditions, possibly due to the availability of dissolved nutrients and their ability to switch to heterotrophic feeding.

Given their sensitivity to environmental conditions, micro-eukaryotic organisms are considered to be useful bioindicators of abiotic variations in their environments (Koenig *et al*. 2015, Geisen *et al*. 2018), and can to some extent reflect soil quality and spatial heterogeneity in different habitats (Mitchell *et al.* 2000). However, they have received little attention in many ecosystems including peatlands. Importantly, assessing the influence of environmental variables on microbial communities of peatlands using climatic gradients is needed to understand and predict the impacts of future climate change on the peatland structure and function (Davidson and Janssens 2006, Bragazza *et al.* 2015, Jassey *et al.* 2015).

In most of the existing studies on this subject, a single region was sampled to address a specific group of micro-eukaryotes. The bioindicator potential of peatland testate amoebae (belong to protists) has been quite well researched in the last few years. For example, Koeing *et al*. (2015) found that testate amoebae were valuable as bioindicators of environmental gradients (temperature, altitude, water table depth, N and C content) in *Sphagnum*-dominated peatlands in Switzerland. In a detailed study, they found that only ten testate amoeba taxa were more powerful indicators of local conditions than the full diversity of vegetation. Lamentowicz *et al*. (2010) also found that such microorganisms were strongly correlated with water table depth and hydrochemistry of mosses in sub-alpine peatlands. Long-term warming experiments in a French peatland have shown a negative response of algal endosymbionts of mixotrophic testate amoebae to temperature increase, which led to decline their abundance and hence reduced peatland C fixation capacity (Jassey *et al.* 2015). The declined in the abundance of mixotrophic organisms due to the disruption of their phototrophic ability under warming is due to the fact that these organisms cannot live as pure heterotrophs without their algal endosymbionts which fail to transmit from mother to daughter cells during cell division under higher temperatures (Jassey *et al.* 2015).

Papadimitriou *et al*. (2010) found that the abundance and diversity of ciliated protozoa increase with increasing dissolved oxygen concentrations and with decreasing electrical conductivity to less than 3.0 mS/cm in wastewater of constructed wetland.

Gilbert *et al*. (1998) observed that increased supply of nutrients resulted in a substantial increase in the ciliates and the micro-algal Bacillariophyceae and a decrease in the abundance of testate amoeba and of other algae. Mieczan (2007) studied ciliate communities and their relationship to environmental factors in the peat-bog reservoirs of Eastern Poland. He found that the abundance of ciliates increased with increasing pH. He added that in the reservoirs

with the lowest pH the lower abundance of ciliates may also result from humic substances that release from the peat and attenuate light penetration, reducing the abundance of autotrophic microorganisms that are a preferred food source for them. The same author (Mieczan 2012) further found that pH, water level, total organic carbon and phosphorus were significant factors constraining testate amoeba and ciliate communities in peatlands of Eastern Poland.

Kang *et al.* (2018) noted that under optimal temperature conditions, increased pH can change fungal communities, stimulating phenol oxidase activity and result in higher dissolved organic carbon (DOC) production. This in turn may elevate DOC concentrations in waters draining peatland areas. The presence of phenolic compounds appeared to be negatively affected the presence of micro-eukaryotic communities possibly indirectly through effects on prey organisms (Fung *et al.* 1985, Opelt *et al.* 2007, Mellegård *et al.* 2009, Pizzolitto *et al.* 2015). Oxygen concentration is also an important factor in structuring fungal populations in peat soils (Lin *et al.* 2012, Bragazza *et al.* 2015).

Mataloni (1999) studied microalgae communities (phototrophic protists) along a hydrological gradient from the open water to the drier surroundings of Argentine peatlands, and found that the species richness of phototrophic protists decreased toward terrestrial drier conditions, lower pH and higher conductivity, with the dominance of all green algae decreasing as a result of replacement by other species that were better adapted to the hostile conditions.

Regarding parasites, water-saturated soils facilitate the transport of parasitic infective stages through the soil to water reservoirs leading to the contamination of water supplies and fresh foods by disease agents. Rainfall is an important natural factor contributing to parasitic infective stages movement horizontally between places and vertically through soil profile (Santamaria and Toranzos 2003). Harvell *et al.* (2002) predicted that increased global temperatures would result in a deterioration in health, because increased temperature and precipitation events resulting from climate change could facilitate disease outbreaks including parasitic diseases. Higher temperatures can compromise the immune system of hosts and increase pathogen growth and transmission rates (Harvell *et al.* 2009). Indeed, warming experiments on a host-parasite system by Studer *et al.* (2010) showed that with increasing temperature, there was an increase in productivity, output and infectivity of parasite transmission stages. Also, the susceptibility of hosts to infection was increased with increasing temperature.

The concentration of DOC in water bodies is another environmental factor affecting parasite abundance. King *et al*. (2008) reported that the inactivation of *Cryptosporidium parvum* oocysts by solar UV irradiation in surface waters decreases with increasing DOC concentration. This is due to the absorption of UV by DOC molecules (mainly humics), a consequence of the presence of chromophores on their aromatic rings (Leenheer and Croue 2003).

A broad diversity of micro-eukaryotes has been identified as typical inhabitants of peatlands; Alveolata, with sequences of Ciliophora are the most numerous (Lara *et al.* 2011, Geisen *et al*. 2015a), photosynthetic Bacillariophyta (Geisen *et al.* 2015a) and the Chrysophyceae within supergroup Stramenopiles (Lara *et al.* 2011), Arcellinida within Amoebozoa and Euglyphida within Rhizaria (Lara *et al.* 2011, Jassey *et al.* 2015) dominated peat soils. Parasitic protist taxa such as Apicomplexa, Oomycetes and Ichthyosporea were ubiquitously detected in soils (Geisen *et al.* 2015a). Up to 55% of fungal species have been identified in the northern peatlands. Fungi, mainly Zygomycota, Ascomycota and Basidiomycota are also peat-associated inhabitants (Gilbert and Mitchell 2006, Lin *et al*. 2012). Microalgae (phototrophic protists) are the most diverse group, estimated to represent 20 to 60% of the total diversity in peatlands (Gilbert and Mitchell 2006).

The spatial heterogeneity of peatland substrate conditions and the associated sampling challenges may in part explain why microorganisms are a relatively under-researched aspect of peatland environments. Few studies have examined microbial abundance and diversity in peat, despite the fact that microbes are recognised as playing a key role in the functioning of the ecosystem (Gilbert and Mitchell 2006).

Clearly, there is still a need for much more baseline data on community composition, function and responses of microorganisms to ecological gradients in contrasting peatlands in order for microbial communities to be a useful monitoring tool in such crucial ecosystems (Gilbert and Mitchell 2006).

1.6. Aims of the study

It is important to study the conditions that influence the activities of peat soil enzymes responsible for organic matter decomposition as a prerequisite for predicting the ecosystem response to climate change, human utilization and management policies (Moore 2002). In

addition, studying the effect of soil variables on microbial communities in peatlands along climatic gradient is useful to predict how peatlands structure and function may respond to climate-driven environmental changes (Bragazza *et al.* 2015).

This study was expanded to include three contrasting peatlands, representing a gradient of environmental conditions, located in three climatic regions, Arctic (Svalbard, Norway), temperate (North Wales, UK) and tropical (Andes mountains, Colombia). Also, the range of metabolic and environmental parameters investigated has been extended, compared with previous studies, to include key hydrolytic enzymes ß-D-glucosidase, arylsulfatase, ß -D-xylosidase, N-acetyl-ß-D-glucosaminidase (chitinase), phosphatase, oxidation enzyme phenol oxidase, the greenhouse gas carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), physicochemical variables pH, electrical conductivity, dissolved organic carbon (DOC), phenolic compounds, soil organic matter (SOM) and water content, cations and anions concentration. In a field scale study, these variables have been identified as important aspects of influencing different microbial processes (Groffman 1991, Pind *et al*. 1994, Kang *et al*. 1998, Kang and Freeman 1999, Kang *et al.* 2018).

In addition, the deoxyribonucleic acid (DNA) from the superficial layers of peat soil was extracted and Illumina MiSeq sequencing was used to identify a broader range of micro-eukaryotes in the three peatlands. The main aims of this study are:

1- To determine the relationship between biogeochemical processes and environmental factors across the peat profile in different climatic regions (chapters 2, 3 and 4).
2- To get an indication of carbon and nutrient cycling in relation to the environmental factors at global scale by comparing the three climatic regions (chapter 5).
3- To determine richness, diversity and relative abundance of micro-eukaryotes along the three different peatlands. Also, attempts were made to relate community composition of micro-eukaryotes to biotic and abiotic properties of studied peatlands by using redundancy analysis (RDA) (chapter 6).

The thesis chapters have been written in the format of publishable papers. As a result, some repetition in the introduction and materials and methods sections was unavoidable.

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Chapter 2

Enzymatic activity and soil respiration linked to physicochemical properties in the Arctic peatland of Svalbard, Norway

2.1. Abstract

Environmental factors that control the functions of microbial communities at different soil depths in Arctic peatlands are still poorly understood. In this study, extracellular enzyme activities (hydrolases and phenol oxidase) and the production of greenhouse gas (carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)) were investigated at different depths in an Arctic peatland in Svalbard, Norway. In addition, the associations between these biogeochemical processes and pH, conductivity, nutrient concentration (cations and anions), phenolic compounds concentration, dissolved organic carbon (DOC) concentration, and soil water and soil organic matter (SOM) content were assessed.

Soil respiration was higher on average in the uppermost layer (0-5 cm) and was associated with enhanced enzymes activity (except for phenol oxidase), higher water and SOM content, and greater labile DOC concentration, indicating that this is the most biologically active layer. The significant decline in hydrolase activities and the fluxes of CO_2 and N_2O with depth suggests a subsequent lack of appropriate microbial substrates and the overall favorable conditions required for microbial activity with increasing depth. The pH values, which ranged from 5.64 to 8.6, seemed unfavorable for hydrolytic enzymes activity. The significantly higher activity of phenol oxidase at the underlying mineral layer (> 20 cm), was attributed to the activating impact of the more favorable pH under anaerobic conditions, as high phenol oxidase activity was accompanied by a significant increase in pH value at this layer and was significantly related to soil pH ($r = 0.431$, $p < 0.01$). Neither extracellular enzyme activity nor greenhouse gas production showed significant negative correlations with phenolic concentrations as would be expected, suggesting that the activities of extracellular enzymes and hence the metabolic end products depend on the form, composition and the source of phenolic compounds rather than their absolute quantities. Contrary to the 'economic model' of microbial enzyme production, phosphatase activity was positively associated with inorganic phosphate concentration, which may indicate that phosphatase production rate was more than microbial demand for phosphate.

The emission of CH_4 and N_2O was significantly correlated with carbon supply (represented by β-glucosidase activity) needed for the growth of heterotrophic microorganisms.

The concentration of DOC showed a significant inverse correlation with ionic strength (conductivity), which is known to negatively affect the solubility of DOC and hence its concentration in peat solution.

2.2. Introduction

Moss tundra peatlands in the High Arctic, Svalbard, dominated by peat-forming bryophytes, are likely to be a hotspot of carbon sequestration, with peat depths of up to 1 m reported in some areas (Nakatsubo *et al.* 2015). Elvebakk (1994) mentioned that the peat-forming moss tundra tends to develop in moist conditions under bird-nesting cliffs where bird manure provides a source of nutrients and stimulating productivity and carbon accumulation. Soils of Arctic peatlands are also large nitrogen stocks, with an estimated of more than 67 billion tonnes of nitrogen accumulated over thousands of years (Harden *et al.* 2012). However, climate change is likely to result in a shift in the the carbon and nitrogen budgets of Arctic peatlands which could result in a shift from sink to a source status (Voigt *et al.* 2017a, b). Under modified climatic conditions, soil gas emissions could increase substantially in this region, though little data in this respect are available in the literature, especially from Norway (Grønlund *et al.* 2008). Furthermore, information concerning the relevant physicochemical parameters and enzymes activity in peatlands of the Arctic region is still limited (Szajdak *et al.* 2016). Various studies have investigated greenhouse gas emissions from Arctic peatlands to evaluate the magnitude of the peat-carbon (Schuur *et al.* 2015, Tveit *et al.* 2015, Schadel *et al.* 2016, Voigt *et al*. 2017a) and nitrogen (Martikainen *et al.* 1993, Marushchak *et al*. 2011, Voigt *et al.* 2017a, b) climate feedback loop. They suggest that enhanced GHGs flux from Arctic soils could represent a strong climate change feedback (Voigt *et al.* 2017b). However, such studies have often overlooked potential enzymatic activities, and the chemical and physical variables that affect them. Additionally and to date, knowledge about the effects of soil physicochemical properties on enzyme activity and soil respiration at different depths within Arctic peat profile is still lacking despite the importance of anaerobic SOM decomposition at deeper layers (Tveit *et al*. 2015). There is a general agreement that the emission of C and N from peatlands is regulating by soil microbial processes which in turn are affected by the surrounding physicochemical conditions such as soil temperature, oxygen concentration, nutrients availability, pH, water content, microbial abundance, inhibitory phenolics, etc. (Freeman *et al.* 1990, Freeman *et al.* 1995, Freeman *et al.* 2001). The present study aims to investigate variations in enzymic decomposition and GHG flux with depth in an Arctic peatland in Svalbard, Norway. Relationships between enzyme activities, soil respiration and abiotic soil conditions, including pH, conductivity, the concentrations of DOC, phenolics, anions and cations, and SOM and water content were also examined. The

hypothesis that the changes in biogeochemical processes with depth are proportional to changes in physicochemical soil properties was tested.

2.3. Materials and methods

2.3.1. Study sites descriptions

The annual rainfall in Svalbard is 400mm, with continuous permafrost and relatively short growing season (40-70 days) (Rozema *et al.* 2006). Annual mean temperature is between -4 and -7 °C (Tveit 2014). Samples of the present study were collected from six peat sites located in the northwest of Svalbard, Norway in late July 2016, two in the Solvatn (site 1 & site 2), two in the Stuphallet (site 3 & site 4), one in the Knudsenheia (site 5) and one in the Storvatnet (site 6) (Figure 2.01). More detailed maps were unavailable for peatland distribution in Svalbard.

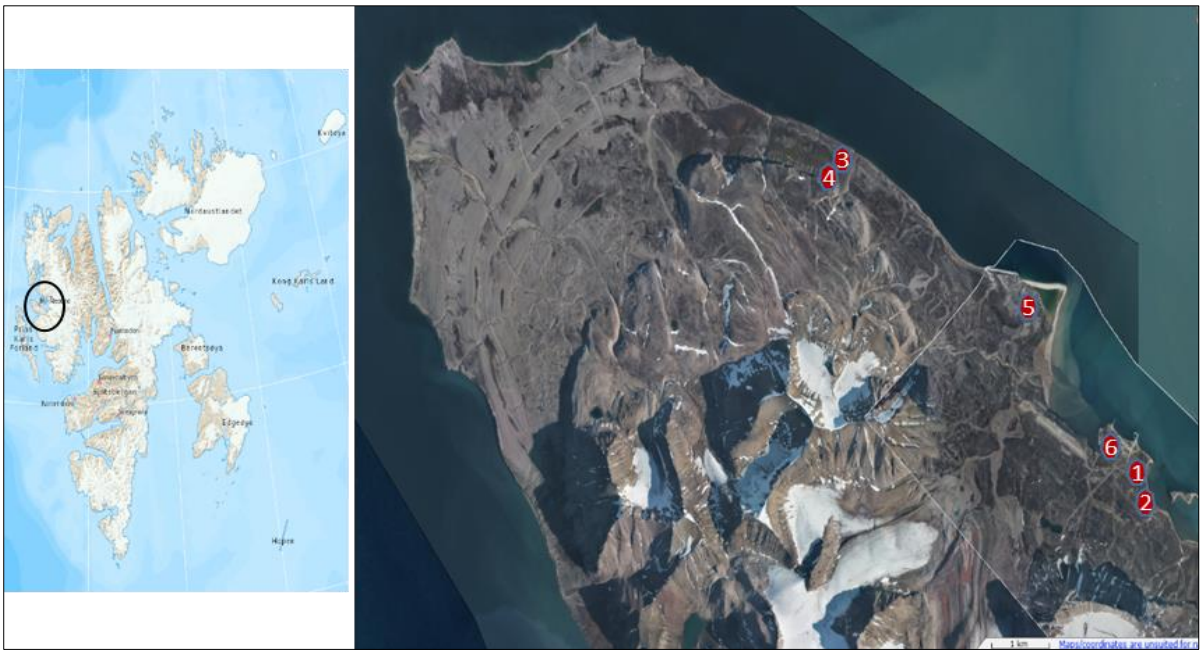

Figure 2.01. Maps showing study site and peat sampling locations in Svalbard, Norway (Source: Google Maps).

The average air temperature at the time of sample collection was 5°C, the water table was at the soil surface during sampling and the moss tundra of the study sites was dominated by the species *Tomenthypnum nitens* and *Calliergon richardsonii*, without vascular plants (Figure 2.02a-d). Peat cores were collected from each of the six sites with three replicates for each site extracted using a Russian corer (50 cm long and 10 cm in diameter). After removing the

vegetation layer, the cores were separated into several layers based on a visual assessment of colour and texture. Figures 2.02e and 2.02f show the stratigraphy of a representative core from the six locations. This approach yielded a total of 51 samples. After removal of stones, roots and macroinvertebrates, all samples were placed separately in labeled plastic bags and transported to the laboratory in Wales in a cooled container, where they were stored at 4°C until further analysis.

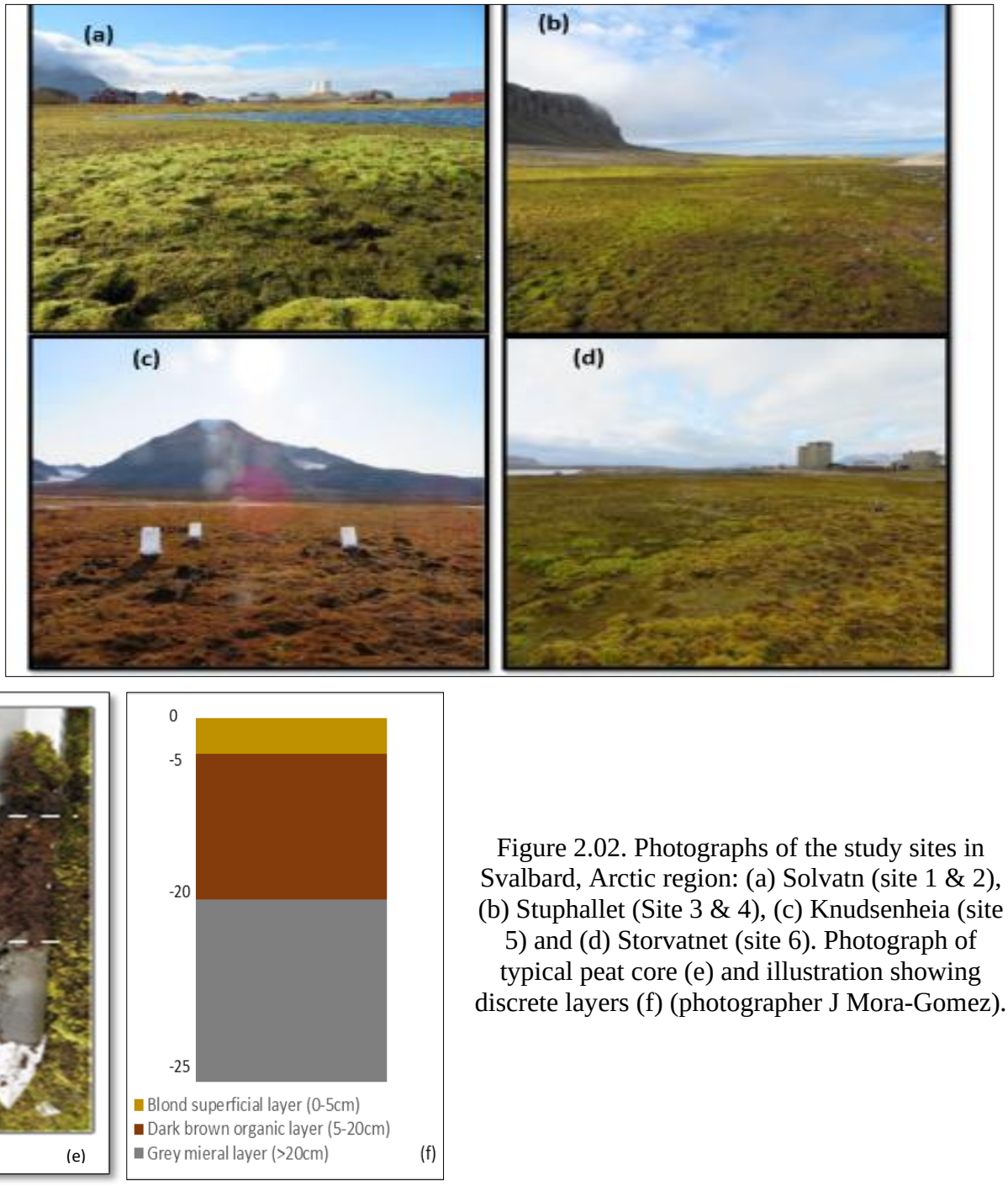

Figure 2.02. Photographs of the study sites in Svalbard, Arctic region: (a) Solvatn (site 1 & 2), (b) Stuphallet (Site 3 & 4), (c) Knudsenheia (site 5) and (d) Storvatnet (site 6). Photograph of typical peat core (e) and illustration showing discrete layers (f) (photographer J Mora-Gomez).

Soil temperature, pH, and oxygen concentration were measured in the field at the time of sampling using a digital thermometer, a Mettler Toledo S20 pH meter and a dissolved oxygen probe, respectively. These data are presented in Table 2.01.

Table 2.01. Peat core locations and conditions. Values are presented as the range of the 3 replicates.

Location	**Soil temp. (°C)**	**pH**	**Soil O_2 Conc. (mg L^{-1})**	**Coordinates**	**Altitude**
1	6.6-7.4	6.9-7.2	3.3-3.9	78°55'30.0"N, 11°56'26.1"E	4 m a.s.l
2	7.0-7.2	6.6-7.6	5.0-5.4	78°55'34.0"N, 11°56'20"E	3 m a.s.l
3	5.5-5.9	7.2-7.5	5.8-6.4	78°57'31.9"N, 11°41'14.2"E	17 m a.s.l
4	7.0-7.4	7.1-7.6	5.0-5.4	78°57'34.8"N, 11°40'38.8"E	16 m a.s.l
5	7.6-8.4	6.2-7.0	6.7-7.3	78°55'33.4"N, 11°54'54.3"E	16 m a.s.l
6	7.0-7.5	7.0-7.2	6.6-7.1	78°55'32.5"N, 11°55'01.3"E	21 m a.s.l

2.3.2. Soil analyses

In the present study, the aim was to estimate the emissions of GHG and the activities of extracellular enzymes that occur under natural conditions within peatlands. Therefore, field conditions were copied as closely as possible by matching gas production and enzyme assay temperature to that recorded in the field.

Analyses were performed within two weeks of collection as previous studies have shown soil enzymes to be stable for this period of time (Dunn *et al*. 2014). Samples were transported by air freight to minimize the potential for changes to occur during transportation and were transported with icepacks to minimize temperature fluctuations during transportation. According to Ross (1965), although different soils can differ in their storage behaviour, storage in a refrigerator at 4°C is generally considered the most suitable. However, while the activities of β-glucosidase and xylosidase have been found not affected by the duration of soil storage, *N*-acetyl-glucosaminidase (chitinase), phosphatase and phenol oxidase activity are more susceptible to change (DeForest 2009) and so some caution must be used when interpreting these data.

2.3.2.1. Enzyme activity analyses

Before conducting any analyses, the samples were kept in the incubator at field temperature for around three hours. The activity of phenol oxidase (POX) in peat soil samples was determined using a procedure adapted from Pind *et al*. (1994) and Dunn *et al*. (2014). Model compound L-DOPA (L-3,4- dihydroxy phenylalanine, Sigma Aldrich Ltd, Dorset, UK) was used as a substrate for the enzyme's degradation. The colour change is determined using a spectrophotometer measuring at 475 nm, from which the activity of phenol oxidase can be calculated. After homogenizing the bag containing the soil sample by hand, two separate 1 g soil samples were weighed and placed in two labelled stomacher bags (Seward, West Sussex, UK). After adding 9 mL of ultrapure water to each bag, these were then placed in a paddle blender (Stomacher® circulator, Seward) to mix the contents for 30 s on a normal speed setting. A 10 mL of ultrapure water was added to one bag, used as a blank. A 10 mL of the substrate (L-DOPA) was added to the other, and both bags were mixed for a further 30 s. All bags were then incubated at field temperature for 10 min before the bags were removed and mixed by hand. For each bag, three 1.5 mL microcentrifuge vials (Eppendorf, Stevenage, UK) were labelled and filled with the bag's solution. The vials were centrifuged at 10,000 rpm for 5 min. A 300 µL of supernatant from each vial was transferred to a well of a clear 96 well microplate (Sterilin, Cambridage, UK). The microplate was then placed on the SpectraMax M2e plate reader spectrophotometer and absorbance values measured at λ= 475 nm. To obtain the average absorbance value, the mean absorbance of the three blanks was subtracted from the mean absorbance of the three samples containing the substrate. The activity of phenol oxidase was then calculated by using Beer-Lambert Law and expressed as µmol of formed diqc per min per g of soil (dry weight).

Hydrolase activities were determined using the procedures of Dunn *et al*. (2014), based on the measurement of fluorescence of methylumbelliferone (MUF). In five 1L volumetric flasks, the relevant amount of MUF-labeled substrates (4-MUF β-D-glucopyranoside for β-D-glucosidase (B), 4-MUF sulfate potassium salt for arylsulfatase (S), 4-MUF β-D-xylopyranoside for β-D-xylosidase (X), 4-MUF N-acetyl-β-D-glucosaminide for N-acetyl-β-D-glucosaminidase (N) and 4-MUF phosphate for phosphatase (P)) were dissolved in 20 mL of cellosolve solvent (Sigma Aldrich Ltd, Dorset, UK), and then ultrapure water was added up to the 1L mark. Magnetic stirrers were used if necessary. Five 1 g subsamples (one for each MUF substrate) were taken from each bag containing soil samples and placed in

separate stomacher bags with 7 mL of the relevant substrate (stored at field temperature before use). These were homogenized using a Seward Stomacher 80 Laboratory Blender for 30 s. The stomacher bags were then incubated at field temperature for 45 min for the enzyme P and 60 min for the other enzymes (B, S, X, and N). The homogenates were then mixed by hand and transferred into separate 1.5 mL centrifuge vials, which were then centrifuged at 10,000 rpm for 5 min. During this time, 50 µL of ultrapure water was added to black microplate wells (Sterilin, Cambridge, UK). At the end of centrifugation, 250 µL of the resulting supernatant was extracted from each sample and added to the relevant wells on the microplate. Fluorescence of the MUF molecule was measured using a microplate fluorometer (Molecular Devices SpectraMax M2e spectrophotometer) at 450 nm emission and 330 nm excitation. Hydrolase activities are expressed as nmol MUF g^{-1} of soil min^{-1}. To prepare the calibration curve, 7 mL of ultrapure water was added to a stomacher bag (labelled as standard) containing 1 gram of soil sample and homogenized as described above. The content from each standard bag was used to fill two 2 mL Eppendorf tubes, which were then centrifuged at 10,000 rpm for 5 mins. Different MUF-free acid (Sigma Aldrich Ltd, Dorset, UK) concentrations (8 in total) were prepared in 2 mL Eppendorf tubes using the MUF stock solution (prepared by dissolving the relevant amount of 4-methylumbelliferone free acid in 10 mL of cellosolve solvent and 500 mL of ultrapure water) and ultrapure water. A column of the 8 standard solutions was prepared per soil sample by pipetting 50µL from each of the standard solution into a series of wells on a black microplate in addition to 250µL from the supernatant of the sample two Eppendorf tubes. The microplate was then analyzed as before.

2.3.2.2. Hydrochemistry analyses

For measurement of pH, conductivity, DOC, phenolic compounds, cations and anions concentrations, a soil solution was prepared by adding 5 g of soil sample and 40 mL of ultrapure water to a 50 mL centrifuge tube, and shaking on a rotary shaker for 24h. This process is not for homogenization, but rather for allowing the listed solutes to leach from the soil to produce an estimate of leachable solutes. Although there is some risk that this could allow changes in the samples (including shifts in microbial composition, consumption of solutes, reproduction, metabolism of solutes to other chemical forms), allowing 24h on a rotary shaker is a common practice for extracting potentially leachable solutes (Dahlgren 1993, Kowalenko 2008).

Measurement of pH and electrical conductivity was conducted on un-filtered samples using a Mettler Toledo S20 pH meter and a Primo 5 handheld conductivity meter, respectively. Samples were then centrifuged at 5000 rpm for 30 min. Finally, the supernatant was filtered through a 0.45µm membrane filter (Whatman, Kent, UK) and collected in 20 mL plastic scintillation vials (Meridian Biotechnologies Ltd) and stored at 4°C until further analysis.

Filtered soil water samples were analyzed for their phenolics content using a version of the spectrophotometric procedures described by Box (1983). The analysis was run in triplicate, 1 mL of the sample was placed in a 1.5 mL microcentrifuge vial with 50 µL of Folin's reagent and 0.15 mL of sodium carbonate (Na_2CO_3 used to rise the pH). The same reagents were added to 1mL of each of the chosen standards (0-10 mg L^{-1} phenol standard solutions were chosen in order to prepare a standard curve). All samples and standards were mixed a few times and allowed to develop a colour for 1 h 15 min at room temperature before 0.3 mL from both standards and samples were transferred to three wells of a clear microplate. Phenolic content was then estimated by measuring absorbance at a wavelength of 750 nm with a BMG Labtech Fluostar Galaxy microplate reader.

The concentration of DOC was measured on filtered and acidified samples using a Thermalox TOC/TN analyser (Analytical Sciences Ltd) equipped with a non-dispersive infrared CO_2 analyser. Anion (chloride, nitrate, phosphate and sulfate) and cation (sodium, ammonium, magnesium and calcium) concentrations were measured using a Metrohm 850 Professional Ion Chromatograph.

2.3.2.3. Soil water and organic matter content measurements

The water content of the soil samples was determined by oven drying a sample of known weight at 105°C for 24 h and then reweighing the samples to determine the mass loss. Organic matter content was estimated by placing soil samples in the muffle furnace at 550°C for 200 min and reweighing the samples to calculate mass loss (Frogbrook *et al.* 2009).

2.3.2.4. GHG flux measurements

For GHG flux measurements, 5 g of soil from each distinct layer within each core was weighed and placed in a 50 mL centrifuge tube with a lid fitted with a rubber septum (Fisher Scientific UK Ltd, Loughborough, UK). Using a 10 cm^3 gas syringe (Sigma Aldrich Ltd, Dorset, UK) with a short hypodermic needle, 10 mL of gas sample was taken from inside the tubes as a starting or background concentration (time 1). The tubes were then sealed and incubated at field temperature. Every 15 min over a 90 min period, gas samples were collected through the pierceable rubber septa. A10 mL of gas sample was extracted from the centrifuge tubes and then injected into labelled 5.9 mL glass screw-cap vials (Labco Exetainers® Ltd, Lampeter, UK), fitted with a rubber septum in their caps. All the exetainers were evacuated three times before use to remove all air, using a 10 mL gas syringe. Calibration curves for the three GHGs (CO_2, CH_4 and N_2O) were prepared using gas chromatograph (GC) standards of known concentrations. All gas samples and standards, in addition to three samples containing atmospheric air as blanks, were placed in the GC rack for analysis using a Varian model 450 GC. The instrument was fitted with two different detectors, an electron capture detector (ECD) to measure the concentration of N_2O and a flame ionization detector (FID) with a methaniser to convert CO_2 to CH_4, for the measurement of CO_2 and CH_4.

2.3.3. Statistical analyses

Where conditions were met by the data, analysis of variance (ANOVA) was performed to analyse potential significant differences in variables between different depths.

Tukey HSD post-hoc test was performed to find where the significant differences among groups lay at a probability level of 0.05. Calculated *F* and degrees of freedom are provided as well. Significant correlations were determined using Pearson correlation analysis. Statistical analysis was carried out using version 22 of the SPSS statistics package.

2.4. Results

As can be seen in Table 2.02 and Figure 2.03, on average, a maximum ß-D-glucosidase (B) enzyme activity was observed in the soil samples of the depth of 0-5cm (15.0 nmol MUF g^{-1} min^{-1}) and the minimum in the depth of >20cm (4.16 nmol MUF g^{-1} min^{-1}). Samples of the depth 0-5cm were significantly higher than samples of all other depths in this respect ($F(2, 48) = 7.547$, $p = 0.001$). Lower arylsulfatase (S) enzyme activity was observed in the soil samples from the depth of 5-20cm (mean 0.54 nmol MUF g^{-1} min^{-1}) and the higher S enzymes activity was observed in the depth of 0-5cm (mean 1.41 nmol MUF g^{-1} min^{-1}). Soil samples from the depth of 0-5cm were significantly higher than those from the other depths. Samples from the depth of >20cm were also significantly higher than those from the depth of 5-20cm ($F(2, 48) = 14.088$, $p = 0.00$). A maximum β-D-xylosidase (X) enzyme activity was observed in the 0-5cm soil samples (mean 2.77 nmol MUF g^{-1} min^{-1}) and the minimum in the 5-20cm samples (mean 1.62 nmol MUF g^{-1} min^{-1}). In this respect, the significant differences between the different depths were found in the first depth (0-5cm), where X enzyme activity of soil samples was significantly higher than all other layers ($F(2, 48) = 6.511$, $p = 0.003$).

Soil samples from the 0-5cm depth exhibited a particularly high phosphatase (P) enzyme activity (mean 33.9 nmol MUF g^{-1} min^{-1}), whereas samples from the 5-20cm depth showed the lowest activity (mean 8.01 nmol MUF g^{-1} min^{-1}). The depth of 0-5cm, which had the highest mean P activity, was significantly higher than all other depths ($F(2, 48) = 16.804$, $p = 0.000$). On average, soil samples of the 0-5cm depth showed higher chitinase (N) enzyme activity (4.06 nmol MUF g^{-1} min^{-1}) compared with the other two depths, which were found to be significantly lower than the 0-5cm samples in this respect ($F(2, 48) = 6.459$, $p = 0.003$).

Mean phenol oxidase (POX) activity was ranging from 1.25 in the 5-20cm depth to 2.45 µmol dicq g^{-1} min^{-1} in the >20cm depth (Table 2.02 and Figure 2.03), which was significantly higher than the 5-20cm depth ($F(2, 48) = 3.41$, $p = 0.032$).

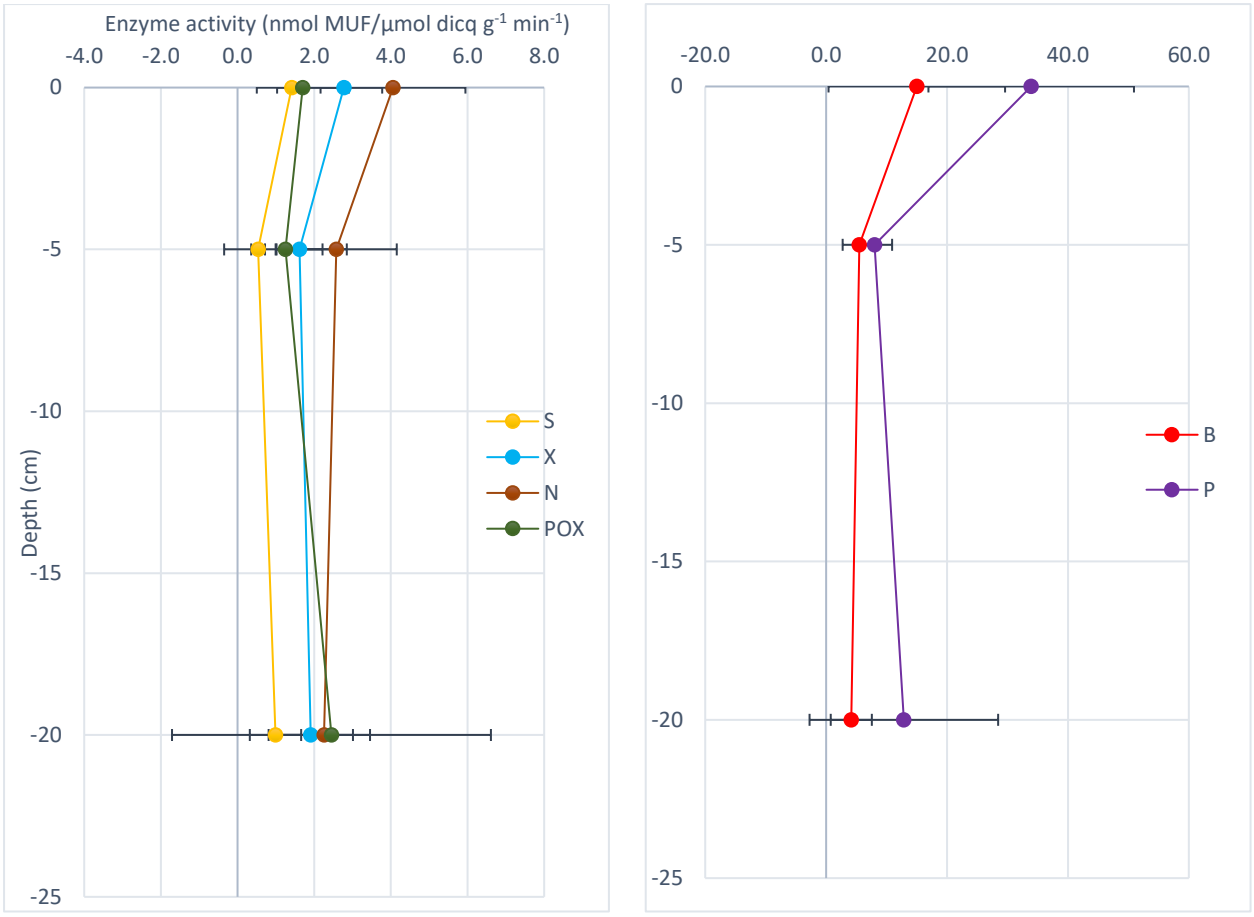

Figure 2.03. Extracellular enzyme activities vary with depth in the soil profile. Each circle represents the mean of the six sampling locations. Error bars represent standard deviation. Abbreviations: ß-D-glucosidase (B), arylsulfatase (S), β-D-xylosidase (X), phosphatase (P), N-acetyl-ß-D-glucosaminidase or chitinase (N) and phenol oxidase (POX).

Table 2.02. The results of measured variables from peatlands in Arctic. The results given as mean ± standard deviation. Letter annotations refer to significantly different means between different depths ($p < 0.05$) identified by Tukey HSD post- hoc test.

Variables \ Layers	0-5cm (a)	5-20cm (b)	>20cm (c)
B nmol MUF g^{-1} min^{-1}	15.0±14.65 bc	5.49±2.76 a	4.16±3.39 a
S nmol MUF g^{-1} min^{-1}	1.41±0.38 bc	0.54±0.18 ac	0.99±0.67 ab
X nmol MUF g^{-1} min^{-1}	2.77±1.0 bc	1.62±0.6 a	1.91±1.1 a
P nmol MUF g^{-1} min^{-1}	33.9±17.1 bc	8.01±2.9 a	12.85±15.67 a
N nmol MUF g^{-1} min^{-1}	4.06±1.89 bc	2.57±1.58 a	2.26±1.2 a
POX µmol dicq g^{-1} min^{-1}	1.7±1.2	1.25±1.6 c	2.45±4.16 b
Phenolics mg L^{-1}	1.88±1.0	2.29±0.8 c	1.49±0.75 b
DOC mg L^{-1}	15.5±6.47 c	11.3±3.5	10.07±6.38 a
pH	6.86±0.5 c	6.96±0.49 c	7.6±0.6 ab
Conductivity µS cm^{-1}	154.36±106.89	215.19±298.8	278.3±244.9
Water %	90.5±1.6 bc	81.8±9.9 ac	37.1±14.0 ab
SOM %	78.46±5.59 bc	57.8±21.48 ac	14.4±16.2 ab
CO_2 µg CO_2 g^{-1} h^{-1}	41.98±37.26 c	31.5±33.2	16.0±18.6 a
CH_4 ng CH_4 g^{-1} h^{-1}	4.03±14.1	-0.95±3.7	2.2±9.4
N_2O ng N_2O g^{-1} h^{-1}	10.34±12.8 b	1.16±2.0 a	4.8±12.9
Chloride mg L^{-1}	4.3±3.5 c	2.8±1.2	1.9±0.77 a
Nitrate mg L^{-1}	8.6±9.4	9.1±9.5	12.6±16.6
Phosphate mg L^{-1}	0.9±3.5	0.02±0.05	0.02±0.03
Sulfate mg L^{-1}	24.0±30.5	79.3±142.5	54.2±48.4
Sodium mg L^{-1}	6.6±5.2 c	4.5±2.3	3.3±2.2 a
Ammonium mg L^{-1}	0.36±0.68	0.04±0.09	0.04±0.08
Calcium mg L^{-1}	46.1±23.3	32.5±17.1 c	52.1±20.9 b
Magnesium mg L^{-1}	8.7±5.7	8.4±9.1	11.7±10.2

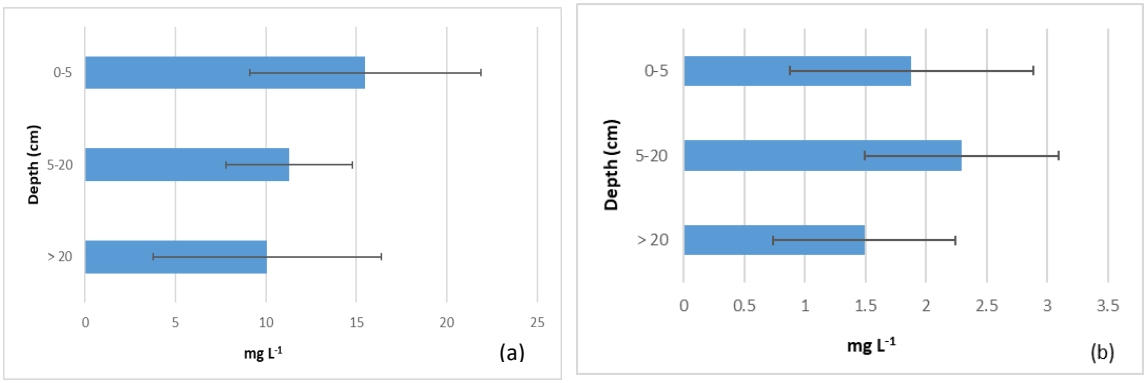

Figure 2.04. Mean DOC (a) and phenolic compound concentration (b) at different depths. Error bars represent the standard deviation of the mean.

Soil samples of the >20 cm depth showed the lowest mean phenolics concentration (1.49 mg L^{-1}) (Figure 2.04b, Table 2.02) and were significantly lower than soil samples of the 5-20 cm depth, which showed the highest mean phenolics concentration (2.29 mg L^{-1}) ($F(2, 48) = 3.392$, $p = 0.042$). Mean DOC concentration was higher in the 0-5cm samples (15.5 mg L^{-1}) and lower in the depth of >20 cm (10.07 mg L^{-1}) (Figure 2.04a, Table 2.02). The concentration of DOC in the soil samples of the 0-5cm depth was significantly higher than in the <20cm depth ($F(2, 48) = 4.45$, $p = 0.017$).

The pH measurements (Table 2.02) were around neutral and ranging between 6.86 and 7.6. Samples of the >20cm depth, which had the highest mean pH value, were significantly higher than both the 5-20cm and the 0-5cm depths, which had the lowest mean pH ($F(2, 48) = 7.65$, $p = 0.001$).

A minimum conductivity measurement (Table 2.02) was observed in samples of the 0-5 cm depth (mean 154.36 µS/cm) and the maximum in the >20 cm (mean 278.3 µS/cm). However, comparisons were not significant between the different layers ($F(2, 48) = 1.347$, $p = 0.270$).

Mean soil water content ranged between 37.1% in the samples of >20 cm depth and 90.5% in the 0-5 cm depth samples (Figure 2.05a). A number of significant differences were identified in this respect (Table 2.02). The depth of 0-5 cm, which had the highest mean water content, was significantly higher than all other layers. The depth of >20cm had significantly lower water content than the other two depth samples ($F(2, 48) = 146.707$, $p = 0.000$).

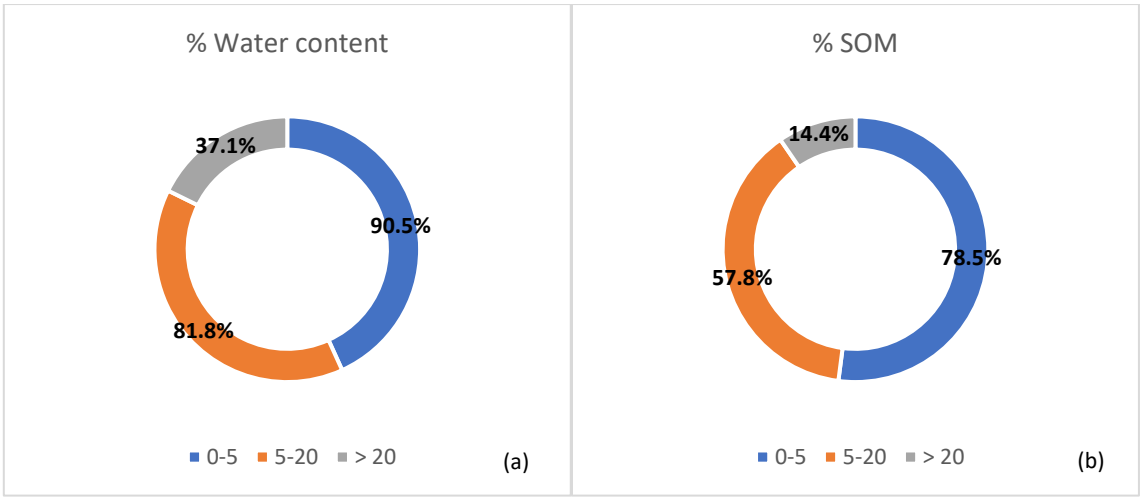

Figure 2.05. Mean water content (a) and soil organic matter (SOM) content (b) at different depths.

The mean soil organic matter content (%SOM) was higher in the soil samples derived from the 0-5 cm depth (78.46%) and lower in the >20 cm samples (14.4%), which were significantly lower than all other layers ($F(2, 48) = 80.220$, $p = 0.000$) (Table 2.02 and Figure 2.05b).

As can be seen in Table 2.02 and Figure 2.06, the flux of CO_2 was gradually reduced with increased depth (ranging from 16.0 in the >20 cm samples to 41.98 µg CO_2 g^{-1} h^{-1} in the 0-5 cm samples). Significant differences were observed in the >20 cm samples which were significantly lower than 0-5 cm samples ($F(2, 48) = 3.280$, $p = 0.04$).

Lower CH_4 flux was observed in the 5-20cm samples (-0.95 ng CH_4 g^{-1} h^{-1}) and the higher CH_4 flux was observed in the 0-5cm samples (4.03 ng CH_4 g^{-1} h^{-1}). However, comparisons were not significant between the different layers ($F(2, 48) = 0.965$, $p = 0.388$).

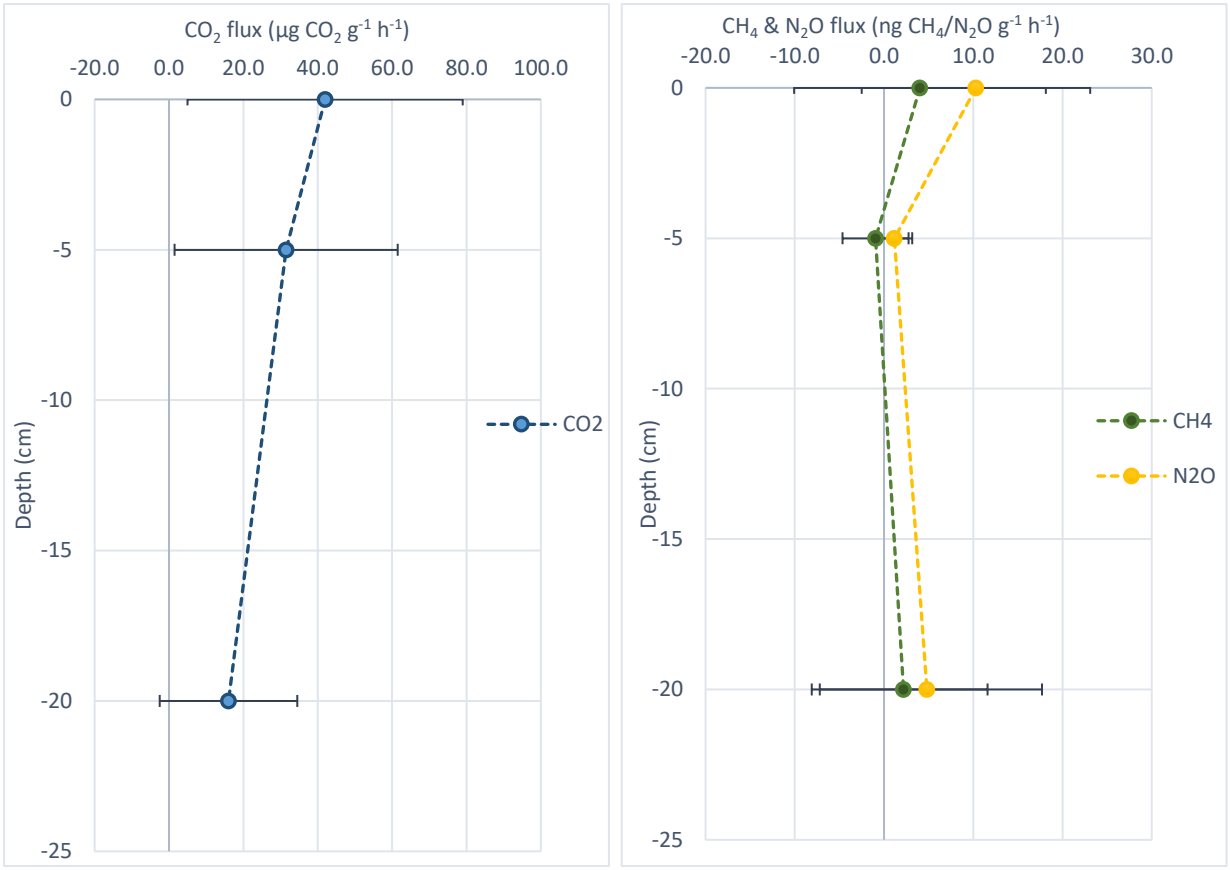

Figure 2.06. Changes in CO_2, CH_4 and N_2O mean fluxes with soil depth. Error bars represent standard deviation.

In terms of N_2O flux, soil samples obtained from the depth of 0-5 cm had the highest mean (10.34 ng N_2O g^{-1} h^{-1}) and were significantly higher than the 5-20 cm samples, which had the lowest N_2O flux mean (1.16 ng N_2O g^{-1} h^{-1}) ($F(2, 48) = 3.991$, $p = 0.04$).

For anion measurements (Table 2.02), mean chloride concentrations ranged from 1.9 mg L^{-1} to 4.3 mg L^{-1}. The depth of 0-5 cm, which had the highest mean chloride concentration, was significantly higher than the depth of >20 cm, which had the lowest mean chloride concentration ($F(2, 48) = 4.974$, $p = 0.011$). The nitrate concentration minimum was spotted in the depth of 0-5cm samples (mean 8.6 mg L^{-1}) and the maximum in the >20cm samples (mean 12.6 mg L^{-1}). However, no statistical differences were found between the different layers ($F(2, 48) = 0.542$, $p = 0.585$).

Mean phosphate concentration was ranging from 0.02 in the samples of both 5-20 cm and >20 cm depths to 0.9 mg L^{-1} in the samples of 0-5cm depth. However, no statistical differences were found between the different layers ($F(2, 48) = 1.016$, $p = 0.370$).

A lower mean sulfate concentration was observed in 0-5 cm samples (24.0 mg L^{-1}) and the higher in 5-20 cm samples (79.3 mg L^{-1}), without any significant differences between the different depths (F (2, 48) = 1.793, p = 0.177).

For cation concentrations (Table 2.02), sodium concentration was highest in the 0-5 cm samples (mean 6.6 mg L^{-1}) and lowest in the >20 cm samples (mean 3.3 mg L^{-1}). Samples of the depth 0-5 cm, which had the highest mean sodium concentration, were significantly higher than the samples of >20 cm depth, which had the lowest mean sodium concentration (F (2, 48) = 3.855, p = 0.028).

The concentration of ammonium was higher in the 0-5 cm samples (mean 0.35 mg L^{-1}) and lower in the other samples from 5-20 cm and >20 cm depths (mean of both 0.04 mg L^{-1}). No significant differences between the different layers were observed here (F (2, 48) = 3.2, p = 0.05).

Mean calcium concentration was higher in the depth of >20 cm (52.1 mg L^{-1}) and lower in the depth of 5-20 cm (32.5 mg L^{-1}), which was significantly lower than the samples of the depth >20 cm (F (2, 48) = 3.733, p = 0.031). Mean magnesium concentration was higher in the samples of depth >20 cm (11.7 mg L^{-1}) and lower in the samples of depth 5-20 cm (8.4 mg L^{-1}), without any significant differences between the different depths (F (2, 48) = 0.763, p = 0.472).

The significant correlations between all the peat soil analyses are shown in Table 2.03. The key observations are that the activities of the five hydrolase enzymes correlated positively with each other. The activity of POX correlated positively with the pH value, which in turn correlated negatively with the activities of B and N enzymes. The activity of N enzyme correlated positively with N_2O flux and with ammonium concentration. The CH_4 and N_2O fluxes correlated positively with B enzyme activity. Phosphate ions concentration correlated positively with all hydrolytic enzyme activities.

Table 2.03. Results of Pearson correlation analysis (n = 51) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: ß-D-glucosidase (B), arylsulfatase (S), ß -D-xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P), phenol oxidase (POX), electrical conductivity (EC), dissolved organic carbon (DOC), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and soil organic matter (SOM). Significant correlations are shown in bold.

	B	S	X	N	P	POX
S	**.437****					
X	**.711****	**.778****				
N	**.760****	**.522****	**.760****			
P	**.658****	**.651****	**.668****	**.568****		
POX	-0.079	**.553****	**.355***	0.066	0.209	
Phenolics	-0.121	-.202	-.256	-.069	-.140	-0.091
DOC	**.348***	.124	0.137	**.332***	0.172	-0.254
pH	**-.430****	.185	-.077	**-.410****	-.160	**.431****
EC	-.125	-.049	-.254	**-.320***	-.026	.205
% water	**.308***	-.034	.113	**.313***	.244	**-.309***
% SOM	**.355***	.032	.196	**.364****	**.281***	**-.306***
CO_2	0.044	0.109	0.004	-0.032	**.335***	.138
CH_4	**.343***	0.011	0.232	0.229	0.145	-0.043
N_2O	**.283***	0.134	0.270	**.408****	**.494****	0.038
Chloride	**.699****	0.233	**.390****	**.411****	**.469****	0.033
Nitrate	-0.168	-0.187	-0.178	-0.217	-0.153	-0.118
Phosphate	**.564****	**.339***	**.289***	**.436****	**.337***	-0.079
Sulfate	-0.146	-0.111	**-.301***	**-.329***	-0.055	0.232
Sodium	**.573****	0.240	0.213	**.378****	**.417****	0.156
Ammonium	0.225	0.234	0.218	**.313***	**.282***	-0.032
Calcium	0.043	0.234	-0.042	-0.190	**.350***	.220
Magnesium	0.041	0.077	-0.074	-0.110	0.051	.121

Continued

	Phenolic	DOC	pH	EC	% Water	% SOM	CO_2	CH_4	N_2O
DOC	**.551****								
pH	-.171	-.197							
EC	-.045	**-.528****	.166						
% water	0.248	**.302***	**-.606****	**-.311***					
% SOM	0.152	**.405****	**-.593****	**-.417****	**.905****				
CO_2	-0.003	-0.242	0.209	**.364****	0.254	0.154			
CH_4	-0.127	-0.080	0.031	0.030	-0.046	-0.034	0.055		
N_2O	0.057	0.116	-0.155	0.003	0.124	0.131	0.095	0.146	
Chloride	-0.009	0.054	**-.467****	0.126	**.319***	**.313***	0.139	0.267	0.228
Nitrate	-0.038	-0.101	-0.181	-0.046	0.052	0.023	-0.230	-0.211	0.229
Phosphate	-0.139	0.004	**-.346***	0.097	0.111	0.133	-0.108	-0.008	0.166
Sulfate	0.196	**-.447****	0.075	**.852****	-0.192	**-.356***	**.390****	0.067	-0.082
Sodium	0.178	0.145	**-.331***	0.216	0.248	0.258	0.112	0.097	0.137
Ammonium	0.087	0.237	-0.225	-0.039	0.221	0.258	-0.054	-0.062	0.238
Calcium	-0.242	**-.461****	0.255	**.661****	**-.296***	**-.326***	**.349***	0.211	0.126
Magnesium	-0.148	**-.458****	0.001	**.791****	-0.249	**-.331***	0.188	0.000	-0.009

2.5. Discussion

The occurrence of highest greenhouse gas (GHG) flux and hydrolytic enzymes activity in the superficial layer (0-5 cm) suggests that this is the most biologically active layer. The increase in extracellular hydrolyse activities was concurrent with decreased phenolic compounds concentration and with elevated water content, soil organic matter availability and DOC concentration, resulting in higher GHGs production rates. Soil organic matter (SOM) provides the substrates for the enzymes and a surface for the immobilisation of extracellular enzymes. Indeed, lots of studies have found a strong relationship between SOM content and extracellular enzyme activities (e.g., Sinsabaugh *et al.* 2008, Schnecker *et al.* 2015). The %SOM and % water content are strongly correlated because the fact that organic matter holds water (Dunn 2013). Soil pore water is another important medium for extracellular enzymes (Song *et al.* 2019). Dissolved organic carbon (DOC) supplies are required by heterotrophic microorganisms as energy sources promoting microbial processes (Kang *et al.* 1998). DOC is reported to have a priming effect on organic matter decomposition and therefore may be expected to exhibit a positive correlation with extracellular enzymes activity (Song *et al.* 2019).

Indeed, significant positive correlations were reported here between SOM and the activities of B, N and P, between water content and the activities of B and N, and between the B and N enzymes activity and DOC concentration.

Phenolic compounds have inhibitory effects on the activities of hydrolytic enzymes (Min *et al.* 2015, Zak *et al*. 2019). However, in the present study, no statistically significant negative correlations between hydrolase activities and phenolics concentration were found that would have confirmed these inhibitory effects, suggesting that the inhibition of extracellular enzymes depends on the form, chemical composition and the source of phenolic compounds, rather than simply their absolute quantities (Hoostal and Bouzat 2008). In this context, Tveit *et al.* (2013) mentioned that unlike *Sphagnum* species that contain lignin-like polymers, tundra mosses have less complex phenolic compound called lignan – clearly a major qualitative difference.

The significant decrease in hydrolase activities, and also the significant decrease in the production of CO_2 and N_2O with increasing depths suggests there to be a gradual decrease in enzyme substrate availability and the overall favourable conditions required for microbial activity at these depths. Conversely, a study conducted by Voigt *et al.* (2017a) showed that

the production of greenhouse gas in the peat profile of a Russian Arctic peatland increased with depth, and they attributed this to downward leaching of DOC that fuelled microbial activity which would thus have stimulated GHG production at depth.

At the same study sites as the present study (near the town Solvatn and Knudsenheia), Tveit *et al.* (2014) investigated the relative abundances of transcripts for microbial enzymes involved in degradation of plant polymers at different depths and they found that the relative abundance of transcripts for hemicellulases increased with depth, while the transcripts for cellulose degradation enzymes decreased with peat depth. They attributed this observations to changes in substrate availability across depths.

Phosphate exhibited a significant positive correlation with all hydrolase activities. This may have arisen because phosphate can be a limiting nutrient for plants. Phosphorus is often present in soil in only small quantities and in a form that cannot be used by plants. If plant metabolism was stimulated by phosphate fertilisation, it would increase rhizodeposition and the release of plant exudates (DOC) into the soil environment (Freeman *et al.* 2004). Such labile DOC is an important resource for microbes, driving de-novo synthesis of enzymes (Shackle *et al*. 2000). However, no significant correlation between phosphate concentration and DOC concentration was found to confirm this, although this should not be unexpected as any exudates could be rapidly consumed by the local microflora (Gough 2014).

Ionic strength (conductivity) is one of the DOC solubility controls and considered as a crucial determinant of peat pore water DOC concentrations (Gough *et al.* 2016). An inverse relationship is reported between DOC concentration in peat pore water and conductivity (Fenner *et al.* 2005). Similarly, in the present study, significant negative correlation was reported between DOC concentration and conductivity. The reason for reduced DOC solubility with increasing ionic strength is not fully understood but may relate to a reduction in the charge density of organic constituents which in turn causes coagulation (Kalbitz *et al.* 2000).

The higher average conductivity in the third mineral layer (depth of > 20 cm) may be a reflection of the elevated mean concentration of nitrate, calcium and magnesium ions. Soil calcium and magnesium originate from weathered bedrock, which explains why highest concentration was found in mineral layer. Significant positive correlation was found between conductivity/ sulfate ($r = 0.852$, $p < 0.01$), conductivity/ calcium ($r = 0.661$, $p < 0.01$) and conductivity/ magnesium ($r = 0.791$, $p < 0.01$).

The significant positive relationships between hydrolase activities suggest that they act together in synergy (Béguin and Aubert 1994) to reduce the structurally heterogeneous biopolymers to constituent monomers obtainable by microbes (Sinsabaugh *et al.* 2008).

A positive relationship was found between the activities of POX/S ($r = 0.553$, $p < 0.01$), and POX/X ($r = 0.355$, $p < 0.05$), which may indicate an indirect catalytic role of POX on hydrolytic enzymes (Jones 2006).

Although there were minor differences in phenolic content, thought to inhibit hydrolase enzymes, between depths, all of the hydrolytic enzymes' activity had large differences between the different depths. There was also no statistically significant correlation between hydrolases/phenolics. In addition, hydrolase activities seemed unaffected positively by the soil pH. On the contrary, pH values correlated negatively with B activity ($r = -0.430$, $p < 0.01$), and with N activity ($r = -0.410$, $p < 0.01$), which may reflect the fact that each enzyme has an optimum pH range (Turner 2010, Robinson 2015) and that there are several factors (e.g., temperature) may affect the response of the enzyme to its optimum pH (Kang *et al.* 2018). The optimum pH of the enzymes B (Robinson 2015) and N (Turner 2010) are 4.8 and 4.2, respectively, at which the activity is maximal. Above and below this point, the enzyme activity decreases (Robinson 2015). In the present study, the pH values ranged from 5.64 to 8.6, which means that they were above the optimum range for the enzymes action.

This suggests that pH value and phenolic concentration were insufficient to explain the significant differences between hydrolase activities at different depths, and that extracellular enzyme activity can be affected by many other edaphic factors such as soil temperature, oxygen, nutrient availability, pH, water content, microbial abundance, inhibitory phenolics, etc. (Freeman *et al.* 1990, Freeman *et al*. 1995, Freeman *et al.* 2001).

According to Chròst (1991), soil nutrient concentrations strongly influence measured enzyme activities since microbes adjust their enzyme production in response to nutrient availability. Therefore, it is believed that a negative correlation would occur between soil enzyme activity and nutrient availability, indicating that if nutrients are limited, enzymes production by microbes increase and vice versa (Sinsabaugh *et al*. 1993, 2008). On this basis and although there were statistically significant differences between the different layers in terms of S enzyme activity, there were no statistically significant differences in sulfate ions concentration between layers and no statistically significant relationship between S/sulfate was found in this study. This finding is in line with what has been observed previously by

Kang and Freeman (1999) in Welsh peatlands. Because all the studied sites were close to the sea, it is possible that sea-salt aerosol represented an important sulfate source in the soils, which could explain the lack of statistically significant negative correlation between S enzyme activity and sulfate concentration (Kang and Freeman 1999).

The significant differences between soil layers in P enzyme activity may have still contributed to the insignificant difference of phosphate concentration. Indeed, significant positive association between P activity/phosphate was reported here and has been identified previously (Senga *et al.* 2015). Similarly, Weintraub and Schimel (2005) noticed a positive relationship between inorganic phosphate and phosphatase activity in Arctic soils. They concluded that this was caused by phosphatase activity that was higher than what was required to meet phosphate demand. This disagrees with the theory that enzymes activity occurs only when there is a demand for nutrients and that the production of enzymes should therefore stop once nutrient availability increases to fulfil demand (economic model of extracellular enzyme production) (Burns *et al.* 2013). One possible explanation is that in situations where enzyme turnover, sorption (to soil particles) and/or deactivation (the enzyme active site changes shape and can no longer bind to the substrate) rates are low, enzymes produced to meet a particular demand for a nutrient may remain active even after that demand has been fulfilled (Allison *et al.* 2007).

Regarding the relationship between chloride ions concentration/enzymes activity and sulfate ions concentration/enzymes activity, Dinesh *et al.* (1995) found that enzymes activity decreased with increasing both ions concentration because they have an inhibitory effect on microbial growth and subsequent enzyme production due to decreased osmotic potential of the soil water leading to microbial cell destruction and/or a salting-out effect on enzyme protein. The positive correlation between chloride and enzymes B, X, N and P activity was therefore unexpected. However, it is likely that the chloride correlation is caused by an unrelated correlation with something else that does genuinely influence enzymes such as ammonium. The chloride might just be there because of dissolution of ammonium chloride. So, the positive correlation is really between ammonium and the enzymes, as found in this study.

Unexpectedly, the activity of POX was higher at the underlying mineral layer (> 20 cm), indicating that the deeper layers warrant attention. The relative increase in POX activity at

this layer was accompanied by the significant increase in pH value (mean 7.6). This was clarified by the activating impact of high pH under anaerobic conditions (Gough *et al.* 2016).

Alongside oxygen, a variety of factors have been appeared recently to weaken the enzymic latch mechanism including pH value (Zak *et al.* 2019). Indeed, a simulation of moisture loss during drought in a peat soil by Williams *et al.* (2000) did not result in increased phenol oxidase activity as would be expected. They attributed this to the low pH that characterized peatland ecosystems. Indeed, statistically significant positive relationship between the activity of POX and pH was found here and has been identified previously (Pind *et al.* 1994, Sinsabaugh 2010, Kang *et al*. 2018). The concentration of hydrogen ions is one of the primary factors controlling decomposition processes through impact on phenol oxidase activity (Pind *et al.* 1994, Williams *et al.* 2000, Sinsabaugh *et al*. 2008).

The pH value may affect the enzyme reaction site, as well as the interaction between immobilised enzymes and their associated matrix (Kang and Freeman 1999). The low pH of wetland soils is cited as one of the main reasons for low decomposition rates in these environments (Clymo 1983).

Another explanation of increased POX activity at depth is that peatlands of Svalbard were shallow, which could create greater influences from fluctuations in water levels which would produce the oxygenation needed for POX activity. Similar high activity of POX at depth has been reported previously from two high-Arctic peatland in Svalbard (Tveit *et al.* 2013), where a genetic potential for POX (genes encoding POX enzyme) was found in all peat layers. This suggests that decomposition of inhibitory compounds can occur in deeper layers whenever the peat soils get oxygenated, such as through the drought-flooding cycles at higher temperatures found before in temperate peatlands (Fenner and Freeman 2011).

Also, this higher activity of POX was accompanied by the relatively decreased phenolic compounds concentration, and water and SOM contents. Lower phenolics and water content with depth was probably relates to the low SOM content and the increasing mineral content with depth (Dunn 2013).

Similar trends in enzymes activity were found in Siberian soil samples where hydrolytic enzyme activities decreased with depth, while phenol oxidase activity in mineral layers was as high as, or higher than in organic topsoil layers (Schnecker *et al*. 2015).

It has been mentioned that the mineralization of N-containing compounds generating mineral nitrogen substrates, mainly ammonium and nitrate, needed for N_2O production (Voigt *et al.* 2017a). In the present study, the superficial layer exhibited the highest N_2O emission, most likely due to the elevated N enzyme activity there. Indeed, N_2O release was positively related to N enzyme activity ($r = 0.408$, $p < 0.01$), which in turn significantly correlated with ammonium concentration. Although N_2O production was most pronounced in the superficial layer, the underlying mineral layer did not display any significant differences with the upper organic layer, suggesting that the leaching of mineral N supplies from the top layer enhanced N_2O production at depth. In this context, a study of Regina *et al.* (1996) in boreal peatlands of Finland concluded that the nitrate (used as electron acceptor during denitrification) leached downwards in the deeper layers of peat profile, where N_2O can be produced by denitrification processes. However, in the present study no statistically significant correlation was found between nitrate and N_2O flux.

It has been mentioned that soluble saccharides produced by hydrolysis of C- containing polymers enhance the growth of heterotrophic methanogens (Béguin and Aubert 1994) and denitrifying bacteria (Senga *et al.* 2015). On this basis, the significant positive relationships identified here between B enzyme activity (used as an indicator of labile carbon availability) and the emissions of CH_4 and N_2O were expected as microbial biomass need C supply for their growth and activity (Kang *et al.* 1998).

As phenolics concentrations increase, a lower decomposition rate is expected because of the inhibitory effects of phenolic compounds on the main agents of decomposition (hydrolase enzymes) (Freeman *et al.* 1990, Fenner and Freeman 2011). Here, neither extracellular enzymes activity nor greenhouse gas productions showed significant correlations with phenolic concentrations, which suggesting that the activities of extracellular enzymes and hence the metabolic end products depend on the form, composition and the source of phenolic compounds, instead of the absolute quantities of them (Hoostal and Bouzat 2008).

The lack of any statistically significant differences in CH_4 flux between studied layers suggests that the activity/biomass of methanogens did not vary significantly with depth or that CH_4 fluxes mitigated by CH_4 oxidation by methanotrophs. In this context, it is worth noting that CH_4 production can be balanced by methanotrophs through methane oxidation (Tveit *et al.* 2013, 2015), which could affect the results of measured CH_4 emission.

The results of significant correlations between the measured variables suggest that soil physicochemical properties that changed at different layers could be account for changes in soil enzymes activity and the potential metabolic end products.

2.6. Conclusions

Information concerning the relevant physicochemical parameters and biogeochemical processes in the peatlands of the Arctic region is still limited. This field survey provides an assessment of the biogeochemical processes in relation to environmental variables along the soil profile in Arctic peatland of Svalbard, Norway.

The occurrence of highest GHG flux and extracellular enzymes activity (with the exception of phenol oxidase activity) in the superficial layer (0-5 cm) indicates that this is the most biologically active layer. This observation was in line with previous studies of peatlands and other soil ecosystems. Overall, the superficial layer was associated with higher hydrolase activities, higher water and SOM contents, higher DOC, nutrients concentrations, and higher CO_2 and N_2O fluxes. All of which relatively decreased with soil depth.

Hydrolase activities were not significantly related to pH, suggesting that the pH values, which ranged from 5.64 to 8.6, were not optimal for hydrolytic enzymes activity. For the B and N enzymes activity, it looks like alkalinity may be exerting some inhibitory effect.

The underlying mineral layer was associated with higher POX activity, indicating that the deeper layers warrant attention. This observation attributed to higher pH in the deeper layer, with a statistically significant positive correlation being found between the two factors. This confirms the activating impact of high pH under anaerobic conditions.

Neither extracellular enzymes activity nor greenhouse gas production showed significant correlations with phenolic concentrations, suggesting that the activities of extracellular enzymes and hence the metabolic end products depend on the form, composition and the source of phenolic compounds, instead of the absolute quantities of them.

Contrary to the ‘economic model ‘of microbial enzyme production, phosphatase activity was positively associated with inorganic phosphate concentration, which may indicate that phosphatase production rate exceeded phosphate demand. Regarding the lack of statistically negative correlation between S enzyme activity and sulfate concentration, it seemed that large

amounts of sulfate was deposited in the soil from the sea instead of being produced by biodegradation of organic sulfur compounds.

DOC concentration showed a significant inverse relationship with conductivity that known to negatively affect the DOC solubility and hence its concentration in peat solution.

The emission of CH_4 and N_2O was significantly correlated with carbon supply (represented by B enzyme activity) needed for the growth of heterotrophic microorganisms. There was no statistically significant change in CH_4 production along the soil depth, possibly due to the activity/biomass of methanogens did not vary significantly with depth or possibly due to the offset by methanotrophs, which occur throughout soil profile. Given the potency of methane as a greenhouse gas, an improved understanding of the biotic and abiotic factors driving its flux in these important carbon sink environments must be a research priority.

Studying biogeochemical processes and the abiotic variables controlling them in peatlands has important implications for predicting the potential impacts of climate change, human intervention and management strategies on peatlands function.

Assessing microbial community composition is recommended, since microbial communities are central to the degradation of SOM and are responsible for a large proportion of soil GHGs emissions.

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Chapter 3

Changes in biogeochemical processes with depth in tropical Colombian peatlands

3.1. Abstract

Despite the importance of tropical peatlands as one of the most important terrestrial carbon stores, the knowledge of their biogeochemical processes and the physicochemical variables influencing them at different depths remains insufficient. This study focussed on tropical Colombian peatlands and aimed to assess whether extracellular enzyme activity and soil respiration vary with depth in response to soil physicochemical characteristics. The activity of extracellular enzymes (hydrolases and phenol oxidase), the fluxes of greenhouse gas (carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)), water and soil organic matter content, pH and conductivity as well as various soil water properties including dissolved organic carbon, phenolic compound and anion and cation concentrations were measured at different depths across six locations in an Andean peatland.

Since the quantity and the quality of soil organic matter decrease with depth, soil enzymes activity is expected to decrease with depth and the microbial assemblages can be expected to change accordingly. In a manner proportional to soil characteristics, the findings of the present study showed a general decrease in soil enzymes activity (including both hydrolases and phenol oxidase) with depth. This observation was in line with decreasing pH and nutrient concentrations and increased phenolic concentrations with increasing depth, suggesting that soil organic matter quality and hence soil metabolism changed with depth. Consequently, CO_2 flux gradually decreased with depth, while the production of CH_4 followed the opposite direction, indicating that methanogens were more common in the deeper peat layers. N_2O fluxes were strongly correlated with enzymatic activities although they showed insignificant variation across the different depths. To a great extent, phenolics concentration, pH and ion concentrations appeared to influence the enzymes activity and CO_2 and N_2O emissions. The content of nitrogen element correlated positively with enzymes activity and soil respiration, indicating that increased nitrogen deposition poses a significant risk to the function of peatlands.

In agreement with the ‘economic model’ of microbial enzyme production, statistically significant negative correlations were found between arylsulfatase activity and sulfate concentration and between phosphatase activity and phosphate concentration.

3.2. Introduction

Previous studies have estimated that nearly 11% (0.44 Mkm^2 (M = million)) of the world's peatlands are located in the tropical areas that spread across Central and South America, Africa, the Caribbean and Southeast Asia. Tropical peatlands are estimated to hold around 18 to 25% of the total global peat volume (1758 Gm^3 (G = 10^9)) and around 15 to 19% of the global peat carbon pool. Southeast Asia is estimated to have the largest area of tropical peatland (247 778 km^2, 56%) and peat volume (1359 Gm^3, 77%) followed by South America (107 486 km^2, 24.4% and 192 Gm^3, 11% peat volume), Africa (55 860 km^2, 13% and 138 Gm^3, 8% peat volume) and the Caribbean and Central America (23 374 km^2, 5.3% and 60 Gm^3, 3% peat volume for both) (Page *et al.* 2011). Interest in Southeast Asian peatlands has increased following extensive land use change for agricultural purposes, which has resulted degradation of soil carbon stores, and significant greenhouse gas (GHG) release (Page *et al.* 2002, Koh *et al.* 2009).

Recently, Gumbricht *et al.* (2017) published a new estimate of peatland extent and peat volume in the tropics. They estimated that tropical peatlands cover 1.7 Mkm^2 and contain 7 268 Gm^3 of peat, of which 750 000 km^2 (44%) and 3 117 Gm^3 (43%) are in South America. These latest figures suggest that Southeast Asian peatlands are surpassed in extent and volume by South American peatlands and highlight the importance of focussing studies of tropical peatlands in this region. Specifically, more research is required to adequately understand tropical peatland biogeochemical processes and the environmental factors affecting them, which will help in developing strategies for the restoration or reconstruction of drained peatlands and for the maintenance of healthy peatlands (Könönen *et al*. 2018). Within South America, Colombia is estimated to have 74 950 km^2 of the total tropical peatlands (Gumbricht *et al.* 2017). Like all tropical peatlands, Colombian peatlands at high elevations are susceptible to the impacts of climate change including global warming (Benavides *et al.* 2013). In the Andes Mountains, temperatures have already increased over the last two decades by 1.4°C with a further temperature rise of 5°C predicted during the next nine decades (Bradley *et al.* 2006, Ruiz *et al.* 2008). The resulting droughts and more oxygenated conditions pose a significant threat to the stability of peatlands, with carbon release also likely to cause positive climate feedbacks (Wieder 2001, Turetsky *et al.* 2002). To date, no data has been published regarding soil metabolism in relation to variations of

abiotic variables in peatland of the Sumapaz National Park, where samples were collected during this project.

Despite the importance of tropical peatlands as one of the largest terrestrial carbon storages, metabolic processes in the deep layers of tropical peat remain poorly understood, with relatively few studies having been conducted in this area (Rieley *et al.* 1996, Jackson *et al.* 2009). In addition, and in contrast to boreal and temperate peatlands, GHG gas fluxes in tropical peatlands have received little attention (Jauhiainen *et al*. 2012). Despite their global importance, South American peatlands have received less attention than other tropical peatlands. This is due partly to methodological and logistical difficulties. However, as climate change and land use changes impact on these environments, their forgotten status could be reversed (Dargie *et al.* 2017, Gumbricht *et al.* 2017).

Despite the fact that soil microorganisms occur throughout the soil profile, their activity and function in deep peat layers remains poorly understood (Schnecker *et al*. 2015). Typically, microbial processes in the top few centimetres of peat, where the oxygen content, fresh litter and hence density of microorganisms is highest, receive most attention (Fierer *et al*. 2003, Senga *et al.* 2015). In the deep layers of the peat profile, partially degraded organic matter decomposes slower due to the dominance of anaerobic conditions and the subsequent accumulation of recalcitrant compounds. However, substantial microbial communities in deep layers of soil have been reported in several studies (Fritze *et al.* 2000, Blume *et al.* 2002, Fierer *et al.* 2003, Jackson *et al.* 2009, Eilers *et al.* 2012, Senga *et al.* 2015, Ko *et al.* 2017), which suggests that even deeper layers of soil contribute to the processes involved in organic matter decomposition, nutrient cycling and GHG emissions (Jackson *et al.* 2009, Senga *et al*. 2015).

In this study, the activities of extracellular enzymes (ß-D-glucosidase, arylsulfatase, ß -D-xylosidase, N-acetyl-ß-D-glucosaminidase (chitinase), phosphatase and phenol oxidase) at various depths were analysed. Greenhouse gas emissions (CO_2, CH_4 and N_2O) were also measured to yield further information on microbial processes. Various physicochemical properties including dissolved organic carbon (DOC) concentration, pH, conductivity, water content, soil organic matter content, phenolic concentration and cations and anions concentrations were measured to test the hypothesis that enzymes activity and soil respiration differ with depth in response to changing soil conditions.

3.3. Materials and methods

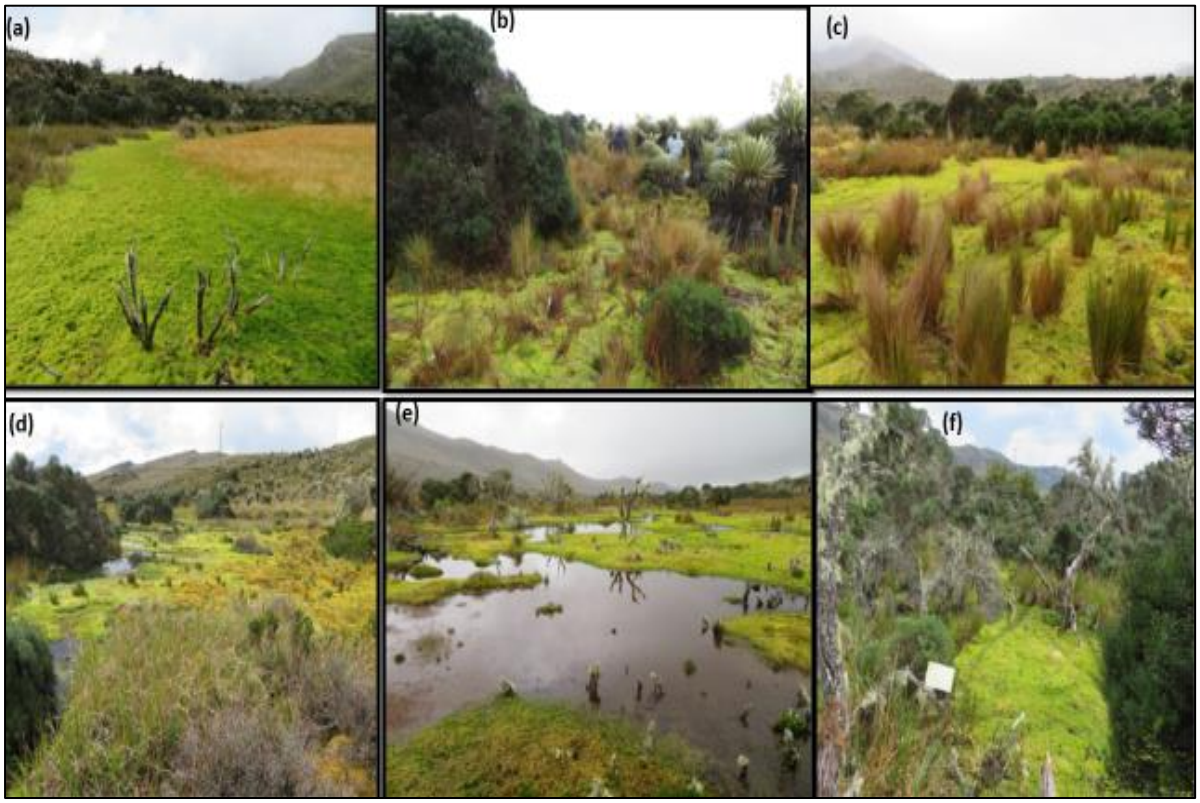
3.3.1. Study site description and sample collection and preparation

More detailed maps were unavailable for peatland distribution in Colombia. The tropical peatland site used in this study is located at high altitude within the Sumapaz National Park in the Andean mountain range, Colombia (Figure 3.01). The area extension is about 154000 ha. The annual rainfall is between 1300 and 2400mm, with continuous and high humidity. The Park is a significant hydrographic center, containing river basins and lakes (Sesana 2006).

Figure 3.01. Maps showing study site and peat core sampling locations in Sumapaz National Park, Colombia (Andean Mountains) (Source: Google Maps).

The dominant vegetation was *Sphagnum* spp, *Carex* spp, *Juncus* spp, *Espeletia* spp and many other shrubs were also more common. The average air temperature was 13.4°C and the water table was at the soil surface at the time of sampling. Soil sampling was performed at the end of July 2017, with peat cores of 100 cm depth were collected from six different *Sphagnum*-dominated locations (Figure 3.02a-f), since vegetation is known to be an important variable affecting microbial communities, and to enable an effective comparison between regions. At each of these locations, three replicate cores were extracted using a Russian corer (50 cm long and 10 cm in diameter). After removing the vegetation layer, the cores were separated into several layers based on a visual assessment of colour and texture. Figures 3.02g and 3.02h show the stratigraphy of a representative core from the six locations. In general,

distinct layers occurred at the following approximate depths: blond superficial layer: 0-10 cm, light brown organic layer: 10-40 cm, dark brown organic layer: 40-90 cm and mineral layer: > 90 cm. This approach yielded a total of 67 samples. After removal of stones, roots and macroinvertebrates, all samples were placed separately in labeled plastic bags and transported to the laboratory in Wales in a cooled container, where they were stored at 4°C until further analysis.



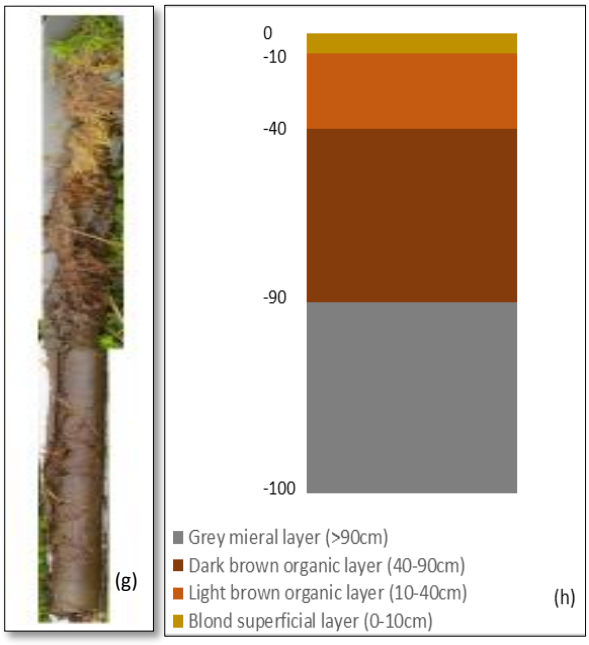


Figure 3.02. Photographs showing six peat core extraction locations (pictures a-f refer to sites 1-6, respectively). Photograph of typical peat core (g) and illustration showing discrete layers (h) (photographer J Mora-Gomez).

Soil temperature, pH, and oxygen concentration were measured in the field at the time of sampling using a digital thermometer, a Mettler Toledo S20 pH meter and a dissolved oxygen probe, respectively. These data are presented in Table 3.01.

Table 3.01. Peat core locations and basic environmental characteristics of pore waters of the studied sites during the time of sampling. Values are presented as the range of the 3 replicates.

Location	Soil temp. (°C)	pH	Soil O_2 conc. (mg L^{-1})	Coordinates	Altitude
1	9.0-9.7	4.4-5.1	1.3-2.7	4°1'23.6" N, 74°12'20.1" W	3714 m a.s.l
2	9.3-10.1	4.0-4.1	2.4-2.6	4°17'22,1" N, 74°12'19" W	3720 m a.s.l
3	10.1-10.3	4.4-4.6	2.0-2.3	4°15'28.3" N, 74°11'28.5" W	3578 m a.s.l
4	9.2-9.6	4.9-5.4	1.3-2.8	4°17'23.1" N, 74°12'12.8" W	3709 m a.s.l
5	8.5-10.6	4.3-4.6	2.0-2.9	4° 16' 25.1" N, 74° 11' 38.1" W	3642 m a.s.l
6	9.0-9.8	4.9-5.0	1.3-2.2	4°15'24" N, 74°11'24.6" W	3573 m a.s.l

3.3.2. Soil analyses

In the present study, the aim was to estimate the emissions of GHG and the activities of extracellular enzymes that occur under natural conditions within peatlands. Therefore, field conditions were copied as closely as possible by matching gas production and enzyme assay temperature to that recorded in the field.

3.3.2.1. Enzyme activity analyses

Analyses were performed within two weeks of collection as previous studies have shown that soil enzymes to be stable for this period of time (Dunn *et al*. 2014). Before conducting any analyses, the samples were kept in the incubator at field temperature for around three hours.

The activity of phenol oxidase (POX) in peat soil samples was determined using a procedure adapted from Pind *et al*. (1994) and Dunn *et al*. (2014). Model compound L-DOPA (L-3,4-dihydroxy phenylalanine, Sigma Aldrich Ltd, Dorset, UK) was used as a substrate for the enzyme's degradation. The colour change is determined using a spectrophotometer

measuring at 475 nm, from which the activity of phenol oxidase can be calculated. After homogenizing the bag containing the soil sample by hand, two separate 1 g soil samples were weighed and placed in two labelled stomacher bags (Seward, West Sussex, UK). After adding 9 mL of ultrapure water to each bag, these were then placed in a paddle blender (Stomacher® circulator, Seward) to mix the contents for 30 s on a normal speed setting. A 10 mL of ultrapure water was added to one bag, used as a blank. A 10 mL of the substrate (L-DOPA) was added to the other, and both bags were mixed for a further 30 s. All bags were then incubated at field temperature for 10 min before the bags were removed and mixed by hand. For each bag, three 1.5 mL microcentrifuge vials (Eppendorf, Stevenage, UK) were labelled and filled with the bag's solution. The vials were centrifuged at 10,000 rpm for 5 min. A 300 µL of supernatant from each vial was transferred to a well of a clear 96 well microplate (Sterilin, Cambridage, UK). The microplate was then placed on the SpectraMax M2e plate reader spectrophotometer and absorbance values measured at λ= 475 nm. To obtain the average absorbance value, the mean absorbance of the three blanks was subtracted from the mean absorbance of the three samples containing the substrate. The activity of phenol oxidase was then calculated by using Beer-Lambert Law and expressed as µmol of formed diqc per min per g of soil (dry weight).

Hydrolase activities were determined using the procedures of Dunn *et al*. (2014), based on the measurement of fluorescence of methylumbelliferone (MUF). In five 1L volumetric flasks, the relevant amount of MUF-labeled substrates (4-MUF β-D-glucopyranoside for β-D-glucosidase (B), 4-MUF sulfate potassium salt for arylsulfatase (S), 4-MUF β-D-xylopyranoside for β-D-xylosidase (X), 4-MUF N-acetyl-β-D-glucosaminide for N-acetyl-β-D-glucosaminidase (N) and 4-MUF phosphate for phosphatase (P)) were dissolved in 20 mL of cellosolve solvent (Sigma Aldrich Ltd, Dorset, UK), and then ultrapure water was added up to the 1L mark. Magnetic stirrers were used if necessary.

Five 1 g subsamples (one for each MUF substrate) were taken from each bag containing soil samples and placed in separate stomacher bags with 7 mL of the relevant substrate (stored at field temperature before use). These were homogenized using a Seward Stomacher 80 Laboratory Blender for 30 s. The stomacher bags were then incubated at field temperature for 45 min for the enzyme P and 60 min for the other enzymes (B, S, X, and N). The homogenates were then mixed by hand and transferred into separate 1.5 mL centrifuge vials, which were then centrifuged at 10,000 rpm for 5 min. During this time, 50 µL of ultrapure water was added to black microplate wells (Sterilin, Cambridge, UK).

At the end of centrifugation, 250 µL of the resulting supernatant was extracted from each sample and added to the relevant wells on the microplate. Fluorescence of the MUF molecule was measured using a microplate fluorometer (Molecular Devices SpectraMax M2e spectrophotometer) at 450 nm emission and 330 nm excitation. Hydrolase activities are expressed as nmol MUF g^{-1} of soil min^{-1}. To prepare the calibration curve, 7 mL of ultrapure water was added to a stomacher bag (labelled as standard) containing 1 gram of soil sample and homogenized as described above. The contents from each standard bag were used to fill two 2 mL Eppendorf tubes, which were then centrifuged at 10,000 rpm for 5 mins. Different MUF-free acid (Sigma Aldrich Ltd, Dorset, UK) concentrations (8 in total) were prepared in 2 mL Eppendorf tubes using the MUF stock solution (prepared by dissolving the relevant amount of 4-methylumbelliferone free acid in 10 mL of cellosolve solvent and 500 mL of ultrapure water) and ultrapure water.

A column of the 8 standard solutions was prepared per soil sample by pipetting 50µL from each of the standard solution into a series of wells on a black microplate in addition to 250µL from the supernatant of the two Eppendorf tubes. The microplate was then analyzed as before.

3.3.2.2. Hydrochemistry analyses

For measurement of pH, conductivity, DOC, phenolic compounds, cations and anions concentrations, a soil solution was prepared by adding 5 g of soil sample and 40 mL of ultrapure water to a 50 mL centrifuge tube, and shaking on a rotary shaker for 24 h. Allowing 24h on a rotary shaker ensures that potentially leachable solutes are fully extracted into solution. There is some risk that this might allow changes in the microbial community (including consumption of solutes, reproduction, metabolism of solutes to other chemical forms), however, previous work has shown that without a 24h period it is impossible to be certain that the extractable solutes reach their maximum.

Measurement of pH and electrical conductivity was conducted on un-filtered samples using a Mettler Toledo S20 pH meter and a Primo 5 handheld conductivity meter, respectively. Samples were then centrifuged at 5000 rpm for 30 min. Finally, the supernatant was filtered through a 0.45µm membrane filter (Whatman, Kent, UK). The supernatant was collected in 20 mL plastic scintillation vials (Meridian Biotechnologies Ltd) and stored at 4°C until further analysis.

Filtered soil water samples were analyzed for their phenolics content using a version of the spectrophotometric procedures described by Box (1983). The analysis was run in triplicate, 1 mL of the sample was placed in a 1.5 mL microcentrifuge vial with 50 µL of Folin's reagent and 0.15 mL of sodium carbonate (Na_2CO_3 used to rise the pH). The same reagents were added to 1 mL of each of the chosen standards (0-30 mg L^{-1} phenol standard solutions were chosen in order to prepare a standard curve). All samples and standards were mixed a few times and allowed to develop a colour for 1 h 15 min at room temperature before 0.3 mL from both standards and samples were transferred to three wells of a clear microplate. Phenolic content was then estimated by measuring absorbance at a wavelength of 750 nm with a BMG Labtech Fluostar Galaxy microplate reader.

The concentration of DOC was measured on filtered and acidified samples using a Thermalox TOC/TN analyser (Analytical Sciences Ltd) equipped with a non-dispersive infrared CO_2 analyser. Anion (chloride, nitrate, phosphate and sulfate) and cation (sodium, ammonium, magnesium and calcium) concentrations were measured using a Metrohm 850 Professional Ion Chromatograph.

3.3.2.3. Soil water and organic matter content measurements

The water content of the soil samples was determined by oven drying a sample of known weight at 105°C for 24 h and then reweighing the samples to determine the mass loss. Organic matter content was estimated by placing soil samples in the muffle furnace at 550°C for 200 min and reweighing the samples to calculate mass loss (Frogbrook *et al.* 2009).

3.3.2.4. GHG flux measurements

For GHG flux measurements, 5 g of soil sample from each distinct layer within each core was weighed and placed in a 50 mL centrifuge tube with a lid fitted with a rubber septum (Fisher Scientific UK Ltd, Loughborough, UK). Using a 10 cm^3 gas syringe (Sigma Aldrich Ltd, Dorset, UK) with a short hypodermic needle, 10 mL of gas sample was taken from inside the tubes as a starting or background concentration (time 1). The tubes were then sealed and incubated at field temperature. Every 15 min over a 90 min period, gas samples were collected through the pierceable rubber septa. A 10 mL of gas sample was extracted from the centrifuge tubes and then injected into labelled 5.9 mL glass screw-cap vials (Labco Exetainers® Ltd, Lampeter, UK), fitted with a rubber septum in their caps.

All the exetainers were evacuated three times before use to remove all air, using a 10 mL gas syringe. Calibration curves for the three GHGs (CO_2, CH_4 and N_2O) were prepared using gas chromatograph (GC) standards of known concentrations. All gas samples and standards, in addition to three samples containing atmospheric air as blanks, were placed in the GC rack for analysis using a Varian model 450 GC. The instrument was fitted with two different detectors, an electron capture detector (ECD) to measure the concentration of N_2O and a flame ionization detector (FID) with a methaniser to convert CO_2 to CH_4, for the measurement of CO_2 and CH_4.

3.3.3. Statistical analyses

Where conditions were met by the data, analysis of variance (ANOVA) was performed to analyse potential significant differences in variables between different depths. Tukey HSD post-hoc test was performed to find where the significant differences among groups lay at a probability level of 0.05. Calculated *F* and degrees of freedom are provided as well. Significant correlations were determined using Pearson correlation analysis. Statistical analysis was carried out using version 22 of the SPSS statistics package.

3.4. Results

For all hydrolase enzymes (Figure 3.03 and Table 3.02), highest mean activity was recorded in the superficial layer (0-10 cm). ANOVA analysis showed that (with the exception of the superficial layer vs. mineral layer X enzyme comparison) the difference in mean enzyme activity between the superficial layer and all other layers was statistically significant ($F(3, 63) = 16.2, 19.2, 11.3, 19.1$and 21.1 for the enzymes B, S, X, N, and P, respectively, $p = 0.000$). Although in general, hydrolase enzymes activity tended to decrease with increasing depth, with four of the five enzymes (B, X, P and N) activity increased in the mineral layer (> 90 cm) (Figure 3.03), though this difference was not statistically significant (Table 3.02). The same trend was observed in POX activity with depth, with highest activity in the superficial layer (3.06 µmol dicq g^{-1} min^{-1}) was significantly higher than the light brown (10-40 cm) and dark brown organic layer (40-90 cm) ($F(3, 63) = 10.5$, $p = 0.000$). The activity of POX in the mineral layer (>90cm) (1.7 µmol dicq g^{-1} min^{-1}) did not differ significantly from the other layers.

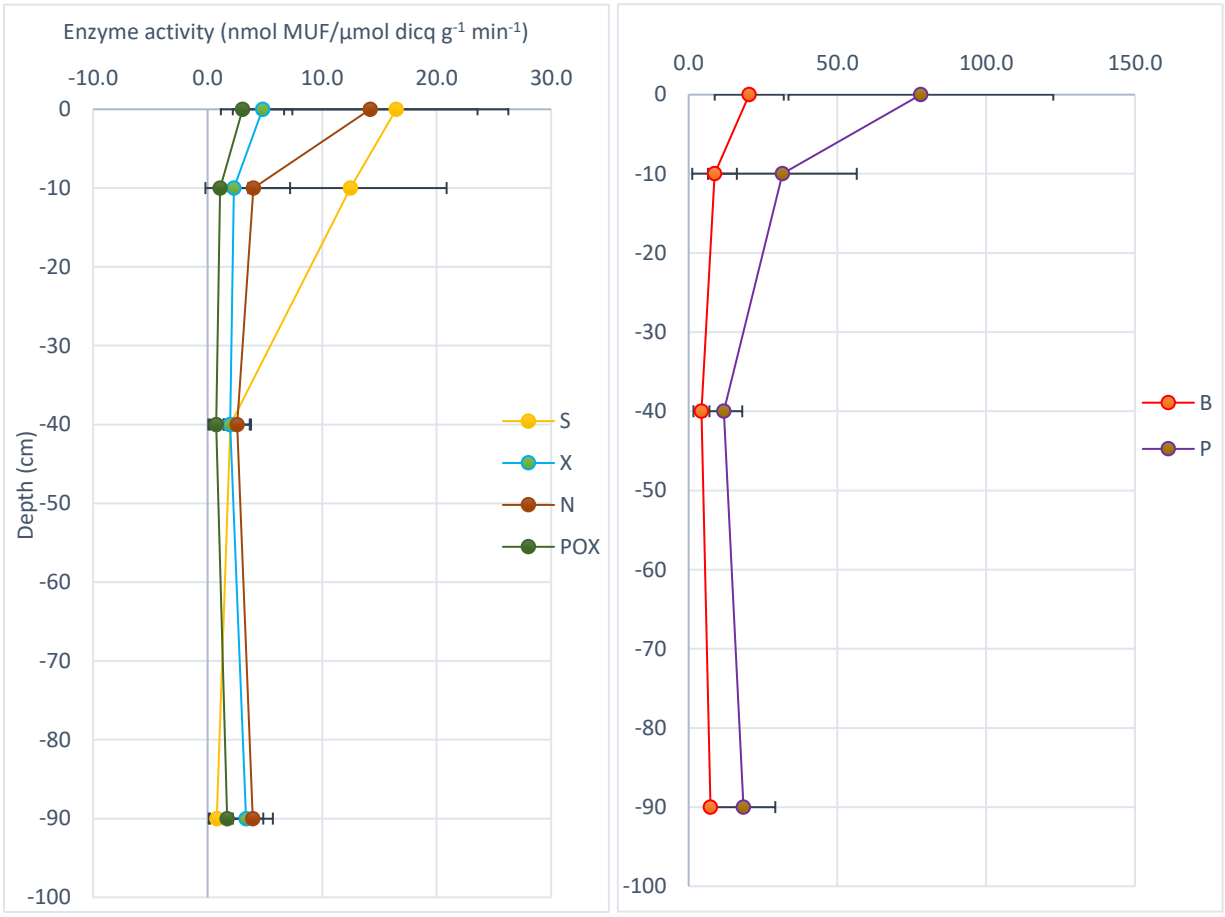


Figure 3.03. Extracellular enzymes activity at different depths in the soil profile. Each circle represents the mean of the six sampling locations. Error bars represent standard deviation. Abbreviations: ß-D-glucosidase (B), arylsulfatase (S), ß -D-xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P), phenol oxidase (POX).

The opposite trend was observed for mean phenolics concentration (Figure 3.04b and Table 3.02), with concentration increasing between the depth of 10-40 cm (3.4 mg L^{-1}) and the depth of 40-90 cm (3.5 mg L^{-1}), and then decreasing at the mineral layer (1.9 mg L^{-1}). One-way ANOVA showed that phenolics concentration differed significantly between the different layers ($F(3, 63) = 17.2$, $p = 0.000$). Post-hoc comparisons using the Tukey HSD test revealed that the depth of 10-40cm and 40-90cm were significantly higher than the other two depths in this regard ($p < 0.05$). Mean DOC concentration (Figure 3.04a and Table 3.02) increased between the superficial layer (13.75 mg L^{-1}) and the dark brown organic layer (23.0 mg L^{-1}) then decreased in the mineral layer (9.2 mg L^{-1}). Mean DOC concentration in the dark brown organic layer was significantly higher than in the superficial and mineral layers. In addition, DOC concentration in the light brown organic layer was significantly higher than in the mineral layer ($F(3, 63) = 4.8$, $p = 0.005$).

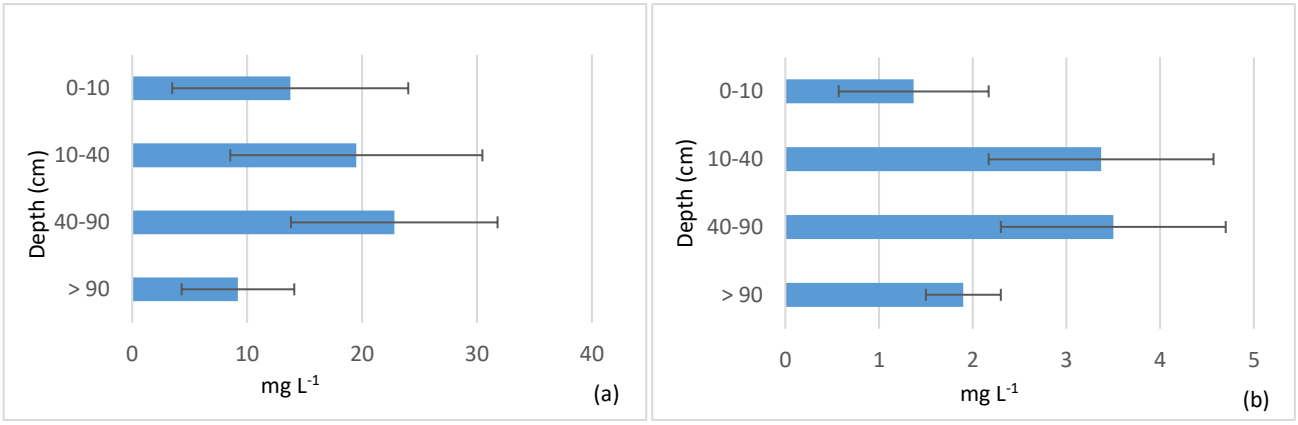


Figure 3.04. Mean DOC (a) and phenolic compound concentration (b) at different depths. Error bars represent standard deviation.

Mean pH level (Table 3.02) was acidic at all depths and ranged between 4.95 in the light brown organic layer and 5.58 in the mineral layer. Mean pH in the mineral and superficial layer were significantly higher than in the light brown organic layer ($F(3, 63) = 6.4$, $p = 0.001$).

Conductivity tended to decrease with depth except for a very slight and statistically insignificant increase between the dark brown organic layer and the mineral layer (Table 3.02). Mean conductivity in the superficial layer (32.4 µS/cm) was significantly higher than all other layers ($F(3, 63) = 15.3$, $p = 0.000$).

Mean soil water content decreased from 92.99% in the superficial layer to 69.5% in the mineral layer (Table 3.02). The mineral layer had significantly lower water content than all other layers and the water content in the dark brown organic layer was significantly lower than in the superficial layer ($F(3, 63) = 16.9$, $p = 0.000$).

Mean soil organic matter content (% SOM) ranged from 57.4% in the light brown organic layer to 23.4% in the mineral layer, which was significantly lower than in all other layers ($F(3, 63) = 7.3$, $p = 0.000$).

Table 3.02. Values for various variables measured at each depth within the peat cores. Results are given as mean ± standard deviation. Letter annotations refer to statistically significant differences between mean values of different depths identified by Tukey HSD post- hoc test ($p < 0.05$).

Variable \ Layer	Superficial (0-10 cm) (a)	Light brown organic (10-40 cm) (b)	Dark brown organic (40-90 cm) (c)	Mineral (>90 cm) (d)
B nmol MUF g^{-1} min^{-1}	20.36±11.62 bcd	8.7±7.5 a	4.3±2.68 a	7.38±0.9 a
S nmol MUF g^{-1} min^{-1}	16.47±9.79 cd	12.48±8.39 cd	2.0±1.79 ab	0.8±0.7 ab
X nmol MUF g^{-1} min^{-1}	4.8±2.6 bc	2.3±1.25 a	1.97±0.58 a	3.36±1.5
P nmol MUF g^{-1} min^{-1}	78.07±44.5 bcd	31.5±25.09 acd	11.81±6.19 ab	18.45±10.67 ab
N nmol MUF g^{-1} min^{-1}	14.2±9.37 bcd	4.0±3.2 a	2.58±1.1 a	3.95±1.75 a
POX µmol dicq g^{-1} min^{-1}	3.06±1.9 bc	1.1±1.29 a	0.75±0.68 a	1.7±1.49
Phenolics mg L^{-1}	1.4±0.8 bc	3.4±1.2 ad	3.5±1.2 ad	1.9±0.4 bc
DOC mg L^{-1}	13.75±10.27 c	19.5±10.97 d	23.0±8.99 ad	9.2±4.9 bc
pH	5.4±0.4 b	4.95±0.36 ad	5.17±0.37	5.58±0.6 b
Conductivity µS cm^{-1}	32.4±17.69 bcd	15.9±6.09 a	12.27±4.0 a	12.65±4.17 a
Water %	92.99±1.55 cd	89.2±2.6 d	83.8±7.08 ad	69.5±20.76 abc
SOM %	48.58±19.6 d	57.4±17.37 d	45.8±14.29 d	23.4±12.9 abc
CO_2 µg CO_2 g^{-1} h^{-1}	24.56±14.56 bcd	11.07±8.97 a	6.87±4.4 a	8.38±5.4 a
CH_4 ng CH_4 g^{-1} h^{-1}	4.49±11.38 bcd	8.0±14.97 ad	9.56±32.4 ad	-1.39±1.7 abc
N_2O ng N_2O g^{-1} h^{-1}	2.6±1.8	1.9±2.37	1.4±1.75	1.7±1.27
Chloride mg L^{-1}	3.6±0.76 bcd	1.19±0.8 a	1.2±0.5 a	1.2±1.86 a
Nitrate mg L^{-1}	0.35±0.69	1.4±2.06 d	0.3±0.58	0.00 b
Phosphate mg L^{-1}	0.07±0.2 cd	0.1±0.19 d	0.36±0.58 a	0.5±0.3 ab
Sulfate mg L^{-1}	2.1±0.7 cd	2.86±0.8 cd	5.19±1.4 ab	4.85±0.68 ab
Sodium mg L^{-1}	4.85±1.58	4.87±1.2	5.58±0.67	5.8±0.85
Ammonium mg L^{-1}	1.0±1.56 bcd	0.03±0.09 a	0.008±0.02 a	0.002±0.006 a
Calcium mg L^{-1}	2.1±4.07	2.9±2.67	2.9±1.86	3.1±0.9
Magnesium mg L^{-1}	0.3±0.5	0.49±0.8	0.46±0.7	0.2±0.1

Mean CO_2 and N_2O flux decreased between the superficial and dark organic layer, then increased slightly at the mineral layer (Figure 3.05 and Table 3.02), though in the case of N_2O, none of the differences were statistically significant ($F(3, 63) = 1.3$, $p = 0.280$). The CO_2 flux in the superficial layer was significantly higher than in the other layers ($F(3, 63) = 12.6$, $p = 0.000$).

The opposite trend was observed for the CH_4, with mean flux increasing between the superficial and dark brown organic layer, then decreasing between the dark brown organic layer and the mineral layer (Figure 3.05 and Table 3.02). Mean CH_4 flux in the superficial and mineral layers was significantly lower than that in each of the other layers ($F(3, 63) = 5.5$, $p = 0.001$).

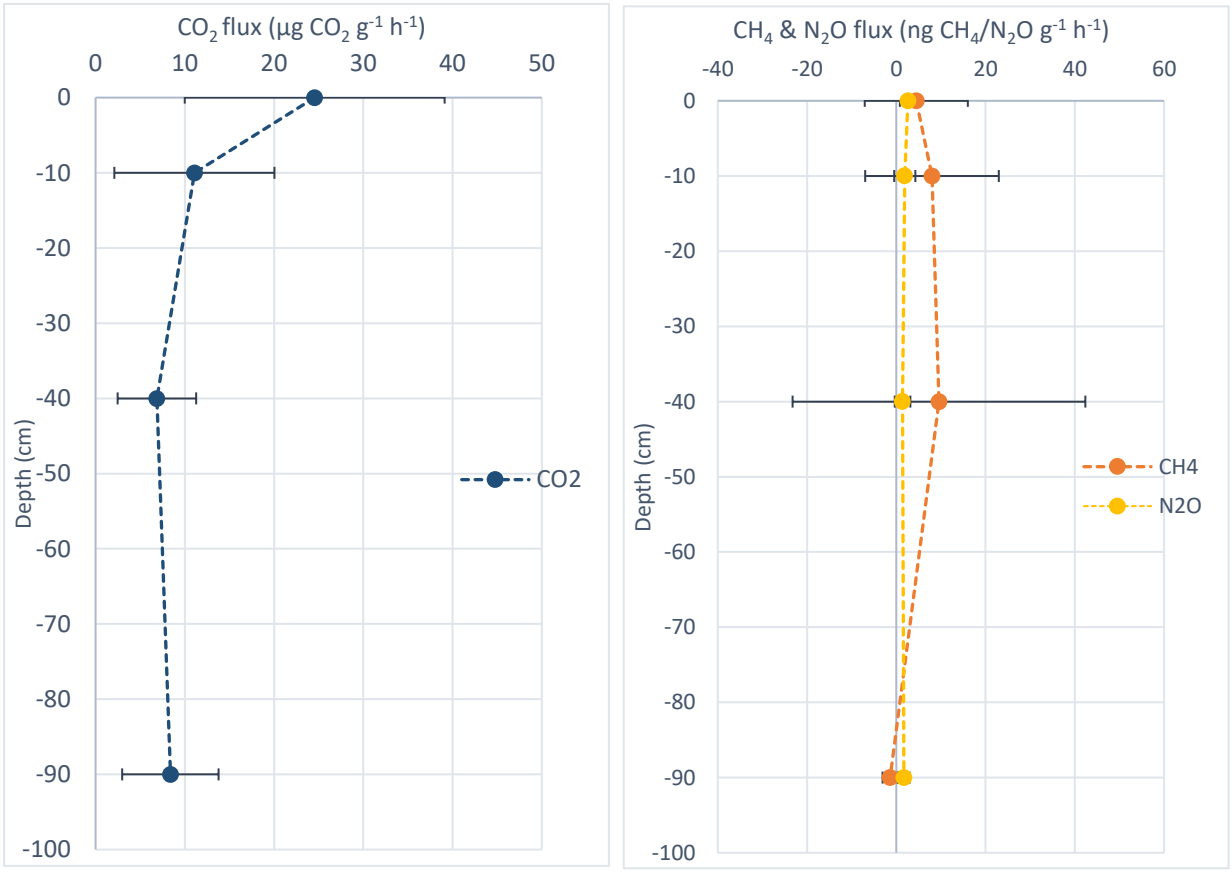


Figure 3.05. Mean CO_2, CH_4 and N_2O fluxes at different depths. Error bars represent standard deviation.

For anion measurements (Table 3.02), mean chloride concentrations ranged from 1.19 mg L^{-1} in the depth of 10-40 cm to 3.6 mg L^{-1} in the superficial layer, which was significantly higher than all other depths ($F(3, 63) = 15.2$, $p = 0.000$). The nitrate concentration was below the limit of detection in the mineral layer and the maximum concentration was found in the light brown organic layer (mean 1.4 mg L^{-1}), which was only significantly higher than the mineral layer ($F(3, 63) = 4.04$, $p = 0.011$). Mean phosphate concentration ranged from 0.07 mg L^{-1} in the superficial layer to 0.5 mg L^{-1} in the mineral layer, which were significantly higher than the light brown organic layer and the superficial layer. The dark brown organic layer was also significantly higher than the superficial layer ($F(3, 63) = 3.97$, $p = 0.012$). Mean sulfate concentration was lowest in the superficial layer (2.1 mg L^{-1}) and highest in the dark brown organic layer (5.19 mg L^{-1}), which was, in addition to the mineral layer, significantly higher than the other depths ($F(3, 63) = 36.9$, $p = 0.000$).

For cation measurements (Table 3.02), mean sodium concentration ranged from 4.85 mg L^{-1} in the superficial layer to 5.8 mg L^{-1} in the mineral layer. No significant differences in sodium concentration were found between depths ($F(3, 63) = 2.55$, $p = 0.064$). Mean ammonium concentration was highest in the superficial layer and substantially and significantly lower (< 0.1 mg L^{-1}) in the remaining depths ($F(3, 63) = 6.65$, $p = 0.001$). Mean calcium concentration was lower in the superficial samples (2.1 mg L^{-1}) and higher in the mineral layer (3.1 mg L^{-1}), without any significant differences ($F(3, 63) = 0.42$, $p = 0.741$). Magnesium concentration was highest in the light brown organic layer (0.49 mg L^{-1}) and lowest in the mineral layer (0.2 mg L^{-1}) although there were no statistically significant differences between the different depths ($F(3, 63) = 0.47$, $p = 0.71$).

Pearson correlation analysis was performed to test for significant relationships between soil physicochemical variables, enzyme activities and GHG fluxes (Table 3.03). The key observations are that the activities of the five hydrolase enzymes correlated positively with each other and with phenol oxidase activity. There were also strong negative correlations between all extracellular enzyme activities and phenolic compound concentration. Figure 3.06 shows the negative correlation between phenol oxidase activity and its phenolic substrate. Figure 3.07 shows the negative correlations between all hydrolase enzyme activities and phenolic compound concentration. The fluxes of CO_2 and N_2O were positively correlated with all enzyme activities, and with conductivity and pH level. Electrical conductivity, chloride and ammonium showed strong positive relationship with all enzyme activities. The pH values also correlated positively with all enzymes activities, with the

exception of arylsulfatase (S) activity. Sulfate ions concentration showed significant negative correlation with all enzyme activities.

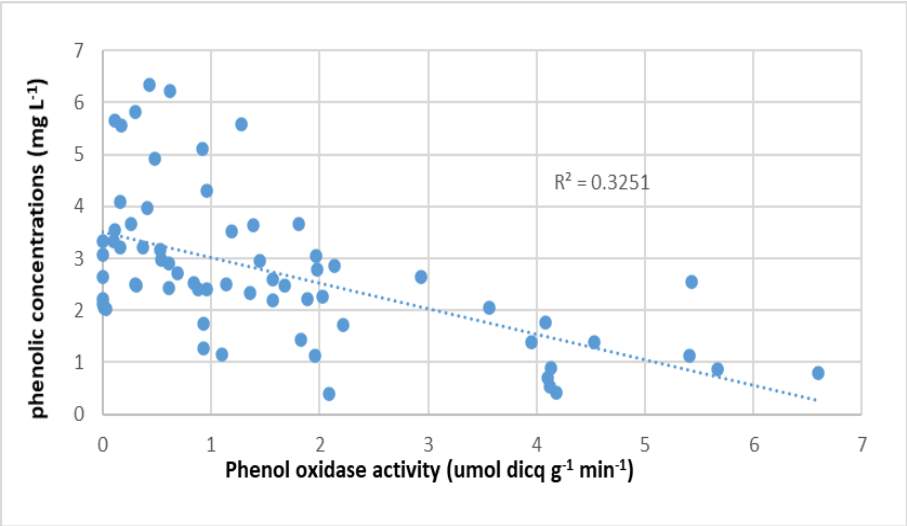


Figure 3.06. Relationship between phenol oxidase activity (POX) and phenolic compound concentration for all samples (n = 67).

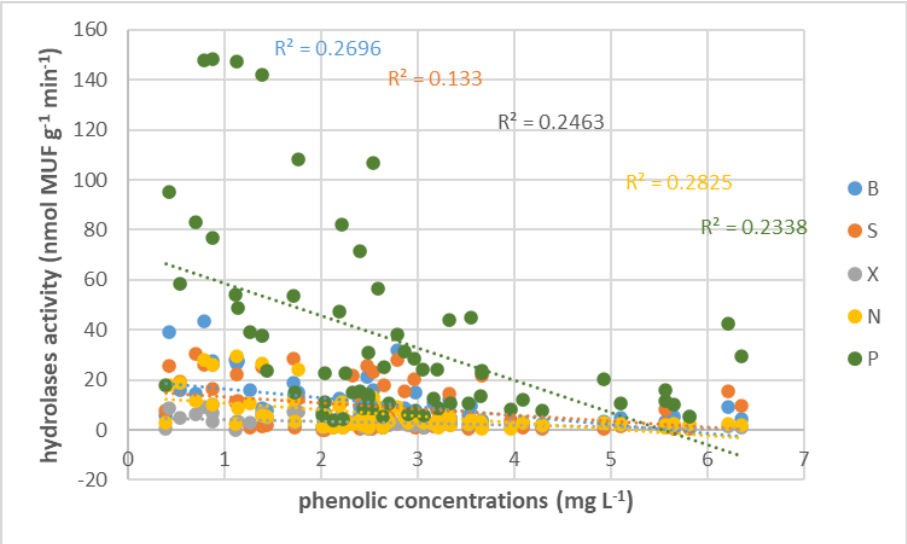


Figure 3.07. Relationship between hydrolase enzyme activities and phenolic compound concentration for all samples (n = 67).

Table 3.03. Results of Pearson correlation analysis (n = 67) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: ß-glucosidase (B), arylsulfatase (S), ß-D-xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P), phenol oxidase (POX), electrical conductivity (EC), dissolved organic carbon (DOC), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and soil organic matter (SOM). Significant correlations are shown in bold.

	B	S	X	N	P	POX
S	**.639****					
X	**.664****	**.486****				
N	**.790****	**.574****	**.793****			
P	**.776****	**.640****	**.746****	**.927****		
POX	**.645****	**.592****	**.705****	**.833****	**.840****	
Phenolic	**-.519****	**-.365****	**-.496****	**-.532****	**-.484****	**-.570****
DOC	.010	.045	-.112	-.001	-.058	.076
pH	**.251***	.195	**.465****	**.435****	**.416****	**.602****
EC	**.617****	**.535****	**.619****	**.803****	**.808****	**.739****
% water	**.311***	**.383****	.121	**.283***	**.312***	.122
% SOM	-.218	-.059	**-.418****	**-.295***	**-.277***	**-.453****
CO_2	**.460****	**.558****	**.472****	**.603****	**.606****	**.695****
CH_4	-.061	.015	-.116	-.044	-.042	.025
N_2O	**.358****	**.322****	**.390****	**.485****	**.446****	**.485****
Chloride	**.626****	**.446****	**.569****	**.788****	**.788****	**.681****
Nitrate	-.038	.194	-.034	.006	.147	.118
Phosphate	-.181	**-.258***	-.219	-.235	**-.241***	-.095
Sulfate	**-.463****	**-.571****	**-.271***	**-.371****	**-.476****	**-.299***
Sodium	**-.354****	-.203	-.215	-.179	**-.250***	-.093
Ammonium	**.448****	**.343****	**.402****	**.737****	**.621****	**.592****
Calcium	-.231	-.089	-.153	-.200	-.154	-.148
Magnesium	-.206	-.046	-.203	-.188	-.132	-.195

Continued

	Phenolic	DOC	pH	EC	% Water	% SOM	CO_2	CH_4	N_2O
DOC	**.256***								
pH	-.240	.140							
EC	**-.484****	-.024	**.467****						
% water	-.121	-.059	**-.400****	.233					
% SOM	**.299***	.114	**-.473****	-.139	**.467****				
CO_2	**-.550****	.067	**.439****	**.722****	**.261***	-.123			
CH_4	.058	-.037	.129	-.036	.034	-.023	-.003		
N_2O	-.189	.114	**.317****	**.295***	.085	-.123	.176	**.245***	
Chloride	**-.449****	.052	**.482****	**.904****	.209	-.176	**.592****	-.053	**.256***
Nitrate	.062	-.173	-.107	.085	.072	-.070	-.014	.040	.094
Phosphate	.025	-.009	-.005	**-.251***	-.128	**-.247***	-.058	.128	-.222
Sulfate	**.486****	**.281***	.179	**-.372****	**-.407****	-.204	**-.320****	.118	-.095
Sodium	.073	.089	.071	-.125	-.080	-.066	.083	-.019	-.215
Ammonium	**-.372****	.121	**.362****	**.651****	.157	-.118	**.582****	-.034	**.336****
Calcium	.180	.020	.008	-.037	-.169	.124	-.031	-.035	-.090
Magnesium	.243	-.070	-.133	-.056	.025	.198	-.046	-.088	-.193

3.5. Discussion

Microbial activity in peatlands is generally thought to be concentrated in the uppermost layer of peat (Fierer *et al.* 2003, Senga *et al.* 2015) more likely due to the higher temperature, aeration, labile organic matter and the fact that this layer corresponds with the rhizosphere and hence highest density of microbes (Moore 2002). As a result, studies of soil microbial processes tend to focus on this upper layer (Page *et al.* 1999, Fierer *et al.* 2003, Senga *et al.* 2015). However, substantial microbial processes are expected in deeper layers of peat as large numbers of microbes have been found well below the surface (Senga *et al.* 2015). Given that limited research has been conducted into South American peatlands (Gumbricht *et al*. 2017), and the importance of microbial processes in affecting the carbon budget of these environments, this study assessed enzymes activity and soil respiration throughout the peat profile and the potential drivers of their variability with depth.

As can be seen in Figure 3.03, the activity of ß-glucosidase (B), arylsulfatase (S), xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P) and phenol oxidase (POX) tended to decrease with depth, with a slight but statistically insignificant increase in activity at the mineral layer. Higher enzymes activity in the upper layers of peat indicate higher rates of enzymic decomposition in this zone. Increased soil depth is corresponding to increasing age of organic matter and hence greater previous decomposition of the peat (Jackson *et al.* 2009).

A reduction in enzymes activity with depth has been previously reported in a tropical Malaysian peatland (Jackson *et al.* 2009), a tropical Indonesian peatland (Könönen *et al.* 2018), a tropical Panamanian peatland (Hoyos-Santillan *et al.* 2015), in temperate peatlands (Pind *et al.* 1994, Freeman *et al.* 1995), in tropical Everglades wetlands (Wright and Reddy 2001), in Japanese wetlands (Senga *et al.* 2015), and in other soil ecosystems (Niemi *et al.* 2005, Jackson and Vallaire 2007, Baldrian *et al.* 2012, Stone *et al.* 2014, Schnecker *et al.* 2015, Ko *et al.* 2017, Hsiao *et al.* 2018). This phenomenon has been attributed to the reduction in oxygen availability and substrates quality with increasing depth (Wright and Reddy 2001, Jackson *et al.* 2009, Jauhiainen *et al.* 2016). In addition to the other edaphic factors such as nutrient availability, moisture content and pH level, all of which were associated with decreased microbial activity as soil depth increased (Ko *et al.* 2017, Könönen *et al.* 2018). In the present study, soil physicochemical variables also varied with depth,

which could explain the variations in the enzyme activity and the associated end products of decomposition processes.

Peaty soil in tropical peatlands is dominated by aromatic compounds, which indicate greater recalcitrance and has been known to severely limit decomposition processes (Hodgkins *et al.* 2018). Since phenolic content, that can inhibit substrate utilization and hence constrain hydrolytic enzymes activity, increase with soil depth, hydrolase activities can be predicted to decrease accordingly. Indeed here, while enzymatic activities were decreasing with depth, phenolic concentrations were increasing.

A significant negative correlation was found between phenolic compound concentrations and the activity of each of the hydrolases, suggesting the phenolics to be exerting a hydrolase-inhibitory effect. This supports the observations of a number of previous studies. For example, Freeman *et al.* (2004) showed that the depletion of phenolics stimulated the activities of a number of hydrolytic enzymes, B, S, X, N and P by 26, 47, 16, 22 and 18%, respectively. The higher hydrolase activities under a lower abundance of phenolic compounds were suggested to indicate that the depletion of phenolics removed inhibitors in the soil solution. In that study, confirmation that the effect was due to the elimination of phenolics and not another component (which might include iron or manganese) was achieved by selectively reducing phenolic abundance in the phenolic-rich water using conditioned polymerised N-vinyl pyrrolidone (PVPP; Carpenter *et al.* 1976). In this study, it is not certain that the inverse relationship between phenolics and hydrolase activity directly indicated causality; to infer causality, it would have been necessary to repeat the selective phenolic-depletion experiment using PVPP carried out by Freeman *et al.* (2004).

This significant negative relationship also helps to explain the decrease in enzyme activity with depth in the present study, since phenolics concentration increased between the superficial and dark brown organic layer. Phenolic compounds have inhibitory effects on hydrolase enzymes and undoubtedly on biodegradation and respiration rates because they can combine with the reactive sites of organic and inorganic substrates, making them resistant to further microbial attack. They can also inhibit litter decomposition by lowering pH (Min *et al.* 2015) and by themselves being strongly resistant to biodegradation (Zak *et al.* 2019).

Increasing phenolic content with depth may be related to decreasing POX activity with depth, allowing phenolic compounds to build up in the deeper layers. Indeed, a significant negative correlation between POX activity and phenolic concentration ($r = -0.570$, $p < 0.01$) was found

in the present study. This relationship has been widely reported previously (e.g., Pind *et al.* (1994) and Freeman *et al.* (2004)). Increasing phenolic compound concentrations with depth is in line with several previous studies (Whitmore 1984, Qualls and Haines 1990, Pind *et al.* 1994, Whitten *et al.* 2000, Könönen *et al.* 2018). This trend may also be related to increasing DOC concentration with depth (since phenolics are a constituent of DOC). Indeed, DOC concentration correlated positively with phenolic concentrations ($r = 0.256$, $p < 0.05$) (Freeman *et al*. 1996, Jones 2006, Kang *et al.* 2018, Wan *et al*. 2018).

Measurement of enzymes activity can reflect microbial nutrient demand as many microorganisms control their enzyme production aligned with the demand for nutrients (Chròst 1991). Therefore, it is believed that a negative correlation would occur between soil enzyme activity and nutrient availability depending on the economic model of microbial metabolism indicating that if nutrients are limited, enzymes production increases and vice versa (Sinsabaugh *et al.* 1993, 2008). In this context, the inverse correlations were found between S enzyme activity and sulfate ions concentration ($r = -0.571$, $p < 0.01$), and between P enzyme activity and phosphate ions concentration ($r = -0.241$, $p < 0.05$), which may indicate that higher nutrient availability, reduces soil enzyme activity. This finding is in agreement with that reported from a tropical peatland in Panama (Sjögersten *et al*. 2011). These relationships may also help to explain the highest S and P activities in the upper layers of peat, where sulfate and phosphate concentrations were lowest.

The strong positive relationships between all hydrolytic enzyme activities and POX activity found in this study have been reported previously (Freeman *et al.* 2004) and reflect the indirect catalytic role of POX on hydrolytic enzymes (Jones 2006). Significant positive correlations between CO_2 and N_2O efflux rates and all extracellular enzymes activities demonstrate the relationship between enzymic decomposition rate and GHGs release. Conversely, a significant negative correlation between CO_2 and phenolic content ($r = -0.550$, $p < 0.01$), highlights the important role of phenolic compounds in the carbon sink status of intact peatlands (Freeman *et al.* 2001).

Contrary to the findings of the present study, Jackson *et al*. (2009) reported undetectable POX levels in all samples obtained from the different depths in a tropical Malaysian peatland, which they linked to low oxygen and pH levels (Pind *et al.* 1994).

Decreasing enzyme activity with depth may also relate to ammonium concentration which was highest in the superficial layer (0-10 cm) and significantly lower in the lower depths. A

significant positive correlation was found between ammonium concentration and all hydrolase activities, and between ammonium/ POX activity, ammonium/ CO_2 flux, ammonium/N_2O flux, and ammonium/ pH. In this context, several studies have shown that elevated N availability in soil often increases hydrolase activity and soil respiration (Waldrop *et al.* 2004, Kim and Kang 2008, Jian *et al.* 2016, Chen *et al.* 2017, Li *et al.* 2018), and can accelerate the loss of C from the soil by activating POX (Bragazza *et al.* 2006). The negative correlation found in the present study between ammonium and phenolics may also signify this. These observations are in line with the findings of Bragazza *et al.* (2006) who found significant positive relationships between N deposition and each of B, P, POX activities and CO_2 emission in peat bogs.

Along with ammonium, high electrical conductivity (indicating higher nutrient loading) in the superficial layer (0-10 cm) is probably linked to high enzyme activity, all analysed extracellular enzymes, CO_2 and N_2O fluxes were strongly correlated with soil conductivity.

Regarding the relationship between chloride concentration/enzymes activity and sulfate concentration/enzymes activity, Dinesh *et al.* (1995) found that chloride and sulfate ions have an inhibitory effect on microbial growth and hence on enzyme synthesis due to an osmotic pressure leading to microbial cell lysis, and/or a salting-out effect on enzyme protein. So, the observation of a positive correlation between chloride concentration and enzymes activity and GHG fluxes was therefore unexpected. However, it is likely that the chloride correlation is caused by an unrelated correlation with something else that does genuinely influence enzymes such as ammonium. The chloride might just be there as a result of dissolution of ammonium chloride. So, the positive correlation is really between ammonium and the enzymes activity and GHG fluxes, as found in this study. Additionally, statistically significant positive relationship between chloride/ ammonium was found ($r = 0.639$, $p < 0.01$, data not shown) which could also prove this.

In the present study, the significant negative relationship with sulfate concentration may be linked to the acidifying effect of sulfate and hydrogen ions, which may originate from dilute sulfuric acid (H_2SO_4) in the soil solutions (Gorham 1958). However, no negative correlation with pH value was found to confirm this.

Phosphatase, which is involved in phosphorus cycling, showed significantly higher activity than the other enzymes. Similar rates of phosphatase activity have been noted in tropical peatlands of Malaysia (Jackson *et al.* 2009) and Panama (Sjögersten *et al.* 2011).

Another important edaphic factor known to affect enzyme activity is pH (Schnecker *et al.* 2015, Kang *et al.* 2018). The declining enzyme activity with depth might therefore be expected to be accompanied by a decrease in pH value. Although no statistically big significant differences in pH were found between different depths, when correlation analysis was conducted on individual measurements, all enzyme activities except S showed a statistically significant positive correlation with pH. The stimulation of extracellular enzyme activity under high pH conditions can also explain the positive correlations found between pH and CO_2 and N_2O flux rates. Regarding the optimum pH for S enzyme activity, Turner (2010) found that soils with a pH of ≤ 3.0 had great rates of S activity, while soils with around an alkaline pH rang, had a little S activity.

About the importance of pH for POX activity, the value of pH emerged to be the first agent in the control of POX activity, subsequently accelerating the release of DOC across a survey of a wide range of peatlands and through field and laboratory manipulation experiments (Kang *et al.* 2018).

Given the decrease in enzyme activity with depth, the increase in DOC concentration between the superficial layer and the dark brown organic layer was unexpected. This could be due to the presence of phenolic compounds which tend to constitute a large proportion (50-75%) of peatland-derived DOC pool (Kang *et al.* 1998), and was increasing between the superficial layer and the dark brown organic layer in the present study. In addition, decreased DOC concentration in the superficial layer compared to the deeper organic layers observed here is likely related to microbial processes under more aerobic conditions (Freeman *et al.* 1993). Also, in the more aerobic conditions of the uppermost layers, the more efficient C metabolism tends to produce CO_2 rather than DOC (Freeman *et al.* 1993).

The low DOC and phenolic compounds concentrations in the mineral layer is likely to be the result of low overall SOM. Aside from DOC production, factors affecting DOC solubility are also known to affect DOC concentrations (e.g., pH, temperature and conductivity) (Gough *et al.* 2016).

The % SOM (a substrate for enzyme activity) decreased significantly with depth. There were significant negative correlations between % SOM and enzymes activity identified. Previous studies have identified positive correlations between soil hydrolytic enzymes and % soil organic matter, since a greater availability of substrate will inevitably support higher microbial activity (Schnecker *et al.* 2015). The absence of a positive relationship between

enzyme activity and SOM in the present study suggests that the chemistry of SOM also strongly influences substrate availability for enzymic decomposition (Schnecker *et al.* 2015).

Globally, altered tropical peatlands are considered as critical carbon sources affecting on the atmospheric GHG composition (Jauhiainen *et al.* 2012, IPCC 2014). International interest in GHG emissions from tropical peatlands is growing, especially in the heavily degraded Asian peatlands, due to the large-scale human disturbance (Gumbricht *et al.* 2017). In contrast to the peatlands of Southeast Asia, where most tropical peatland studies of GHG emissions have occurred so far, the tropical peatlands of South America remain poorly understood.

Jackson *et al*. (2009) examined the structure and the enzymatic activity of the prokaryotic community at several depths (between 0 to 50 cm) in tropical peatlands in Malaysia and found that each depth hosted different prokaryotic assemblages, with the upper few centimetres being more populated than the deeper layers. They only excluded archaeal community that were limited to the deeper layers (20-50 cm), where anoxic conditions are thought to facilitate their growth. In this study, mean CH_4 flux varied with depth, with higher flux recorded in the deeper organic layers (10-40 and 40-90 cm), suggesting that the abundance of methanogenic *Archaea* was low in the superficial layer (0-10 cm) and increased with depth. This finding is similar to that shown in other peatland studies (Kotsyurbenko *et al*. 2004, Dedysh *et al.* 2006). Unlike CO_2, CH_4 fluxes seemed unaffected by phenolics concentration, perhaps because the inhibitory effect of phenolic compounds is mainly on the breakdown of more labile C compounds in the soil litter (Dunn 2013), while CH_4 can also be generated through the hydrogenotrophic pathway (CO_2 reacting with H_2 directly to form the CH_4), which does not involve the breakdown of organic matter (Dunn 2013).

Regarding N_2O flux, which showed insignificant variation with depth, strong positive correlations were found between chitinase, (relating to the degradation of N-rich structural compounds) and ammonium concentration, between chitinase and N_2O flux and between N_2O flux and ammonium concentration. This suggests that the nitrogen released in N-rich chitin degradation could contribute to the N_2O production processes (Könönen *et al.* 2018).

Organic carbon supplies are required by heterotrophic microorganisms as energy sources promoting microbial processes such as denitrification (Kayranli *et al.* 2010). In this regard, strong positive correlations between N_2O fluxes and both oxidative (POX) and hydrolytic enzymes (B, X and N) were found. Similarly, Kang *et al.* (1998) found that the N_2O emission

increased with increasing β-glucosidase activity (used as an indicator of labile carbon availability) in a Welsh peatland.

3.6. Conclusions

Our study aimed to investigate and explain variations in extracellular enzyme activities and soil respiration with depth within a Colombian peatland in the Andean mountains of south American continent, which have received relatively little attention in the literature, despite the fact that they represent a major contribution to global tropical peatland coverage (750 000 km^2) and volume (3 117 Gm^3). In this study, extracellular enzymes activity (hydrolases and phenol oxidase) and the production of greenhouse gas (CO_2, CH_4 and N_2O) were investigated at different depths in a tropical peatland of the Andes, Colombia. In addition, the associations between these variables and pH, conductivity, nutrient concentration (cations and anions), phenolic compound concentration, dissolved organic carbon (DOC) concentration, and soil water and soil organic matter (SOM) content were assessed.

In agreement with previous studies of tropical peatlands and other soil ecosystems, a general decrease in extracellular enzymes activity with depth was found and attributed to a combination of factors including decreasing pH, oxygen content, utilizable organic matter and increasing recalcitrant compounds concentration. Strong associations were found between enzyme activities and electrical conductivity, pH, water content and the concentrations of some nutrients.

Given the decrease in enzymes activity with depth, the increase in DOC concentration between the superficial layer and the dark brown organic layer was unexpected. This could be due to the presence of phenolic compounds which are known to constitute a large proportion (50-75%) of the peatland-derived DOC pool, and was increasing between the superficial layer and the dark brown organic layer in the present study, with a significant positive correlation being found between the two factors (DOC concentration and phenolics concentration).

The strong link between enzyme activities and decomposition rate (as indicated by CO_2 and N_2O flux) was also evident in the data of this study. Variations in GHG flux with depth can also be attributed to differences in the composition of the microbial community (e.g., the abundance of methanogens) in response to depth-related variations in abiotic conditions.

The strong positive relationships between all hydrolytic enzyme activities and POX activity found in this study have been reported previously and reflect the catalytic role of POX on hydrolytic enzymes. The significant negative relationships with phenolic concentrations support the suppressing role of phenolics on enzymes activities and the resulting soil respiration. Altogether could support the enzymic latch mechanism.

A significant positive correlation was found between ammonium concentration and all extracellular enzymes activity and soil respiration (CO_2 and N_2O), indicating that increased N content poses a significant risk to peatlands function.

The significant inverse correlations found between S enzyme activity and sulfate ions concentration and between P enzyme activity and phosphate ions concentration support the economic model of microbial enzymes production.

The statistically significant correlations with pH confirm the importance of pH in affecting decomposition rates of peatlands.

This baseline information could contribute to the environment of moss-dominated peatlands in Colombian Andes, and more specifically will provide important knowledge for use by Sumapaz National Park employers in managing natural parks.

Studying biogeochemical processes and the abiotic variables controlling them in peatlands has important implications for predicting the potential impacts of climate change, human intervention and management strategies on peatlands function. Further and more thorough studies are needed for better understanding the patterns in microbial community composition and function at each depth in light of the dominant environmental variables.

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Chapter 4

Environmental controls on biogeochemical processes in a temperate Welsh peatland

4.1. Abstract

Improving the knowledge of the physicochemical factors affecting enzymic decomposition processes in peatlands is essential in order to predict the potential impacts of climate change and human exploitation and hence enable the development of effective peatland management strategies. Temperate peatlands are one of threatened peat-rich area due to the on-going climate change and human exploitation, which indicates that they warrant attention. In the present study, the effect of substrate depth on enzymes activity, greenhouse gas (GHG) production rates and different abiotic variables were examined and the relationships between them were investigated.

The quantity and the quality of soil organic matter decrease with depth, and so soil enzyme activity was likewise expected to decrease with depth. Indeed, and in agreement with previous studies of temperate peatlands and other soil ecosystems, the highest mean enzyme activity tended to occur in the superficial layer and lowest enzyme activity was consistently found in the depth of > 60 cm. Interestingly, pH values, which ranged from 3.8 to 5.4, showed a significant positive correlation with each of the hydrolytic enzymes measured.

Phenol oxidase (POX) activity did not vary significantly between depths despite presumed differences in oxygen concentration with depth. This observation could be related to the pH (which more strongly influences POX activity), not differing significantly between the different layers, although no statistically significant correlation was found between POX activity and pH. Additionally, with phenol oxidase not varying between depths it is unsurprising that phenolic concentrations (substrate for the enzyme) did not vary significantly between depths. However, no statistically significant correlation was found between POX activity and phenolics concentration.

The activities of enzymes (except chitinase) and gaseous fluxes appeared unaffected by the concentration of phenolic compounds. These results suggest that phenolics are not always efficient inhibitors of microbial metabolism and that the forms, source and the chemical composition of phenolic compounds could play an important role in affecting the activities of soil extracellular enzymes.

The absence of a positive association between enzyme activities and metabolic end products suggests that the measured activities were not due to de-novo enzyme synthesis but rather due to “legacy enzymes”. Legacy enzymes are enzymes immobilised in humus-enzyme or clay-

enzyme complexes. These enzymes are very stable and resistant to biological, physical and chemical degradation. They may persist independently in soils for far longer than the soil microbes from which they were synthesised.

The mean flux of methane (CH_4) was highest in the deeper soil layers, suggesting that methanogens are better adapted to the anaerobic conditions of the deeper peat layers. The production of soil enzymes is often driven by poor nutrient availability, as observed by an inverse correlation between enzyme activity and nutrients. However, the lack of statistically significant negative relationships between enzyme activity and nutrients in the present study confirms the importance of legacy enzymes that are present in large amounts in the soil and are independent of microbial regulation and thus do not respond to nutrient availability.

4.2. Introduction

An imbalance between the rates of organic matter decomposition and production means that peatlands are an extremely important store of terrestrial carbon (Potter *et al.* 2017) and are therefore an important component of the global carbon cycle (Yu *et al.* 2011). However, climate change threatens to alter the biogeochemical processes that occur in peatland ecosystems (Moore 2002) including temperate peatlands (Potter *et al*. 2017). Climate change including global warming is predicted to increase water loss and drought in peatlands. The associated oxygen ingress, combined with higher temperatures will result in higher enzymic decomposition rates, threatening their carbon sink status and causing the contraction of other peatland services such as drinking water production. The switching of peatlands from carbon sinks to carbon sources will also add a positive feedback to the global warming problem. In this context, it is important to study the conditions that influence the activities of soil enzymes responsible for peat decomposition as a prerequisite for predicting the ecosystem response to climate change, human utilization and management policies (Moore 2002). Indeed, rising concern about the potential consequences of climate change on soil metabolic processes in peat-forming ecosystems has stimulated a significant amount of experimental research focussed on the development of methods for enhancing ecosystem services and carbon sequestration (Freeman *et al.* 2012).

In the UK, where temperate climatic conditions exist, peatlands cover around 24,640 km^2 (15% of the total area), store 2,302 Megatons (Mt) of carbon (Billett *et al*. 2010) and

represent over 10% of the global peat store (IUCN 2014). Over 80% of the UK's peatlands have been the focus of protection and restoration efforts, following damage resulting from cultivation, drainage, peat extraction for fuel, grazing and burning (Holden *et al.* 2007). A report by the Committee on Climate Change (2013) went as far as to say that only 4% of peatlands in the UK are considered to be in good condition and are actively accumulating peat (Committee on Climate Change 2013). In Wales, where the present study is focused, 732 km^2 of land is occupied by peatlands, which are reported to store 119 Mt of carbon (Billett *et al.* 2010).

Over several decades, scientists have made significant progress in understanding the role of enzymes in soil fertility and plant growth. More recently, the role of enzymes in carbon cycling and climate change has attracted growing attention (Dunn *et al.* 2014).

The effects of soil properties including physical and chemical conditions on soil enzymes activity in peatland ecosystems are frequently debated (Pind *et al.* 1994, Freeman *et al.* 1995, 1996, Moore 2002, Freeman *et al.* 2004, Freeman *et al.* 2012, Kang *et al.* 2018) in order to relate specific microbial processes to a narrow range of environmental properties. As the climate continues to change, understanding the relationship between biogeochemical processes and environmental variables in temperate peatlands, which are extensive and important pools for atmospheric carbon, is especially important (Moore 2002, Leifeld and Menichetti 2018).

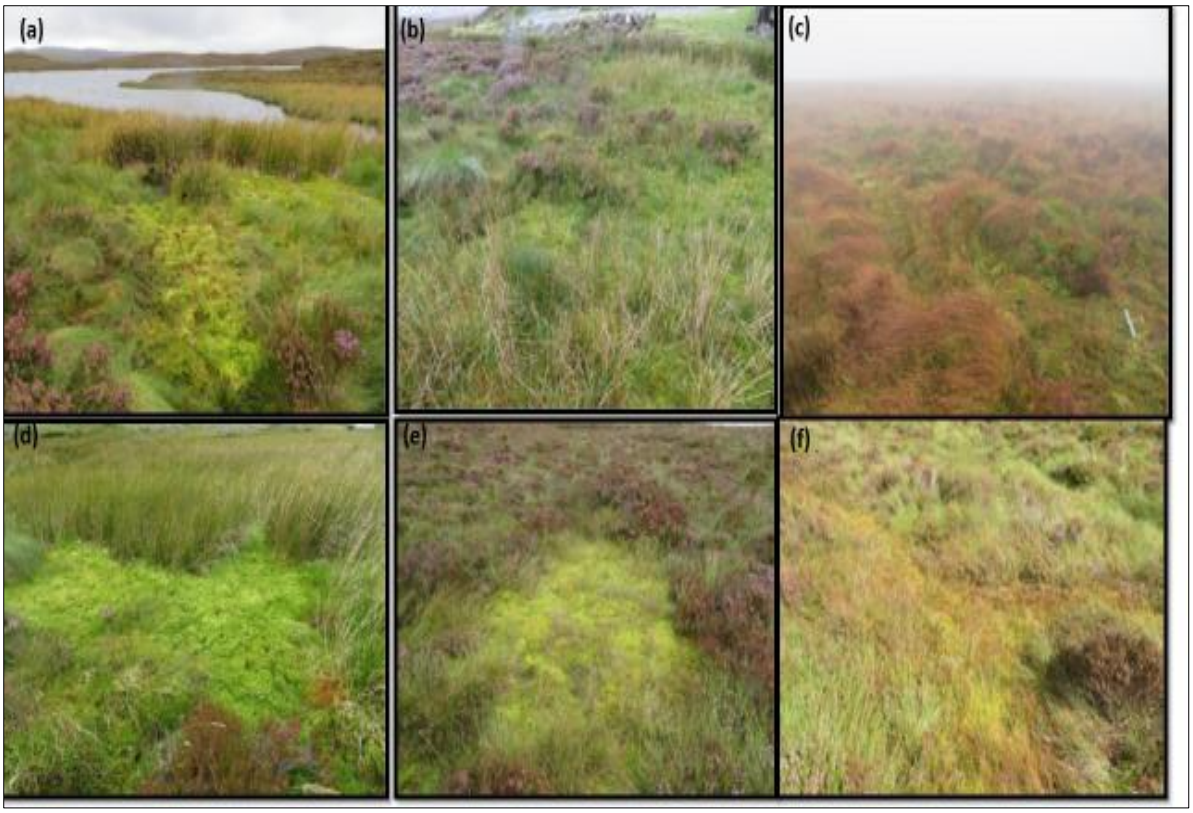
Compared with previous studies, the range of biogeochemical and environmental parameters investigated here has been extended. All of these parameters were measured across different depths. The statistically significant relationships between all measured parameters in a Welsh peatland in the Snowdonia National Park, UK were assessed. The activities of ß-glucosidase (B), arylsulfatase (S), xylosidase (X), phosphatase (P), N-acetyl-ß-D-glucosaminidase or chitinase (N) and phenol oxidase (POX) within different substrate layers were analysed. Greenhouse gas (CO_2, CH_4 and N_2O) emissions were also measured. Various physicochemical properties including dissolved organic carbon (DOC) concentration, pH, conductivity, soil water content, soil organic matter content, phenolic concentration and cation and anion concentration were measured to test the hypothesis that the variation in enzymes activity and GHG production with depth is driven by soil physicochemical properties.

4.3. Materials and methods

4.3.1. Study site

Six *Sphagnum*-dominated peatland sites located in the Migneint Valley (Snowdonia National Park), North Wales, UK were sampled at the beginning of September 2016 (Figure 4.01). More detailed maps were unavailable for peatland distribution in Wales. Annual rainfall in this area is 2,400 mm. The study sites were covered by *Sphagnum* spp, *Juncus* spp, and *Carex* spp interspersed with some cotton grasses such as *Eriophorum* spp and some *Calluna vulgaris* (common heather). Peat cores were collected from six sites with three replicates for each site (Figure 4.02).

Figure 4.01. Maps of the sampled peatland in North Wales (Source: Google Maps).



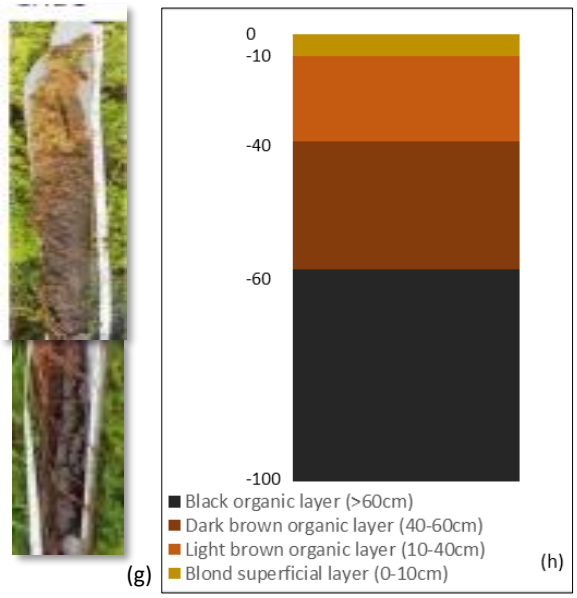


Figure 4.02. Photographs showing six peat core extraction locations (pictures a-f refer to sites 1-6, respectively). Photograph of typical peat core (g) and illustration showing discrete layers based on the colour and texture (h) (photographer J Mora-Gomez).

The average air temperature was 14.3°C and the water table level was at the soil surface at the time of sampling. Abiotic variables such as soil temperature, pH and oxygen concentration were measured directly in pore water at the time of sampling using a digital thermometer, a Mettler Toledo S20 pH meter and a dissolved oxygen probe, respectively. All the measurements were recorded in the field and are provided in Table 4.01.

Table 4.01. Peat core locations and basic environmental characteristics of pore waters of the studied sites during the time of sampling. Results are presented as the range of the 3 replicates.

Location	**Soil temp. (°C)**	**pH**	**Soil O_2 conc. (mg L^{-1})**	**Coordinates**	**Altitude**
1	12.3-12.6	2.2-3.5	5.0-5.2	52°59'45.0"N, 3°48'54.6"W	459 m a.s.l
2	14.5-15.1	2.7-3.0	3.8-4.3	52°59'42.4"N, 3°48'58.1"W	456 m a.s.l
3	12.3-12.7	3.5-3.8	4.0-4.6	52°58'51.8"N, 3°50'28.5"W	468 m a.s.l
4	12.7-13.2	3.3-3.6	3.3-3.9	52°58'18.5"N, 3°51'38.1"W	462 m a.s.l
5	13.0-13.3	3.3-4.0	4.3-5.2	52°58'24.1"N, 3°51'41.2"W	470 m a.s.l
6	13.0-13.1	3.4-3.5	2.0-3.1	52°59'38.6"N, 3°49'03.6"W	455 m a.s.l

4.3.2. Sample collection and preparation

At each site, a Russian corer (50 cm long and 10 cm in diameter) was used to extract three peat cores from a depth of 1m. After removing the vegetation layer, the cores were separated into blond superficial (0-10 cm), light brown (10-40 cm), dark brown (40-60 cm) and black organic layers (> 60 cm), based on the degree of decomposition, which was inferred from visual assessment of the colour and the texture of the layers (Bayley *et al.* 2005) (4.02g&h).

After removal of stones, roots and macroinvertebrates, all samples were placed in separate labeled plastic bags and transported to the laboratory in cooled container, where they were stored in at 4°C until further analysis.

4.3.2. Soil analyses

In the present study, the aim was to estimate the emissions of GHG and the activities of extracellular enzymes that occur under natural conditions within peatlands. Therefore, field conditions were copied as closely as possible by matching the gas production and enzyme assay temperature to that recorded in the field.

4.3.2.1. Enzyme activity analyses

Analyses were performed within two weeks of collection as previous studies have shown that soil enzymes to be stable for this period of time (Dunn *et al*. 2014). Before conducting any analyses, the samples were kept in the incubator at field temperature for around three hours.

The activity of phenol oxidase (POX) in peat soil samples was determined using a procedure adapted from Pind *et al*. (1994) and Dunn *et al*. (2014). Model compound L-DOPA (L-3,4-dihydroxy phenylalanine, Sigma Aldrich Ltd, Dorset, UK) was used as a substrate for the enzyme's degradation. The colour change is determined using a spectrophotometer measuring at 475 nm, from which the activity of phenol oxidase can be calculated. After homogenizing the bag containing the soil sample by hand, two separate 1 g soil samples were weighed and placed in two labelled stomacher bags (Seward, West Sussex, UK). After adding 9 mL of ultrapure water to each bag, these were then placed in a paddle blender (Stomacher® circulator, Seward) to mix the contents for 30 s on a normal speed setting. A 10 mL of ultrapure water was added to one bag, used as a blank. A 10 mL of the substrate (L-DOPA) was added to the other, and both bags were mixed for a further 30 s. All bags were then incubated at field temperature for 10 min before the bags were removed and mixed by hand. For each bag, three 1.5 mL microcentrifuge vials (Eppendorf, Stevenage, UK) were labelled and filled with the bag's solution. The vials were centrifuged at 10,000 rpm for 5 min. A 300 µL of supernatant from each vial was transferred to a well of a clear 96 well microplate (Sterilin, Cambridage, UK). The microplate was then placed on the SpectraMax M2e plate reader spectrophotometer and absorbance values measured at λ= 475 nm. To obtain the average absorbance value, the mean absorbance of the three blanks was subtracted from the mean absorbance of the three samples containing the substrate. The activity of phenol oxidase was then calculated by using Beer-Lambert Law and expressed as µmol of formed diqc per min per g of soil (dry weight).

Hydrolase activities were determined using the procedures of Dunn *et al*. (2014), based on the measurement of fluorescence of methylumbelliferone (MUF). In five 1L volumetric flasks, the relevant amount of MUF-labeled substrates (4-MUF β-D-glucopyranoside for β-D-glucosidase (B), 4-MUF sulfate potassium salt for arylsulfatase (S), 4-MUF β-D-xylopyranoside for β-D-xylosidase (X), 4-MUF N-acetyl-β-D-glucosaminide for N-acetyl-β-D-glucosaminidase (N) and 4-MUF phosphate for phosphatase (P)) were dissolved in 20 mL of cellosolve solvent (Sigma Aldrich Ltd, Dorset, UK), and then ultrapure water was added up to the 1L mark. Magnetic stirrers were used if necessary.

Five 1 g subsamples (one for each MUF substrate) were taken from each bag containing soil samples and placed in separate stomacher bags with 7 mL of the relevant substrate (stored at field temperature before use). These were homogenized using a Seward Stomacher 80 Laboratory Blender for 30 s. The stomacher bags were then incubated at field temperature for 45 min for the enzyme P and 60 min for the other enzymes (B, S, X, and N). The homogenates were then mixed by hand and transferred into separate 1.5 mL centrifuge vials, which were then centrifuged at 10,000 rpm for 5 min. During this time, 50 µL of ultrapure water was added to black microplate wells (Sterilin, Cambridge, UK).

At the end of centrifugation, 250 µL of the resulting supernatant was extracted from each sample and added to the relevant wells on the microplate. Fluorescence of the MUF molecule was measured using a microplate fluorometer (Molecular Devices SpectraMax M2e spectrophotometer) at 450 nm emission and 330 nm excitation. Hydrolase activities are expressed as nmol MUF g^{-1} of soil min^{-1}. To prepare the calibration curve, 7 mL of ultrapure water was added to a stomacher bag (labelled as standard) containing 1 gram of soil sample and homogenized as described above. The content from each standard bag was used to fill two 2 mL Eppendorf tubes, which were then centrifuged at 10,000 rpm for 5 mins. Different MUF-free acid (Sigma Aldrich Ltd, Dorset, UK) concentrations (8 in total) were prepared in 2 mL Eppendorf tubes using the MUF stock solution (prepared by dissolving the relevant amount of 4-methylumbelliferone free acid in 10 mL of cellosolve solvent and 500 mL of ultrapure water) and ultrapure water.

A column of the 8 standard solutions was prepared per soil sample by pipetting 50µL from each of the standard solution into a series of wells on a black microplate in addition to 250µL from the supernatant of the two Eppendorf tubes. The microplate was then analyzed as before.

4.3.2.2. Hydrochemistry analyses

For measurement of pH, conductivity, DOC, phenolic compounds, cations and anions concentrations, a soil solution was prepared by adding 5 g of soil sample and 40 mL of ultrapure water to a 50 mL centrifuge tube, and shaking on a rotary shaker for 24 h. Allowing 24h on a rotary shaker ensures that potentially leachable solutes are fully extracted into solution. There is some risk that this might allow changes in the microbial community (including consumption of solutes, reproduction, metabolism of solutes to other chemical forms), however, previous work has shown that without a 24h period it is impossible to be certain that the extractable solutes reach their maximum.

Measurement of pH and electrical conductivity was conducted on un-filtered samples using a Mettler Toledo S20 pH meter and a Primo 5 handheld conductivity meter, respectively. Samples were then centrifuged at 5000 rpm for 30 min. Finally, the supernatant was filtered through a 0.45µm membrane filter (Whatman, Kent, UK). The supernatant was collected in 20 mL plastic scintillation vials (Meridian Biotechnologies Ltd) and stored at 4°C until further analysis.

Filtered soil water samples were analyzed for their phenolics content using a version of the spectrophotometric procedures described by Box (1983). The analysis was run in triplicate, 1 mL of the sample was placed in a 1.5 mL microcentrifuge vial with 50 µL of Folin's reagent and 0.15 mL of sodium carbonate (Na_2CO_3 used to rise the pH). The same reagents were added to 1 mL of each of the chosen standards (0-30 mg L^{-1} phenol standard solutions were chosen in order to prepare a standard curve). All samples and standards were mixed a few times and allowed to develop a colour for 1 h 15 min at room temperature before 0.3 mL from both standards and samples were transferred to three wells of a clear microplate. Phenolic content was then estimated by measuring absorbance at a wavelength of 750 nm with a BMG Labtech Fluostar Galaxy microplate reader.

The concentration of DOC was measured on filtered and acidified samples using a Thermalox TOC/TN analyser (Analytical Sciences Ltd) equipped with a non-dispersive infrared CO_2 analyser. Anion (chloride, nitrate, phosphate and sulfate) and cation (sodium, ammonium, magnesium and calcium) concentrations were measured using a Metrohm 850 Professional Ion Chromatograph.

4.3.2.3. Soil water and organic matter content measurements

The water content of the soil samples was determined by oven drying a sample of known weight at 105°C for 24 h and then reweighing the samples to determine the mass loss. Organic matter content was estimated by placing soil samples in the muffle furnace at 550°C for 200 min and reweighing the samples to calculate mass loss (Frogbrook *et al.* 2009).

4.3.2.4. GHG flux measurements

For GHG flux measurements, 5 g of soil sample from each distinct layer within each core was weighed and placed in a 50 mL centrifuge tube with a lid fitted with a rubber septum (Fisher Scientific UK Ltd, Loughborough, UK). Using a 10 cm^3 gas syringe (Sigma Aldrich Ltd, Dorset, UK) with a short hypodermic needle, 10 mL of gas sample was taken from inside the tubes as a starting or background concentration (time 1). The tubes were then sealed and incubated at field temperature. Every 15 min over a 90 min period, gas samples were collected through the pierceable rubber septa. A 10 mL of gas sample was extracted from the centrifuge tubes and then injected into labelled 5.9 mL glass screw-cap vials (Labco Exetainers® Ltd, Lampeter, UK), fitted with a rubber septum in their caps.

All the exetainers were evacuated three times before use to remove all air, using a 10 mL gas syringe. Calibration curves for the three GHGs (CO_2, CH_4 and N_2O) were prepared using gas chromatograph (GC) standards of known concentrations. All gas samples and standards, in addition to three samples containing atmospheric air as blanks, were placed in the GC rack for analysis using a Varian model 450 GC. The instrument was fitted with two different detectors, an electron capture detector (ECD) to measure the concentration of N_2O and a flame ionization detector (FID) with a methaniser to convert CO_2 to CH_4, for the measurement of CO_2 and CH_4.

4.3.3. Statistical analyses

Where conditions were met by the data, analysis of variance (ANOVA) was performed to analyse potential significant differences in variables between different depths.

Tukey HSD post-hoc test was performed to find where the significant differences among groups lay at a probability level of 0.05. Calculated *F* and degrees of freedom are provided as well. Significant correlations were determined using Pearson correlation analysis. Statistical analysis was carried out using version 22 of the SPSS statistics package.

4.4. Results

As can be seen in Table 4.02, highest mean ß-glucosidase (B) enzyme activity was observed in the superficial layer (0-10 cm) (8.80 nmol MUF g^{-1} min^{-1}) and decreased with depth to a minimum of 4.55 nmol MUF g^{-1} min^{-1} in the depth of > 60 cm (Figure 4.03). B enzyme activity in the depth of > 60 cm was significantly lower than in all other layers F (3, 64) = 4.536, P = 0.006. Lowest mean arylsulfatase (S) enzyme activity was observed in the depth of > 60 cm (0.4 nmol MUF g^{-1} min^{-1}) and highest activity in depth 10-40 cm (1.37 nmol MUF g^{-1} min^{-1}) (Figure 4.03). Activity in depth of 10-40 cm was significantly higher than that in the other layers, except the first layer (0-10 cm), which had significantly higher activity than in depth of > 60 cm, F (3, 64) = 6.402, P = 0.001. Mean ß-D-xylosidase (X) enzyme activity decreased with depth from 2.96 nmol MUF g^{-1} min^{-1} in the superficial layer to 2.0 nmol MUF g^{-1} min^{-1} in > 60 cm (Figure 4.03). No statistically significant differences were found between layers F (3, 64) = 2.008, P = 0.122. Mean phosphatase (P) enzyme activity also decreased with depth, from 46.4 nmol MUF g^{-1} min^{-1} in superficial layer to 8.2 nmol MUF g^{-1} min^{-1} in > 60 cm (Figure 4.03). Mean P enzyme activity was significantly higher in the superficial layer than all the other layers. In addition, mean P enzyme activity in the depth of 10-40 cm (26.2 nmol MUF g^{-1} min^{-1}) was significantly higher than > 60 cm, F (3, 64) = 13.417, P = 0.000. In comparison, differences in chitinase (N) enzyme activity between layers were relatively small, mean N enzyme activity was highest in the depth of 10-40 cm (4.59 nmol MUF g^{-1} min^{-1}) and lowest in > 60 cm (3.0 nmol MUF g^{-1} min^{-1}) (Figure 4.03, Table 4.02). No statistically significant differences were found between soil layers in this respect, F (3, 64) = 1.956, P = 0.130.

Mean phenol oxidase (POX) activity was fairly similar across all layers, ranging from 0.19 in the > 60 cm layers to 0.29 µmol dicq g^{-1} min^{-1} in the superficial layer (Figure 4.03, Table 4.02). No significant differences between the different layers were identified, F (3, 64) = 0.260, P = 0.854.

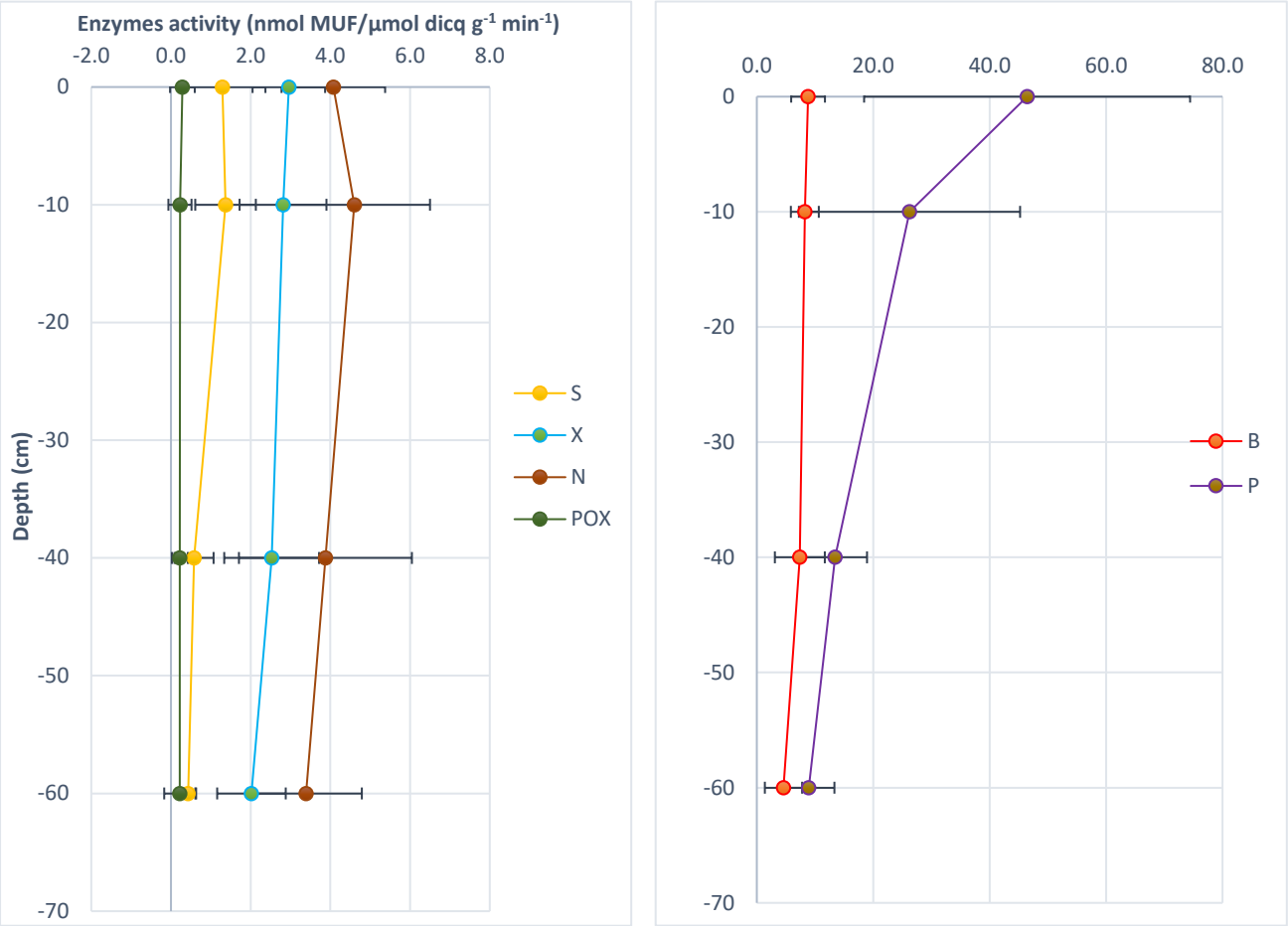


Figure 4.03. Extracellular enzymes activity at different depths in the soil profile. Each circle represents the mean of the six sampling locations. Error bars represent standard deviation. Abbreviations: ß-glucosidase (B), arylsulfatase (S), xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P), phenol oxidase (POX).

Lowest mean phenolics concentration (2.4 mg L^{-1}) was recorded in the superficial layer and the highest in 40-60 cm (3.76 mg L^{-1}). No significant differences were found between the different layers, F (3, 64) = 2.354, P = 0.080 (Figure 4.04b, Table 4.02). Highest mean dissolved organic carbon (DOC) concentration was found in > 60 cm (19.8 mg L^{-1}) and the lowest (10.07 mg L^{-1}) in the superficial layer (Figure 4.04a, Table 4.02). Mean DOC concentration in layer > 60 cm was significantly higher than in layers 0-10 cm and 10-40 cm. Mean DOC concentration in layer 40-60 cm (mean 18.4 mg L^{-1}) was significantly higher than in the superficial layer F (3, 64) = 8.370, P = 0.000.

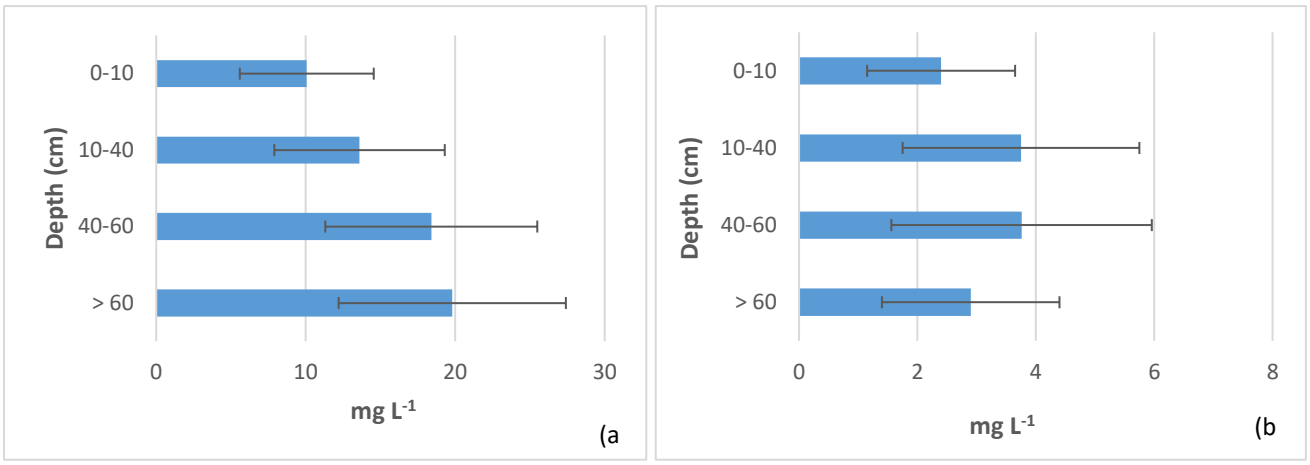


Figure 4.04. Mean DOC (a) and phenolic compound concentration (b) at different depths. Error bars represent standard deviation.

Mean pH was acidic in all layers and ranged from 4.6 in layer 0-10 cm to 4.36 in layer > 60 cm. No statistically significant differences were found between layers, F (3, 64) = 2.326, P = 0.083.

Mean conductivity measurements were similar across all layers (Table 4.02). Highest conductivity was observed in the superficial layer (20.2 µS/cm) and the lowest in layer > 60 cm (mean 17.7 µS/cm). However, no significant differences were found between the different layers, F (3, 64) = 0.408, P = 0.748.

Mean soil water content was highest in the superficial layer (92.7%) and lowest (84.4%) in the depth of > 60 cm, which had significantly lower water content than all other layers, except layer 40-60 cm, F (3, 64) = 6.793, P = 0.000 (Table 4.02).

Mean soil organic matter content (%SOM) was highest in layer 10-40 cm (77.7%) and lowest in layer > 60 cm (70.68%). Differences between the layers were not statistically significant, F (3, 64) = 0.461, P = 0.710 (Table 4.02).

Mean CO_2 flux decreased with increasing depth (Figure 4.05, Table 4.02), ranged from 9.19 in the depth > 60 cm to 17.0 µg CO_2 g^{-1} h^{-1} in the superficial layer. The only statistically significant difference was between these two depths (F (3, 64) = 3.036, P = 0.03). By contrast, mean CH_4 flux tended to increase with increasing depth, with lowest flux observed in the first two layers (-1.3 ng CH_4 g^{-1} $h^{-1}$and -1.6 ng CH_4 g^{-1} h^{-1}, respectively) and the higher CH_4 flux observed in layer > 60 cm (4.9 ng CH_4 g^{-1} h^{-1}) (Figure 4.05, Table 4.02). However,

no statistically significant differences were found between the different layers, F (3, 64) = 1.310, P = 0.279. Mean N_2O flux tended to be higher in the upper two layers (1.69 and 2.15 ng N_2O g^{-1} h^{-1} in layers 1 and 2, respectively) and decreased with increasing depth to a minimum of 0.62 ng N_2O g^{-1} h^{-1} in layer > 60 cm. Differences between layers were not statistically significant, F (3, 64) = 0.661, P = 0.579 (Figure 4.05, Table 4.02).

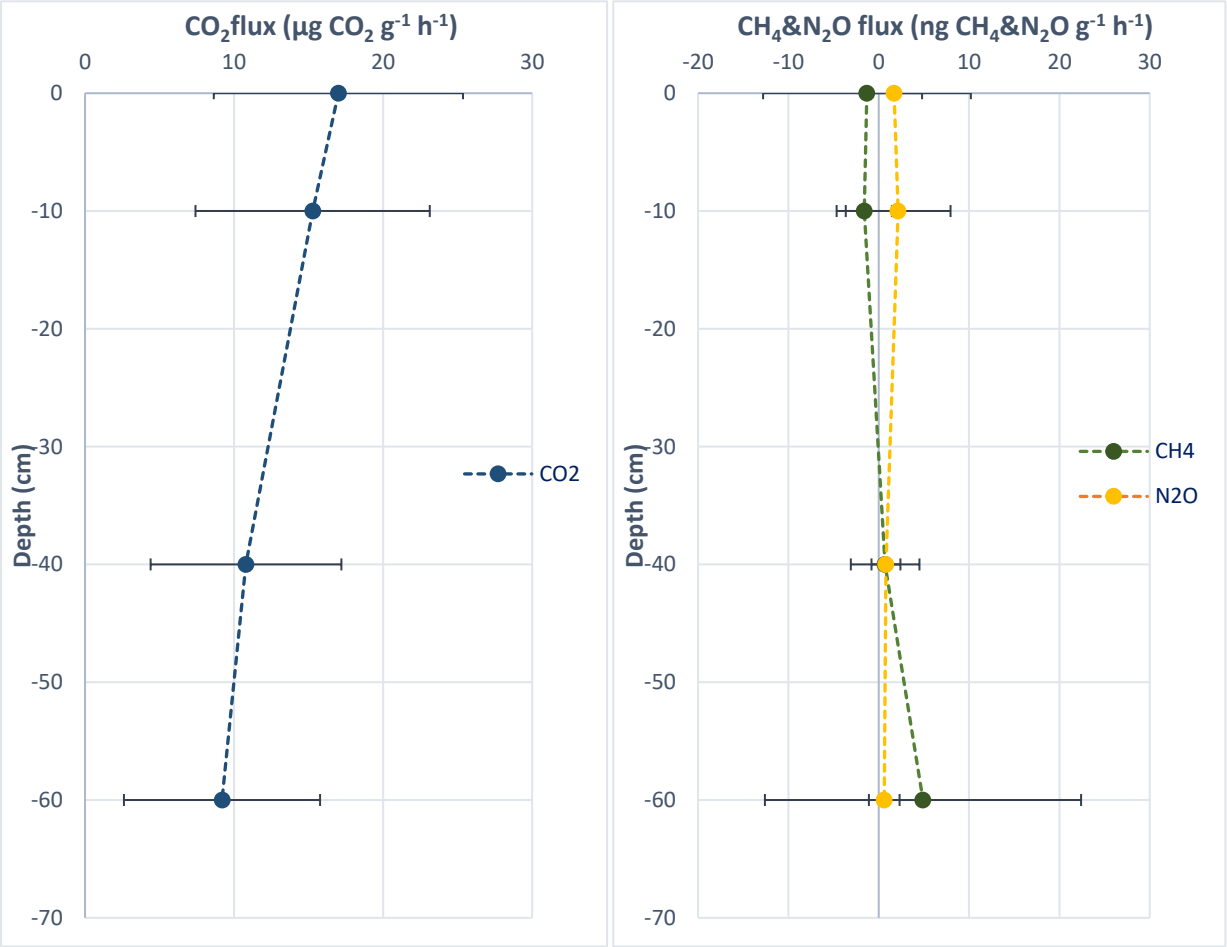


Figure 4.05. Mean CO_2, CH_4 and N_2O fluxes at different depths. Error bars represent standard deviation.

Mean chloride concentrations were similar across all layers, ranging from 1.69 mg L^{-1} in layer 10-40 cm to 1.93 mg L^{-1} in layer > 60 cm. No statistically significant differences were identified between layers F (3, 64) = 0.277, P = 0.842. Phosphate concentration also showed no statistically significant variation between layers F (3, 64) = 0.311, P = 0.818. The mean ranging from 0.17 mg L^{-1} in layers 40-60 cm and > 60 cm to 0.27 mg L^{-1} in layer 10-40 cm. For nitrate and sulfate, the lowest mean concentration was observed in superficial layer (0.24 and 1.6 mg L^{-1}, respectively). For nitrate, the highest concentration was observed in the depth

of > 60 cm (0.8 mg L^{-1}), which was significantly higher than the mean concentrations in the first two layers (F (3, 64) = 3.8, P = 0.014).

For sulfate, the highest concentration was observed in layer 40-60 cm (4.14 mg L^{-1}). The concentration in superficial layer was significantly lower than in all other layers (F (3, 64) = 12.7, P = 0.000).

For all cations except ammonium, highest mean concentration was observed in the depth of > 60 cm. Mean sodium concentration ranged from 1.12 mg L^{-1} in the depth of 10-40 cm to 1.8 mg L^{-1} in layer > 60 cm. No statistically significant differences were found between the different layers *(*F (3, 64) = 0.56, P = 0.64). Mean ammonium concentration ranged from 0.77 mg L^{-1} in layer > 60 cm to 2.4 mg L^{-1} in layer 10-40 cm. The mean concentration in layers 10-40 cm and 40-60 cm was significantly higher than in 0-10 cm and > 60 cm (F (3, 64) = 10.0, P = 0.000). Mean calcium concentration ranged from 1.2 mg L^{-1} in superficial layer to 1.6 mg L^{-1} in layer > 60 cm. No statistically significant differences were identified between the different layers (F (3, 64) = 0.114, P = 0.95). Mean magnesium concentration ranged from 0.39 mg L^{-1} in superficial layer to 0.8 mg L^{-1} in layer > 60 cm. The concentration in this layer was significantly higher than in the first two layers (F (3, 64) = 4.64, P = 0.005).

The results of the Pearson's correlation analyses are shown in Table 4.03. Several significant correlations were found between hydrolase enzyme activities and other measured variables. A significant weak positive correlation was identified between POX activity and B activity (p < 0.05). Significant positive correlations were found between pH and the activities of the five hydrolase enzymes (p < 0.01 for the pH/B, pH/X and pH/N correlations and p < 0.05 for the pH/S and pH/P correlations). These correlations were weak in the case of B, S and P activity and moderate in the case of X and N activity. Figure 4.06 shows the positive correlations between pH and hydrolase activity. Significant weak positive correlations were also found between soil water content and all hydrolase activities (p < 0.05 for all except the % water/S activity correlation, which was significant at the p < 0.01 level). A number of significant correlations were found between cation concentration and hydrolase enzyme activity: weak positive correlations between ammonium concentration and B and X activity (both p < 0.05), a moderate positive correlation between ammonium concentration and N activity (p < 0.01), and weak negative correlation between magnesium concentration and P enzyme activity (p < 0.05).

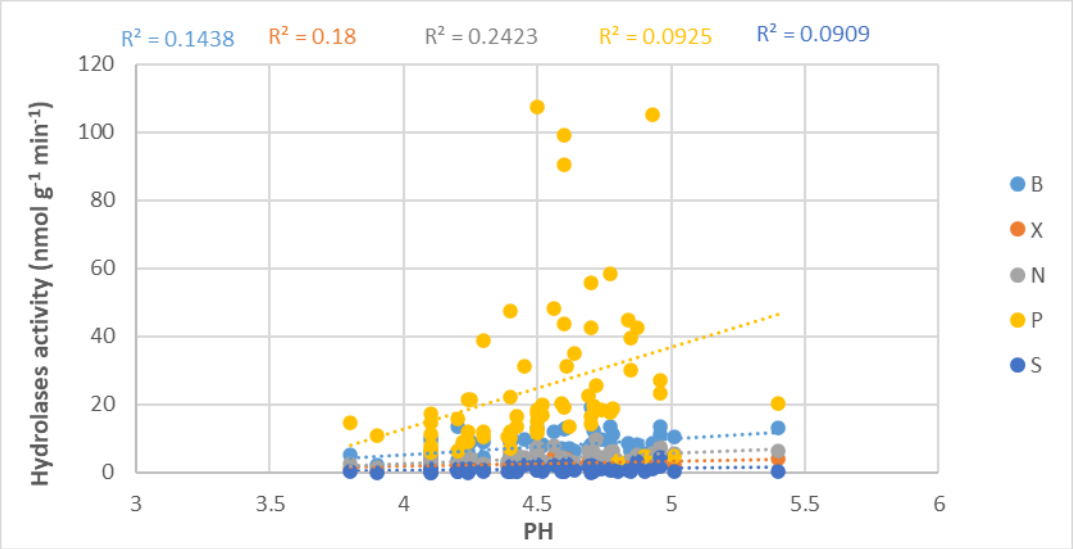


Figure 4.06. Regression analysis exhibited the positive correlation between all hydrolytic enzyme activities and pH values within Welsh peatland (n = 68).

Table 4.02. The results of environmental variables from peatlands in Wales based on the different depths. The results given as mean ± standard deviation. Letter annotations refer to significantly different means between different depths identified by Tukey HSD post- hoc test ($p < 0.05$).

Layer (cm) / Variable	Superficial (0-10 cm) (a)	Light brown organic (10-40 cm) (b)	Dark brown organic (40-60 cm) (c)	Black organic (> 60 cm) (d)
B nmol MUF g^{-1} min^{-1}	8.80±2.9 d	8.25±2.48 d	7.99±4.47 d	4.55±2.66 abc
S nmol MUF g^{-1} min^{-1}	1.29±1.08 d	1.37±0.76 cd	0.6±0.5 b	0.4±0.2 ab
X nmol MUF g^{-1} min^{-1}	2.96±0.91	2.81±1.09	2.6±1.27	2.0±0.7
P nmol MUF g^{-1} min^{-1}	46.4±28.6 bcd	26.2±19.9 ad	14.8±4.98 a	8.2±3.7 ab
N nmol MUF g^{-1} min^{-1}	4.07±1.36	4.59±1.9	4.2±2.2	3.0±1.3
POX µmol dicq g^{-1} min^{-1}	0.29±0.31	0.22±0.29	0.23±0.2	0.19±0.3
Phenolics mg L^{-1}	2.4±1.25	3.75±2.0	3.76±2.2	2.9±1.5
DOC mg L^{-1}	10.07±4.48 cd	13.6±5.7 d	18.4±7.09 a	19.8±7.6 ab
pH	4.6±0.2	4.5±0.2	4.46±0.38	4.36±0.28
Conductivity µS cm^{-1}	20.2±8.9	18.1±6.0	18.87±6.8	17.7±4.4
Water %	92.7±2.36 d	91.97±2.5 d	87.5±5.75	84.4±11.0 ab
SOM %	76.1±17.2	77.7±15.3	73.0±14.96	70.68±26.8
CO_2 µg CO_2 g^{-1} h^{-1}	17.0±8.36 d	15.27±7.86	10.8±6.4	9.19±6.58 a
CH_4 ng CH_4 g^{-1} h^{-1}	-1.3±11.5	-1.6±3.0	0.71±3.8	4.9±17.5
N_2O ng N_2O g^{-1} h^{-1}	1.69±3.1	2.15±5.8	0.79±1.6	0.62±1.7
Chloride mg L^{-1}	1.88±0.76	1.69±0.63	1.92±1.0	1.93±1.2
Nitrate mg L^{-1}	0.24±0.14 d	0.34±0.23 d	0.4±0.3	0.8±1.06 ab
Phosphate mg L^{-1}	0.26±0.49	0.27±0.29	0.17±0.36	0.17±0.39
Sulfate mg L^{-1}	1.6±0.6 bcd	3.36±1.26 a	4.14±1.4 a	3.25±1.7 a
Sodium mg L^{-1}	1.58±1.28	1.12±0.30	1.65±1.3	1.8±1.75
Ammonium mg L^{-1}	0.8±0.67 bc	2.4±1.5 ad	1.8±1.2 ad	0.77±0.7 bc
Calcium mg L^{-1}	1.2±1.7	1.5±2.25	1.5±2.0	1.6±2.0
Magnesium mg L^{-1}	0.39±0.24 d	0.44±0.35 d	0.6±0.36	0.8±0.47 ab

Table 4.03. Pearson correlation analysis between soil properties, enzymes activity and soil respiration (n = 68) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: ß-glucosidase (B), arylsulfatase (S), xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N), phosphatase (P), phenol oxidase (POX), electrical conductivity (EC), dissolved organic carbon (DOC), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and soil organic matter (SOM). Significant correlations are shown in bold.

	B	S	X	N	P	POX
S	**.422****					
X	**.648****	**.714****				
N	**.521****	**.558****	**.738****			
P	0.145	0.225	0.166	0.227		
POX	**.263***	-.027	.053	.079	-.047	
Phenolic	.144	.103	.166	**.397****	.072	-.171
DOC	-0.078	-0.190	-0.190	0.047	-.360	0.049
pH	**.379****	**.301***	**.424****	**.492****	**.304***	.155
EC	.058	-.036	-.046	-.099	-.187	.156
% water	**.278***	**.312****	**.291***	**.307***	**.307***	.037
% SOM	-.055	.116	-.030	-.019	.045	-.003
CO_2	0.140	0.140	0.168	0.084	0.231	0.083
CH_4	-0.149	-0.206	-0.236	-0.184	-.326	-0.003
N_2O	0.060	.248	0.076	-0.012	.251	-0.042
Chloride	.168	-.049	.038	.068	-.105	.068
Nitrate	-.207	-.160	-.170	-.340	-.297	-.077
Phosphate	0.127	-0.056	0.045	0.012	-0.163	-0.120
Sulfate	0.075	-0.159	0.084	0.173	**-.420****	0.030
Sodium	.282	0.037	0.207	0.039	-0.153	0.057
Ammonium	**.248***	0.211	**.307***	**.494****	-0.180	0.152
Calcium	0.032	-0.198	-0.193	-0.112	-0.070	-0.035
Magnesium	-0.075	-0.231	-0.095	-0.094	**-.253***	0.097

Continued.

	Phenolic	DOC	pH	EC	% water	% SOM	CO_2	CH_4	N_2O
DOC	**.519****								
pH	.190	-0.170							
EC	**-.406****	-0.066	**-.309***						
% water	.193	**-.279***	**.492****	-.130					
% SOM	**.280***	0.112	**.251***	-.229	**.572****				
CO_2	-0.012	-0.097	**.252***	0.054	**.297***	0.151			
CH_4	-0.134	0.138	-0.196	0.082	-0.195	-0.037	-0.172		
N_2O	0.085	-.250	-0.020	-0.167	0.090	0.036	**.278***	-0.016	
Chloride	.033	0.201	.024	**.347****	-.092	-.230	.193	-0.073	-0.217
Nitrate	**-.310***	-0.006	**-.346****	.083	**-.391****	**-.380****	-0.067	.188	-0.030
Phosphate	-0.018	0.006	0.005	0.151	0.042	-0.232	0.099	-0.059	-0.235
Sulfate	0.048	0.227	-0.141	0.200	-0.113	**-.305***	0.011	0.022	-0.182
Sodium	-0.032	0.072	-0.011	0.197	-0.032	-0.094	0.126	-.036	-0.162
Ammonium	**.447****	**.300***	0.160	-0.040	**.278***	0.221	-0.066	-0.100	-0.089
Calcium	-0.057	0.155	-0.046	**.351****	-0.225	**-.382****	0.156	0.032	-0.146
Magnesium	0.150	**.342****	-0.224	0.101	-0.123	-0.022	-0.047	0.065	-0.077

4.5. Discussion

The extracellular enzyme activities reported in the present study are within the range of enzyme activities reported previously from Welsh peatlands (Pind *et al.* 1994, Freeman *et al.* 1995, Kang *et al.* 1998, Kang and Freeman 1999) using a MUF-substrate-based method and L-DOPA-substrate-based method for measurement of soil hydrolase and phenol oxidase activities, respectively.

The activities of ß-glucosidase (B), arylsulfatase (S) and phosphatase (P) decreased at depths of greater than 40 cm, while for xylosidase (X), N-acetyl-ß-D-glucosaminidase (chitinase) (N) and phenol oxidase (POX) activities, data analysis demonstrated that there were no significant differences between the different depths. Generally, the highest mean enzymes activity tended to occur in the superficial layer and the lowest was consistently found in the depth of > 60 cm. These results are consistent with previous studies showing a reduction in enzyme activities with depth. Specifically, in Welsh peatland soils, Freeman *et al*. (1995) followed the depth-dependent variations in the activities of *ß*-glucosidase, arylsulfatase and phosphatase. They observed that the activities of the three enzymes declined with increasing depth. The *ß*-glucosidase, arylsulfatase and phosphatase data reinforce their finding of a depth-dependent decrease in enzymes activity.

A previous study on Welsh peat cores also showed a gradual reduction in POX activity with depth, resulting in higher phenolics concentrations at greater depths, thereby further inhibiting hydrolytic enzymes activity (Pind *et al.* 1994). Although oxygen concentration did not measure at each depth, this is thought to relate to a decrease in oxygen concentration with depth, which inhibits enzyme activity (Pind *et al*. 1994, Freeman *et al.* 2004, Fenner and Freeman 2011, Bonnett *et al.* 2017, Potter *et al.* 2017). The inhibitory effects of phenolic compounds on hydrolytic enzymes activity and microbial growth are widely known (Fung *et al.* 1985, Freeman *et al.* 2004, Opelt *et al*. 2007, Mellegård *et al.* 2009, Fenner and Freeman 2011, Pizzolitto *et al.* 2015). In the present study however no statistically significant difference in POX activity were identified between depths. Nor were there any negative correlations between hydrolytic enzymes activity and phenolics concentration as would be expected according to the enzyme latch theory (Freeman *et al.* 2001). In fact, the only significant correlation between hydrolase enzyme activity and phenolics concentration was a positive correlation involving N enzyme activity, which could suggest a high demand for N element involved in phenolics chemical structure (e.g., lignin) (Li *et al.* 2018). This

observation is in line with other studies that have identified a significant stimulation in hydrolase activities after low molecular weight phenolics were added (e.g., Dunn 2013). This observation suggests that at the time of this study, the phenolics measured in Wales may have lower molecular weight, and therefore they served as an enzyme substrate. In agreement with this suggestion, Fierer *et al.* (2001) observed that low molecular weight phenolics represented a labile substrate, inducing organic matter mineralization.

In addition, the fluxes of CO_2, CH_4 and N_2O were unaffected by phenolic compounds, suggesting variations in the form, composition and the source of phenolic compounds, may be driving variations in enzyme activities and the resultant metabolic end products (Hoostal and Bouzat 2008, Min *et al.* 2015).

A study of the effect of POX additions on hydrolase activities has shown that the addition of POX to Welsh peat slurries appeared to increase the activity of the enzymes B, S and P concurrent with a marked decline in inhibitory phenolics (Freeman *et al.* 2004). Similarly, Jones (2006) found strong relationships between the activities of B, S and P and the activity of POX at Welsh bog soils. The data regarding the positive relationship between POX and B activity supported their finding. However, no inverse correlation with phenolic concentrations was recorded here to confirm this (Bending and Read 1997, Tian and Shi 2010).

In the present study, the concentrations of phenolic compounds were on average higher in the deeper depths than the upper layer. This observation could be due to POX activity, which is responsible for the transformation of phenolics (Fenner and Freeman 2011), as this enzyme did not differ significantly between the different layers. The lack of variation in POX activity between depths despite presumed differences in oxygen concentration with depth, could be due to the pH, which more strongly influences POX activity (Tahvanainen and Haraguchi 2013, Kang *et al.* 2018), did not differ significantly between the different layers.

Although some statistically significant differences in mean nutrient concentrations were found between different depths, no consistent pattern was identified. A number of statistically significant correlations were found between anion and cation concentrations and enzyme activities, the relative rise in S enzyme activity at the depths of 0-10 cm was concurrent with the relatively low sulfate concentrations at the same depth. Nevertheless, no inverse correlation was found between S enzyme activity and sulfate concentration. Also, no inverse correlation was found between P enzyme activity and phosphate concentration to confirm the economic model of enzymes production, which predicts that the lesser the nutrient

availability, the more soil microbial enzyme activity and vice versa (Chròst 1991, Li *et al.* 2019). This result is in line with the findings of Kang and Freeman (1999) who did not find an inverse correlation between S activity/sulfate concentration and between P activity/phosphate concentration in each studied sites of Welsh peatland. The lack of these relationships was attributed to the legacy enzymes which are defined as immobilised enzymes usually presented in large amounts in the soil and are independent of microbial regulation and thus do not respond to nutrient availability. These enzymes are very stable and resistant to biological, physical and chemical degradation. They may persist independently in soils for far longer than the soil microbes from which they were synthesised (Burns 1982, Kang and Freeman 1999).

A higher N enzyme activity was observed consistent with an increase in the concentration of ammonium ions (Bayley *et al.* 2005, Jones 2006). Indeed, a positive relationship between N activity/ ammonium concentration was identified in this study ($r = 0.494$, $p < 0.01$).

According to Bragazza *et al.* (2006), when more nitrogen is available, living *Sphagnum* moss will synthesize proteins over polyphenols and thus eventually lowering phenolics level in the litter, which in turn limit enzyme inhibition and promote soil metabolism. Similarly, Lu *et al*. (2011) mentioned that N element availability stimulated the activities of hydrolytic C-acquiring enzymes, leading to elevated soil organic matter mineralisation and the release of C in gaseous and aqueous forms. Indeed, a positive correlation was identified here between ammonium/B activity, ammonium/X activity and ammonium/phenolics and DOC concentrations, suggesting that (although N cycling processes could change during the duration of soil storage; DeForest 2009) N enrichment may have had a stimulatory effect on the activities of hydrolytic enzymes, potentially leading to greater decomposition of labile compounds and weakening the C sequestration capacity of peatlands (Jian *et al.* 2016, Chen *et al.* 2017, Li *et al*. 2018).

The pH did not vary significantly with depth, but significant positive correlations were identified between pH and each of the hydrolytic enzymes measured, suggesting that pH values, ranged from 3.8 to 5.4, were somewhat optimal for hydrolase activities in Wales. In addition, a positive correlation was found between pH and CO_2 release ($r = 0.252$, $p < 0.05$). Overall, the positive correlations between hydrolyses/%water, hydrolyses/pH, CO_2/pH and CO_2/%water suggest that despite waterlogged conditions, labile carbon was mineralised hydrolytically under the favourable pH, resulting in high fluxes of CO_2 (Guggenberger *et al.*

1994). However, no positive correlation was found between hydrolase enzyme activities and CO_2 fluxes to confirm this.

Various studies have reported enhanced phenol oxidase activity under high pH conditions (Pind *et al.* 1994, Williams *et al.* 2000), which in turn leading to increased DOC production (Fenner and Freeman 2011, Kang *et al.* 2018). In the present study, neither phenol oxidase activity nor DOC concentrations showed significant correlations with pH. The most likely explanation for the lack of responses of POX activity to the pH is that all the samples of the present study were acidic, creating suboptimal condition for the action of oxidative enzymes, which require a pH level between around neutral to alkaline (Pind *et al*. 1994, Kang *et al.* 2018). Also, the range of pH values in the samples was relatively narrow, which may give another explanation for the lack of a significant correlation between the two variables.

The statistically significant positive correlations between pH and each of the hydrolytic enzymes and the absence of a correlation between pH and POX activity are agreed with a previous study on acidic soils by Xue *et al*. (2016).

Mean DOC concentration increased with depth in the present study. This result was unexpected given that some previous studies report a decrease in pore water DOC concentration with depth (e.g., Freeman *et al*. 1993). Decreased DOC concentration in the superficial layer compared to the deeper layers observed here is likely attributed to microbial processes under more aerobic conditions (Freeman *et al.* 1993). Additionally, in the more aerobic conditions of the uppermost layers, the more efficient C metabolism tends to produce CO_2 rather than DOC (Freeman *et al.* 1993). The range of DOC concentration (10.07-19.8 mg L^{-1}) in this study is higher than what has been previously reported from Welsh peatland (Freeman *et al.* 1993).

The positive correlation observed here between DOC concentration and phenolic concentrations is expected since phenolic compounds are a constituent of DOC (Wetzel 1992, Freeman *et al.* 1996).

The emissions of CO_2, CH_4 and N_2O were not significantly affected by the activities of measured enzymes. A possible explanation for the lack of any positive correlations between enzyme activity and microbial respiration may be that the measured activities were for the legacy enzymes that were already present in the soil rather than the generalised measurement of microbial metabolic process that includes de-novo enzyme synthesis, which is usually

associated with microbial respiratory activity. This finding is supported up by a previous study of a Welsh peat soils (Freeman *et al.* 1996).

An expected positive correlation was found between conductivity and chloride ($r = 0.347$, $p < 0.01$), and conductivity/ calcium ($r = 0.351$, $p < 0.01$) since both are a measure of ionic strength.

A reduction in % water content with depth may reflect decreasing % SOM with depth. In the present study, a significant strong positive correlation was found between % water and % SOM contents ($r = 0.572$, $p < 0.01$), because, and unlike mineral content, organic matter can hold substantial amounts of water (Dunn 2013). A significant positive correlation was found here between the percentage of water content and all the activities of hydrolytic enzymes, indicating that water unavailability may constrain the activities of hydrolase and the drought conditions could reduce the production of hydrolytic enzymes, as microbial biomass declines (Allison and Vitousek 2005, Sardans and Penuelas 2005, Sowerby *et al*. 2005, Steinweg *et al.* 2013). In agreement with the findings of this study, a very recent study in temperate Chinese peatlands has described a strong dependence of soil enzymes activity on soil moisture (Song *et al.* 2019).

Jobbágy and Jackson (2000) observed that the content of SOM varied vertically and horizontally in soil. However, no significant differences were recorded here between the different depths in this respect, probably due to the lack of variation in most of the enzyme activities along the Welsh soil profile in addition to there was little influence from mineral material (Schnecker *et al.* 2015).

A general decrease in CO_2 flux was observed, and an increase in CH_4 flux with increasing depth, consistent with a shift towards methanogenic *Archaea* as a result of increasingly anoxic conditions (Kotsyurbenko *et al.* 2004, Dedysh *et al.* 2006). Similarly, DOC concentration was significantly higher in the deeper layer, all of which suggest that under anaerobic conditions of deep layers of peat, fermentative metabolisms are prevalent, inducing the production of CH_4 and DOC (Bonnett *et al.* 2017). Indeed, Fenner and Freeman (2011) and Gough *et al.* (2016) reported that under anoxic conditions, anaerobic metabolism becomes dominant, switching metabolic end products towards CH_4, DOC and CO_2 rather than principally CO_2.

4.6. Conclusions

It is important to understand the conditions that influence the activities of soil enzymes that mediate key processes as a prerequisite for predicting ecosystem responses to climate changes, human utilization and management strategies.

In the present study, extracellular enzymes activity (hydrolases and phenol oxidase) and the production of greenhouse gas (CO_2, CH_4 and N_2O) were investigated at different depths in a temperate peatland of the Snowdonia National Park, Wales. In addition, the associations between these variables and pH, conductivity, nutrient concentration (cations and anions), phenolic compound concentration, dissolved organic carbon (DOC) concentration, and soil water and soil organic matter (SOM) content were assessed. There were statistically significant differences between depths in terms of most hydrolase activities and several abiotic variables, in addition to a number of significant correlations between measured parameters.

Since the quantity and the quality of soil organic matter decrease with depth, soil enzymes activity is expected to decrease with depth. Indeed, the average activity of extracellular enzymes decreased with increasing soil depth, in agreement with previous studies that followed the depth-dependent variations in enzymes activities of peatlands.

Also of interest, pH values, which ranged from 3.8 to 5.4, showed a significant positive correlation with each of the hydrolytic enzymes measured, meaning that as pH increases, hydrolase activities increase.

The variation of phenol oxidase (POX) activity was not significant between depths despite presumed differences in oxygen concentration with depth. This observation could be due to the pH, which more strongly influences POX activity, not differing significantly between the different layers. However no statistically significant correlation was found between POX activity and pH, possibly due to the acidic and narrow range of soil pH.

Additionally, the variation of phenolics concentration did not vary significantly between depths perhaps because POX activity, which is responsible for the transformation of phenolics, did not differ significantly between the different layers. However no statistically significant correlation was found between POX activity and phenolics concentration.

The activities of hydrolytic enzymes, with the exception of chitinase, and phenol oxidase, in addition to the atmospheric end products appeared to be unresponsive to the concentrations of phenolic compounds. These results suggest that phenolics cannot all be considered as efficient inhibitors of microbial metabolism and that the forms, source and the chemical composition of phenolic compounds could play an important role in affecting the activities of soil extracellular enzymes.

The absence of a positive association between enzyme activities and the metabolic end products suggests that the measured activities were not due to de-novo enzyme synthesis but rather due to legacy enzymes. The lack of statistically negative relationships between S activity/sulfate and between P activity/phosphate here can give further confirmation of measurement of legacy enzymes which are present in large amounts in the soil and are independent of microbial regulation and thus do not respond to nutrient availability.

The mean flux of CH_4 was highest in the deeper soil layers, suggesting that methanogens are better adapted to the anaerobic conditions of the deeper peat layers.

Although some statistically significant differences in mean nutrient concentrations were found between different depths, no consistent pattern was identified. A positive correlation was identified here between ammonium and the activities of hydrolytic C-acquiring enzymes, alongside with N-acquiring enzyme and DOC concentration, indicating that increased N deposition poses a significant risk to the function of peatlands.

Assessing microbial communities' composition is recommended, since microbial communities are central to the mineralization of SOM and are responsible for a large proportion of soil GHGs emissions.

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Chapter 5

A meta-analysis of biogeochemical data collected in Svalbard, Colombia and Wales.

5.1. Abstract

The comparison of soil extracellular enzyme activities on a global scale provides an opportunity to identify the global patterns of biogeochemical processes and the dominant controlling factors. Studying biogeochemical processes and the abiotic variables controlling them in peatlands has important implications for predicting the potential impacts of climate change, human intervention and management strategies on peatlands stability. In this study, the significant differences in measured parameters between different regions (Arctic (Svalbard, Norway), tropical (Sumapaz National Park in Andes mountains, Colombia) and temperate (Snowdonia National Park in Wales, United Kingdom)) were identified.

Soil enzyme activities, with the exception of ß-glucosidase activity, and the fluxes of greenhouse gas (GHG), with the exception of methane (CH_4), varied across the three climatic peatlands in relation to measured physicochemical variables. Relatively high hydrolase activities were observed in Colombia, possibly due to the higher demand for nutrients and/or higher energy supply. The activity of phenol oxidase was relatively high in Svalbard, where significantly higher pH and concentrations of most inorganic nutrients, and lower concentration of phenolic compounds was also observed. Conversely, the Welsh peatland exhibited the lowest phenol oxidase activity, consistent with lowest pH and consequently highest phenolic concentrations. The pH values were significantly different for each of the three regions, Svalbard (mean 7.1) > Colombia (mean 5.2) > Wales (mean 4.5). Guano is known to have an alkaline pH, and this may have contributed to the unexpectedly high pH observed in Svalbard. Phenolic concentration was highest in the Welsh and Colombian peatlands possibly due to the dominant vegetation being *Sphagnum* moss.

The greater dominance of *Sphagnum* moss in Colombia and Wales may also provide a reason for the lower pH value in these regions compared to Svalbard. The higher mean dissolved organic carbon (DOC) concentration in Colombia and Wales compared with Svalbard peatland could be related to the higher primary productivity towards the equator where temperatures are warmer and the growing season longer. In addition, decreased DOC concentration has been linked to increased sea salt, all of which were high in Svalbard possibly due to the proximity of the sampling sites to the sea. The fluxes of carbon dioxide (CO_2) and nitrous oxide (N_2O) were all highest in the Svalbard samples. Additionally, Svalbard peatland had the highest nutrient concentrations possibly due to its close proximity to the sea, the influence of the underlying mineral layer and nutrient supply from bird guano.

5.2. Introduction

Peatlands represent a significant terrestrial carbon pool, holding at least 550 Gt of atmospheric carbon as a result of the imbalance between organic matter production rates and decomposition rates in these persistently water-saturated environments (Mitsch and Gosselink 2000, Moore 2002, Holden 2005), which raising concerns over their potential to become a carbon source and contribute to climate change.

Due to the importance of soil enzymes in the processing of organic matter in wetland ecosystems, many researchers have investigated the factors influencing enzyme activities and biogenic greenhouse gas (GHG) emissions (Dunn *et al.* 2014). Studying biogeochemical processes and the abiotic variables controlling them in peatlands can be of value for predicting the potential impacts of climate change, human intervention and management strategies on peatlands function (Dunn *et al.* 2014). However, most studies in this field have been conducted in a single region with a limited number of studied variables.

In the present study, the most widely measured soil enzyme activities, metabolic end products and various physicochemical variables of soil were compared across three climatic regions, Arctic, temperate and tropical. The description of soil extracellular enzyme activity on a global basis reveals the different ranges of variation and different distributions of enzymes activities in relation to ecosystem variables, which provides an opportunity to compare contrasting ecosystems and to relate soil microbial function to global patterns of microbial community composition, nutrient ratios and soil organic matter storage (Sinsabaugh *et al.* 2008). Differences in microbial function between peatlands in different climatic regions are expected since vegetation composition, peat chemical composition and hence decomposition rates, which depend on microbial community composition, will inevitably vary between climates (Preston *et al.* 2012). Indeed, Hodgkins *et al.* (2018) examined plant and peat chemistry across a latitudinal transect from the Arctic to the tropics and found that peaty soils in temperate to tropical peatlands are more chemically recalcitrant, having higher aromatic content and lower carbohydrate content than those from Arctic and boreal peatlands. This recalcitrance towards the tropics may allow peats to persist despite warmer temperatures. Understanding the relationship between enzyme activities and physicochemical variables in peatlands, which are extensive sinks of carbon and nitrogen, is especially important (Moore 2002, Voigt *et al.* 2017, Leifeld and Menichetti 2018) and necessary for anticipating the ecosystem response to climate change, land use change and restoration attempts (Moore

2002). Peatlands along the transect showed large differences in climate and vegetation cover and presumably in the chemical composition of litter entering the soil and in the physicochemical variables of soil. The hypothesis of the present study proposed that biogeochemical processes show greater differences between peatlands of the three contrasting regions in relation to soil variables. This chapter aimed to compare enzyme activities (hydrolases and phenol oxidase) and production of GHG (CO_2, CH_4 and N_2O) in relation to the potential controlling factors (pH, conductivity, water and soil organic matter contents, the concentrations of phenolic compounds, dissolved organic carbon, anions and cations) at regional and global scale.

5.3. Materials and methods

5.3.1. Study sites and soil sampling

In this study, moss-dominated peatlands from three climatic regions were chosen. They were located in the Arctic region (Svalbard, Norway), a temperate region (Snowdonia National Park in North Wales, UK), and a tropical region (Sumapaz National Park in Andean mountains, Colombia). In each peatland, soil cores were collected from six different sites, and three replicates of soil cores were extracted from each site. Samples were collected from Svalbard, Norway in late July 2016. The average air temperature was 5°C, the water table was at the soil surface during sampling and the moss tundra of the study sites was dominated by the species *Tomenthypnum nitens* and *Calliergon richardsonii*, without vascular plants.

Six *Sphagnum*-dominated peatland sites located in the Snowdonia National Park, North Wales, UK were sampled at the beginning of September 2016. The study sites were covered by *Sphagnum* spp, *Juncus* spp, and *Carex* spp interspersed with some cotton grasses and some *Calluna vulgaris* (common heather). The average air temperature was 14.3°C and the water table level was at the soil surface at the time of sampling.

The tropical peatland sites were located at high altitude within the Sumapaz National Park in the Andean mountain range, Colombia. Sampling was performed at the end of July 2017. The dominant vegetation was *Sphagnum* spp, *Carex* spp, *Juncus* spp, *Espeletia* spp and many other shrubs were also more common. The average air temperature was 13.4°C and the water table was at the soil surface during the time of sampling.

Each soil core (100 cm depth) was examined and boundaries between distinct layers identified based on the colour and the texture. These individual layers were then placed in individual plastic bags and transported in ice to the laboratory in Wales. Distinct layers in the soil cores were handled and analysed separately. Subsamples of soil for enzymes activity measurement and chemical analysis were stored at 4°C. Because the time between soil collection and enzyme assay can vary greatly between studies, it is possible that data variability in these studies can be explained, in part, by this variation (DeForest 2009). The soil temperature in the Arctic was 7.1°C while that in Colombia was 9.8 °C. The storage temperature was 4°C, which means that the storage temperature was slightly below those of the field conditions, which would minimise the risk that storage could detrimentally affect microbial activities. The largest difference between storage temperature and field temperature was in Wales where the soil temperature was 13.2 °C. However, these samples were collected locally, and so it was more feasible to minimise the delay between sample collection and storage. Work by Fenner *et al.* (2005) suggests that short term storage of samples at higher or lower than field conditions can potentially result in declines in activity over a two-week period. The work of Fenner *et al.* (2005) suggests that there is minimal risk with small temperature differences however.

Soil temperature and pore water pH and dissolved oxygen concentration were measured in the field using portable instruments. Measurements of soil enzyme activity, greenhouse gas fluxes, organic matter content and water content, pH value, electrical conductivity, and soil water extraction in order to measure phenolic compound, dissolved organic carbon and ions concentrations were performed in the laboratory following standard protocols.

5.3.2. Soil analyses

The activities of extracellular enzymes were determined following a protocol described by Dunn *et al*. (2014). Briefly, the phenolic amino acid L-3,4- dihydroxy phenylalanine (L-DOPA, Sigma Aldrich Ltd, Dorset, UK) was used as a target substrate for assay phenol oxidase activity. The colour change is determined using a spectrophotometer at 475 nm and the activity of phenol oxidase was then calculated by using Beer-Lambert Law and expressed as µmol of formed (diqc) per minute per gram of soil (dry weight). Hydrolase enzyme activities were determined using the procedures of Freeman *et al.* (1995) and Dunn *et al*. (2014), based on the measurement of fluorescence of methylumbelliferone (MUF). Fluorescence of the MUF molecule was measured using a microplate fluorometer (Molecular

Devices SpectraMax M2e spectrophotometer) at 450 nm emission and 330 nm excitation. Hydrolase activities are expressed as nmol MUF g^{-1} of soil min^{-1}.

Soil subsamples were oven-dried for 24 h at 105° C in order to determine soil water content. Soil organic matter was determined by muffle-burning dried subsamples for 4 h at 550° C.

To determine the amount of water-extractable carbon in the peat samples, 5 g subsample of peat was taken and homogenising with 40 ml of water on a rotary shaker for 24 h. The resulting slurry was centrifuging at 5000 rpm for 30 minutes. The supernatant was filtered through a 0.45µm membrane filter (Whatman, Kent, UK) to separate particulate organic carbon (POC) from dissolved organic carbon (DOC). The filtered samples were then collected in 20 mL plastic scintillation vials (Meridian Biotechnologies Ltd) and stored at 4°C until measurement. A pH meter and a Primo 5 handheld conductivity meter were inserted into peat slurry before filtration in order to measure the pH and the conductivity of the peat, respectively.

The concentration of phenolics was assayed using Folin-Ciocalteu Reagent, as described in detail by Box (1983).

Cation and anion concentrations were analyzed on extracted, filtered water by using a Metrohm 850 Professional Ion Chromatograph.

The concentration of DOC was measured on filtered and acidified samples using a Thermalox TOC/TN analyser (Analytical Sciences Ltd) equipped with a non- dispersive infrared CO_2 analyser. Microbial respiration (CO_2, CH_4 and N_2O) was also measured in peat samples using Gas Chromatography equipped with flame ionization detector (FID) for measuring the concentration of CO_2 and CH_4 and electron capture detector (ECD) for measuring the concentration of N_2O.

5.3.3. Statistical analyses

Where conditions were met by the data, analysis of variance (ANOVA) was performed to analyse potential significant differences in variables between different regions. Tukey HSD post-hoc test was performed to find where the significant differences among groups lay at a probability level of 0.05. Calculated *F* and degrees of freedom are provided as well. Significant correlations were determined using Pearson correlation (r) analysis. Linear

regression analysis (r^2) was also used. Statistical analysis was carried out using version 22 of the SPSS statistics package.

5.4. Comparing results at global scale:

As can be seen in Figure 5.01 and Table 5.01, mean ß-glucosidase (B) activity was lowest in the Welsh samples (7.67 nmol MUF g^{-1} min^{-1}) and highest in Colombia (10.26 nmol MUF g^{-1} min^{-1}). However, one-way ANOVA analysis indicated no statistically significant differences between the three regions, F (2,183) = 1.7, p = 0.17. ANOVAs were carried out using each of the individual replicate measurements rather than simply the mean values, and so reflected fully the variability in the samples.

Mean arylsulfatase (S) activity was lowest in Wales (0.97 nmol MUF g^{-1} min^{-1}) and highest in Colombia (8.6 nmol MUF g^{-1} min^{-1}). One-way ANOVA indicated that S differed significantly between the three contrasting regions, F (2,183) = 41.9, p = 0.000. Tukey HSD post-hoc test revealed that Colombian samples had significantly higher S activity than both Wales and Svalbard soil samples ($p < 0.05$), but that the Wales/ Svalbard comparison was not significant.

Mean xylosidase (X) activity ranged from 2.1 nmol MUF g^{-1} min^{-1} in the Svalbard samples to 3.0 nmol MUF g^{-1} min^{-1} in the Colombian samples. One-way ANOVA showed that X activity differed significantly between the three locations, F (2,183) = 4.9, p = 0.008. Post-hoc comparisons using the Tukey HSD test showed that Colombian peat samples had significantly higher X activity than in the Svalbard ($p < 0.05$). The Welsh samples, which had an intermediate mean X activity, did not vary significantly from the other two regions.

Mean phosphatase (P) activity ranged from 18.86 nmol MUF g^{-1} min^{-1} in the Svalbard samples to 35.0 nmol MUF g^{-1} min^{-1} in Colombia. One-way ANOVA showed that P activity varied significantly between studied regions, F (2,183) = 5.79, p = 0.004. Tukey post-hoc comparisons test revealed that Colombian peat samples had significantly higher P activity than in the Svalbard ($p < 0.05$). Wales's peat samples, which had an intermediate mean P activity, did not vary significantly from the other two regions.

Mean N-acetyl-ß-D-glucosaminidase (N) activity was lowest in Svalbard (2.98 nmol MUF g^{-1} min^{-1}) and highest in the Colombian samples (6.2 nmol MUF g^{-1} min^{-1}). One-way ANOVA indicated that N activity differed significantly between the three regions, F (2,183) = 8.5, p =

0.000. Tukey HSD post-hoc test revealed that Colombian soil samples had significantly higher N activity than both Wales and Svalbard soil samples ($p < 0.05$), but that the Wales/ Svalbard comparison was not significant.

Mean phenol oxidase (POX) activity was highest in the Svalbard (1.83 µmol dicq g^{-1} min^{-1}) and lowest in Wales (0.24 µmol dicq g^{-1} min^{-1}). One-way ANOVA showed that POX differed significantly between regions, F (2,183) = 15.4, p = 0.000. Tukey HSD post-hoc test revealed that Wales peat samples had significantly lower POX activity than both Colombia and Svalbard ($p < 0.05$), but that the Colombia/ Svalbard comparison was not significant (Figure 5.01 and Table 5.01).

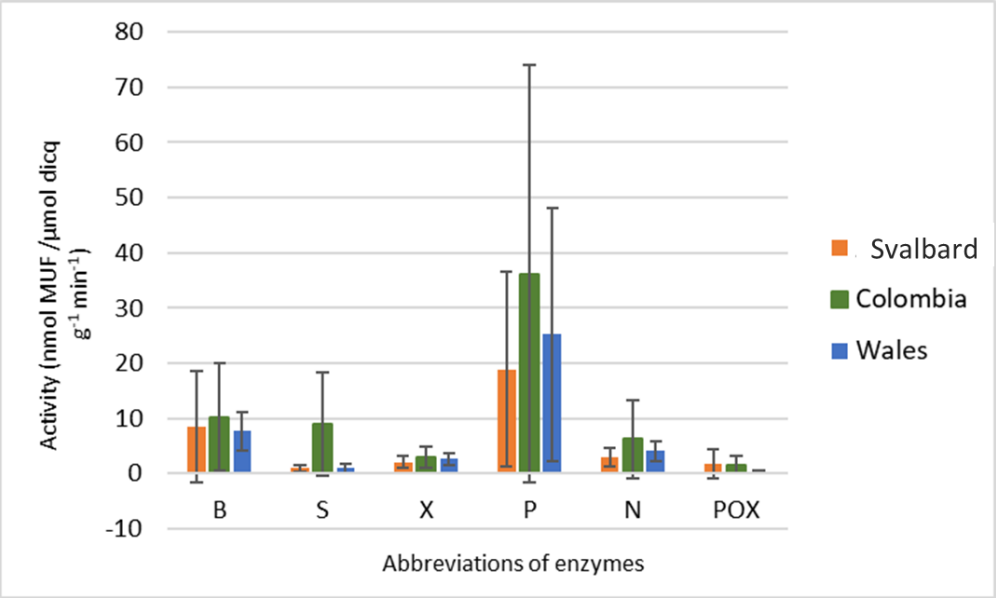


Figure 5.01. Extracellular enzymes activity in Svalbard, Colombia and Wales. Abbreviations, B = ß-glucosidase, S = arylsulfatase, X = xylosidase, N = N-acetyl-ß-D-glucosaminidase (chitinase), P = phosphatase and POX = phenol oxidase. Enzyme activities are reported as mean along with ± standard deviation.

Table 5.01 Mean values for measured variables in Svalbard (S), Colombia (C) and Wales (W). The results represent the mean of all replicates, across all depths ± standard deviation. Letter annotations refer to significantly different means ($p < 0.05$) identified by Tukey HSD post-hoc test.

Regions / variables	Svalbard (S)	Colombia (C)	Wales (W)
B nmol MUF g^{-1} min^{-1}	8.39±10.2	10.26±9.7	7.67±3.5
S nmol MUF g^{-1} min^{-1}	1.0±0.58 C	8.6±9.4 WS	0.97±0.8 C
X nmol MUF g^{-1} min^{-1}	2.1±1.07 C	3.0±2.0 S	2.66±1.07
P nmol MUF g^{-1} min^{-1}	18.86±17.7 C	35.0±37.8 S	25.2±22.9
N nmol MUF g^{-1} min^{-1}	2.98±1.7 C	6.2±7.1 WS	4.07±1.8 C
POX µmol dicq g^{-1} min^{-1}	1.83±2.7 W	1.58±1.6 W	0.24±0.28 SC
Phenolics mg L^{-1}	1.86±0.9 WC	2.7±1.4 S	3.33±1.8 S
DOC mg L^{-1}	12.3±6.1 C	16.38±10.5 S	15.12±7.2
pH	7.1±0.6 WC	5.2±0.46 WS	4.5±0.29 SC
Conductivity µS cm^{-1}	216.0±228.0 WC	18.8±13.0 S	18.8±6.8 S
Water %	69.8±26.0 WC	83.9±10.2 S	89.6±6.5 S
SOM %	50.2±31.5 W	43.8±18.9 W	74.75±17.9 SC
CO_2 µg CO_2 g^{-1} h^{-1}	29.7±31.9 WC	12.7±11.8 S	13.4±7.9 S
CH_4 ng CH_4 g^{-1} h^{-1}	1.9±10.3	5.5±21.2	0.26±9.8
N_2O ng N_2O g^{-1} h^{-1}	5.7±11.3 CW	1.9±1.9 S	1.4±3.6 S
Chloride mg L^{-1}	3.0±2.4 WC	1.8±1.6 S	1.85±0.89 S
Nitrate mg L^{-1}	10.2±12.4 WC	0.6±1.3 S	0.4±0.5 S
Phosphate mg L^{-1}	0.33±2.1	0.2±0.4	0.2±0.38
Sulfate mg L^{-1}	52.5±85.4 WC	3.6±1.66 S	3.1±1.56 S
Sodium mg L^{-1}	4.8±3.8 W	5.2±1.2 W	1.55±1.2 SC
Ammonium mg L^{-1}	0.15±0.4 W	0.28±0.9 W	1.5±1.2 SC
Calcium mg L^{-1}	43.6±21.8 WC	2.7±2.7 S	1.47±1.97 S
Magnesium mg L^{-1}	9.7±8.5 WC	0.4±0.66 S	0.5±0.38 S

Mean phenolic compound concentration ranged from 1.86 mg L^{-1} in Svalbard samples to 3.33 mg L^{-1} in Wales (Figure 5.02). One-way ANOVA showed that phenolic concentration varied significantly between regions, F (2,183) = 12.5, p = 0.000. Tukey post-hoc comparison test indicated that phenolics concentration was significantly lower in the Svalbard soil samples than in Wales and Colombia ($p < 0.05$). No significant difference was found between the Welsh and Colombian peat samples (Table 5.01).

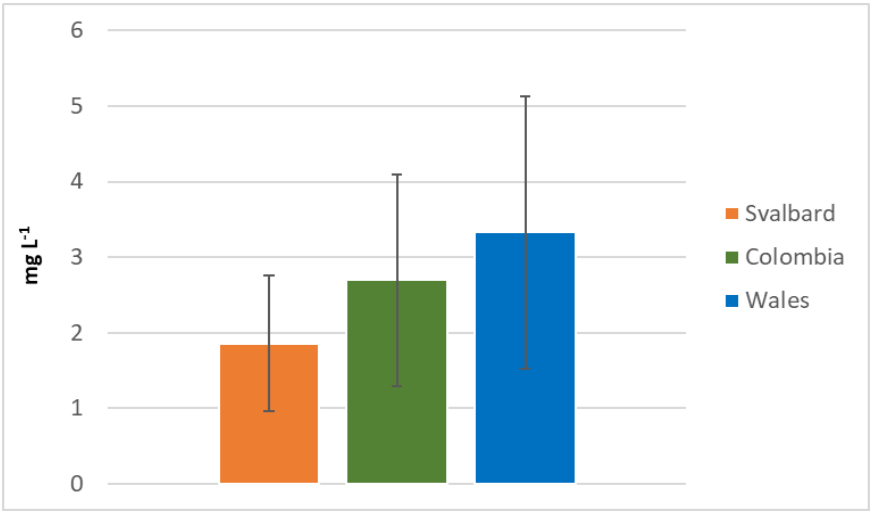


Figure 5.02. Phenolic compound concentration in the Svalbard, Colombian and Welsh samples. Concentrations are reported as the mean along with ± standard deviation.

Mean DOC concentration (Figure 5.03) was lowest for the Svalbard (12.3 mg L^{-1}) and highest in the Colombian samples (16.38 mg L^{-1}). One-way ANOVA showed that DOC concentration differed significantly between the three regions, F (2,183) = 5.28, p = 0.006. Tukey HSD post-hoc test revealed that the Svalbard soil samples had significantly lower DOC concentration than Colombia ($p < 0.05$). The Welsh samples, which had an intermediate mean DOC concentration, did not differ significantly from the other two regions (Table 5.01).

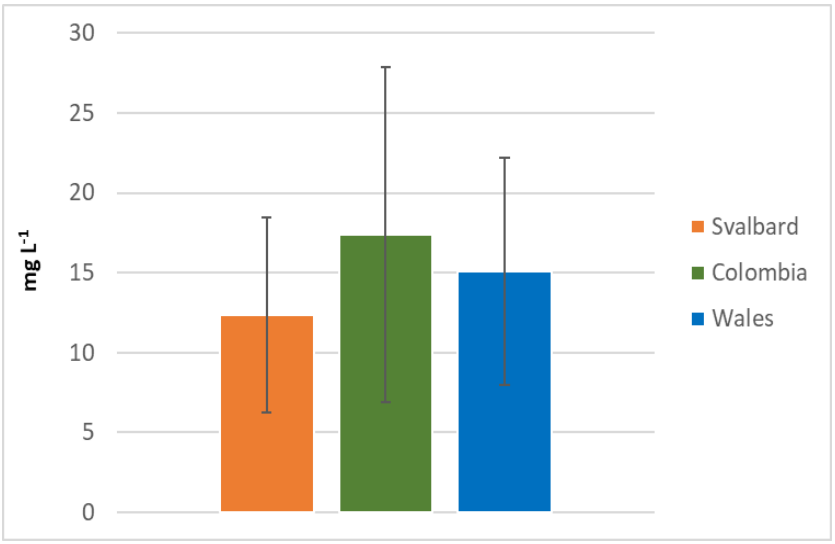


Figure 5.03. DOC concentrations of the Svalbard, Colombian and Welsh soil samples. The concentrations are reported as mean along with ± standard deviation.

Mean pH (Table 5.01) was highest in the Svalbard soil samples (7.1) and lowest in Wales (4.5). One-way ANOVA indicated that pH differed significantly between the three regions, F (2,183) = 475.6, p = 0.000. Tukey HSD post-hoc comparison test revealed that the Svalbard samples had a significantly higher pH than the other two regions ($p < 0.05$), and the Wales soil samples had significantly lower pH than both Svalbard and Colombia ($p < 0.05$).

Mean conductivity was extremely high in the Svalbard samples (216 µS/cm) and substantially lower in Wales and Colombian samples (both measuring 18.8 µS/cm). One-way ANOVA showed that electrical conductivity differed significantly between the three locations F (2,183) = 50.3, p = 0.000. Post-hoc comparisons using the Tukey HSD test revealed that the Svalbard samples had significantly higher conductivity than the other two regions ($p < 0.05$). The Colombia/Wales comparison was not significant (Table 5.01).

Mean soil water content ranged from 69.8% in the Svalbard peat samples to 89.6% in Wales (Figure 5.04). One-way ANOVA showed that water content varied significantly between regions, F (2,183) = 28.5, p = 0.000. Tukey post-hoc comparison test indicated that water content was significantly lower in Svalbard compared with Wales and Colombia ($p < 0.05$). No significant difference was found between the Welsh and Colombian samples (Table 5.01).

Mean soil organic matter content (% SOM) was highest in Wales (74.75%) and lowest in Colombia (43.8%). One-way ANOVA showed that %SOM differed significantly between regions, F (2,183) = 28.5, p = 0.000. Tukey HSD post-hoc test revealed that Wales had significantly higher %SOM than both Colombia and the Svalbard ($p < 0.05$), but that the Colombia/ Svalbard comparison was not significant (Table 5.01).

As can be seen in Figure 5.04 and Table 5.01, CO_2 flux was highest in Svalbard (29.7 µg CO_2 g^{-1} h^{-1}), with the flux measurements in Wales and Colombia substantially lower (13.4 and 12.7 µg CO_2 g^{-1} h^{-1}, respectively). One-way ANOVA showed that CO_2 flux differed significantly between the three locations F (2,183) = 14.3, p = 0.000. Post-hoc comparison using the Tukey HSD test revealed that the Svalbard samples had significantly higher CO_2 flux than the other two regions ($p < 0.05$). The Colombia/Wales comparison was not significant.

The Welsh samples showed the lowest mean CH_4 flux (mean 0.26 ng CH_4 g^{-1} h^{-1}), with intermediate flux (1.9 CH_4 g^{-1} h^{-1}) in Svalbard and highest flux (5.5 ng CH_4 g^{-1} h^{-1}) in Colombia. However, no significant differences were found between the three regions, F (2,183) = 2.15, p = 0.045.

Mean N_2O flux was lowest in the Welsh samples (1.4 ng N_2O g^{-1} h^{-1}) and highest in the Svalbard (5.7 ng N_2O g^{-1} h^{-1}). One-way ANOVA showed that N_2O flux varied significantly between regions, F (2,183) = 7.46, p = 0.001. Tukey post-hoc comparison test indicated that the N_2O flux were significantly higher in Svalbard compared with Wales and Colombia ($p < 0.05$). No significant difference was found between the Welsh and Colombian samples.

While many interesting features were found in the studies, it is worth noting that there is the potential for storage and transportation to have influenced the results in the same way that was described regarding the enzyme activities and solute concentrations (see page 41&137).

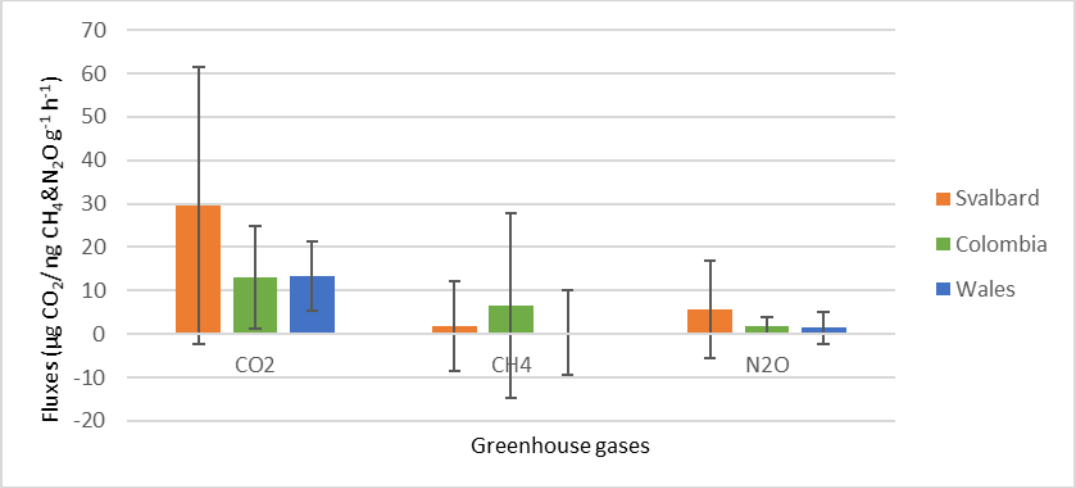


Figure 5.04. Mean GHG flux for Svalbard, Colombian and Welsh samples. Error bars represent the standard deviation of the mean.

All mean anion concentrations (Table 5.01) were highest in the Svalbard samples (3.0, 10.2, 0.33 and 52.5 mg L^{-1} for chloride, nitrate, phosphate and sulfate, respectively). One-way ANOVA showed that chloride, nitrate and sulfate concentrations varied significantly between the three regions. Tukey HSD post-hoc comparison test showed that the concentrations of all anions, except phosphate, were significantly higher in the Svalbard samples than in Wales and Colombia ($p = 0.000$). No significant differences were found between Wales and Colombia.

The concentration of phosphate varied slightly and insignificantly between the three contrasting regions (F (2, 183) = 0.143, $p = 0.866$).

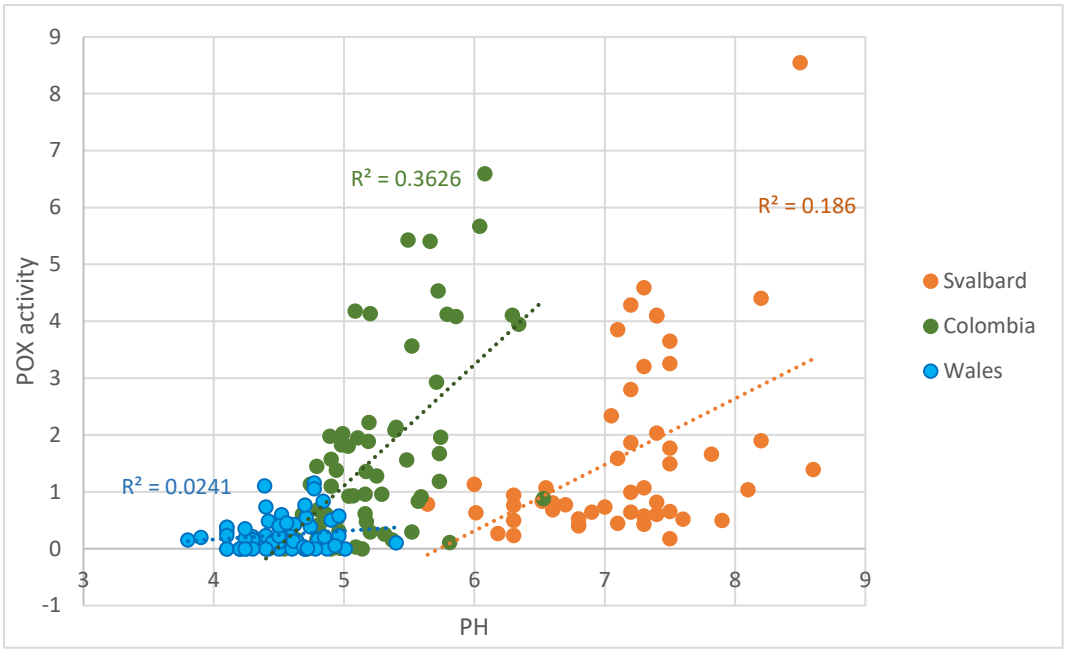
Mean sodium concentration was lowest in the Welsh samples (1.55 mg L^{-1}) and highest in Colombia (5.2 mg L^{-1}). One-way ANOVA showed that sodium concentration varied significantly between the three regions, F (2,183) = 52.3, $p = 0.000$. Tukey HSD post-hoc comparison indicated that the Svalbard and Colombian soil samples had significantly higher sodium concentration than Wales ($p < 0.05$). The Svalbard /Colombia comparison was not significant.

Mean ammonium concentration was lowest for the Svalbard samples (0.15 mg L^{-1}) and highest for Wales (1.5 mg L^{-1}). One-way ANOVA showed that ammonium concentration varied significantly between the three regions, F (2,183) = 40.46, $p = 0.000$. Tukey HSD post-hoc test indicated that ammonium concentration was significantly higher in the Welsh samples compared with both Svalbard and Colombia ($p < 0.05$), but that the Svalbard /Colombia comparison was not significant (Table 5.01).

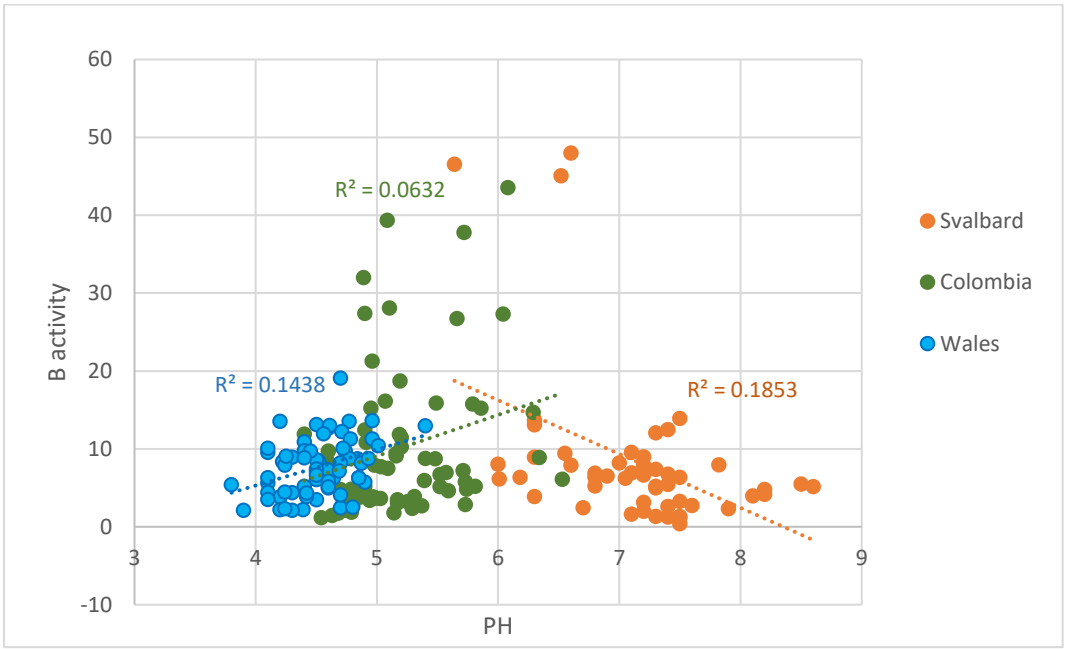
Calcium and magnesium concentrations were significantly higher in the Svalbard samples compared with the other two regions ($p < 0.05$), but that the Wales/Colombia comparison was not significant (Table 5.01).

Figure 5.05 shows the correlation between the *in situ* sample pH and extracellular enzyme activity. The activity of POX correlated positively with pH in the Svalbard and Colombia and no statistically significant correlation between pH and POX was found in Wales (Figure 5.05 a). The activity of all five hydrolase enzymes correlated positively with pH in Wales and in Colombia, with the exception of S in Colombia, whilst no statistically significant positive correlations were recorded for the Svalbard samples (Figures 5.05 b, c, d, e and f). Figure 5.06 displays the correlation between phenolic compounds and the activity of POX. POX activity correlated negatively with the concentration of phenolics in the Colombian peat, and no statistically significant correlation between phenolics and POX was found in Svalbard and Wales.

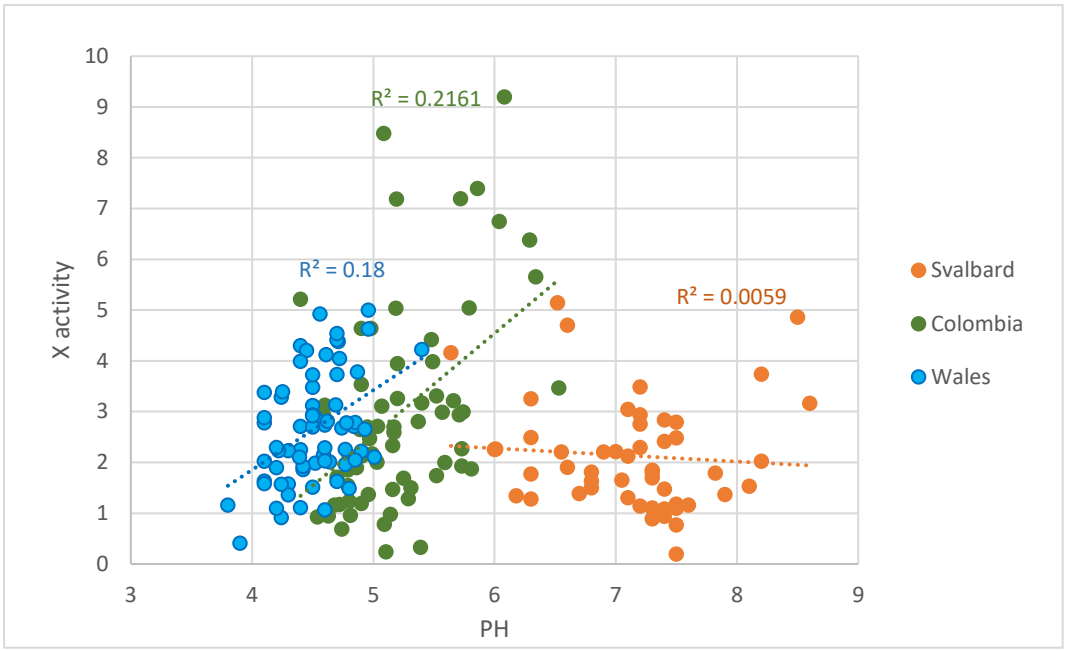
(a)


POX activity
PH
R² = 0.3626
R² = 0.186
R² = 0.0241
Svalbard
Colombia
Wales

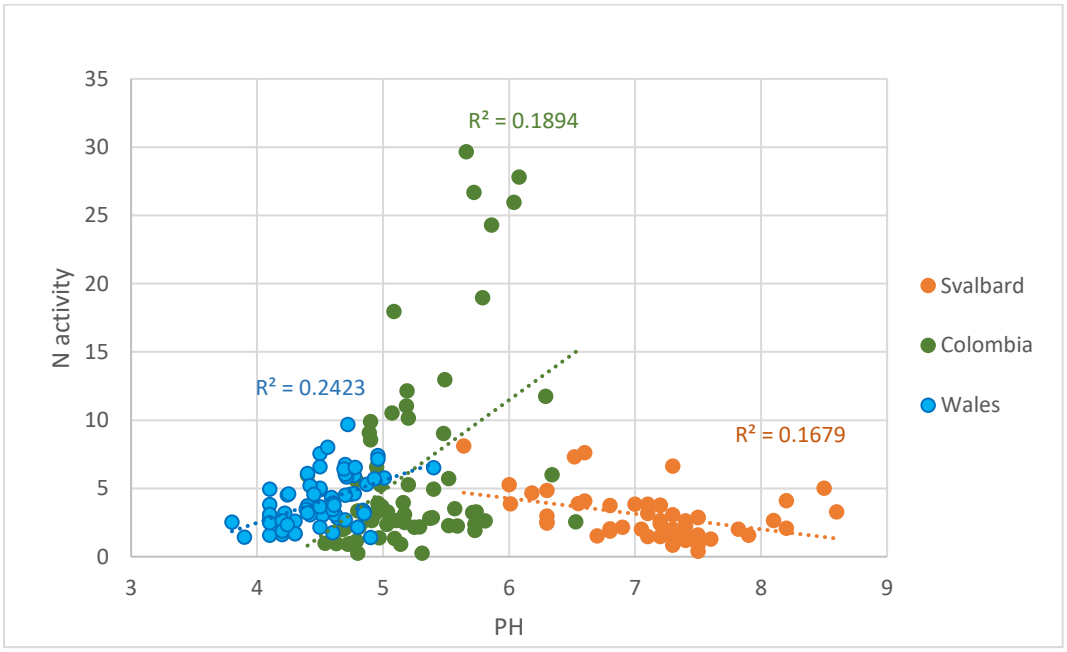
(b)


B activity
PH
R² = 0.0632
R² = 0.1438
R² = 0.1853
Svalbard
Colombia
Wales

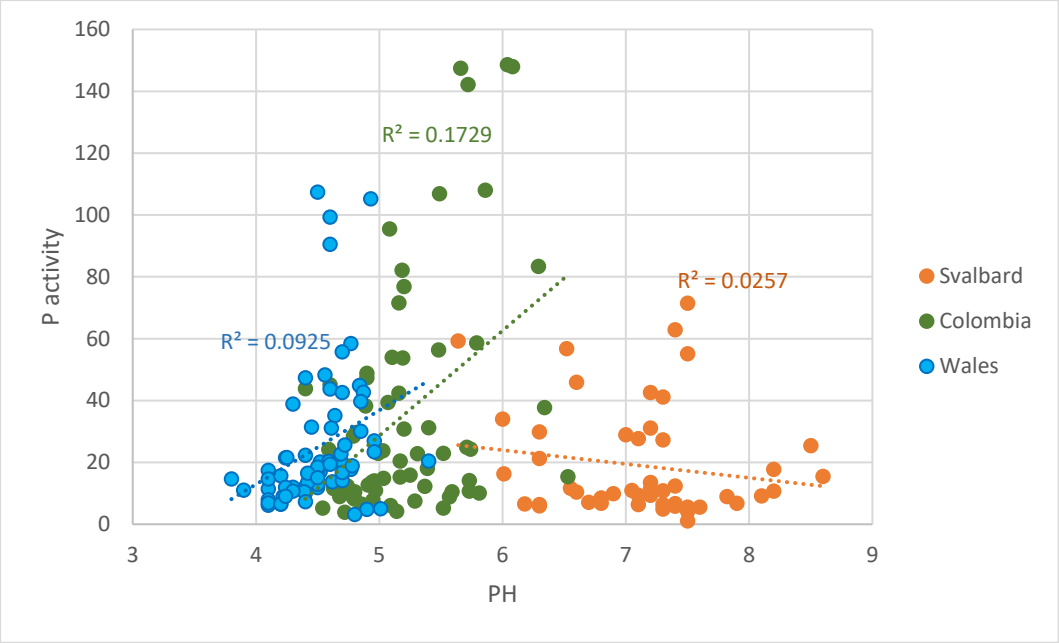
(c)


X activity
PH
R² = 0.2161
R² = 0.18
R² = 0.0059
Svalbard
Colombia
Wales

(d)


N activity
PH
R² = 0.1894
R² = 0.2423
R² = 0.1679
Svalbard
Colombia
Wales

(e)



(f)

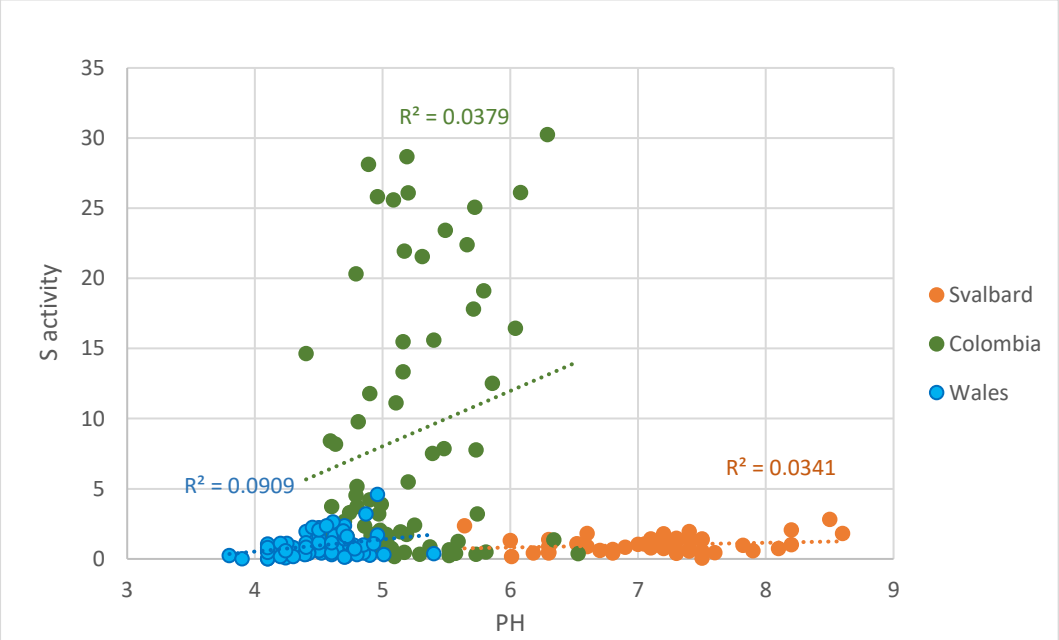


Figure 5.05. The relationship between pH and the activity of (a) phenol oxidase (POX), (b) β-glucosidase (B), (c) xylosidase (X), (d) chitinase (N), (e) phosphatase (P) and (f) arylsulfatase (S).

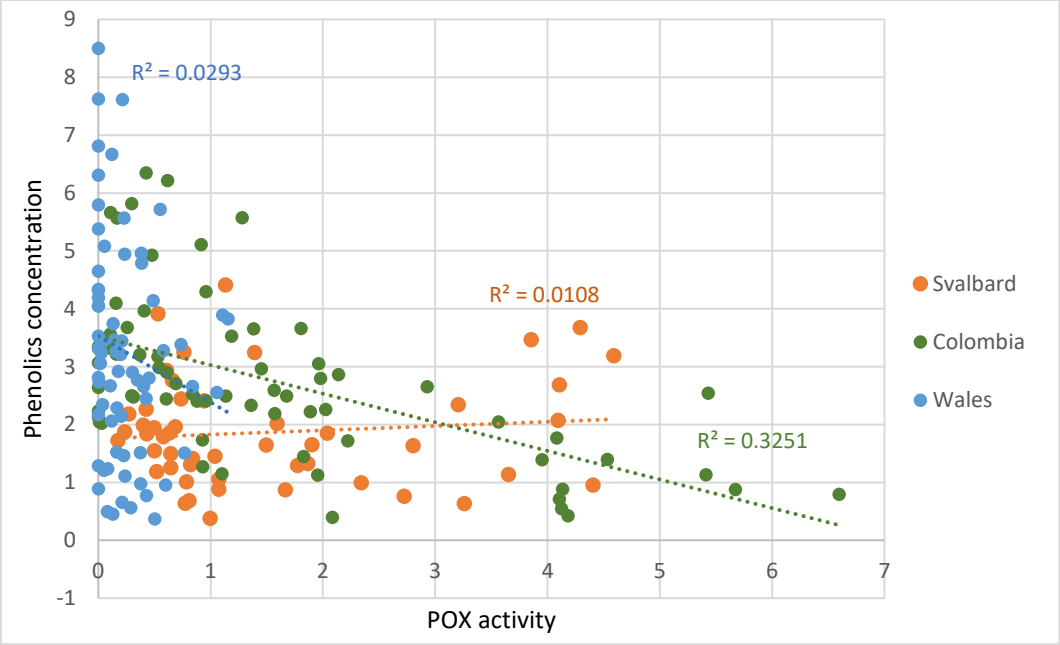


Figure 5.06. The relationship between phenolic compounds and the activity of phenol oxidase (POX).

Principal component analysis (PCA) was used to show the tendency of all measured variables within the different regions and to discover the links between the variables (Appendix A Figure 1).

5.5. Discussion

This study presents a comparison of peatland soil parameters within and between different climatic regions (Arctic, temperate and tropical), and provides insight into the physicochemical controls on soil enzyme activity and the rate of greenhouse gas production based on field observation.

Across the three climatic regions, enzyme activities and GHG fluxes exhibited different relationships with soil physicochemical variables. Generally, significant positive relationships between hydrolase activities were observed at a regional scale indicating that they act together in synergy (Béguin and Aubert 1994) to reduce the structurally heterogenous biopolymers to constituent monomers obtainable by microbes (Sinsabaugh *et al.* 2008).

Several studies have demonstrated the indirect positive effect of POX activity on hydrolase enzyme activities (Freeman *et al.* 2001, 2004, Jones 2006, Fenner and Freeman 2011) due to the decline in inhibitory phenolics. The data of this study in this regard supported their finding as significant positive correlations were found between the activities of all hydrolytic enzymes and POX activity which in turn correlated negatively with its substrate phenolics. Phenolic compounds have an inhibitory effect on microbial functions involved in SOM decomposition, nutrient cycles and GHGs flux (Kim and Kang 2008) because they can combine with the reactive sites of organic and inorganic substrates, making them resistant to further microbial attack. They can also inhibit litter decomposition by lowering pH (Min *et al.* 2015) and by themselves being strongly resistant to biodegradation (Zak *et al.* 2019).

The response of hydrolytic enzymes activity and GHG flux to phenolics concentration varied across the three peatlands, all hydrolytic activities in addition to CO_2 flux were negatively related to phenolics concentration in Colombia. Such activities and fluxes showed no significant relationship with phenolics concentration in Svalbard. This observation suggests that the three climatic peatlands differ in parent plant chemistry that controls peat formation (peat chemical composition) and peat preservation across a latitudinal transect from the Arctic to the tropics. Indeed, Hodgkins *et al*. (2018) found that peaty soils from tropical peatlands are more chemically recalcitrant, having higher aromatic content and lower carbohydrate content than those from Arctic and boreal peatlands. Increasing peat recalcitrance towards the equator mirrors the increasing aromatic content in peat-forming plants with warmer climates and longer growing season (Hodgkins *et al.* 2018). Additionally,

Tveit *et al.* (2013) mentioned that unlike *Sphagnum* species that contain lignin-like polymer, tundra mosses have less complex phenolic compound called lignan.

In Wales the only significant correlation between hydrolase enzyme activity and phenolics concentration was a positive correlation involving N enzyme activity (all other correlations were non-significant), which could suggest a high demand for N element associated with polyphenolic compounds (Li *et al.* 2018). Generally, variations in the form, composition and the source of phenolic compounds, may be driving variations in enzyme activities and the resultant metabolic end products (Hoostal and Bouzat 2008, Min *et al*. 2015).

The Colombian peatland showed the highest arylsulphatase and chitinase activities of the three peatlands, and the highest xylosidase and phosphatase activities compared with Svalbard. The relatively high hydrolase activities in Colombia possibly due to the higher energy supply. Indeed, Bragazza *et al.* (2015) found that greater biomass of woody species toward the tropics enhanced the availability of low-molecular weight carbon through temperature-induced root exudation, fuelling a greater microbial biomass, which mirrors the increasing enzyme activities.

The higher DOC concentration towards tropics compared with Arctic peatland may be related to the higher primary productivity towards the equator where warmer temperatures and longer growing season exist (Hodgkins *et al.* 2018). In addition, decreased DOC concentration has been linked to increased sea salt (Gough 2014), all of which were high in Svalbard possibly due to the proximity of the sampling sites to the sea. Ionic strength (conductivity) is one of the controls on DOC solubility and may be a significant factor affecting peat pore water DOC concentrations (Gough *et al.* 2016), an inverse relationship has been reported between DOC concentration in peat pore water and conductivity (Fenner *et al.* 2005). Similarly, in the present study, significant negative correlation was reported between DOC concentration and conductivity in the Svalbard peatland. The explanation for reduced DOC solubility with higher ionic strength is not fully understood but may relate to the reduced charge density of organic substances, which in turn causes coagulation (Kalbitz *et al*. 2000).

Phenolic concentration was highest in the Welsh and Colombian peatlands possibly due to the dominant vegetation being *Sphagnum* moss, which dies back rapidly during warm periods resulting in large inputs of dead plant material into the soil. A large proportion of this plant

material is comprised of phenolic compounds (Wetzel 1992, Worrall *et al.* 2002, Jones 2006, Sinsabaugh *et al.* 2008).

Compared with Colombia, the activity of hydrolase enzymes in Svalbard was significantly lower, despite the soil's higher conductivity, pH and lower phenolics concentration. This limited activity may be the result of low temperature (Kang *et al.* 2018). Hodgkins *et al*. (2018) reported that more recalcitrant organic matter is the key factor at the tropics, while cold temperature is the key factor at the Arctic.

Compared with Wales, the activity of POX was significantly higher in Svalbard and Colombian soils. This is likely due to the higher pH, suggesting that around neutral to alkaline pH values are optimal for POX activity. Indeed, a strong positive correlation between POX activity and pH is frequently observed (Pind *et al*. 1994, Sinsabaugh *et al.* 2008, Kang *et al.* 2018) and was observed in the present study in Colombia ($n = 67$, $r = 0.602$, $p < 0.01$), and Svalbard ($n = 51$, $r = 0.431$, $p < 0.01$).

Regarding the relationship between POX activity and the concentrations of its substrate phenolics, many contradictory results have been reported, while some studies demonstrated a negative relationship (Pind *et al.* 1994, Waldrop and Zak 2006, Stursova and Sinsabaugh 2008, Waldrop and Harden 2008, White *et al.* 2011), still others reported a positive (Freeman *et al.* 2001, Fenner *et al*. 2005, Yao *et al.* 2009, Theuerl *et al.* 2010, Kang *et al.* 2018) or no relationship (Bending and Read 1997, Tian *et al.* 2010). Such conflicting evidence could be due to the dual functions of phenolic compounds that enable them to serve as an enzyme substrate and/or as a product of enzyme action (Min *et al.* 2015). In this study, and as illustrated in Figure 5.06, a significant negative correlation between the activity of POX and phenolic concentrations was detected only in Colombian soil samples, while no statistically significant relationship was observed in Wales and in Svalbard.

The remarkably higher conductivity measurements in the Svalbard samples observed in the present study were likely to be due to the high inputs of the major sea ions (from sea spray aerosols containing Na^+, Cl^-, Ca^{2+}, Mg^{2+} and SO_4^{2-}), the presence of which was likely to be resulting from the close proximity to the sea, in addition to the greater influence of the mineral substrate layer. Conversely, the lower conductivity levels in the Welsh and Colombian soils may reflect a lesser marine influence in these upland sites (Jones 2006, Pilson 2012).

Measurement of enzyme activities can reflect microbial nutrient demand and it is believed that a negative correlation would occur between soil enzyme activity and nutrient availability depending on the economic theory of microbial metabolism indicating that if nutrients are limited, enzymes production increase and vice versa (Chròst 1991, Sinsabaugh *et al.* 1993, 2008, Zhang *et al.* 2019). Based on this, the lower sulfatase activity in the Svalbard soil compared with the other regions could be explained by the markedly higher sulfate concentration, and the higher S enzyme activity in Colombian soils could also be explained by the lower sulfate concentration. Indeed, a significant negative correlation between S enzyme activity and sulfate concentration was identified at the Colombian samples ($n = 67$, $r = -0.571$, $p < 0.01$). In the Svalbard, the negative correlation between sulfatase activity/sulfate concentration was not significant. Because all the studied sites were close to the sea, it is possible that sea-salt aerosol represented an important sulfate source in the soils, which could explain the lack of statistically significant negative correlation between S activity and sulfate concentration (Kang and Freeman 1999) in Svalbard.

Mean phosphatase activity showed the highest activity of all the enzymes in each region probably because of the phosphate ions, on average, being the lowest concentration at all regions. It is therefore unsurprising that a significant negative correlation was observed between phosphate ions and phosphatase activity in Colombia ($n = 67$, $r = -0.241$, $p < 0.05$). Conversely, there was a statistically significant positive correlation between phosphatase activity and phosphate concentration in the Svalbard peatland ($n = 51$, $r = 0.337$, $p < 0.05$), which means that the phosphate measurement could represent the product of phosphatase activity (Senga *et al*. 2015). Possibly, this was caused by phosphatase activity that was higher than what was required to meet phosphate demand (Weintraub and Schimel 2005). This disagrees with the theory that enzymes activity occurs only when there is a demand for nutrients and that the production of enzymes should therefore stop once nutrient availability increases to fulfil demand (economic model) (Burns *et al.* 2013). One possible explanation is that in situations where enzyme turnover, sorption (to soil particles) and/or deactivation (the enzyme active site changes shape and can no longer bind to the substrate) rates are low, enzyme produced to meet a particular demand for a nutrient may remain active even after that demand has been fulfilled (Allison *et al.* 2007).

In Wales, no inverse correlation was reported between S enzyme activity and sulfate concentration. Also, no inverse correlation was reported between P enzyme activity and phosphate concentration to confirm the economic model of enzyme production. According to

Kang and Freeman (1999), the lack of a significant inverse correlation between enzyme activity and nutrient concentration could be due to the legacy enzymes (immobilised enzymes) that are already present in large amounts in the soil, and are known to be independent of microbial regulation.

Despite previous research suggesting that the availability of N element in soils could decrease the production of N-acquiring enzymes (Bragazza *et al.* 2006, Steinweg *et al.* 2013), a statistically significant positive correlation between N enzyme activity and ammonium concentration was identified in each of the three peatlands, in the Welsh soils ($n = 68$), $r = 0.494$, $p < 0.01$, in the Colombian soils ($n = 67$), $r = 0.737$, $p < 0.01$, and in the Svalbard ($n = 51$), $r = 0.313$, $p < 0.05$. These results suggest that ammonium was due to the decomposition, as it is end product of the mineralisation of nitrogen-containing compounds (Bayley *et al.* 2005, Jones 2006).

N enrichment significantly increased hydrolase activity (Min *et al.* 2015), DOC concentration and soil respiration (Lu *et al.* 2011) due to the lower release of polyphenols by *Sphagnum* mosses when N content is high, which would in turn limit enzyme inhibition and promote soil metabolism (Bragazza *et al.* 2006). In addition, N deposition can promote C loss from peatlands by activating phenol oxidase (Bragazza *et al.* 2006). Indeed, in the present study, many significant positive relationships have combined ammonium concentration with extracellular enzymes activity, GHG flux and DOC at regional scale, which indicate that increased N content poses a significant risk to peatlands function (Bragazza *et al.* 2006).

Moreover, a significant positive correlation was found at a regional scale between percentage soil water content and the activities of all hydrolytic enzymes, suggesting that the aqueous medium is better for the activity of hydrolase enzymes providing a medium for enzymes and the drought conditions could reduce the production of hydrolytic enzymes, as microbial biomass declines (Allison and Vitousek 2005, Sardans and Penuelas 2005, Sowerby *et al.* 2005, Steinweg *et al.* 2013).

The lower percentage of SOM content in the Svalbard and Colombian samples compared with Wales is due to the influence from mineral inputs which were identified in most of the studied sites of the Svalbard and Colombian peatlands.

The pH value may affect the enzymes active sites, as well as the interaction between immobilised enzymes and their associated matrix. Thus, pH is an important control on soil enzyme activity (Kang and Freeman 1999). The findings of this study suggest that higher pH

is the dominant controlling factor for POX activity, but not for hydrolase enzyme activities, enzyme activity varied in relation to soil pH for all regions (Figure 5.05 a-f), in Colombia all activities, with the exception of S activity, were significantly related to soil pH which ranged from 4.4 to 6.5. Regarding the optimum pH for S activity, Turner (2010) found that arylsulfatase activity had a very acidic optimum pH in all soils (pH ≤3.0).

In Wales, all hydrolase activities were significantly correlated with pH (which ranged from 3.8 to 5.4), and POX activity showed no correlation, suggesting that acidic pH was to a great extent optimal for hydrolase activities. In the Svalbard peatland, where pH ranged from around neutral (5.64) to alkaline (8.6), only POX activity showed a significant positive correlation with soil pH, while the activity of B and N showed a significant negative relationship with soil pH which may reflect the fact that each enzyme has a different optimum pH range (Turner 2010, Robinson 2015). The optimum pH of the enzymes B (Robinson 2015) and N (Turner 2010) are 4.8 and 4.2, respectively, at which the activity is maximal. Above and below this point, the enzyme activity decreases (Robinson 2015). In Svalbard, the pH values ranged from 5.64 to 8.6, which means that they were above the optimum range for the enzymes action.

The average soil pH values differed significantly between each of the three regions, the pH of which was ranked: Svalbard > Colombia > Wales. Guano was found in Svalbard and is a material known to influence various physical and chemical parameters, including the concentration of nitrogen, phosphate, magnesium and potassium ions. Guano has an alkaline pH (Zwolicki *et al.* 2013), and this may have contributed to the unexpectedly high pH observed in Svalbard. The large divergence of the peatland in Svalbard from the other two locations, makes it more difficult to compare the data on a latitudinal basis. The findings create uncertainty about whether the differences observed were due to latitude or simply the unusual ecosystem inputs. However, while Svalbard may have been suboptimal as a representative site at which to carry out a latitudinal comparison, it has nevertheless suggested a need for further research to evaluate the extent to which guano creates an overriding influence on wetland ecosystem characteristics.

The lower pH observed in Wales may have contributed to the significant lower POX activity, as acidic conditions are known to be sub-optimal for POX activity (Pind *et al.* 1994). Low pH is one of the reasons why organic matter degradation is very low in wetlands (Clymo 1983).

Fluxes of greenhouse gas from wetlands are related to soil enzymes activity and hydrochemistry (Kang *et al.* 1998). At a global scale, the higher flux of CO_2 from the Svalbard peat soils was concurrent with high POX activity and pH and with the low phenolics concentration.

Significant positive correlations were found in Colombia between all enzyme activities and the production of CO_2 and N_2O. Several significant relationships were also found between enzyme activities and GHG flux in the Svalbard. By contrast, enzyme activities in Wales did not correlate with GHG flux, possibly because the enzyme activity measured in Wales was from the legacy enzymes that were already present in the soil rather than the generalised measurement of microbial metabolic process that includes de-novo enzyme synthesis, which is usually associated with soil microbial respiration (Freeman *et al.* 1996, Preston *et al.* 2012).

It is important to note here that there was a significant amount of bird guano found at the Svalbard sites, which probably explains the high concentration of nitrate, a readily utilizable source of nitrogen and in turn may explain the significant N_2O emission from the Svalbard peatlands (Kang *et al.* 1998, Thomson *et al.* 2012). At a global scale, CH_4 flux did not vary significantly between the three peatlands suggesting that the activity/biomass of methanogens did not vary across studied regions.

5.6. Conclusions

In this study, the responses of soil enzyme activities and soil respiration to physical and chemical properties were assessed within peat soils obtained from three climatic regions representing a gradient of environmental conditions: Arctic (Svalbard, Norway), temperate (Snowdonia National Park in Wales, UK) and tropical (Sumapaz National Park in Andean mountains, Colombia).

When the data of each variable within each region was amalgamated and compared between the different regions, enzymes activity (except for β-glucosidase) and GHGs flux (except for CH_4), varied in relation to soil physicochemical variables which in turn extremely varied across the three contrasting peatlands. The Colombian peatland showed the highest arylsulphatase and chitinase activities of the three peatlands, and the highest xylosidase and phosphatase activities compared with Svalbard. For phenol oxidase, the lowest activity was

observed in the Welsh peatland consistent with the lowest pH. The activity of POX was relatively high at Arctic soils in conjunction with this site having higher pH, conductivity and inorganic nutrients and lower concentration of inhibitory phenolic compounds. Interestingly, phosphatase activity was the highest recorded enzyme activity in all three peatlands, most likely due to higher demand for phosphorus, which is often less available in terrestrial environments. The higher mean DOC concentration in peatland of Colombia and Wales compared with Svalbard may be related to the higher primary productivity towards the equator where warmer climates and longer growing season exist. Phenolic concentration was highest in the Welsh and Colombian peatlands possibly due to the dominant vegetation being *Sphagnum* moss, which dies back rapidly during warm periods resulting in large inputs of dead plant material into the soil. A large proportion of this plant material is comprised of phenolic compounds. The dominance of *Sphagnum* moss in Colombia and Wales may also provide a reason for the lower pH value in these regions compared to Svalbard. *Sphagnum* acidifies its surroundings by cation exchange. It adsorbs base cations such as calcium and magnesium from the soil water and exchanges or releases hydrogen ions.

The Arctic peatland had higher nutrient concentrations than the other two regions, most likely due to the close proximity to the sea and nutrient inputs from birds manure as well as the influence from the underlying mineral layer.

The present study is the first, to current knowledge, giving a comparative view of biogeochemical processes in relation to the surrounding physicochemical factors in peatlands of three different climatic regions. This could have an implication for predicting how peatland biogeochemical processes may respond to climate change and human exploitation, which help in development of management strategies for maintain carbon storage function of peatlands.

5.7. Acknowledgments

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Chapter 6

Using environmental DNA metabarcoding to compare micro-eukaryote populations in Arctic, temperate and tropical peatlands

6.1. Abstract

Peatlands arise because primary production rates exceed decomposition rates, leading to the importance of the ecosystem in global carbon sequestration. Ongoing climate change represents a threat both for the composition (biotic communities) and function (carbon storage) of peatlands. Alongside prokaryotes, eukaryotic microorganisms are key regulators of nutrients cycling within soil food webs and through their multiple functions, they can directly and/or indirectly influence the function of peatlands. They are used as bioindicators of abiotic changes in various environments. However, they have received relatively little attention in many ecosystems including peatlands. Studying the structure and function of micro-eukaryotes in peatlands along a climatic gradient could allow us to anticipate how peatland may respond to future climate change.

In this study, for the first time, richness, diversity, relative abundance and the structure of micro-eukaryotic community were assessed in peat samples collected from three contrasting climatic regions (Arctic, temperate and tropical) representing a gradient in climate-driven environmental variables. In the present study, the general pattern of peat soil micro-eukaryote abundance was consistent with other studies of peatlands, where the supergroups Alveolata, Opisthokonta, Rhizaria and Stramenopiles were dominant. The findings based on redundancy analysis revealed that the community composition of micro-eukaryotes, which are presented here based on the functional groups (phagotrophs, phototrophs, mixotrophs, osmotrophs and parasites), changed significantly between the peatlands of the three climatic regions in response to the selected abiotic and metabolic variables of the soil; increased pH, conductivity, soil organic matter (SOM) content, oxygen concentration and lower temperature, structured micro-eukaryote community, which dominated by phagotrophs, phototrophs and mixotrophs, in the Arctic. Higher temperature and phenolic compound concentrations and lower pH and nutrient concentrations explained community composition, which represented by high relative abundance of parasites, in the temperate Welsh peatland. Micro-eukaryotic community in the Colombian peatland was dominated by osmotrophs and explained by high activities of the extracellular enzymes glucosidase, arylsulfatase, chitinase, phosphatase and phenol oxidase, which were also consistent with lower phenolic concentrations, and higher pH and dissolved organic carbon (DOC) concentration. Furthermore, the occurrence of predator and host invertebrates was noted as well as prey organisms and considered their potential significance for micro-eukaryotic organisms.

6.2. Introduction

In the microbiomes found in soils, eukaryotic microbial communities are the most species rich (Geisen *et al.* 2015). These organisms, including fungi and protists, are functionally diverse (Araujo *et al.* 2018), which enables them to play a number of essential roles within the ecosystem's food webs (Adl and Gupta 2006). In soils, phototrophs containing photosynthetic pigments are the primary producers fixing carbon during photosynthesis (Seppey *et al.* 2017). As phagotrophs, they feed mainly on bacterial and fungal communities in addition to other eukaryotic microorganisms and thus they contribute to elemental cycling, nutrient release and soil fertility, which in turn stimulating plant growth (Geisen 2016, Geisen *et al.* 2018). Through predation, phagotrophs also control their microbial prey populations (Geisen *et al.* 2018). Mixotrophic organisms are those that benefit from both trophic behaviours (phototrophy and phagotrophy), during both of these functions the mixotrophic organisms can directly and indirectly affect the carbon balance of the ecosystem (Jassey *et al.* 2015). Osmotrophs absorb soluble nutrients through the cell membrane and are mostly found within the fungal group (Adl and Gupta 2006). These have been identified as the main decomposers of organic substrates, which they achieve by releasing extracellular enzymes (Min *et al.* 2015). Parasitic protists of animals and plants are likely to drive the diversity of animal (Mahé *et al.* 2017) and plant (Schwelm *et al.* 2018) communities, respectively. Additionally, micro-eukaryotic organisms are distributed ubiquitously and are known to respond to changes in environmental conditions including water content, oxygen availability, temperature, pH, light intensity, nutrient availability, vegetation type and anthropogenic disturbances. They are therefore considered to be useful bioindicators of abiotic variations in their environments (Koenig *et al*. 2015), and can to some extent infer spatial heterogeneity in different habitats and reflect soil quality (Mitchell *et al.* 2000). For example, testate amoebae respond quickly to hydrological changes and thus their distribution is mainly controlled by soil water content (Booth *et al.* 2010) and water chemistry (Mitchell *et al*. 2000). Rose and Caron (2007) showed that temperature is the main factor affecting algal community, while nutrient availability can affect diatom distributions (Gilbert *et al*. 1998). Min *et al*. (2015), Fierer (2017) and Kang *et al.* (2018) noted that pH plays a key role in shaping fungal communities, under optimal temperature conditions (Kang *et al.* 2018), with higher pH stimulating phenol oxidase activity, resulting in higher dissolved organic carbon (DOC) production (Kang *et al.* 2018).

Electrical conductivity, pH and total organic carbon concentration appear to be the dominant drivers of phagotroph abundance (Mieczan 2007). In addition, dissolved oxygen concentration has been identified as another important factor affecting the abundance of both phagotrophic and phototrophic microorganisms (Papadimitriou *et al.* 2010).

The presence of phenolic compounds appears to negatively affect the presence of microbial communities (Fung *et al.* 1985, Opelt *et al.* 2007, Mellegård *et al.* 2009, Pizzolitto *et al.* 2015), which will in turn affect many essential processes such as decomposition (Freeman *et al*. 2012, Min *et al*. 2015) and photosynthesis (Mieczan 2007). Conversely, the darkness created by elevated phenolic concentrations in soil pore waters provides a natural protective shield for parasitic infective stages from harmful sunlight (Thomas *et al*. 1995, King *et al.* 2008). Furthermore, studying the effect of environmental variables on community composition of micro-eukaryotes along a climatic gradient is useful for predicting how peatland may respond to the future climate change (Jassey *et al.* 2015).

The structure and function of peatlands is threatened by ongoing global warming which has been attributed to high emissions of greenhouse gas into the atmosphere (IPCC 2013). Through their multiple functions, micro-eukaryotic communities can directly and/or indirectly affect the carbon balance of peatlands and hence possibly contribute to the climate change (Dedysh *et al*. 2006, Jassey *et al*. 2015).

Among protists, the Ciliophora within supergroup Alveolata (Mieczan 2007, Papadimitriou *et al.* 2010, Geisen *et al.* 2015), Arcellinida and Euglyphida testate amoebae within supergroups Amoebozoa and Rhizaria, respectively (Gilbert *et al.* 1998, Mitchell *et al.* 2000, Lara *et al.* 2011, Mieczan 2012), and Chrysophytes within supergroup Stramenopiles (Lara *et al.* 2011) are known to be peatland associated. In addition, Fungi within supergroup Opisthokonta (Lara *et al.* 2011) are considered typical inhabitants of peatlands. Recently, high throughput sequencing-dependent approaches revealed that parasitic protists are abundantly present in soils (Geisen *et al.* 2015, Mahé *et al.* 2017), suggesting that contaminated soil can be an important source of pathogens to food, groundwater and surface water. As such, the potential for a role of soil in parasite transmission should not be disregarded (Santamaria and Toranzos 2003).

Wetland systems are ecotones between fully aquatic and terrestrial habitats, and so tend to support high levels of biodiversity (Mitsch and Gosselink 2000). Richer biodiversity,

including more diverse hosts, vectors and parasites, may by implication lead to increased predation and parasite-host encounter rates, increasing the probability of transmission of parasites with complex life cycles between hosts (Thomas *et al.* 1997) due to consumption of infected intermediate hosts (Dobson *et al.* 2008).

Thomas *et al.* (1997) in their study of parasitism in wetlands, concluded that in addition to the pathogenic effects of parasites, they can also affect the distribution, behaviour and diversity of their hosts. Such consequences of parasitism suggest that parasites are important players in ecosystems structuring and functioning (Thomas *et al.* 2005).

Soil microbial communities, including prokaryotes and micro-eukaryotes, are an essential component of soil ecosystems, as they are responsible for nutrients cycling and ecosystem processes (Andersen *et al.* 2013). Prokaryotic and eukaryotic communities in peatlands are important in the net sequestration of atmospheric carbon and therefore play a unique role in global carbon cycling (Winsborough and Basiliko 2010). However, unlike prokaryotes, eukaryotic microorganisms have received relatively little attention in many ecosystems including peatlands. These are globally significant environments since they represent huge carbon storage, sequestering about one third of global soil carbon in the form of peat (Kang *et al.* 2018). To date, no published data giving a comparative view of micro-eukaryotic community composition in relation to the surrounding environmental factors in peatlands of different climatic regions. Also, no data has been published so far about micro-eukaryotes in the chosen field sites of this study.

In the present study, the aim was to compare richness, diversity, relative abundance and composition of the micro-eukaryotic community between comparable peatlands in three distinct climatic regions, namely Arctic, temperate and tropical with contrasting biogeochemical conditions. Soil DNA metabarcoding of the hypervariable V4 region of the 18S SSU ribosomal RNA gene was used to assess the micro-eukaryotic community. Redundancy analysis (RDA) was used to test the hypothesis that the micro-eukaryotic community differs significantly between climatic regions in a manner predictable based on the biogeochemical soil conditions present. This analysis also enables a visualisation of the effect of the most important soil variables and metabolic factors on micro-eukaryote community composition. In order to more easily analyse and interpret the data, micro-eukaryotes were organized into five functional groups according to their nutritional mode: phagotrophs, phototrophs, mixotrophs, parasites and osmotrophs. The biotic interactions of

eukaryotic microorganisms were also considered with their potential hosts, predators and prey organisms.

6.3. Materials and methods

6.3.1. Soil sampling and laboratory analysis

In this study, moss-dominated peatlands along a latitude gradient and from three climatic regions were chosen. They were located in the Arctic region (Svalbard, Norway), a temperate region (Snowdonia National Park in North Wales, UK), and a tropical region (Sumapaz National Park in Andean mountains, Colombia). In each peatland, soil cores were collected from six different sites, and three replicates of soil cores were extracted from each site. Samples were collected from Svalbard, Norway in late July 2016. The average air temperature was 5°C, the water table was at the soil surface during sampling and the moss tundra of the study sites was dominated by the species *Tomenthypnum nitens* and *Calliergon richardsonii*, without vascular plants.

Six *Sphagnum*-dominated peatland sites located in the Snowdonia National Park, North Wales, UK were sampled at the beginning of September 2016. The study sites were covered by *Sphagnum* spp, *Juncus* spp, and *Carex* spp interspersed with some cotton grasses and some *Calluna vulgaris* (common heather). The average air temperature was 14.3°C and the water table level was at the soil surface at the time of sampling.

The tropical peatland sites were located at high altitude within the Sumapaz National Park in the Andean mountain range, Colombia. Sampling was performed at the end of July 2017. The dominant vegetation was *Sphagnum* spp, *Carex* spp, *Juncus* spp, *Espeletia* spp and many other shrubs were also more common. The average air temperature was 13.4°C and the water table was at the soil surface during the time of sampling.

Each soil core was examined and boundaries between distinct layers identified based on the colour and the texture. These individual layers were then placed in individual plastic bags and transported in ice to the laboratory in Wales. Distinct layers in the soil cores were handled and analysed separately. Subsamples of soil for molecular analysis were frozen at - 20°C, and for enzymes activity measurement and chemical analysis were stored at 4°C. Molecular analysis was conducted on soil samples of the superficial layer (from 0-5 cm in the Arctic, and from 0-10 cm in Wales and Colombia). Soil temperature, pH and dissolved oxygen concentration were measured in the field using portable instruments. Measurements of soil enzyme activity, greenhouse gas fluxes, organic matter content and water content, pH value, electrical conductivity, and soil water extraction in order to measure phenolic compound, dissolved organic carbon and ions concentrations were performed in the laboratory following standard protocols.

6.3.2. Soil analyses

The activities of extracellular enzymes were determined following a protocol described by Dunn *et al.* (2014). Briefly, the phenolic amino acid L-3,4- dihydroxy phenylalanine (L-DOPA, Sigma Aldrich Ltd, Dorset, UK) was used as a target substrate for assay phenol oxidase activity. The colour change is determined using a spectrophotometer at 475 nm and the activity of phenol oxidase was then calculated by using Beer-Lambert Law and expressed as µmol of formed (diqc) per minute per gram of soil (dry weight). Hydrolase activities were determined using the procedures of Dunn *et al*. (2014), based on the measurement of fluorescence of methylumbelliferone (MUF). Fluorescence of the MUF molecule was measured using a microplate fluorometer (Molecular Devices SpectraMax M2e spectrophotometer) at 450 nm emission and 330 nm excitation. Hydrolase activities are expressed as nmol MUF g^{-1} of soil min^{-1}.

Soil subsamples were oven-dried for 24 h at 105° C in order to determine soil water content. Soil organic matter was determined by muffle-burning dried subsamples for 4 h at 550° C.

To determine the amount of water-extractable carbon in the peat samples, 5 g subsample of peat was taken and homogenising with 40 ml of water on a rotary shaker for 24 h. The resulting slurry was centrifuging at 5000 rpm for 30 minutes. The supernatant was filtered through a 0.45µm membrane filter (Whatman, Kent, UK) to separate particulate organic carbon (POC) from dissolved organic carbon (DOC). The filtered samples were then

collected in 20 mL plastic scintillation vials (Meridian Biotechnologies Ltd) and stored at 4°C until measurement.

A pH meter and a Primo 5 handheld conductivity meter were inserted into peat slurry before filtration in order to measure the pH and the conductivity of the peat, respectively.

The concentration of phenolics was assayed using Folin-Ciocalteu Reagent, as described in detail by Box (1983).

Cation and anion concentrations were analyzed on extracted, filtered water by using a Metrohm 850 Professional Ion Chromatograph.

The concentration of DOC was measured on filtered and acidified samples using a Thermalox TOC/TN analyser (Analytical Sciences Ltd) equipped with a non- dispersive infrared CO_2 analyser. Microbial respiration (CO_2, CH_4 and N_2O) was also measured in peat samples using Gas Chromatography equipped with flame ionization detector (FID) for measuring the concentration of CO_2 and CH_4 and electron capture detector (ECD) for measuring the concentration of N_2O.

6.3.3. Nucleic acid extraction and polymerase chain reactions (PCR) amplification

DNA was isolated from soil samples of the superficial layer (54 samples) using the PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA). The amount of extracted DNA was checked using a Thermo Scientific NanoDrop 2000c machine.

Eukaryote communities were assessed by amplifying the V4 regions of the 18S rDNA gene using the broad-spectrum eukaryotic primers (TAReuk454FWD1 and TAReukREV3) (Stoeck *et al.* 2010).

The PCR reaction mixture (25 µL) contained 12.5 µL 2X Phusion Master Mix (New England BioLabs Inc.), 0.4 µM of each of the primers, approximately 10 ng of isolated DNA as template, and 9.5 µL of nuclease-free water. The PCR reactions basically comprised an initial denaturation step at 95°C for 5 minutes, followed by 10 three-step cycles consisting of 94ºC for 30 seconds, 60 ºC for 45 seconds, and 72 ºC for 1 minute, followed by 25 further three-step cycles consisting of 94º C for 30 seconds, and primer annealing temperature of 50 ºC for 45 seconds, and 72º C for 1 minutes and a final two minutes elongation at 72 ºC (ProFlex™ 3 x 32-well PCR System, Thermo Scientific).

High throughput sequencing was performed externally by the Research Technology Support Facility Genomics Core in Michigan State University (USA).

6.3.4. Sequence processing

Raw sequences were processed using the DADA2 R package and following a standard pipeline of paired end Illumina MiSeq data analysis (https://benjjneb.github.io/dada2/tutorial.html) to produce the final taxa and sequence tables. In short, primers were eliminated from sequences and the quality of reads was checked before trimming them at 230 bases to avoid low quality scores present at the end of the reads, a common problem in illumine sequencing. Reads were denoised (to combine all identical sequencing reads into unique sequences), paired reads were merged, and chimeras were checked and removed. The amplicon sequence variant table (ASV) (a higher-resolution version of the OTU table) was constructed. Sequences were clustered into operational taxonomic units (OTUs) based on the RDP naive Bayesian classifier method (97% sequence similarity). Sequences were compared with the PR^2 database using the *assignTaxonomy* function of DADA2 package.

Generally, it is worth noting that amplicon sequence data are semi-quantitative. For example, in prokaryotes for 16S rRNA-based data, it has been shown that due to fluctuation of ribosomal operon copy numbers between microbial species, an absolute quantification of a given OTU is not feasible (Jacquiod *et al.* 2016). This same issue would also be relevant in eukaryotes where 18S rRNA is used as a genetic marker (Boers *et al*. 2019).

A primary goal of microbiome studies can be to determine which microbial taxa are present and, by implication, that may play roles in ecosystem functioning. These studies may also entail assessment of differential abundance between different taxonomic groups (differentially abundant taxa). Whilst these abundance surveys are often achieved through amplification of the ribosomal RNA (rRNA) gene amplicon with “universal” primer sets before high-throughput sequencing, the fact that the nucleic acids are amplified experimentally means that they can only be illustrative of potential differential abundance of each taxon in each sample. Whilst a range of standard statistical tests or microbiome-specific packages can be used to assess differential abundance, it is important to note that these measurements are at best semiquantitative as they cannot enumerate the absolute number of individuals of a given species due to the amplification stage. Hence, data reported in this

study are semiquantitative illustrations of likely relative abundances, and must be treated cautiously.

6.3.5. Data analyses

Firstly, *phyloseq* package was used to import and visualize the data (sequences and taxonomy tables). The complete OTUs database generated after sequencing analysis was used for diversity analysis of the micro-eukaryote community, while community composition analysis was conducted only on the taxa with > 0.01% of relative abundance in order to reduce the effect of rare species in the ordination analysis.

Redundancy analysis (RDA) was used to discover which soil physicochemical properties were the most important in determining the community structure of micro-eukaryotes, as well as which microbial metabolic factors were most strongly correlated with protistan and fungal community composition (*vegan* package). Before conducting RDA, a presence-absence transformation was performed to calculate species number per site and then the rare species were removed. Next, gradient length was checked using detrended correspondence analysis (DCA). Community data was Hellinger transformed (Legendre and Gallagher 2001). The significant subset of the explanatory variables was selected using forward selection. Briefly, an initial global test was conducted, and model significance was tested using the Monte Carlo permutation test. If significant, forward selection was conducted using the *ordistep* function from *vegan* package. RDA plots represent only the selected subset of variables. Alpha diversity was calculated from an abundance matrix using Shannon's index (*vegan* package). Barplot and boxplot were performed to determine the relative abundance by *ggplot2* package, venn diagram was performed to determine the number of exclusive and shared OTUs by *vennDiagram* package.

Statistically significant differences between the three climatic regions in terms of richness, diversity and relative abundances were analysed using ANOVA with Tukey's HSD post hoc test ($p < 0.05$) (*nlme* and *multcomp* packages). All statistical analyses and graphs were carried out using R V.3.5.1.

6.4. Results

6.4.1. Eukaryotic community structure

After quality filtering, a total of 7,519,493 sequences and 18555 operational taxonomic units (OTUs) were obtained based on 97% similarity. More than half of the eukaryotic OTUs belonged to the protist group (64%), followed by animal (15%), fungi (7%) and plant (1%). In terms of the relative abundance of reads, most eukaryotic reads belonged to the animal group (42%), followed by protist (39%), fungi (9%) and plant (7%) (Figure 6.01&Table 6.01).

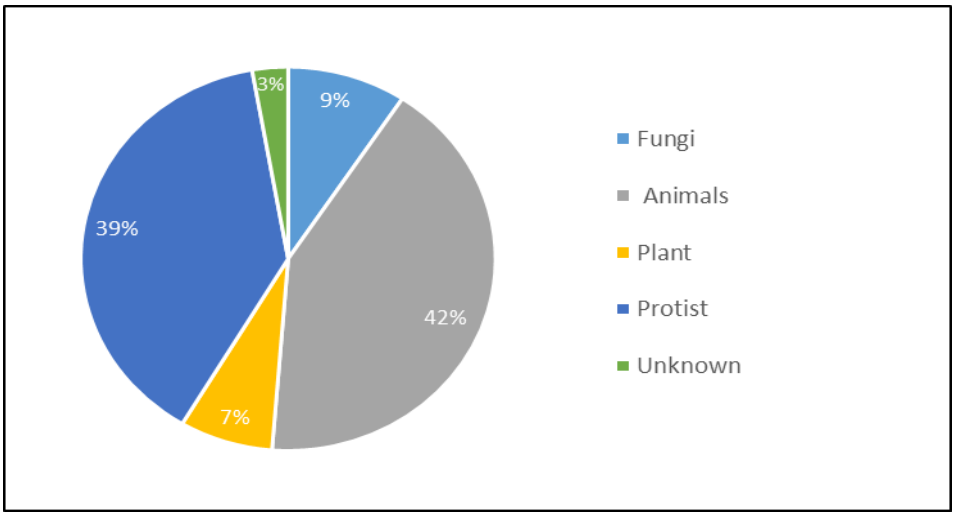

Figure 6.01. General relative abundance of reads associated with eukaryote groups. The animal group includes the Metazoa (Annelida, Arthropoda, Cnidaria, Gastrotricha, Mollusca, Nematoda, Platyhelminthes, Rotifera and Tardigrada taxa). The plant group includes the Streptophyta (Embryophyceae taxa).

The relative abundance of the protistan group was higher in the Svalbard peatland than peatlands in both Colombia and Wales ($F = 20$, $P < 0.001$). The fungal group was higher in the Colombian peatland than in the other two regions ($F = 19.54$, $P < 0.001$). The relative abundance of the animal group was higher in Wales than in the other two regions ($F = 10.8$, $P < 0.001$. Finally, the relative abundance of the plant group was lower in Colombia than in the other two regions ($F = 7.094$, $P < 0.01$) (Table 6.01).

Table 6.01. The relative abundance of eukaryote groups. The different letters denote statistically significant differences identified using the Tukey's HSD post-hoc test ($p < 0.05$).

Eukaryote groups	reads	OTUs	Svalbard (S)	Colombia (C)	Wales (W)
Protist	39.0%	64.0%	49.5% CW	21.8% SW	28.7% SC
Animal	42%	15%	20.1% W	33.2 W	46.7% SC
Plants	7.0%	1.0%	37.0% C	14.0% SW	49.0% C
Fungi	9.0%	7.0%	9.5% C	80.0% SW	10.4% C

6.4.2. Micro-eukaryotic community patterns across studied peatlands

After removing the OTUs of the Metazoa (animals) and Streptophyta (plants), 3,828,089 sequences were obtained and 15507 OTUs were found to be affiliated to micro-eukaryote organisms.

40.4% (6265) of obtained OTUs were observed in Colombia, 37.4% (5801) in Svalbard, whereas the fewest were found in the Welsh peatland (27.3%, 4229 OTUs). Only 55 of the OTUs (0.4%) were shared between all the three regions (Figure 6.02).

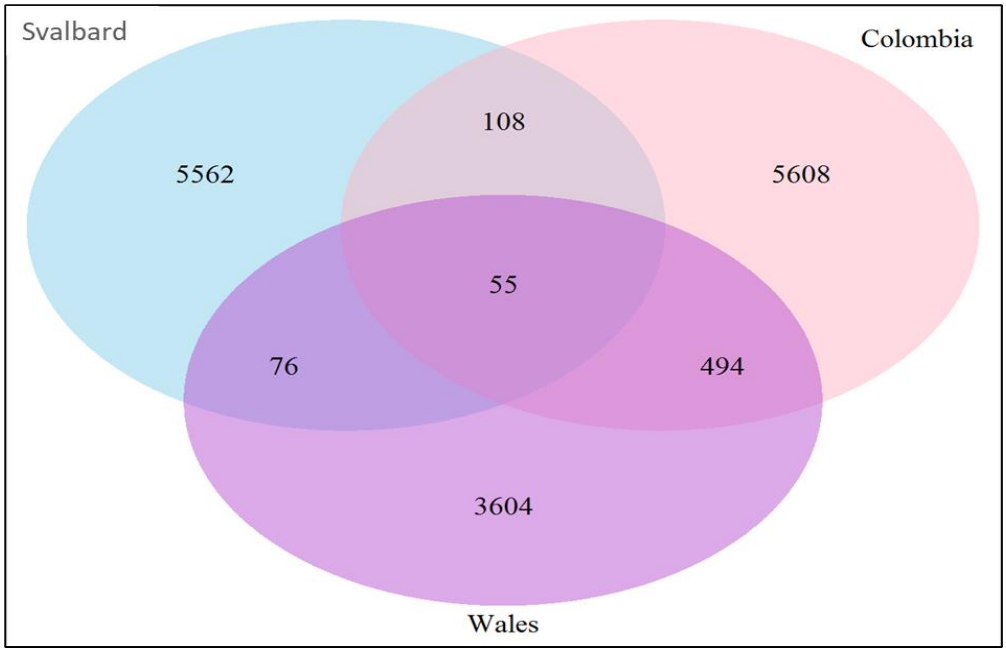

Figure 6.02. Venn diagram representing the number of exclusive and shared micro-eukaryote OTUs between the three different climatic regions.

Micro-eukaryotic alpha diversity and richness did not differ significantly between the three climatic regions *(F* = 1.614, *P* = 0.210 and *F* = 1.45, *P* = 0.247, respectively) (Figure 6.03).

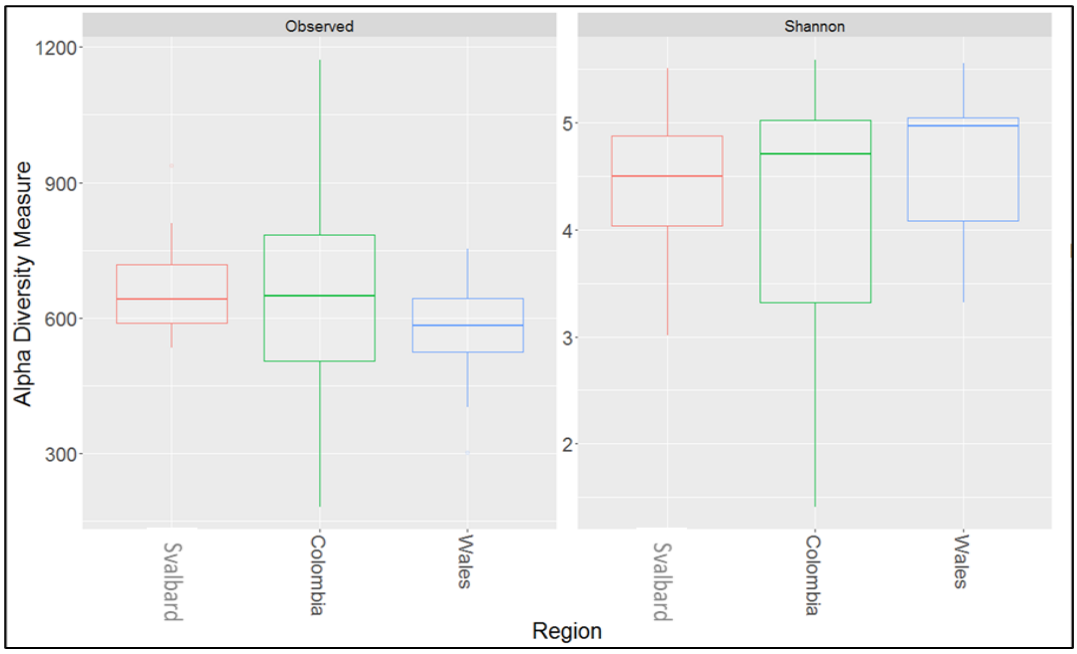

Figure 6.03. Richness and diversity of micro-eukaryote OTUs in the contrasting regions.

6.4.3. Comparison of micro-eukaryote taxonomic and functional groups between peatlands

Alveolates (44.3% of all micro-eukaryotes reads, 22.6% of all OTUs) dominated the micro-eukaryotic community, followed by Opisthokonta (reads: 21.8%, OTUs: 18.3%), Rhizaria (reads: 11%, OTUs: 30.2%), Stramenopiles (reads: 11%, OTUs: 16%) and Amoebozoa (reads: 6.8%, OTUs: 7.6%). The else groups, that is, Hacrobia (reads: 2.8%, OTUs: 1.3%), Chlorophyta (reads: 2.1%, OTUs: 2.8%), Excavata (reads: 0.2%, OTUs: 0.6%) and Apusozoa (reads: 0.14%, OTUs: 0.5%) were generally less numerous (Figure 6.04&Table 6.02).

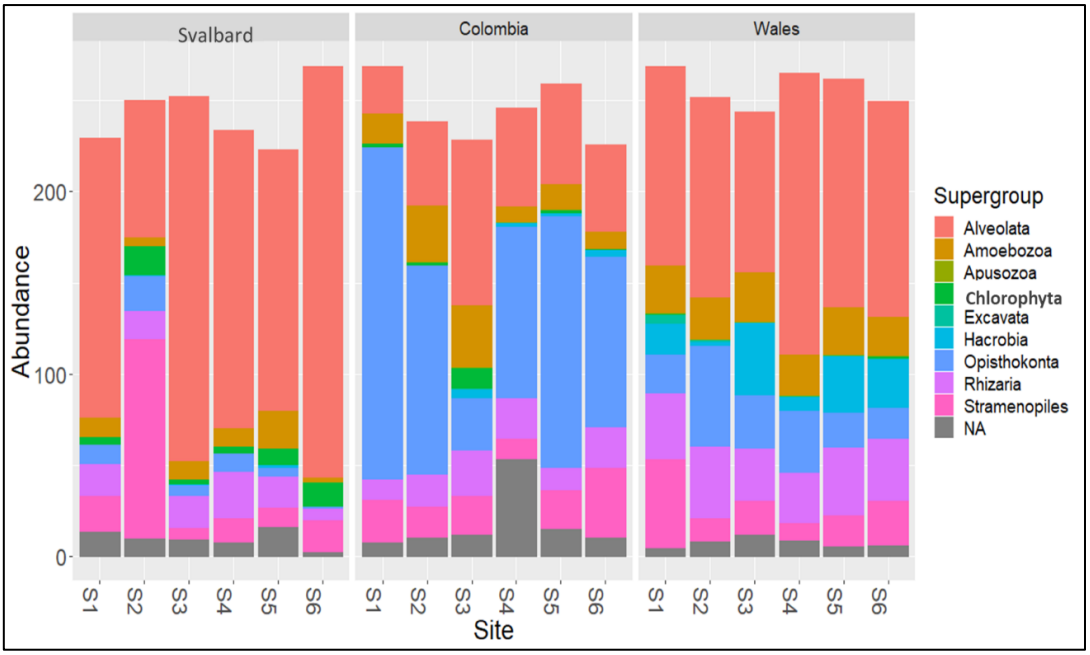

Figure 6.04. Stacked barplot representing the community composition of micro-eukaryote supergroups at each site within the different climatic regions. NA, not available.

All the nine micro-eukaryote supergroups and the five functional groups were found at each region (Figures 6.04& 6.05, Appendix B Figures 8&9, Tables 6.02& 6.03).

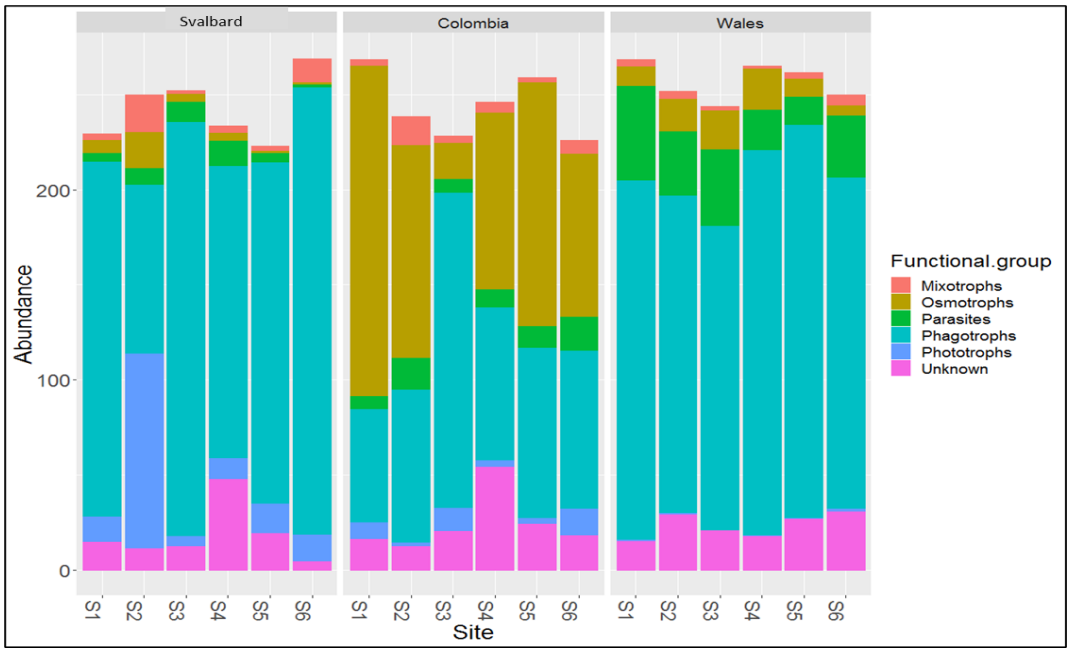

Figure 6.05. The relative abundance of the five functional groups of micro-eukaryotes at each site within each climatic region.

Alveolates and Chlorophyta were significantly more abundant in Svalbard compared with the others (F = 21.4, P = 0.000 and F = 7.95, P = 0.001, respectively). Opisthokonta organisms were more abundant in Colombian peat (F = 12.3, P = 0.000), while Hacrobia and Excavata were highest in Wales (F = 5.03, P = 0.01 and F = 5.5, P = 0.007, respectively). No differences in the relative abundance of Amoebozoa, Apusozoa, Rhizaria and Stramenopiles were observed (Table 6.02, $p < 0.05$).

Our results indicated significant differences in the richness, diversity and the relative abundance of the functional groups ($p < 0.05$). The OTUs richness of mixotrophic group was higher in the Arctic region than Wales, while no significant differences between the three regions in the mixotrophic diversity (Appendix B Figure 2, $p < 0.05$). The relative abundance of mixotrophs, dominated by Chrysophyceae (represented 90.5% of mixotrophic reads), was highest in Svalbard than Colombia and Wales (Table 6.03, $p < 0.05$).

Table 6.02. The relative abundance of micro-eukaryotic supergroups. The different letters denote statistically significant differences identified using the Tukey's HSD post-hoc test ($p < 0.05$).

Supergroup	reads	OTUs	Svalbard (S)	Colombia (C)	Wales (W)
Alveolata	44.3%	22.6%	59.1% CW	15.6% S	25.3% S
Amoebozoa	6.8%	7.6%	28.8%	37.4%	33.8%
Apusozoa	0.14%	0.5%	27.5%	29.1%	43.4%
Chlorophyta	2.10%	2.8%	78.3% CW	19.1% S	2.6% S
Excavata	0.16%	0.6%	9.2% W	24.25%	66.6% S
Hacrobia	2.8%	1.3%	4.4% W	10.0% W	85.6% SC
Opisthokonta	21.8%	18.3%	10.4% C	75.2% SW	14.4% C
Rhizaria	11.0%	30.2%	34.4%	32.0%	33.6%
Stramenopiles	11.0%	16.0%	53.0%	27.0%	20.0%

Osmotrophs group was more abundant in Colombia than both Svalbard and Wales (Table 6.03, $p < 0.05$), and was dominated by Mucoromycota (represented 41.2% of osmotrophic reads), Ascomycota (represented 18.23% of osmotrophic reads) and Basidiomycota

(represented 14.98% of osmotrophic reads) (Appendix B Table 1). The OTUs richness of osmotrophs was higher in Colombia than both Arctic and Wales, while no differences in the diversity were detected (Appendix B Figure 3, $p < 0.05$). The relative abundances of phagotrophs, which dominated by ciliated Spirotrichea (represented 29.6% of phagotrophic reads) and Oligohymenophorea (represented 19.0% of phagotrophic reads), and phototrophs, which dominated by Bacillariophyta (represented 56.1% of phototrophic reads) and Chlorophyta (represented 34.6% of phototrophic reads), were highest in Svalbard (Table 6.03, Appendix B Table 1, $p < 0.05$). The OTUs richness of phagotropic group was higher in Svalbard than Wales and the diversity of phagotrophs was higher in Colombia than the other two regions (Appendix B Figure 4, $p < 0.05$). The richness of phototropic OTUs was higher in Svalbard than Wales and Colombia. The latter was significantly higher than Wales. The phototrophs diversity was higher in Svalbard than both Colombia and Wales (Appendix B Figure 5, $p < 0.05$). The OTUs richness of parasitic group was higher in Wales than the two other regions, and the diversity was higher in Wales than Svalbard (Appendix B Figure 6, $p < 0.05$). The relative abundance of parasitic group was significantly higher in Wales than both Svalbard and Colombia (Table 6.03, $p < 0.05$), and was dominated by apicomplexan parasites (represented 52.4% of parasitic reads). These are widespread parasites of humans and animals. Plant-parasitic Oomycetes were the second more abundant parasitic group (represented 12.6% of parasitic reads), followed by Phytomyxa (represented 6.3% of parasitic reads), Perkinsea and Ichthyosporea (represented 1.3% and 1.2% of parasitic reads, respectively). All effects are outlined in Table 6.03& Appendix B Table 1.

Table 6.03. The relative abundance of micro-eukaryotic functional groups. The different letters denote statistically significant differences identified using the Tukey's HSD post-hoc test ($p < 0.05$).

Functional group	reads	OTUs	Svalbard (S)	Colombia (C)	Wales (W)
Mixotrophs	3.0%	4.0%	57.0% CW	29.1% SW	13.8% SC
Osmotrophs	17.2%	5.6%	7.8% C	84.0% SW	8.2% C
Parasites	6.0%	8.2%	22.8% W	30.1% W	47.1% SC
Phagotrophs	59.3%	52.0%	49.7% CW	21.0% SW	29.4% SC
Phototrophs	5.7%	5.0%	81.7% CW	16.4% SW	2.0% SC

As can be seen in Table 6.04, Ciliophora (within Alveolata) and Cercozoa (within Rhizaria) were the most popular and OTU-rich groups (reads: 38.5%, OTUs: 14% and reads:11.0%, OTUs: 25.5%, respectively) (Figure 6.06). Ciliophora was the highest in the Arctic compared to the other regions ($p < 0.05$). Within Rhizaria, phytomyxean parasites represented 14.6% of the Cercozoa reads. The typical inhabitants of peat bogs within Rhizaria such as orders Euglyphida (represented 31.5% of Filosa reads), Limnofilida (represented 30.0% of Filosa reads) and Vampyrellida (represented 99.0% of Endomyxa reads) were widely observed in all the samples of the present study.

Fungi represented 19.8% of Opisthokonts reads (8.5% of OTUs), with greater relative abundance in the Colombian peat than the others. Fungi group was dominated by Mucoromycota (38.6% of reads), Ascomycota (17.06% of reads) and Basidiomycota (14.03% of reads). Parasitic Apicomplexa (reads: 4%, OTUs: 3%) was comprised 86.5% Gregarines and 8.4% Coccidia. Parasitic Mesomycetozoa (reads: 0.1%, OTUs: 0.5%) was dominated by Ichthyosporea (98.3% of reads). All of these parasitic groups were higher in the Welsh peat than the other two peatlands (Table 6.04, $p < 0.05$). Stramenopiles (reads: 3.8%, OTUs: 5.3%) were more abundant in Wales and Colombia. Ochrophyta (reads: 7.2%, OTUs: 8.2%) was more abundant in Svalbard than both Wales and Colombia.

In addition, less abundant groups differed between the three regions such as Amoebozoa, Chlorophyta, Perkinsea and Telonemia being more abundant in Svalbard than the others, Choanoflagellida, Hilomonadea, Katablepharidophyta, Lobosa and Metamonada being higher in Wales than the others, Conosa being more abundant in the Colombian peat than Wales, while the opposite pattern was observed for Dinoflagellate (Table 6.04, $p < 0.05$). Within Amoebozoa, Variosea was the dominant Conosa taxa (represented 99.7% of Conosan reads) and Tubulina was the dominant Lobosa (represented 26.36% of Lobosan reads). Order Arcellinida (represented 54.1% of Tubulina reads) was higher in the Welsh peat than the other two peatlands ($p < 0.05$).

A statistically significant positive correlation was found between parasite and animal relative abundance in Wales ($n = 18$, $r = 0.740$, $p < 0.01$), while in Colombia and the Arctic there was no statistically significant correlation (Figure 6.07).

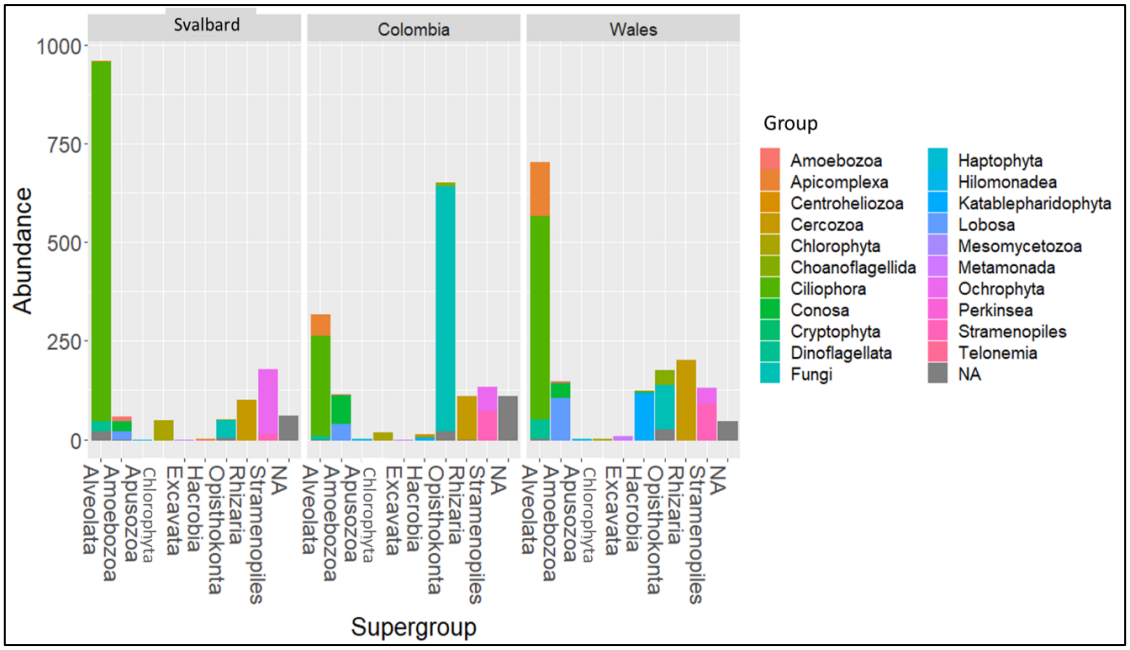

Figure 6.06. Stacked barplot representing the community composition of micro-eukaryote groups within each supergroup at each region. NA, not available.

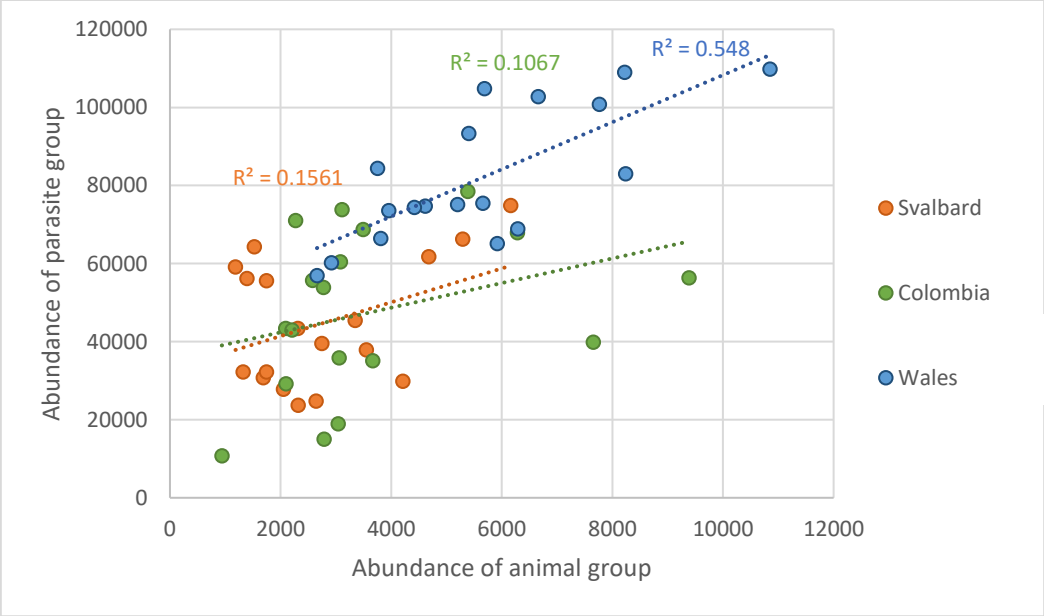

Figure 6.07. Animal vs. parasite relative abundance in Svalbard, Colombia and Wales (n = 18 for each region).

Table 6.04. The relative abundance of micro-eukaryotic groups. The different letters denote statistically significant differences identified using the Tukey's HSD post-hoc test ($p < 0.05$).

Supergroup	Group	reads	OTUs	Svalbard (S)	Colombia (C)	Wales (W)
Alveolata	Apicomplexa	4.0%	3.0%	1.6% CW	38.0% SW	60.3% SC
	Ciliophora	38.5%	14.0%	64.7% CW	14.0% S	21.3% S
	Dinoflagellata	1.7%	1.5%	35.0%	12.0% W	53.0% C
	Perkinsea	0.1%	0.45%	67.2% CW	7.0% S	25.7% S
Amoebozoa	Amoebozoa	0.3%	0.3%	88.3% CW	4.3% S	7.5% S
	Conosa	3.0%	3.0%	27.5%	52.4% W	20.0% C
	Lobosa	3.5%	2.8%	23.5% W	27.7% W	48.8% SC
Apusozoa	Hilomonadea	0.1%	0.2%	4.8% CW	39.3% SW	55.8% SC
Chlorophyta	Chlorophyta	2.1%	2.3%	78.3% CW	19.1% S	2.6% S
Excavata	Metamonada	0.1%	0.4%	1.6% W	24.5% W	74.0% SC
Hacrobia	Centroheliozoa	0.2%	0.5%	34.5% C	54.4% SW	11.2% C
	Cryptophyta	0.1%	0.1%	9.0% W	21.3%	69.5% S
	Haptophyta	0.1%	0.25%	2.0% C	68.0% S	30.0%
	Katablepharidophyta	2.4%	0.3%	0.0% W	3.0% W	97.0% SC
	Telonemia	0.03%	0.01%	98.0% CW	2.0% S	0.0% S
Opisthokonta	Choanoflagellida	1.0%	2.1%	14.6% W	19.6% W	65.8% SC
	Fungi	19.8%	8.5%	9.5% C	80.0% SW	10.4% C
	Mesomycetozoa	0.1%	0.5%	32.3% W	22.3% W	45.4% SC
Rhizaria	Cercozoa	11.0%	25.5%	34.4%	32.0%	33.6%
Stramenopiles	Ochrophyta	7.2%	8.2%	71.0% CW	18.6% S	10.5% S
	Stramenopiles	3.8%	5.3%	18.2% CW	43.8% S	38.0% S

6.4.4. Abiotic parameters and metabolic factors

The selected soil variables (8 variables of 20), that significantly influenced micro-eukaryote community composition, were soil temperature ($F = 312.7$, $P < 0.001$), pH ($F = 130$, $P < 0.001$), conductivity ($F = 25.1$, $P < 0.001$), dissolved oxygen concentration ($F = 72.2$, $P < 0.001$), SOM content ($F = 30$, $P < 0.001$), DOC concentration ($F = 3.34$, $P = 0.001$), phenolics concentration ($F = 4.4$, $P = 0.006$), and water content ($F = 9.3$, $P < 0.001$) (Figure 6.08a). The selected metabolic factors were the enzymes ß-glucosidase (B) ($F = 5.04$, $P = 0.010$), arylsulfatase (S) ($F = 42.3$, $P < 0.001$), chitinase (N) ($F = 20.0$, $P < 0.001$), phosphatase (P) ($F = 9.02$, $P < 0.001$), phenol oxidase (POX) ($F = 19.7$, $P < 0.001$) and nitrous oxide gas (N_2O) ($F = 8.8$, $P = 0.001$) (Figure 6.08b).

Results of the redundancy analysis (RDA) showed that the differences in abiotic and metabolic factors could explain the differences in the structure of micro-eukaryotic communities between the three contrasting peatlands. In the ordination space, samples within each region appeared close together because they had similar community structure. The first two axes of the graph explained 22% (Figure 6.08a) and 15.8% (Figure 6.08b) of the total variance and separated the samples in 3 groups, Svalbard, Colombia and Wales ($F = 1.992$, $P = 0.001$).

Microbial community structure in Svalbard was characterized by an increasing dominance of phagotrophs, phototrophs and mixotrophs with increased pH, conductivity, SOM content, and oxygen concentration and decreased temperature, while higher temperature and phenolics concentration and lower pH, nutrient availability and oxygen concentration structured the community composition in the temperate Welsh peatland, which represented by an increasing dominance of parasitic group and decreasing dominance of other groups. Micro-eukaryotic communities in the tropical Colombian peatland were dominated by osmotrophs and determined by lower phenolic concentration, and higher soil moisture and DOC concentration (Figure 6.08a, Appendix B Table 2a, $p < 0.05$). In addition to the metabolic factors (ß-glucosidase (B), N-acetyl-ß-D-glucosaminidase or chitinase (N), arylsulfatase (S), phosphatase (P) and phenol oxidase (POX)) that correlated with the community composition of soil micro eukaryotes in Colombia (Figure 6.08b, Appendix B Table 2b, $p < 0.05$).

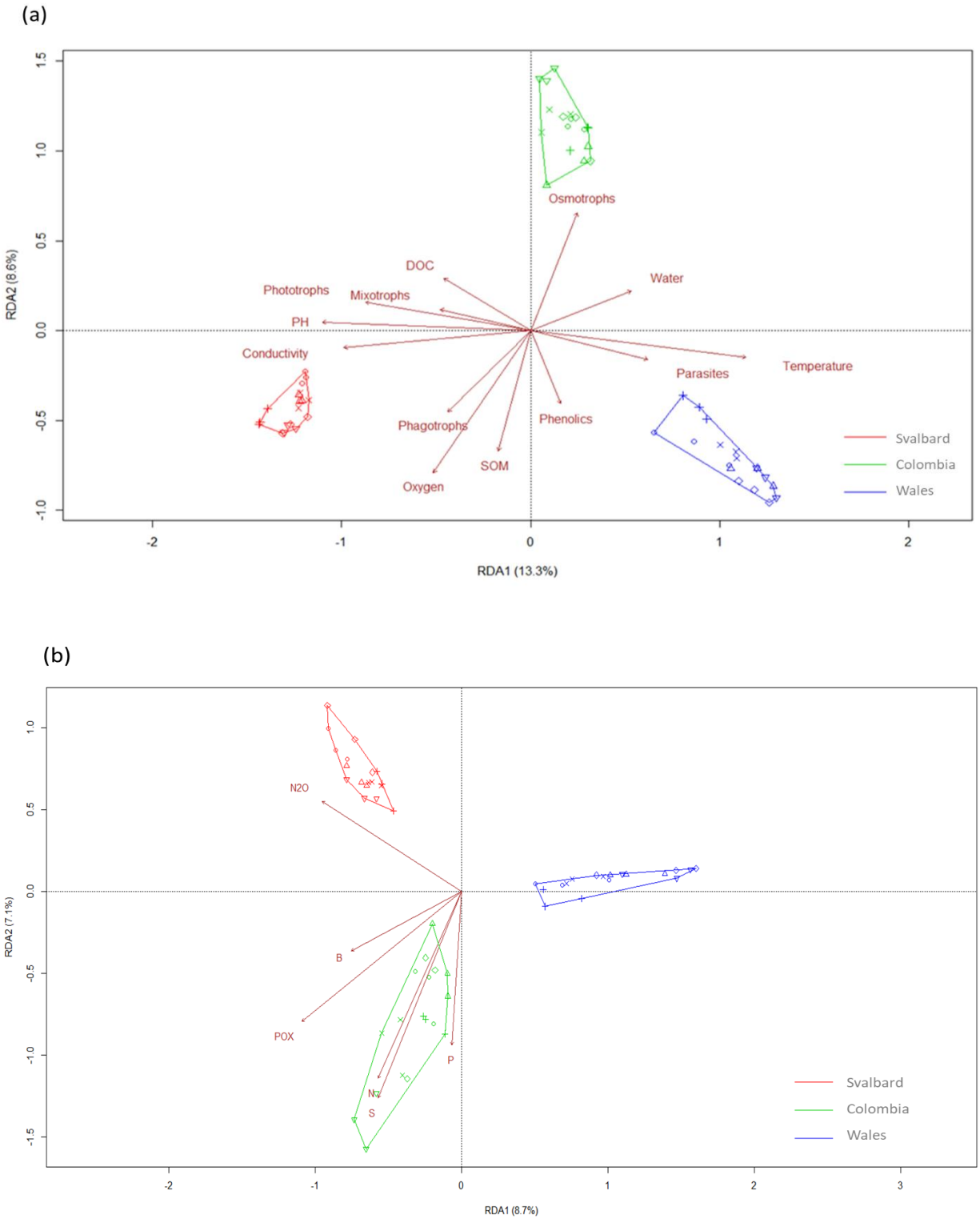

Figure 6.08. Redundancy analysis (RDA) based on the selected soil variables with the functional groups of micro-eukaryotes (a) and the selected metabolic factors (b). The different shapes represent the different sampling sites in each peatland. Abbreviations: dissolved organic carbon (DOC), soil organic matter (SOM), ß-glucosidase (B), N-acetyl-ß-D-glucosaminidase or chitinase (N), arylsulfatase (S), phosphatase (P), phenol oxidase (POX) and nitrous oxide gas (N_2O).

6.5. Discussion

Peatlands at a global scale occur in a wide range of environmental conditions, which makes them suitable areas for studying different environmental determinants of microbial community structure (Więcek *et al.* 2013). Understanding these factors is required as a prior condition for using soil micro-eukaryotes in bioindication to reflect soil quality, heterogeneity of different habitats and for predicting the ecosystem response to future environmental changes (Geisen *et al.* 2018). Clearly, the communities of microbial eukaryotes varied greatly between the three compared regions included in the present study as they shared only 0.4% of OTUs. However, the observed differences in the relative abundances and the community composition were not mirrored in OTUs richness and diversity, as both analyses were similar across the three regions.

6.5.1. Common inhabitants of peatland and the consideration of biotic factors

In this study, the general patterns of peat soils micro-eukaryote abundances were in line with those reported previously. For example, the recording of highest relative abundances for the supergroup Alveolata (with the Ciliophora being the most abundant), followed by Opisthokonta (dominated by Fungi), Rhizaria (exclusively Cercozoa) and Stramenopiles (mostly Ochrophyta), was consistent with a number of other peatland studies (Gilbert and Mitchell 2006, Lara *et al.* 2011, Geisen *et al.* 2015).

Micro-eukaryotic parasites are highly diverse and widespread across the eukaryotic tree of life (Bass *et al.* 2015). In the present study, parasitic organisms were distributed across the supergroups Alveolata, Opisthokonta, Rhizaria and Stramenopiles. Apicomplexan parasites (within supergroup Alveolata) were dominated by Gregarines and were more abundant in the temperate Welsh soil compared with the other two regions. Gregarines are widespread parasites of arthropods and other invertebrates (Mahé *et al.* 2017). This dominating parasite can be attributed to the higher relative abundance of the animal group, including potential invertebrate hosts which were found in the Welsh peatland. Indeed, a statistically positive correlation was found in Wales between parasites and animals' relative abundances. This pattern is supported by several previous studies (Grabda 1991, Mendonca 2011, Hong *et al.* 2014, Dupont *et al.* 2016, Mahé *et al.* 2017, Araujo *et al.* 2018). Similarly, Opisthokonta Ichthyosporea (Mesomycetozoan parasites) are targeting the metazoan taxa as hosts (Mendoza *et al.* 2002, Marshall *et al.* 2008), and they were most common in the Welsh peatland. Animal parasitic Ichthyosporeas have been described as parasites of aquatic

animals, but recently have also been detected as inhabitants of terrestrial animals (Glockling *et al.* 2013, Geisen *et al.* 2015). As the Ichthyosporean taxa were found in all soil samples from Wales, Svalbard and Colombia, this suggests that they are also parasites of animals in terrestrial ecosystem. Additionally, parasitic Perkinsea within the Alveolata were more abundant in the Arctic peat samples probably as a result of a greater host availability (Arneberg *et al.* 1998, Lara *et al.* 2011).

Arctic peat was also dominated by phytomyxean parasites (within supergroup Rhizaria), which are considered pathogens of the primary producers in terrestrial, marine and freshwater habitats and have a negative effect on carbon fixation process (Neuhauser *et al.* 2011). Terrestrial Oomycetes (within supergroup Stramenopiles) that contain a variety of parasites of plants (Latijnhouwers *et al.* 2003) and other organisms (Phillips *et al.* 2008) were the second most abundant parasitic group, suggesting an important role in structuring higher plant communities. In this context, Thomas *et al.* (1997) who reviewed parasitism in wetlands, concluded that in addition to the pathogenic effects of parasites, they can also affect the distribution, behaviour and diversity of their hosts.

It should be noted that it is not intended that references to Alveolata and Rhizaria should be considered synonymous with parasites as both also contain non-parasites. For example, ciliates are a taxon-rich group of the Alveolata. Amongst all ciliates taxa, Colpodea and Haptoria are notably more diverse and well represented in soils. Most members of soil Rhizaria possess flagella and pseudopodia, and can therefore be associated to both amoebae and flagellates. This combined morphological variability appears particularly well suitable for foraging between soil aggregates. This group within Rhizaria includes the Glissomonads and Cercomonads, which are among the most abundant protists in soils (Geisen *et al.* 2018). In the present study, although supergroups Alveolata and Rhizaria contain parasitic organisms, there were diverse species of free-living protozoan organisms within these supergroups (e.g., ciliated Spirotrichea and Oligohymenophorea within Alveolata and Euglyphida and Limnofilida within Rhizaria).

The lower relative abundance of ciliates and most amoebas in Wales possibly due to the grazing effects of rotifers and arthropods (within the animal group) on protozoan communities. Indeed Francez (1986), Gilbert *et al*. (1998), Mitchell *et al*. (2003) and Mieczan *et al*. (2015), found that both rotifers and arthropods (copepods) play an important role in controlling the abundance of protozoa in peatland ecosystems.

The biodiversity of Opisthokonta comprises metazoans, fungi and several additional microbial eukaryote lineages. Recent molecular studies suggest that Opisthokonta should be expanded to include a diverse collection of primitively single-celled eukaryotes previously classified as Protozoa. In the present study, the biodiversity range of Opisthokonta included examples of Metazoa, Choanoflagellida, Mesomycetozoa and Fungi. After removing the OTUs of the Metazoa, soil Fungi were the dominant group. The fungal group was more abundant in tropical Colombian peatland, in agreement with the findings of Field *et al.* (2015) and Singer *et al*. (2016). This probably due to the low grazing by animal group (invertebrates), which were present in low abundance in the Colombian peatland (Crowther *et al.* 2012).

In agreement with Geisen *et al.* (2014, 2015), Variosea and Tubulinea (within Amoebozoan supergroup) were the dominant conosan and lobosan taxa, respectively. In particular, order Arcellinida (within Tubulina) had highest relative abundance in the Welsh peatland which had the highest field temperature at time of sampling, in agreement with earlier studies on *Sphagnum* bogs by Heal (1964) and Lara *et al.* (2011), who found the Arcellinida in higher numbers in the warm months. Other common peatland inhabitants such as those affiliated to rhizarian orders Euglyphida, Limnofilida and Vampyrellida were widely observed in all the samples, in agreement with earlier studies of peat bog environments (Strüder-Kypke and Schönborn 1999, Kreutz and Foissner 2006, Bass *et al.* 2009, Lara *et al.* 2011).

6.5.2. Soil variables driving micro-eukaryotic functional groups

Assessing the variation in soil micro-eukaryotes using environmental DNA metabarcoding is one of the most important aims of protists community ecology (Geisen *et al.* 2018).

As shown by the Redundancy Analysis (RDA), samples from Svalbard, Colombia and Wales did not overlap in the multivariate ordination space, meaning that each region has different community structure. In addition, the findings based on RDA indicated that the distribution and the relative abundance of peatland micro-eukaryotic communities were predictable based on eight soil variables; soil temperature, pH, electrical conductivity, water content, soil organic matter (SOM) content and the concentrations of oxygen, phenolic compounds and dissolved organic carbon (DOC). In addition to six metabolic factors that can be used as indicators for their producers; the enzymes ß-glucosidase (B), N-acetyl-ß-D-glucosaminidase or chitinase (N), arylsulfatase (S), phosphatase (P), phenol oxidase (POX) and nitrous oxide gas (N_2O). To easily visualize and explain the effects of soil variables on microbial

eukaryotes, all micro-eukaryotic organisms were organized into five functional groups on the basis of their trophic behaviour (nutritional mode); osmotrophs, phototrophs, phagotrophs, mixotrophs and parasites.

In line with the hypothesis of this study, the structure of micro-eukaryotes community based on the functional groups differed greatly between the three contrasting peatlands. This is likely to be attributed to the differences in soil abiotic factors, which strongly drive the above-ground plant species (Amorim and Batalha 2006, Maracahipes-Santos *et al.* 2017), and therefore potentially, below-ground soil biota as well (Araujo *et al.* 2017, 2018).

In general, and compared with all other environmental variables, soil water content is considered to be one of the main factors affecting key microbial processes in peatlands (Bonnett *et al.* 2017), and it is a major factor shaping richness, abundance and community composition of soil micro-eukaryotes along a transect from the Arctic to the tropics (Tsyganov *et al*. 2013). Given the soil water content as one of the factors chosen in the present study, soil micro-eukaryotes permanently need water for their mobility and functions. Consequently, their activities are constrained by lack of water. In this context, the biodiversity of rainforest soil ciliates decreased significantly under the influence of air drying (Geisen *et al*. 2014, Geisen *et al.* 2018). On the other hand, excess soil water content causes anaerobic conditions, the presence of which contribute to lower micro-eukaryotic growth rates to less than 25% of that associated with more ventilated environments (Fenchel and Finlay 1990).

Micro-eukaryotes show a wide range of responses to soil temperature depending on the species studied (Geisen *et al.* 2018). In this study, the high relative abundance of phototrophic Chlorophyta in the Arctic region is consistent with other studies from cold environments and is thought to relate to the high growth rate of micro-eukaryotic algae in low temperature conditions (Rose and Caron 2007). A significant influence of nutrient availability on the relative abundance of phototrophic Bacillariophyta was also found in the Arctic peat. A similar observation was reported by Gilbert *et al.* (1998) who found that the pigmented autotrophic Bacillariophyceae attained greater biomass concentrations in nutrient-rich environments and were closely correlated with total inorganic nutrients and conductivity. Amblard (1991) reported that the development ability of many phototrophic diatoms has not impeded by dark conditions, possibly due to the availability of dissolved nutrients and their ability to switch to heterotrophic feeding.

The parasitic group (dominated by apicomplexans) was most abundant in the Welsh peatland, most likely due to the higher temperature. This finding is in line with previous studies that found positive correlations between the spread of parasites and temperature but in the tropics zone (Singer *et al.* 2016, Mahé *et al.* 2017). In this context, Studer *et al*. (2010) stated that intermediate temperatures ranging from 20 to 25°C are optimal for the development and transmission of parasites. Also, the susceptibility of hosts to infection was increased with increasing temperature. Overall, among apicomplexan orders, Gregarines were dominant in both reads and OTUs, a finding consistent with a study of soil protist diversity in tropical rainforests, where apicomplexan Gregarines represented the majority of the total protist abundance (Mahé *et al.* 2017).

A number of researchers (e.g., Wynn-Williams 1982, Nilsson *et al.* 1992, Czeczuga 1993, Zvyagintsev 1994, Lara *et al.* 2010) noted that the fungal group is a characteristic inhabitant of peatlands. In the present study, the osmotrophic group (with the Mucoromycota, Ascomycota and Basidiomycota being the most abundant groups), dominated the samples from the tropical peatland possibly due to the relatively high pH conditions and hence the high extracellular enzymes activity. This in turn suggests higher rates of phenolic compound degradation (Min *et al.* 2015) and higher DOC release as reported in the present study and as confirmed by Kang *et al*. (2018). Additionally, it is well known that the composition of fungal community is affected by litter type (Andersen *et al.* 2013). According to Hodgkins *et al.* (2018) plant litter towards the tropics is dominated by recalcitrant compounds due to the expansion of woody trees and shrubs, which influence microbial communities and favour fungal growth (Andersen *et al.* 2013). Therefore, the vegetation cover in Colombia may be another reason for the presence of recalcitrant compound decomposers in large quantities.

A relatively low abundance of Fungi and hence low extracellular enzymes activity, in particular phenol oxidase, in the Arctic despite the significantly higher pH may be due to the low temperatures. Indeed, at each pH level tested, phenol oxidase activity and DOC concentration was higher at 15º C than at 5º C (Kang *et al.* 2018). The other possible reason for lower fungal relative abundance despite the higher pH in the Arctic may be consumption by phagotrophic organisms (Coûteaux and Dévaux 1983) which were found to be particularly abundant in the Arctic peatland.

Phagotrophs and phototrophs were abundantly observed in the Arctic most likely because of higher dissolved oxygen concentrations (Papadimitriou *et al.* 2010), the absence of which

leads to decrease the growth rates of eukaryotic microorganisms. A predator-prey relationship is another possible reason behind the higher relative abundance of phagotrophs and phototrophs in the Arctic peat, as demonstrated by Coûteaux and Pussard (1983). Gilbert *et al.* (1998) reported that the abundance of phagotrophic amoebas was strongly correlated with the abundance of phototrophic microorganisms such as Bacillariophyceae and Cyanobacteria and with other phagotrophic organisms such as flagellates and ciliates. All these organisms are likely the preferred food sources of phagotrophic amoebas. An additional reason for the high relative abundance of phagotrophs in the Arctic could be high abundance of their bacterial prey (Geisen *et al*. 2018). Also, the emission of N_2O gas from the Arctic soils may indicate the presence of bacterial prey there (Fierer 2017).

A relatively low abundance of phagotrophs in the phenolic-rich Welsh peatland could be due to low bacterial and fungal prey abundance. The negative effect of phenolic compounds on the growth rate of bacterial and fungal communities has been widely reported (Fung *et al.* 1985, Opelt *et al.* 2007, Mellegård *et al.* 2009, Pizzolitto *et al.* 2015), with the occurrence of predatory phagotrophs also likely to be inhibited. Other possible reasons for the low relative abundance of phagotrophic organisms in Wales is low pH, conductivity and DOC concentration, as provided by Mieczan (2007), who found positive relationship between pH, conductivity and total organic carbon concentration and the abundance of phagotrophic ciliates in the peat-bog reservoirs of eastern Poland. In this context, Papadimitriou *et al.* (2010), found high protozoan's abundance in a wetland with low electrical conductivity soils (< 3.0 mS/cm), and attributed this to the destructive effect of high conductivity on cells as a result of the osmotic phenomenon.

The nutrient-poor status of the Welsh peatland may also be responsible for the low relative abundance of phototrophs. Indeed, the density of phototrophic protists was increased by the addition of experimental fertilisers in nutrient depleted peatlands (Gilbert *et al.* 1998). A relatively low abundance of phototrophs in Wales could be also attributed to the high concentrations of phenolic compounds which stain soil water a dark colour (Lim *et al.* 2017), limiting the penetration of sunlight, and thus likely reducing the abundance of phototrophic microorganisms (Mieczan 2007). On the other hand, this darkness resulting from elevated phenolics concentrations could provide a natural protective shield for parasitic infective stages from harmful sunlight (Thomas *et al.* 1995, King *et al.* 2008).

It was observed that the abundance of reads related to mixotrophic organisms (mostly belonging to Chrysophyceae) were higher in the Arctic samples. This is likely due to the lower temperature (Lara *et al.* 2011) in this site that favours a higher growth rate of mixotrophic organisms. In this context, Jassey *et al.* (2015) discussed direct and indirect impacts of mixotrophic protists on peatland carbon cycle; as primary producers they directly contribute to carbon fixation through photosynthesis using chloroplasts of endosymbiotic algae, while as heterotrophs they can indirectly affect organic matter decomposition by feeding on microbial decomposers. They found through field and microcosm experiments that the mixotrophs were extremely sensitive to relatively high temperature, which negatively affected the phototrophic ability of mixotrophic organisms and caused a significant decline in their abundance. This suggests that the decreased abundance of mixotrophs in response to climate warming may lead to decreased peatland carbon fixation (Jassey *et al.* 2015). The declined in the abundance of mixotrophs due to the disruption of their phototrophic ability under warming is due to the fact that these organisms cannot live as pure heterotrophs without their algal endosymbionts that fail to transmit from mother to daughter cells during cell division under higher temperatures (Jassey *et al.* 2015).

Our findings conflict with a short-term warming experiment conducted by Wilken *et al.* (2012) who studied the response of mixotrophic organism *Ochromonas* sp. (within Chrysophyceae) to warming in freshwater samples and concluded that with rising temperatures, mixotrophs tended to change their trophic behaviour towards the heterotrophic mode, which was followed by an increase in their abundance. This inconsistency of results suggests that the responses of microbial communities to environmental factors are complex, a reality attributed to the type of environmental samples and micro-eukaryote species under study (Geisen *et al.* 2018).

6.6. Conclusions

The peatlands included in this study, representing a range of climatic conditions, all showed diverse micro-eukaryotic communities, with all major micro-eukaryotic supergroups present in each region. Overall, the following four micro-eukaryotic supergroups emerged as dominant: Alveolata (dominated by Ciliophora), Opisthokonta (mostly Fungi), Rhizaria (exclusively Cercozoa) and Stramenopiles (with Ochrophyta being the most abundant) were dominated the peat samples of the present study, in agreement with previous peatland studies.

The observations based on the redundancy analysis revealed that micro-eukaryotic functional groups differed significantly between the three regions and are strongly influenced by the prevailing environmental conditions. This supported the hypothesis that the micro-eukaryotic community composition can in part be predicted by several abiotic soil variables. For example, high temperature and phenolic compound concentration, acidic pH, and low nutrient availability and oxygen concentration in the Welsh peatland were found to be optimal for parasites and sub-optimal for phototrophs, phagotrophs, mixotrophs and enzyme-producing organisms. By contrast, the edaphic factors associated with the Arctic peatlands (low temperature, high pH, conductivity, SOM content, oxygen concentration) were relatively more favourable and produced higher relative abundances of mixotrophic, phototrophic and phagotrophic microorganisms. In the Colombian peatland, the relatively high abundance of osmotrophs (mostly Fungi) was consistent with low phenolic concentrations and high pH, extracellular enzyme activities and DOC concentrations. This finding could improve knowledge of micro-eukaryotic communities in peatlands from a global perspective focusing on the physicochemical constraints upon them, which allow to predict how peatlands structure and function may respond to the potential climate change.

Parasites were ubiquitous in all the studied samples, which could have potential implications for public health and economic issues since peatlands are a source of drinking water in many areas. An investigation of the extent to which peat soils contribute to the transmission of parasites to drinking water sources is recommended. The presence of prey organisms and the important roles of micro-metazoans were also considered as potential host and/or predator invertebrates.

In the present study, there was an emphasis on variations in soil properties as a driver of micro-eukaryotic community composition. Further studies consider how micro-eukaryotic communities might in turn impact on soil properties are recommended.

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Chapter 7

Final Discussion

7.1. Overview

Peatlands represent a significant terrestrial carbon pool, holding at least 550 Gt of atmospheric carbon as a result of the imbalance between organic matter production rates and decomposition rates in these persistently water-saturated environments (Mitsch and Gosselink 2000, Moore 2002, Holden 2005), which raising concerns over their potential to become a carbon source and contribute to climate change.

Due to the importance of soil enzymes in the processing of organic matter in wetland ecosystems, many researchers have investigated the factors influencing enzymes activity and biogenic greenhouse gas emissions (Dunn *et al*. 2014). Studying biogeochemical processes and the abiotic variables controlling them in peatlands has important implications for predicting the potential impacts of climate change, human intervention and management strategies on peatlands function. However, most studies in this field have been conducted in a single region with a limited number of studied variables and were focused on the uppermost layers of peat. This is problematic since soil microorganisms, which are central to the decomposition of soil organic matter and are directly responsible for a large proportion of soil respiration, occur throughout the soil profile (Jackson *et al*. 2009, Senga *et al*. 2015).

In line with this, the present study includes three peatlands along a climatic gradient, Arctic (Svalbard, Norway), temperate (North Wales, UK) and tropical (Andean mountains, Colombia). Also, the range of biotic and abiotic variables investigated was deliberately broad and included key hydrolytic enzymes (ß-D-glucosidase, arylsulfatase, ß -D-xylosidase, N-acetyl-ß-D-glucosaminidase (chitinase) and phosphatase), the oxidative enzyme phenol oxidase, the greenhouse gas (GHG), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), and the physicochemical variables, pH, electrical conductivity, dissolved organic carbon (DOC), phenolic compounds, soil organic matter (SOM), soil water content, and cations and anions concentration. Several aspects were considered in this study: depth-dependent enzymes activity was assessed in relation to a range of physicochemical variables in the three contrasting peatlands, fluxes of GHGs were also compared to physicochemical factors and enzyme activities, enzyme activities, metabolic end products and all other soil variables were compared across the three climatic regions.

The description of soil extracellular enzyme activities on a global basis reveals the different ranges of variation and different distributions of enzyme activities in relation to ecosystem variables, which provides an opportunity to compare contrasting ecosystems and to relate soil

microbial function to global patterns of microbial community composition, nutrient ratios and soil organic matter storage (Sinsabaugh *et al.* 2008). Differences in microbial function between peatlands in different climatic regions are expected since vegetation composition, peat chemical composition and hence decomposition rates, which depend on microbial community composition, will inevitably vary between climate zones (Preston *et al.* 2012). Indeed, Hodgkins *et al*. (2018) found that peaty soils from tropical peatlands are more chemically recalcitrant, having higher aromatic content and lower carbohydrate content than those from Arctic and boreal peatlands. This recalcitrance may allow tropical peats to persist despite warmer temperatures.

Additionally, at a global scale, peatlands occur across a wide range of environmental conditions, which makes them suitable areas for studying different structuring factors that shape microbial communities (Więcek *et al*. 2013). Peatlands are water-saturated environments thanks to the spongy layer of *Sphagnum* moss on their surface. This condition allows planktonic organisms to be mobile and dynamic. These organisms include prokaryotes, microbial eukaryotes and small metazoan taxa that together form the microbial food web where nutrients and energy are transferred through the trophic interactions (Jassey *et al.* 2015). Unlike prokaryotes, eukaryotic microorganisms have received little attention in many ecosystems including peatlands despite their importance as bioindicators of abiotic variations in their environments (Koenig *et al.* 2015). Peatlands structure (biotic communities) and function (carbon storage) are threatened due to ongoing global warming, caused by the accumulation of greenhouse gas in the atmosphere (IPCC 2013). Micro-eukaryotic communities, through their diverse functions, can directly and/or indirectly affect the carbon balance function of peatlands and hence possibly contribute to climatic warming (Dedysh *et al*. 2006, Jassey *et al.* 2015).

These communities are including fungi, the main decomposers of SOM, phagotrophic protozoa, the main consumers of decomposers (bacterial and fungal communities) and hence influence decomposition process and nutrient cycling, phototrophic algae which contribute to carbon sequestration through photosynthesis, mixotrophic protists which have photosynthetic and phagotrophic functions, and parasitic protists which contribute to animal and plant diversity (Araujo *et al.* 2018). Understanding potential controlling factors, using a climatic gradient, is required as a prior condition for using soil micro-eukaryotes in bioindication and for predicting the response of peatland structure and function to future environmental changes (Geisen *et al.* 2018).

In the present study, the deoxyribonucleic acid (DNA) from the superficial layers of peat soil was extracted and a broader range of micro-eukaryotes was covered in the three peatlands by amplifying V4 region of 18S SSU rRNA gene. The richness, diversity and relative abundance of micro-eukaryotes in the three peatlands were determined. Also, a redundancy analysis (RDA) was carried out in which attempts were made to relate micro-eukaryote community composition to biogeochemical variables in order to improve understanding of the environmental factors that influence their distribution at a global scale. To easily visualize and explain the effects of these soil variables on microbial eukaryotes, all micro-eukaryotic organisms were organized into five functional groups on the basis of the trophic behaviour (nutritional mode), osmotrophs, phototrophs, phagotrophs, mixotrophs and parasites. Further, the occurrence of predator and host invertebrates was noted as well as prey organisms and recognise their potential significance for the relative abundance of micro-eukaryotic organisms.

7.2. Environmental controls on the biogeochemistry of peatlands: a comparison of three regions.

Our study has shown contrasting results concerning the relationships between measured parameters across the three peatlands. Generally, within each of the three peatlands, the concentrations of phenolic compounds increased with depth and no further increase was observed at the deeper mineral layers in Svalbard and Colombia. Enzyme activities decreased with depth in all the three regions, in agreement with the majority of previous studies. The occurrence of highest GHG flux and enzymes activity in the superficial layer indicates that this is the most biologically active layer (Moore 2002). However, in the deeper mineral layer of the Svalbard peatland, phenol oxidase activity was as high as in the superficial layer, with higher pH and nutrient concentrations, and lower phenolic concentration also notable in this zone. Also, N_2O flux in the deeper layer did not differ significantly from that measured in the upper layers, further highlighting the importance of the deeper layers of peats. The CH_4 production in the three contrasting peatlands increased gradually with soil depth, indicating a shift from aerobic to anaerobic metabolic pathways with depth (Kotsyurbenko *et al.* 2004, Dedysh *et al.* 2006). The pH values were significantly different for each of the three regions, Svalbard (mean 7.1) > Colombia (mean 5.2) > Wales (mean 4.5). The dominant controlling factor for phenol oxidase activity in the Svalbard and Colombian peatlands was pH, while in Wales only

hydrolytic enzyme activities were positively correlated with pH. The response of hydrolytic enzymes activity and GHGs flux to phenolics concentration also varied across the three climatic regions, indicating that the peatlands in the three climatic zones differ in parent plant chemistry that controlling peat chemical composition and preservation across a latitudinal transect from the Arctic to the tropics (Hodgkins *et al*. 2018).

A negative correlation could be anticipated between soil enzyme activity and nutrient availability according to the economic model of enzyme production (Chròst 1991). In the present study, however, there were contradictory results concerning this issue. This may have been related to the soil samples obtained from the Svalbard, Colombian and Welsh peatlands having different nutrient concentrations and therefore microbial communities in these contrasting regions would be expected to have different nutrient (and therefore enzyme production rates) requirements.

The lack of significant correlation between enzymes activity and nutrients and between enzymes activity and GHG flux could be due to the measurement of independent legacy enzymes (Kang and Freeman 1999), as it was in Wales.

The comparison of soil extracellular enzyme activity on a global scale provides an opportunity for identification of the global patterns of biogeochemical processes and the dominant controlling factors. In this study, significant differences in measured parameters between different regions were identified. Soil enzymes activity (except for β-glucosidase) and the fluxes of GHG (except for CH_4) varied across the three climatic peatlands in relation to physicochemical variables. Relatively high hydrolase enzyme activities were observed in Colombia, possibly due to the higher demand for nutrients and/or higher energy supply. The present study showed that the activity of phenol oxidase was relatively high in the Svalbard site, where significantly higher pH and concentrations of most inorganic nutrients, and lower concentration of phenolic compounds was also observed. Conversely, the Welsh peatland exhibited the lowest phenol oxidase activity, consistent with lowest pH and consequently highest phenolic concentrations. Phenolic concentration was significantly higher in the Welsh and Colombian peatlands possibly due to the dominant vegetation being *Sphagnum* moss, which may also has contributed to the lower pH values in both regions. The higher mean DOC concentration in Colombia and Wales compared with Svalbard can be attributed to the higher primary productivity towards the equator where warmer temperatures and longer growing season (Hodgkins *et al.* 2018). In addition, decreased DOC concentration has been linked to

increased sea salt (Na^+, Cl^-, Ca^{2+}, Mg^{2+} and SO_4^{2-}) (Pilson 2012, Gough 2014), all of which were high in Svalbard possibly due to the proximity of the sampling sites to the sea.

Among measured enzymes, phosphatase activity was the highest in all three peatlands and was attributed to low phosphorus availability. The fluxes of CO_2 and N_2O were all highest in the Svalbard samples despite the lower temperature. Additionally, these sites had the highest nitrate concentration, possibly due to nutrient supply from bird guano that covered the sampling areas in Svalbard. The latter may mean that the chosen sampling location was less representative of Arctic sampling locations than assumed.

7.3. Biotic and abiotic variables driving micro-eukaryotic communities in peatlands

As would be expected, the communities of microbial eukaryotes varied greatly between the peatlands of the three regions. Data of micro-eukaryotic functional groups were coupled with abiotic and metabolic data using redundancy analysis (RDA) which showed that 22% and 15.8% of variance in micro-eukaryotic density was explained by abiotic and metabolic factors, respectively. The higher pH, conductivity, soil organic matter (SOM) content, oxygen concentration, and the lower temperature were the most important factors structuring micro-eukaryote communities in Svalbard, while higher temperature and phenolic compound concentrations and lower pH and nutrients structured the community composition in the Welsh peatland, and micro-eukaryotic communities in the Colombian peatland were distinguished by the high activities of the extracellular enzymes glucosidase, arylsulfatase, chitinase, phosphatase and phenol oxidase, which were consistent with the decreased phenolic concentrations, and with the high pH and dissolved organic carbon (DOC) concentrations.

The high relative abundance of phototrophic microorganisms (Chlorophyta) in the Arctic is likely to be linked to their adaptation to cold environments (Rose and Caron 2007). In addition, a significant influence of nutrient availability on the relative abundance of phototrophic Bacillariophyta (within supergroup Stramenopiles) was found in the Arctic peat. Similar observations have been reported in a French peatland by Gilbert *et al.* (1998), the pigmented autotrophic Bacillariophyceae attained greater biomass in nutrient-rich environments and were closely correlated with total inorganic nutrients and conductivity. Phagotrophs and phototrophs were more abundant in the Arctic peatland probably because of relatively high dissolved oxygen concentrations (Papadimitriou *et al*. 2010). High temperatures influence the

phototrophic ability of mixotropic organisms, thus decreasing abundance and growth rates (Jassey *et al.* 2015). Indeed, in the present study, it was observed that sequences related to mixotrophic organisms (mostly taxon Chrysophyceae) were more common in the Arctic samples, where the preferred lower temperature environment.

Kang *et al.* (2018) linked greater fungal abundance with the greater DOC release under rising pH conditions that accordingly enhanced key enzyme activities contributing to peat soil decomposition. In the present study, high fungal relative abundance in Colombia also appeared to be linked to high DOC concentrations, which may be driven by favourable (high pH) conditions for extracellular enzymes.

A relatively low abundance of fungi and hence low extracellular enzymes activity, in particular phenol oxidase, in the Arctic peatland despite the high pH may be due to the effect of low temperature on fungal relative abundance, enzyme activities and hence DOC release. Indeed, over a range of pH conditions, phenol oxidase activity and DOC concentrations were consistently higher at 15º C than at 5º C (Kang *et al.* 2018). The other possible reason for lower fungal relative abundance in the Arctic peatland may have been their consumption by phagotrophic organisms (Coûteaux and Dévaux 1983) that were found to be very abundant in the Arctic peatland.

A relatively low abundance of phagotrophs in the phenolic-rich Welsh peatland could be due to low bacterial and fungal prey. The inhibitory effects of phenolic compounds on the growth rates of bacterial and fungal communities have been widely reported (Fung *et al.* 1985, Opelt *et al*. 2007, Mellegård *et al.* 2009, Pizzolitto *et al.* 2015). The relatively low abundance of phototrophs in the Welsh peatlands may be due to their nutrient poor status. Indeed, the density of phototrophic protists has been shown to increase following fertilizer addition in nutrient depleted peatlands (Gilbert *et al.* 1998).

A relatively low abundance of phototrophs in Wales could be also attributed to the high concentrations of phenolic compounds. These compounds stain water in dark colour (Lim *et al*. 2017), limiting the penetration of sunlight, and thus likely reducing the abundance of phototrophic microorganisms (Mieczan 2007). On the other hand, this darkness resulting from elevated phenolics concentrations could provide a natural protective shield for parasitic infective stages from harmful sunlight (Thomas *et al.* 1995, King *et al.* 2008). The presence of hosts around the sampling areas could be the most important factor influencing parasite abundance since their presence is host availability-dependent (Hong *et al.* 2014, Mahé *et al*. 2017, Araujo *et al.* 2018). Parasitic groups (Apicomplexan and Mesomycetozoan parasites)

were more abundant in the temperate Welsh soil compared with the other two regions. This may be related to the higher relative abundance of the animal group, which includes the potential invertebrate hosts in Wales as confirmed in this study. This pattern (the tendency to find more abundant parasites where populations of host animals are higher) is supported by several previous studies (Grabda 1991, Mendonca 2011, Hong *et al*. 2014, Dupont *et al*. 2016, Mahé *et al.* 2017, Araujo *et al.* 2018).

The lower relative abundance of ciliates and most amoebas in Wales may be due to the grazing effects of rotifers and arthropods (within the animal group) on protozoan communities. Indeed, Francez (1986), Gilbert *et al*. (1998), Mitchell *et al*. (2003) and Mieczan *et al*. (2015), reported that both rotifers and arthropods (copepods) play an important role in controlling the abundance of protozoa in peatland ecosystems.

The general patterns of peat soil micro-eukaryote relative abundances observed in the present study were in line with other studies applied on peatlands, where the supergroups Alveolata (with group Ciliophora being the most abundant), followed by Opisthokonta (mostly Fungi), Rhizaria (exclusively contained the Cercozoa), and Stramenopiles (mostly Ochrophyta), were the dominant micro-eukaryote groups (Gilbert and Mitchell 2006, Lara *et al.* 2011, Geisen *et al*. 2015).

7.4. Conclusions

Understanding the relationship between enzyme activities, GHG emissions and physicochemical variables in peatlands, which are extensive sinks of carbon and nitrogen, is especially important (Moore 2002, Voigt *et al.* 2017, Leifeld and Menichetti 2018) and necessary for anticipating the ecosystem response to climate change, land use change and restoration attempts (Moore 2002).

In this study, the responses of soil enzymes activity and biogenic GHG emissions to physical and chemical properties were assessed within peat soils obtained from three climatic regions: Arctic (Svalbard, Norway), temperate (Snowdonia National Park in North Wales, UK) and tropical (Sumapaz National Park in Andes mountains, Colombia). Several aspects were considered in the present study: depth-dependent enzyme activity and the resultant metabolic end products in relation to the physicochemical variables were assessed in each of the three

peatlands, enzymes activity, metabolic end products and all other soil parameters across the three climatic regions were compared.

This study is the first study giving a comparative view of biogeochemical processes in relation to the surrounding physicochemical factors in peatlands of three different climatic regions. Compared with previous studies, the range of biotic and abiotic parameters investigated has been extended to include key hydrolytic enzymes (ß-D-glucosidase, arylsulfatase, ß-D-xylosidase, N-acetyl-ß-D-glucosaminidase (chitinase), phosphatase) and oxidative enzyme (phenol oxidase), the GHGs (CO_2, CH_4 and N_2O), and the physicochemical variables pH, conductivity, DOC concentration, phenolics concentration, SOM and water content, and cation (sodium, ammonium, magnesium and calcium) and anion (chloride, nitrate, phosphate and sulfate) concentrations. All together were measured across the peat profile. In addition, the biogeochemical processes and the abiotic factors controlling them are still relatively unknown in peatlands of Svalbard and Sumapaz National Park, both of which were included in the present study.

This baseline information could contribute to the environment of moss-dominated peatlands in Colombian Andes, and more specifically will give important knowledge for use by Sumapaz's employers in managing natural parks.

Our finding, together with a body of information from other studies applied on peatlands can enable us to gain an understanding of how environmental properties are affecting decompositional processes, which may allow us to identify strategies capable of slowing down biodegradation and improving carbon sequestration in peatlands. However, by field survey only, these results cannot be generalized. Manipulation experiments are recommended considering the different layers to determine the impact of a particular abiotic factor on enzyme activity. In addition to using contemporary molecular techniques to determine genes encoding key functions in soil organic matter degradation in order to expand the knowledge on how microbial communities functionally interact and how they may respond to environmental changes.

Peatlands at a global scale have wide ranges in environmental conditions, which makes them ideal system for studying different structuring factors that can shape microbial communities. In the microbiomes found in soils, microbial eukaryotic communities are the most species rich. These organisms including fungi and protists, both of which are functionally diverse, which enables them to play a number of essential roles within the ecosystem food webs.

Additionally, micro-eukaryotic organisms are distributed ubiquitously and are known to respond to environmental changes. They are therefore considered to be useful bioindicators of abiotic variations in their environments, inferring to some extent the heterogeneity of different habitats and reflect soil quality. Importantly, linking micro-eukaryotic communities to environmental variables along a climate gradient can help in predicting the effects of climate-driven changes in the structure and function of peatland. However, information on micro-eukaryotic communities of peatlands remains sparse. Specifically, no data has been published yet about micro-eukaryotic communities at any of the present study field sites.

This study is the first, to current knowledge, giving a comparative view of micro-eukaryotic community composition in relation to the surrounding environmental factors in peatlands of three different climatic regions. The range of micro-eukaryotic organisms and abiotic factors investigated have been extended as well, compared with previous studies. The findings of this study could improve the understanding of micro-eukaryotic communities in peatlands from a global perspective, focusing on the physicochemical constraints upon them. This could allow to predict how peatland structure and function may respond to climate-driven environmental changes.

In the present study, there was an emphasis on soil properties as drivers of community composition of micro-eukaryotes. Further studies to consider how micro-eukaryotic communities might in turn impact on soil properties are recommended.

As a limited field survey of only one site from each region, these results cannot be generalized. Further field studies and experimental manipulations of environmental factors are needed to better explore the effects of soil variables on community composition of micro-eukaryotes.

Some studies have been addressed the effects of soil type on parasite movement and survival for public water supplies risk assessment (Zopp *et al.* 2016). As parasitic groups were found to be ubiquitous in peat soils, and since peatlands areas are a source of drinking water in many areas, an investigation of the extent to which peat soils contribute to the transmission of parasites to drinking water sources are recommended.

7.5. References

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Appendices

Appendix A

(for chapter 5)

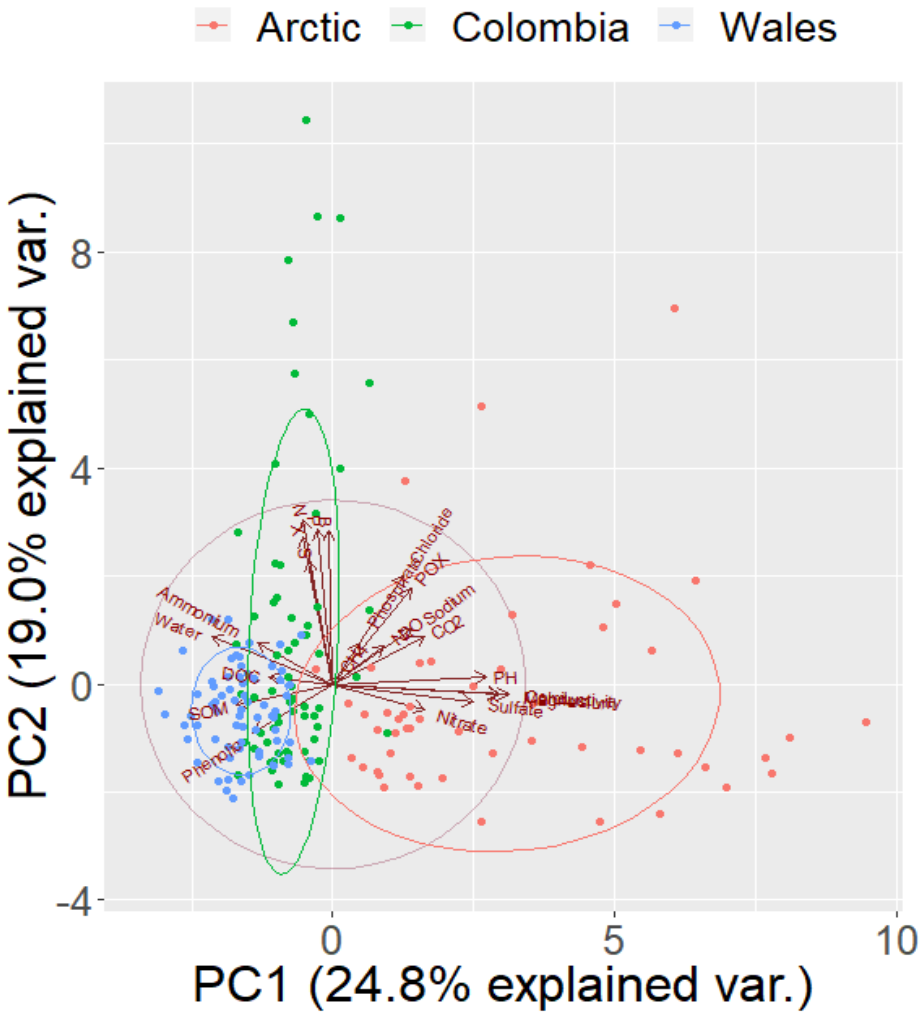

Figure 1. Principal component analysis (PCA), biplot chart represents the soil variables in the Arctic, temperate and tropical regions.
The arrows represent the soil variables, ovals represent the three contrasting regions and the points represent the samples in each region. R 3.5.0.

Appendix B

(for chapter 6)

Table1. The relative abundance of reads of the dominant taxa within each functional group in Arctic, Colombia and Wales.

	Taxa	Arctic	Colombia	Wales
The dominant parasites	Apicomplexa	1.6%	38.0%	60.3%
	Phytomyxa	85.95%	9.5%	4.6%
	Ichthyosporea	38.26%	20.6%	41.11%
	Perkinsea	67.2%	7.0%	25.7%
	Oomycota	40.56%	19.9%	39.5%
The dominant Osmotrophs	Mucoromycota	0.3%	98.6%	1.1%
	Ascomycota	0.1%	99.5%	0.3%
	Basidiomycota	3.88%	91.6%	4.47%
The dominant Phagotrophs	Spirotrichea	72.8%	5.04%	22.1%
	Oligohymenophorea	58.8%	23.3%	17.9%
The dominant Phototrophs	Bacillariophyta	84.5%	14.4%	1.14%
	Chlorophyta	78.3%	19.1%	2.6%
The dominant Mixotrophs	Chrysophyceae	62.98%	24.87%	12.14%

Table 2a. Averages of the selected soil physicochemical properties in Arctic, temperate and tropical peatlands. The different letters denote the significant differences between the Arctic (A), Colombia (C) and Wales (W) based on Tukey's HSD test ($p < 0.05$). Abbreviations: dissolved organic carbon (DOC), soil organic matter (SOM).

Region	Soil temperature (°C)	Water content (%)	SOM content (%)	Conductivity (µS/cm)	DOC (mg/L)	Phenolics (mg/L)	Oxygen (mg/L)	pH
Arctic	7.1 CW	90.5 CW	78.4 C	154.4 CW	15.5 W	1.88 C	5.72 CW	6.86 CW
Colombia	9.8 AW	93.0 A	48.6 AW	32.4 A	13.75 W	1.4 AW	2.1 AW	5.4 AW
Wales	13.2 AC	92.7 A	76.1 C	20.2 A	10.1 AC	2.4 C	4.1 AC	4.6 AC

Table 2b. Averages of the selected soil metabolic factors along Arctic, temperate and tropical peatlands. The different letters denote the significant differences between the Arctic (A), Colombia (C) and Wales (W) (Tukey's HSD test, $p < 0.05$). Abbreviations: ß-D-glucosidase (B), N-acetyl-ß-D-glucosaminidase or chitinase (N), arylsulfatase (S), phosphatase (P), phenol oxidase (POX) and nitrous oxide gas (N_2O).

Region	B (nmol MUF g^{-1} min^{-1})	S (nmol MUF g^{-1} min^{-1})	N (nmol MUF g^{-1} min^{-1})	P (nmol MUF g^{-1} min^{-1})	POX (µmol dicq g^{-1} min^{-1})	N_2O (ng N_2O g^{-1} h^{-1})
Arctic	15.0	1.4 C	4.1 C	33.9 C	1.7 CW	10.3 CW
Colombia	20.4 W	16.5 AW	14.2 AW	78.1 AW	3.06 AW	2.6 A
Wales	8.8 C	1.3 C	4.1 C	46.4 C	0.3 AC	1.69 A

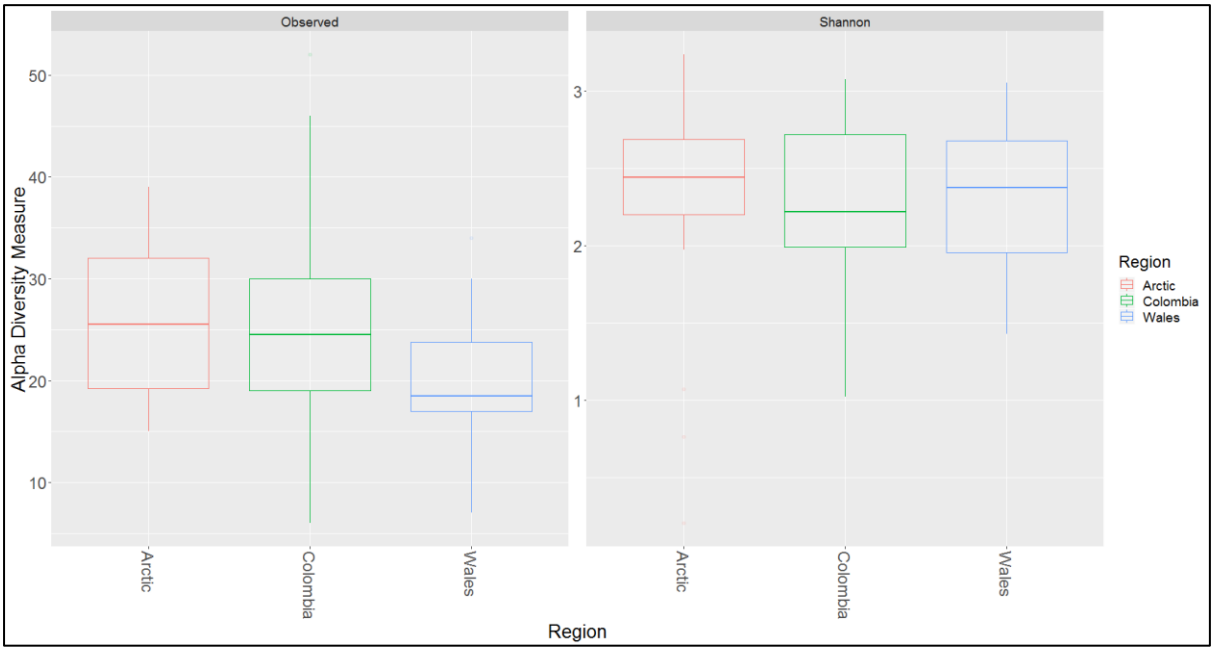

Figure 2. Mixotrophs richness and diversity.

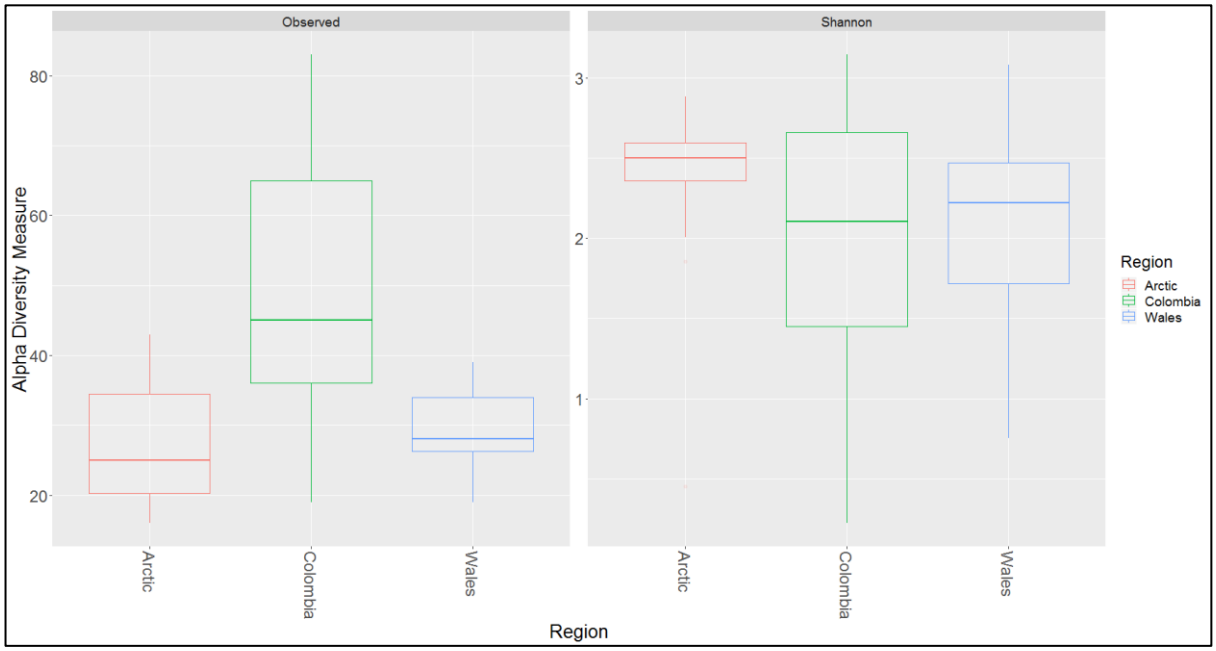

Figure 3. Osmotrophs richness and diversity.

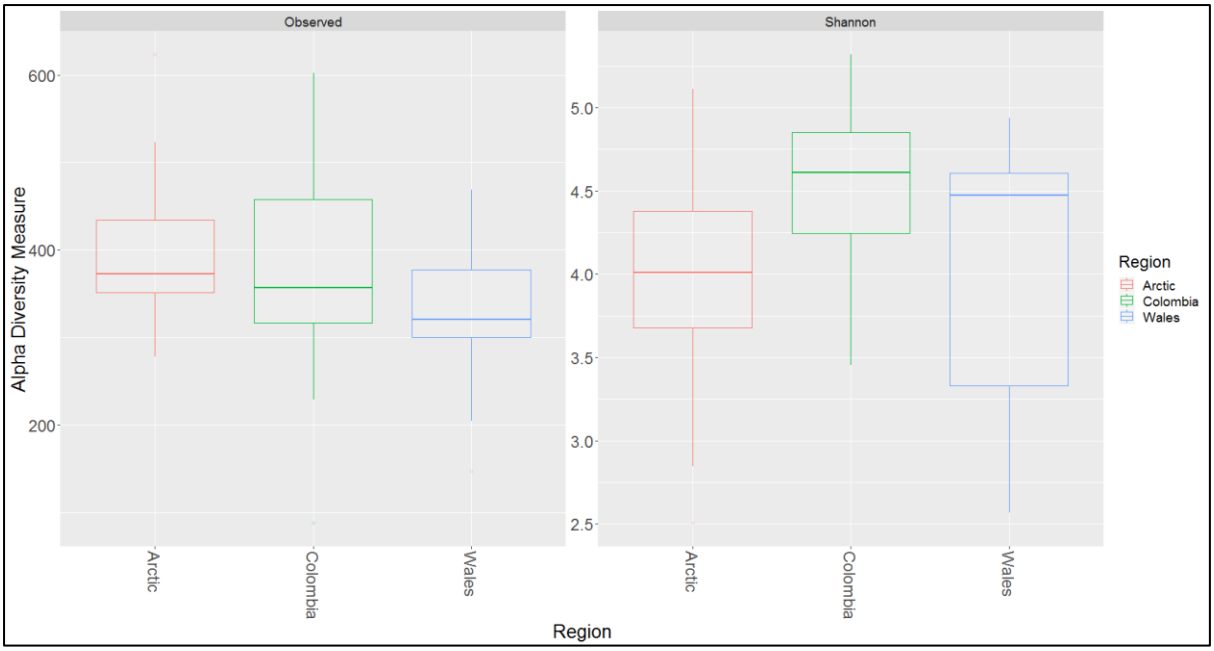

Figure 4. Phagotrophs richness and diversity.

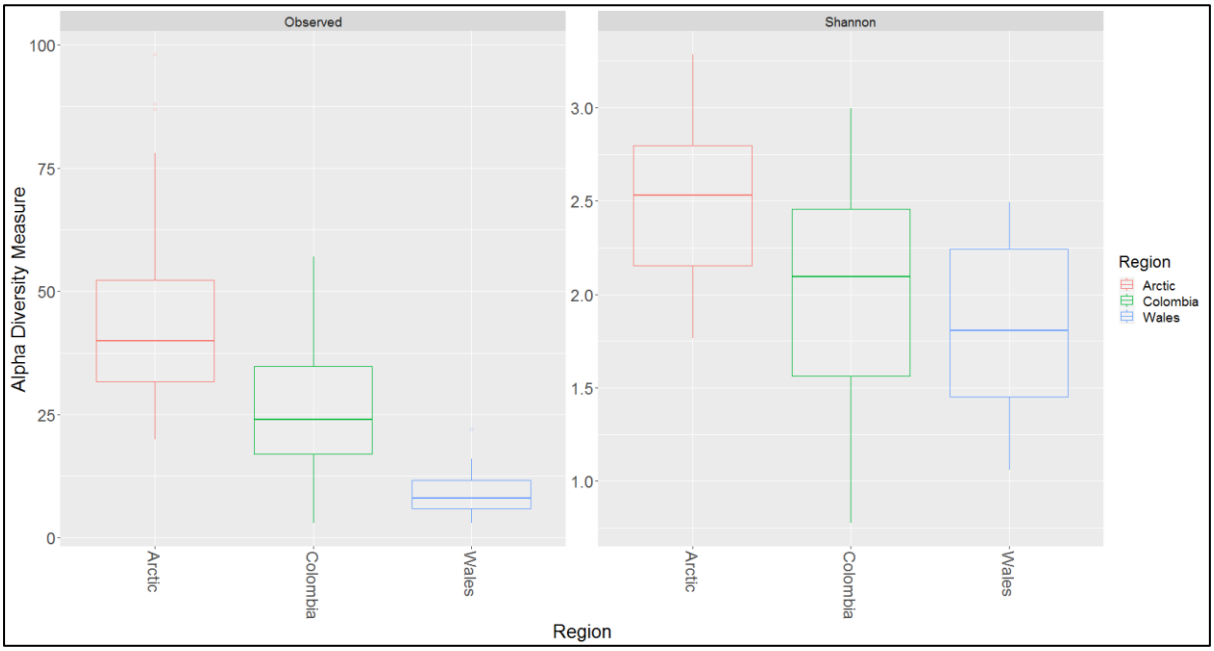

Figure 5. Phototrophs richness and diversity.

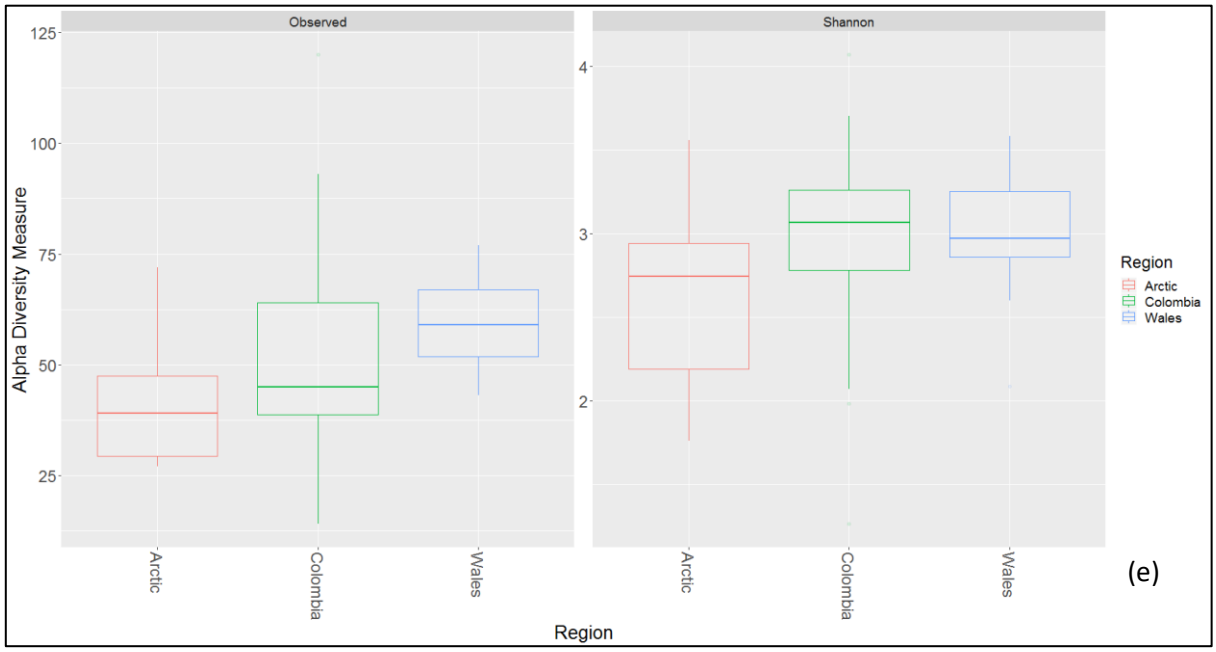

Figure 6. Parasites richness and diversity.

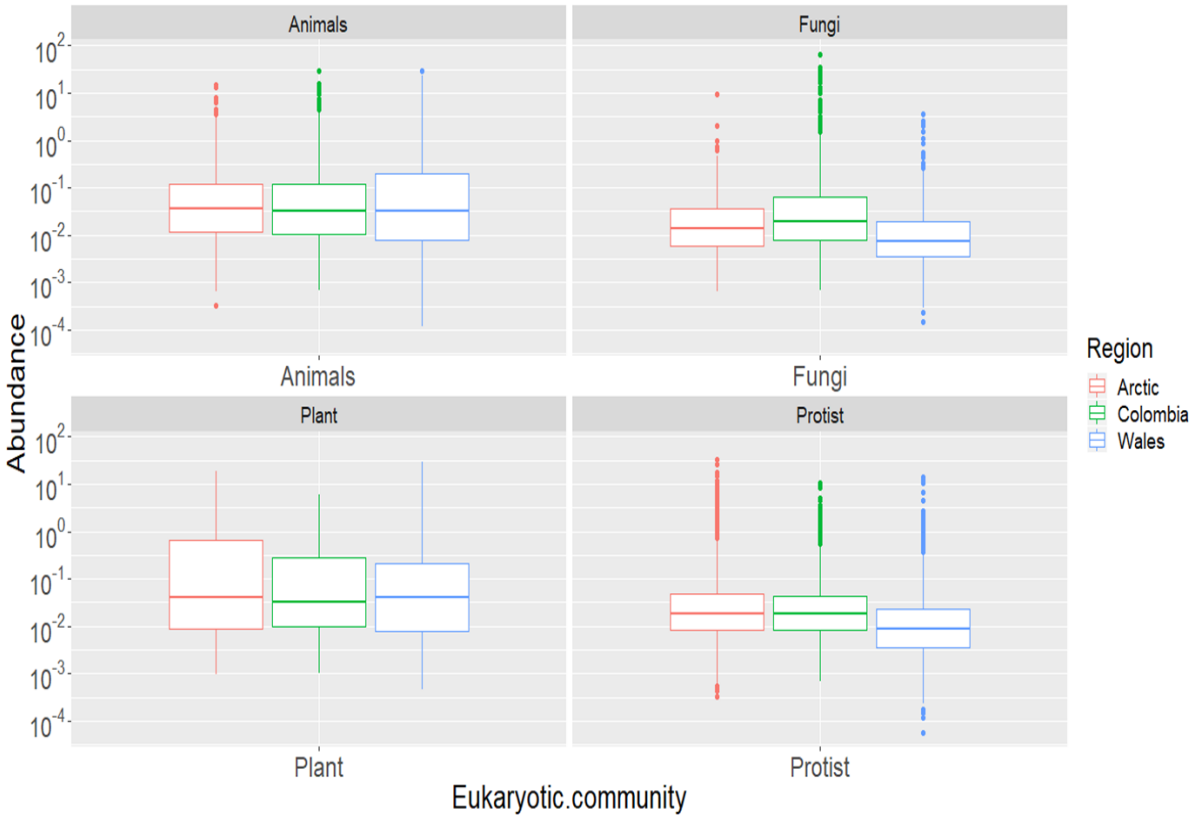

Figure 7. Boxplots represent the comparison of the relative abundances of reads (log scale) of eukaryotes between the different climatic regions.

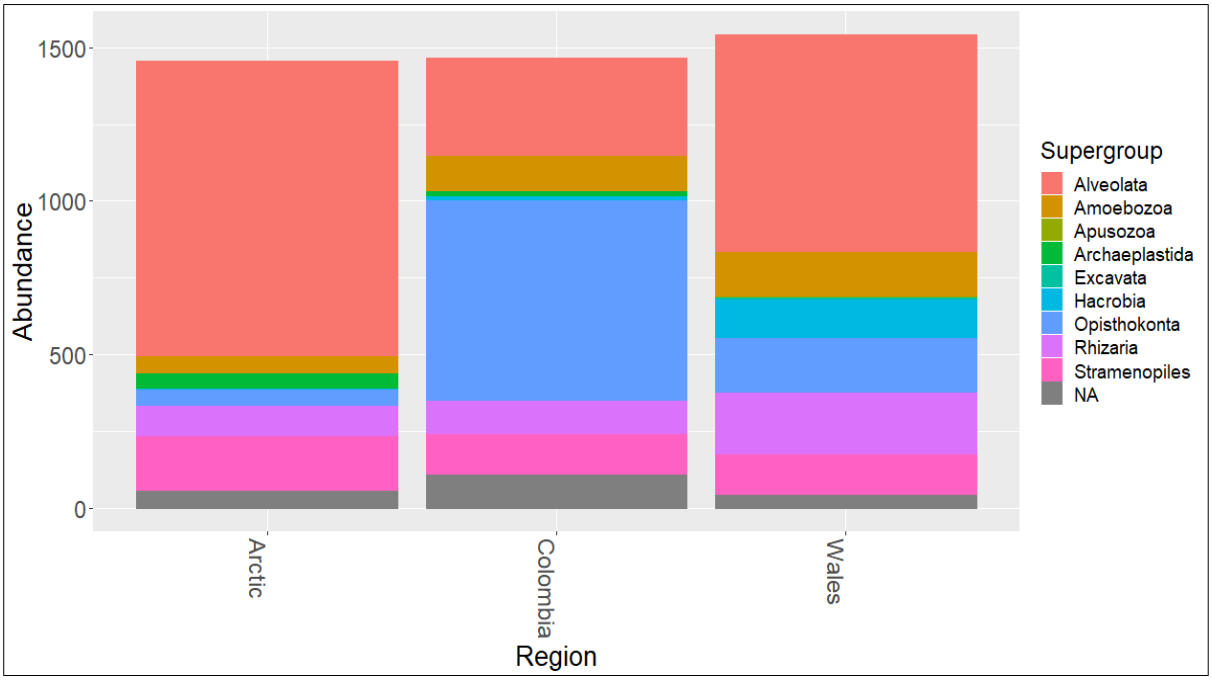

Figure 8. The relative abundance of the supergroups of micro-eukaryotes at each region.

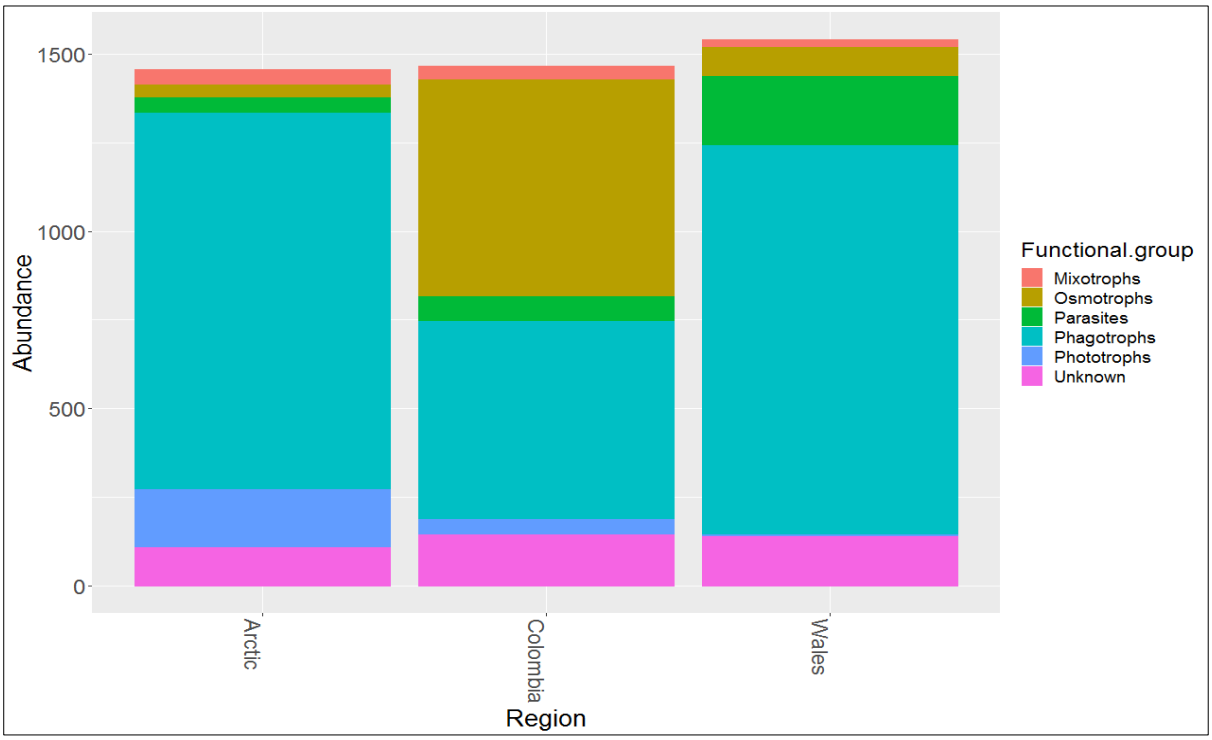

Figure 9. The relative abundance of the five functional groups of micro-eukaryotes at each region.