Microbial Controls on Carbon Fluxes in Forest-to-Bog Restoration

BY

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THESIS

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Abstract

Northern temperate peatlands are globally efficient terrestrial carbon stores subject to large-scale afforestation in the 20th century. Afforestation of open peatland compromises their ability to function as a net sink of atmospheric carbon dioxide (CO_2) and may become sources of CO_2 and other greenhouse gases (GHGs). Microorganisms control GHG fluxes through both direct (e.g. decomposition) and indirect (e.g. predation) and some are responsible for the fixation of atmospheric carbon.

Forest-to-bog restoration aims to recover key ecosystem functions, however, there is little evidence to show whether restoration can recover microbial community structure and the carbon balance. Key knowledge gaps exist calling for further investigation from a range of different restoration techniques, sites and peatland types. This study provides empirical evidence on the balance between carbon uptake through photosynthesis and soil respiration, because this will essentially determine the C sink potential and inform policy and future management of these ecosystems.

This project is highly applied, contributes novel data on the important role of microorganisms in forest-to-bog restoration and presents an annual gaseous C budget over a chronosequence of forest-to-bog restoration areas. This study is the first to highlight the potential of testate amoeba communities and functional traits as bioindicators of the progress of forest-to-bog restoration in both blanket and raised bog. Using a space for time substitution approach, findings here suggest forest-to-bog restoration can be beneficial for the carbon balance over timescales of around ten to fifteen years.

These data add to the limited existing evidence base on the carbon balance for UK peatland undergoing forest-to-bog management and are the first such evidence for raised bog in the UK. In addition, this study further demonstrates how published soil respiration models can be improved by accounting for plant function and provides the first evidence of the potential links between testate amoebae and in-situ GHG fluxes from forest-to-bog sites.

Keywords: Peatland, forest-to-bog restoration, blanket bog, raised bog, carbon dioxide, methane, testate amoebae, vegetation.

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This thesis is dedicated in memory of Dr Richard Payne.

Declaration: Publications

This thesis is written and presented in a manuscript format. For each manuscript that forms part of this thesis details of my contributions and that of co-authors are included. There may be minor editorial differences between the published papers and the thesis chapters.

Chapter two is published as:

Creevy, A.L., Andersen, R., Rowson, J.G. and Payne, R.J. (2018) Testate amoebae as functionally significant bioindicators in forest-to-bog restoration. *Ecological Indicators*, **84**, 274-282.

This manuscript was solely prepared by A. Creevy; R Andersen provided training and guidance on statistical analysis using the R software. This chapter investigated the impacts of afforestation and forest-to-bog restoration on Testate amoeba communities, and the potential for this group of microorganisms to act as bioindicators of forest-to-bog restoration. This work developed an indicator-based approach for monitoring forest-to-bog restoration. The manuscript was reviewed by the co-authors.

Chapter three is published as:

Creevy, A.L., Payne, R.J., Andersen, R. and Rowson, J.G. (2020) Annual gaseous carbon budgets of forest-to-bog restoration sites are strongly determined by vegetation composition. *Science of the Total Environment,* **705**, 135863.

This manuscript was also solely prepared by A. Creevy with final edits from the co-authors. J. Rowson also provided support during data analysis for modelling gaseous carbon budgets by suggesting ways to improve model fit using the Generalised

Reduced Gradient method. This chapter presents an annual gaseous carbon budget of forest-to-bog restoration areas of raised bog and investigates the role of vegetation in driving GHG fluxes. Data usage: in this chapter measured fluxes were used to estimate annual carbon budgets for the three study areas and for different vegetation groups.

Chapter four is prepared for submission to *Journal of Environmental Management*:

This manuscript was also fully prepared by A. Creevy with final edits from the co-authors. R. Andersen provided statistical advice on variation partitioning and redundancy analysis using the R software. This chapter explores drivers of GHG fluxes at forest-to-bog sites and examines how afforestation and subsequent restoration has altered edaphic factors. Data usage: in this chapter measured fluxes for four visits (August 2015, November 2015, February 2016 and May 2016) were used to explore the drivers of GHG fluxes.

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Chapter One

General Introduction

1.1 Importance of Northern peatlands

Peatlands are wetland ecosystems that play a fundamental role in the maintenance of biodiversity and global carbon (C) storage and cycling (Rydin and Jeglum, 2013). As such, they are increasingly recognised for providing a variety of ecosystem services such as climate mitigation, flood prevention, provision of fresh water, support of biodiversity, as historic archives and for recreation (Bonn et al., 2014). Peat is an organic soil that accumulates when plant litter inputs exceeds C outputs from soil respiration in nutrient-poor, anoxic, acidic environments (Clymo 1984). In their natural, active state peatlands emit methane (CH_4) and sequester atmospheric carbon dioxide (CO_2). Undisturbed peatlands are the only terrestrial ecosystem to have accumulated C continuously since the end of the last glacial maximum ~10,000 years ago (Clymo et al., 1998) and have acted as a net sink of CO_2 throughout the Holocene (Frolking and Roulet, 2007). This highlights the long-term development of peatlands which has had a strong influence on the global C cycle over millennia, regulated by a network of interacting feedbacks between plant ecology, soil biogeochemistry and ground/soil water hydrology (Limpens et al., 2008).

Peatlands store disproportionally more organic C compared with other terrestrial ecosystems. To put this in context, they only cover a small proportion of Earth's surface (~3 %) but store similar amounts of C than global forest cover, which is about ten times greater than the global extent of peatland (Frolking et al., 2011). Northern peatlands are estimated to store 500 ± 100 (approximate range) gigatons of C (Gt C)

(Yu *et al* 2012) as peat, however, the stability of this C is a major uncertainty in predictions of future climate change (Lenton and Huntingford, 2003; Limpens et al., 2008; Dise 2009; Leifield et al., 2019). For instance, it is probable that increased atmospheric CO_2 and temperature will affect plant production, microbial decomposition with indirect controls on carbon/nutrient interactions, altered hydrology and storage of organic matter (OM) and C loss (Gorham 1991). A combination of climatic variability and the effects of various direct and indirect anthropogenic threats make peatlands particularly vulnerable to environmental change (Billet et al., 2010; Leifield et al., 2019).

1.2 Peatlands in the British Isles

The British Isles contains blanket bog, raised bog (or mire) and fen peatlands. This study focuses on ombrotrophic (rain fed) raised and blanket bog type peatlands. These peatlands have formed by the processes of terrestrialisation and paludification (Lindsay 2010) and the largest distribution of ombrogenous bogs are found in Scotland and Ireland. In contrast with raised bogs which features elevated peat domes often several kilometres in diameter, blanket bog features peat deposits that cover the landscape sometimes over gentle slopes (Moore 2002). Ombrotrophic blanket bog predominates and the British Isles hold a significant proportion (~15 %) of the global blanket bog resource (Tallis 1998). Blanket bog forms under cool, extremely wet oceanic conditions and peat thickness ranges widely between 0.3 m and 6 m (Lindsay, 2010), peat thickness at raised bogs is potentially greater (~10 m) and these bogs are most frequent in oceanic regions (Moore 2002).

In the UK, blanket and raised bog peatlands cover around 23,000 km^2 or around a tenth of the UK land area (Montanarella et al., 2006) and are estimated to store 3.2

billion tonnes of C (Bain et al., 2011). The extreme development of blanket bog in Caithness and Sutherland in Scotland demonstrates how initiation of ombrotrophic blanket bogs relies on suitable climate, relief and hydrogeology factors which govern physical processes. Importantly, these peatlands (referred to as the ‘Flow Country’) are an extensive example (4,000 km^2) of a near-natural landscape still surviving in Britain and is the largest expanse of blanket mire in Europe (Lindsay et al., 1988; Andersen et al., 2017). The importance of this landscape is reflected by its nomination for UNESCO World Heritage status (Gewin 2020). These globally rare ecosystems provide vital habitat for biodiversity and ecosystem service provision. The dominant vegetation consists of bryophytes, sedges and dwarf shrubs which have preferentially established either in wetter hollows with a higher water table or on drier hummocks with a lower water table position. Raised bog contains similar plant species to blanket bog.

The International Union for Conservation of Nature (IUCN) estimates around eighty percent of peatlands in the UK are in a damaged or deteriorated condition and is strongly related to land-use change (IUCN 2018). Past land management activities of UK peatlands, including drainage for agricultural improvement/forestry and extraction for horticulture have shifted healthy peatlands into a damaged, often eroded state and are at increased risk of fire and burning. Damaged or drained peatlands can have a negative impact on biodiversity and the carbon balance (IUCN 2018).

1.3 Peatlands and the carbon cycle

Peatland gaseous C dynamics are a balance of carbon dioxide (CO_2) uptake by green tissues (i.e. plants and endosymbiotic algae) at the peatland surface during photosynthesis, CO_2 release through plant and soil respiration, and methane (CH_4)

production and consumption (Figure 1.1). The C cycle in peatlands is mainly controlled by soil temperature, plant community structure, position of redox boundaries associated with the water table and the chemical composition of plant tissues and peat (Bubier et al., 1993). There are various pathways for the release of these gases including diffusion, ebullition, or root and stem aerenchyma.

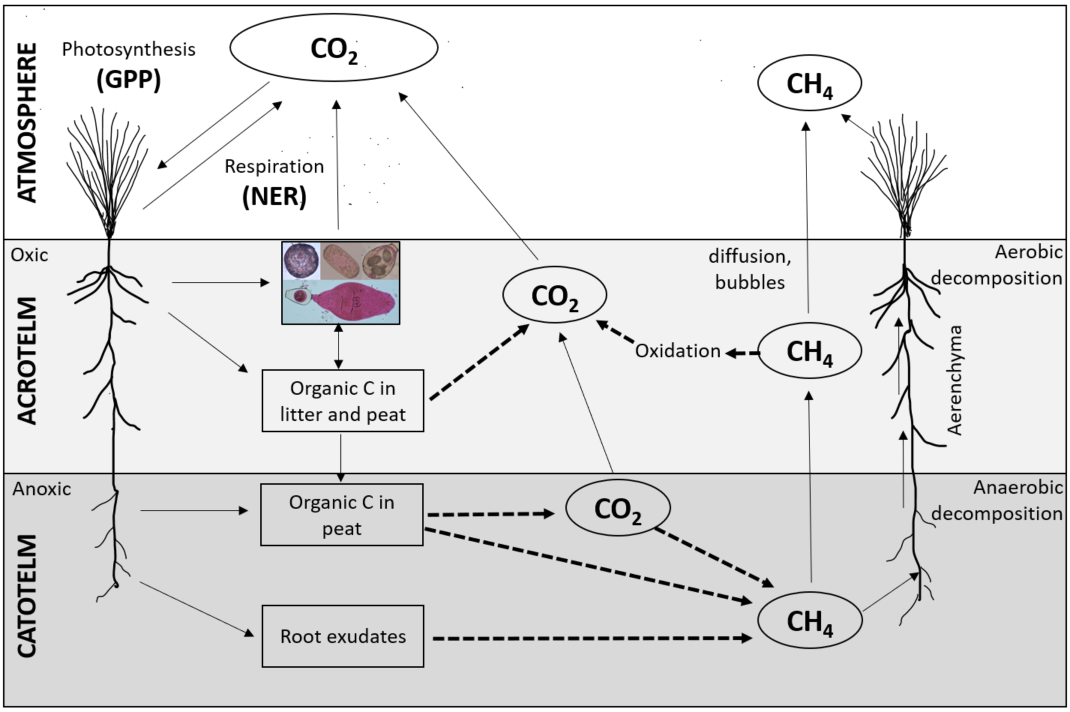


Figure 1.1. Gaseous carbon flow in a peatland with an oxic upper part and an anoxic layer beneath. Encircled symbols represent the movement of the gases studied; dashed arrows show microbial processes. NER (net ecosystem respiration, GPP (gross primary productivity). Recreated and adapted from Rydin and Jeglum, (2013).

Intact peatlands (or bogs) are often termed '*diplotelmic'*, that is, they are conceptualised of consisting of two distinct zones: the acrotelm (variably saturated upper oxic layer) and the catotelm (permanently saturated lower anoxic layer). The term '*mesotelm'* was later introduced to describe the layer where the water table fluctuates in the acrotelm (Clymo and Bryant, 2008), thus highlighting the non-static nature of the vertical peat profile. The position of the water table therefore is the key abiotic factor which influences the redox potential, and in turn, the type of microbial respiration i.e. aerobic respiration in the acrotelm versus anaerobic respiration in the catotelm. In general, CO_2 emissions increase when the water table is lowered whilst CH_4 usually shows an inverse relationship with emissions decreasing under drier conditions and vice-versa (Rydin and Jeglum, 2013). Bellisario et al. (1999) though report CH_4 emission decreasing with a higher water suggesting controls other than temperature and water table may be important at wetland sites where the water is close (<15 cm) to the surface.

The net primary production (NPP) of CO_2 is photosynthesis minus plant respiration, the remainder of which is added to the living plant biomass and supplies labile C for microbial decomposition (Blodau 2002). The upper aerobic oxic layer is a zone of high CO_2 productivity related to increased soil microbial CO_2 production, due to the supply of labile C from plant root exudates, decomposition of plant litter and root respiration (Rowson et al., 2013). In the lower anoxic layer, some of the litter will be decomposed to CO_2 and CH_4, but in intact peatlands most will be stored as peat. Methanotrophs that exist in the hyaline cells of *Sphagnum* oxidise CH_4 as it diffuses upwards from the anoxic peat. In drier ecosystems, Hartley et al, (2015) report CH_4 uptake when methanotrophic bacteria utilise CH_4 as their energy source and release CO_2.

As wetlands, peatlands are natural biogenic source of CH_4, produced by methanogens. Methanogens produce CH_4 in the anaerobic catotelm some of which is consumed by methanotrophs in the aerobic acrotelm. The growth of methanogens is primarily controlled by the volume of fresh litter and root exudates reaching the anoxic zone, therefore plant primary production and depth to water table are the two most important factors with alternative electron acceptors, temperature and pH as secondary controls (Rydin and Jeglum, 2013). Over a 100-year cycle CH_4 has twenty-eight times greater global warming potential (GWP) than CO_2 (Myhre et al., 2013) and emissions are controlled by the topography, which, in turn influences the hydrologic regime (i.e. proximity of water table) and vegetation composition. Studies have shown relationships between net ecosystem productivity and dominating plant species when water table position is closer than about 20 cm to the peatland surface (Bubier et al., 1995). This indicates that plant communities supply substrates for CH_4 production, may act as a short-circuit for transport of CH_4 and are indicative of average table position (Bubier 1995).

The first stage in predicting whether peatlands will be a source or sink of GHGs involves creating a C budget (or balance) by measuring C fluxes. To understand C budgets gas fluxes are measured either continuously using eddy covariance (EC) techniques or over short periods of time using chamber-based approaches. Chamber-based approaches are preferred at the plot scale whereas EC provide landscape scale estimation of fluxes (Alm et al., 2007). The largest C flux from peatland ecosystems to the atmosphere is Net Ecosystem Respiration (NER) (Rowson et al., 2013). Net Ecosystem Exchange (NEE) is used to determine whether a peatland functions as a C source or sink. NEE is defined as the sum of the NER of CO_2 and the uptake of CO_2 by gross primary productivity (GPP). The source/sink strength is dependent on the balance between NER and GPP (Eq. 1).

$$\mathrm{NEE} = \mathrm{GPP} + \mathrm{NER} \tag{1}$$

Several chamber-based studies (Alm et al., 2007; Rowson et al., 2013; Goud et al., 2017) have measured NEE in light conditions and NER by covering the chamber with a light impenetrable cover, with GPP calculated from the difference (NEE – NER).

Many studies use the micro-meteorological sign convention which defines a negative NEE as a C sink with positive NEE indicating a C source.

It is becoming increasingly recognised that studying changes in soil respiration is crucial for understanding the future of soil as a C sink, demonstrated by a noticeable rise in the number of published ‘soil respiration’ studies over the past three decades (Figure 1.2). This rise probably coincides with the need to better understand the effects of drainage and increased temperature on terrestrial C stocks (Wilkinson 2008; Crowther et al., 2016). Temperature is one of the most important variables regulating the rate at which chemical and biological processes occur, affecting the rate at which CO_2 is produced. The Arrhenius equation was used by Lloyd and Taylor, (1994) to link soil respiration to temperature for different ecosystems. The novel work of Rowson et al. (2013) improved soil respiration models by linking NER to plant function by accounting for the feedback between plant root exudates, season (i.e. plant senescence) and respiration as well as temperature and water table position. To improve those models further, there is a need for empirical understanding and quantification of fine-scale microbial controls on these fluxes.

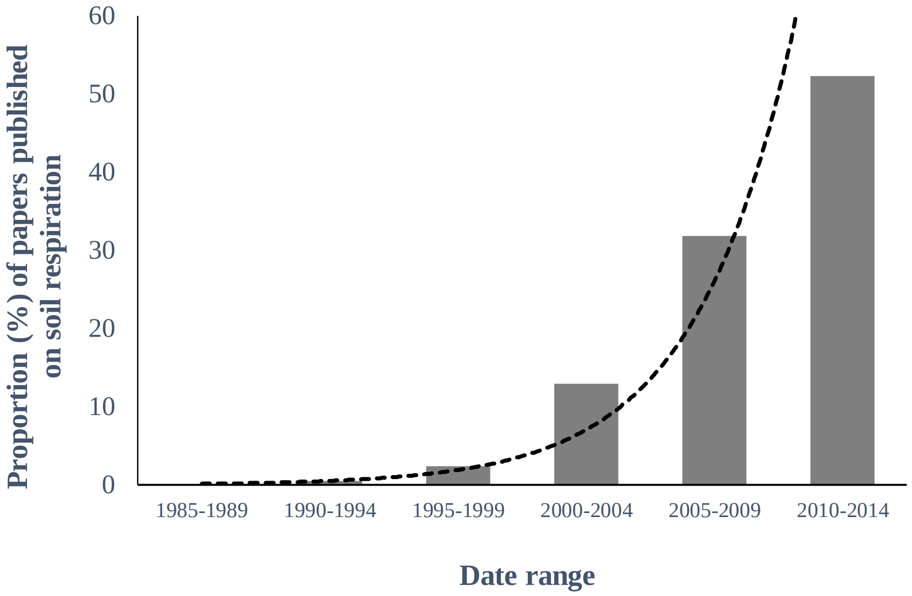


Figure 1.2. The relative proportion (%) of peer-reviewed published papers on soil respiration since 1985. The numbers were obtained from an advanced literature search for the key terms “soil respiration”, “soil CO2 efflux” and “belowground respiration” in the Web of Science database.

1.4 Microbial controls

Microorganisms mediate the C cycle, yet the mechanistic understanding of the links between measured processes (e.g. fluxes of CO_2 and CH_4) and the biology of the organisms responsible for those processes in the soil environment (microorganisms) is currently lacking. Microorganisms control GHG fluxes through both direct (e.g. decomposition) and indirect (e.g. predation) mechanisms and are responsible for the fixation of atmospheric carbon (Lara and Gomaa, 2017). Because of the enormous microbial diversity in soils (Geisen et al., 2017), functional classifications are often used to describe how they obtain C for growth and metabolism and how they capture energy (for example, Table 1). The term ‘protist’ is the informal grouping of single celled phototrophic, mixotrophic and heterotrophic eukaryotes (with the exception of fungi) and are the most diverse group of keystone organisms of soil ecosystems (Geisen et al., 2017). Testate amoebae are particularly abundant protists in peatlands.

Some protists termed ‘mixotrophs’ include some species that are known to permanently harbour green algal symbionts and are able to combine auto- and heterotrophic C uptake and are increasingly recognised for their significant contribution to C cycling in Northern peatlands (Lara and Gomaa, 2017; Jassey et al., 2015).

Table 1.1. Descriptions of the different respiration terms

Term	Description
Autotrophic respiration	Carbon assimilated through photosynthesis by trees and ground vegetation and released by respiration of above-ground biomass and roots.
Heterotrophic respiration	CO_2 released from microbial biomass and animals during decomposition of carbon substrates.
Rhizosphere respiration	Operationally defined term, which includes underground autotrophic respiration and heterotrophic respiration of carbon substrates originating from newly assimilated C e.g. root exudates and recent dead root biomass (so called rhizodeposition) mainly in the rhizosphere. Decomposition of extrametrical hyphal exudates and biomass may or may not be included depending upon the method employed.
Total soil respiration	Sum of autotrophic and heterotrophic respiration. Sometimes reported as sum of rhizosphere respiration and bulk soil heterotrophic respiration.

Recreated and adapted From Van Hees et al., 2005

Most of the C loss from soil is regulated by the metabolic activity of microorganisms and soil respiration is estimated to contribute ~50% of the annual CO_2 emissions from terrestrial environments (Martins et al., 2015). In peatlands, Rydin and Jeglum (2013) report a substantial proportion of above and below-ground litter (~90%) is released as CO_2 through autotrophic and heterotrophic respiration. The rate of OM turnover in soil is dependent on both the microbial community and environmental conditions, e.g. temperature, pH, soil water capacity, which, in turn, govern the biochemical activities of the microorganisms (Rousk and Bengtson, 2014).

Microbial food-webs are a key factor in the functioning of peatland ecosystems (Gilbert et al., 1998; Gilbert and Mitchell, 2006; Jassey et al., 2013), driving the flow of matter and energy and recycling nutrients for plant uptake. In turn, plants support microbial activities by connecting above and below-ground biogeochemical processes through rhizodeposition, and for above and below-ground biomass productivity (Bragazza et al., 2015). In terms of biomass, Gilbert et al. (1998) have shown four dominant group of microorganisms at the peatland surface: testate amoeba (48% of the total biomass), heterotrophic bacteria (15%), cyanobacteria (14%) and Bacillariophyceae (13%). However, Gilbert et al. (1998) did not quantify fungi which are generally thought to play a more dominant role than bacteria in the process of aerobic decomposition in the oxic upper layers (Andersen et al., 2013). In association with ericoid shrubs, Bragazza et al. (2015) reported increasing fungal dominance over bacteria under conditions of increased temperature, reduced water content and with improved soil oxygenation.

Some researchers have predicted that a rise in temperature due to climate change will increase the heterotrophic respiration of soil OM and the C flux from soils to the atmosphere (Knorr et al., 2005; Bellamy et al., 2005), contributing to a positive climate feedback effect (Crowther et al., 2016). On the other hand, since its expected that climate warming will also lead to lowered water levels in peatlands, others (e.g. Laine et al., 1995; Mäkiranta et al., 2009) have highlighted the importance of soil moisture regulating heterotrophic respiration, suggesting that low water levels could hinder soil decomposition. To better predict how peatlands will respond to environmental change, it is crucial that we deepen our understanding of the different microbial groups and their role in driving fluxes of key biogenic GHGs. There are several approaches that can be used to quantify microbial groups in soil, but few differentiate between different groups of microorganisms (Kirk et al., 2004) and others fail to capture the active microbes driving fluxes (Blagodatskaya and Kuzyakov, 2013).

1.5 Peatland management and impacts

Largescale and often rapid land-use change of peatlands has led to the degradation of around 80 % of UK peatlands (Glenk et al., 2014). Land management of peatlands includes drainage for agriculture/forestry, livestock grazing, managed burning and extraction of peat for fuel, gardening and horticulture. Lowland raised bogs have been particularly vulnerable to land-use change, now only about 6 % of raised bog habitat is in an undamaged state (Trinder et al., 2008). Large losses of peatland have occurred directly, for example, from drainage for forestry (~18 - 20% of total UK peat area) and drainage for use as cropland (~7 % of total UK peat area) and peat extraction for horticulture and fuel (IUCN 2018). Indirect losses have occurred, for example, as a result of nitrogen (N) deposition (Gorham 1991). Undisturbed *Sphagnum* peatlands are naturally nutrient-poor ecosystems and are usually N-limited, except in areas with high rates of N deposition resulting from human activities (Mitchell et al., 2003). Human activities have severely degraded many peatlands in England which suffer legacy effects of pollution and subsequent erosion, for example, Southern Pennines. Drainage and degradation of UK peatlands makes them vulnerable to wildfire damage. This thesis considers the impacts of afforestation and restoration of afforested peatlands.

1.5.1 Afforestation

Trees can be a natural component of peatlands in the temperate climatic zone (Hommeltenberg et al., 2014). However, there is a distinct difference between treed continental bogs and bogs in colder, oceanic climates which are almost treeless (Lindsay 2010; ; Rydin and Jeglum, 2013; Payne and Jessop, 2018). In the boreal and temperate zones, it is estimated that around 15 Mha of peatlands have been drained for forestry (Paavilainen and Päivänen, 1995). Afforestation of previously open peatland was widespread in the British Isles in the late 20th century, and by the 1990's > 800,000 ha (circa 20%) was afforested in the UK, and > 200,000 ha (circa 16%) in Ireland (Andersen et al., 2017). The largest impact of afforestation occurred on blanket bog and between the 1940s and 1980s almost 20 % of UK blanket bogs were planted with non-native conifers (Stroud et al., 1987). In open peatlands, forestry requires the drainage of the peat surface before trees are planted. Afforestation of open peatlands in the UK often involved closely spaced plough furrows between deeper drainage systems, creating ridges sufficiently raised above the drained water table before fertilising and planting conifers Lodgepole pine (*Pinus contorta)* and Sitka spruce (*Picea sitchenisis)* (see Anderson et al., 2000).

Afforestation of open peatland involves multiple different disturbance mechanisms such as: 1) shading by the forest canopy; 2) fertilisation during forest planting; 3) water table drawdown; 4) physical disturbance in ploughing; 5) input of large quantities of conifer needle litter and 6) changes in microbial communities which may interact with e.g. the introduction of conifer root specific mycorrhizas. When peatlands are drained and re-profiled prior to afforestation, alterations in the structure of the vertical peat profile can potentially increase microbial activity and respiration. This is because when peatlands are drained for forestry, the acrotelm is no longer water-logged with higher potential for CO_2 emissions due to aerobic decomposition of exposed peat and a likely reduction in CH_4 emission (Figure 1.3). Afforestation increases the depth of the oxic layer increasing water table drawdown and in turn increasing the available space for CH_4 to be oxidised by methanotrophic bacteria (Payne and Jessop, 2018). Drier conditions as a result of drainage and water table drawdown also changes vegetation composition. For instance, there may be a reduction in *Sphagnum* cover with drier conditions more favourable for brown mosses such as *Pleurozium* spp. and *Polytrichum* spp.

1.5.2 Forest-to-bog restoration

Large-scale forest-to-bog restoration was first initiated in the UK because of - and is still - largely driven by conservation concerns (Stroud et al., 1987; Wilson et al., 2014). The impacts of forestry on wading birds and associated habitat were the reason why afforestation of deep peat was essentially prohibited in the 1990s with tax incentives withdrawn (Sloan et al., 2018). However, there is now a growing body of evidence to suggest that restoration of peatlands for biodiversity is also beneficial for climate mitigation (Table 1.2). With this, the climate mitigation role of forest-to-bog restoration became a priority and funding allowed for research to determine whether forest-to-bog restoration could also provide benefits as shown in other peatland restoration management (Andersen et al., 2017).

Studies so far have shown that forest-to-bog restoration can be beneficial for microbial community structure (Creevy et al., 2018), for climate mitigation (Hermans et al., 2019 and references therein) and potentially for carbon stocks (Sloan et al., 2018). However, management techniques are evolving rapidly, as recognised in a recent article in Nature (Gewin 2020), therefore, there is a lack of evidence for newer approaches to restoration. Furthermore, long-term studies are needed to assess the magnitude of these benefits provided by forest-to-bog restoration, over decadal timescales. On a large scale, direct measurements of GHG fluxes are too expensive to implement and therefore alternative approaches are needed, for example proxies, if we can demonstrate strong relationships with GHG emission in a predictable and consistent way. Estimation of the carbon benefits provided by forest-to-bog restoration are particularly important if these GHG emission reductions are included in Intergovernmental Panel on Climate Change (IPCC) reporting under Land use, Land-Use Change and Forestry (LULUCF) in the future. The IPCC calculates total greenhouse gas emissions from the land-use sector for the National Inventory Report (IPCC 2006). For peatlands however, inconsistencies in relation to peatland climate zone and soil type definitions has hampered the accurate estimation of peatland GHG emissions (Couwenberg 2011). Perhaps more importantly, a workshop run by the IUCN UK Peatland Programme (IUCN 2014) highlighted that emissions from peatlands under restoration are not currently included in inventories. However, more recently, the first UK-wide inventory of peatland GHG emissions has been implemented (Evans et al., 2017).

A critical question of interest to the current project is: how long does it take to return the C balance and microbial communities to conditions prior to drainage and afforestation? Research suggests that some degraded peatland ecosystems will likely return to a net C sink approximately ten years post restoration (Strack and Zuback et al 2013), however these results are from sites where peat extraction has taken place and are not necessarily comparable to forest-to-bog restoration sites. Emerging research at forest-to-bog sites in the UK (Table 1.2) suggests timescales of between 5 and 15 years for the gaseous C flux/balance to return to pre-disturbance conditions, although, C loss from other pathways (Figure 1.3), for example, dissolved organic carbon (DOC) may take longer (Gaffney et al., 2018).

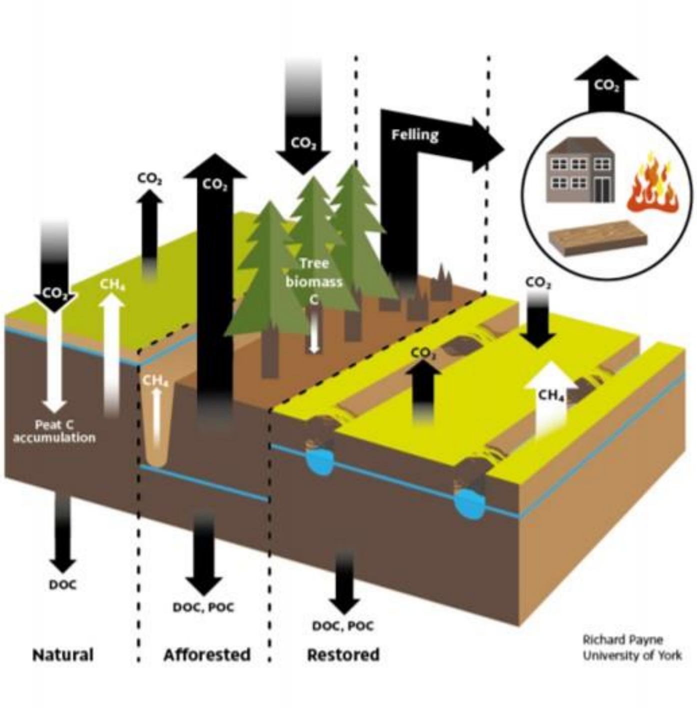


Figure 1.3. Conceptual diagram of key carbon cycle pathways and changes with afforestation and restoration. Payne and Jessop, (2018).

Limited studies monitoring vegetation change at forest-to-bog restoration sites in blanket bog have shown a dominance of *Carex* sedges (Hancock et al., 2018), which is associated with higher CH_4 emission (positive feedback). Nwaishi (2016) highlights how in developing peatlands the biochemical composition of decomposing litter combines with rhizodeposition to alter the water chemistry and biogeochemical processes (see Strack et al., 2006). Forest-to-bog restoration sites could also have legacy effects from forestry practices, for example fertilisation (Konings et al., 2019), potentially creating minerotrophic conditions and increasing the NPP of *Carex* sedges (Dise 2009; Nwaishi 2016) and subsequently, increasing the opportunities for aerenchymatous species to act as a short-circuit for CH_4 flux to the atmosphere.

Table 1.2 Current published research on the C flux/balance of forest-to-bog restoration sites in the UK.

Peatland type	**Location**	**Measurement**	**Method**	**Key finding**	**Reference**
Raised/ombrotrophic blanket bog	County Clare and County Tipperary, Ireland	CO_2, CH_4, N_2O	Chamber flux	Annual C budgets suggest sites were C source 7 and 12 years after restoration.	Rigney et al., 2018
Ombrotrophic blanket bog	Forsinard Flows National Nature Reserve, Scotland	CO_2, CH_4, N_2O	Chamber flux	CH_4 flux increased with restoration age (17 yrs), CO_2 flux driven by peat quality and nutrient availability, small sink for N_2O.	Hermans 2018
Ombrotrophic blanket bog	Forsinard Flows National Nature Reserve, Scotland	DOC	Catalytic combustion	DOC concentration remained elevated 17 years post restoration, recovery time of DOC may take in excess of 17 years.	Gaffney et al., 2018
Ombrotrophic blanket bog	Forsinard Flows National Nature Reserve, Scotland	CO_2, CH_4, DOC	Incubation study	Restoration sites showed lower CO_2 flux but no change in CH_4 flux or DOC concentration.	Hermans et al., 2019
Ombrotrophic blanket bog	Forsinard Flows National Nature Reserve, Scotland	CO_2	Eddy covariance	Annual C budgets suggest sites were C sink 16 years and C source 10 years after restoration.	Hambley et al., 2019
Ombrotrophic blanket bog	Forsinard Flows National Nature Reserve, Scotland	CO_2	Eddy covariance	C assimilation (GPP) reached between 5 and 10 years using satellite data.	Lees et al., 2019
Raised bog	Fenns and Whixall Moss, Shropshire, England	CO_2, CH_4	Chamber flux	Annual C budgets suggest sites were C sink 17 years and C source 6 years after restoration.	Creevy et al., 2020

* Restoration in this context relates to the time since felling of plantation forestry.

1.6 Bioindicators or proxies of forest-to-bog restoration

Bioindicators which effectively monitor the trajectory of restoration and proxies of GHG fluxes are needed at forest-to-bog restoration sites, as measuring these processes can often be complex and labour intensive (Artz et al., 2018). Vegetation composition coupled with the thickness of the unsaturated zone are reported to be promising predictors for soil respiration at the local scale (Limpens et al., 2008). Because vegetation is in turn controlled by water level, nutrient availability and pH, local scale vegetation can potentially be used for up-scaling plot level heterogeneity of fluxes to landscape level (Couwenberg et al., 2011). Although, Limpens et al. (2008) illustrate some of the problems of scale-dependency.

The question of scale would be important for studies investigating the role of microorganisms in driving fluxes. According to Basiliko et al. (2013), information about microbial ecology and whether microbial community structure influences GHG production is severely lacking in peatlands heavily affected by anthropogenic disturbances. For forest-to-bog sites in the UK, there are currently no existing published studies combining flux measurements with quantification of microbial communities. Published research investigating microbial groups as determinants of GHG fluxes had tended to focus on prokaryotes (e.g. Basiliko et al., 2013; Martins et al., 2015), compared with eukaryotic microorganisms. Using molecular methods Basiliko et al. (2013) found no apparent strong linkages between prokaryote (bacteria and archaea) community structure and CO_2 or CH_4 production. The same authors suggest a possible explanation is that different organisms exhibit functional redundancy and/or that the same taxa function at very different rates when exposed to different peat substrates. Studies investigating active microbial biomass would be useful to investigate functional redundancy in response to changing substrates.

1.6.1 Vegetation

Changes in plant communities are known to be an important determinant of ecosystem scale C fluxes, for example, the colonisation of early successional species such as *Eriophorum vaginatum* has been shown to temporarily increase CH_4 flux in the short-term after peatland restoration (Komulainen et al., 1999). There are many studies that have investigated the role of vegetation in the exchange of GHG's at the peatland surface of restoration sites (Waddington and Day, 2007; Ward et al., 2009; Ward et al., 2013). However, less is known from forest-to-bog restoration sites. Pioneer studies in Scotland have shown that the process of forest-to-bog restoration and raising the water table creates a shift in vegetation communities, often a dominance of sedges, particularly *Eriophorum angustifolium* (Hancock et al., 2018), with potential for higher CH_4 fluxes in the short term (Waddington and Day, 2007). Plant traits could also be a co-driver of GHG fluxes of forest-to-bog restoration sites as different traits (e.g. leaf area and chlorophyll content) have been observed at afforested and forest-to-bog sites compared with undisturbed open bog, Konings et al. (2019) suggested that legacy effects of the forestry may persist even after vegetation communities have returned.

1.6.2 Testate amoebae

Testate amoebae form a polyphyletic group of unicellular eukaryotes exhibiting a shell (test), traditionally placed in the phylum Rhizopoda (Margulis and Chapman, 2009) now split between three major unrelated groups (Kosakyan et al., 2016). They are large (5 – 300 µm approximate range) in terms of microorganisms, and their shells are composed of proteinaceous, siliceous or 'agglutinated' tests which incorporate material from the environment, for example diatoms, sand grains and siliceous plates of others testates.

Microorganisms such as testate amoebae respond more rapidly to environmental change compared with other components of an ecosystem, and certain groups could have the potential to be useful in monitoring peatland restoration (Davis and Wilkinson, 2004; Valentine et al., 2013; Swindles et al., 2016; Secco 2019). Testate amoebae are shelled protists that contribute a significant proportion (~48%) of the microbial biomass in peatlands (Gilbert et al., 1998). Like other groups of protists such as diatoms, identification is based on morphological characteristics and they can be counted directly using a light microscope. This technique has the advantage of producing community structure and population size data similar to studies of macroscopic organisms (Wilkinson et al., 2012).

Because of their predatory role sitting at the top of the microbial food-web (Wilkinson and Mitchell, 2010), testate amoebae could be representative integrators of the wider microbial community (Payne 2013). These protists are widely-used as sensitive indicators of peatland surface moisture conditions and have been the focus of contemporary (Koenig et al., 2015; Sullivan and Booth, 2011; Lamentowicz and Mitchell, 2005; Lamentowicz and Obremska, 2010) and palaeoenvironmental studies (Booth 2002; Payne et al., 2006; Charman et al., 2007). While testate amoebae have been used as peatland restoration indicators in several previous studies (Buttler et al., 1996; Jauhiainen 2002; Davis and Wilkinson, 2004; Vickery 2006; Laggoun-Défarge et al., 2008; Valentine et al., 2012), fewer studies have evaluated the use of testate amoebae as bio-indicators following blanket bog restoration (Swindles et al., 2016).

1.7 Aims and Objectives

The overall aim of this research is to broaden our knowledge of the microbial (Testate amoebae) and plant response and the carbon balance of a peatland after the removal of coniferous forestry plantations. Additionally, to present an indicator-based approach for assessing the ecological functioning and trajectory of forest-to-bog restoration sites. The three main objectives were to:

1) Develop a bioindicator-based approach for evaluating the progress and trajectory of forest-to-bog restoration management practices.
2) Examine changes in carbon exchange over a chronosequence of forest-to-bog restoration areas and compare fluxes with an ‘unafforested’ (open) area.
3) Identify whether functional traits and/or indicator species within plant and/or microbial groups could be used as a proxy for GHG fluxes at forest-to-bog restoration areas.

1.8 Structure of thesis

The thesis consists of five chapters. The first chapter provides a general introduction and background to the three major objectives designed to address key knowledge gaps. The three manuscripts therein address each of the specific objectives. The fifth chapter summarises the research findings and suggests ideas for future research to better our understanding of the biotic and abiotic response of forest-to-bog restoration practices.

Chapter Two

Testate amoebae as functionally significant bioindicators in forest-to-bog restoration

2.1 Introduction

Afforestation is one of the most widespread vegetation changes currently occurring in peatlands worldwide (Lachance et al., 2005). While large areas of the world's peatlands are naturally forested, many areas of naturally tree-less peatlands are being drained for forestry. In north-west Europe much of this afforested peatland is blanket bog. Blanket bogs are predominantly tree-less peatland ecosystems mostly occurring in temperate, hyperoceanic regions (Gallego-Sala and Prentice, 2012; Lindsay et al., 1988; Moore 2002). These geographically restricted ecosystems store globally significant quantities of carbon (Gorham 1991) and are important for biodiversity by providing habitat for unique assemblages of species adapted to the wet and acidic conditions (Bonn et al., 2016). Like many peatlands, blanket bogs have been widely exploited for fuel, agriculture and forestry threatening biodiversity and carbon storage (IUCN, 2014)

The British Isles hold a significant proportion (~20%) of the global blanket bog resource (Tallis, 1998). Between the 1940s and 1980s almost 20% of UK blanket bogs were planted with non-native conifers (Stroud et al., 1987). Afforestation involved drainage by ploughing and ditch-cutting and planting with the conifers Lodgepole pine (*Pinus contorta)* and Sitka spruce (*Picea sitchenisis)* (Anderson et al., 2000). This afforestation of deep peat was essentially prohibited in the 1990s primarily due to concerns about impacts on birds (Stroud et al., 1987). For instance, losses of key conservation-priority species such as the European Golden Plover (*Pluvialis apricaria*) have been associated with edge effects surrounding forestry plantations (Wilson et al., 2014). More recently the impact of drainage on peatland carbon stock has also become an increasing concern. It is likely that water table drawdown following afforestation may promote peat mineralisation leading to carbon losses to the atmosphere and watercourses (Lindsay, 2010).

It is now widely recognised that peatlands in relatively good condition deliver a range of benefits to society, including climate mitigation, flood prevention, provision of fresh water, support of biodiversity, as historic archives and for recreation opportunities (IUCN 2014). Land use changes such as the afforestation of blanket bogs have the potential to erode these benefits, with significant costs to society (Bonn et al., 2014). Large investments are now being made in peatland restoration with some of the most intensive schemes focused on the restoration of afforested blanket bog back to a more natural tree-less state. Forest-to-bog restoration typically involves tree-felling and ditch-blocking to raise the water table, with more recent trials also including whole tree harvest and furrow blocking.

Impacts of peatland afforestation on macroscopic organisms are relatively well-known with birds and plants particularly well-studied (Stroud et al., 1997; 1998; Lachance et al., 2005; Wilson et al., 2014). However, impacts below-ground have seldom been explored. Many studies emphasize the need to integrate microbial communities in the evaluation of restoration in peatlands yet, our understanding of responses to these often large and rapid land-use changes is still fragmentary (Andersen et al., 2013a; Andersen et al., 2013b; Elliott et al., 2015; Swindles et al., 2016; Nwaishi et al., 2016). This is surprising given that peatland restoration is often justified in terms of carbon storage and biodiversity. Microorganisms constitute both the vast majority of species in peatlands and play crucial roles in carbon and nutrient cycling. Reduction of carbon losses can only be achieved if the imbalance between higher net primary productivity (NPP) by plants and lower decomposition by microbial communities can be restored (Andersen et al., 2013).

Testate amoebae form a polyphyletic group of unicellular eukaryotes exhibiting a shell (test), traditionally placed in the phylum Rhizopoda (Margulis and Chapman, 2009) now split between three major unrelated groups (Kosakyan et al., 2016). As microbial consumers considered to feed on bacteria, fungi, microalgae, ciliates, rotifers and nematodes (Yeates and Foissner, 1995; Gilbert et al., 1998; Jassey et al., 2013a) they are a key element in the functioning of peatland ecosystems playing an important role in carbon and nutrient cycling (Wilkinson and Mitchell, 2010; Rydin and Jeglum, 2013; Jassey et al., 2013b). These protists are widely-used as sensitive indicators of peatland surface moisture conditions and have been the focus of contemporary (Koenig et al., 2015; Sullivan and Booth, 2011; Lamentowicz and Mitchell, 2005; Lamentowicz and Obremska, 2010) and palaeoenvironmental studies (Booth 2002; Payne et al., 2006; Charman et al., 2007). While testate amoebae have been used as peatland restoration indicators in several previous studies (Buttler et al., 1996; Jauhiainen 2002; Davis and Wilkinson, 2004; Vickery 2006; Laggoun-Défarge et al., 2008; Valentine et al., 2012), we are aware of only one study which evaluates the use of testate amoebae as bio-indicators following blanket bog restoration (Swindles et al., 2016), and none which consider forest-to-bog restoration.

Functional traits (FT’s) in testate amoebae have potential as restoration indicators (Fournier et al., 2012) and indicators of past environmental change in peatlands (Fournier et al., 2015). Recently, traits such as mixotrophy and aperture position/size have been reported to be potentially useful proxies of disturbance in

Sphagnum peatlands (Marcisz et al., 2016). Recent research investigating FT's in naturally forested peatlands suggests afforestation could lower the trophic level of testate amoeba communities and reduce the contribution of mixotrophic taxa to primary production (Payne et al., 2016). Jassey et al, (2015d) highlight the potential magnitude of such effects on peatland carbon dynamics. However, the functional response of testate amoebae to afforestation is not known, nor whether these changes are reversible.

The aims of this study were 1) to evaluate how testate amoebae respond to forest-to-bog restoration and 2) to assess whether they could be used as microbial indicators of disturbance and recovery. A multi-site approach was used to encompass the natural variability in blanket bog at the landscape scale, and within the blanket bog between different micro-topographic features. The following hypotheses were tested: **[H1].** There are distinct testate amoeba communities associated with afforested bog, open-bog and forest-to-bog restoration sites. **[H2].** Peatland restoration has shifted testate amoeba community composition back towards that of undisturbed open-bog habitat. **[H3**]. Testate amoebae are sufficiently specific (i.e. differ between open and afforested) and responsive (i.e. affected by disturbance and restoration) to be used as a bio-indicators in peatlands undergoing forest-to-bog restoration.

2.2 Materials and Methods

2.2.1 Study sites

The study was undertaken in the blanket peatlands of Caithness and Sutherland, northern Scotland (the 'Flow Country') (Fig 2.1). This region comprises the largest single area of blanket bog in the UK (~27% total) and is perhaps the most important expanse of intact blanket bog worldwide (Holden 2005). Several sites were selected ranging from 90 – 210 m elevation above sea level across the central area of the Flow Country (58^0 25’ N, 3^0 34’ W). The mean annual precipitation of this region is around 1196 mm with a minimum annual temperature of 3.6°C and a maximum annual temperature of 11.7°C (Turner et al., 2016).

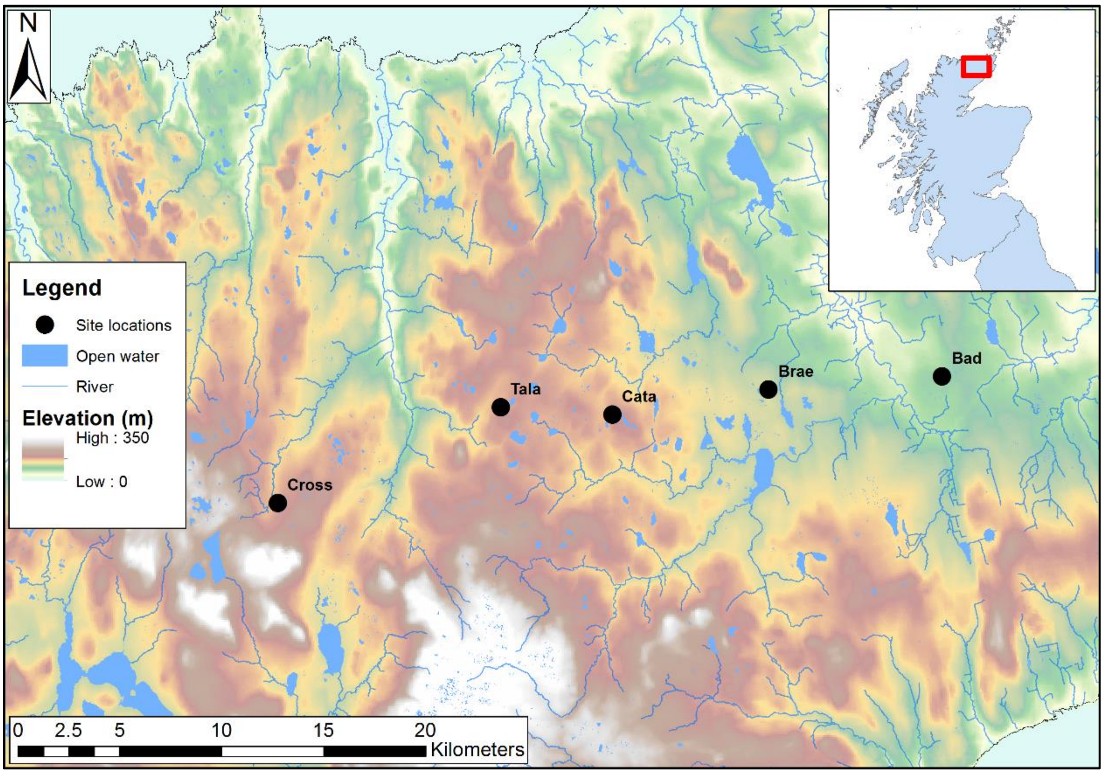


Figure 2.1. Location of sampling sites in this study.

Four paired afforested and un-afforested (‘open’) sites were selected along a West-East gradient: Cross Lochs (‘cross’), Catanach (‘cata’), Braehour (‘brae’), and Bad a’ Cheo (‘bad’) (Fig 2.1). All forested sites were drained, ploughed and planted with Sitka spruce *Picea sitchenisis* and Lodgepole pine *Pinus contorta* from the 1960s to the 1980’s, usually at 2 metre spacings (Anderson et al., 2000). In addition to these plantations, the forest-to-bog restoration site Talaheel (‘tala’) was sampled. At the restoration site trees were felled, rolled into furrows to slow drainage and main drains were blocked in 1997-1998. This is the oldest forest-to-bog restoration site in our study region and therefore provides a good opportunity to assess long-term restoration trajectories.

In all sites, there was strong micro-topographical variability. In open bog, the vegetation of hummocks consisted of *Sphagnum capillifolium*, lawns of *S. papiliosum* (*S. palustre* at Braehour), and hollows/pools with *S. cuspidatum* (*S. fallax* at Braehour). In the forested and forest-to-bog sites, regularly spaced microforms were created by the planting process: ploughing at a depth of ca 1m created linear "furrows", the "plough throw" which created ridges on either sides of the furrows, leaving an untouched strip in the middle where the trees were planted, referred to as the 'original surface'. Forested areas were generally covered with needle litter and non-*Sphagnum* bryophytes such as *Plagiothecium undulatum* and *Hylocomnium splendens*. At the forest-to-bog site the original surface consisted of sparse cushions of *S. capillifolium, Calluna vulgaris* and *Eriophorum sp.* with *S. cuspidatum* in the wetter furrows. The plough throw had *Polytrichum* spp. and *Cladonia* spp. colonising the bare peat along with *Calluna vulgaris, Deschampsia flexuosa* and *Eriophorum spp.*

2.2.2 Field sampling and laboratory analyses

Samples for testate amoeba and micro-environmental analyses (n = 108) were collected in October 2013. Sampling encompassed the natural variability between sites (n=4) and within sites, i.e. hummocks, lawns and pools in the open sites and plough throw, original surface and furrows in the forested and forest-to-bog sites. In the four paired open/forested sites (Cross, Cata, Brae, Bad) a quadrat (1 x 1 m) was randomly placed within each of the three microforms (hummock/plough throw, lawn/original surface, hollow/furrow) and three subsamples were taken at random within the quadrat. Open sites were sampled at least 100 metres from the forest edge to eliminate unwanted edge effects. Four replicate sets of samples were collected in the forest-to-bog site (tala), which were considered independent as they were at least 50m apart and separated by a series of ridges, furrows and/or rides (i.e. wider unplanted areas separating forestry blocks). At each sampling location, surface samples of approximately 5 x 5 x 10 cm^3 were collected for testate amoeba analysis and frozen prior to laboratory preparation. Samples for measurement of micro-environmental variables (bulk density, moisture content, loss on ignition, pH and conductivity) were extracted from the same locations as the testate amoeba samples and taken back to the laboratory.

Testate amoebae were prepared using a modified version of the method of Booth et al., (2010). For each sample the upper (photosynthetic portion) of each moss was removed and the top 3 – 5 cm was used for preparation (see Mitchell and Gilbert, 2004). To extract testate amoebae the sample was soaked and disaggregated in 50 ml de-ionised water and stirred occasionally. The material was washed through a 250 µm sieve to remove plant and coarse particulate matter and centrifuged at 3000 rpm for three minutes. Amoeba tests were identified and counted at 200 x and 400 x magnification using an Olympus CK2 inverted microscope. Search effort was restricted to 100 individuals per sample following Payne and Mitchell (2009). Morphological identification of TA was based on the guides of Charman et al., (2000), Clarke, (2003), Ogden and Hedley, (1980) and Mazei and Tsyganov, (2006). We did not differentiate between dead and alive individuals.

Samples for measurement of micro-environmental variables were prepared according to standard methods (Chambers et al., 2010). Bulk density samples were carefully extracted by cutting into the surface vegetation/peat using an open cylinder of known volume (400 ml). Samples were weighed, oven-dried at 105°C and reweighed to determine bulk density and moisture content. Organic matter content was determined by loss on ignition at a temperature of 550°C (Chambers et al., 2010). . Samples for measurements of pH and conductivity were prepared by mixing 20 ml surface sample with 25 ml de-ionised water. Samples were shaken at 400 rpm for 30 minutes and centrifuged at 3000 rpm for 5 minutes. Measurements were obtained using a Hanna HI991300 multiparameter probe.

2.2.3 Statistical analyses

Differences between open, forested and forest-to-bog sites were analysed using both multivariate community data and several indices. To investigate diversity patterns, taxon richness and the Shannon Diversity Index (SDI) were calculated for each sample (Shannon, 1948). In order to detect changes in community functioning two functional indices were calculated: mixotrophy ratio and aperture size. Mixotrophy was determined as the proportion of mixotrophic species within a community (Fournier et al., 2015). Aperture sizes were classified into three size categories: 1 = < 20 µm, 2 = < 40 µm and 3 = > 41µm following Payne et al. (2016). These data were log-transformed to homogenise variance prior to analysis. ANOVA was used to test for differences in diversity and functional indices between open, forested and forest-to-bog sites. Kruskal-Wallis test was used for data which did not satisfy the assumptions of ANOVA.

Multivariate techniques were used to investigate controls on testate amoeba communities and differences between open bog, forested and forest-to-bog sites. Taxa present in less than ten samples were eliminated from the testate amoeba community dataset in order to reduce the influence of rare taxa. The species abundance data were Hellinger transformed prior to all multivariate analyses (Legendre and Gallagher, 2001). Nonmetric Multidimensional Scaling (NMDS; function '*'isoMDS'*) was used to compare testate amoeba assemblages between the three treatments using the Bray-Curtis dissimilarity index. Nested Permutational Multivariate Analysis of Variance (PERMANOVA, function '*adonis'*) was used to determine the significance of treatments, sites and microforms in determining testate amoeba community composition.

Redundancy analysis (RDA) was used to test the relationship between testate amoeba communities and environmental variables with the testate amoeba dataset as the response variable. The constraining variables were selected based on available environmental variables: vegetation (presence/absence of key taxa: pH, electrical conductivity (EC), moisture content, bulk density (BD) and organic matter content (OM). The significant association of explanatory variables to the testate amoeba community data was tested using ANOVA (by "axis").

The IndVal approach (Dufrene and Legendre, 1997) was adopted to identify indicator species of open bog, forested and forest-to-bog sites. The clusters were categorised by type (open, forested, forest-to-bog). Indicator species for each cluster were identified using the 'indval' function in the package 'labdsv' (Roberts, 2016). For each community, taxa with a p-value = < 0.01 and IndVal > 0.30 were selected as potential indicator species. IndVal analysis was performed on the full testate amoeba dataset.

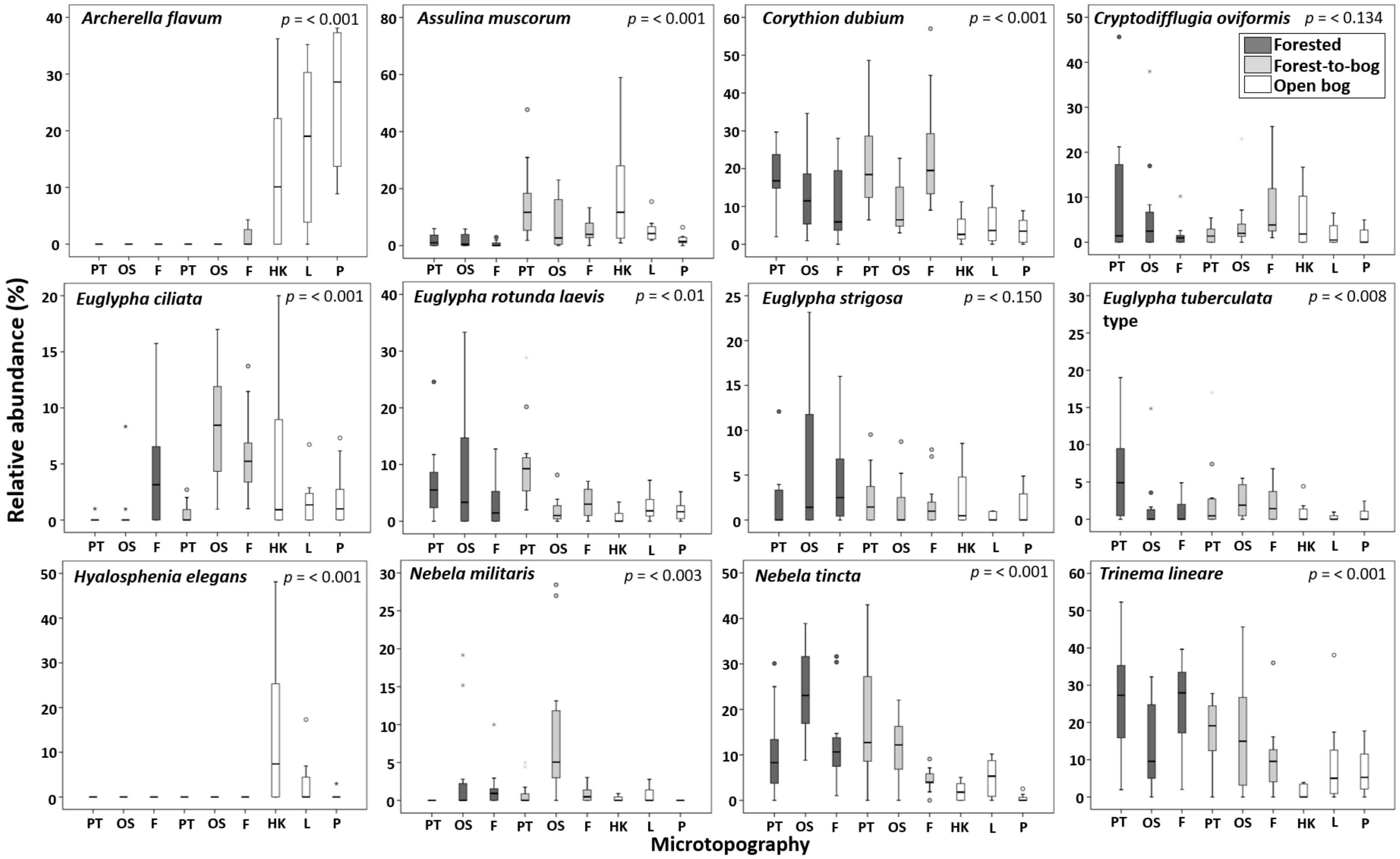
Statistical analyses were performed with R (R Core Team, 2013) using the packages "vegan" (Oksanen et al., 2012), "MASS" (Venables and Ripley, 2002) and "labdsv" (Roberts, 2016).

2.3 Results

2.3.1 Testate amoebae community structure

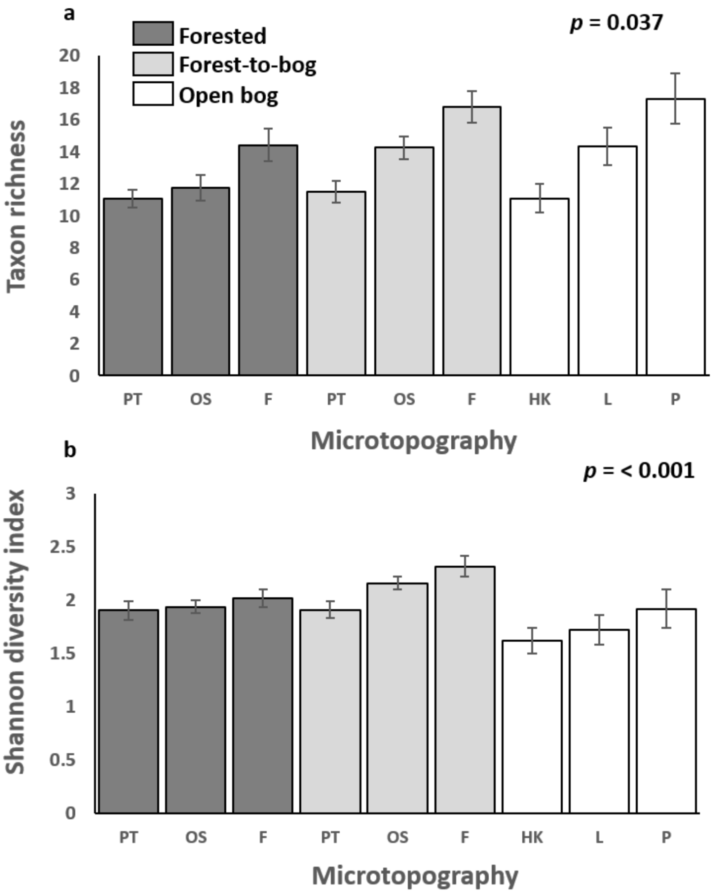
The overall testate amoeba community showed high diversity with 66 taxa identified from 11,599 individuals in the 108 samples. The most abundant taxa in decreasing order of abundance were: *Trinema lineare* (14.2% of all tests), *Corythion dubium* (12.0%), *Archerella flavum* (11.9%), *Nebela tincta* (10.0%), *Assulina muscorum* (6.4*%), Cryptodifflugia oviformis* (4.3%), *Euglypha rotunda/laevis* (4.3%), *Euglypha ciliata* (3.0%), *Euglypha strigosa* (2.4%), *Euglypha tuberculata* type (2.2%), *Hyalosphenia elegans* (2.2%) and *Hyalosphenia papilio* (1.9%). These taxa accounted for a relatively high proportion (> 70%) of the overall assemblage (Figs 2.2 a – l).

There were significant differences (Kruskal-Wallis test, $p = < 0.05$) in relative abundance (%) between open, forested and forest-to-bog sites for all taxa except for *C. oviformis* and *E. strigosa*. Certain taxa were present only in certain treatments, sites or microforms. For instance, *Archerella flavum* was completely absent in forested sites and *Bullinaria indica* was only observed in forest-to-bog habitats. *Hyalosphenia elegans* and *Hyalosphenia papilio* were observed in open sites, notably, taxa from this genus were also completely absent in both the forested and forest-to-bog sites.



Figures 2.2 a-l. Boxplots of the relative abundance (%) of dominant testate amoebae taxa in forested, forest-to-bog and open bog (site categories) between different microtopographies (PT = plough throw, OS = original surface, F = furrow, HK = hummock, L = lawn, P = pool). The lower boundary of the box indicates the 25th percentile, the line within the box marks the median and the upper boundary indicates the 75th percentile. The whiskers above and below the box indicates the 10th and 90th percentiles. Significant values represent differences between site categories (Kruskal-Wallis test).

Taxon richness was significantly higher in open sites than forested and forest-to-bog sites (F = 3.393, *p* = 0.037). In contrast, diversity (Shannon's index) was significantly lower in open sites (F = 9.304, *p* = < 0.001) and was highest in forest-to-bog sites (Figs 2.3a and b). Diversity was influenced by peatland microtopography, for instance, taxon richness was significantly greater in open pools than open hummocks (*p* = 0.002) and different between the plough throw and furrows in forest-to-bog sites (*p* = 0.01). There were no significant different in mean taxon richness in forested areas.



Figures 2.3a and b. Taxon richness and Shannon Diversity Index between site categories and microtopography. Values are mean with standard errors. Significant values represent differences between sites categories (ANOVA test).

The NMDS suggested a clear distinction between forested and undisturbed open sites at the community level (Fig 2.4). Testate amoeba communities in the forest-to-bog sites appeared intermediate but tended to display more similarity with forested sites compared to open bog. Furrows in forest-to-bog sites clustered closer to drier open bog microhabitats. PERMANOVA showed that community composition was significantly different between open bog, forested and forest-to-bog sites (F=13.227, *p* = 0.001). The testate amoeba community also differed significantly with microtopography (F=3.746, *p* = 0.001) and between sites (F=7.820, *p* = 0.001).

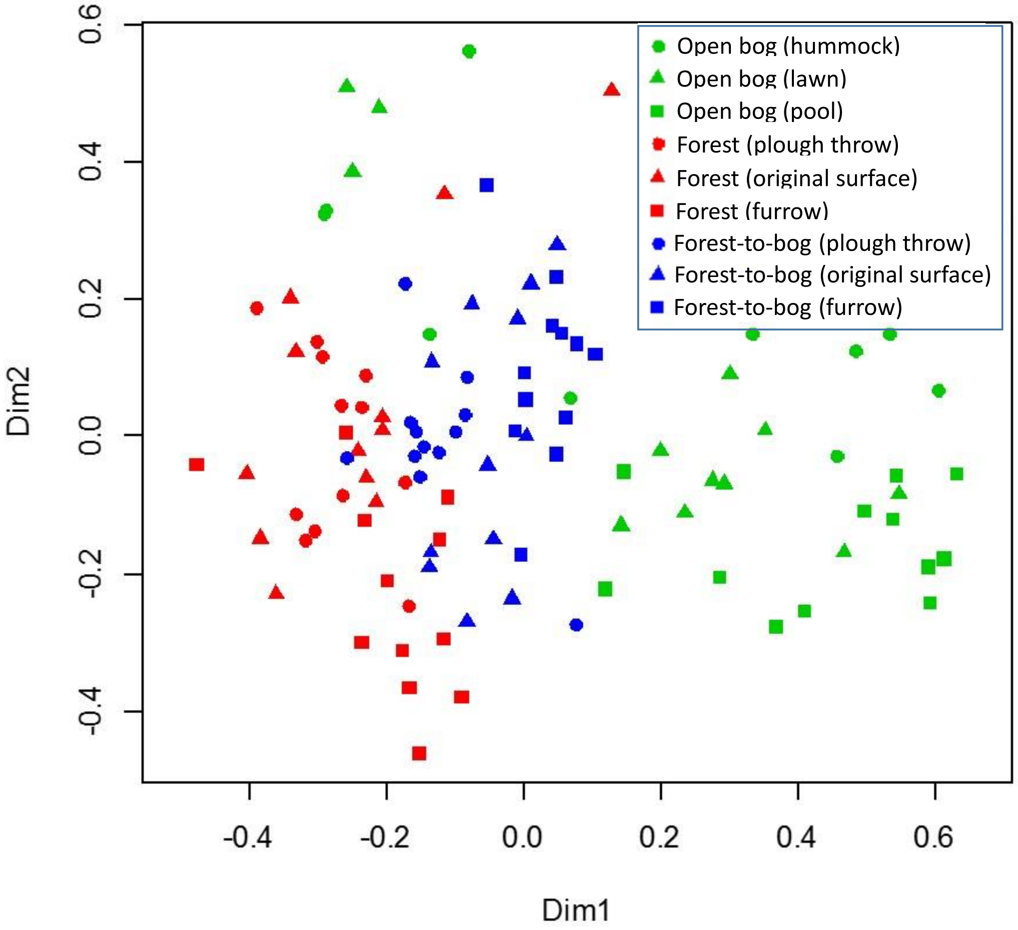
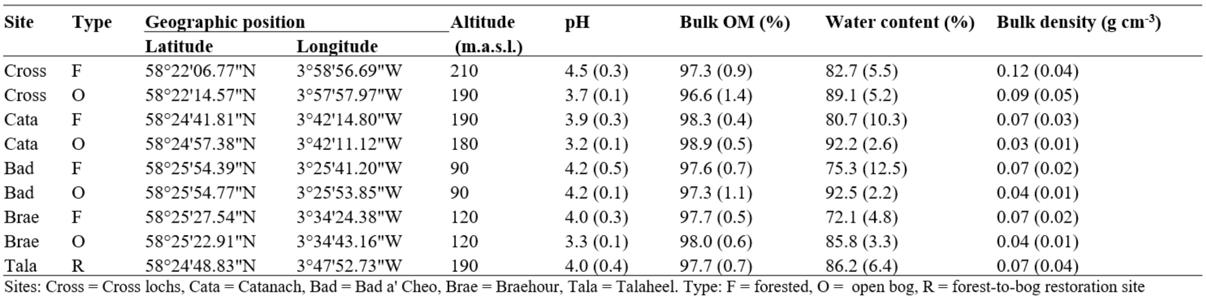


Figure 2.4. Non-Metric Multidimensional Scaling (NMDS) of Hellinger transformed testate amoeba community dataset.

2.3.2 Environmental factors

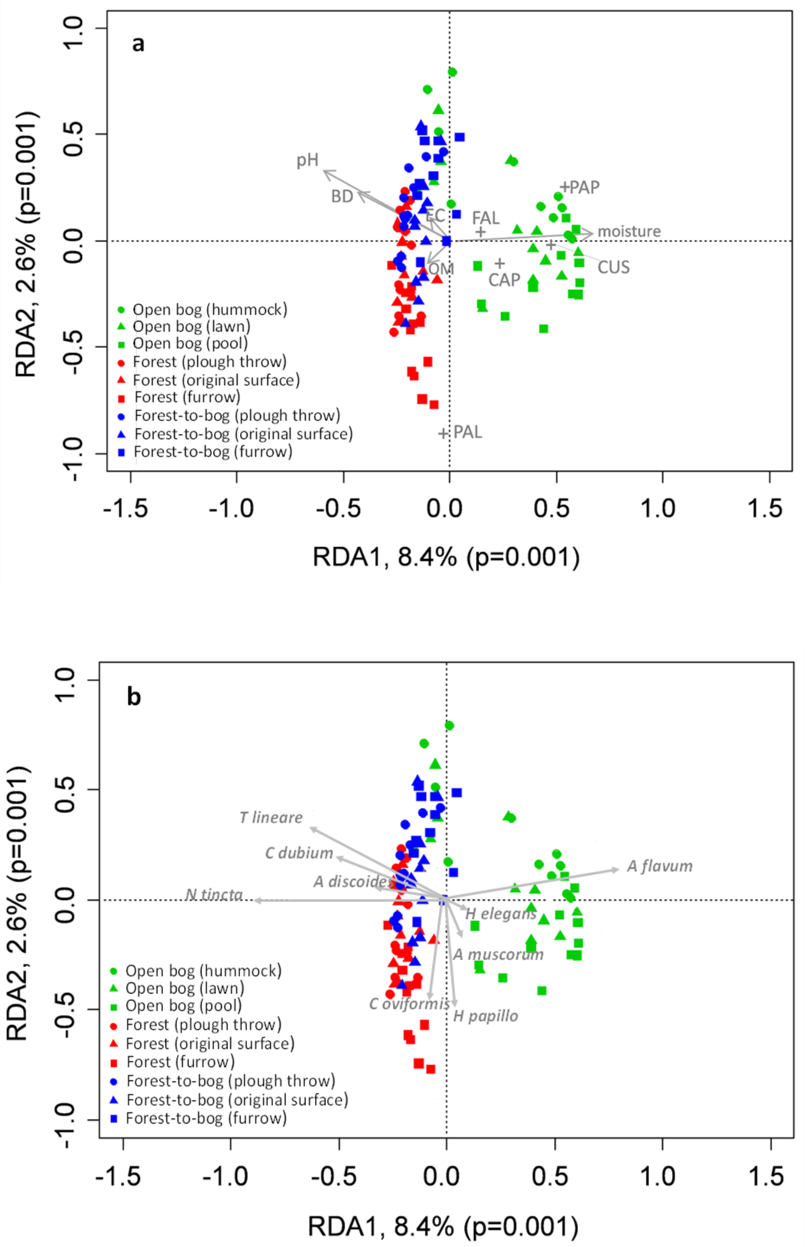
In the redundancy analysis environmental factors accounted for 34% of the variation in the testate amoeba community data. The first axis explained 8.4% of the variation (p = 0.001) and indicated a moisture-acidity gradient with the wetter, least acidic end associated with open bog and the drier, more acidic end mostly associated with the forested and forest-to-bog sites (Fig 2.5). The second axis explained 2.6% of the variation (p = 0.001) and indicated a bulk density-moisture gradient with higher bulk density mostly associated with the drier forested sites. The environmental variables measured were shown to be significant controls on the testate amoeba community: vegetation (presence/absence of key taxa: *Sphagnum*/non-*Sphagnum* bryophytes) (F = 7.43, p = 0.001), pH (F = 3.51, p = 0.003), conductivity (F = 2.15, p = 0.02), moisture (F = 3.03, p = 0.004) and bulk density (F = 2.22, p = 0.02). No significant association was found for organic matter and all sites were shown to be high in organic matter (Table 2.1).

Table 2.1. Site characteristics, geographic positions and physico-chemical properties. Given are means and standard deviations.



Site	Type	Geographic position		Altitude (m.a.s.l.)	pH	Bulk OM (%)	Water content (%)	Bulk density (g cm^{-3})
		Latitude	Longitude					
Cross	F	58°22'06.77"N	3°58'56.69"W	210	4.5 (0.3)	97.3 (0.9)	82.7 (5.5)	0.12 (0.04)
Cross	O	58°22'14.57"N	3°57'57.97"W	190	3.7 (0.1)	96.6 (1.4)	89.1 (5.2)	0.09 (0.05)
Cata	F	58°24'41.81"N	3°42'14.80"W	190	3.9 (0.3)	98.3 (0.4)	80.7 (10.3)	0.07 (0.03)
Cata	O	58°24'57.38"N	3°42'11.12"W	180	3.2 (0.1)	98.9 (0.5)	92.2 (2.6)	0.03 (0.01)
Bad	F	58°25'54.39"N	3°25'41.20"W	90	4.2 (0.5)	97.6 (0.7)	75.3 (12.5)	0.07 (0.02)
Bad	O	58°25'54.77"N	3°25'53.85"W	90	4.2 (0.1)	97.3 (1.1)	92.5 (2.2)	0.04 (0.01)
Brae	F	58°25'27.54"N	3°34'24.38"W	120	4.0 (0.3)	97.7 (0.5)	72.1 (4.8)	0.07 (0.02)
Brae	O	58°25'22.91"N	3°34'43.16"W	120	3.3 (0.1)	98.0 (0.6)	85.8 (3.3)	0.04 (0.01)
Tala	R	58°24'48.83"N	3°47'52.73"W	190	4.0 (0.4)	97.7 (0.7)	86.2 (6.4)	0.07 (0.04)

Sites: Cross = Cross lochs, Cata = Catanach, Bad = Bad a' Cheo, Brae = Braehour, Tala = Talaheel. Type: F = forested, O = open bog, R = forest-to-bog restoration site



Figures 2.5 a and b. Redundancy analysis of testate amoeba dataset showing (a) environmental drivers of community change and (b) selected major species. PAP = *Sphagnum papiliosum*, CAP = *S. capillifolium*, FAL = *S. fallax*, CUS = *S. cuspidatum*,

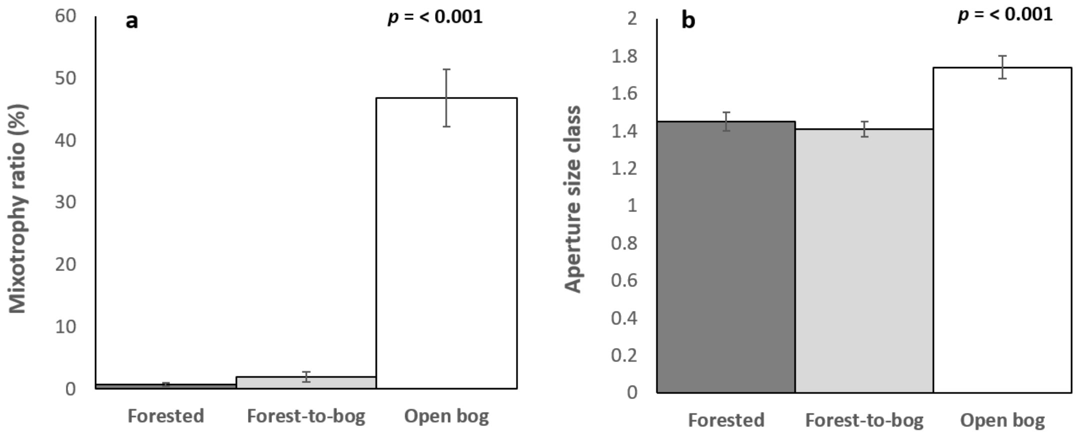
2.3.3 Indicator species and functional traits

Indicator species analysis revealed twelve significant indicator species (Table 2.2). The highest indicator values (IndVal) were found for *A. flavum* (0.79), *N. tincta* (0.51) and *T. lineare* (0.51). The best indicators of forested areas were *N. tincta, T. lineare* and *Trachelocorythion pulchellum.* In contrast, the best indicators of open bog were *Archerella flavum* (0.79), *Amphitrema wrightianum* (0.44), *Assulina seminulum* (0.33) and *Hyalosphenia elegans* (0.33). The third group indicative of forest-to-bog sites had the largest proportion of indicator species (41.6%) with *C. dubium, E. ciliata, N. tincta f. galeata*, *N. militaris* and *N. collaris* identified as significant indicators. Although not a significant indicator at our cut-off point (IndVal > 0.30), notably, *Arcella discoides* was shown to be a significant indicator of forested sites (IndVal 0.17, $p = 0.02$).

Table 2.2. Indicator species for 3 clusters characterised by the categorical variables site categories and environment showing taxa with Indval > 0.30, significant at p < 0.01.

Forested	**Forest-to-bog**	**Open bog**
Nebela tincta (0.51)	*Corythion dubium* (0.49)	*Archerella flavum* (0.79)
Trinema lineare (0.51)	*Euglypha ciliata* (0.42)	*Amphitrema wrightianum* (0.44)
Trachelocorythion pulchellum (0.46)	*Nebela tincta f. galeata* (0.36)	*Assulina seminulum* (0.33)
	Nebela militaris (0.35)	*Hyalosphenia elegans* (0.33)
	Nebela collaris (0.33)	

The functional trait analysis suggested some functional impacts of the differences in testate amoeba community composition between open bog, forested and forest-to-bog sites (Figs 2.6a and b). Kruskal-Wallis test showed mixotrophs were a significantly larger proportion of the community in open undisturbed habitats (χ^2 (2) = 35.233, p = < 0.001) compared with forested and forest-to-bog sites. Open bog sites also supported taxa with significantly larger aperture sizes (χ^2 (2) = 26.665, p = < 0.001. Mixotrophy ratios and aperture sizes in forested and forest-to-bog sites were not significantly different (χ^2 (2) = 622.0, p = 0.770; χ^2 (2) = 38.500, p = 0.270).



Figures 2.6 a and b. Functional traits (a) mixotrophy ratio (%) and (b) aperture size class. Aperture sizes were classified into 3 size categories: 1 = < 20 µm, 2 = < 40 µm, 3 = > 41 µm. Values are means with standard errors. Significant values represent differences between site categories (Kruskal-Wallis test).

2.4 Discussion

2.4.1 Impact of afforestation

Results here demonstrate significant effects of commercial forestry practices on testate amoeba communities. Multivariate analysis revealed that whilst there was some variability between individual sampling points, the first hypothesis could be accepted because there was a clear distinction in testate amoeba communities between relatively undisturbed open-bog, forested and forest-to-bog habitats.

Functional trait analysis revealed striking differences in testate amoeba communities between forested and undisturbed open bog. Taxa typifying the forested sites were mostly smaller and bacterivorous (e.g. *Trinema lineare; Corythion dubium*) and displayed smaller aperture sizes whereas the open bog microforms contained taxa with significantly larger aperture sizes and frequent mixotrophic taxa (e.g. *Archerella flavum* and *Hyalosphenia papilio*). These mixotrophs were completely absent in the forested sites. This parallels the loss of mixotrophs observed along transects across natural open-to-forested bog ecotones in Russian peatlands (Payne et al., 2016). These findings suggest that afforestation of blanket bog has led to a total loss of mixotrophic taxa in forested sites with a corresponding loss of mixotrophic primary production. Constraining the magnitude of this loss will require further studies.

It has been known for some time that mixotrophic testate amoebae respond to light conditions (Heal 1964; Schönborn 1965) and dense plantations will reduce ground-level light levels. Therefore, it is perhaps not surprising that mixotrophic taxa such as *A. flavum* and *H. papilio* were only found in undisturbed open bog as they cannot survive without photosymbionts and require light to survive. In contrast, *T. lineare* and *N. tincta* were the most dominant taxa in the forested sites and to our knowledge there are no records of photosynthetic endosymbionts in these taxa. Other studies have found taxa such as *T. lineare* and *N. tincta* able to thrive in shaded habitats (e.g. Marcisz et al., 2014). These findings could suggest mixotrophs are less competitive against strict heterotrophs in forested areas with extremely low light levels. In contrast, mixotrophs have the competitive advantage of its algal symbionts in *Sphagnum*-rich open bog when the density of its preferential prey is low (Jassey et al., 2013b). Light was the most important limiting factor for mixotrophs in this study, however, it is recognised that multiple disturbance mechanisms may also be important limiting factors for mixotrophs. The response of mixotrophs to drained and drained forested communities may provide further insights into the limiting factors affecting mixotrophic abundance and distribution in disturbed peatlands.

Moisture content is reported to be a significant control on testate amoebae in forested peatlands (Charman and Warner 1992). These results indicated a moisture-acidity gradient with the wetter, least acidic end associated with open bog and the drier, more acidic end mostly associated with the forested and forest-to-bog sites.

Moisture content was considered a more appropriate measure of surface wetness than water table depth as the water table in forested areas was > 1 metre below the surface in most locations and was therefore considered independent of surface moisture. Swindles et al. (2016) show that water table was not a significant control on testate amoebae in blanket peatlands under restoration, reporting a poor relationship between water table and surface moisture. Whilst water table depth is widely known to be a significant control on testate amoeba communities in *Sphagnum*-rich open bog (Lamentowicz and Mitchell, 2005; Marcisz et al., 2014), measurements of surface moisture may be more useful in biomonitoring peatlands undergoing forest-to-bog restoration.

This study considered the impacts of commercial forestry practices on peatland testate amoebae. These practices include multiple different disturbance mechanisms which encompass: 1) shading by the forest canopy; 2) fertilisation during forest planting; 3) water table drawdown; 4) physical disturbance in ploughing; 5) input of large quantities of conifer needle litter and 6) changes in microbial communities which may interact with testate amoebae, particularly the introduction of conifer root specific mycorrhizas. There may be further secondary impacts (e.g. change in pH) which will arise as a result of these direct impacts or their interactions. As these processes are complex and involve many components of the ecosystem which were not directly studied the exact mechanisms cannot be identified. However, this work highlights that there are good reasons to believe that the mechanisms are more complex than simply an impact of, for instance, water table drawdown alone.

2.4.2 Impact of restoration

Whilst restoration has shifted the testate amoeba community, communities in forest-to-bog sites still more closely resembled forested sites, therefore, the second hypothesis was rejected. Results suggest that the seventeen-year period between restoration and sampling has only seen relatively modest recovery in testate amoeba communities. Studies of microbial community change following peatland forest-to-bog restoration are rare and there has been no previous study of testate amoebae in this context. A study investigating the restoration of microbial processes following restoration of raised bog suggests timescales in excess of ten years are needed for microbial processes to be fully re-established and resemble near-natural conditions (Andersen et al., 2013). Other studies from degraded blanket bare peat report timescales in excess of twenty-five years for restoration to deliver the desired outcome (Elliott et al., 2015).

There was a clear differentiation in the ecology of indicator species in open bog and forested sites. This perhaps reflected the contrasting abiotic and biotic controls, for instance, moisture conditions and the size and availability of prey (Jassey et al., 2013b). The ecology of forest-to-bog indicators nevertheless showed more similarity with forested indicators with small, bacterivorous generalists such as *Trinema lineare* and *Corythion dubium* identified as powerful indicators of afforestation and restoration sites. Certain taxa from the genus *Nebela* were also prominent indicators of afforestation (e.g. *N. tincta*) and restoration (e.g. *N. collaris*) and could perhaps reflect the feeding behaviour and availability of food sources. For instance, fungal spores and mycelia have been shown to be important food sources for *Nebela tincta* (Jassey et al., 2013c), although, currently there is not enough evidence to extend this generalisation to *N. collaris* nor to other taxa from this genus. It was speculated that afforestation and restoration has created a legacy of environmental conditions which has altered the availability of food sources and hence microbial feeding strategies. Other studies have assessed the links between peatland restoration of cutover bogs and *Sphagnum* presence (Buttler et al., 1996; Grosvernier et al., 1997). Arguably, one of the main barriers to recovery depends on creating conditions conducive to *Sphagnum* re-establishment, which in turn, will shift the microbial food web towards pre-disturbance conditions.

Recovery of testate amoeba communities will be influenced by the availability of food sources, which, in turn, will be strongly influenced by vegetation composition and density (Sullivan and Booth, 2011; Jassey et al., 2013a). The partial recovery of testate amoebae in this study follows a similar trend to that visible in the vegetation community in Talaheel, which has still not fully recovered to be comparable to nearby reference sites. This is particularly the case on plough throws where conditions are dry (Hancock, M. personal communication). Similarly, it was evident from the multivariate analysis that testate amoeba communities in drier microforms in forest-to-bog sites resembled the forested sites. In contrast, testate amoeba in furrows where *Sphagnum* had established were beginning to show some resemblance to pool microforms in undisturbed open bog, except for the driest site, Braehour. These findings highlight the need for using a wide range of reference sites to encompass natural variability in blanket bog.

Restoration methods in this study region have moved on from felling-to-waste, and more recent technical development mean that the whole tree can be harvested. In some sites including Talaheel, further management is also being undertaken where furrows are being blocked, leading to a step-change in the recovery of the water table which could potentially accelerate re-colonisation by *Sphagnum sp.* Given the range of management practices being trialled, there are now interesting opportunities to further test the potential of testate amoebae as indicator species and use them in combination with hydrological conditions and vegetation assemblages to assess trajectories of recovery and inform management decisions.

2.4.3 Testate amoebae as bioindicators in forest-to-bog restoration

This is the second study to highlight the potential of testate amoebae as bioindicators in restoration of blanket bog and the first in the context of forest-to-bog restoration. Based on the variability between sampling points, it is recommended that future sampling designs encompass microtopography. The importance of microtopography was demonstrated in the indicator species analysis which showed *Arcella discoides* to be a potential indicator in forested sites. This taxon was most abundant in wetter furrows compared with open bog microhabitats and in forested sites it was frequently found together with dry indicators *Trachelocorythion pulchellum* and *Trinema lineare*. *A. discoides* is considered a key wet indicator in contemporary (Swindles et al., 2016) and palaeohydrological reconstructions of testate amoebae (Charman et al., 2007). Lamentowicz et al. (2008) propose that samples with a high abundance of both *A. discoides* and taxa indicative of dry conditions, as in the present study, should be interpreted with caution as they indicate highly variable, fluctuating water tables (e.g. temporary flooding of dry peat surfaces), resulting in the mixing of testate amoebae with contrasting wetness requirements.

Compared with groups of macroscopic organisms such as plants and animals, microorganisms have seldom been explored in afforested peatlands or those undergoing forest-to-bog restoration. Other studies recently highlight the potential of testate amoebae in biomonitoring peatlands (Koenig et al., 2015; Swindles et al., 2016). Koenig et al, (2015) demonstrated how ten easily identifiable testate amoeba taxa could be useful in biomonitoring of peatlands. All these reported ‘easily identifiable’ taxa were observed in the indicator species analysis in this study with the exception of *Heleopera rosea*, These findings therefore strongly support the idea that testate amoebae could be a good bio-indicator for the Flow Country because key species could be easily identified by practitioners and/or those with little taxonomic expertise in monitoring forest-to-bog restoration. It is promising that this study revealed 90% of the same indicator species published by Koenig et al., (2015), especially as we look to provide peatlands managers with sound tools for following restoration processes.

As well as species themselves, this study has shown that functional traits in testate amoeba are specific and responsive enough to act as bioindicators in peatlands undergoing forest-to-bog restoration. Perhaps the most striking evidence was the complete loss of mixotrophic taxa observed in drier afforested sites compared with the wetter, oligotrophic conditions in open bog sites. Mixotrophy is an important foraging strategy in nutrient poor open bog habitats. Mixotrophs feed on preys through phagocytosis (heterotrophy) and use their algal symbionts to acquire food and energy through photosynthetic activity (Jassey et al., 2013a; Jassey et al., 2013b; Fournier et al., 2015). Endosymbiotic algae are considered to provide mixotrophs with a competitive advantage over heterotrophic testate amoeba when density of preferential prey is low (Jassey et al., 2013b) and are suggested to influence the competitive hierarchy amongst species through a decrease of the predation pressure of testate amoebae on lower trophic levels (Fournier et al., 2015). In other words, mixotrophs can potentially modify the functioning of the microbial food web by influencing the imbalance between primary productivity and lower decomposition by microbial communities. Thus, mixotrophs may have potential to indicate changes at lower trophic levels (Payne 2013) and as such could be useful bioindicators.

2.5 Conclusion

This research has shown that whilst afforestation has significantly changed testate amoeba communities in blanket bog, recovery is at least partially evident seventeen years after restoration management. Results suggest that the “threshold” conditions which will lead to the recovery of the testate amoeba community are strongly dependent on the recovery of *Sphagnum*, which in turn, relies on a hydrological regime conducive to saturated conditions. Therefore, in order to recover microbial ecosystem function in blanket bog, it is essential to ensure that conditions are suitable for *Sphagnum* to regenerate, which in turn, may also trigger changes in the microbial communities, such as the recovery of mixotrophic taxa. Modern restoration techniques will provide new and interesting opportunities for future studies investigating the effects of forest-to-bog restoration on different groups of microorganisms. An indicator species approach using species traits may be particularly useful with potential for application in bio-monitoring peatlands under restoration.

Chapter Three

Annual gaseous carbon budgets of forest-to-bog restoration sites are strongly determined by vegetation composition

3.1 Introduction

Peatlands only cover a small proportion (~3%) of Earth's surface (Xu et al., 2018), yet store about 500 ± 100 (GtC) (Gorham, 1991; Yu et al., 2010), representing an important stock (~25-40%) of global soil carbon (Yu, 2012; Rydin and Jeglum, 2013; Cooper et al., 2014). Over millennia, intact peatlands have had a net cooling effect on the global radiation balance through C sequestration and storage (Frolking et al.,2011). However, when peatlands are drained, C can be released from peat into the atmosphere through aerobic decomposition at far more rapid rates than it was accumulated (Gorham 1991; Lindsay 2010). In northern temperate peatlands, it is currently uncertain whether contemporary C accumulation rates offset C emissions from degraded peatlands (Billet et al., 2010). Recent research suggests plant communities have potential to significantly affect C accumulation (Mathijssen et al., 2019), and hence, the C sink function of ombrotrophic peatlands, i.e. raised bogs and blanket bogs.

Peatland gaseous C dynamics are a balance of carbon dioxide (CO_2) uptake by plants during photosynthesis (gross primary productivity, GPP) and CO_2 release through plant and soil respiration (net ecosystem respiration, NER), and methane (CH_4) production and consumption. The Intergovernmental Panel on Climate Change (IPCC) defined the global warming potential (GWP) of CH_4 over a 100-year cycle and is reported to be 28 times greater than CO_2 (Myhre et al., 2013). Trade-offs between the balance of CO_2 and CH_4 uptake and release have important climate implications, and human actions can alter the greenhouse gas (GHG) balance in peatlands (Luo and Zhou, 2006; Dise 2009; Laine et al., 2019). Determining whether peatlands are functioning as a net C sink requires the calculation of a C budget (or balance), which can be estimated by measuring fluxes of C exchanges at land-atmosphere interface (Rowson et al., 2013) and export of dissolved organic C (DOC) into the aquatic environment (for example, Worrall et al., 2011; Gaffney et al., 2018). Several studies have calculated annual gaseous C budgets of peatlands degraded by human activity (for example, Strack and Zuback, 2013; Worrall et al., 2009), with results strongly dependent on land-use and peatland type (Hommeltenberg et al., 2014).

It is estimated that around 15 Mha of peatlands have been drained for forestry in the boreal and temperate zones (Paavilainen and Päivänen, 1995). In the British Isles, afforestation of previously open peatland was widespread in the late 20th century, and by the 1990's > 800,000 ha (circa 20%) was afforested in the UK, and > 200,000 ha (circa 16%) in Ireland (Andersen et al., 2017). Whilst trees are a natural component of many peatland ecosystems in continental climates (Hommeltenberg et al., 2014), in more oceanic or colder climates most peatlands are treeless (Lindsay 2010; Payne and Jessop, 2018a). In open peatlands, forestry requires the drainage of the peat surface before trees are planted. Forestry practices in the UK often involved using closely spaced plough furrows between deeper drainage systems, creating ridges sufficiently raised above the drained water table to encourage tree establishment and survival. It is generally accepted that drainage of peatlands leads to higher peat decomposition with substantial C losses to the atmosphere (Couwenberg, 2011), yet, some studies have shown that forest drainage does not necessarily change a peatland from a C sink to a C source (Minkkinen and Laine, 1998; Hommeltenberg et al., 2014). Net ecosystem exchange (NEE) is used to determine the sink–source status of peatlands drained for forestry (Hommeltenberg et al., 2014; Laine et al., 2019; Hambley et al., 2019).

Considerable investments have been made in the UK to restore peatland, including those damaged by drainage and afforestation (Andersen et al., 2017; Payne et al., 2018a; Payne et al., 2018b). Restoration of an afforested peatland typically involves the removal of trees and the blocking of drainage ditches and/or plough furrows to raise the water table. Trials of different restoration techniques are occurring rapidly, especially in Scotland, where older restoration techniques (e.g. felling to waste where small trees are rolled into furrows, and only collector drains are blocked) are being superseded by newer, more novel restoration practices (Hancock et al., 2018), such as whole tree harvest, reprofiling, etc. All these techniques involve a significant initial disturbance. In the case of forest-to-bog restoration, these efforts are largely driven by biodiversity incentives, to halt losses associated with afforestation and habitat fragmentation (see Wilson et al., 2014). However, a key question remains whether forest-to-bog restoration also deliver the climate benefits widely associated with peatland restoration (Bonn et al., 2016).

Data on the C balance of forest-to-bog restoration sites are limited (Sloan et al., 2018), and in the UK, research has focused on blanket bog (Hermans, 2018; Hambley et al., 2019; Hermans et al., 2019; Lees et al., 2019). Despite some uncertainties, this research has shown that forest-to-bog restoration sites older than 15 years can have climate benefits, by storing more GHG’s than they emit (Hermans et al., 2019). However, apart from one study in Ireland (Rigney et al., 2018), there is currently a lack of knowledge of whether forest-to-bog restoration can restore the C balance of raised bog. Using an eddy covariance (EC) technique on blanket bog, Hambley et al, (2019) report C sink sixteen years post restoration and C source ten years post restoration, although this study did not capture the CH_4 flux. In contrast, using a closed chamber technique, eight years post restoration, Rigney et al, (2018) found the C sink function in a raised bog in Ireland had not recovered to pre-disturbance conditions. In their study, microsites dominated by *Cladonia* sp. and *Calluna* sp. were reported to act as strong sinks for CO_2-C.

Vegetation is known to play an important role in the exchange of GHG's at the peatland surface of restoration sites (Waddington and Day, 2007; Ward et al., 2009; Ward et al., 2013), but less is known in relation to forest-to-bog restoration. Initial studies in Scotland have shown that the process of forest-to-bog restoration and raising the water table creates a shift in vegetation communities, often a dominance of sedges, particularly *Eriophorum angustifolium* (Hancock et al., 2018), with potential for higher CH_4 fluxes in the short term (Waddington and Day, 2007). Plant traits could also be a co-driver of GHG fluxes of forest-to-bog restoration sites. For example, Konings et al (2019) found intra-specific variation in plant species, specifically, significantly higher leaf area and chlorophyll content in afforested and forest-to-bog sites compared with undisturbed open bog and suggested that legacy effects of the forestry may persist even after vegetation communities have returned.

The aims of this paper are to present annual gaseous carbon budgets for a chronosequence of forest-to-bog restoration areas of a raised bog and to compare these to unafforested open conditions, using a space-for-time substitution approach (Pickett 1989; Walter et al., 2007). The hypotheses were: 1) Restoration of afforested peatlands returns the CO_2 sink function, 2) There will be a higher CH_4 efflux from vegetation dominated by *Eriophorum angustifolium* and, 3) Annual net ecosystem exchange of

CO_2 and CH_4 (therefore total C sequestration) will be lower in the youngest restoration areas than oldest and unafforested areas.

3.2 Materials and Methods

3.2.1 Study area

This study was conducted on a large raised bog complex spanning the border of England and Wales. Fenns, Whixall and Bettisfield Mosses National Nature Reserve (52°92’24’’N, 2°76’94’’W) is a lowland raised mire in the counties of Shropshire and Wrexham at an altitude of 72 m above sea level (Fig. 3.1). The raised bog complex includes several distinct but inter-connected peat domes with peat initiation occurring in the early Holocene (Hardy 1939; Turner, 1964). The site was drained during the 18th century for commercial peat cutting, which persisted throughout the nineteenth and twentieth centuries (see Leah et al., 1998). Commercial peat extraction ceased in 1990 when the site was acquired by statutory nature conservation organisation the Nature Conservancy Council. Historically, the site has also been managed for commercial forestry, particularly around the periphery of the site. Marginal forestry plantings at the study areas were circa 1960 with Lodgepole pine *Pinus contorta*, Norway spruce *Picea abies* and Scots pine *Pinus sylvestris* planted at 2 metre spacings. Since the late 1990’s, management efforts have focused on removing existing plantations to reduce interception, evapotranspiration, shading and tree seed sources combined with damming ditches to raise the water table (Joan Daniels, pers comm). Monthly average air temperature ranges from 0.6^{O}C (min) to 21.0^{O}C (max) and mean annual rainfall is 659.9 mm (1981 – 2010 average at Shawbury Meteorological Station, 22 km from the study site).

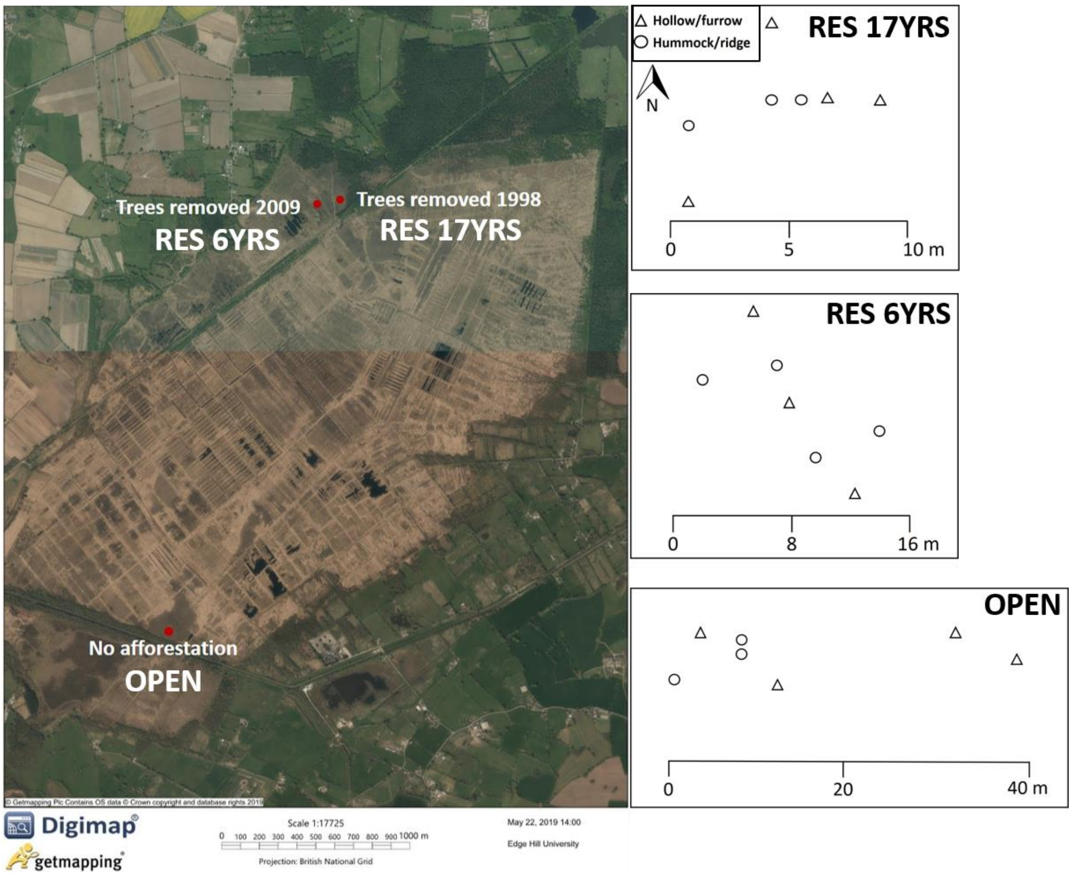


Figure 3.1. Location of the three study areas at Fenns, Whixall and Bettisfield Moss with sampling point (collar) distribution in each of the three study areas.

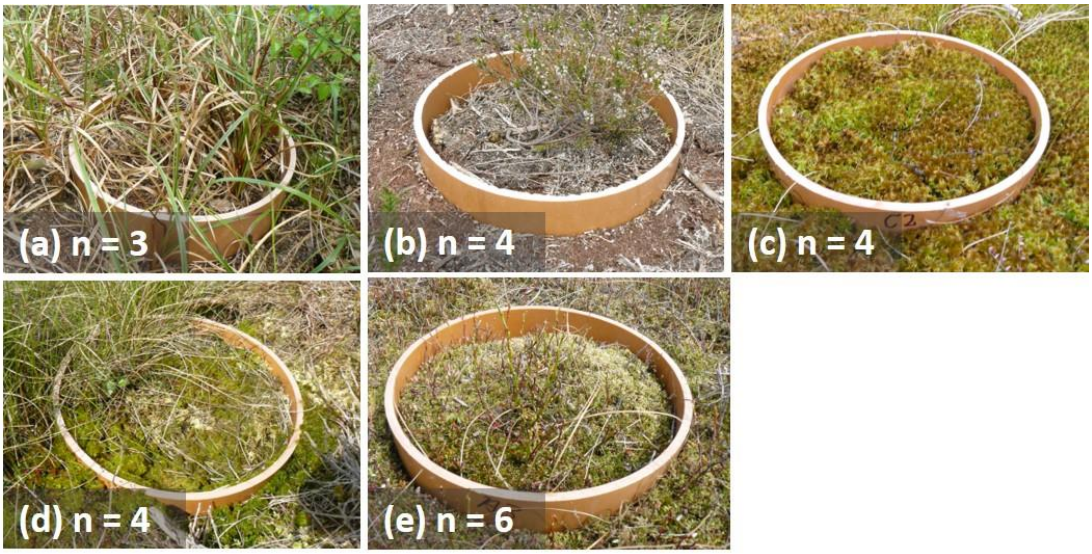
3.2.2 Experimental design

This study focused on a chronosequence design considering non-afforested peatland and two phases of restoration. In April 2015, a restoration chronosequence was identified, consisting: 1) trees planted in ~1960 and removed in 1998 (17 years post-restoration – referred to as 'RES 17YRS'), 2) trees planted in ~1960 and removed in 2009 (6 years post-restoration – referred to as 'RES 6YRS'), 3) control area (no afforestation – referred to as 'OPEN'). All study areas were adjacent and were not impacted by historical peat cutting. Both afforested areas were planted with trees at the same time, using the same practices and can be considered to have been equivalent prior to restoration. Restoration involved whole tree removal (motor-manually felled), scrub control and brash removal coupled with damming drainage systems to promote a high-water table. At the oldest restoration area (RES 17YRS) the trees were manually carried off, whereas at the younger restoration area (RES 6YRS) trees were mechanically removed. This is a different technique to mechanised harvesting because in both cases it was whole tree removal rather than processing on site (Joan Daniels, pers comm).

Unafforested areas were characterised by hummock-hollow microtopography and supported typical peatland plant species such as: *Sphagnum* spp., *Aulacomnium palustre*, *Vaccinium oxycoccos*, *Erica tetralix* and *Eriophorum vaginatum.* Restoration areas were characterised by ridge-furrow microtopography. Vegetation/cover in the oldest restoration areas were dominated by ericaceous species such as: *Calluna vulgaris*, *Vaccinium oxycoccos* and *Erica tetralix* on the drier ridges with *Eriophorum vaginatum* and *Sphagnum* spp., colonising the wetter furrows. In the youngest restoration areas, ridges consisted of bare peat and brash (e.g. conifer needle litter and woody debris) with *Calluna vulgaris* and furrows were colonised by *Eriophorum angustifolium* on bare peat and brash. Photographs of the five main vegetation communities identified across the three study areas are shown (Figs. 3.2a-e).

Seven sampling points (n = 7) were distributed in hummock/ridge and hollow/furrow microtopography in each study area to give a total of 21 sampling points. Sampling points were categorised based on the dominant vegetation and five groups were identified (see Table 3.3). At each sampling point, PVC gas chamber base rings, also referred to as collars (31.5 cm diameter and 10 cm height) and dipwells were permanently installed to allow for repeated gas flux and water table measurements. Collars were left for at least one month prior to gas flux measurements.

Soil and air temperature loggers (HOBO Pro v2 U23-00x) and water level loggers (HOBO U20L-0x) were installed in each of the three study areas logging at one-hour intervals. Soil temperature loggers were installed at 5 cm depth. Hourly daily air temperature and solar radiation data obtained from the UK meteorological office from Shawbury meteorological station (22 km from the study site) were used to supplement the data logged data for modelling C fluxes.



Figures 3.2 a-e. Photographs showing the five main different vegetation types identified across the three study areas: (a) *Eriophorum angustifolium* and bare peat (ERIANG), (b) *Calluna vulgaris* and bare peat/brash (CALVUL) (c) *Sphagnum* dominant (SPH), (d) mixed community of *Sphagnum* and *Eriophorum vaginatum* dominant and ericaceous shrubs (SPH ERIVAG) and (e) Ericaceous shrubs and non-*sphagnum* moss (SHRUB).

3.2.3 Vegetation composition

Sampling points were surveyed in August 2015 at the peak biomass period using the line-point intercept (LPI) method (Rochefort et al., 2013), and re-surveyed in August 2016 to assess vegetation change. For this, a 50 x 50 cm frame was placed carefully over each collar and 25 measurements were obtained at equal 5 cm intervals based on the number of species touches with a vertically placed pin (1mm diameter). The LPI method was preferred over a percentage cover characterisation of the plant species within the collars because it did not involve subjective estimates of plant cover. Furthermore, this method is preferred to spatially assess the impact of restoration actions in the early stages post-restoration (Rochefort et al., 2013), which was the case in the youngest restoration area (RES 6YRS). Plant species composition for both surveys for each sampling point were averaged and frequencies (expressed as percentages) were calculated for each species, providing a quantitative measurement of cover. The nomenclature follows Atherton et al. (2010) for mosses and Stace (2019) for vascular plants.

3.2.4 GHG flux measurements

Sampling points were monitored during seventeen visits from June 2015 to June 2016 with measurements taken between 1000 and 1600 hours. The closed chamber method (Alm et al., 2007; Rowson et al., 2013; Goud et al., 2017) was used for surface exchange of CO_2 and CH_4. This method involves circulating air between a transparent closed chamber and a gas analyser (Los Gatos Research Greenhouse Gas Analyser Model 915-0011) which also measured ambient air temperature. The chamber was fitted with a fan for headspace air mixing and a rubber seal was used to prevent the exchange of gases between the chamber and the surrounding environment. Solar radiation was measured using an Apogee Silicon Pyranometer (model SP-212-L).

This study measured net ecosystem exchange (NEE) and ecosystem respiration (NER) with GPP calculated from the difference (NEE – NER). NEE was measured as the flux in light conditions and NER was measured by covering the chamber with a light impermeable cover. Fluxes were monitored in real time which allowed measurements to be corrected if equilibrium or a poor chamber seal was detected. Typically, light and dark measurements could be taken in two minutes with a one-minute flush in between measurements until ambient concentrations of CO_2 and CH_4 were reached. This study used the micro-meteorological sign convention which defines a negative NEE as a C sink with positive NEE indicating a C source. Therefore, CO_2 taken in from the atmosphere (via photosynthesis) is presented as a negative value, CO_2 emitted (via respiration) is presented as a positive value. Gas fluxes were calculated and processed according to Dossa et al. (2015), with slight modifications. Flux values were calculated based on the most linear change of CO_2 and CH_4 within the two-minute measurement period.

3.2.5 Modelling annual GHG fluxes

Annual NER was modelled for each sampling point based on measured fluxes using the 2z + S model (Rowson et al., 2013). When the data was unavailable to parameterise the Rowson et al. (2013) model (specifically from June 2015 – September 2015 due to a delay installing the loggers), the widely used Lloyd and Taylor (1994) model was adopted (Eq. 2).

$$\mathrm{NER} = \mathrm{R}_{10}{}^{\mathrm{E}_0\left(\left(\frac{1}{283.15-\mathrm{T}_0}\right)-\left(\frac{1}{\mathrm{T}-\mathrm{T}_0}\right)\right)} \tag{2}$$

Where R_{10} is the amount of CO_2 produced at 10 °C, E_0 = 308.56 J mol^{-1} K^{-1}, T_0 = 227.13 K and T = temperature in Kelvin (K).

The Lloyd and Taylor model accounted for twenty four percent of the calculated annual NER budget whilst the Rowson model made up the largest proportion (~76%). The Rowson et al. (2013) 2z model (Eq. 3) is an extension of the more widely used Lloyd and Taylor, (1994) equation based upon the Arrhenius relationship between soil temperature and measured NER. This model hypothesises that peat respiration can be divided into two distinct zones which modifies the R_{10} value to be linearly scaled with changes in WTD. Therefore, as the aerobic zone increases in volume CO_2 release also increases given the same temperature, stimulated by plant root exudates which stimulate microbes, soil respiration and root respiration. The model assumes there is a constant rooting depth that determines the depth of these 2 zones. If the WTD was greater than the rooting depth constant zones 1 and 2 were summed. If the WTD had a smaller value than the rooting depth constant then only zone 1 equations were used.

Therefore:

When WTD < rooting depth constant:

$$\text{Zone 1a} = (a\text{WTD} + b)^{E_0\left(\left(\frac{1}{283.15 - T_0}\right) - \left(\frac{1}{T - T_0}\right)\right)} \tag{3}$$

When WTD > rooting depth constant:

$$\text{Zone 1b} + \text{Zone 2b} \tag{4}$$

Where:

$$\text{Zone 1b} = (a\text{Rooting depth constant} + b)^{E_0\left(\left(\frac{1}{283.15 - T_0}\right) - \left(\frac{1}{T - T_0}\right)\right)} \tag{5}$$

$$\text{Zone 2b} = (c(\text{WTD} - \text{Rooting depth constant}) + d)^{E_0\left(\left(\frac{1}{283.15 - T_0}\right) - \left(\frac{1}{T - T_0}\right)\right)} \tag{6}$$

Where a, b, c and d and rooting depth constant are fitted constants, WTD = water table depth, all other terms as defined in equation 2.

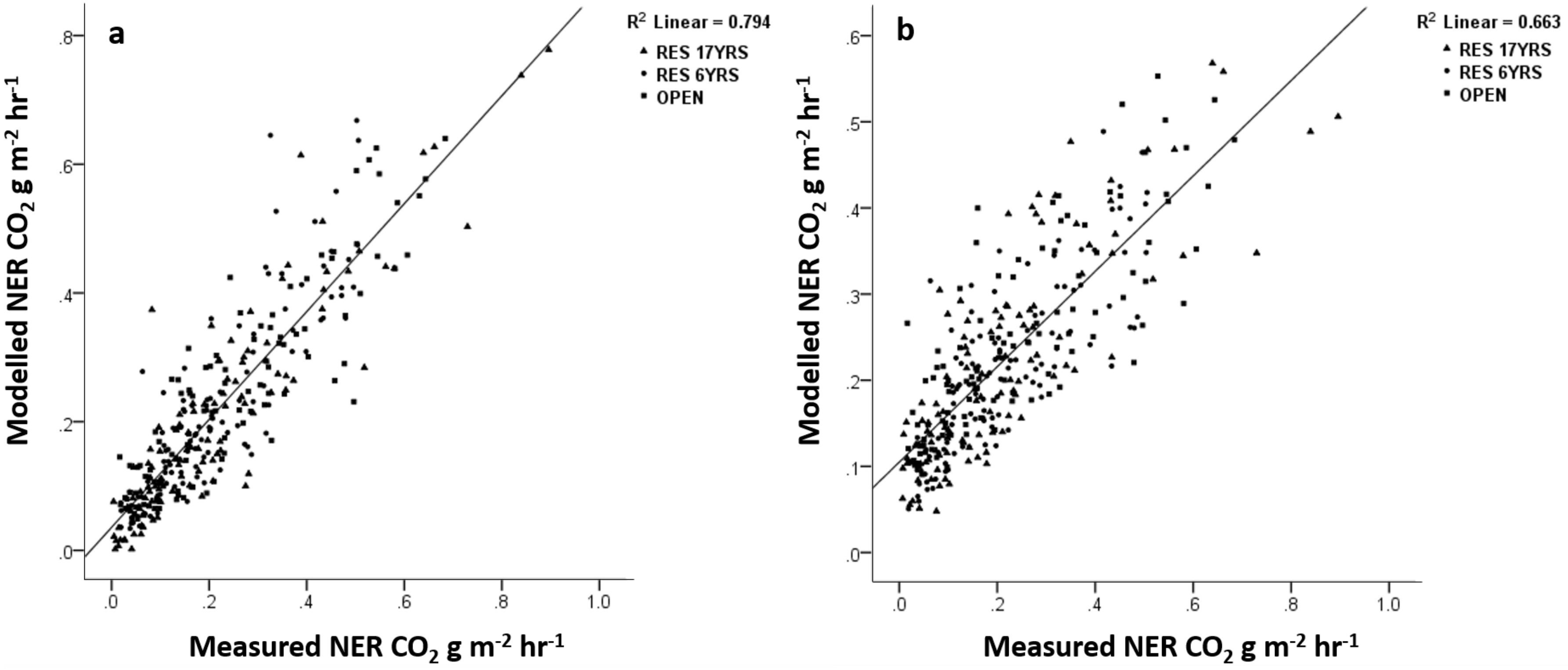
In addition, the 2z + S model links NER to plant function by accounting for the feedback between plant root exudates, season (i.e. incorporates plant senescence) and respiration. This model assumes zone 1 will only produce CO_2 when plants are growing. When plants start to senesce, this was called the start month (i.e. September). When plants finish senescing, this was called the end month (i.e. April).

Therefore, when the month number is greater than the start month, or less than the end month:

$$\mathrm{Zone\,2a} = (\mathrm{cWTD} + \mathrm{d})^{\mathrm{E_0}\left(\left(\frac{1}{283.15}\right) - \left(\frac{1}{\mathrm{T} - \mathrm{T_0}}\right)\right)} \tag{7}$$

Or else, equations 3 – 6 were used.

Accounting for plant function in this way (i.e. using equations 3 – 7) improved the average R^2 from 0.66 to 0.79 (Figs. 3.3a and b) and reduced the standard error of the estimate (SSE) from 0.01 to 0.006. Therefore, the model proposed by Rowson et al. (2013) was deemed more robust for modelling annual NER due to the better fit and lower error terms.



Figures 3.3 a and b. Modelled and measured estimates of NER of CO_2 from the three study areas (a) estimated using the model proposed by Rowson et al. (2013) and (b) estimated using Lloyd and Taylor (1994) model. Linear trend lines and R^2 values are displayed for both models.

The parameters of the modelled NER (Rowson et al., 2013) derived for each sampling point were fitted to temperature and WTD data, constants were identified using the generalised reduced gradient (GRG2) nonlinear optimisation code (Lasdon et al., 1978), with 7 constants defined for each sampling point. From each equation, the modelled values were plotted against the measured NER values. The regression line for each sampling point had a relatively high coefficient of determination (range $R^2 = 0.544 – 0.928$). Having found no significant relationships between measured CO_2 flux and temperature or solar irradiance, GPP was modelled based upon a second order (quadratic) polynomial relationship (Eq. 8) with NER – a standard approach for modelling curvilinear regressions (Solkal and Rohlf, 2012). Solar radiation data (MET office) was used to eliminate GPP fluxes in the absence of solar radiation.

$$\mathrm{ax2} + \mathrm{bx} + \mathrm{c} = \mathrm{GPP} \tag{8}$$

The interpolation approach which linearly integrates fluxes between measurement periods (Green and Baird, 2017) was adopted for CH_4 because, in contrast with other studies (e.g. Laine et al., 2007), no significant relationships between measured CH_4 flux and soil/air temperature or WTD were observed, which eliminated the use of a modelling approach for CH_4. Therefore, annual CH_4 flux was calculated based on linear interpolation during the time periods between flux-chamber measurements (see Dise et al., 1993). These values were then summed to predict the annual flux. GWP was calculated by converting CH_4 fluxes to CO_2 equivalent emissions (CO_2-eq). For this, CO_2-eq was determined by multiplying annual CH_4 flux by a factor of 28, which is reported as the GWP of CH_4 over 100 years (Myhre et al., 2013). The total gaseous C budget was calculated by summing the CH_4 CO_2-eq and CO_2 NEE values.

3.2.6 Statistical analyses

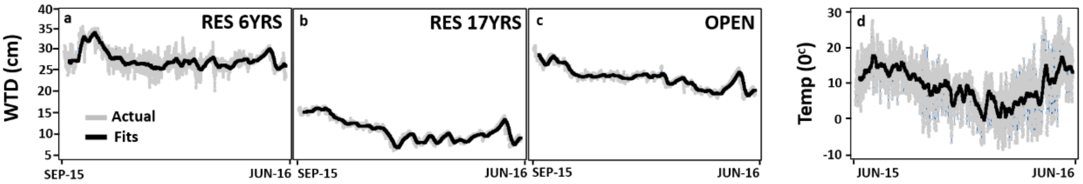
Measured flux data (NER, GPP and NEE of CO_2 and CH_4) were compared between the three study areas over the year. Linear mixed models (LMM) were used, fitted using restricted maximum likelihood (REML) estimation of parameters, to analyse the effect of study area (restoration age), microtopography, vegetation/cover and time of year on CO_2 fluxes (NEE, NER and GPP). Area, microtopography, and time of year were included as categorical fixed factors, vegetation/cover was included as a random factor and air temperature and WTD were included as covariates in all analyses. Solar radiation was included for GPP and NEE.

For modelled flux data, one-way analysis of variance (ANOVA) was used to test for differences between study areas, microtopography, collars and vegetation/cover. Differences between study areas and vegetation/cover were analysed using Tukey post-hoc tests. The non-parametric Kruskal–Wallis (KW) test was used for continuous data which did not satisfy the assumptions of normality and homogeneity of variance after data transformation (as was the case for CH_4). All data were analysed using statistical package IBM SPSS statistics (version 25).

3.3 Results

3.3.1 Water table depth and temperature

Water table depths for all three study areas showed variable seasonal patterns (Figs. 3.4a-c). Restoration areas generally showed higher autumn/winter levels with high spring levels decreasing in summer months. Water table position was closer to the peatland surface at the oldest restoration area (RES 17YRS) compared with the unafforested area (OPEN). Temperature across all three study areas followed a similar pattern (Fig. 3.4d) with a sharp decline in temperature in autumn 2015 and gradually increasing from spring 2016.



Figures 3.4 a-d. Weekly moving average (length 168) water table depths (cm) for each of the three study areas from Sep 15 – Jun 16 (4a-c) and temperature (4d) from Jun 15 – Jun 16.

3.3.2 Measured GHG fluxes

There was a clear annual cycle of CO$_2$ fluxes observed in all study areas and time of year was a significant factor in all CO$_2$ models, with significantly higher fluxes from June to October 2015. LMM analysis of net ecosystem CO$_2$ exchange showed that vegetation/cover, collar, time of year and microtopography were significant factors in CO$_2$ fluxes (p = < 0.05), but study area (restoration age) was not found to be a significant factor (p = 0.945) (Table 3.1). However, there was a significant interaction between study area and time of year which means patterns of uptake and release vary over the year between the three study areas. In all 3 study areas NEE of CO$_2$ was higher in hummock/ridge microtopography with lower water table and lower in furrows/hollows with high water table. NER of CO$_2$ followed a similar trend and was greater in hummock/ridge microtopography and was best explained by WTD and temperature. There was a significant interaction among study area and collar for NER of CO$_2$ which means that there is a significant factor within the collars which influences fluxes among the study areas and is perhaps a more important factor than restoration age.

Table 3.1. Linear mixed models for net ecosystem exchange (NEE), net ecosystem respiration (NER) and gross primary productivity (GPP) of CO_2 at forest-to-bog restoration sites, measured from June 2015 - June 2016. Model is based on Type 1 sums of squares.

	NEE			**NER**			**GPP**		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Sunlight	1	3.688	0.056				1	0.411	0.522
Air temperature	1	5.395	0.021	1	15.088	<0.001	1	0.285	0.594
WTD	1	0.969	0.326	1	6.368	0.012	1	2.984	0.086
Restoration area	2	0.056	0.945	2	4.909	0.008	2	1.994	0.139
Microtopography	1	29.367	<0.001	1	19.800	<0.001	1	4.340	0.039
Vegetation/cover	2	6.071	0.003	2	28.245	<0.001	2	11.353	<0.001
Collar	18	16.993	<0.001	18	22.301	<0.001	18	25.939	<0.001
Time of year	15	3.566	<0.001	16	36.664	<0.001	15	8.657	<0.001
Area * time of year	29	2.501	<0.001	32	1.272	0.164	29	1.462	0.071
Cover * Time of year	42	1.179	0.229	46	1.251	0.151	42	0.896	0.655
Area * cover	2	2.389	0.095	2	11.837	<0.001	2	1.575	0.21
Area * cover * time of year	25	0.695	0.857	27	0.951	0.539	25	0.460	0.988
Total d.f.	139			148			139		

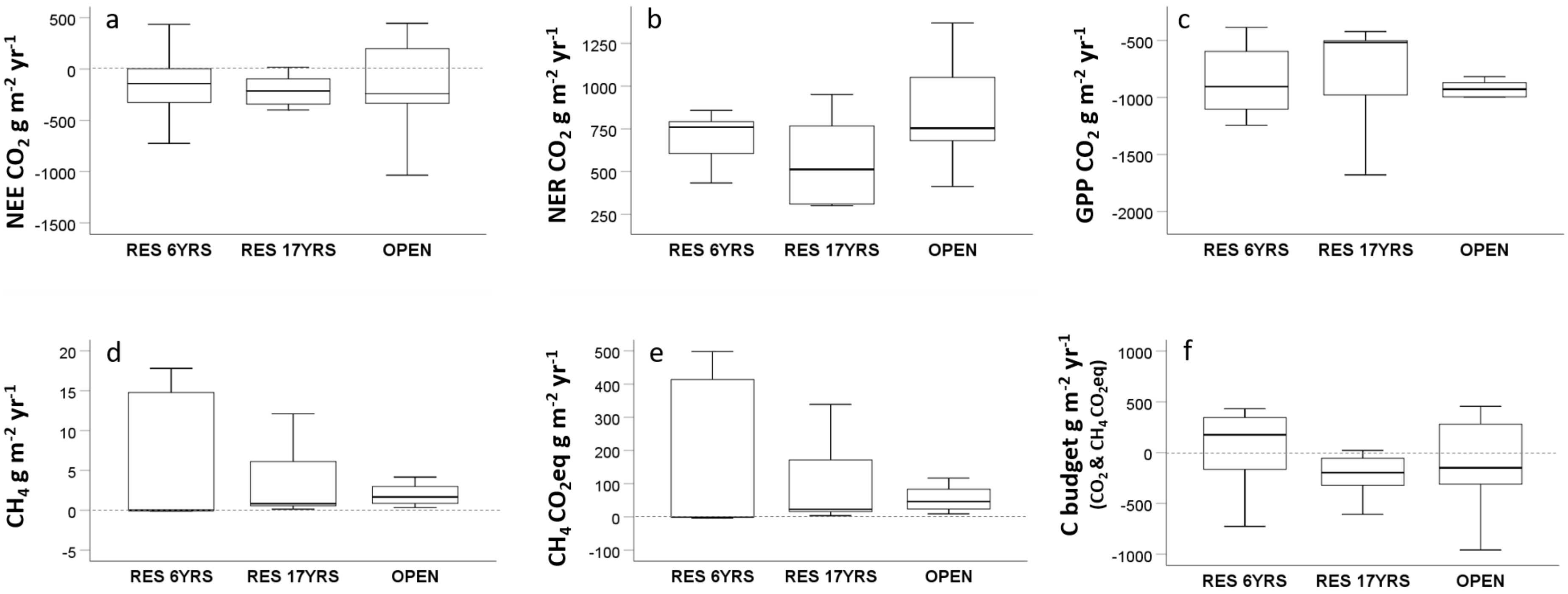
In contrast with CO_2, CH_4 fluxes showed no clear annual cycle and time was not observed to be a significant factor (X^2 (16) =4.656, p = 0.997). Collar (X^2 (20) = 238.338, p <0.001) and microtopography (X^2 (1) = 85.40, p <0.001) were significant factors in CH_4 fluxes compared with study area (X^2 (2) =5.227, p 0.073), with highest fluxes found in hollows/furrows with a high-water table and significantly lower fluxes observed from hummocks/ridges with a low water table. Vegetation/cover was observed to be the best predictor of CH_4 fluxes with higher fluxes observed from collars composed of *Eriophorum* species. Measured fluxes were not subject to further analyses and were used for modelling annual gaseous C budgets.

3.3.3 Modelled GHG fluxes and annual budgets

During the time period (June 2015 to June 2016) temperature ranged from -5.6 °C to 28.7 °C with an average annual temperature of 10.6 °C. Temperature for this time period was similar to national average temperature for 2014 and 2016. In contrast, winter 2015 was a wetter year compared with 2014 and 2016. Notably, December 2015 was exceptionally warm with a mean monthly temperature of 9.9 °C compared with the following month when temperatures in January 2016 averaged 5.7 °C (Met Office).

The three study areas were net CO_2 sinks (Fig. 3.5a), with mean annual NEE of CO_2 ranging from -237 ± 153 CO_2 g m^{-2} yr^{-1} in the oldest restoration area to -154 ± 136 CO_2 g m^{-2} yr^{-1} in the youngest restoration area. The oldest restoration area was a significantly stronger sink than the youngest restoration area ($F_{2,195045} = 178.01$, $p < 0.001$) (Table 3.2), whilst no significant difference in NEE of CO_2 were found between the youngest restoration area and the unafforested area ($p = 0.779$). Mean NER of CO_2 (Fig. 3.5b) was significantly greater in the unafforested area (857 ± 120 g CO_2 m^{-2} yr^{-1}) compared with restoration areas ($F_{2,195045} = 4778.67$, $p < 0.001$), lowest NER was found at the oldest restoration area (560 ± 102 g CO_2 m^{-2} yr^{-1}). The unafforested area also had significantly greater GPP (-1015 ± 194 CO_2 g m^{-2} yr^{-1}) than the restoration areas ($F_{2,195045} = 659.74$, $p < 0.001$) (Fig. 3.5c).

In contrast with CO_2, all three study areas were CH_4 source (Fig. 3.5d), with mean fluxes significantly greater in the youngest restoration area (6.73 ± 3.27 g CH_4 m^{-2} yr^{-1}) ($F_{2,195045} = 6318.75$, $p < 0.001$). Lowest mean annual CH_4 fluxes were found at the unafforested area (1.98 ± 0.55 g CH_4 m^{-2} yr^{-1}). When modelled annual CH_4 fluxes were converted to CO_2-eq, and included in the total gaseous C budget, the unafforested and oldest restoration areas were C sinks (Fig. 3.5f), whereas the youngest restoration area was an overall C source, estimated to be emitting on average 35 g C m^{-2} yr^{-1}. Study area, collar, microtopography and vegetation/cover were all significant factors on modelled fluxes (NEE, NER and GPP of CO_2 and CH_4) (Table 3.2). Modelled CO_2 fluxes demonstrated that collars distributed in hollows/furrows acted as a sink for atmospheric CO_2 whilst CH_4 fluxes were significantly greater in collars composed of *E. angustifolium* and bare peat in the youngest restoration area.



Figures 3.5 a-f. Mean (± SE) annual carbon budgets for forest-to-bog restoration areas (n=7) and open (control) area (n=7). Annual carbon budgets calculated between June 2015 to June 2016 are reported in g m^{-2} yr^{-1} for: (a) NEE of CO_2, (b) NER of CO_2, (c) GPP of CO_2, (d) CH_4, (e) CH_4 CO_2eq and (f) CO_2 and CH_4 CO_2eq.

3.3.4 Vegetation controls

The mixed vegetation communities within each collar were categorised based on the dominant vegetation/cover, these were: 1*) E. angustifol*ium and bare peat/brash, 2) *Calluna vulgaris* and bare peat/brash, 3) *Sphagnum* dominated, 4) *Sphagnum* and *E. vaginatum* dominated and 5) Ericaceous shrubs and non-*sphagnum* mosses (Table 3.3).

Vegetation significantly influenced all modelled fluxes (Figs. 3.6a-d). Modelled results for NEE of CO_2 indicated that all vegetation groups were a net sink, the strongest sink was the *Sphagnum* - *E. vaginatum* group sequestering -372 ± 214 g CO_2 m^{-2} yr^{-1}. The mean NEE flux of -0.04 g CO_2 m^{-2} hr^{-1} for the *Sphagnum* - *E. vaginatum* group was significantly greater compared with the ericaceous shrub – non-Sphagnum moss group, which was estimated to sequester -0.013 g CO_2 m^{-2} hr^{-1} (Table 3.3). There were noticeable differences in fluxes between the groups that contained aerenchymatous species, for instance, the mean NEE flux of CO_2 from *Sphagnum* - *E. vaginatum* was significantly greater than *E. angustifolium* – bare peat/brash. NER flux of CO_2 was significantly lower in the *Sphagnum* dominated community compared with the ericaceous shrub – non-*Sphagnum* moss community which showed the highest NER flux of CO_2 to the atmosphere.

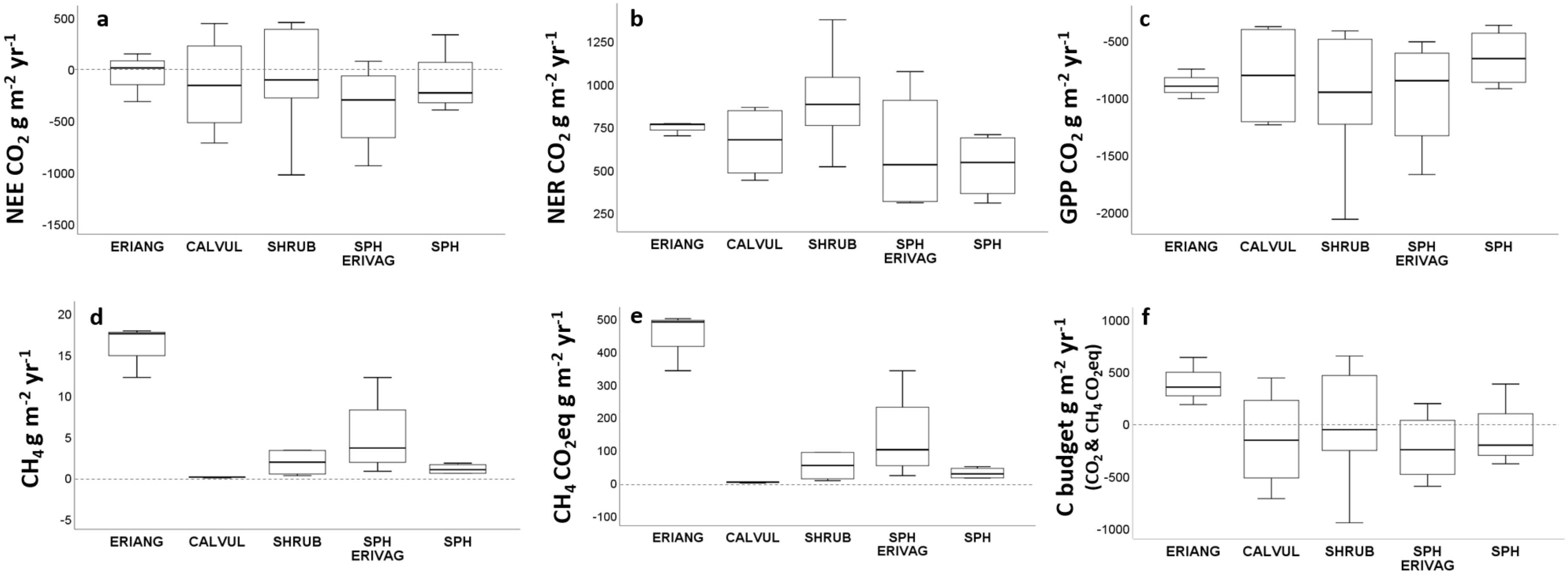
Table 3.2. ANOVA table of *F* and *P* values on the effects of area (restoration), collar (sampling point), microtopography and cover/vegetation community on modelled CO_2 (NEE, NER and GPP) and CH_4.

	NEE CO_2			**NER CO_2**			**GPP CO_2**			**CH_4**		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Restoration area	2, 195045	178.01	<0.001	2, 195045	4778.67	<0.001	2, 195045	659.74	<0.001	2, 195045	6318.75	<0.001
Collar	20, 195027	2349.08	<0.001	20, 195027	2853.1	<0.001	20, 195027	1602.99	<0.001	20, 195027	10470.8	<0.001
Microtopography	1, 195046	529.89	<0.001	1, 195046	6037.25	<0.001	1, 195046	389.96	<0.001	1, 195046	19570.98	<0.001
Vegetation/cover	4, 195043	529.07	<0.001	4, 195043	3350.47	<0.001	4, 195043	689.69	<0.001	4, 195043	32497.44	<0.001

Table 3.3 Vegetation grouping community composition and characteristics of flux measurements. Values reported are mean ± SE.

Microtopography	**Dominant vegetation/cover**	**Number of collars**	**WTD (cm)**	**Sedge density (% cover)**	**Vegetation composition (% cover)**	**NEE (g CO_2 m^{-2} hr^{-1})**	**GPP (g CO_2 m^{-2} hr^{-1})**	**NER (g CO_2 m^{-2} hr^{-1})**	**CH_4 (g CH_4 m^{-2} hr^{-1})**
Furrow	Eriophorum angustifolium - bare peat/brash	3	26.87 ± 1.96	48.1 ± 6.02	Eriophorum angustifolium (48), bare peat/brash (49), Betula pubescens (1), Calluna vulgaris (<1)	-0.016 ± 0.0005	-0.096 ± 0.0006	0.080 ± 0.0003	0.002 ± 0.000
Ridge	Bare peat/brash - Calluna vulgaris	4	35.11 ± 1.49	0	Bare peat/brash (59), Calluna vulgaris (33), Molinia caerula (2), Betula pubescens (2}	-0.017 ± 0.0005	-0.087 ± 0.0006	0.071 ± 0.0002	-0.000005 ± 0.000
Hollow/furrow	Sphagnum dominated	4	11.07 ± 0.99	4.08 ± 1.31	Sphagnum angustifolium (67), Vaccinium oxycoccos (22), Eriophorum vaginatum (7), Empetrum nigrum (2), Eriophorum angustifolium (1), Erica tetralix (<1), Calluna vulgaris (<1)	-0.015 ± 0.0003	-0.071 ± 0.0004	0.056 ± 0.0002	0.0001 ± 0.000
Hollow/furrow	*Sphagnum - Eriophorum vaginatum*	4	9.38 ± 1.0	14.33 ± 4.60	Sphagnum angustifolium (53), Eriophorum vaginatum (24), Vaccinium oxycoccos (13), Eriophorum angustifolium (5), Empetrum nigrum (4), Aulacomnium palustre (<1), Erica tetralix (<1), Calluna vulgaris (<1), Betula pubescens (<1)	-0.040 ± 0.0005	-0.105 ± 0.0007	0.065 ± 0.0003	0.0005 ± 0.000
Hummock/ridge	Ericaceous shrub - non Sphagnum moss	6	21.25 ± 0.79	2.54 ± 1.33	Vaccinium oxycoccos (29), Pleurozium schreberi (19), Aulacomnium palustre (18), Vaccinium mytillus (11), Erica tetralix (6), Sphagnum angustifolium (4), Eriophorum vaginatum (4), Calluna vulgaris (3), Polytrichum sp. (2), Eriophorum angustifolium (1), Betula pubescens (1), Sphagnum capillifolium (<1), Rubus sp. (<1), Cladonia sp. (<1)	-0.013 ± 0.0005	-0.110 ± 0.0006	0.097 ± 0.0003	0.0003 ± 0.000

* Microtopographic classifications: furrow and ridge refers to afforested topography, hummock and hollow refers to equivalent topography in unafforested areas. Vegetation groupings refer to the cross-study area communities of dominant plant categories identified from the cluster analysis.



Figures 3.6 a-f. Mean (± SE) annual carbon budgets for different vegetation types. Annual budgets calculated between June 2015 to June 2016 are reported in g m^{-2} yr^{-1} for: (a) NEE of CO_2, (b) NER of CO_2, (c) GPP of CO_2, (d) CH_4, (e) CH_4 CO_2eq and (f) CO_2 and CH_4 CO_2eq.

All vegetation communities were found to be a CH_4 source except for the bare peat/brash – *C. vulgaris* community which was observed be a negligible CH_4 sink (Table 3.3). The *E. angustifolium* – bare peat/brash community showed the highest annual CH_4 emission (15.8 ± 1.84 g CH_4 m^{-2} yr^{-1}) and was significantly greater than the *Sphagnum* dominated community (0.96 ± 0.31 g CH_4 m^{-2} yr^{-1}). The calculation of CH_4 CO_2 equivalent clearly highlighted the vegetation controls on NEE CO_2eq, with *E. angustifolium* – bare peat/brash becoming a relatively larger source of C in comparison with other vegetation groups (Fig. 3.6f). This result had a noticeable influence on the calculation of the overall annual C budget (CO_2 and CH_4 CO_2eq), all vegetation groups remained C sinks apart from the *E. angustifolium* – bare peat/brash group which was estimated to emit on average 383 g C m^{-2} yr^{-1}. The *Sphagnum* - *E. vaginatum* group was the strongest sink sequestering on average -234 g C m^{-2} yr^{-1}.

3.4 Discussion

3.4.1 Forest-to-bog restoration and CO_2 sink function

In the absence of longer time series data, the present study adopted a commonly used space-for-time substitution approach (Pickett 1989; Walter et al., 2007) to compare early and more advanced stages of forest-to-bog restoration. These estimates of NEE of CO_2 suggests that forest-to-bog restoration is effective in restoring the CO_2 sink function. Findings here show six- and seventeen-years post restoration, study areas were functioning as net CO_2 sinks. Therefore, the first hypothesis was partially accepted because the unafforested area was not found to be a stronger sink compared with the oldest restoration area.

These data add to the limited existing evidence base for UK peatland undergoing forest-to-bog management and are the first such evidence for raised bog in the UK. In a study assessing the impacts of forest-to-bog restoration in Northern Scotland, Hambley et al, (2019) found that restoration efforts were successful in restoring the CO_2 sink function sixteen years post restoration, although, in contrast with these findings, their youngest restoration area (10 years), was still a source, emitting 80 g CO_2 m^{-2} yr^{-1}. In comparison, the youngest restoration area (6 years) in this study was estimated to sequester -154 g CO_2 m^{-2} yr^{-1}. A key difference was the technique employed in Hambley et al, (2019) study, specifically, the sites had been "felled-to-waste" meaning that large amounts of brash remained on site and contributed to the emission of CO_2. Further, differences in peatland types, i.e. raised versus blanket bog and climatic condition at the time of the study may further explain the differences. After felling of plantation forestry and re-wetting in Ireland, Rigney et al, (2018) found both blanket bog (8 years after felling) and raised bog (3 years after felling) microsites were also CO_2 sources, suggesting fluxes were largely determined by plant community type, which varied along the water table gradient.

In this study a strong positive correlation between GPP of CO_2 and soil respiration was observed (Olajuyigbe et al., 2012). Results for GPP could suggest a potential legacy effect of previous conifer plantations and forest-to-bog restoration practices on C fluxes, potentially altering the balance between autotrophic and heterotrophic respiration (Olajuyigbe et al., 2012). For example, highly variable GPP estimates in restoration areas could be explained by increased nutrient availability from initial fertilisation i.e. phosphate and conifer felling/needle and brash decomposition (Gaffney et al., 2018). In other words, higher nutrient availability in forest-to-bog restoration areas leads to increased plant growth and production, and hence, higher rates of photosynthesis (e.g. Konings et al., 2019). Surprisingly, the present study found NER of CO_2 significantly higher in open areas with no afforestation, with lowest NER of CO_2 observed in the oldest restoration area. Other large-scale studies on blanket bog have shown the opposite, with annual NER of CO_2 lower in unafforested open sites compared to forest-to-bog restoration sites (Hambley et al., 2019). Differences in NER could be explained by differences in WTD, peatland type (i.e. blanket bog vs raised bog) and vegetation composition. For instance, lower NER at the oldest restoration area is perhaps best explained by WTD which was closer to the surface compared with the open areas. Highest NER of CO_2 was associated with ericaceous shrubs, which were dominant at the reference (OPEN) areas. Whilst the 'OPEN' area in this study has never been disturbed by afforestation, it is worth noting that it is probably hydrologically degraded given the extent of the surrounding disturbance (Fig 3.1).

Net Ecosystem Respiration is the second largest flux from terrestrial ecosystems, with rates expected to increase with higher temperatures, potentially diminishing the CO_2 sink strength (Luo and Zhou, 2006). Importantly, results here show improved accuracy in estimating NER flux by accounting for the feedback between plant root exudates, season and NER, as well as temperature and WTD (Rowson et al., 2013). The Lloyd and Taylor model (which made up twenty four percent of the calculated annual NER budget reported here) tended to overestimate annual NER (~2400 CO_2 g m^{-2} yr^{-1}, (estimated from September 2015 – June 2016), compared with the Rowson et al, (2013) model. In this study, the main model adopted to predict NER accounted for different NER production with varying peat depths, whilst also accounting for season to prevent under-predicting NER over summer months and over-predicting NER during winter months (Alm et al., 2007). We should state however that one cannot accurately say whether these NER values are a product of decomposition of old peat and/or increased root respiration and decomposition of fresh litter, because this study did not distinguish between autotrophic and heterotrophic respiration. A root trenching experiment carried out in forestry plantations on deep peat has shown that newly created dead root biomass can contribute a significant proportion (~27%) of the total soil CO_2 flux of forest plots in the first year of decomposition (Hermans, 2018), which could be potentially contributing to the NER flux of CO_2 in the restoration areas in the present study.

This study presents an annual gaseous CO_2 budget (NEE, NER and GPP) of forest-to-bog restoration areas and compare to unafforested conditions. It should be noted however that C can be released by other pathways not measured in this study. For example, fluvial C fluxes: dissolved organic carbon (DOC), particulate organic carbon (POC), dissolved inorganic carbon (DIC) and dissolved CO_2 (Worrall et al., 2009; Gaffney et al., 2018; Payne and Jessop, 2018b) were not quantified. Quantification of fluvial C fluxes would be more appropriate at the unafforested areas compared with restoration areas, where there are no obvious drains/water flow due to the modified surface topography. However, Limpens et al. (2008) report that DOC concentrations are significantly greater where woody *Calluna* dominates when compared to *Eriophorum* dominated sites or *Sphagnum* – *Eriophorum* sites. Furthermore, the present study focused on the movement of two gases CO_2 and CH_4, other GHG's e.g. nitrous oxide (N_2O) were not measured in this study. Whilst N_2O fluxes can be substantial in peatlands (Couwenberg et al., 2011), other studies suggest N_2O fluxes could be smaller and less important than CO_2 and CH_4 in forest-to-bog restoration sites (Rigney et al., 2018, Hermans et al., 2019). A further limitation of this study was that the collars had to be distributed within proximity of each other due to the practicalities of using the equipment, for instance, it was not possible to monitor twenty-one collars widely spread in a 6-hour time slot with travelling between sites. It would also not be possible to have more areas with these restoration efforts because most of the bog has been commercially extracted.

3.4.2 The influence of vegetation on GHG flux

This study has indicated that vegetation was a stronger influence on all fluxes compared with time since restoration. One possible explanation for this is that restoration of vegetation communities is unlikely to be a linear process, and will vary spatially (Hancock et al., 2018). For instance, it has been reported that forest-to-bog restoration may be more successful in the wetter furrows and stall in drier microhabitats such as plough ridges and steeper slopes (Hancock et al., 2018). In this study comparison of peatland vegetation groups showed that all vegetation groups were net sinks (CO_2 and CH_4 CO_2-eq), except for vegetation composed of *Eriophorum angustifolium* on bare peat which was estimated to emit 383 ± 132 g C m^{-2} yr^{-1}. This supports the idea that the relative proportion of the different plant assemblages will have a strong influence on the net C emissions at the site scale (Gray et al., 2013). Similar findings of the influence of vegetation composition on fluxes in peatlands undergoing restoration is reported in other similar studies. For instance, Urbanová et al. (2012) conclude the C balance of pristine, drained and bogs undergoing restoration was largely determined by plant community type which ultimately reflects long-term hydrology.

As predicted, CH_4 fluxes were significantly greater from the genus *Eriophorum* compared with other vegetation groups, therefore accepting the second hypothesis. Some studies have found CH_4 emissions increase post-restoration (Cooper et al., 2014), as a higher water table position results in conditions conducive for enhanced methanogenesis and decreased CH_4 oxidation (Komulainen et al., 1998).

However, these effects are not always observed, and other studies have found no consistent effect of drain blocking (Green et al., 2018) or forest-to-bog restoration (Hermans et al., 2019). In the present study higher CH_4 fluxes were observed from *E. angustifolium* on bare peat compared with other mixed vegetation communities including *Sphagnum – E. vaginatum* dominated community (Table 3.3). Frenzel and Rudolf (1998) show greater CH_4 fluxes from tussocks of *E. vaginatum* compared with *Sphagnum* lawns, suggesting that the lack of CH_4 oxidation associated with *Eriophorum* is one reason for the high emission rate from peatlands. When these sedges dominate re-wetted peatlands, they fuel methanogenesis by supplying labile C or supress methanogenesis by transferring oxygen into the rhizosphere through aerenchyma (Bubier 1995). The importance of plant mediated CH_4 transport is a well-known phenomenon in wetland plants (Bubier 1995; Frenzel and Rudolph, 1998).

At the plot scale, these results agree with previously published studies which show lower CH_4 fluxes from high hummocks with low water table and highest fluxes in wetter areas with high sedge cover (Kettunen et al., 2000; Couwenberg et al., 2011). In this study, bare peat/brash – *C. vulgaris* hummocks were observed as either not releasing CH_4 or functioning as small sinks, a similar trend was observed in *Aulacomnium palustre* hummocks in the unafforested areas. Rigney et al, (2018) report hummock vegetation types, for instance, *Cladonia-Calluna* hummocks acting as a strong sink for CO_2-C. In accordance with these findings, Hartley et al, (2015) report CH_4 uptake in drier ecosystems when methanotrophic bacteria utilise CH_4 as their energy source and release CO_2. It is also worth noting the known symbiotic relationship between *Sphagnum* and methanotrophs (Chen and Murrell, 2010). For instance, in sampling points composed of *Sphagnum* there are more opportunities for methane oxidation in the *Sphagnum* layer, in comparison with sampling points composed of non-*Sphagnum* mosses and vascular plants.

Globally, one of the main goals of peatland restoration is to halt C emissions associated with degradation (Bonn et al., 2016). Ultimately, to return the C sink function means bringing back conditions that lead to peat formation and for raised bogs, this involves encouraging the establishment of peat-forming vegetation (Andersen et al., 2017). In contrast with other vegetation groups studied, sampling points dominated by *Sphagnum* were shown to have relatively smaller effect on fluxes (NEE, NER, GPP of CO_2 and CH_4). Mean NER of CO_2 from the *Sphagnum* dominated community was low (Table 3.3), compared with vegetation composed of ericaceous shrubs and non-*sphagnum* mosses. Therefore, these results agree with the suggestion made by Kivimäki et al, (2008), that reintroduction of *Sphagna* is recommended after the colonisation of vascular plants to speed up the C sink function of peatlands under restoration. In terms of vascular plants, findings here agree with other studies (Hancock et al., 2018), that *Eriophorum vaginatum* and *E. angustifolium* increase markedly in forest-to-bog restoration areas, highlighting that *Eriophorum* taxa can promote peat formation by reducing nutrient cycling and facilitating *Sphagnum* spread. Additionally, in order to ensure *Sphagnum* survival in forest-to-bog restoration sites, it may be necessary to remove the microtopographic differences i.e. plough ridges, in order to create a flat, moist surface conducive to *Sphagnum* establishment and survival. In many blanket bogs impacted by afforestation, a technique known as ground smoothing, where plough ridges are flattened, furrows are blocked and brash is buried in the peat, is being trialled to achieve these conditions.

Numerous studies highlight the potential for vegetation to be used as a proxy for CO_2 and CH_4 fluxes in peatlands (Bubier 1995; Ward et al., 2009; Couwenberg et al., 2011; Urbanová et al., 2012; Gray et al., 2013; Ward et al., 2013). These results are partly consistent with Gray et al, (2013), who suggest that CO_2 uptake and turnover is greater for graminoids, however, our results do not fully conform to the idea that the presence of dwarf-shrubs reduces C uptake and gross CO_2 fluxes. A possible explanation for discrepancies in the literature could be because very few studies have considered plant traits/functional groups and GHG fluxes in response to forest-to-bog restoration. In a pilot study that aimed to begin to identify plant traits in sites undergoing forest-to-bog restoration, Konings et al, (2019) report that afforestation and restoration has led to greater specific leaf area and chlorophyll content and lower leaf dry matter content. Recent research (Laine et al., 2019) also suggests gas fluxes are not only primarily controlled by temperature and WTD, but also by leaf area. Clearly, these relatively small yet significant changes in plant traits in forest-to-bog restoration sites will have an impact on C fluxes and the overall C budget and require better understanding.

3.4.3 The impact of forest-to-bog restoration on the GHG balance

Published studies reporting annual C budgets of forest-to-bog restoration sites in the UK have focused on landscape-scale CO_2 emissions in blanket bog (e.g. Hambley et al., 2019; Lees et al., 2019), with results suggesting the C balance of forest-to-bog restoration sites can be achieved between 5- and 10-years post-restoration. However, these studies did not measure methane. Results shown here demonstrate the importance of incorporating CH_4 measurements into annual estimates. In contrast to CO_2, CH_4 fluxes were significantly greater in the youngest restoration area where *E.*

angustifolium was established in the wetter furrows. When methane fluxes were averaged for each study area, the annual CH_4 flux in the youngest restoration area was almost a two-fold increase from CH_4 fluxes in the oldest restoration areas. Lowest CH_4 fluxes were observed in areas not planted with trees. Interpolated CH_4 estimates presented here were comparable to annual CH_4 fluxes in other ombrotrophic peatlands (see Dise et al., 1993; Laine et al., 2007). When these fluxes are expressed as CO2-eq, the oldest restoration area and unafforested area were a significantly stronger sink than the younger restoration area, as hypothesised.

The climate impact of peatlands has been the net result of reduced radiative forcing due to CO_2-C uptake and storage as peat and enhanced radiative forcing due to CH_4 emissions (Frolking and Roulet, 2007). Because the GWP of CH_4 for a 100-year time horizon is twenty-eight times greater than CO_2 (Myhre et al., 2013), increases in CH_4 flux from forest-to-bog restoration sites may offset increased CO_2 sequestration. Results here clearly demonstrate the GWP of forest-to-bog restoration sites. Studies have shown that whilst CH_4 only represents a small (~1%) of the total C emissions from peatlands undergoing restoration, in terms of GWP, CH_4 represented 14% of the total CO_2 equivalent losses from the site (Waddington and Day, 2007). Results of the present study are consistent with these findings. It should also be noted however that peatlands are a natural source of CH_4 and can possibly be a promising indication that key processes are returning. From a C management perspective, the challenge is to design forest-to-bog restoration plans that do not create large hotspots of CH_4 (Cooper et al., 2014), whilst also accepting that CH_4 emissions are inevitable. Importantly, novel restoration techniques are currently being trialled across the UK for which there is little empirical evidence of whether techniques promote *Sphagnum*. Clearly, if certain techniques promote the re-establishment of *Sphagnum* whilst limiting the spread of *Eriophorum*, this might have more of a positive impact on the C balance.

This study demonstrated that forest-to-bog restoration can be successful in restoring the GHG balance over time. The advantage of the closed chamber approach used here is that it focuses on quantifying the movement of different forms of carbon (i.e. CO_2 and CH_4) with differing greenhouse warming potential, thus providing insights into the underlying mechanisms and complementing other approaches such as stock-based approaches or eddy covariance (Payne et al., 2018; Hambley et al., 2019). In terms of forest-to-bog restoration, future research should quantify flux measurements (such as those reported here) from a range of sites and time periods, particularly targeting newer restoration techniques that may result in different restoration trajectories. In doing so, this would address the potential issue of pseudoreplication in the present study due to a shortage of forest-to-bog restoration areas at the study site. Ideally, some sites should be established for long-term monitoring such that space-for-time substitutions can eventually be replaced by time-series. Future work should also pay consideration to vegetation composition, plant traits and microtopography as an integrator of processes controlling CO_2, and especially CH_4 fluxes at forest-to-bog restoration sites. Findings of the present study support the suggestions reported by others (Gray et al., 2013: Urbanová et al, 2012: Couwenberg et al., 2011), that robust predictive gas flux models can be derived from plant species data, which can be used as indicators of a positive or negative C balance.

3.5 Conclusion

In this study, the estimated annual gaseous C budget for forest-to-bog restoration areas was compared to areas with no afforestation. From chamber-based CO_2 and CH_4 flux measurements, total ecosystem respiration (NER) and gross primary productivity (GPP) was modelled based on hydrological, temperature and light responses. Findings show that forest-to-bog restoration can restore the C sink function, however, this is strongly dependent on vegetation composition. In terms of vegetation most conducive to C sequestration, these findings suggest a ground cover of *Sphagnum* with vascular plants could be most effective. Importantly, these results highlight the necessity of incorporating vegetation composition as an integrator of processes controlling C fluxes in forest-to-bog restoration sites. The development of models which predict fluxes based upon specific vegetation (and/or plant traits) and representative vegetation communities would be extremely useful for upscaling heterogenous plot-scale measurements to landscape-scale.

Chapter Four

Testate amoebae and vegetation as bioindicators and proxies for GHG fluxes in peatland restoration

4.1 Introduction

Northern peatlands sequester about one-third of the global soil organic carbon (C) pool, driven by higher plant C uptake through photosynthesis and lower microbial decomposition in anoxic, nutrient deficient, waterlogged conditions (Gorham, 1991). These ecosystems store about ninety percent (547 Gt) of the total peatland C pool (Yu et al., 2010). The fate of this terrestrial C store however is currently uncertain due to both climate change and large-scale rapid land use change of peatlands (Leifield et al., 2019; Lenton and Huntingford, 2003; Billet et al., 2010). Increased drought and drying in peatlands may favour microbial decomposition of peat by lowering the water table and introducing oxygen into previously anaerobic soil (Bardgett et al., 2008), which in turn will potentially shift these ecosystems from being stores of C in their intact form, to accelerating climate change by releasing more carbon dioxide (CO_2) and other greenhouse gases (GHG’s) into the atmosphere (Dise 2009). Composition of the aboveground vegetation plays an important role in C and nutrient cycling in northern peatlands, with importance for the global C cycle (Lindo and Gonzalez, 2010). Alongside their microbial counterparts, certain plant species have been found to stimulate organic matter decomposition (Kaštovská et al., 2018), whilst others may be more beneficial for C sequestration (Gorham and Rochefort, 2003). As global efforts towards peatland restoration heighten (Gewin 2020), it is more important than ever to deepen our understanding of the feedbacks between plant-microbial interactions and GHG fluxes.

Restoration of degraded peatlands is argued to be among the most cost-effective means for climate change mitigation, described as a ‘low-hanging fruit’ (Bain et al., 2011; Bonn et al., 2016). However, climate mitigation is not always the primary driver of peatland restoration. For instance, between the 1960s and the 1980s, nearly 20% of UK peatlands were afforested with non-native conifer plantations, including 67,000 ha within the “Flow Country” peatlands of Caithness and Sutherland, a site of global significance (Gewin 2020). There, concerns about the negative effects of forestry on breeding waders and loss of specialist peatland species led to the first large-scale attempts of “forest-to-bog” restoration (see Wilson et al., 2014). Several other restoration projects around the UK – for examples funded through EU LIFE programme – are still largely driven by conservation concerns but could still provide climate benefits through net reduction of GHG’s. In the case for forest-to-bog restoration in the UK emerging research showed that indeed, such net benefits were achievable within decadal timescales (Hermans, 2018; Hambley et al., 2019; Hermans et al., 2019; Lees et al., 2019). However, newer restoration techniques are rapidly replacing older approaches for which those findings apply, generating the need for further quantification of fluxes from different forest-to-bog sites (Hermans et al., 2019), particularly if forest-to-bog restoration sites are to be included under IPCC reporting in the future. Given that the measurement and monitoring of GHG fluxes from forest-to-bog restoration sites is complex and labour intensive (Artz et al., 2018) and that it requires specialist equipment and skills unlikely to be rolled out over a large number of sites, alternatives are needed. One solution is to identify proxies (i.e. a quantitative figure that can be used to represent the value of something in a calculation) which can be used for up-scaling plot-level heterogeneity of fluxes to landscape level (Couwenberg et al., 2011). In this sense, a good proxy would have a predictable and consistent relationship with GHG's, such that you can predict the GHG flux from a known community composition.

Vegetation and microbial composition have both been shown to be useful bioindicators of forest-to-bog restoration trajectories (Creevy et al., 2018; Hancock et al., 2018), but these studies have not specifically related those plants and/or microbes to GHG fluxes from forest-to-bog sites or determined whether they can act as GHG proxies. Most research identifying biological drivers of GHG fluxes in peatland ecosystems has focused on vegetation - as opposed to microbes - and many studies highlight the potential for vegetation to be used as a proxy for CO_2 and CH_4 fluxes in peatlands (Bubier 1995; Ward et al., 2009; Couwenberg et al., 2011; Urbanová et al., 2012; Gray et al., 2013; Ward et al., 2013). On the other hand, the potential of microbial proxies of GHG fluxes is much less understood. Contemporary research examining the role of microbial communities in the *in-situ* release of soil respiratory CO_2 has often focused on quantifying bacteria and fungi (e.g. Martins et al., 2017). Remarkably, the role of protists has so far been largely ignored (Anderson 2011). However, more recently, Gabilondo et al, (2019) assessed ciliates as bioindicators of soil emissions using direct observations, reporting that biodiversity of these protists (Equitability, Margalef and Shannon indices) decreased with the increase in CO_2 fluxes. Although time consuming, such direct observations using light microscopy can be useful to identify active (or living) microbial taxa and to then relate them to specific biogeochemical functions, recognised as an important area for future research (Basiliko et al., 2013; Nwashi, 2015; Jassey et al., 2013). One group of protists that have received comparatively less attention in peatlands are diatoms. Like testate amoebae, diatoms produce a decay resistant shell that can be identified based on morphology using light microscopy.

Testate amoebae are an informal grouping of protists which possess many traits making them suitable candidates for assessing their potential as proxies for estimating C fluxes in peatlands undergoing restoration. As heterotrophic microbial consumers - sitting at the top of the microbial food web in peatlands (Mitchell el al., 2003; Payne 2013), they are important for nutrient and C cycling in organic-rich soils (Wilkinson and Mitchell, 2010), and have been shown to be sensitive to changes in elevated CO_2 in peatlands (Mitchell et al., 2003; Jassey et al., 2013). Using traditional microscopy techniques, populations can be enumerated - like data collected in the study of the ecology of macroscopic organisms such as plants (Wilkinson 2008; Wilkinson et al., 2012) - and living individuals can be quantified seasonally (Marcisz et al., 2014). Mixotrophic testate amoebae (MTA) are increasingly recognised for their dual role in peatland C cycling at different trophic levels (Jassey et al., 2015; Payne et al., 2016; Herbert et al., 2019). MTA contribute to C fixation (primary production) through photosynthesis (phototrophy) and obtain energy though organic compounds (heterotrophy). They could be potential contenders as bioindicators for forest-to-bog restoration sites because they have been shown to: be sensitive to changes in temperature (Jassey et al., 2015), display seasonal trends in abundance (Heal 1964; Marcisz et al., 2014) and are impacted by reduced light penetration of natural forested bog (Payne et al., 2016) and afforestation of formerly open peatland (Creevy et al., 2018). In order to better understand the role of testate amoebae in general and especially MTA in peatland C cycling, more work is needed to examine community structure and attributes/traits and to begin to relate with *in situ* fluxes of energy in peatland ecosystems.

Chamber-based flux measurements used in this study provide a useful approach to investigate peatland gaseous C dynamics and relate to finer-scale biological processes (Alm et al., 2007). Measurements of carbon dioxide (CO_2) uptake by plants during photosynthesis (gross primary productivity, GPP) and CO_2 release through plant and soil respiration (net ecosystem respiration, NER), and methane (CH_4) production and consumption can be measured in the field, coupled with direct observations of vegetation composition and testate amoeba communities. This research is novel because there are no other studies investigating potential links between these in situ fluxes, vegetation and testate amoebae. Using a space-for-time substitution approach (Pickett 1989), this study aims to examine the impact of forest-to-bog restoration on testate amoeba communities and identify key controls on GHG fluxes at forest-to-bog restoration areas. The key hypotheses to be tested were: H1) Forest-to-bog restoration of raised bog restores the testate amoebae community to pre-disturbance conditions, H2) Testate amoebae and vegetation can be used as proxies for GHG fluxes at forest-to-bog restoration sites, H3) Testate amoebae and vegetation respond similarly to environmental drivers at forest-to-bog restoration areas.

4.2 Materials and Methods

4.2.1 Field site and experimental design

The experimental site Fenns, Whixall and Bettisfield Mosses National Nature Reserve (Fig 3.1) is a raised bog under restoration situated on the border between England and Wales (52°92’24’’N, 2°76’94’’W). Below-ground (testate amoeba) communities and GHG fluxes (CO_2 and CH_4) were measured across a forest-to-bog restoration chronosequence in August 2015, November 2015, February 2016 and May 2016. Above-ground vegetation (vascular plants, mosses) was measured in August 2015 and 2016. The study considered two phases of forest-to-bog restoration: trees removed in

2009 ~ 6 years post restoration (RES6YRS) and trees removed in 1998 ~ 17 years post restoration (RES17YRS) and compared with a reference area not subject to afforestation (OPEN). Within each of the three study areas seven sampling points (n = 7) were located at the interface of micro-topographic features, these were ridges and furrows at the restoration areas and hummocks and hollows were the equivalent microforms at the reference 'OPEN' area. Collars and dipwells were permanently installed at each sampling point to allow for repeated GHG flux and water table measurements (Fig.4.1). Collars were left for at least one month prior to gas flux measurements. Study areas were considered representative, for instance, closely adjacent and not impacted by historical peat cutting, and comparable, for example, both afforested areas were planted with trees at the same time, using the same practices and can be considered to have been the same prior to restoration. At these restoration areas trees were motor-manually felled and whole trees were removed. Other management included scrub control, brash removal coupled with damming drainage systems to promote a high-water table. The only difference in management between restoration areas were at the oldest restoration area the trees were manually carried off in contrast with the younger restoration area where trees were mechanically removed (Joan Daniels, pers comm).

Vegetation composition in OPEN areas supported typical peatland plant species such as: *Sphagnum* spp., *Aulacomnium palustre*, *Vaccinium oxycoccos, Erica tetralix* and *Eriophorum vaginatum.* At RES17YRS *Sphagnum* spp. had established in wetter furrows along with *Eriophorum vaginatum* and ridges were dominated by ericaceous species such as *Calluna vulgaris, Vaccinium oxycoccos* and *Erica tetralix*. In RES6YRS, ridges consisted of bare peat and brash (e.g. conifer needle litter and woody debris) with *Calluna vulgaris* and furrows were colonised by *Eriophorum angustifolium* on bare peat.

4.2.2 Testate amoebae sampling and preparation

For the analysis of testate amoeba communities, surface vegetation/peat samples were collected every three months (August 2015, November 2015, February 2016 and May 2016) from three permanently marked plots located within one metre of each of the collars, giving a total of 84 samples (Fig. 4.1). This experimental field design allowed for multiple sampling over time and collection of a composite sample from each plot to encompass small-scale variation. In-situ samples for testate amoebae analysis were collected at the same time as flux measurements and all samples were frozen in the laboratory prior to preparation. Testate amoebae samples were prepared using a modified version of the method of Booth et al. (2010), specifically, the boiling stage was omitted to allow quantification of the living community. Communities were enumerated by direct counts using light microscopy at x 400 magnification (Prior Scientific Advanced Laboratory Microscope). Search effort was restricted to 100 individuals per sample following Payne and Mitchell (2009). Morphological identification of testate amoebae was based on the guides of Charman et al., (2000), Ogden and Hedley, (1980) and Mazei and Tsyganov, (2006). This study differentiated between dead and alive individuals using Rose Bengal which tends to preferentially stain the cytoplasm. Rose Bengal solution was prepared by mixing 300 ml ethanol with 700 ml distilled water and adding 1.5 g Rose Bengal and 1.5 g $NaHCO_3$.

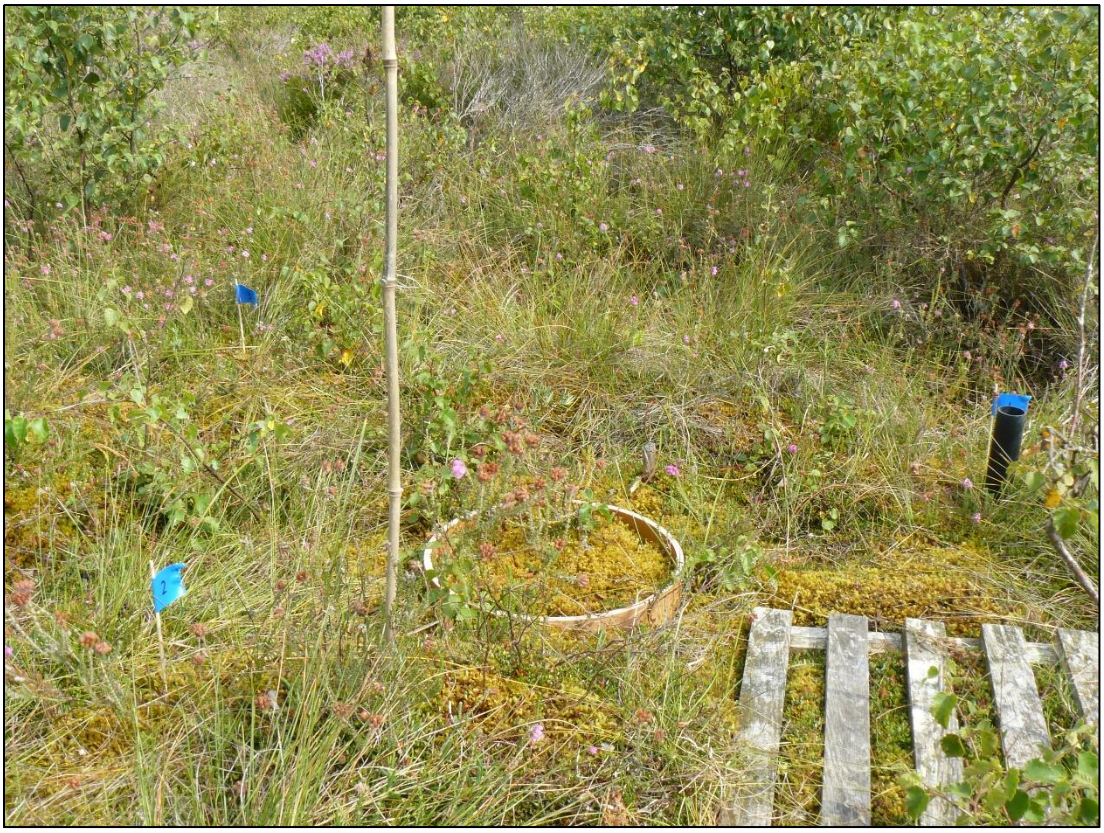


Figure 4.1. An example of the experimental field set up for one sampling point for GHG flux monitoring (collar), three microbial plots (blue flags) for the collection of a composite sample from each sampling point and measurements of water table position (black dipwell).

4.2.3 Vegetation composition

Vegetation surveys of the collars was performed at the peak biomass period in August 2015 and August 2016 using the line point-intercept (LPI) method (Rochefort et al., 2013). This involved carefully placing a 50 x 50 cm frame over each collar and taking 25 measurements at equal 5 cm intervals based on the number of species touches with a vertically placed pin (1 mm diameter). Plant species composition for both surveys was averaged and the and moss and vascular plant abundances were expressed as percentage of mean number of hits (%). The nomenclature followed Atherton et al. (2010) for mosses and Stace (2019) for vascular plants.

4.2.4 GHG flux measurements

The closed chamber method was used was used for surface exchange of CO_2 and CH_4 (Alm et al., 2007; Luo and Zhou, 2006). This technique involves circulating air between a transparent closed chamber and a gas analyser (Los Gatos Research Greenhouse Gas Analyser, Model 915-0011) which also measured ambient air temperature. The chamber was fitted with a fan for headspace air mixing and a rubber seal was used to prevent the exchange of gases between the chamber and the surrounding environment. The advantage of using this approach is that fluxes can be monitored in real time allowing for measurements to be corrected if equilibrium or a poor chamber seal was detected (Fig. 4.2). Net ecosystem exchange (NEE) and ecosystem respiration (NER) was measured with GPP calculated from the difference (NEE – NER). NEE was measured as the flux in light conditions and NER was measured by covering the chamber with a light impermeable cover. Each light and dark measurements could usually be taken in two minutes each with a one-minute flush in between measurements until ambient concentrations of CO_2 and CH_4 were reached. This study used the micro-meteorological sign convention which defines a negative NEE as a C sink with positive NEE indicating a C source (Rowson et al., 2013). Therefore, CO_2 taken in from the atmosphere (via photosynthesis) is presented as a negative value, CO_2 emitted (via respiration) is presented as a positive value. Gas fluxes were calculated and processed mostly according to Dossa et al. (2015). Flux values were calculated based on the most linear change of CO_2 and CH_4 within the two-minute measurement period.

Figure 4.2. Field equipment at the youngest restoration area (RES6YRS). Los Gatos GHG analyser and chamber (left) demonstrating real-time fluxes of CO_2 and CH_4 (right).

4.2.5 Edaphic and environmental variables

Samples for the measurement of pH, conductivity, moisture content, bulk organic matter content and bulk density were collected in December 2015. Three replicate sub-samples were collected from each sampling point/collar (n = 63). Samples for measurement of micro-environmental variables were prepared according to standard methods (Chambers et al., 2010). Bulk density samples were carefully extracted by cutting into the surface vegetation/peat using an open cylinder of known volume (400 ml). Samples were weighed, oven-dried at 105°C and reweighed to determine bulk density and moisture content. Organic matter content was determined by loss on ignition at a temperature of 550°C (Chambers et al., 2010). Samples for measurements of pH and conductivity were prepared by mixing 20 ml surface sample with 25 ml de-ionised water. Samples were shaken at 400 rpm for 30 minutes and centrifuged at 3000 rpm for 5 minutes. Measurements were obtained using a Hanna HI 98128 multiparameter probe for pH and conductivity was measured using a Hanna HI 98311 probe. Water table position was measured at the same time as flux measurements measured using dipwells located within one metre of the collar.

4.2.6 Statistical analyses

Statistical analyses were performed with R (R Core Team, 2013) except for mixed model analysis which was carried out using IBM SPSS statistics (version 25).

Multivariate techniques were used to analyse influences on testate amoeba communities and vegetation and to investigate differences between study areas. Species data were Hellinger transformed prior to all multivariate analyses in order to give low weights to rare species (Legendre and Gallagher, 2001). Principal components analysis was used to investigate the structure of the full testate amoeba dataset between restoration areas, sampling month and microtopography. Nested Permutational Multivariate Analysis of Variance (PERMANOVA, function '*adonis'*) was used to determine the significance of restoration area, microtopography and sampling month in determining testate amoebae community composition. The IndVal approach (Dufrene and Legendre, 1997) was adopted to identify indicator species of forest-to-bog and open (unafforested) study areas on the full testate amoeba dataset. The clusters were categorised by type (RES6YRS, RES17YRS and OPEN). Indicator species for each cluster were identified using the 'indval' function in the package 'labdsv' (Roberts, 2010). For each community, taxa with a p-value = <0.01 and IndVal >0.30 were selected as potential indicator species. Linear mixed models fitted using maximum likelihood (ML) estimation of parameters were used to determine the best parameters that predict CO_2 fluxes (NEE, NER and GPP) and CH_4 fluxes. Study area (restoration age), microtopography, vegetation/cover and time of year were included as categorical fixed factors. Environmental variables, testate amoebae composition (Indval species), traits and diversity indices and percentage cover of vegetation (AERENCOV, SPHCOV and SHRUBCOV) were tested as predictors in all analyses.

Redundancy analysis (RDA) (function “RDA”, package “vegan”) was used to identify whether vegetation and testate amoebae respond similarly to edaphic and environmental drivers. To reduce the influence of rare species on the RDA, taxa present in less than ten samples were eliminated, except for *Hyalosphenia papilio* which was included because of its high abundance at certain sampling points. Variation partitioning (function “varpart”, package “vegan” (Peres-Neto et al. 2006)) was used to test the proportion of variation in testate amoebae and vegetation explained by edaphic and environmental variables. The non-parametric Kruskal–Wallis (KW) test was used for continuous data which did not satisfy the assumptions of normality and homogeneity of variance after data transformation.

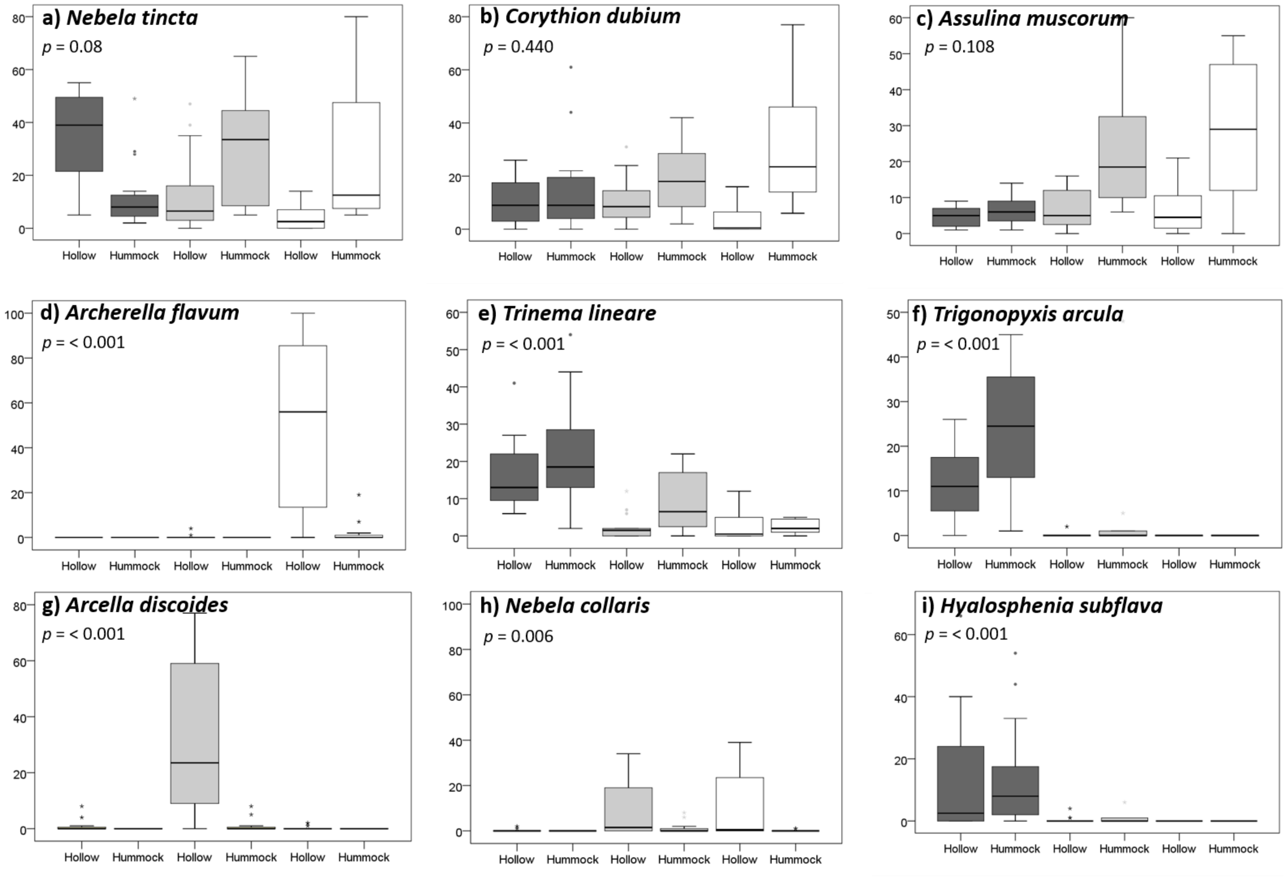
4.3 Results

4.3.1 Testate amoebae community structure

Testate amoeba communities displayed relatively high diversity with 54 taxa identified from 8495 individuals in the 84 samples. The most abundant taxa in decreasing order of abundance were: *Nebela tincta* (15.7 % of all tests), *Corythion dubium* (12.2 %), *Assulina muscorum* (10.0 %), *Archerella flavum* (8.5 %), *Trinema lineare* (7.8 %), *Trigonopyxis arcula* (5.9 %), *Arcella discoides* (5.5 %), *Nebela collaris* (4.5 %), *Hyalosphenia subflava* (4.0 %) and *Cryptodifflugia oviformis* (3.7 %). These taxa accounted for a relatively high proportion (>77 %) of the overall assemblage (Figs 4.3 a-i). There were significant differences (Kruskal-Wallis test, p =

<0.05) in relative abundance (%) between restoration areas and OPEN areas for all taxa except for: *Nebela tincta, Corythion dubium* and *Assulina muscorum*. Large variations were observed between certain taxa and microtopography, for instance, *A. discoides* was present only in hollows at RES17YRS in contrast with *A. flavum* whose presence was largely observed in OPEN areas.

Testate amoeba communities were distinctive between the three study areas as shown by the PCA (Fig 4.4). The first two PCA axes explained 41 % of the variability in the unconstrained species data and cumulatively PCA axes 1-4 accounted for 62 % of the variability in the unconstrained species data. Communities in RES6YRS were quite distinct compared with communities observed at RES17YRS and OPEN areas, which displayed some overlap between microtopography. For instance, hummocks in RES17YRS clustered closer to OPEN hummocks with some overlap displayed. In contrast, communities in the OPEN hollows appeared distinct compared with hollows in both restoration areas, primarily because of the high abundance of *Archerella flavum* which was largely absent in restoration areas (Fig 4.3d). PERMANOVA showed that community composition was significantly different between forest-to-bog restoration areas and the OPEN area (F=21.010, p = 0.001). The testate amoebae community also differed significantly with microtopography (F=9.180, p = 0.001) but not with sampling month (F=1.328, p = 0.067).



Figs 4.3 a-i. Boxplots of the relative abundance (%) of dominant testate amoeba taxa in restored and unforested study areas between hummocks and hollows. Dark grey (RES6YRS), Light grey (RES17YRS) and white (OPEN). The lower boundary of the box indicates the 25th percentile, the line within the box marks the median and the upper boundary indicates the 75th percentile. The whiskers above and below the box indicates the 10th and 90th percentiles. Significant values represent differences between study areas (Kruskal-Wallis test).

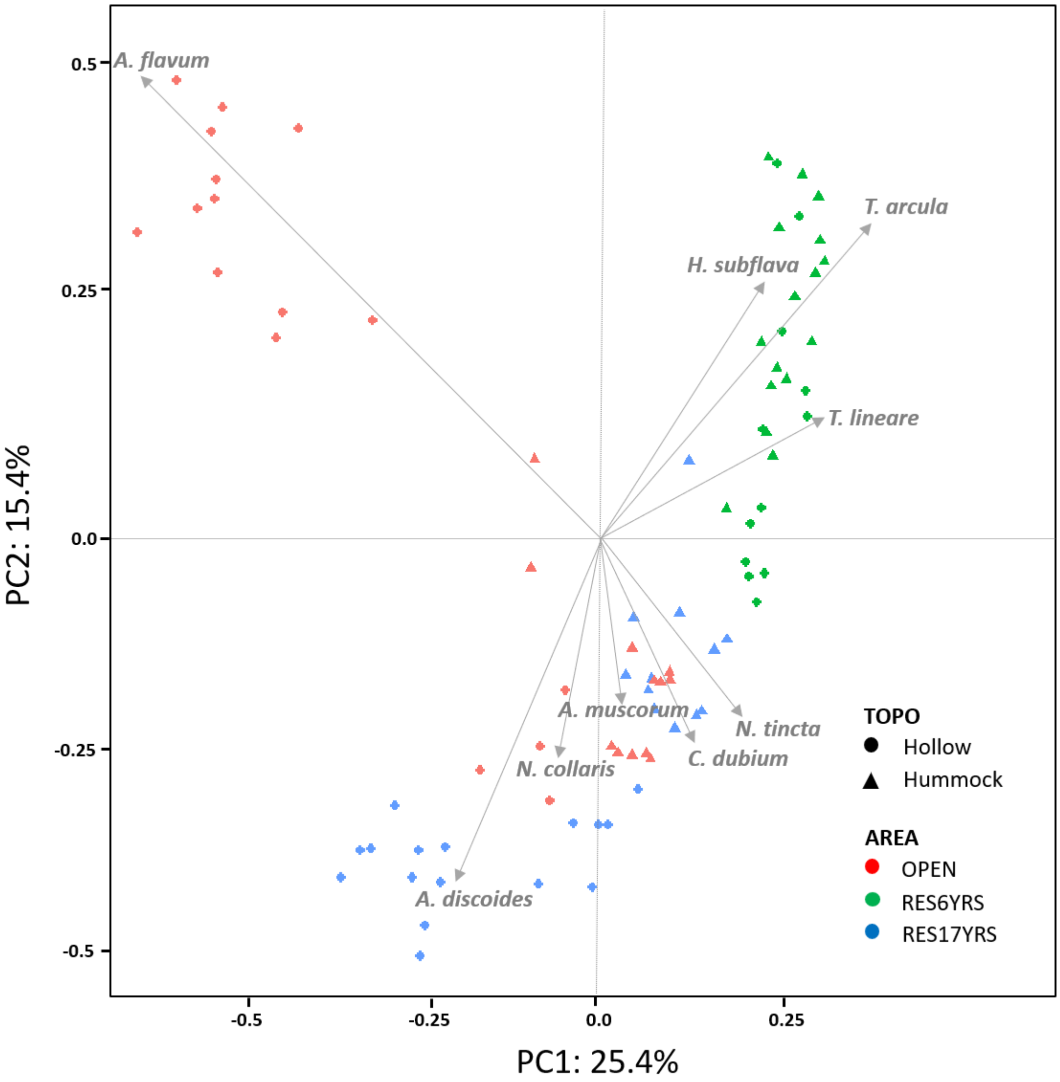
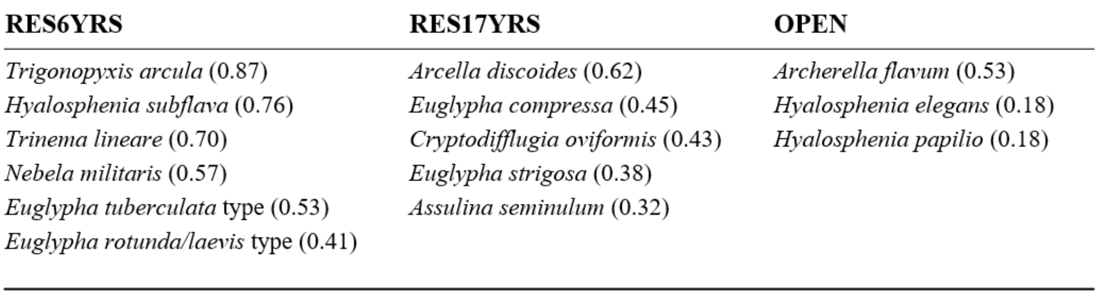


Figure 4.4. Principal Components Analysis on full testate amoeba dataset showing the effect of restoration age and microtopography on testate amoeba communities. Dominant taxa are displayed.

4.3.2 Testate amoebae indicator species and functional traits

Fourteen significant indicator species were identified (Table 4.1), with the highest indicator values (IndVal) were found for *T. arcula* (0.87), *H. subflava* (0.76) and *T. lineare* (0.70). The best indicators of RES17YRS were *A. discoides* (0.62), *Euglypha compressa* (0.45) and *C. oviformis* (0.43). RES6YRS contained the largest proportion of indicator species (43%) with *T. arcula*, *H. subflava*, *T. lineare*, *N. militaris*, *E. tuberculata* type and *E. rotunda/laevis* identified as significant indicators. The strongest indicator for OPEN areas was *A. flavum*. Although not significant indicators at the defined cut-off point (IndVal > 0.30), *H. elegans* and *H. papilio* were shown to be significant indicators of OPEN areas (IndVal 0.18) respectively.

Table 4.1. Indicator species for 3 clusters characterised by the categorical variables site categories and environment showing taxa with IndVal > 0.30 (except for *H. elegans* and *H. papilio*), significant at p < 0.01.



RES6YRS	**RES17YRS**	**OPEN**
Trigonopyxis arcula (0.87)	*Arcella discoides* (0.62)	*Archerella flavum* (0.53)
Hyalosphenia subflava (0.76)	*Euglypha compressa* (0.45)	*Hyalosphenia elegans* (0.18)
Trinema lineare (0.70)	*Cryptodifflugia oviformis* (0.43)	*Hyalosphenia papilio* (0.18)
Nebela militaris (0.57)	*Euglypha strigosa* (0.38)	
Euglypha tuberculata type (0.53)	*Assulina seminulum* (0.32)	
Euglypha rotunda/laevis type (0.41)		

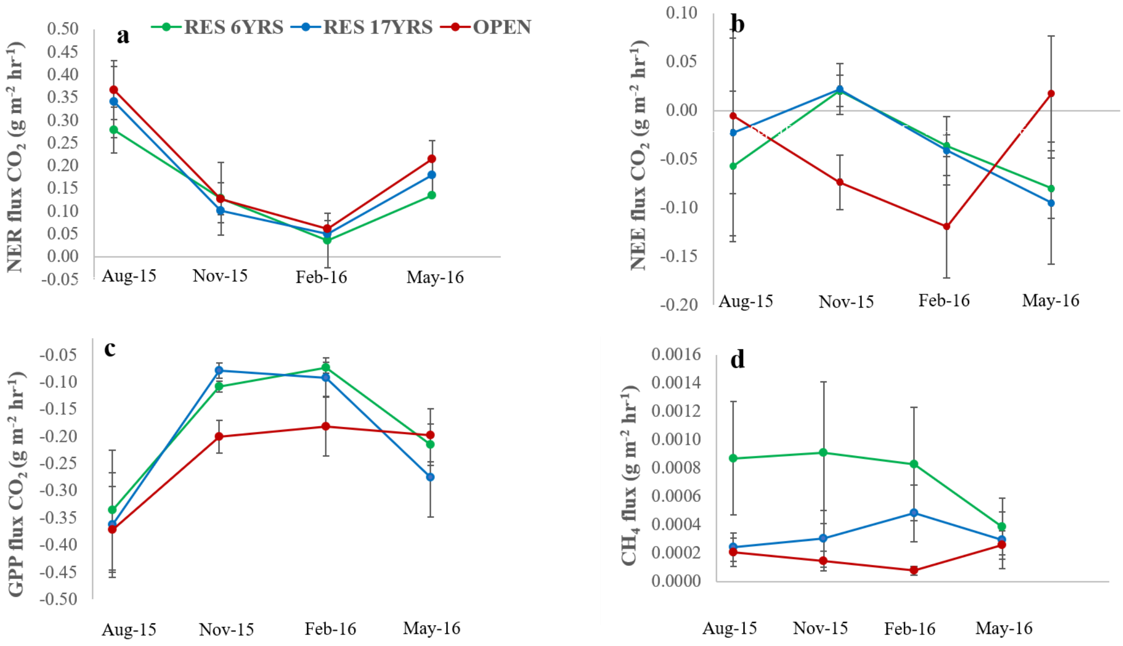
The functional trait analysis showed the effects of forest-to-bog restoration on certain functional traits within the testate amoeba communities. Notably, mixotrophs (*Archerella flavum* and *Hyalosphenia papilio*) made up a significantly larger proportion of the community in OPEN areas ($\chi2$ (2) = 26.429, *p* = < 0.001), compared with forest-to-bog restoration areas where mixotrophs were largely absent. In contrast, there was no difference in the proportion of mixotrophs between sampling months ($\chi2$ (3) = 1.632, *p* = 0.652). Sampling months also had no effect on aperture size (F = 0.977, *p* = 0.408), and aperture sizes were not significantly different between the three study areas (F = 2.734, *p* = 0.071).

4.3.3 Vegetation and testate amoebae controls on GHG fluxes

Vegetation cover of plant groups differed between restoration areas (Table 4.2). *Sphagnum* cover was significantly greater in OPEN areas and at RES17YRS ($F_{2,77}$ = 17.403, *p* = <0.001), coverage of aerenchymas species was significantly higher in hollows and furrows compared with hummocks and ridges ($F_{1,78}$ = 89.175, *p* = <0.001). Measured fluxes followed a relatively similar trend across study areas, with greater NER and GPP of CO_2 during summer and declining during winter (Figs 5a and c). Seasonal patterns of NEE of CO_2 and CH_4 however were variable between restoration areas and OPEN areas (Figs 4.5b and d).

Mixed model analysis (Table 4.3) showed vegetation composition and some testate amoebae indicator species and traits may be useful in predicting gas fluxes. Amongst all measured fluxes (Table 4.2), microtopography was a statistically powerful predictor, especially for CH_4 ($F_{1,79}$ = 132.77, *p* = <0.001) and NEE of CO_2 ($F_{1,80}$ = 41.34, *p* = <0.001). In order of relative importance, strongest predictors of CH_4 flux were: microtopography, vegetation cover, restoration age and *Sphagnum* cover. For NER and GPP of CO_2 fluxes were best explained by sampling month and vegetation cover, followed by microtopography. Vegetation cover classification (as a categorical fixed factor) was a significant factor influencing all fluxes except for NEE of CO_2 in which aerenchymas and *Sphagnum* cover were better predictors. Aerenchymas, *Sphagnum* and shrub cover were all significant factors for NER of CO_2 and *Sphagnum* cover was shown a better predictor for CH_4 fluxes.

Five out of the fourteen indicator testate taxa included in the analysis were found to be potential predictors of fluxes (Table 4.3). Testate taxa appeared to better predict NEE of CO_2 compared with other measured fluxes. In terms of testate amoebae functional traits, aperture size and proportion of alive testates were better predictors for CO_2 fluxes, whilst mixotrophy ratio was a significant factor for CH_4. Along with mixotrophy ratio, WTD, pH and bulk density were also important factors for $CH_{4,}$ in contrast, OM content was shown to be more important for CO_2 fluxes. The influence of testate amoebae diversity indices on fluxes was not significant with only species richness statistically significant for NEE of CO_2.



Figures 4.5 a-d. Mean (± SE) in situ flux measurements for a) NER of CO_2, b) NEE of CO_2, c) GPP of CO_2 and d) CH_4. Flux measurements are reported in g m^{-2} hr^{-1} taken at the same time as testate amoebae sampling.

Table 4.2. Relative abundance of key plant groups and flux values for NEE, NER and GPP of CO_2 and CH_4 at the three study areas. Values reported are means (± SE).

	AERENCOV	**SPHCOV**	**SHRUBCOV**	**NEE** (g CO_2 m^{-2} hr^{-1})	**NER** (g CO_2 m^{-2} hr^{-1})	**GPP** (g CO_2 m^{-2} hr^{-1})	**CH_4** (g CH_4 m^{-2} hr^{-1})
RES6YRS							
Ridge	0.000 (± 0.000)	0.000 (± 0.000)	0.491 (± 0.043)	-0.004 (± 0.017)	0.129 (± 0.022)	-0.133 (± 0.017)	0.0000 (± 0.0000)
Furrow	0.736 (± 0.020)	0.000 (± 0.000)	0.007 (± 0.002)	-0.084 (± 0.042)	0.165 (± 0.038)	-0.249 (± 0.074)	0.0017 (± 0.0002)
RES17YRS							
Ridge	0.038 (± 0.007)	0.000 (± 0.000)	0.563 (± 0.036)	0.046 (± 0.051)	0.229 (± 0.064)	-0.183 (± 0.055)	0.0002 (± 0.0001)
Furrow	0.379 (± 0.032)	0.782 (± 0.055)	0.193 (± 0.022)	-0.106 (± 0.036)	0.124 (± 0.026)	-0.230 (± 0.058)	0.0004 (± 0.0001)
OPEN							
Hummock	0.069 (± 0.019)	0.077 (± 0.017)	0.474 (± 0.003)	0.028 (± 0.047)	0.235 (± 0.051)	-0.207 (± 0.038)	0.0002 (± 0.0001)
Hollow	0.155 (± 0.020)	0.951 (± 0.021)	0.450 (± 0.040)	-0.095 (± 0.032)	0.184 (± 0.040)	-0.279 (± 0.049)	0.0002 (± 0.0001)

Table 4.3. Linear mixed models for net ecosystem exchange (NEE), net ecosystem respiration (NER), gross primary productivity (GPP) of CO2 and CH4 at forest-to-bog restoration areas measured seasonally in August 2015, November 2015, February 2016 and May 2016. Sampling month (referred to as season), *Restoration age, *microtopography and *vegetation cover were fixed factors other variables were included as covariates. Model is based on Type 1 sums of squares using Maximum Likelihood estimation of parameters. Significant values are in bold.

	NEE				**NER**				**GPP**				**CH_4**			
Source	d.f.	d.f.	*F*	*P*	d.f.	d.f.	*F*	*P*	d.f.	d.f.	*F*	*P*	d.f.	d.f.	*F*	*P*
Season	3	80	1.298	0.281	3	80	86.301	**<0.001**	3	80	24.713	**<0.001**	3	79	2.629	0.056
Restoration age	2	80	0.018	0.982	2	80	5.042	**0.009**	2	80	1.57	0.214	2	79	29.482	**<0.001**
Microtopography	1	80	41.344	**<0.001**	1	80	16.318	**<0.001**	1	80	9.727	**0.003**	1	79	132.774	**<0.001**
Vegetation cover	2	80	1.413	0.249	2	80	27.661	**<0.001**	2	80	5.394	**0.006**	2	79	81.720	**<0.001**
WTD	1	80	0.444	0.507	1	80	0.263	0.61	1	80	0.074	0.787	1	79	8.215	**0.005**
AERENCOV	1	80	5.077	**0.027**	1	80	4.852	**0.03**	1	80	9.563	**0.003**	1	79	0.039	0.844
SPHCOV	1	80	7.125	**0.009**	1	80	7.689	**0.007**	1	80	14.090	**<0.001**	1	79	19.490	**<0.001**
SHRUBCOV	1	80	0.011	0.918	1	80	12.671	**0.001**	1	80	3.540	0.064	1	79	0.391	0.534
Arcella discoides	1	80	8.890	**0.004**	1	80	0.068	0.795	1	80	6.893	**0.01**	1	79	1.237	0.269
Euglypha strigosa	1	80	5.749	**0.019**	1	80	1.779	0.186	1	80	1.584	0.212	1	79	0.001	0.97
Euglypha tuberculata type	1	80	0.065	0.800	1	80	4.496	**0.037**	1	80	0.921	0.34	1	79	0.130	0.719
Hyalospehnia elegans	1	80	7.097	**0.009**	1	80	9.165	**0.003**	1	80	0.293	0.588	1	79	2.933	0.091
Trigonopyxis arcula	1	80	6.665	**0.012**	1	80	0.092	0.763	1	80	3.925	0.051	1	79	0.414	0.522
Aperture size	1	80	24.787	**<0.001**	1	80	7.215	**0.009**	1	80	7.071	**0.009**	1	79	0.002	0.964
Alive testates	1	80	6.054	**0.016**	1	80	0.281	0.598	1	80	5.477	**0.022**	1	79	2.004	0.161
Mixtrophy ratio	1	80	0.272	0.604	1	80	0.454	0.502	1	80	0.650	0.423	1	79	4.530	**0.036**
Testate species richness	1	80	4.795	**0.031**	1	80	0.569	0.453	1	80	1.976	0.164	1	79	0.121	0.729
Bulk density	1	80	0.463	0.498	1	80	2.105	0.151	1	80	1.870	0.175	1	79	14.267	**<0.001**
Bulk organic matter content	1	80	4.936	**0.029**	1	80	12.534	**0.001**	1	80	0.012	0.915	1	79	0.181	0.672
pH	1	80	0.092	0.762	1	80	4.264	**0.042**	1	80	0.789	0.377	1	79	3.976	**0.05**
Moisture content	1	80	0.713	0.401	1	80	3.035	0.085	1	80	0.068	0.795	1	79	2.078	0.153

* Restoration age refers to 2 stages of restoration (trees removed in 1998 and trees removed in 2009)and an open area not subject to afforestation. Microtopography refers to hummock/hollow in open areas and equivalent microforms ridges/furrows in restorations areas. Vegetation cover refers to five broad categories: ERIANG, CALVUL, SPH, SPH_ERIVAG and SHRUB based upon the dominant vegetation within each collar.

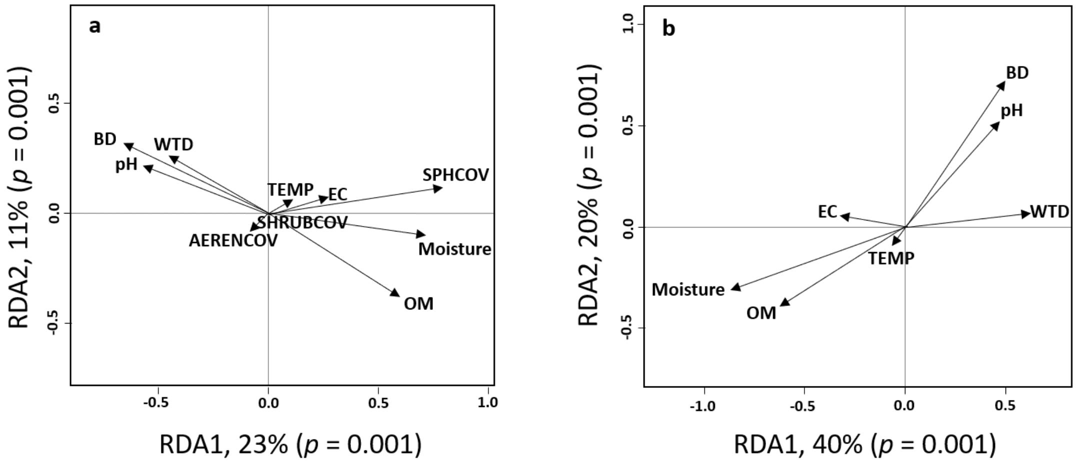
Table 4.4. Study area characteristics, geographic positions and physico-chemical properties. Given are means and standard errors (SE)

Site name	**Geographic position**		**pH**	**Conductivity**	**Bulk Organic Matter (%)**	**Moisture (%)**	**Bulk density (g cm^{-3})**	**WTD (cm)**
	Latitude	**Longitude**						
RES6YRS	52°93’52’’	02°75’86’’	5.56 (± 0.07)	14.64 (± 1.19)	77.41 (± 2.22)	75.06 (± 0.85)	0.10 (± 0.005)	28.32 (± 2.68)
RES17YRS	52°93’49’’	02°76’00’’	5.22 (± 0.04)	13.48 (± 0.80)	93.31 (± 1.61)	85.42 (± 1.98)	0.06 (± 0.004)	12.59 (± 2.14)
OPEN	52°91’44’’	02°77’23’’	4.95 (± 0.06)	18.40 (± 1.25)	89.88 (± 2.39)	88.61 (± 1.08)	0.05 (± 0.003)	17.08 (± 1.58)

4.3.4 Vegetation and testate amoebae response to environmental drivers

Edaphic and environmemtal factors were variable among the three study areas (Table 4.4). Moisture content ($F_{2,57}$ = 24.902, p = <0.001) and conductivity ($F_{2,60}$ = 5.449, p = <0.007) were significantly higher in OPEN areas relative to restoration areas. Significantly higher organic matter content ($F_{2,60}$ = 15.876, p = <0.001) was observed in RES17YRS relative to RES6YRS and OPEN areas. RES6YRS had higher bulk density ($F_{2,60}$ = 48.957, p = <0.001) and was least acidic ($F_{2,60}$ = 28.889, p = <0.001).

In the RDA (Fig 4.6), edaphic (OM, BD, pH, EC and moisture) and environmental (WTD and temperature) variables accounted for 60 % variation in vegetation (percentage cover of AERENCOV, SPHCOV and SHRUBCOV) (Fig 4.6b). The first axis explained 40 % of the variation and indicated a bulk density - moisture – acidity gradient. The second axis explained 20 % of the variation in the vegetation dataset and indicated an organic matter – moisture gradient. Edaphic factors and environmental variables measured were found to be significant controls on vegetation compsition (p = 0.001). The results of the RDA for the testate community (which also included vegetation cover as a covariate) explained a smaller proportion (47 %) of the variation. The first axis explained 23 % variation and the contribution of RDA axis one to the variation in the model was significant (p = 0.001) and indicated strong influences of moisture, *Sphagnum* cover and organic matter content on the testate communities (Fig 4.6a). The contribution of the second axis to the variation in the model was also significant and explained 11 % variation indicating pH, BD and WTD were stronger drivers. *Sphagnum* cover was shown to have a greater influence on testate communities compared with shrub and aerenchymas cover.



Figures 4.6 a and b. RDA biplots of Hellinger transformed (a) testate and (b) vegetation to address the hypothesis whether TA and vegetation respond similarly to the same edaphic and environmental drivers.

Analysis to determine the proportion of the variation in testate communities that is explained by environmental factors demonstrated that edaphic variables were stronger drivers than vegetation shaping the testate amoeba communities (Fig 4.7). Overall, the model explained about 40 % of the variation in the testate communities, edaphic and environmental variables explained a larger proportion (16 %) compared with vegetation (13 %) and the remaining 11 % was explained by the interaction between edaphic/environmental factors and vegetation composition.

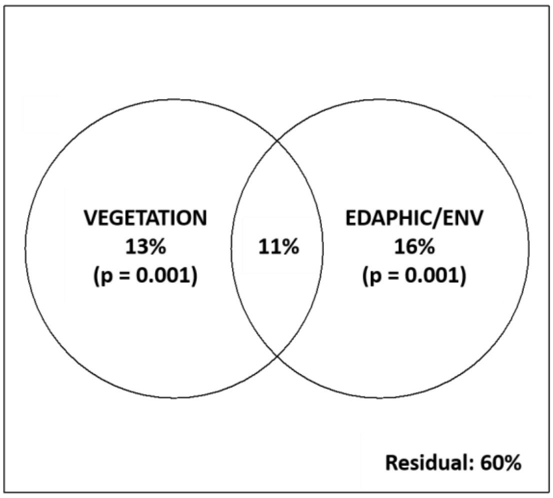


Figure 4.7. Variation partitioning Venn diagram showing the percentages of individual contributions of vegetation cover (AERENCOV, SPHCOV and SHRUBCOV) and abiotic (WTD and Temperature) and edaphic (BD, OM, pH, EC, moisture) factors to testate amoeba communities.

4.4 Discussion

4.4.1 Does forest-to-bog restoration recover testate amoeba communities?

These results demonstrate significant effects of forest-to-bog restoration on testate amoeba communities in a raised bog. The first hypothesis that forest-to-bog restoration recovers the testate amoebae community to unafforested conditions was rejected because whilst there was some overlap between individual sampling points at RES17YRS and OPEN, there was a clear distinction in testate amoeba communities between the three study areas. These trends reflect the slow recovery of microbial communities and absence of mixotrophic testate amoebae (MTA) at forest-to-bog restoration areas. Patterns of testate amoebae community structure in this study agree - in part - with similar studies on forest-to-bog restoration sites on blanket bog (Creevy et al., 2018), however, indicator species and traits showed a somewhat different response in raised compared with blanket bog.

In addition to restoration age, microtopography was also shown to be an important factor for the recovery of testate amoeba communities. For example, testate communities found in ridges at RES17YRS clustered closer to hummocks in OPEN areas whereas communities observed in ridges at RES6YRS were still quite distinct and supported taxa indicative to a dry bog surface such as *Hyalosphenia subflava* (Valentine et al., 2013) and *Trigonopyxis arcula* (Gauthier et al., 2019). Taxa typifying furrows at RES6YRS were smaller and bacterivorous (e.g. *Trinema lineare*) whereas the open bog hollows supported MTA, specifically, a high abundance of *Archerella flavum* (Fig 4.3d) and wet-related taxon *Hyalosphenia papilio* (*sensu lato*) (Heger et al., 2013). *Nebela collaris* (*sensu lato*) (Kosakyan et al., 2015) was comparably abundant in furrows at RES17YRS and OPEN bog hollows (Fig 4.3h) and the recovery of this taxon in hollows at RES17YRS is perhaps best explained by the feeding habits. *N. collaris* is a heterotroph and has been shown to feed on a wide variety of material including diatoms, fungal spores, rotifers, ciliates and smaller testate amoebae (Gilbert et al., 2003). In contrast with RES6YRS, *Sphagnum* mosses were established in furrows at RES17YRS. In these areas, the water content of *Sphagnum* mosses plays an important role in determining the ability of predators to catch their prey (Gilbert et al., 2003), and could further explain the comparable abundance of *N. collaris*.

The recovery of MTA at forest-to-bog restoration sites however is more complex. There is growing evidence of an absence of MTA at forested peatlands (Payne et al., 2016; Creevy et al., 2018; Lamentowicz et al., 2020), with potential consequences for the global carbon cycle (Jassey et al., 2015; Lara and Gomaa, 2017). Results here demonstrate a lack of recovery of MTA seventeen years after tree removal of a raised bog, despite having a higher water table than the OPEN reference area. Furthermore, these results demonstrate that recovery of light conditions (i.e. removal of trees) is not enough to recover the MTA over a decadal timescale. This is an important finding because it has been shown that decreasing MTA abundance may affect bryosphere photosynthesis and/or respiration (Jassey et al., 2015) because green algal symbionts contained within MTA may be responsible for the fixation of a significant part of global atmospheric carbon in ombrotrophic northern peatlands (Lara and Gomaa, 2017). Recent research (Hamard et al., 2019) suggests species-specific *Sphagnum* leachate may be an important factor influencing the survival and/or reproduction of MTA, which could explain why they are poorly represented in certain *Sphagnum* biomes. Further, MTA are dependent on their green algal symbionts (Lara and Gomaa, 2017) and cannot survive solely by means of heterotrophy (Jassey et al., 2015). It is also likely that the fluctuating water table at RES17YRS may not favour mixotrophs such as *A. flavum* as decreases in MTA abundance has been linked to prolonged periods of drought (Jassey et al., 2015 and references therein). The high abundance of *A. discoides* in the present study agreed with comments made by Lamentowicz et al. (2008) and was indicative of the fluctuating water table position at RES17YRS. Results here agree with recent findings and the suggestions made by Lamentowicz et al, (2020), that mixotrophic species obviously need light, but they also require stable conditions and wet *Sphagnum* to survive and reproduce. Importantly, the slow recovery of MTA at forest-to-bog sites is an important area for future research, as despite over five decades of research investigating mixotrophic protists (e.g. Schönborn 1965), we still do not fully understand the energetic benefits of endosymbiotic algae of mixotrophs and their potential role in the global carbon cycle (Jassey et al., 2015). Indeed, despite MTA possibly occurring simultaneously with the first establishment of Northern oligotrophic *Sphagnum* dominated peatlands, we currently no idea of how much atmospheric carbon has been fixed by green, algal symbionts of MTA (Lara and Gomaa, 2017).

In contrast with other studies (e.g. Marcisz et al., 2014), sampling month was not found to be a significant control on testate communities, including MTA. These findings could support the suggestion made by Mazei and Tsyganov, (2007), that seasonal trends in testate amoebae dynamics are probably variable in different biotopes. Marcisz et al. (2014), using different sampling methodologies from those employed in the present study, found the relative abundance of mixotrophic species increased between spring and summer and linked this to light intensity changes over the season. The cranked wire method (Clymo 1970) which distinguishes the new growth of *Sphagnum* mosses is a better approach if the aim of the study is to analyse the living testate community, however, this methodology maybe problematic in younger forest-to-bog restoration areas, for example RES6YRS, where *Sphagnum* has not established. Other observational studies have shown mixotroph abundance higher on the upper portions of *Sphagnum* stems (Mitchell and Gilbert, 2004) and higher in open as opposed to forested peatlands (Payne et al., 2016), providing further evidence of light dependence in mixotrophs. Although, using stable isotopes Herbert et al. (2019) show that mixotroph abundance increased in shaded plots, highlighting the need for further research to assess the impacts of light variability on peatland microbial communities. As well as mixotrophs, sampling month was not shown to have a significant control on aperture size. Aperture size is an effect trait (Fournier et al., 2015) and can be used as an indicator of the trophic position in the microbial food web i.e. taxa with smaller apertures feed on smaller prey and vice-versa (Jassey et al., 2013). For future trait-based studies, shell type could be useful at forest-to-bog sites as it has been reported to show a clear response to light availability (Lamentowicz et al., 2020).

4.4.2 Testate amoebae and vegetation as proxies for GHG fluxes

Testate amoebae and vegetation are key biotic components of C cycling in peatlands, contributing to both photosynthesis and respiration. Vegetation has been shown to respond to forest-to-bog restoration (Hancock et al., 2018). In addition, it has recently been shown that annual gaseous C budgets of forest-to-bog restoration areas are significantly influenced by vegetation (Creevy et al., 2020), thus highlighting the potential of vegetation to be used as a proxy for GHG fluxes at forest-to-bog restoration sites. However, in terms of testate amoebae, we currently have little idea whether certain functional traits or species could act as a proxy for measured in situ fluxes. This study demonstrated the potential for vegetation as a proxy for GHG fluxes at forest-to-bog sites, in addition traits and certain species within testate amoebae showed potential to predict GHG fluxes. Additional studies would be useful to build on this work and ascertain taxa or traits that can reliably predict fluxes. As an alternative to species and traits, it may be more appropriate to link microbial functional diversity (Andersen et al., 2013) or microbial biomass (Jassey et al., 2015) with in situ GHG fluxes. The second hypothesis that testate amoebae and vegetation can be used as proxies of GHG fluxes from forest-to-bog sites was therefore accepted.

Mixed model analysis indicated vegetation was a stronger driver on fluxes compared with testate amoebae, with *Sphagnum* cover significantly influencing all fluxes (NEE, NER, GPP of CO_2 and CH_4). In contrast with other findings (e.g. Tuittila et al., 2000), in this study percentage aerenchymas cover was not a significant factor of CH_4 fluxes. One possible explanation is because the category 'AERENCOV' included both *Eriophorum angustifolium* and *E. vaginatum*. Results have shown areas of *E. angustifolium* release significantly greater CH_4 fluxes compared with cover of *E. vaginatum* and *Sphagnum* at forest-to-bog sites (Creevy et al., 2020), vegetation cover composed of *Sphagnum* and *E. vaginatum* has been shown to have a positive influence on the carbon balance. The powerful influence of microtopography on all fluxes agrees - in part - with findings from other forest-to-bog restoration sites. For instance, Hermans (2018) observed in the period post-felling that CO_2 fluxes from the plough throw (comparable to ridges in the present study) were significantly lower. The same authors also found some of the most extreme CH_4 flux values occurring in wetter furrows. In the present study, lowest mean NER of CO_2 was observed from ridges at RES6YRS which were composed of *Calluna vulgaris* and bare peat and highest CH_4 flux observed from furrows dominated by *E. angustifolium*.

Restoration age was shown to have a significant control on NER of CO_2 and CH_4 fluxes. This is perhaps associated with differences in vegetation establishment over time after tree removal, for example, *Sphagnum* mosses showed no recovery six years following tree removal (Table 4.2) and vegetation composition of key plant groups was highly variable. In addition to restoration age and vegetation cover, results here demonstrate the proportion of MTA was shown to have a significant association with CH_4 flux. Mixotrophy is both a trait of prokaryotes and eukaryotes. Eukaryotic microorganisms - including testate amoebae – form symbiotic relationships between heterotrophic and/or photosynthetic organisms (Lara and Gomaa, 2017). However, most studies investigating such relationships has mainly taken place for prokaryotes, notably bacteria (e.g. O'Brien et al., 1984). Mixotrophic species can use both CO_2 and organic carbon as a source of C (Mitchell et al., 2003), therefore, an association with NER and GPP of CO_2 may not be surprising because of their dual functional role as phototrophs and heterotrophs. The links between MTA and CH_4 flux observed in the present study could indicate the strong control of *Sphagnum* on CH_4 (e.g. oxidation by methane oxidising bacteria in *Sphagnum*) and the high abundance of mixotrophs present in *Sphagnum*. Nevertheless, this is the first report of a potential link between the proportion of MTA and CH_4 flux and deserves further study, because whilst it is well known that CH_4 oxidation is a microbial process (Fig 1.1), microbial interactions under natural conditions in the oxic zone are far from understood. Studies linking microbial functional diversity with GHG fluxes may be more useful to disentangle microbial interactions (Martins et al., 2015). Notably, the sampling methodology in this study is perhaps more suited to vegetation than testate amoebae species or traits. Obviously, sampling testate amoebae from within the collars would be more representative to link with GHG fluxes, however, this would create disturbance to the vegetation which would in turn impact on the *in-situ* flux measurement. Therefore, similar future studies should address the problem of scale found in the present study.

Drainage and compaction at RES6YRS increased bulk density by about fifty percent (Table 4.3), therefore reducing the diffusion of CH_4 and oxygen (Ball et al., 1997). Bulk density was shown to have a significant control on CH_4 flux with higher bulk density generally leading to lower CH_4 flux, as was the case for *Calluna* dominated ridges at RES6YRS. These results suggest that at the microscale, large variations can occur between rates of methane production and consumption and the ability of plants and microbes to transport the gas to the surface. Higher bulk density would similarly increase the micropores potentially resulting in an increase in moisture retention. Roulet et al. (1993) report that only a small negative change in soil moisture would be required to significantly reduce CH_4 flux from northern peatlands. The mean water table position (also a significant control on CH_4 flux) at RES6YRS (28.3 cm) is reported as the critical water table at which these peatlands convert from a CH_4 source to sink (Roulet et al., 1993). Surface ridge/furrow topography at the restoration areas was remnant of forestry practices with restoration areas showing greater CH_4 fluxes from furrows composed of *E. angustifolium* and negligible sink from ridges composed of bare peat and *Calluna vulgaris*. In many blanket bogs impacted by afforestation, a technique known as ground smoothing, where plough ridges are flattened, furrows are blocked and brash is buried in the peat, is being trialled which may reduce CH_4 emissions and have more of a positive influence on the C balance of forest-to-bog restoration sites.

In general, testate amoebae indicator species were shown to be better predictors of CO_2 fluxes compared with CH_4. Interestingly, most of the significant testate indicator species were associated with NEE of CO_2 measured under light conditions, in contrast, vegetation cover had a stronger association with NER of CO_2. Testates of interest in this study were *Trigonopyxis arcula* (Fig 4.3f) and *Arcella discoides* (Fig 4.3g) as, in contrast to blanket bog (Creevy et al., 2018), they were both abundant and important indicators at forest-to-bog restoration areas on raised bog at different stages of restoration. This is interesting in terms of restoration techniques. For instance, *T. arcula* is a relatively large testate which appears to be a fungal specialist, either directly consuming hyphae or possibly feeding on exudates from the hyphae (Wilkinson and Mitchell, 2010). In this study this taxon was largely confined to RES6YRS and was more abundant in ridges as opposed to hollows. Six years post-restoration, these ridges had barely been colonised by plants other than a patchy cover of *Calluna vulgaris*. A possible explanation for the dominance of *T. arcula* is that the dry bare peat is being decomposed by fungi providing an important food source for *T. arcula*. Seventeen years post-restoration ridges generally consisted of shrubs *Vaccinium oxycoccos* and *V. myrtillus* with a ground cover of *Pleurozium schreberi* and were associated with typical peatland testates such as *Nebela tincta* and *Assulina muscorum*.

Overall, these findings suggest vegetation is perhaps a more powerful predictor of GHG fluxes at forest-to-bog restoration sites compared with testate amoebae, which are perhaps better used as bio-indicator species for monitoring the progress of restoration. From a management perspective, it is certainly easier and less time consuming to quantify the surface vegetation composition than testate amoeba communities, with results for vegetation more easily upscaled to landscape level (Couwenberg et al., 2011). Nevertheless, given that testate amoebae operate at a different spatio-temporal scale than vegetation, (for instance, testate amoebae have been shown to respond faster than plant communities in peatlands under restoration

(Secco 2019), this study may not have fully captured how testates could be possibly altering fluxes. Other studies (Basiliko et al., 2013) have found no strong linkages between prokaryote community structure and CO_2 or CH_4 production and suggest functional redundancy could be one explanation, and/or that the same taxa function at very different rates when exposed to different peat substrates.

4.4.3 Testate amoebae and vegetation response to edaphic and environmental drivers

Given that vegetation and microbial communities operate at completely different spatial and temporal scales, an important question in the present study is whether they respond similarly to the same environmental drivers – and if so – indirectly, how would that relate to fluxes? These results suggest that vegetation composition and testate amoebae communities show opposite trends in their response to environmental drivers at forest-to-bog restoration areas. The third hypothesis that testate amoebae and vegetation respond similarly to the same environmental drivers was therefore rejected. Edaphic variables were a stronger driver than vegetation shaping testate communities, suggesting their potential role as underlying determinants of the GHG contribution of forest-to-bog restoration sites.

Results here demonstrate that afforestation of previously open bog and subsequent restoration to open conditions has altered edaphic factors. These results agree with other findings (Anderson and Peace, 2017), that forest-to-bog restoration treatments resulted in a decrease in bulk density and an increase in water content. These directional changes could indicate that forest-to-bog restoration can be successful in returning physical peat properties to pre-disturbance conditions, but this study suggests timescales in excess of ten years may be required. Bulk density of approximately 0.2 g cm^{-3} was recently reported as the critical threshold for maintaining hydraulic properties of peat soils, with higher values tending to behave more like mineral soils with respect to hydraulic parameters (Liu and Lennartz, 2019). Higher bulk density estimates – as found at RES6YRS (0.1 g cm^{-3}) - can be attributed to greater microbial decomposition and lower porosity and could partly explain why edaphic variables were shown to be stronger drivers than vegetation shaping the testate amoeba communities. Decomposition at RES17YRS on the other hand is impeded by saturated conditions owing to the high-water table in furrows leading to greater organic matter content (Moore 1987). Results here provide evidence for this as RES17YRS was shown to have highest OM content and water table position (Table 4.4) and mean NER flux of CO_2 was lowest in furrows (Table 4.2). Andersen et al (2013) found the capacity of microorganisms in using different C sources (microbial functional diversity) highest at restoration sites and lowest at the reference site, suggesting that vegetation may override peat properties (e.g. bulk density, moisture, degree of decomposition), regulating belowground processes through changes in microbial community structure. Future studies should quantify microbial functional diversity and relate to *in situ* fluxes alongside taking detailed measurements of the chemical and physical environment.

4.5 Conclusion

This research demonstrated partial recovery of testate amoeba communities at forest-to-bog restoration sites of a raised bog seventeen years after restoration management. Results here suggest edaphic factors are more important than vegetation for the recovery of testate communities, including mixotrophic testate amoebae. These findings suggest that whilst testate amoebae are useful bioindicators of the progress of forest-to-bog restoration, vegetation is perhaps a better predictor of CO_2 and CH_4 fluxes, especially the cover of *Sphagnum* mosses and aerenchymas species, which in turn reflect water table position. As discussed, this is a promising finding because quantification of vegetation is easier and less time-consuming than quantifying testates. Future research should therefore focus on the development of models which estimate fluxes based upon specific vegetation (and/or plant traits) and representative vegetation communities. Modern spatial analysis techniques could then be used to upscale heterogenous plot-scale measurements to landscape-scale, thus providing a proxy for direct GHG flux measurements that would be less time consuming and more cost effective to use on a large-scale.

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Chapter Five

General Summary and Conclusion of Thesis

5.1 Summary

This inter-disciplinary research project aimed to broaden our knowledge of the microbial (Testate amoebae) response, plant response and the carbon balance of a peatland after planted conifers have been removed. Studies investigating the balance between C uptake and release and the vegetation and microbial response are of primary interest in forest-to-bog restoration research. The thesis addresses three main objectives:

1) Develop a bioindicator-based approach for evaluating the progress and trajectory of forest-to-bog restoration management practices (Chapter two).

2) Examine changes in carbon exchange over a chronosequence of forest-to-bog restoration areas and compare fluxes with an ‘unafforested’ (open) area (Chapter three).

3) Identify whether functional traits and/or indicator species within plants and/or microbial groups could be used as a proxy for GHG fluxes at forest-to-bog restoration areas (Chapter four).

The main findings of this thesis are:

1) Testate amoebae community structure and functional traits in testate amoeba communities are specific and responsive enough to act as bioindicators in peatlands undergoing forest-to-bog restoration on both blanket and raised bog.

2) Significant reductions in GHG fluxes can be achieved around ten to fifteen years after conifer removal. Short-term increases in CH_4 flux are strongly influenced by

vegetation colonisation, however, if the conditions are suitable for *Sphagnum* to re-establish, then this can have a more positive benefit on the carbon balance.

3) Vegetation better predicted all measured fluxes (NEE, NER, GPP of CO_2 and CH_4), which in turn reflects water table position. The ratio of mixotrophs was linked with CH_4 flux and testate amoebae were mostly driven by edaphic factors after conifer removal.

This project is highly applied (Figure 5.2) and relevant to current peatland policy and practice, addressing important knowledge gaps. For instance, one of the main questions that emerged from a recent afforested peatland stakeholder consultation was how the GHG budget of a peatland changes with restoration (Payne and Jessop, 2018). Data on the carbon balance presented here are particularly important in relation to forest-to-bog management in the UK, because most research investigating the impacts of peatland afforestation and restoration on the carbon balance has taken place in countries where techniques are not directly comparable to UK sites. Therefore, this study provides much needed data for forest-to-bog emission factors and shows that peatlands can be successfully restored in terms of carbon emissions after planted conifers have been removed, however, this process is likely to take between 10 – 15 years. Recovery of microbial communities may take longer. This is especially the case for mixotrophic testate amoebae at forest-to-bog restoration areas, where more work is needed to investigate their slow recovery. Further, more work is needed to develop the status of MTA as C fixers in peatlands in general.

Chapter two addressed the first objective and presented the results of a landscape-scale field study undertaken on blanket bog which examined the impacts of afforestation and forest-to-bog restoration on testate amoeba communities and functional traits. This was an important area for research because alterations in microbial food-web structure can affect ecosystem function, but this has been seldom explored in afforested peatlands and those under forest-to-bog restoration management. Findings of this study suggested afforestation had reduced the trophic level of testate amoeba communities, recovery was partially evident seventeen years post-restoration in the wettest areas where *Sphagnum* had re-colonised. Based on these results, it was hypothesised that the ‘threshold’ conditions that lead to the recovery of the testate amoebae community - including mixotrophic testate amoebae – are strongly dependent on the recovery of *Sphagnum*, which in turn, relies on a hydrological regime conducive to saturated conditions. Afforestation of previously open bog is shown to result in a non-trivial loss of mixotrophic testate amoebae, notably *Archerella flavum* and *Hyalosphenia papilio*, and recovery of these mixotrophs to pre-disturbance conditions is a slow process. These taxa contribute to both C fixation and release in peatlands and so are particularly important in C cycling. Constraining the magnitude of loss of mixotrophic testate amoebae on the C exchange of afforested peatlands and forest-to-bog restoration sites deserves further study. From a monitoring perspective, these results demonstrated ten easily identifiable testate amoebae taxa which could potentially make them useful for practitioners and/or those with little taxonomic expertise in monitoring the progress of forest-to-bog restoration. This study provided the first quantification of testate amoeba communities at forest-to-bog restoration areas and the second study to highlight the potential for testate amoebae as bioindicators of blanket bog restoration.

Chapter three addressed the second objective and presents an annual gaseous carbon budget of a raised bog over a chronosequence of restoration areas. This work examined the role of vegetation and environmental factors driving GHG fluxes from forest-to-bog areas. GHG fluxes are the products of biogeochemical transformation functions, therefore, fluxes and annual budgets presented here are quantifiable proxies providing insight into how these functions (for example, the balance between microbial decomposition and photosynthesis) change over time at forest-to-bog restoration sites. From chamber-based CO_2 flux measurements, this research further demonstrates that soil respiration models are more accurate when accounting for plant function, specifically, the feedback between plant root exudates, season (i.e. plant senescence) and respiration at forest-to-bog sites. Research findings show that forest-to-bog restoration can recover the carbon balance over timescales of around ten to fifteen years, however, this is strongly dependent on vegetation establishment. Based on these findings, it was hypothesised that plants most beneficial for the carbon balance were a ground cover of *Sphagnum* with vascular plants. These data add to the limited existing evidence base for C dynamics of forest-to-bog restoration in the UK and are the first such evidence for raised bog. Results here demonstrated how a combination of the legacy effects of forestry plantations, subsequent restoration and resulting vegetation establishment had increased CH_4 fluxes at the youngest (~ 6 years) restoration area. Methane emissions from forest-to-bog restoration sites are inevitable, but from a C management perspective the results of this study suggest the challenge is to design restoration plans that do not create large hotspots of CH_4. New restoration techniques being currently trialled in the UK, such as ground-smoothing, will provide new insights into whether management promotes the re-establishment of *Sphagnum* whilst limiting the spread of aerenchymas species such as *Eriophorum angustifolium*, as this might have more of a positive impact on the C balance of forest-to-bog sites (Fig. 5.1). From these data, robust predictive gas flux models can be developed from plant species data, which can be upscaled to landscape level and used as indicators of a positive or negative C balance.

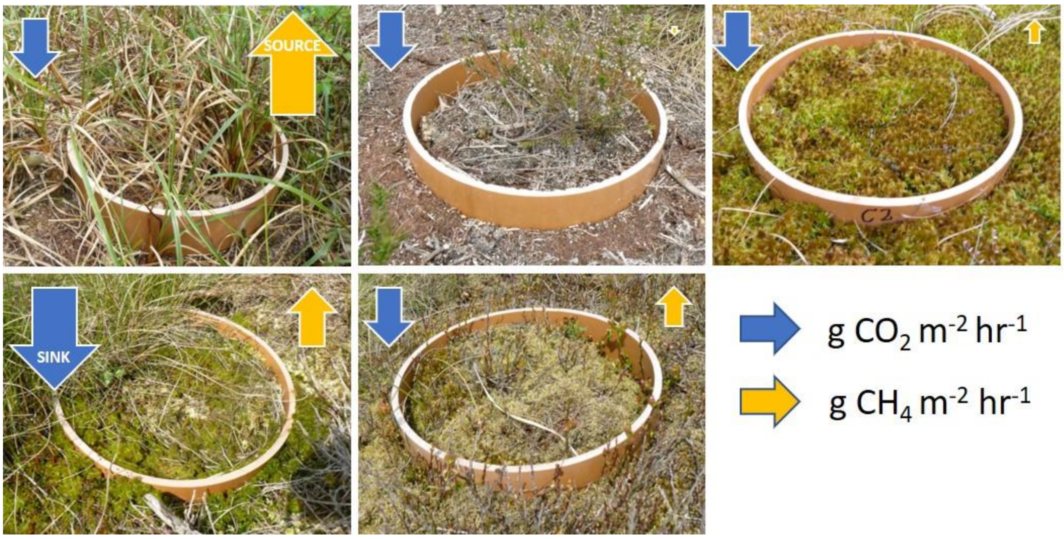

Figure 5.1. Schematic diagram of average fluxes for the five main different vegetation types identified across the three study areas: (a) *Eriophorum angustifolium* and bare peat (ERIANG), (b) *Calluna vulgaris* and bare peat/brash (CALVUL) (c) *Sphagnum* dominant (SPH), (d) mixed community of *Sphagnum* and *Eriophorum vaginatum* dominant and ericaceous shrubs (SPH ERIVAG) and (e) Ericaceous shrubs and non-*sphagnum* moss (SHRUB).

Chapter four addressed the third objective and was an important area for research from an applied perspective. Chamber-based flux measurements are labour intensive and expensive to roll-out on a large scale, therefore, we need to develop proxies to predict GHG fluxes from a known community in a reliable and consistent way. On the whole, vegetation - especially the cover of *Sphagnum* mosses and vascular plants - which in turn represents water table position, was deemed a better predictor of fluxes. Nevertheless, testate amoebae showed potential as proxies for fluxes, perhaps most interesting was the potential link between MTA and CH_4 flux, which is not reported elsewhere in the literature. It is generally believed that CH_4 production and consumption is driven mainly by prokaryotes. These results challenge this idea and suggest future studies should investigate microbial interactions between higher and lower microbial trophic groups and examine their influence on CH_4 flux. This the first study to investigate potential direct links between in situ fluxes of CO_2 and CH_4 and testate amoebae community structure and functional traits. It is worth noting that the study design was perhaps more suited to vegetation than testate amoebae, which operate at a different spatial and temporal scale to vegetation. Given that vegetation and microbial communities operate at completely different spatial scales, an important question in the present study was whether they respond similarly to the same environmental drivers – and if so – indirectly, how that would relate to fluxes. Based on these results, it was hypothesised that edaphic factors are more important than vegetation for the recovery of testate communities, including mixotrophic testate amoebae.

In addition, chapter four tested some of the hypothesises from chapter two by examining testate amoebae as bioindicators of forest-to-bog restoration of a raised bog. In contrast with testate amoebae indicator species of blanket bog (Table 2.2), testate amoebae indicator species were somewhat different on raised bog (Table 4.1). Four species were shown to be consistent indicators of both blanket and raised bog: *Trinema lineare*, *Nebela militaris*, *Archerella flavum* and *Hyalosphenia elegans*. *Nebela collaris* was a notable indicator of restoration sites in blanket bog and despite not being a significant indicator in raised bog, it was locally abundant in hollows seventeen years after conifer removal. This chapter demonstrated that edaphic factors are particularly important for the recovery of testate amoeba communities at forest-to-bog sites. Edaphic factors differed between blanket (Table 2.1) and raised bog (Table 4.4), which could explain the differences in indicator species, except for *Archerella flavum* which was both common and frequently abundant in open areas of blanket and raised bog. This taxon was found to be the best indicator that microbial processes are returning at forest-to-bog sites. Importantly, both chapters provided additional evidence for the loss of mixotrophic testate amoebae associated with afforestation and slow recovery following forest-to-bog restoration. Results here have indicated that

MTA need light, but they also require a stable water table position and wet *Sphagnum* to survive and reproduce. This chapter speculates on the factors potentially influencing MTA at forest-to-bog sites and suggests multiple disturbance mechanisms.

Despite differences in testate amoebae indicator species, similar conclusions could be drawn from recovery of testate amoeba communities and edaphic factors ~ seventeen years after conifer removal of both raised and blanket bog:

- Forest-to-bog restoration slowly recovers testate amoeba communities in this timescale, except mixotrophic testate amoebae which show limited recovery in wetter furrows where *Sphagnum* has established.
- Testate amoebae community composition is largely driven by edaphic factors.
- Forest-to-bog restoration decreases bulk density and increases moisture.

5.2 General conclusion and recommendations

This study has shown that forest-to-bog management can recover microbial communities and the carbon balance. The recovery of microbial communities to pre-disturbance ‘open’ conditions on blanket and raised bog is a slower process requiring timescales in excess of twenty years. Recovery of the C balance is a faster process taking between ten – fifteen years depending on vegetation establishment after tree removal. At younger restoration areas (e.g. ~5 years), short-term increases in CH_4 fluxes are inevitable with a dominance of *Eriophorum angustifolium*. As the position of the water table rises with restoration, vegetation composed of *Sphagnum* and *Eriophorum vaginatum* can have a more positive influence of the C balance (Figure 5.2) .

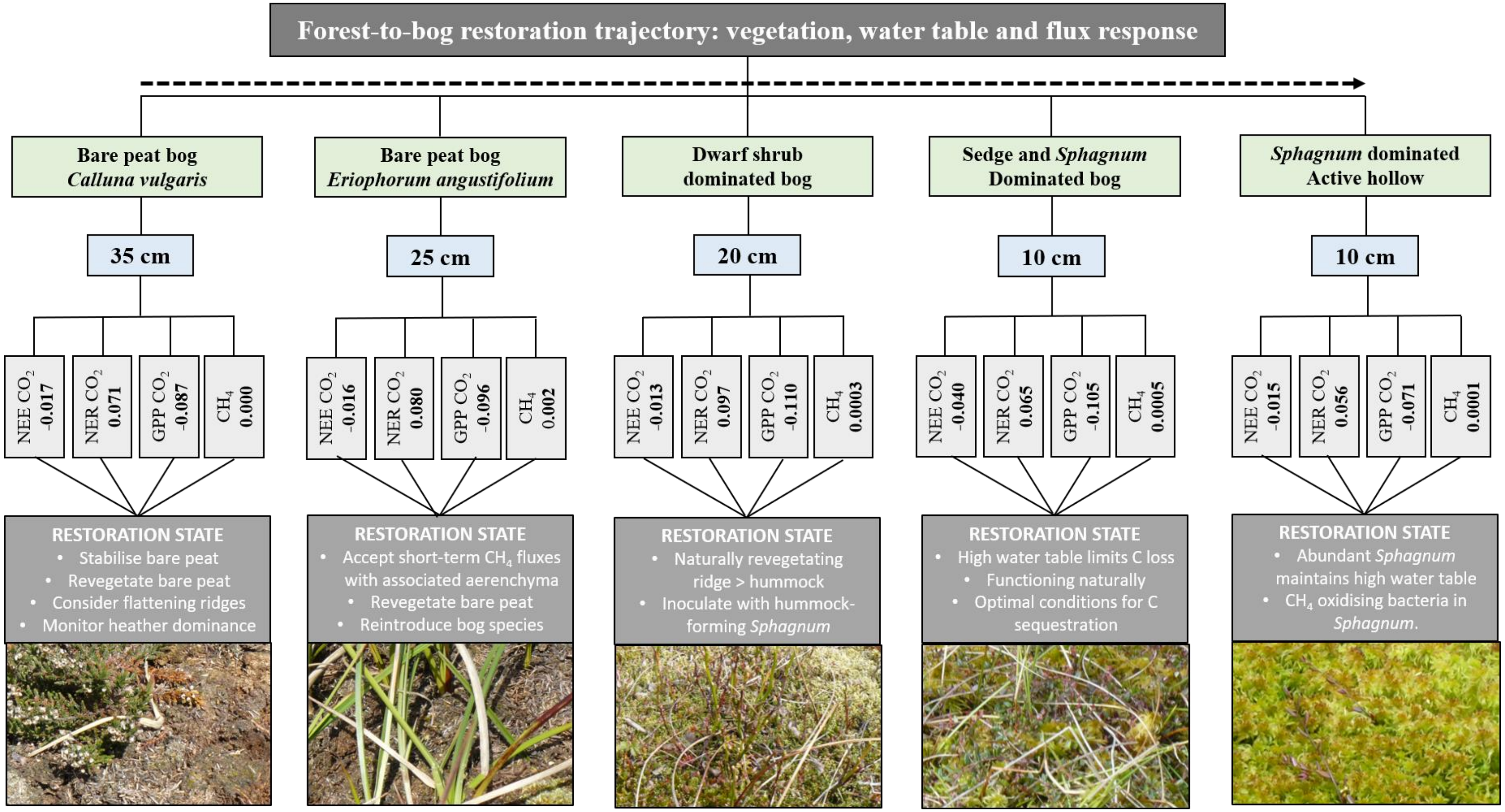

Figure 5.2. Forest-to-bog restoration state toolkit based on dominant vegetation. Blue boxes demonstrate approximate water table position. Fluxes are reported in g m^{-2} hr^{-1}. Dashed arrow indicates the restoration trajectory over time. This toolkit assumes that drains have been blocked and trees have been removed off site.

Forest-to-bog restoration aims to make peatlands more resilient ecosystems (Gewin 2020). This study has shown that the removal of coniferous plantations on peatlands can have a positive effect on the carbon balance by storing more GHG's than they emit. Because these surface gaseous fluxes are strongly dependent on vegetation, which in turn reflects water table position, these results (Fig. 5.2) can potentially be applicable to both blanket and raised bogs with similar environmental characteristics and vegetation composition. Nevertheless, it should be noted that forest-to-bog restoration techniques often differ between sites. For instance, at the Scottish sites' trees were 'felled-to-waste' (see Fig. 5.3), whereas at the raised bog at Fenns and Whixall trees were removed off site. Newer, more novel restoration techniques, such as ground smoothing to flatten ridges and encourage *Sphagnum* establishment and inoculation with *Sphagnum* at bare peat sites could be considered to try and speed up the progress of restoration (Fig. 5.2). Overall, findings of this study show that raising the water table alone is not enough to recover key ecosystem processes, blocking drains and damming ditches often needs to be coupled with additional management such as revegetating and restabilising bare peat depending on the surface composition. Moreover, management intervention should be followed up with monitoring water table position and vegetation response.

A particularly interesting area for future research is how specific plant traits (for example, leaf area) can alter the GHG balance as such data could further improve existing models used to estimate carbon budgets. A common theme that emerged from each chapter was the effectiveness of *Sphagnum* on the functioning of sites under forest-to-bog management in relation to both recovery of microbial communities in blanket and raised bog and the C balance of raised bog. In both bog systems studied *Sphagnum* had successfully established in the wetter furrows and it was evident that management was shifting edaphic factors towards pre-disturbance conditions, for instance, with a decrease in bulk density and an increase in moisture. Edaphic factors were found to be strong drivers of testate amoeba communities and differences in indicator species reflect the differences in edaphic factors between blanket and raised forest-to-bog sites.

Fig. 5.3. An area under forest-to-bog management on Forsinard Flows Nature Reserve in August 2013 demonstrating ‘felled-to-waste’

Based on these findings, this study recommends that future forest-to-bog research and management should consider the following:

- Adopting an indicator-based approach for evaluating the progress and trajectory of forest-to-bog restoration management practices, using testate amoebae as model organisms.
- Establishing sites for long-term monitoring of GHG fluxes such that space-for-time substitutions can eventually be replaced by time-series.
- Consideration of vegetation composition, plant traits and microtopography as an integrator of processes controlling CO_2, and especially CH_4 fluxes at forest-to-bog restoration sites.
- Relating microbial functional diversity with in-situ GHG fluxes alongside partitioning auto and heterotrophic respiration through collar/experimental design.
- From a C management perspective, the challenge for managers of forest-to-bog restoration sites is to design restoration plans that do not create large hotspots of CH_4 whilst accepting that short-term fluxes are inevitable and could indicate the return of key ecosystem functions.
- Ecological monitoring of newer, more novel forest-to-bog restoration techniques (such as ground smoothing of ridges and new trenching techniques to prevent water loss from peat cracks) on the C balance and microbial structure and function.
- Using data such as those reported here to develop robust predictive gas flux models derived from plant species data, which can be upscaled and used as indicators of a positive or negative C balance of forest-to-bog restoration sites.

- Finally, a co-ordinated, inter-disciplinary approach is required to try and disentangle the linkages between microbial and vegetation community structure, GHG fluxes and the physical and chemical environment of peatlands heavily influenced by human disturbance.

5.3 Future research

Forest-to-bog restoration is an emerging theme in UK peatlands heavily impacted by drainage and afforestation. Newer restoration techniques, such as ground-smoothing and new trenching techniques to prevent water loss from peat cracks, are providing interesting opportunities to further test the potential of testate amoebae as indicator species and use them in combination with hydrological conditions and vegetation assemblages to assess trajectories of recovery and inform management decisions. Importantly, this research has demonstrated how afforestation has reduced the trophic level of testate amoeba communities, with a loss of mixotrophic taxa which do not appear to re-establish successfully when conifers are removed and light levels are reinstated. Because mixotrophic testate amoebae contribute to both carbon fixation and release in ombrotrophic peatlands, constraining the magnitude of loss of mixotrophs on the C exchange of afforested peatlands and forest-to-bog restoration sites is an important area for future research. Specifically, we need to develop the status of mixotrophic testate amoebae as peatland C fixers. For the first time this study has demonstrated potential links between MTA and CH_4 flux. These finding highlights how little we know about the relative role of different microbial groups in driving peatland CH_4 flux.

In the absence of longer time series data, chapters three and four adopted a commonly used space-for-time substitution approach. Future similar research should quantify GHG flux measurements over decadal timescales from a range of forest-to-bog restoration sites and time periods, particularly targeting newer restoration techniques that may result in different restoration outcomes. Ideally, some sites should be established for long-term monitoring such that space-for-time substitutions can eventually be replaced by time-series. To improve understanding of the different drivers of the carbon balance, it is strongly recommended that future work considers vegetation composition, plant traits and microtopography as an integrator of processes controlling CO_2, and especially CH_4 fluxes at forest-to-bog restoration sites. Data, such as those reported in chapter three, should contribute to the development of robust predictive gas flux models, which can potentially be derived from plant species data. This would then allow fine-scale mapping of plant species data which can then be upscaled and used as indicators of a positive or negative C balance of forest-to-bog restoration sites.

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