Micro-propagated *Sphagnum* introduction to a degraded lowland bog: photosynthesis, growth and gaseous carbon fluxes

A T KEIGHTLEY

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Micro-propagated *Sphagnum* introduction to a degraded lowland bog: photosynthesis, growth and gaseous carbon fluxes

ANNA THERESA KEIGHTLEY

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Faculty of Science and Engineering
Manchester Metropolitan University

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Abstract

Degraded peatlands are significant sources of carbon greenhouse gases, and their recovery can make significant contributions to UK climate change mitigation responsibilities, as well as deliver biodiversity benefits to BAP priority habitats. *Sphagnum* mosses are key species for northern peatland formation, and re-introduction is seen as an essential factor in successful restoration, but natural sources are scarce and protected. Micropropagated *Sphagnum* moss products (BeadaMoss®) have been developed to provide the *Sphagnum* necessary for new acrotelm development, peatbog recovery and hence carbon greenhouse gas (CGHG) sequestration following degradation. However, the properties and performance of BeadaMoss® *Sphagnum*, now being produced on an industrial scale, have not been scientifically assessed. This study made a detailed investigation of the performance of BeadaMoss® *Sphagnum* and its potential for growth and CGHG sequestration under laboratory and field conditions.

In the laboratory (Chapter 2), maximum photosynthesis (P_{max}) rates, and the ratio of P_{max} to respiration, of BeadaMoss® *Sphagnum* were higher than those of wild-sourced *Sphagnum*. There were positive relationships between P_{max} and macronutrients levels, and BeadaMoss® *Sphagnum* Nitrogen content reached 30 mg g^{-1} with no signs of toxicity. There were few anatomical or morphological differences, but generally more chloroplasts were recorded in BeadaMoss® than wild-sourced *Sphagnum*.

Productivity of 11 species of BeadaGel™ (strands of developing BeadaMoss® *Sphagnum* in a hydrocolloidal gel, applied to a substrate) as both individual species and in a commercial mix, were studied in indoor and outdoor conditions (Chapter 3). The *Sphagnum* developed many growth points and grew rapidly in indoor conditions especially, and species traits developed as expected, particularly outdoors. Some suggestions are made for further increasing productivity in the commercial mix.

Ecosystem CGHG flux was measured using closed chambers at plot scale on a degraded lowland bog undergoing restoration with and without application of BeadaGel™ *Sphagnum* to areas of both mature and immature *Eriophorum angustifolium* (Chapter 4).

Studies were conducted over two-years of contrasting weather patterns (September 2016 to August 2018). In year 1 there was a mean net CGHG uptake of -264.39 ± 368.95 g CO_{2e} m^{-2} yr^{-1} (all vegetated monitoring points, assuming equal distribution), with progression from CGHG emission from bare peat to increasing CGHG uptake as vegetation matured. In year 2, gross photosynthesis reduced significantly during a summer drought but there was still a mean net CGHG uptake of -99.01 ± 339.59 g CO_{2e} m^{-2} yr^{-1}, demonstrating some resilience to climate change scenarios in this early-stage restoration site, particularly with *Sphagnum* application. CGHG emission from bare peat (341.10 ± 75.47 g CO_{2e} m^{-2} yr^{-1}) showed the magnitude of avoided losses. *Sphagnum* introduction reduced *E. angustifolium* density within mature vegetation, and increased both *E. angustifolium* density and CGHG uptake within immature vegetation. Methane flux contributed significantly to CGHG emission but was not closely related to water table depth.

A study of physical and chemical peat characteristics (Chapter 5) showed that the site had legacy effects from long-term degradation, reducing capacity for hydrological stability and resilience to anticipated climate changes, particularly more regular episodes of drought.

In summary, BeadaMoss® materials showed potentially rapid proliferation, essential for surface moisture retention in the early stages of restoration and for promoting acrotelm development, and hence application is likely to deliver good outcomes for degraded lowland bog recovery and CGHG uptake.

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Chapter 1: Introduction

1. 1 Peatlands – global, local and restoration perspectives

1.1.1 Peatlands - Carbon storage and carbon greenhouse gas (GHG) flux

Intact, functioning peatlands sequester more atmospheric carbon per hectare than other terrestrial habitats, (estimated by Alonso *et al*. (2012) to be 0.1 to 0.46 t C ha^{-1} yr^{-1} globally) making them a vital resource for anthropogenic climate change mitigation (Parish *et al*., 2008; Lindsay, 2010; Wilson *et al*., 2013; Joosten *et al.*, 2016a; Renou-Wilson *et al*., 2019). Although peatlands only cover 3% of northern hemisphere land (Gorham, 1991) they are estimated to contain 500 ± 100 Gt C (Yu, 2012) which is more carbon than in all forests globally (Joosten *et al*., 2016a). But an estimated 15% of the world's peatlands are damaged (Joosten *et al*., 2012) and account (with peat fires) for around 5% of all anthropogenic CO_2 emissions (Crump, 2017), and at around 2 Gt CO_2 yr^{-1} (Joosten, 2016a) almost twice that from global aviation (Greifswald Mire Centre, 2019). Europe holds 13.2% of peatlands globally, 43.7% of which are degraded – higher proportionally than any other continent, and related to greater density of population and demand for land use (2008 data source: IMCG Global Peatland Database in Joosten, 2016). Indeed, Rydin and Jeglum (2013) state that 'several European countries have lost more than 80% of their original peatland areas.'

The wide-ranging inventory of UK emissions (Evans *et al*., 2017) estimates that UK GHG emissions from peatlands currently exceed 23 Mt CO_{2e} yr^{-1}. Arable cropland accounts for 32%, due to drainage and fertilization, even though this occupies only 7% of UK peatlands. Grasslands, primarily drained lowland improved grassland, occupy only 8% of land but account for 27% of emissions. Peatland forestry could be responsible for 20% of emissions, although CO_2 uptake and retention in trees and timber could not be accounted for. Areas of peatland classed as 'semi-natural' (mostly upland peats), have been subject to damage through drainage, burning, grazing and erosion, and emit lower levels of GHG than other land uses, but account for 15% of all emissions due to their large area. Peat extraction and abandonment accounts for around 5% of emissions; highest per area from peat extraction sites, but the greatest amount comes from domestic cut sites as these cover a much greater area.

The gaseous exchange between soil surfaces and atmosphere, or Net Ecosystem Exchange (NEE) is largely dependent on uptake of CO_2 by plant photosynthesis and emission through respiration in soils and plants (Worrall *et al*., 2011). However, on peatlands CH_4 emission due to anaerobic microbial processes in saturated substrate can be a significant contributor to climate forcing (Glatzel *et al*., 2004; van Winden *et al*., 2012; Haddaway *et al*., 2014; Turetsky *et al*., 2014). Evans *et al*. (2017) currently estimate that natural UK peatlands are 'climate neutral' due to climate forcing of CH_4 emissions counteracting CO_2 uptake. Worrall *et al*. (2011) found previously that natural peatlands, and damaged peatlands undergoing restoration, are not always gaseous carbon sinks (or 'modified' peatlands always gaseous carbon sources) due to the climate forcing of methane emission. However, more recently, Günther *et al*. (2020) explore the dichotomy between CO_2 emission from drained peatlands and the CH_4 emission from rewetted peatlands and conclude that 'CH_4 radiative forcing does not undermine the climate change mitigation potential of peatland rewetting'. Evans *et al*. (2016) conducted and reported on wide-ranging and co-ordinated research into the GHG budgets of lowland bogs in the UK as a baseline for further research, concluding that they are major sources of land-based GHGs due to drainage and modification, particularly arable agriculture, with water table depth being an over-riding factor, and that more data is needed to refine emissions factors in the UK.

1.1.2 UK Peatlands – extent and condition

Peatlands are estimated to cover almost 3 million hectares (approximately 12%) of UK land; the majority in Scotland (about two-thirds) and around 23% (682,200 ha) in England (Artz *et al*., 2019). Although authors agree that damage and loss of peatlands has been wide-spread in the UK (Gosselink and Maltby, 1990; Lindsay and Immerzi, 1996; Haslam, 2003; Worrall *et al*., 2011; Alonso *et al*., 2012), exact figures and parameters have varied widely. There is still uncertainty about the original and remaining peatland stocks in the UK, and recognition that clear and consistent mapping of peatlands globally (Joosten *et al*., 2012) and in the UK (Lindsay *et al*., 2014) is necessary to quantify carbon storage potential, and better inform, direct and support restoration efforts. The use of earth observation techniques, supported initially by fine-scale data to inform and 'train satellite

classification algorithms', have potential for future large-scale assessment of the extent of peatlands and their condition, and resilience of peatlands in climate change models (Artz *et al*., 2019).

The JNCC (2019) lists peatland habitats (specifically Blanket Bogs, Lowland Raised Bogs, Lowland Fens, and Upland Flushes, Fens and Swamps) as 'priority habitat', originally designated under the UK Biodiversity Action Plan (UK BAP), which identified habitats that were 'the most threatened and requiring conservation'. This designation was carried over to subsequent legislation, and is split for lowland raised bogs on the 'EU Habitats Directive Annex I habitats' into 'H7110 Active Raised Bogs' (accumulating peat) (Williams, 2006) and 'H7120 Degraded raised bogs capable of natural regeneration' (not currently accumulating peat) (Williams, 2006), to protect peat stocks where there is some chance of recovery.

The Joint Nature Conservation Committee (2011) reported that UK peatlands (with UK BAP Priority Habitat status) were made up primarily of blanket bogs (96%), 53,347 ha were lowland raised bogs (just over 2%), the remainder being lowland fens (although upland flushes, fens and swamps were then a new classification and not reported on), and all were assessed (on 'expert judgement') as declining or probably declining, slowly. One third of UK lowland raised bogs are in England (17,411 ha), with the majority in Northern Ireland.

The Joint Nature Conservation Committee (2011) assessed active peat-formation through the presence of typical bog vegetation, adapted to waterlogged, low-nutrient conditions. In England, only 5,803 ha (less than 1%) of all peatland was undamaged (actively peat-forming) in 2011, 3,263 ha was *Molinia caerulea*-dominated (mostly blanket bogs), which will form peat, albeit slowly, and 203,048 ha (just under 30%) was semi-natural but not peat-forming. Only 338 ha of English raised bog was classed as undamaged. There were many land management practices listed as causing damage to peatland of various types, such as forestry, agriculture, burning, over-grazing, peat extraction and development, but the major damaging factor was pollution, cited as exceeding NH_4-Nitrogen deposition thresholds for peat-forming vegetation (nearly 60% of English peatlands), the vast majority on blanket bog (Joint Nature Conservation Committee, 2011). However,

condition assessment of UK peatlands has been constrained by inconsistencies in surveying, reporting and classification regimes, and there is no common framework for peatland condition assessment in place (Artz *et al*., 2019).

1.1.3 Policy considerations in peatland restoration

It is important to reliably quantify carbon capture and storage, and ecosystem services benefits (see section 1.1.5) in a range of peatland-use scenarios to justify the advantages of peatland restoration (Bonn *et al*., 2014; Andersen *et al*., 2017; Evans *et al*., 2017). Generally, peatlands have higher stocks of carbon compared to other natural capital, and reducing further loss is less expensive than restoration, but there is currently little global capital invested (Griscom *et al.*, 2017) and compliance with Kyoto Protocol has been sporadic, partly due to its complicated nature (Joosten *et al*., 2016b). However, in the UK there appears to be increasing political will for climate mitigation strategies with calls from the Governmental Committee on Climate Change (Committee on Climate Change, 2019) to set more ambitions targets (zero GHG emissions by 2050) than those set out in the Climate Change Act 2008 (2050 GHG emissions 80% lower than 1990 baseline), due to increasingly dire global climate change scenarios and lack of time in which to act on them (IPCC, 2018). Projects for peatland restoration on a large scale have been ongoing since 2012 through the 'Peatland ACTION' programme (NatureScot, 2020), where the Scottish Government have already invested over £40M to put 25,000 ha of peatlands 'on the road to recovery' with a pledged £120M to fund partner-led projects over the next ten years. Defra has recently released a more modest £10M in grants for 6,580 ha of upland and lowland peatland restoration (DEFRA 2018b) as part of its 25-year environment plan (DEFRA, 2018a) commitment.

Peatland recovery and restoration and are seen as the most readily available and achievable climate change mitigation activities (Bain *et al.,* 2011; Joosten *et al*., 2012) and given the extensive area of damaged peatlands, the potential for carbon off-setting is huge (Waddington and Warner, 2001). The European Union (EU) Habitats Directive LIFE programme (EU, 2019) has supported large peatland restoration projects in the UK (e.g., Cumbria BogLIFE project, MoorLIFE 2020, LIFE Blanket Bog in Wales, and many more), although only an estimated 2% of peatlands in Western Europe have benefitted

(Andersen *et al*., 2017). The Scottish 'Peatland ACTION' programme appears to be the only model offering government funding for peatland restoration projects that propose a 'nature-based solution to the climate crisis' (NatureScot, 2020). Other schemes are developing but are often on a voluntary basis and are not linked to governmental policy. The UK 'Peatland Code' (IUCN UK Peatland Programme, 2017) facilitates financial support for validated carbon-capture peatland projects through voluntary investment by businesses and individuals, and in Germany, Moorfutures (MoorFutures, 2019) has been in operation since 2012. This scheme allows individuals and companies to voluntarily offset their emissions by buying MoorFuture® credits, although they are not valid for the country's mandatory CO_2 emission reduction targets. MoorFutures support a number of wetland restoration projects which have a value in terms of tonnes of CO_2 sequestered over 50 years: 1 MoorFuture® is equivalent to 1 tonne of CO_2. Other options for peatland recovery may be through providing alternative agricultural uses for degraded peatlands, such as paludiculture (wetland farming e.g., *Sphagnum* farming), which will prevent further erosion while still providing viable businesses for landowners (Clarke and Rieley, 2010; Joosten *et al.*, 2016a; Greifswald Mire Centre, 2019; Gaudig *et al*., 2017).

Locally to this thesis study, peatland losses have been avoided as applications for further extraction licences to 2025 on Chat Moss lost on appeal in 2012 (Department for Communities and Local Government, 2012). This was due to Government commitments to reduce peat use and to mitigate climate change, as well as the suitability of the site for conservation and hydrological benefit of restoration on adjacent peatlands. So, there is increasing recognition of the value of peatlands in climate change mitigation and for ecosystem services, and greater willingness of policy-makers and carbon-emitters to allocate various monies, but accountability in terms of monitoring and reporting, is still lacking (Andersen *et al*., 2017; Artz *et al*., 2019). More evidence of the climate mitigation benefits of peatland restoration may release greater sources of funding. Moreover, restoration techniques and benefits of lowland peatlands are presently under-researched compared to upland systems in the UK (Haddaway *et al*., 2014) and there is currently a lack of data on carbon fluxes from degraded lowland raised bogs (Evans *et al*., 2017) so this study will add to the body of knowledge in this area.

1.1.4 Upland Bog Damage (England)

Anthropogenic impacts on upland bogs in England, such as managed burning (for grouse-shooting), drainage, grazing (Haslam, 2003; Schumann and Joosten, 2008), air pollution (Malmer *et al*., 2003, Caporn and Emmett, 2008; Evans *et al*., 2014) and visitor footfall (Stevens *et al*., 2008, Cris *et al*., 2012) have caused extensive erosion of peat and vegetation (Carroll *et al*., 2009), leading to deep gullying, loss of carbon stocks and biodiversity, reduction in catchment water quality (Stevens *et al*., 2008; Evans *et al*., 2014; Pilkington, 2015) and greater risk of flooding (Pilkington, 2015). If these pressures are minimised, however, it is possible for some human activities to co-exist with a healthy landscape. Lee *et al*. (2013) found that short-rotational burning had beneficial effects in the uplands for *Eriophorum* spp. and *Sphagnum* abundance, and light grazing had little impact.

1.1.5 Lowland Bog Damage (England)

English lowland bogs have been extensively drained, mainly for the purposes of traditional agriculture, urbanisation, forestry and extraction for horticulture (Lindsay and Immerzi, 1996; Waddington *et al.*, 2002; Haslam, 2003; Alonso *et al*., 2012), eliminating their carbon sequestration function and resulting in peat shrinkage, compaction, oxidisation, and loss of regulating (climate and flooding), and cultural (aesthetic, educational, recreational and heritage), ecosystem services (Rochefort, 2000; Joosten, 2016; JNCC, 2019). Bog damage may also happen naturally, through 'bog burst' (Clymo, 1984). When peatlands are drained, stored carbon is oxidised and emitted as CO_2, contributing to global warming. When the acrotelm is removed from a cut-over bog the remaining catotelm is said to become hostile to *Sphagnum* moss establishment as the peat becomes compressed, its capacity for water storage, permeability and infiltration is lost, and moisture becomes unavailable to support *Sphagnum* growth and maintenance (Price and Whitehead, 2001; Price *et al*., 2003; Quinty and Rochefort, 2003). (The importance of *Sphagnum* moss in bog formation and recovery is outlined in section 1.2) Moreover, the surface peat can become dry, friable, degraded and hydrophobic on re-wetting, particularly if restoration measures are delayed (Thompson and Waddington, 2008; Worrall *et al*., 2011; Joosten *et al*., 2016c). Peat harvesting also destroys the

archaeo-environmental record held in the peat profile (Coles, 1991; Chapman *et al*., 2003; Haslam, 2003; Gearey and Fife, 2016) and the specialised biodiversity in the eradicated habitat (Parish *et al*., 2008; Minayeva *et al*., 2016).

1.1.6 Peatland restoration

Artz *et al.* (2019) suggest that the current lack of definition and standardisation around what constitutes peatland restoration, in terms of targets and methodology, makes success difficult to gauge. Restoration of bogs in both uplands and lowlands is generally based on the same principles of restoration ecology – repairing habitat degraded by human and other actions to achieve good environmental, functional and stakeholder outcomes, although methods differ due to past usage, topographical differences, and stakeholder needs (Aber *et al*., 2012; Cris *et al*., 2012). However, maintaining a permanently high and stable water table level is an agreed essential factor in aiding the recovery of damaged peatlands (Joosten *et al.*, 2012; Gonzáles and Rochefort, 2014). Nevertheless, the potential for improving biodiversity and sequestering carbon both depend on site conditions and previous management, particularly historic impacts in terms of drainage, peat harvesting type and extent, remaining peat depth and character, usage and nutrient addition, and therefore sites are often not directly comparable, making restoration decisions difficult and results of restoration through re-wetting unpredictable (Alonso *et al*., 2012; Zając *et al*., 2018; Renou-Wilson *et al*., 2019). Nonetheless, Evans *et al*. (2017) report that peatland restoration efforts since 1990 on an estimated 95,000 ha of peatland in the UK, the majority of which has been on blanket bog and has included rewetting, has delivered an emissions reduction of 423 kt CO_2e yr^{-1}.

1.1.7 Upland bog restoration in England

Restoration on the uplands requires a different approach to lowland restoration due to obvious differences in topography, but also pressures from current and historic damage. The Uplands Management Group (2017) describe standard restoration techniques used on large-scale upland restoration projects in England to provide multiple-benefit outcomes for the environment and landowners. Efforts are concentrated initially on stabilising the peat surface to prevent further erosion and improve catchment water

quality through application of heather brash or geotextile, and gully- and drain-blocking using various techniques and materials to reduce surface run-off, and re-vegetation of bare peat areas. Initial re-vegetation uses heather brash protection for utility grasses, with lime and fertilizer to encourage establishment and good surface cover, then introduction of more typical upland bog vegetation such as *Eriophorum* spp., *Vaccinium myrtillus*, *Empetrum nigrum*, *Erica tetralix*, and subsequently *Sphagnum*. Over-dominant vegetation such as *Molinia caerulea* and *Calluna vulgaris* may be managed through cutting, rotational burning or grazing management to encourage greater biodiversity. Prevention of peat-fires and reducing grazing pressure are ongoing challenges, along with climate change (Yeloff *et al*., 2006) and a partnership approach has been vital to success in English upland restoration projects (Cris *et al*., 2012).

1.1.8 Lowland bog restoration - general principles

There are several generally accepted techniques for repairing damaged and/or drained lowland bogs, which are removal of scrub and invasive plants (Zając *et al*., 2018), relevelling and retention of water on site (Money and Wheeler, 1999; Quinty and Rochefort, 2003; Bönsel and Sonneck, 2011; Worrall *et al*., 2011; Gonzáles and Rochefort, 2014) and re-introduction of peatland plants (Gorham and Rochefort, 2003; Quinty and Rochefort, 2003).

Scrub on peatlands adds nutrients, lowers the water table through evapotranspiration, and outcompetes bog species (Money and Wheeler, 1999; Zając *et al*., 2018), although a tree-line may be a beneficial windbreak (Schumann and Joosten, 2008). Alonso *et al*. (2012) state that unfavourable condition assessment of around half of the high percentage of lowland raised bog SSSIs was due to levels of scrub and invasive weed cover, and much of the rest was related to problems of hydrological control which, no doubt, influenced scrub and weed proliferation. Water can be retained on site through ditch-blocking, peat-bunding and/or plastic piling (Money and Wheeler, 1999; Quinty and Rochefort, 2003; Bönsel and Sonneck, 2011; Worrall *et al*., 2011; Gonzáles and Rochefort, 2014) to encourage the growth of peat-forming vegetation, and low-nutrient irrigation or water storage reservoirs may be necessary (Schumann and Joosten, 2008), but it is essential not to create large pools of standing water, which hamper vegetation

establishment (Schumann and Joosten, 2008; Joosten *et al*., 2012), and so appropriate hydrological management is necessary. Re-wetting needs to happen quickly after peat-harvesting finishes to improve climate benefits of restoration (Nugent *et al*., 2019). The porosity of the peat surface on cut-over bogs diminishes over time with no intervention, and so becomes increasingly dense and hostile to seed germination and establishment of bog vegetation, particularly *Sphagnum* mosses, due to low capability for water retention and availability (Quinty and Rochefort, 2003; Zając *et al*., 2018), which also makes reintroduction of peatland plants generally necessary (Quinty and Rochefort, 2003). This hostile peat surface is also more susceptible to scrub proliferation, and Zając *et al*. (2018) and Rydin and Jeglum (2013) advise that evaluation of site characteristics, particularly surface peat quality and moisture content are essential pre-cursors to restoration decisions and interventions.

Because UK lowland bogs are generally situated alongside farmland or plantation (Lindsay and Immerzi, 1996), a degree of artificial management may always be necessary. However, paludiculture (wetland farming) can be utilized to re-wet peatlands under agricultural use where economic returns and local employment needs dictate, and also acts as a restoration buffer-zone, reducing levels of management intervention (Joosten *et al*., 2012; Wichtmann *et al*., 2016; Crump, 2017).

1.2 Peatland formation and the role of *Sphagnum* mosses

1.2.1 Peat and Bog formation

Lindsay and Immerzi (1996) and Schumann and Joosten (2008) make the distinction that 'peatlands' are areas containing peat, and 'mires' are peatlands which are accumulating peat, and Schumann and Joosten (2008) highlight the interrelation between peat, water and vegetation in peatland formation and function. Peat is formed when 'plant production exceeds decay' (Joosten and Clarke, 2002), which largely depends on reduced temperature and limitation of oxygen-dependent decomposing organisms through waterlogged conditions, and the carbon assimilated by the plants during growth is stored (Joosten and Clarke, 2002; Haslam, 2003; Lindsay *et al*., 2014). The generic term 'peatland' is used to collectively describe the wide range of habitats that are underlain

with peat, whose characteristics are determined by nutrient content, acidity and plant assemblage (Joosten and Clarke, 2002). Rydin and Jeglum (2013) state that classification as a peatland usually depends on the depth of peat, which in Canada is a minimum of 40 cm (citing National Wetlands Working Group, 1997) and more generally is 30 cm (citing Joosten and Clarke, 2002). Worrall *et al.* (2011) outline the accepted definition of peat from Avery (1980): 'a deposit of at least 40 cm depth (50 cm in Scotland) which contains greater than 20-25% organic material within the top 80 cm of the soil profile'.

Peatlands are found throughout the world, supporting diverse flora and fauna and providing a wide range of ecosystem services (Crump, 2017). The majority are found where the water table is at or near the peat surface in the northern hemisphere, creating cool, wet conditions favouring growth of bryophytes (particularly *Sphagnum* mosses) over vascular plants (Joosten, 2016). Less than one fifth of global peatlands are found in tropical and subtropical areas, primarily in south-east Asia and the Amazon and Congo river basins, where peat accumulation is due to high rainfall coupled with poor drainage (Rydin and Jeglum, 2013). This can result in swamp forests and a woodier peat, much older and deeper in some areas than northern peats (Rydin and Jeglum, 2013). There are a wide range of peatland types, separated by features related to topography, nutrient inputs, mineral inputs, hydrological characteristics and species richness, and Joosten (2016) (representing the International Mire Conservation Group) outlines proposals for division into 11 recognised mire types globally, so that identification and conservation efforts can be better co-ordinated.

In the UK, a large proportion of peatlands are blanket bogs, which form directly on higher ground and shallow slopes in cool, oceanic climates where annual rainfall is greater than evaporation, nutrients are leached and ombrotrophic vegetation accumulates (Rydin and Jeglum, 2003). Some authors have opined that early farming communities shaped this landscape through tree-removal and hydrological manipulation (Moore, 1993) but later authors challenge this and suggest that humans merely adapted to the paludified landscape already in place (Tipping, 2004). The theory persists, however (MFFP, 2020a).

Lowland bogs can be preceded by fens, which expanded principally 9000 to 7000 years BP (Almquist-Jacobson and Foster, 1995). Fens can initiate in flat areas (e.g., Flood-plain fen,

Basin fen) and on slopes (e.g., Spring fen, Ladder fen) (JNCC 1989). Fens in flat areas are connected to groundwater where there is influence of nutrients and mineralised water (so they are termed 'minerotrophic') (Lindsay and Immerzi, 1996; Worrall, *et al*., 2011; JNCC, 2018) until they reach topographical barriers (i.e., steep slopes) (Almquist-Jacobson and Foster, 1995). JNCC broadly separates fens by the nature of water movement within them: vertical - 'topogeneous' and lateral - 'soligenous'; the latter describes fens that remain in lowland bog systems as 'lagg fen' at the lower, outer margins (Worrall, *et al*., 2011; JNCC, 2018). Fens can be broadly termed 'poor' or 'rich' depending on the floral diversity, which is related to nutrient inputs and pH. JNCC provide a full description of different fen types and associated flora and fauna: http://jncc.defra.gov.uk/page-5853. Poor fens have a pH < 5 and minimal nutrient input, are generally species-poor and may be associated with *Sphagnum* mosses (see section 1.2.3), sedges, and a few species of marsh plants. Rich fens have a pH > 5 with mineral and nutrient-rich inputs, with a species-rich carpet of mosses, sedges and herbs, or a mix of taller reeds and marsh plants (Worrall, *et al*., 2011; JNCC, 2018).

Lowland bog formation can occur through plant litter accumulation in a fen until the surface is no longer connected with mineral soils or ground water, and receives water and nutrients only through precipitation, when it is termed 'ombrotrophic' (Lindsay and Immerzi, 1996; Hughes and Barber, 2003; Worrall *et al*., 2011; Rydin and Jeglum, 2013, JNCC, 2019). Almquist-Jacobson and Foster (1995) found that transition from fen to ombrotrophic mire conditions in the UK appeared to depend on a relatively sudden change in the climate to drier conditions between 4000 and 5000 years BP, allowing *Sphagnum* mosses to colonise previously sedge-dominated fens, and also, on a local level, the expansion of fens and the accumulation of litter in the centre of these areas, creating domes which became increasingly hydrologically separate from the fen and surrounding groundwater. Hughes and Barber (2003) expand this to suggest there were two distinct fen to bog (via oligotrophic mire) transition routes; one during periods of increased precipitation, particularly after around 8300 BP which was a period of climate cooling, and another during periods of decreased precipitation (but still within effective levels for mire support) or episodes of 'river capture', where substantial water supply to the fen was diverted elsewhere.

The growth of a lowland bog where the surface becomes domed (Lowland Raised Bog) is determined by several factors – the radius and height of the mound, the amount of moisture it receives and the permeability of the peat body, and the growth is limited by the amount and compaction of plant material accumulated (Clymo, 1984; Almquist-Jacobson and Foster, 1995). When the optimum height and width is achieved, and the bog is in equilibrium, expansion can occur laterally, particularly downslope, or the surface can flatten, encouraging pool formation, and several localised bogs may coalesce into a larger bog complex (Almquist-Jacobson and Foster, 1995).

A functioning lowland bog is generally considered to be 'diplotelmic' (Money and Wheeler, 1999; Vasander and Kettunen, 2006). The active 'acrotelm' of living and partially decomposed and collapsing plant material which, through hummock or hollow habitat may be between 30 cm and 50 cm thick, has a fluctuating water table, high water-holding capacity, and aerated conditions. The increasingly compacted 'catotelm' of dead plant material is below the lowest water level in the acrotelm, and so is entirely waterlogged and therefore anaerobic, with slower microbial decomposition rates and minimal lateral movement of water due to a greater bulk density (Clymo, 1984; Price and Whitehead, 2001; Quinty and Rochefort, 2003; Lindsay, 2010). The transition area between the acrotelm and catotelm is termed the 'mesotelm', which is 'usually anoxic but periodically oxic' (Clymo and Bryant, 2008). The mesotelm depth can vary considerably, changing the rate of peat accumulation at the surface of the catotelm, depending on microtopography and hydrological, climatic and plant assemblage dynamics (Rydin and Jeglum, 2013). Orme (1990) estimated a net vertical peat accumulation rate in bogs of 0.34 cm yr^{-1}, with a range of 0.01 – 0.98 cm yr^{-1} although Strivens *et al*. (2017) suggested raised bog peat accumulation in the last 150 years in a relatively undisturbed bog complex (Latvia) to be 0.35 cm yr^{-1} reducing to 0.28 cm yr^{-1} in the last 50 years due to increased indirect drainage, although rates vary widely across Europe due to vegetation composition, climate and anthropogenic disturbance.

1.2.2 Peatland plant assemblage and adaptations to bog conditions

Rydin and Jeglum (2013) suggest that an open bog is classified as having < 10% cover of woody vegetation, and a microtopography of carpet, lawn and hummocks with

Sphagnum mosses, low sedges, dwarf shrubs and lichens. The loose acrotelmic structure of the *Sphagnum* layer allows active rooting of vascular plants (Quinty and Rochefort, 2003). The specialised vascular plant community growing on bogs is able to tolerate the stress of acidic (usually about pH4), low-nutrient, waterlogged conditions through various adaptive measures (Aerts, 1995; Aerts, 1999; Haslam, 2003; Aber *et al*., 2012; Rydin and Jeglum, 2013), here described. Plants such as *Eriophorum spp.* have aerenchymatous tissue to allow air to reach the roots (Schimel, 1995; Videmšek *et al*., 2006). Some shrubby plants may develop 'adventitious' roots to deal with *Sphagnum* competition by layering, and short-rooted plants such as *Calluna vulgaris*, *Erica tetralix, Andromeda polifolia* and *Vaccinium oxycoccos* grow on hummock tops to allow root aeration, but have leathery leaves to reduce evapotranspiration; many peatlands plants are semi-evergreen for long-term activity and retention of nutrients. Carnivorous plants such as *Drosera spp.* absorb nutrients by attracting and consuming insects. Nutrients may be conserved by perennial and slow growth, and clonal behaviour (as in *Eriophorum* and *Carex* species) stores energy and nutrients in rhizomes or stolons for new plant growth, and also reduces the expenditure needed for seed production and the difficulty of seed germination in a generally hostile environment (Haslam, 2003).

1.2.3 Sphagnum moss physiology and distribution

Sphagnum mosses are an intrinsic part of lowland bog formation and development, and bioengineer the environment for continuously favourable ecohydrological conditions (van Breemen, 1995; Quinty and Rochefort, 2003) through a capacity for capillarity of water around external plant structures which buffer against evapotranspiration (Spieksma, 1999; Thompson and Waddington, 2008; Mazziotta *et al*., 2018) and raise the water table to the growing surface of the moss, along with chemical processes (outlined below in section 1.2.4) (Haslam, 2003; Quinty and Rochefort, 2003; Rydin and Jeglum, 2013).

Sphagnum, like all bryophytes, is poikilohydric; that is, it cannot regulate water content as vascular plants do through stomata and other mechanisms but, unlike other bryophytes, it has developed strategies to expand its habitat, such as external capillarity and plastic growth habits to avoid desiccation and resulting loss of growth (Hájek, 2014). *Sphagnum* mosses are a separate class within Division Bryophyta due to their discrete morphology

(Atherton *et al*., 2010). Their branches develop within a tightly-packed head (capitulum) at the top of the single stem, which is the growing point of the plant, and emerge in clusters (fascicles, with divergent and pendent branches) rather than singly, spirally along the stem as it elongates, and the leaves on stems and branches differ morphologically.

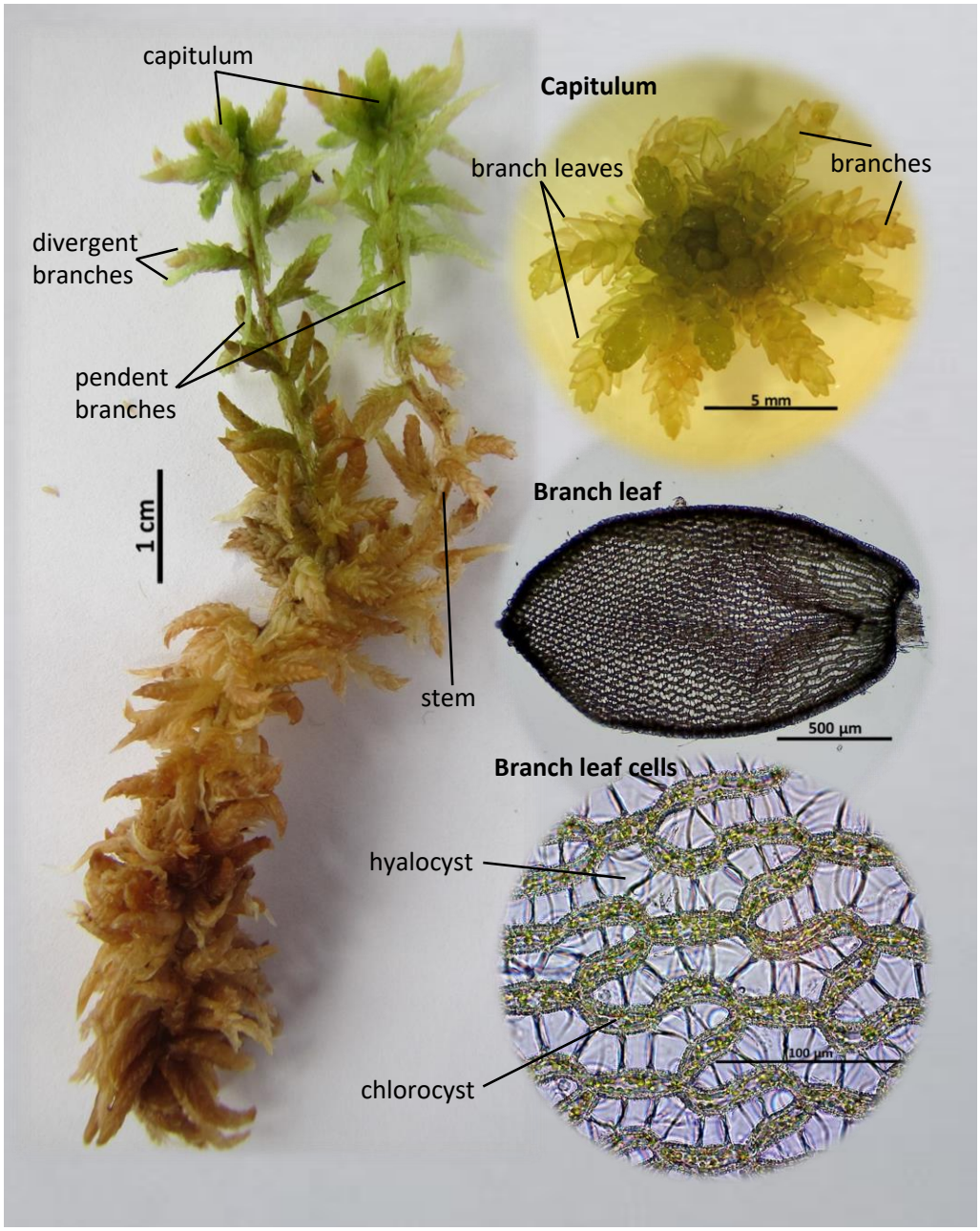


Figure 1.1. Basic *Sphagnum* anatomy. Full plant image: *S. palustre*; other images: *S. papillosum*. All images: A Keightley.

Branch leaves have distinctive features, depending on species, but all are unistratose (one cell thick) and made up of two types of cell: large, dead (on maturity), water-filled hyaline cells (hyalocysts) generally containing pores, surrounded by 'walls' containing

chlorophyllous cells (chlorocysts) (Smith, 2004; Atherton *et al*., 2010; Laine *et al*., 2018). The stem has a central cylinder surrounded by hyalocysts and has no rhizoids (van Breemen, 1995) to anchor the plant to a substrate and only the mass of the plants grouped together keeps them in place, the bulk of which is water held between the plant structures. At the time of writing, there are 35 species of *Sphagnum* in the UK (Atherton *et al*., 2010) and around 250 globally (Smith, 2004), grouped by collective features into Sections: *Acutifolia*, *Cuspidata, Rigida, Sphagnum, Squarrosa* and *Subsecunda* (UK Sections). T heir characteristics and forms can be plastic, depending on habitat, shade and season (Table 1.1), making identification often difficult in the field (Smith, 2004; Atherton *et al*., 2010; Laine *et al*., 2018). Physiologically, *Sphagnum* bioengineers the environment to be wet, acid and nutrient-poor, principally through the action of phenolic compounds (Malmer *et al*., 2003).

1.2.4 Sphagnum moss - bog environment engineer

Phenolic compounds are produced by plants to facilitate stress-tolerance, and are found in all *Sphagna* (predominantly the monophenolic *p*-hydroxy-β-(carboxymethyl)-cinnamic acid, termed '*sphagnum* acid' by Rudolph, 1972) in varying quantities depending on species and season, in cell fluids and as polymers in cell walls (Verhoeven and Liefveld, 1997). Some *Sphagnum* phenols are excreted in bog water and may affect vascular plants allelopathically, hence slowing their growth and reducing competition and evapotranspiration (van Breemen, 1995; Verhoeven and Liefveld, 1997).

The accumulation of carbohydrates occurs in environments which restrict plant growth and are stored as carbon, and those in *Sphagnum*, especially uronic acids (sphagnan), also form polymers in cell walls, responsible for ionic exchange, and are reckoned by Clymo (1987) to make up 10-30% of fresh *Sphagnum* dry weight. Cation exchange between H^+ and Ca^{2+} and Mg^{2+} especially, but also K^+ and NH_4^+, traps nutrients for *Sphagnum* growth and creates an acid, nutrient-poor environment, most efficiently in the upper acrotelm (Verhoeven and Liefveld, 1997; Rydin and Jeglum, 2013). Freeman *et al*. (2001) suggest the primary factor in the capacity of peatlands to store carbon is the waterlogged, anaerobic conditions which prevent phenol oxidase from destroying the phenolic

compounds that inhibit microbial degradation of organic material, and thus preventing aeration of peatlands through drainage or drought is vital to protect carbon stocks.

Sphagnum does not contain lignin for rigidity, as do vascular plants, but polymeric phenols give strength to cell walls by retaining cellulose (polysaccharides), which maintains the integrity of hyaline cells and makes the plants unpalatable, preventing herbivory (Verhoeven and Liefveld, 1997). Cell wall polysaccharides are also superficially coated by lipids, and together with tannins and other phenolics, these mechanisms inhibit microbial pathogens and hamper microbial decomposition of *Sphagnum* (Verhoeven and Liefveld, 1997), with variable decomposition rates depending on the species rather than the micro-environment (Rochefort *et al*., 1990; Rydin and Jeglum, 2013; Bengtsson *et al*., 2018). This leads to peat storage and accumulation, and improves the efficiency of nutrient recycling to the living part of the plant (Malmer *et al*., 2003; Laine *et al*., 2011). However, *Sphagnum* also forms 'mutualistic associations with N-fixing cyanobacteria' (Rydin and Jeglum, 2013), contributing to its nutrient content.

Within the permanently water-logged catotelm there is low redox potential, hence slow anaerobic decomposition with a low pH and accumulation of *Sphagnum* litter, which releases tannins through hydrolysis over time, and exacerbates inhibition of microbial activity (Verhoeven and Liefveld, 1997). This slow decomposition also preserves many organic natural and anthropogenic relics which provide an 'archaeological and paleo-environmental record' of long-term climactic and environmental changes and human activity (Gearey and Fyfe, 2016).

Table 1.1. The majority of UK *Sphagnum* species (very rare omitted), their Section, general characteristics, form, habitat and nutrient requirements/tolerance. Adapted from Atherton *et al*. (2010) and Laine *et al*. (2018).

Section	Taxon	Alternate Name	Capitulum Size	Capitulum Colour	Capitulum Shape	Form	Habitat	Nutrient
Acutifolia	*Sphagnum capillifolium* subsp. *capillifolium*	*S. capillifolium* , Acute-leaved Bog Moss	small, medium	green, red	convex-hemispherical	dense hummock	varied	ombrotrophic, weakly minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum capillifolium* subsp. r*ubellum*	Red Bog-moss	small, medium	yellow-green, deep red	flat, branch leaves in 5-ranked rows	hummock, lawn, carpet	bog, poor fen	ombrotrophic
Acutifolia	*Sphagnum fimbriatum*	Fringed Bog-moss	small	bright green, pale green	apical bud	soft hummock, loose carpet	mire, wet woodland, ditch, fen, open-shaded	minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum fuscum*	Rusty Bog-moss	small	orange-brown, deep brown, brown-green, green	flat	dense, low hummock	open raised-blanket bog, undisturbed sites	ombrotrophic
Acutifolia	*Sphagnum girgensohnii*	Girgensohn's Bog-moss	medium	green, yellow-green	stellate, apical bud	loose mat/cushion, stiff-stemmed	shaded, damp woodland, fen	minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum quinquefarium*	Five-ranked Bog-moss	medium	pale green, yellow-green, pink, red	convex-hemispherical,branch leaves in 5-ranked rows	soft carpet, hummock	well-drained, wooded banks, rock surfaces	minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum russowii*	Russow's Bog-moss	medium	pale green, red	flat, stellate, apical bud	soft hummock, loose carpet	open-shaded, wooded mire, heath, flush	ombrotrophic, weakly-minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum subnitens*	Lustrous Bog-moss	medium	yellow-brown, brown, pinkish, red, green centre	flat, outer branches covering	moderately dense cushion, small hummock	varied	ombrotrophic, minerotrophic, nutrient-tolerant
Acutifolia	*Sphagnum warnstorfii*	Warnstorf's Bog-moss	medium, small	green, red-green, purplish, red	flat, stellate, branch leaves in 5-ranked rows	soft carpet, low cushion	medium-rich fen, flush, open-shaded, wooded fen	minerotrophic, nutrient-tolerant
Cuspidata	*Sphagnum angustifolium*	*S. recurvum* var. *tenue* , Fine Bog-moss	small, medium	green, yellow-green, brown-green, yellow-brown	convex-hemispherical	lawn, hummock	open-shaded	ombrotrophic, minerotrophic
Cuspidata	*Sphagnum cuspidatum*	Feathery Bog-moss	medium	green, yellow-green, brown, yellow-brown	untidy/feathery, flat	aquatic, semi-aquatic	open bog, poor fen	ombrotrophic
Cuspidata	*Sphagnum fallax*	*S. recurvum* , *S. brevifolium* , *S. isoviitae* , Flat-topped Bog-moss	medium, large	pale green, yellow-green, brown	stellate, convex, branch leaves in 5-ranked rows	lawn, carpet, open-shaded	bog, poor-intermediate fen	ombrotrophic, weakly minerotrophic
Cuspidata	*Sphagnum flexuosum*	*S. recurvum* var. *amblyphyllum* , Flexuous Bog-moss	small, medium	green, yellow-green, pale green, yellow-brown	rounded, compact, stellate	lawn, carpet	poor-intermediate fen, wet woodland	minerotrophic

Section	Taxon	Alternate Name	Capitulum Size	Capitulum Colour	Capitulum Shape	Form	Habitat	Nutrient
Cuspidata	*Sphagnum pulchrum*	Golden Bog-moss	medium, large	orange, yellow-brown, red-brown, green, brown-green	broad, densely branched	carpet, lawn, robust	open, bog pool, flush, intermediate fen	ombrotrophic, minerotrophic
Cuspidata	*Sphagnum tenellum*	Soft Bog-moss	small, delicate	pale green, yellow-orange, yellow-brown, brown-green	small, delicate, untidy	small flat patches, low cushion, single shoots	open bog, bare peat, wet heath	ombrotrophic, weakly minerotrophic
Rigida	*Sphagnum compactum*	Compact Bog-moss	small	green, pale green, yellow-green, orange-green, brown-green	outer branches covering	compact, dense, lawn, low cushion	poor fen, heath, disturbed wet ground, rocky flush	ombrotrophic
Sphagnum	*Sphagnum affine*	*S. imbricatum* subsp. *affine* , Imbricate Bog-moss	medium	green, yellow, orange-brown, yellow-brown, purplish	compact, rounded	cushion, low hummock, compact, loose	poor-intermediate fen, ditch, flush, wooded mire, heath	minerotrophic
Sphagnum	*Sphagnum austinii*	*S. imbricatum* subsp. *austinii* , Austin's Bog-moss	medium	orange, orange-brown, green centre, yellow-brown	flat, blunt branches	tall, dense hummock	raised bog, blanket bog, undisturbed	ombrotrophic
Sphagnum	*Sphagnum divinum*	prev. *S. magellanicum*	large	purple-red/wine-red, mottled green and pink, green	flat, tapering branches	low hummock, lawn, carpet	mire margin, wet heath, forested peatland	minerotrophic, nutrient-tolerant
Sphagnum	*Sphagnum medium*	prev. *S. magellanicum*	large	purple-red, wine-red	flat, blunt branches	low hummock, lawn, carpet	varied ombrotrophic habitats	ombrotrophic
Sphagnum	*Sphagnum palustre*	Blunt-leaved Bog-moss	large	green, yellow-green, yellow-brown, pink	compact, rounded	cushion, mat, untidy	open-shaded, wooded fen, flush	ombrotrophic, nutrient-tolerant
Sphagnum	*Sphagnum papillosum*	Papillose Bog-moss	large	green, yellow-green, brown-green, yellow-brown	compact, rounded	hummock, lawn, carpet	open, raised bog, blanket bog, poor-rich fen	ombrotrophic, nutrient-tolerant
Squarrosa	*Sphagnum squarrosum*	Spiky Bog-moss	large	bright green, pale green	spiky, apical bud	untidy, small cushion, mat	shaded, wet woodland, wooded fen, flush, ditch	minerotrophic, nutrient-rich
Squarrosa	*Sphagnum teres*	Rigid Bog-moss	medium, small	green, orange, red-brown	stellate, apical bud	carpet	open-shaded, open-wooded fen, flush	minerotrophic, nutrient-tolerant
Subsecunda	*Sphagnum denticulatum*	*S. auriculatum* , Cow-horn Bog-moss	medium, large	green, yellow-green, copper	rounded	carpet, aquatic, semi-aquatic	bog pool, flush, spring, ditch	acidic, nutrient-poor
Subsecunda	*Sphagnum inundatum*	*S. auriculatum* var. *inundatum* , *S. subsecundum* subsp. *inundatum* , Lesser Cow-horn Bog-moss	medium, large	orange-brown, yellow-brown, dark-brown, red-green, green, yellow-green	stellate, rounded	carpet	edge of flush, poor fen, ditch, mire	ombrotrophic, minerotrophic
Subsecunda	*Sphagnum subsecundum*	Slender Cow-horn Bog-moss	small	orange, yellow-brown, yellow, green	untidy, curved	loose carpet	flush, intermediate fen, ditch, swamp	minerotrophic, nutrient-tolerant

1.3 *Sphagnum* mosses in the peatland restoration process

1.3.1 Introduction of Sphagnum moss to damaged lowland peatlands

Establishment of peat-forming *Sphagnum* mosses is vital for returning a surface acrotelm to a damaged peatland, to resemble the moisture-retaining properties of near-natural peatlands (Quinty and Rochefort, 2003) and reduce peat respiratory carbon losses (Waddington and Warner, 2001). In establishment phases of bog restoration vascular plants can 'nurse' and promote *Sphagnum* moss growth by providing scaffolding, environmental protection and a beneficial microclimate (Grosvernier *et al*., 1995; Ferland and Rochefort, 1997; Quinty and Rochefort, 2003; Pouliot *et al.*, 2011) and other mosses such as *Polytrichum strictum* can reduce plant displacement through frost heaving (Price *et al*., 2003). It is recommended that these nurse plants are introduced together with *Sphagnum*, as long as a balance can be maintained between evapotranspiration, light stress and nutrient enrichment caused by vascular plants, and reduced mineralisation, decomposition and nutrient levels in the environment resulting from *Sphagnum* proliferation (Rochefort, 2000; Quinty and Rochefort, 2003; Groeneveld *et al.*, 2007; Landry and Rochefort, 2009; Pouliot *et al.*, 2011). However, Groeneveld *et al.* (2007) warn this cannot remediate for poor hydrological conditions. Moreover, Thompson and Waddington (2008) state that it is vital to provide resistance to evaporation with vegetation to maintain lower water tension in near-surface peat, making water available to support *Sphagnum* moss growth (Landry and Rochefort, 2009). Further benefits are that, as vegetation develops, open water areas are reduced (Spieksma, 1999), and the plants also trap wind-borne seeds, increasing germination and proliferation (Groeneveld *et al.*, 2007).

Eriophorum species are early colonisers, and also perhaps the species of choice to nurse *Sphagnum* moss re-colonisation (Pouliot *et al.,* 2011; Nugent *et al*., 2018) as they provide environmental protection and help stabilize the peat surface without out-competing *Sphagnum* or smothering it with plant litter (Guêné-Nanchen *et al.,* 2017). Additionally, decomposition of this minimal litter may provide the necessary metabolites and environment for *Sphagnum* spore establishment (Sundberg and Rydin, 1998). However,

aerenchyma in *Eriophorum* species, designed to bring air to the roots and rhizosphere of wetland plants, act as conduits for CH_4 from the anaerobic peat, through the plant and out through the leaf blades to the atmosphere (Schimel, 1995; Videmšek *et al*., 2006). This will raise concerns that peatlands undergoing restoration may become GHG sources rather than sinks due to elevated CH_4 emissions (Lindsay, 2010; Evans *et al*., 2016), particularly with pool-formation (Worrall *et al*., 2011).

Nevertheless, oxic conditions created in the rhizosphere of aerenchymatous plants also allow methanotrophic bacteria to oxidise CH_4 and distance roots from the methane store (Fritz *et al*., 2011), so perhaps, on balance, the role of such vegetation is advantageous for climate mitigation in peatlands if it facilitates successful restoration. Rapid colonisation by *Sphagnum* mosses is needed to reduce vascular plant cover and to restore an effective, ecohydrological function in the short term (Lindsay, 2010). Carbon sequestration benefits of peatland restoration are likely to increase over time, through avoided losses (e.g., prevention of drainage and erosion) and new carbon gains (e.g., through re-vegetation) (Worrall *et al*., 2011; Renou-Wilson *et al*., 2019), with development of a functioning acrotelm resembling those of near-natural peatlands, necessary for peat accumulation (Quinty and Rochefort, 2003; Lucchese *et al*., 2010; Waddington *et al*., 2011; Worrall *et al*., 2011). Lucchese *et al.* (2010) consider the timescale for recovery of carbon storage to be decades, although Nugent *et al*. (2018) report their Canadian study site being a consistent gaseous carbon sink within 14 years despite fluctuating water table levels, which they assert will stabilise once the *Sphagnum* depth increases.

Peatland ecohydrological function is only restored with the establishment of a new acrotelm layer thick enough to moderate seasonal and inter-annual variations in the water table (Quinty and Rochefort, 2003; Lucchese *et al.*, 2010). Quinty and Rochefort (2003) suggest that, with intervention, a full plant carpet and water table stabilization can be achieved in 5 years, and Lucchese *et al.* (2010) found that restoration to an approximation of a natural system is possible in 8 years. It is generally agreed that typical peatland species, particularly *Sphagnum* mosses, do not readily colonise the bare peat surfaces of abandoned extraction sites. Therefore, self-regeneration to a functioning sustainable peatland is highly unlikely (Money and Wheeler, 1999; Quinty and Rochefort,

2003) and intervention with active restoration is needed to re-establish ecological function and peat accumulation (Rochefort, 2000; Chirino *et al*., 2006; Lucchese *et al.*, 2010).

Re-introduction of *Sphagnum* moss for bog restoration relies on availability. Canada has large, active, peatland reserves (Global Peatland Database, 2017) but they face the same threats as peatlands elsewhere (extraction [mostly in the south], agricultural conversion and forestry) with the addition of hydroelectric dam construction (Pouliot *et al*., 2004), and protected areas are considerable but still small compared to the whole peatland extent of around 125M ha (Graf and Rochefort, 2016). Small bogs or remnants of harvested peatlands are often available for harvesting donor plant material for restoration of nearby abandoned extracted sites (Quinty and Rochefort, 2003). The most well-known Canadian restoration methods broadly involve large-scale site re-wetting, active re-introduction of *Sphagnum* diaspores (any part of plants capable of producing a new individual – seeds, spores, roots, stems, leaves, branches, etc.) and application of a protective mulch (straw) cover – the 'moss layer transfer technique' (Rochefort, 2000; Quinty and Rochefort, 2003). A lack of humidity has a severe effect on both *Sphagnum* establishment and long-term extent and density (Chirino *et al.,* 2006), and mulch protects the *Sphagnum* fragments during the establishment phase from heat, desiccation and displacement by high rainfall (Landry and Rochefort, 2009) although Waddington *et al.* (2003) caution that during the first few seasons of restoration a mulch layer of straw significantly increases the capacity of the peatland as a carbon source, due to decomposition. *Sphagnum* diaspores (plant fragments) to be re-introduced are harvested from the donor site to a depth of 5 - 10 cm (below 10 cm the material is essentially dead) at one-fifteenth the area of the host restoration site (Chirino *et al.*, 2006) although Quinty and Rochefort (2003) suggest that one-tenth will compensate for material losses or failures, and that the recommended collection depth allows rapid recovery of the remaining moss (within 4-6 years).

Western European peatland restoration projects have historically involved retaining rain water at the surface only, to re-wet the peat and encourage spontaneous *Sphagnum* growth but the *Sphagnum* carpets produced by this method are reportedly species-poor (principally *S. cuspidatum* and *S. fallax*) and acrotelm development stagnates (Quinty and

Rochefort, 2003, Robroek *et al.,* 2009). They also tend to be colonised by invasive vascular species and do not naturally regenerate to 'functioning peatland' in the short-term (< 25 years) (Rochefort, 2000). More recent work in Germany, utilising the Canadian method of restoration, found that cut mosses regenerated almost completely after 2.5 years with water levels consistently high (Gaudig *et al.,* 2018).

1.3.2 Sphagnum – the need for propagation

As *Sphagnum* is a key component of peatland ecohydrological function and peat accumulation, at least in the northern hemisphere, restoration in conservation areas to *Sphagnum*-dominated lowland bog, and *Sphagnum* farming outside of conservation areas, are arguably the optimum routes for peatland recovery (Pouliot *et al*., 2015; Gaudig *et al*., 2017). Farmed *Sphagnum* could have a range of horticultural uses (Aubé *et al*., 2015; Pouliot *et al*., 2015) but its use as a replacement for peat in growing media is an urgent consideration for compliance with the UK Government's recommendation to end peat use by 2030 (DEFRA, 2013) and to prevent potential damage from harvesting alternative sources outside of the UK.

1.3.3 Sphagnum species selection

Selection of *Sphagnum* species for restoration is important. Section *Acutifolia* species (such as *S. subnitens*, *S. fimbriatum, S. capillifolium*) are preferred over others due to their ability to colonize bare peat surfaces and form hummocks and dense colonies, thereby retaining water efficiently and developing resilience to unstable water levels. Section *Cuspidata* species (e.g., *S. cuspidatum*, *S. fallax*) form loose colonies in lawns and hollows, and are not adapted to retain water (Rochefort, 2000; Quinty and Rochefort, 2003). Landry and Rochefort (2009) also found that Section *Acutifolia* species perform best (comprising 53% of the resulting carpet) followed by Section *Sphagnum* (e.g., *S. palustre*, *S. papillosum*, *S. medium*) (30%) then Section *Cuspidata* (5%). Selection should also be adapted to site conditions and previous use and inputs which may affect *Sphagnum* growth rates (Hájek *et al.,* 2006). Robroek *et al.* (2009) found that species-poor communities of *Sphagnum* are generally 'less effective in sequestering carbon and are more sensitive to environmental changes' than *Sphagnum* mixtures, and *Sphagnum*

species can be highly competitive, although establishment may be positively affected by competition between species, particularly if species tolerant of dry conditions are included (Chirino *et al.,* 2006). It appears that to create and develop a more diverse *Sphagnum* community an aggregate of species should be transplanted where each competes for its optimum hydrological niche. The size of the aggregate may be important ('positive self-association') but is not yet understood (Robroek *et al.*, 2009), nor which mix of species and optimum conditions for greatest biomass production (Gaudig *et al*., 2018).

1.3.4 Barriers to natural propagation

Due to large, long-term losses of peatland, active peatlands tend to be fragmented patch habitat niches in a non-like (usually agricultural) matrix, with significant effects on long-term regional biodiversity resilience (Rochefort, 2000). Habitat fragmentation leads to decreased population dispersal (Lawton *et al*., 2010) resulting in a loss of genetic diversity and local extinctions (Gunnarsson and Söderström, 2007). Colonisation barriers limit dispersal of *Sphagnum* spores (sexual propagules) (Quinty and Rochefort, 2003) and diaspores in fragmented sites (Quinty and Rochefort, 2003; Chirino *et al*., 2006). Moreover, within some species a low genetic diversity may cause low sexual output, and so artificial dispersal of *Sphagnum* fragments (asexual propagules) is necessary (Gunnarsson and Söderström, 2007). However, in many areas (as for this study) natural peatlands are designated for conservation and collection of *Sphagnum* is not only undesirable, but prohibited (Gahlert *et al*., 2012; Caporn *et al*., 2018), and so other methods of propagation are needed.

1.3.5 Sphagnum propagation techniques

Gaudig *et al*. (2018) reported on *Sphagnum* propagation trials in Germany (specifically for paludiculture), which used fragments of *Sphagnum* on stripped agricultural peats (no mulch application) with ditch irrigation to propagate sufficient quantities for harvest and further propagation after 5 years of growth, although recharge of ditches with nutrient-rich water created some challenges with vascular plant growth and *Sphagnum* species competition. Other trials used controlled, shaded conditions with overhead irrigation, which improved multiplication rate 10-fold and reduced weed growth compared to field

conditions. Comparable trials of *Sphagnum* propagated on floating rafts had slightly lower productivity rates compared to field conditions (Gaudig *et al.,* 2014).

Gahlert *et al*. (2012) experimented with propagation from spores, seen as a 'clean' base, of *S. fimbriatum*, *S. palustre* and *S. papillosum*, on a range of sterile, non-sterile and artificial substrates in controlled conditions, fertilised with either rainwater-equivalent or a x20 concentration. They had successful outcomes, although labour-intensive, with sterile substrates and nutrient agar, concentrated rainwater-equivalent fertilization, and subsequent field transplantation, but not with direct seeding onto the field environment, even with shading. Beike *et al*. (2015) sterilized spores and germinated on petri dishes in controlled conditions, transferred as protonema to new petri dishes, and resulting gametophores were cultivated in vitro on solid culture with microelements, and in liquid culture media with microelement supplementation, sucrose, ammonium nitrate and pH adjustment, with larger-scales trials in bioreactors. Authors reported a 30-fold increase in *Sphagnum* mass in bioreactors and suggested such in vitro methods could supply enough material for 40,000 ha of degraded peatland, although Gaudig *et al*. (2018) suggested the methods were 'difficult to accomplish' and spore collection impractical. Sundberg and Rydin (2002) state that *Sphagna* spore prolifically and suggest, after conducting growth chamber and field experiments, that P-limitation in bogs inhibits spore germination where *Sphagnum* is already present, but as spore dispersal has occurred over long distances, particularly in disturbed peats, that germination is facilitated by phosphorus inputs from animal faeces and plant litter. However, judging by the few trials of propagation by spores, artificially or manually, it does not appear to be a viable or sufficiently controlled process for large-scale restoration or *Sphagnum* farming.

1.3.6 Sphagnum micropropagation – background and development

Micropropagation Services (EM) Ltd (the BeadaMoss® company) produces large quantities of *Sphagnum* from tiny amounts of wild-sourced material, using standard tissue-culture techniques involving plant division in a sterile, controlled environment, and growing on under greenhouse conditions (Caporn *et al.*, 2018). The company was originally approached by Moors for the Future Partnership (MFFP) to supply large numbers of peatland plants for South Pennines and Peak District blanket bog restoration,

with a future need for bulk quantities of *Sphagnum* once the vascular plant cover had stabilised the peat surface (Wittram *et al.*, 2015). The decision on a mix of *Sphagnum* species, and the proportions in the mix, was made collaboratively by the BeadaMoss® company and MFFP, who requested development of a product for broad application. Selection was based on a greater percentage of species most likely to grow on enriched moorland (limed and fertilized to promote vascular plant growth) along with those suited to a range of environmental niches found in the uplands, and a small percentage of species which would add diversity if successful but not likely to hamper restoration efforts if they did not survive. Further products have been adapted to suit habitat-specific areas (Neal Wright, BeadaMoss®, personal communication).

The first product developed (Figure 1.2) was BeadaMoss® - tiny fragments of immature *Sphagnum* in a hard gel 'bead', which was partially successful (Wittram *et al*., 2015; Caporn *et al*., 2018). More mature *Sphagnum* in the form of plug plants (BeadaHumok™ - 'plugs') was developed, whereby strands of developing *Sphagnum*, suspended in a hydrocolloidal gel, are applied to growing media and grown on into plug plants in the greenhouse. Subsequently, BeadaGel™ ('gel') was developed as a cheaper alternative (because it is applied earlier in the process), with the aim of giving a more rapid ground cover than the beads, and a more even spread of *Sphagnum* than plugs. Research and development into the products continue, to optimise effectiveness and ease of application, and to cut costs.

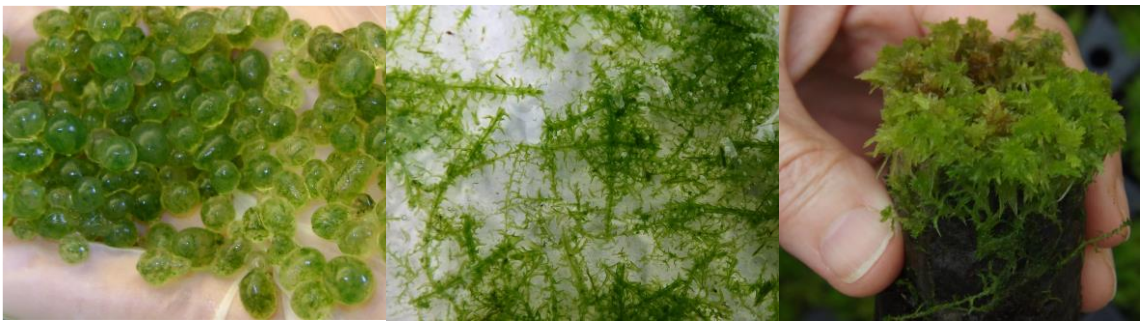


Figure 1.2. Examples of BeadaMoss® products: BeadaMoss®, BeadaGel™ and BeadaHumok™

1.3.7 Micropropagated Sphagnum species selection

The *Sphagnum* species in the BeadaMoss® 11-species mix (applied on the Cadishead Moss trial plots in this study – Chapter 4, and used in species comparative growth studies in Chapter 3) is the mix originally requested by MFFP. The species were in the following proportions: *S. capillifolium* (Ehrh.) Hedw. (ssp. *capillifolium)* ~10%, *S. cuspidatum* Ehrh. ex Hoffm. ~10%, *S. denticulatum* Brid. ~1%, *S. fallax* (H.Klinggr.) H.Klinggr. ~25%, *S. fimbriatum* Wilson ~10%, *S. medium/divinum* (originally designated as *S. magellanicum* Brid.) ~1%, *S. papillosum* Lindb. ~10%, *S. squarrosum* Crome ~1%, *S. palustre* L. ~20%, *S. tenellum* (Brid.) Pers. ex Brid ~1%, *S. subnitens* Russow & Warnst. ~5%. *S. magellanicum* is now recognised as a species specific to the southern hemisphere, and European species similar to *S. magellanicum* are separated into *S. medium and S. divinum* (Hassel *et al*., 2018), which have some morphological differences and are generally found respectively in ombrotrophic and minerotrophic conditions (Laine *et al*., 2018). The *Sphagnum* sourced for BeadaMoss® material could be either or a mixture of these, and so will be referred to as *S. medium/divinum*. A few strands of species were sourced from the Peak District National Park, apart from *S. medium/divinum* and *S. tenellum*, sourced from Cumbria (Caporn *et al.*, 2018). The species are from five *Sphagnum* Sections: *Acutifolia, Cuspidata, Sphagnum, Squarrosa* and *Subsecunda*, which thrive in a range of microhabitats (Atherton *et al*., 2010; Laine *et al*., 2018) from pools to hummocks across the peatland landscape (Table 1.1). Therefore, the species mix provides the opportunity for *Sphagnum* to grow wherever it is placed, as each species is adapted to a particular environmental niche where its productivity is greater than that of other species (Clymo and Hayward, 1982). This study has included detailed examination of differences in photosynthesis rates and in cell structures between different species of both wild-sourced and BeadaMoss® *Sphagnum*, which is novel research, exploring the potential benefits of micropropagated *Sphagnum* in the field.

1.4 The focus of this thesis

1.4.1 Chat Moss complex and Cadishead Moss study site

Daniel Defoe in his book of letters, '*A tour through the whole island of Great Britain*', first published 1724 – 1727, described Chat Moss thus:

> '*…the great bog or waste call'd Chatmos … The surface, at a distance, looks black and dirty, and is indeed frightful to think of, for it will bear neither horse or man, unless in an exceeding dry season, and then not so as to be passable, or that any one should travel over them … We saw it in some places eight or nine foot thick, and the water that dreins from it look'd clear, but of a deep brown, like stale beer. What nature meant by such a useless production, 'tis hard to imagine; but the land is entirely waste, except, as above, for the poor cottagers fuel, and the quantity used for that is very small.'*

Local people told Defoe that the extent of it was,

> *'…on the left-hand of the road for five or six miles east and west, and ... in some places, seven or eight miles from north to south.'*

which gives a potential estimated area of perhaps between 8,500 and 10,682 ha.

Lindsay and Immerzi (1996) reported that the extent of peat mass in the Chat Moss area was originally 2,587 ha (considerably less than estimated by Dafoe's sources, but allowances have to be made for local exaggeration). Hall *et al*. (1995) report that Chat Moss is approximately 9 km long and 4.5 km wide (i.e., 4,050 ha), and bounded by 'the Glaze Brook to the west, the M63 (sic: M62) to the east, the A580 in the north and the A57 in the south'. Hall *et al*. (1995) state that peat formation in the Greater Manchester area is due to impermeable sub-surface clays (and sands) from the last glacial period creating a 'perched water-table' that initiated formation of fen-carr and subsequently lowland bog but that, due to the undulating nature of the underlying geology, Chat Moss as a whole cannot be seen as a typical lowland raised bog, and is best regarded as an 'intermediate' or 'ridge-raised mire': a coalition of several peat bodies that have grown across the landscape, as described by Lindsay *et al*. (2014).

Drainage works to reclaim land for agriculture on the Manchester Mosses were on a large scale by the late 18th century, with fertilisation initially by paring and burning the surface vegetation, then with the addition of marl and sand and later with night soil brought by rail from Manchester, so that by 1849 'a third of Chat Moss had been brought under cultivation' (Hall *et al*., 1995). At the time of Lindsay and Immerzi's 1996 inventory of lowland raised bogs in Great Britain, classification for the Chat Moss area was 'revegetated or regenerating cutover' which was predominantly in agricultural use, although a small proportion (92 ha or 3.5%) was designated SSSI (Astley and Bedford Mosses) which was upgraded to a Special Area of Conservation (SAC) in April 2005 under the name 'Manchester Mosses' and covers 170.49 ha (JNCC, undated) (Figure 1.3). This was prior to the acquisition and subsequent restoration by the Lancashire Wildlife Trust (LWT) of 8 ha Cadishead Moss (in 2009) and 107 ha Little Woolden Moss (in 2012), currently with no designation, although Cadishead Moss is nominated a Site of Biological Importance (SBI) for 'HB1 – Heathland and Bog' and 'Br5 – Birds, UK Priority Species' (data.gov.uk, 2018). OS maps to 1991 show Little Woolden and Cadishead Mosses as having peat deposits > 1 m depth and covered with scrub, with woodland on the western edge of Little Woolden (Hall *et al*., 1995). Only fragments of Chat Moss remain in a semi-natural condition (Figure 1.3) and LWT estimate that only 2% of the original peatland complex remains in a 'salvageable condition' (LWT, 2019).

1.4.2 Local restoration implications

Renou-Wilson *et al*. (2019) state that restoration of damaged bogs through re-wetting is hugely challenging, and carbon sink function is more rapidly achieved than establishment of *Sphagnum* mosses. Restoration of UK peatlands is currently delivering emissions reductions (Evans *et* al., 2017), but papers reviewed by Worrall *et al*. (2011) suggest that, even if overall GHG benefits are not easily achieved this is outweighed by ecosystem services and habitat and wildlife conservation benefits (JNCC, 2019) in the short term. However, practices are improving over time as new data and techniques are shared and implemented.

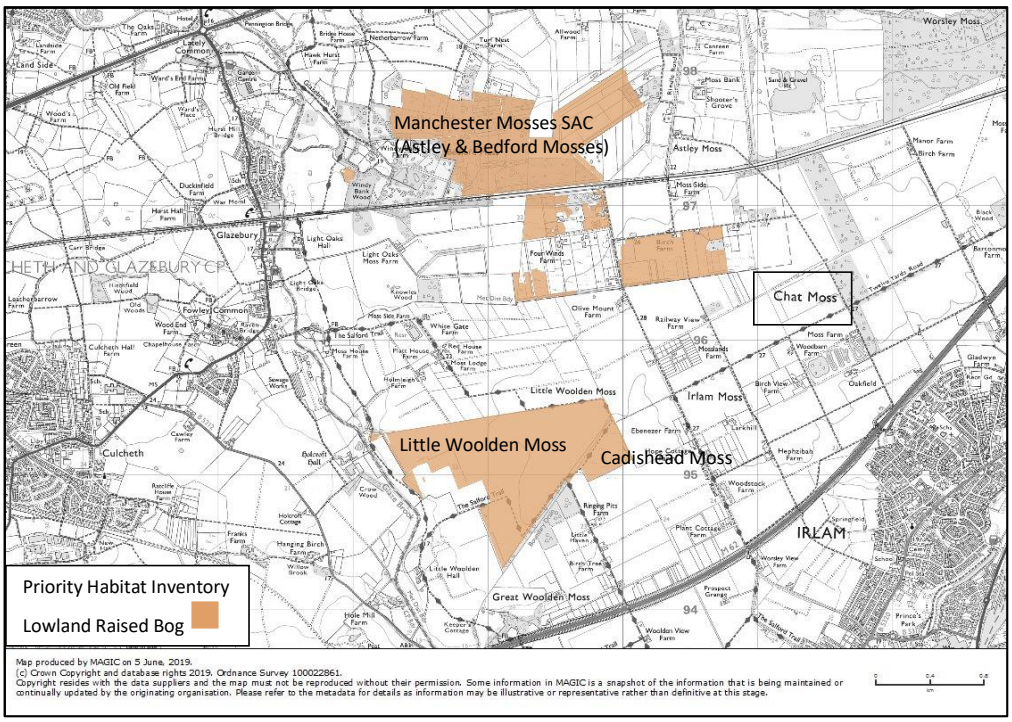


Figure 1.3. Map of Chat Moss Lowland Raised Bog fragments. Multi-Agency Geographic Information for the Countryside (MAGIC) map downloaded from JNCC.

Large-scale restoration work is currently ongoing on Chat Moss. Revegetation on the ex-peat-harvested Little Woolden Moss with vascular plants (*Eriophorum angustifolium* and *E. vaginatum* in the first instance) is a more recent aspect to restoration. Plants are translocated from areas of abundance into bare peat areas. Seeds are collected for either direct dispersal or germination and growing on and then planting out. More recently, *Eriophorum spp.* and *Sphagnum* plug plants primarily, but also *Erica tetralix* and *Empetrum nigrum* to increase biodiversity, grown by micropropagation (purchased from the BeadaMoss® company), have been planted out in large numbers (tens of thousands) for revegetation of approximately 30 ha of mainly bare peat areas more recently under restoration management after lapse of a pre-existing peat-harvesting tenure. Data gathered for this study on Cadishead Moss (8 ha) is already informing the restoration practices on the adjoining larger Little Woolden Moss (107 ha) both in terms of *Sphagnum* establishment and potential carbon GHG sequestration.

1.4.3 Basis for this study

BeadaMoss® *Sphagnum* has been successfully introduced on this study site in the past (Caporn *et al*., 2018), and broadly applied in the uplands (Crouch, 2018). However, the products have had little study in themselves, as the need for *Sphagnum* introduction for restoration was clear, naturally-sourced material was limited and protected, and BeadaMoss® materials were ready for purpose and available in large quantities. This thesis explores properties of BeadaMoss® *Sphagnum* in terms of photosynthesis rates, morphology and productivity, and compares their performance with that of wild-sourced *Sphagnum*, to find out whether they are in fact an effective substitute, particularly for use in restoration of degraded peatlands where re-construction of *Sphagnum*-dominated acrotelm is key to recovering their potential for carbon sequestration.

1.4.4 Chapter navigation

Chapter 2: 'Study into comparative photosynthesis rates of BeadaMoss® and wild-sourced *Sphagnum*' explores the potential productivity and CO_2 uptake of a range of *Sphagnum* species, of tissue-cultured and naturally-sourced origin, primarily through studies of photosynthesis and respiration rates. This tests the key hypothesis that BeadaMoss® *Sphagnum* has similar photosynthetic capacity and productivity to wild-sourced *Sphagnum*, and so BeadaMoss® *Sphagnum* is suitable for wide-scale introduction on degraded sites for restoration purposes.

Rates of photosynthesis of six different BeadaGel™ species, and of wild-sourced counterparts, were measured and analysed in controlled conditions, and some inferences drawn from chemical analysis of the *Sphagnum* samples. Samples of the same six species of micropropagated *Sphagnum* from BeadaMoss® were also compared microscopically with equivalent samples from natural sources for any obvious morphological differences that could account for any disparity in rates of photosynthesis.

Chapter 3: 'Investigation of BeadaMoss® *Sphagnum* growth in a commercial mix of species' researches the productivity of micropropagated *Sphagnum* in indoor and more natural environments through growth trials of eleven individual *Sphagnum* species in

'BeadaGel™' form, and the BeadaMoss® company's commercial BeadaGel™ mix of those eleven species. This tests the key hypotheses that BeadaMoss® *Sphagnum* species display the same phylogenetic traits as *Sphagnum* in natural settings, but productivity is enhanced in benign conditions and remains proportionally consistent when grown in a species mix.

Chapter 4: 'Carbon greenhouse gas fluxes on a degraded lowland peatland using micropropagated *Sphagnum* moss in the restoration process' researches how introduced, micropropagated *Sphagnum* (as BeadaGel™) and naturally occurring *Eriophorum angustifolium* influences gaseous carbon fluxes on degraded peatlands undergoing restoration as vegetation matures. The key hypotheses were that CO_2 uptake would increase as vegetation matured, particularly with the addition of *Sphagnum*, but that greater volumes of *E. angustifolium* would increase CH_4 emissions.

Carbon greenhouse gas fluxes (CO_2 and CH_4) were measured over a period of two years on field plots containing mature and immature vegetation: *E. angustifolium with* BeadaGel™ and *E. angustifolium*-only, and bare peat (i.e., five treatments).

Chapter 5: 'Analysis of surface peat chemistry and peat cores at carbon GHG flux trial plots' explores whether past site disturbance is demonstrated through peat chemistry, and whether there is a residual influence on current carbon GHG flux. The hypothesis was that degradation of the site had resulted in poor-quality peat, which exacerbated hydrological instability and influenced change in carbon GHG fluxes.

Chapter 6: 'Study synthesis' draws together elements from the previous chapters and proposes recommendations based on findings.

Chapter 2: Study into comparative photosynthesis rates of BeadaMoss® and wild-sourced *Sphagnum*

2.1 Introduction

Sphagnum is instrumental in bioengineering the cool, wet, acidic, low-nutrient conditions in northern peatbogs which leads to the formation of peat, through chemical processes and recalcitrant plant tissues (van Breemen, 1995; Verhoeven and Liefveld, 1997; Malmer *et al*., 2003; Rydin and Jeglum, 2013; Lindsay *et al*., 2014). Different species have developed a range of adaptive or phylogenetic traits to specific ecological stressors of light, shade and moisture (Rice *et al.*, 2008; Hájek *et al*., 2009; Bonnett *et al*., 2010; Laine *et al*., 2011), allowing them to outcompete vascular plants in the resulting hostile bog environment (Malmer *et al*., 2003; Rydin and Jeglum, 2013). Traits assigned to species in terms of their tolerance to a range of environmental factors, such as shade, pH, nutrient content and moisture (Ellenberg values) have been assigned to bryophytes, and values (from Hill *et al.*, 2007) for the species in this study are outlined in Table 2.1.

Table 2.1. Ellenberg values for the range of species studied.

Ellenberg Values	Light	Moisture	Reaction	Nitrogen
S. capillifolium	7	7	2	1
S. fallax	7	9	2	3
S. medium/divinum	8	8	1	1
S. palustre	7	8	3	2
S. papillosum	8	8	1	1
S. squarrosum	6	9	4	3

Light = 1 (deep shade) to 9 (full light)
Moisture = 1 (extreme dryness) to 12 (submerged plant)
Reaction = 1 (extreme acidity) to 9 (basic reaction/high pH)
Nitrogen = 1 (extremely infertile) to 9 (extremely rich fertility/pollution)
From Hill *et al.* (2007)

A wide-ranging review of global measurements found *Sphagnum* stem-length growth and photosynthetically active radiation (PAR) were strongly correlated, and the latter was a more important growth indicator than moisture levels, albeit for only two species assessed, *S. magellanicum* (probably *S. medium* or *S. divinum*) and *S. fuscum* (Loisel *et al.*,

2012). However, photosynthesis is constrained by moisture levels in bryophytes, as the plants are poikilohydric: too much moisture causes CO_2 diffusion and reduced carboxylation, and too little causes loss of cellular pressure and subsequently damages photosynthetic 'apparatus' (Hajek, 2014).

The *Sphagnum* genus occupies a wide range of peatland ecological niches, and *Sphagnum* species vary widely in their photosynthetic rates, but rates generally decline following the 'successional gradient' of species in bog development towards ombrotrophic conditions (Laine *et* al., 2011). Species with metabolic strategies such as high density and carotenoid concentration to tolerate drier, unshaded conditions, tend to have reduced rates of growth and photosynthesis (Rice *et al.*, 2008), and shade-adapted species tend to have high photosynthesis rates (Laing *et al*., 2014). Moreover, Hájek *et al*. (2009), in laboratory conditions and under a range of light intensities, found a clear rank of CO_2 uptake between species, with those sourced from shaded habitats tending to rank higher than those sourced from open habitats. The authors surmised that species from open habitats suffered persistent photodamage, which reduced photosynthetic capacity, despite photoprotective pigments such as sphagnorubin.

Additionally, low levels of nutrients, particularly nitrogen, can support the processes of photosynthesis in mosses, although higher levels can be toxic and promote shading from vascular plants (Bubier *et al*., 2007; Mazziotta *et al*., 2019). *Sphagna* utilize the same nutrient elements that all plants use for photosynthesis, respiration and growth, but absorb them directly into cells (Bragazza *et al*., 2004), as they have no vascular transportation system for uptake from the soil, and allocate them differently as nutrient resources are limited in an ombrotrophic bog system (Aldous, 2002; Rice *et al*., 2008).

To capture the range of photosynthetic response to light of diverse *Sphagnum* species, a decision on study parameters was needed. Haraguchi and Yamada (2011) found that optimum light levels for photosynthesis for a range of *Sphagnum s*pecies were between 300 and 500 µmol photons m^{-2} s^{-1} of PPFD (Photosynthetic Photon Flux Density). However, Rice *et al.* (2008) found that photoinhibition (to prevent high-light damage) in *Sphagnum* occurs at 800 µmol photons m^{-2} s^{-1} and Loisel *et al.* (2012) reported an optimum of 500 to 900 µmol photons m^{-2} s^{-1}. Hájek *et al*. (2009) found that the light

saturation point for all *Sphagna* they studied was similar at an average of 2124 ± 86 (SE) µmol photons m^{-2} s^{-1}, much higher than for other studies. Therefore, it seemed appropriate in this study to use 800 µmol photons m^{-2} s^{-1} as the highest light level.

A study into photosynthesis of different species of *Sphagnum* moss is pertinent to understanding production and, therefore, carbon sequestration in bogs (Loisel *et al*., 2012). However, for peatland sites undergoing restoration, wild sources of *Sphagnum*, a key species for bog restoration, are scarce and protected in the UK (Caporn *et al*., 2018) and therefore unavailable for harvesting and application to restoration sites. Therefore, BeadaMoss® *Sphagnum* has been developed to fill that gap in resources. Studies of BeadaMoss® *Sphagna* also present unique opportunities to compare the photosynthesis rates and growth potential of species based purely on their phylogenetic properties, as they have been cultured and grown under the same optimum light, moisture and nutrient levels, and they are at the same stage of development.

The aims of this chapter were to compare the photosynthesis and respiration rates, and examine phylogenetic traits, morphological differences, and chemical composition of six *Sphagnum* species of both tissue-cultured (BeadaMoss®) and wild-sourced (Wild) plants. This comparison is novel. Additionally, a greater understanding of the potential carbon sequestration of each BeadaMoss® species will help direct both product development and restoration efforts where these products are used.

The objectives were, firstly, to measure the CO_2 uptake (photosynthesis), emission (respiration) and species traits of samples of *S. capillifolium*, *S. fallax, S. medium/divinum, S. palustre, S. papillosum* and *S. squarrosum* in controlled conditions. These represent species from a broad environmental range (Table 1.1) and were readily available in BeadaMoss® greenhouses. Wild-sourced samples were taken from established naturally-occurring colonies in a range of peatland environments. Secondly, BeadaMoss® and wild-sourced samples of the same six species used for photosynthesis rate studies were examined under a microscope and measurements made of chlorocyst (cells containing chloroplasts) size and number of chloroplasts. Thirdly, the physical and chemical properties of samples used for photosynthesis measurements were analysed to assess whether levels of chemical elements within tissues of BeadaMoss® *Sphagnum*, which is a

product of horticultural processes, and wild-sourced *Sphagnum*, had a bearing on their capacity for photosynthesis.

These objectives tested the hypotheses that:

1) there is no difference in the rates of photosynthesis and respiration between BeadaMoss® and naturally-occurring *Sphagnum*;
2) BeadaMoss® and wild-sourced *Sphagnum* are morphologically similar, and so are likely to have a similar photosynthetic capacity;
3) BeadaMoss® *Sphagnum* is appropriate for use in peatland restoration projects to promote acrotelm development and CO_2 uptake;
4) BeadaMoss® and wild-sourced *Sphagnum* have a dissimilar chemical composition, likely to have a bearing on their photosynthesis and respiration rates.

2.2 Methods

2.2.1 Sphagnum photosynthesis and respiration

Small amounts (approximately 2 litres) of wild-sourced *Sphagnum* species were harvested in August 2017 from ombrotrophic mires and heaths in the north of England and Wales: *S. capillifolium, S. fallax,* and *S. palustre* from Chat Moss Remnants Site of Biological Importance (SBI) (53°27'53.0"N, 2°26'56.4"W), *S. medium/divinum* from Borth Bog (Cors Fochno) (52°30'18.7"N, 4°00'43.5"W) and *S. papillosum* from Ruabon Moor (53°00'11.5"N, 3°08'21.6"W), apart from *S. squarrosum*, from Alderley Edge (53°17'52.1"N, 2°12'18.0"W), a wooded valley mire flush.

The same species were harvested from BeadaMoss® greenhouses in June-July 2017 (see section 1.3.6 and 1.3.7 for background, development and species origin of BeadaMoss® material). BeadaMoss® *Sphagnum* is cultivated in controlled and optimum light, humidity and temperature conditions in greenhouses, and the samples used were grown from BeadaGel™ - tissue-cultured *Sphagnum* suspended in a hydrocolloidal gel, and applied directly onto the growing-media surface.

All *Sphagnum* was acclimated in a Fitotron growth chamber manufactured by Weiss Technik (previously Weiss Gallenkamp) for a minimum of 5 days, set to typical summer-time environmental conditions in the local area: 20˚C during the day (0600 - 2200 hrs), 12˚C during the night, day-time light intensity of 750-800 µmol m^{-2} s^{-1} (values calculated from mean Astley Moss Weather Station data, 2012 – 2015), 85% humidity. Humidity levels were difficult to maintain, but the *Sphagnum* was misted with rainwater as necessary to keep it well-hydrated.

Five samples of each species from each origin (i.e., five replicates) were cut from the 2L bulk amounts of *Sphagnum* at growing density and placed in a 5 cm diameter 3 cm high clear acrylic cylinder fitted with a mesh base to allow air circulation through the samples (examples in Figure 2.1), with the top surface of the sample level with the top of the cylinder. The cylinder was used as a cutting guide. An LGR™, Ultraportable Greenhouse Gas Analyser (UGGA), Model 915-0011 (Los Gatos, Research, Palo Alto, CA, USA) (LG) was fitted to 500 ml sealable clear-glass chamber via tubing through air-tight ports in the lid, and samples placed in the chamber for analysis (Figure 2.2). Change in CO_2 concentration within the chamber was measured over 2 minutes and a light response curve determined for each species starting from light intensities of 800 µmol photons m^{-2} s^{-1} to zero in increments of 50 µmol photons m^{-2} s^{-1} for each sample.

The samples were photographed prior to measurements and the number of capitula per sample counted later on a PC screen. Samples were weighed to check for water loss between measurements, and the chamber was closed between each light level measurement to reduce drying. The reduction in light transmission through the chamber (92%) was accounted for by increasing the light intensity in the cabinet accordingly for each light intensity measurement. The LG flow rate was 0.8 litres min^{-1} with space between gas inlet and outlet points (Figure 2.2); air was released into the chamber along a small pipe with holes along the length to encourage mixing (LG low flow rate threshold for analysis is ~0.35 litres min^{-1} [personal communication, Lewis John, LG Sales Representative]). After photosynthesis measurements, samples were dried overnight at 105 ^{o}C to obtain dry weight for calculations and chemical analysis.

Net photosynthesis or respiration rate was calculated (via data analysis tools in Microsoft Excel, 2019) from the rate of CO_2 depletion or increase and further expressed on the basis of surface area of the plant chamber (A_s) and of total plant dry weight (DW). Values are expressed using the leaf gas exchange sign convention whereby plant uptake of CO_2 from the atmosphere is expressed as positive and loss to the atmosphere is expressed as negative. The calculation to determine the rate of photosynthesis/CO_2 flux (adapted from Dossa *et al.*, 2015) is:

$$Photosynthesis/Respiration = \frac{\Delta CO2}{t} * \frac{PV}{RT} * \frac{1}{As} * \left(\frac{44*60*60}{1000}\right) \quad [1]$$

(P (atm) = atmospheric pressure; V (m^3) = chamber volume; R (L atm mol^{-1} K) = universal gas constant; T (K) = gas temperature in Kelvin; A_s (m^2) = sample surface area; 44 g mol^{-1} = molecular weight of CO_2); Photosynthesis/Respiration = g CO_2 m^{-2} h^{-1}.

S. capillifolium *S. fallax* *S. medium/divinum* *S. palustre* *S. papillosum* *S. squarrosum*

Figure 2.1. Examples of BeadaMoss® (top of pair) and wild-sourced *Sphagnum* species samples for analysis of photosynthesis rate, showing typical visual differences in pigmentation and form.

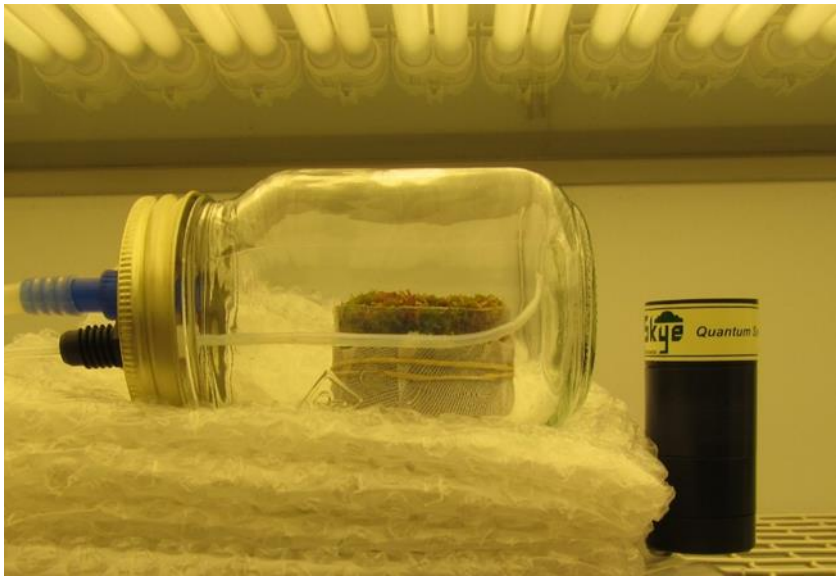


Figure 2.2. Glass chamber containing *Sphagnum* sample in the growth cabinet with PAR sensor.

2.2.2 Sphagnum samples chemical analysis

Sphagnum %N values were obtained using a LECO FP628 elemental analyser. A minimum of 0.05 g of dry *Sphagnum* was used per sample. Each sample used for photosynthesis measurements (n = 60) was analysed, plus 4 replicates of one species from each treatment (BeadaMoss® and Wild) to check for experimental error (total n = 68). The LECO analyser was calibrated on the first carousel with 4 samples of EDTA powder plus 2 further samples on each subsequent carousel (0.1503 ± 0.0003 g [mean amount ± SD] n = 8).

For other elements, samples were prepared for ICP-OES through acid and microwave digest, using HNO_3 S.G. 1.42 (> 68%) PrimerPlus-Trace analysis grade in a CEM Mars Xpress 5 Microwave, first putting microwave tubes through a cleaning cycle with 7 ml acid to 7 ml DI water, rinsing with deionised (DI) water and leaving to air-dry before use. Each sample of 0.25 ± 0.1 g was weighed into prepared dry tubes and 8 ml acid plus 2 ml DI water added, sufficient to digest samples; blank samples (10% of the sample number) were prepared in the same way. Samples were microwaved for one hour and then filtered through Whatmans No. 1. paper into conical flasks, first adding 5ml of DI water,

then making up to 25 ml volume. The solute was decanted into 30 ml universal tubes for ICP-OES analysis.

2.2.3 Sphagnum cell measurements and analysis

Small samples (several strands only) of *Sphagnum* for microscopic analysis were harvested from BeadaMoss® unheated greenhouses (daytime temperature ~20°C, personal communication, Neal Wright, BeadaMoss®) and from natural sources, and assessed over the following week during September 2019. Wild-sourced *Sphagnum* was from sites in the north of England: *S. fallax*, *S. medium/divinum* and *S. palustre* from Cadishead Moss (53°27'07.9"N, 2°27'18.9"W); *S. capillifolium* and *S. papillosum* from Astley Moss (53°28'32.2"N, 2°27'15.5"W), and *S. squarrosum* from Windy Bank Wood (53°28'15.3"N, 2°28'59.6"W). Divergent branch leaves just below the capitula are generally used for microscopic observation of *Sphagnum* (Smith, 2004; Laine *et al*., 2018). The cell structure changes across the leaf from proximal to distal ends and from edge to centre, and between convex and concave aspects (Atherton *et al*., 2010; Laine *et al*., 2018; previous personal observation). For standardisation of results in this study, leaves from branches immediately surrounding the capitulum (as indicated in Figure 2.3) were observed and measured centrally, on the concave aspect.

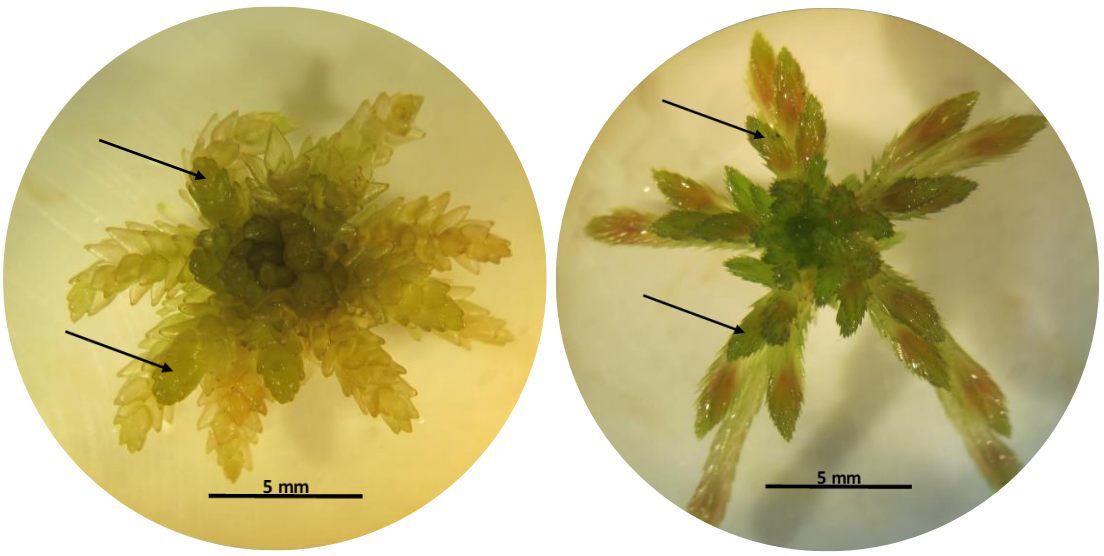


Figure 2.3. Examples of capitula used for microscopic study; arrows indicate typical branch selection.

Leaves from three branches from three capitula of each sample (i.e., 9 leaves) were removed onto a slide and photographed using a Brunel Eyecam Plus attached to a compound microscope at 1000X magnification; cell dimensions were measured after calibration at the same magnification. The collection of chlorocysts (cells containing chloroplasts) surrounding a hyalocyst (a dead, thin-walled and hollow cell with a water storage function) are generally made up of six segments of varying size, which are sometimes subdivided. The width (rather than length, because of sub-division of some cells) of six appropriate segments was measured centrally (Figure 2.4), and the number of green-pigmented cells (chloroplasts) counted in each. A mean value was calculated from each set of measurements for each leaf, and thus there were 9 values for chlorocyst width and for number of chloroplasts for each species. Where a chlorocyst was subdivided it was treated as a single entity, and only chloroplasts in the chlorocysts immediately surrounding a hyalocyst were included in measurements.

2.2.4 Data analysis

Data were prepared through Microsoft Excel (2019) and analysed statistically using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp and also PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer *et al*., 2001) where indicated. Data were tested for normality using Shapiro Wilk tests. Data for maximum photosynthesis (P_{max}), respiration rates, number of capitula per sample, fresh (FW) and dry weight (DW) density and also data for microscopic measurements of chlorocyst width and number of chloroplasts, were found to be normally distributed, and so parametric t-tests were used to test differences between *Sphagnum* types (BeadaMoss® and wild-sourced), and two-way ANOVA with post-hoc Tukey HSD to test differences between types for each species. Dependency of respiration rates on P_{max} rates was tested using linear regression. Data for chemical elements, were not normally distributed, and so non-parametric independent variable tests (Mann-Whitney U) were used to test differences in distribution between *Sphagnum* types (BeadaMoss® and wild-sourced). Associations between nutrient levels, P_{max} and respiration rates, and *Sphagnum* type and species, were tested with a correlation matrix through Principal Component Analysis using PAST software (Hammer *et al*., 2001).

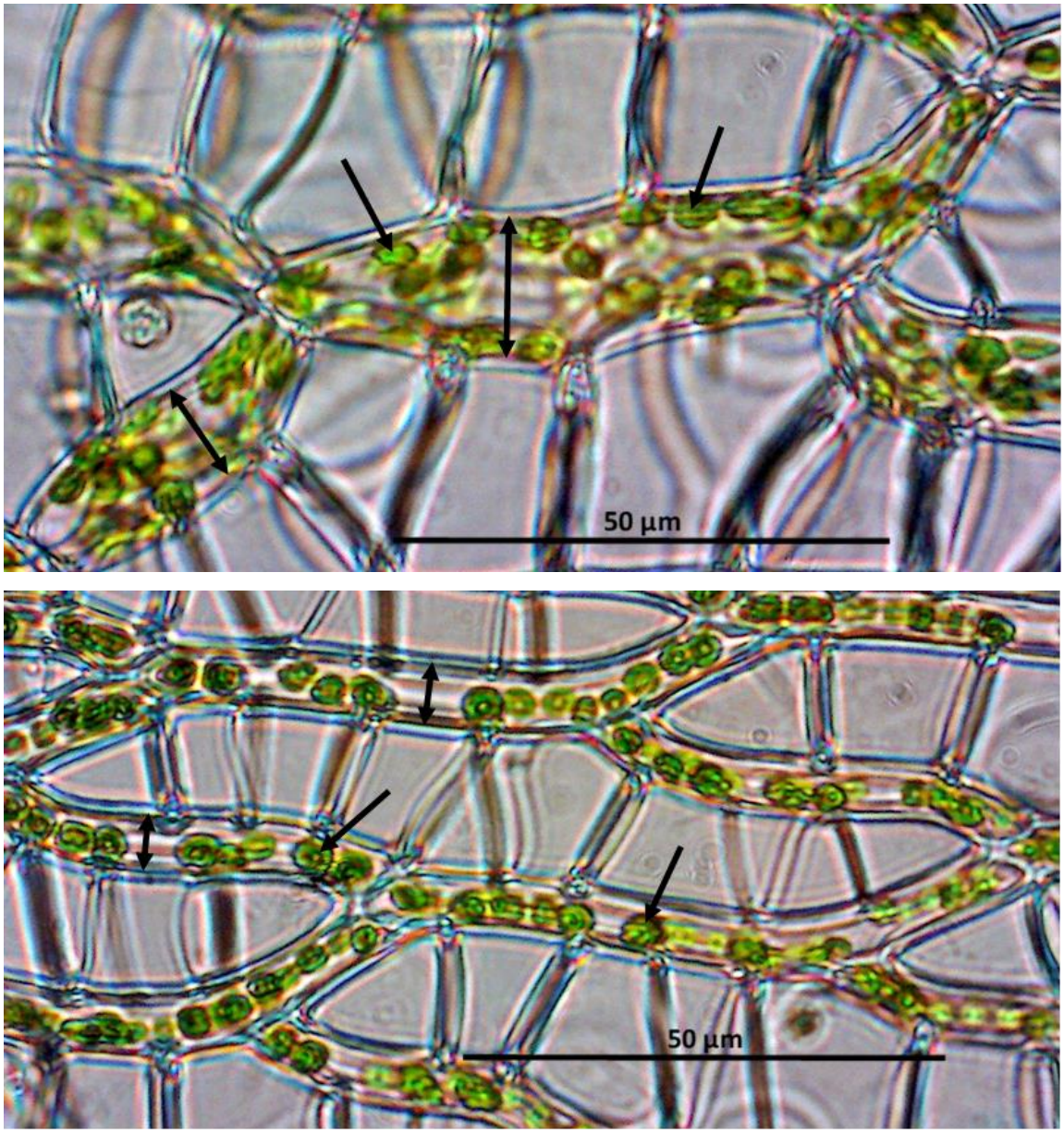


Figure 2.4. Examples of chlorocyst width measurement locations (double-ended arrows) and chloroplasts (single-arrowed) (*S. palustre* and *S. squarrosum*).

2.3 Results

2.3.1 Sphagnum photosynthesis and respiration

The response of Net Photosynthesis, (P_n) to changing light levels showed a similar pattern across all species in both BeadaMoss® and wild-sourced samples (Figure 2.5), reaching a maximum P_n (P_{max}) between 400 and 650, and 400 and 750 µmol $m^{-2}s^{-1}$ respectively (Tables 2.2 and 2.3) and either levelling or reducing thereafter. P_{max} rates on both an area and DW basis were significantly higher in BeadaMoss® than in wild-sourced samples overall (t = 7.312, p < 0.001, df = 58 and t = 8.647, p < 0.001, df = 58 respectively). Respiration rates were significantly higher in BeadaMoss® than in wild-sourced samples overall on a DW basis only (t = 5.816, p < 0.001, df = 58). P_{max} rates of each species were higher in BeadaMoss® than wild-sourced types on an area basis and particularly on a DW basis (significant differences from t-tests are indicated in Tables 2.2 and 2.3) (NS for *S. capillifolium* and *S. squarrosum* on an area basis), whereas respiration rates were similar on an area basis (although significantly higher for wild-sourced than BeadaMoss® *S. capillifolium*), but higher in most BeadaMoss® than wild-sourced types on a DW basis (not *S. fallax* or *S. palustre*). The P_{max} rates across BeadaMoss® samples were less variable than wild-sourced samples (coefficient of variation of 20.1% and 46.7% by area and 32.2% and 74.7% by DW respectively).

BeadaMoss® species are ranked from highest to lowest P_{max} in Table 2.2, but the ranking for wild-sourced species differs: *S. squarrosum > S. fallax > S. capillifolium >/< S. palustre > S. papillosum > S. medium/divinum* on an area and DW basis. The ranking of respiration rates of wild-sourced species also differs from that of BeadaMoss® species: *S. capillifolium > S. fallax > S. squarrosum > S. papillosum > S. palustre > S. medium/divinum* on an area basis, and *S. squarrosum > S. fallax > S. palustre > S. capillifolium > S. papillosum > S. medium/divinum* on a DW basis. BeadaMoss® and wild-sourced species with the highest (*S. squarrosum*) and lowest (*S. medium/divinum*) P_{max} and respiration rates were the same on a DW basis.

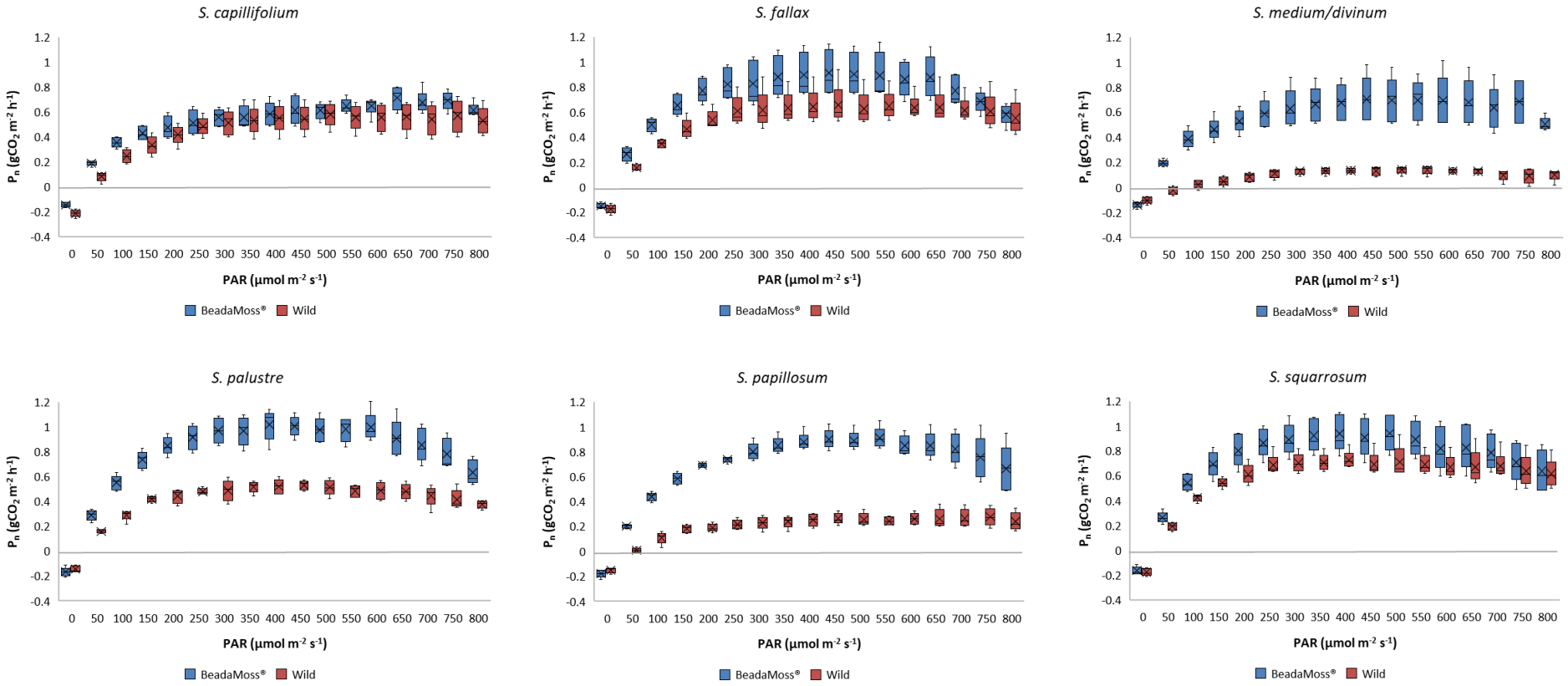


Figure 2.5. Comparison of BeadaMoss® and Wild *Sphagnum* photosynthetic response to a light intensity range from 0 to 800 µmol m^{-2} s^{-1} for each species. P_n = Net Photosynthesis. Crosses indicate the mean, lines indicate the median, and interquartile range is exclusive.

Table 2.2. P_{max} (with associated PAR level) and respiration rates of samples, expressed on an area basis (g CO_2 m^{-2} s^{-1}) ordered greatest to least P_{max} BeadaMoss® species, paired with wild-sourced equivalent. Significant differences (two-way ANOVA Tukey post-hoc tests) between each pair are indicated: *$p < 0.05$; **$p < 0.001$.

BeadaMoss®	PAR (µmol m^{-2} s^{-1})	P_{max} (g CO_2 m^{-2} h^{-1})	Respiration (g CO_2 m^{-2} h^{-1})	**Wild-sourced**	PAR (µmol m^{-2} s^{-1})	P_{max} (g CO_2 m^{-2} h^{-1})	Respiration (g CO_2 m^{-2} h^{-1})
S. palustre	400	1.02 ± 0.13 **	-0.17 ± 0.04	*S. palustre*	450	0.53 ± 0.04	-0.15 ± 0.02
S. squarrosum	500	0.94 ± 0.14	-0.16 ± 0.03	*S. squarrosum*	400	0.73 ± 0.07	-0.17 ± 0.03
S. fallax	450	0.91 ± 0.18 *	-0.15 ± 0.02	*S. fallax*	450	0.65 ± 0.16	-0.17 ± 0.03
S. papillosum	550	0.91 ± 0.08 **	-0.18 ± 0.03	*S. papillosum*	750	0.28 ± 0.07	-0.15 ± 0.02
S. capillifolium	650	0.71 ± 0.09	-0.15 ± 0.02 *	*S. capillifolium*	500	0.58 ± 0.09	-0.21 ± 0.03
S. medium/divinum	450	0.70 ± 0.18 **	-0.14 ± 0.02	*S. medium/divinum*	550	0.14 ± 0.03	-0.10 ± 0.03

Leaf gas exchange sign convention used (i.e. CO_2 uptake positive and CO_2 emission negative). Values are mean (n = 5) ± SD.

Table 2.3. P_{max} (with associated PAR level) and respiration rates of samples, expressed by dry weight (nmol CO_2 g^{-1} s^{-1}) ordered greatest to least P_{max} BeadaMoss® species, paired with wild-sourced equivalent. Significant differences (two-way ANOVA Tukey post-hoc tests) between each pair are indicated: *$p < 0.05$; **$p < 0.001$.

BeadaMoss®	PAR (µmol m^{-2} s^{-1})	P_{max} (nmol CO_2 g^{-1} s^{-1})	Respiration (nmol CO_2 g^{-1} s^{-1})	**Wild-sourced**	PAR (µmol m^{-2} s^{-1})	P_{max} (nmol CO_2 g^{-1} s^{-1})	Respiration (nmol CO_2 g^{-1} s^{-1})
S. squarrosum	500	46.94 ± 4.74 **	-8.15 ± 1.77 *	*S. squarrosum*	400	25.32 ± 3.75	-6.05 ± 1.11
S. palustre	400	37.69 ± 11.58 **	-5.88 ± 0.66	*S. palustre*	450	15.23 ± 1.00	-4.15 ± 0.60
S. papillosum	550	36.28 ± 3.65 **	-7.03 ± 0.47 **	*S. papillosum*	750	3.55 ± 1.27	-1.91 ± 0.49
S. fallax	450	32.54 ± 6.17 **	-5.42 ± 0.82	*S. fallax*	450	16.14 ± 1.25	-4.37 ± 0.72
S. capillifolium	650	24.12 ± 2.53 **	-4.97 ± 0.55 *	*S. capillifolium*	500	6.97 ± 1.64	-2.54 ± 0.31
S. medium/divinum	450	20.57 ± 5.51 **	-4.07 ± 0.78 **	*S. medium/divinum*	550	1.59 ± 0.42	-1.17 ± 0.29

Leaf gas exchange sign convention used (i.e. CO_2 uptake positive and CO_2 emission negative). Values are mean (n = 5) ± SD.

The ratio of P_{max} to respiration on area and DW basis was consistently higher in BeadaMoss® samples (5.51 and 5.58 respectively) than wild-sourced samples (3.03 and 3.41 respectively). However, there was a strong positive linear regression between P_{max} and respiration rates in wild-sourced samples ($R^2 = 0.46$, $p < 0.001$ by area; $R^2 = 0.90$, $p < 0.001$ by DW) which was not as evident in BeadaMoss® samples ($R^2 = 0.09$, p = NS by area; $R^2 = 0.48$, $p < 0.001$ by DW).

Weight (moisture) loss from samples during assessment through the range of light intensities was 6.9 ± 2.4% and 7.4 ± 2.9% for BeadaMoss® and wild-sourced respectively; minimum and maximum were 4.4% (*S. medium/divinum*) and 10.5% (*S. squarrosum*) in BeadaMoss® samples and 4.6% (*S. papillosum*) and 9.7% (*S. fallax*) in wild-sourced samples. Moisture content of samples at P_{max} ([Sample P_{max} fresh weight - sample dry weight] / sample dry weight x 100) was 2335 ± 420% (CV = 18%) (BeadaMoss®) and 1551 ± 320% (CV = 21%) (wild-sourced).

There were significantly more capitula per sample in BeadaMoss® than in Wild *Sphagnum* overall (mean ± SD = 41.8 ± 24.2 and 27.0 ± 18.7 respectively; $t = 2.635$, $p = 0.011$, $df = 58$) (Examples in Figure 2.1). Moreover, there were more capitula in BeadaMoss® than in Wild samples of each species, although (on two-way ANOVA post-hoc Tukey HSD) this was not significant for *S. fallax*, *S. medium/divinum* and *S. squarrosum* (*S. capillifolium* and *S. papillosum* $p < 0.001$; *S. palustre*: $p < 0.01$, $n = 10$).

2.3.2 Density

The FW and DW density were greater in wild-sourced than in BeadaMoss® samples (not *S. fallax* or *S. palustre* by FW) ($F = 22.3$ [FW], $F = 73.6$ [DW]; $p < 0.001$, $df = 11$ for both) (Figure 2.6). Differences in types were significant (ANOVA post-hoc Tukey HSD) for *S. capillifolium* (FW $p = 0.019$, DW $p < 0.001$) *S. medium/divinum* (FW $p = 0.003$, DW $p < 0.001$) and *S. papillosum* (FW and DW $p < 0.001$) [$n = 10$ throughout]. Within types, the density across BeadaMoss® samples was less variable than wild-sourced samples (coefficient of variation of 34.7% and 45.3% by FW and 25.0% and 44.5% by DW respectively) with greater density in *S. capillifolium, S. medium/divinum* and *S. papillosum*

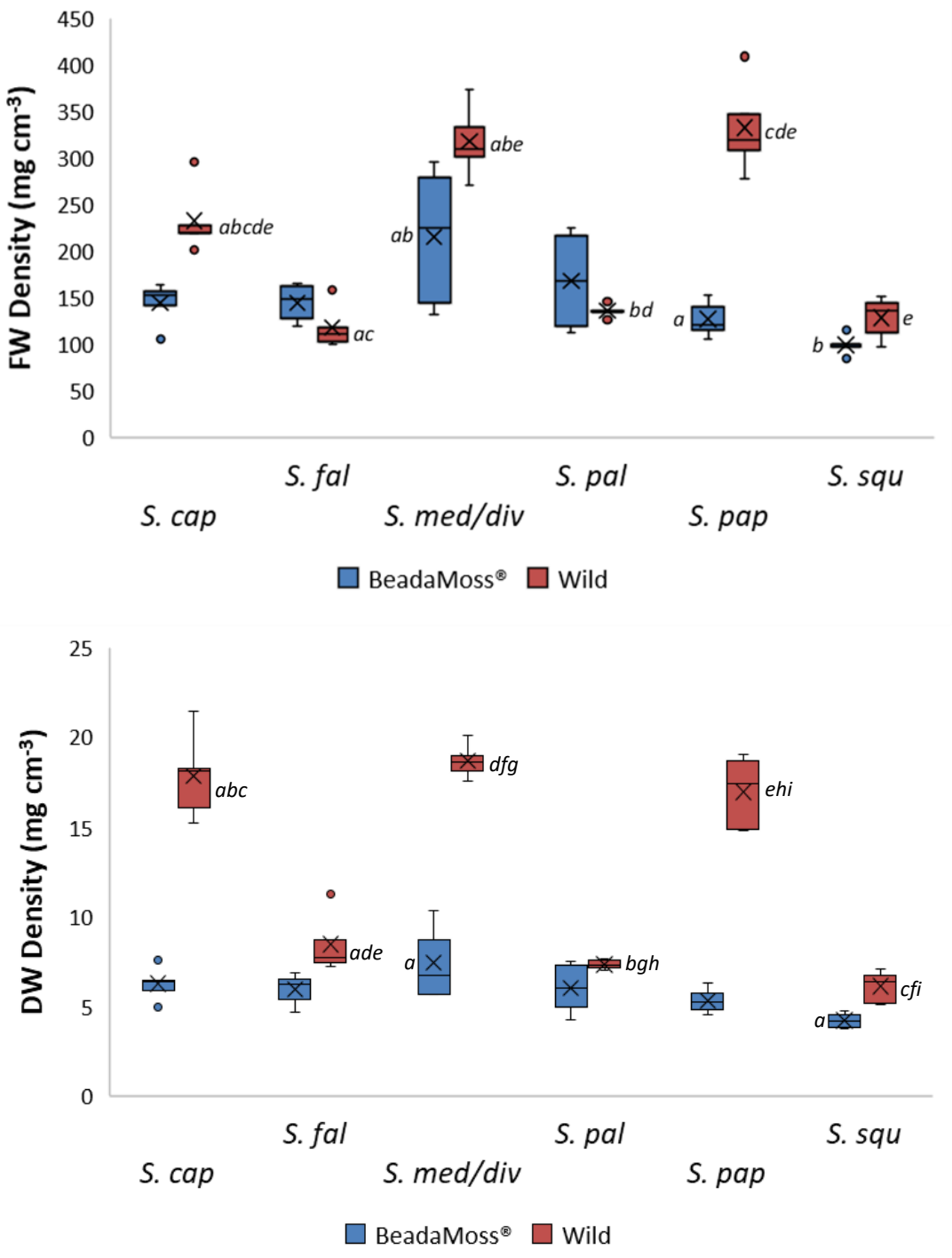


Figure 2.6. Comparison of BeadaMoss® and Wild *Sphagnum* density by fresh weight (FW) and dry weight (DW). Crosses indicate the mean, lines indicate the median, and interquartile range is inclusive. Shared letters within types (BeadaMoss® and Wild) indicate significant differences in density between species (one-way ANOVA post-hoc Tukey HSD).

than other species in wild-sourced samples. There was a statistically significant difference between species within both BeadaMoss® and wild-sourced samples as determined by one-way ANOVA (FW: $F = 4.71$, $p = 0.004$, $F = 44.65$, $p < 0.001$ and DW: $F = 3.99$, $p = 0.009$, $F = 70.37$, $p < 0.001$ respectively). One-way ANOVA post-hoc Tukey HSD statistically significant differences are shown on Figure 2.6.

There were significant negative relationships on linear regression between DW density and both P_{max} (DW) and respiration (DW) (P_{max} and respiration rates decreased as density increased) of BeadaMoss® and particularly wild-sourced samples (P_{max}: $R^2 = 0.573$ and 0.827 respectively; respiration: $R^2 = 0.503$ and 0.789 respectively; $p < 0.001$, $df = 29$ throughout).

2.3.3 Chemical elements

Macronutrient (Ca, K, Mg, N, P, S) levels were significantly higher in BeadaMoss® than in wild-sourced samples overall (Mann-Whitney U test: S: $p = 0.001$, all other elements: $p < 0.001$; $n = 60$ throughout) (Table 2.4). Nitrogen made up the largest proportion of the macronutrient content in all species throughout, and there was a high proportion of potassium in all BeadaMoss® samples and in most of the wild-sourced samples. There was a noticeable clustering and disassociation with macronutrients in wild-sourced samples (Figure 2.7a) apart from *S. squarrosum*, which was more closely associated with BeadaMoss® *Sphagnum* and with S. BeadaMoss® *Sphagnum* was correlated with macronutrients, although there were differences in the associations between macronutrients and species. N, P and K are closely associated, and there is a correlation between these macronutrients and BeadaMoss® *S. palustre, S. papillosum and S. squarrosum.* P_{max} was most closely correlated with N and K, and respiration with S.

There was a lower N:P and N:K ratio and a lower variation between species in BeadaMoss® than in and wild-sourced samples: N:P = 9.61 ± 1.24 (CV = 12.9%) and 31.18 ± 19.54 (CV = 62.7%) respectively, and N:K = 1.85 ± 0.09 (CV = 4.9%) and 3.13 ± 1.40 (CV = 44.8%) respectively. The N:P and N:K ratios in wild-sourced *S. squarrosum* (11.41 and 1.54), and to a lesser extent, *S. palustre* (17.51 and 2.05) were the most similar to their

BeadaMoss® equivalents, and those of wild-sourced *S. medium/divinum* (64.59 and 5.24) and *S. papillosum* (42.56 and 3.86) the most dissimilar.

Levels of trace elements Al, Fe, Mn, Na, Ni and Pb were higher in wild-sourced than BeadaMoss® samples overall (Al, Fe, Pb: $p < 0.001$; Mn: $p = 0.01$; Na and Ni: p NS; $n = 60$ throughout) (Table 2.5) with the highest levels of Al, Fe, Mn and Na in *S. papillosum*. Levels of micronutrients Cu and Zn were higher in BeadaMoss® than wild-sourced samples overall ($p < 0.001$; $n = 60$) (although not in *S. squarrosum*). Na made up the largest proportion of the micronutrient and trace element content in all samples. There was a clustering and association with Cu and Zn in BeadaMoss® samples together with wild-sourced *S. squarrosum* (Figure 2.7b). Other samples of wild-sourced *Sphagnum* were only loosely grouped by species and more closely correlated with micronutrients other than Cu and Zn. Cu and Zn were closely associated, as were Fe and Pb, and Mn and Na. *S. papillosum* appeared to have a strong correlation with Na and Al, and *S. capillifolium, S. medium/divinum* and *S. fallax* with Fe and Pb. P_{max} and respiration had a negative correlation with trace elements and a weak positive correlation with micronutrients Cu and Zn.

2.3.4 Microscopic analysis

There were significant differences between the groups of species by type (BeadaMoss® or wild-sourced) in both chlorocyst width and number of chloroplasts ($F = 49.9$, $F = 33.7$ respectively; $p < 0.001$, $df = 11$ for both). There were no significant differences (ANOVA post-hoc Tukey HSD) in chlorocyst width between BeadaMoss® and wild-sourced species (Table 2.6) except for *S. squarrosum* (Wild > BeadaMoss®). There were more chloroplasts in BeadaMoss® compared to wild-sourced species in all but *S. squarrosum* (Table 2.6) and differences were significant (ANOVA post-hoc Tukey HSD) for *S. capillifolium, S. palustre* and *S. papillosum* . The widest chlorocysts were recorded in *S. palustre* (BeadaMoss® and wild-sourced samples), and the greatest chloroplast numbers were found in *S. palustre* and *S. papillosum* (BeadaMoss®) and *S. palustre* (wild-sourced). Morphological differences between BeadaMoss® and wild-sourced samples are limited to reduced colour expression (*S. capillifolium* and *S. medium/divinum*) and maturity (*S. papillosum* cell papillae) in BeadaMoss® *Sphagnum* (Figure 2.8).

Table 2.4. Macronutrient content of *Sphagnum* samples (values in mg g^{-1} DM ± SD)

Sphagnum origin and species	Ca	K	Mg	N	P	S
BeadaMoss® *S. capillifolium*	4.30 ± 0.38	11.58 ± 0.52	1.51 ± 0.08	21.08 ± 0.72	2.90 ± 0.16	0.68 ± 0.03
Wild-sourced *S. capillifolium*	1.74 ± 0.27	3.32 ± 0.56	0.65 ± 0.13	12.63 ± 1.43	0.44 ± 0.09	0.69 ± 0.08
BeadaMoss® *S. fallax*	4.53 ± 1.17	10.25 ± 1.30	1.19 ± 0.19	19.68 ± 1.71	1.84 ± 0.27	0.59 ± 0.08
Wild-sourced *S. fallax*	0.84 ± 0.29	5.34 ± 0.51	0.39 ± 0.04	12.28 ± 2.63	0.55 ± 0.07	0.51 ± 0.06
BeadaMoss® *S. medium/divinum*	3.48 ± 0.49	10.32 ± 1.21	1.06 ± 0.11	20.41 ± 1.43	2.09 ± 0.32	0.57 ± 0.07
Wild-sourced *S. medium/divinum*	1.31 ± 0.33	2.05 ± 0.49	0.70 ± 0.09	10.77 ± 1.35	0.17 ± 0.01	0.49 ± 0.04
BeadaMoss® *S. palustre*	4.47 ± 0.58	15.99 ± 1.51	1.55 ± 0.14	29.27 ± 0.78	2.82 ± 0.17	0.92 ± 0.10
Wild-sourced *S. palustre*	1.34 ± 0.11	6.80 ± 0.92	0.72 ± 0.11	13.94 ± 2.33	0.80 ± 0.08	0.52 ± 0.03
BeadaMoss® *S. papillosum*	3.14 ± 0.28	15.40 ± 1.28	1.26 ± 0.06	28.69 ± 2.14	2.80 ± 0.18	0.85 ± 0.07
Wild-sourced *S. papillosum*	1.60 ± 0.37	2.75 ± 0.36	0.58 ± 0.05	10.63 ± 1.21	0.25 ± 0.06	0.53 ± 0.04
BeadaMoss® *S. squarrosum*	2.82 ± 0.20	15.55 ± 1.43	1.19 ± 0.13	26.66 ± 3.11	2.86 ± 0.33	0.90 ± 0.12
Wild-sourced *S. squarrosum*	1.21 ± 0.19	13.61 ± 0.75	0.90 ± 0.07	20.90 ± 2.05	1.83 ± 0.16	0.90 ± 0.08

S. capillifolium, S. fallax, S. palustre - Chat Moss Remnant SBI; *S. medium/divinum* - Borth Bog; *S. papillosum* - Ruabon Moor; *S. squarrosum* - Alderley Edge.

Table 2.5. Micronutrient and trace element content of *Sphagnum* samples (values in μg g^{-1} DM ± SD)

Sphagnum origin and species	Al	Cu	Fe	Mn	Na	Ni	Pb	Zn
BeadaMoss® *S. capillifolium*	9.75 ± 0.38	5.88 ± 0.50	52.74 ± 5.10	39.12 ± 2.37	337.99 ± 35.03	0.15 ± 0.46	0.12 ± 0.03	47.92 ± 9.82
Wild-sourced *S. capillifolium*	91.66 ± 9.86	4.48 ± 0.87	143.67 ± 12.63	64.30 ± 12.54	470.65 ± 90.91	0.60 ± 0.71	2.27 ± 0.26	24.89 ± 7.55
BeadaMoss® *S. fallax*	15.58 ± 2.53	5.65 ± 0.78	35.44 ± 3.99	28.21 ± 5.38	505.29 ± 120.34	0.33 ± 0.37	0.25 ± 0.04	66.08 ± 17.08
Wild-sourced *S. fallax*	83.05 ± 10.78	2.68 ± 0.64	198.86 ± 21.32	19.32 ± 2.95	256.82 ± 29.67	0.18 ± 0.13	1.34 ± 0.50	7.81 ± 2.19
BeadaMoss® *S. medium/divinum*	18.20 ± 5.43	7.43 ± 0.89	47.35 ± 4.18	27.95 ± 3.62	430.03 ± 76.53	0.89 ± 0.94	0.33 ± 0.09	76.96 ± 8.94
Wild-sourced *S. medium/divinum*	62.51 ± 12.35	3.29 ± 0.66	90.15 ± 17.61	29.50 ± 13.02	899.72 ± 195.32	0.32 ± 0.24	0.70 ± 0.06	10.74 ± 2.74
BeadaMoss® *S. palustre*	13.32 ± 3.32	8.87 ± 0.62	66.89 ± 3.66	35.81 ± 4.45	497.86 ± 110.86	0.14 ± 0.22	0.17 ± 0.03	57.57 ± 5.83
Wild-sourced *S. palustre*	36.95 ± 4.80	4.68 ± 0.89	46.62 ± 6.59	89.78 ± 9.53	372.11 ± 51.82	0.54 ± 0.69	0.63 ± 0.14	18.53 ± 4.28
BeadaMoss® *S. papillosum*	11.20 ± 2.65	7.79 ± 0.90	50.42 ± 3.84	29.56 ± 2.01	360.70 ± 55.17	0.34 ± 0.49	0.24 ± 0.03	38.47 ± 8.75
Wild-sourced *S. papillosum*	303.86 ± 268.44	3.72 ± 1.06	245.47 ± 175.15	171.48 ± 102.64	1158.98 ± 387.53	1.08 ± 0.34	1.65 ± 0.88	16.92 ± 8.92
BeadaMoss® *S. squarrosum*	7.87 ± 1.44	6.29 ± 1.65	83.87 ± 83.46	28.30 ± 2.71	303.77 ± 39.22	0 ± 0.07	0.20 ± 0.15	40.44 ± 5.45
Wild-sourced *S. squarrosum*	35.17 ± 3.67	6.33 ± 1.01	52.79 ± 5.91	92.32 ± 11.15	448.22 ± 97.71	0.24 ± 0.10	0.70 ± 0.10	67.41 ± 6.03

S. capillifolium, S. fallax, S. palustre - Chat Moss Remnant SBI; *S. medium/divinum* - Borth Bog; *S. papillosum* - Ruabon Moor; *S. squarrosum* - Alderley Edge.

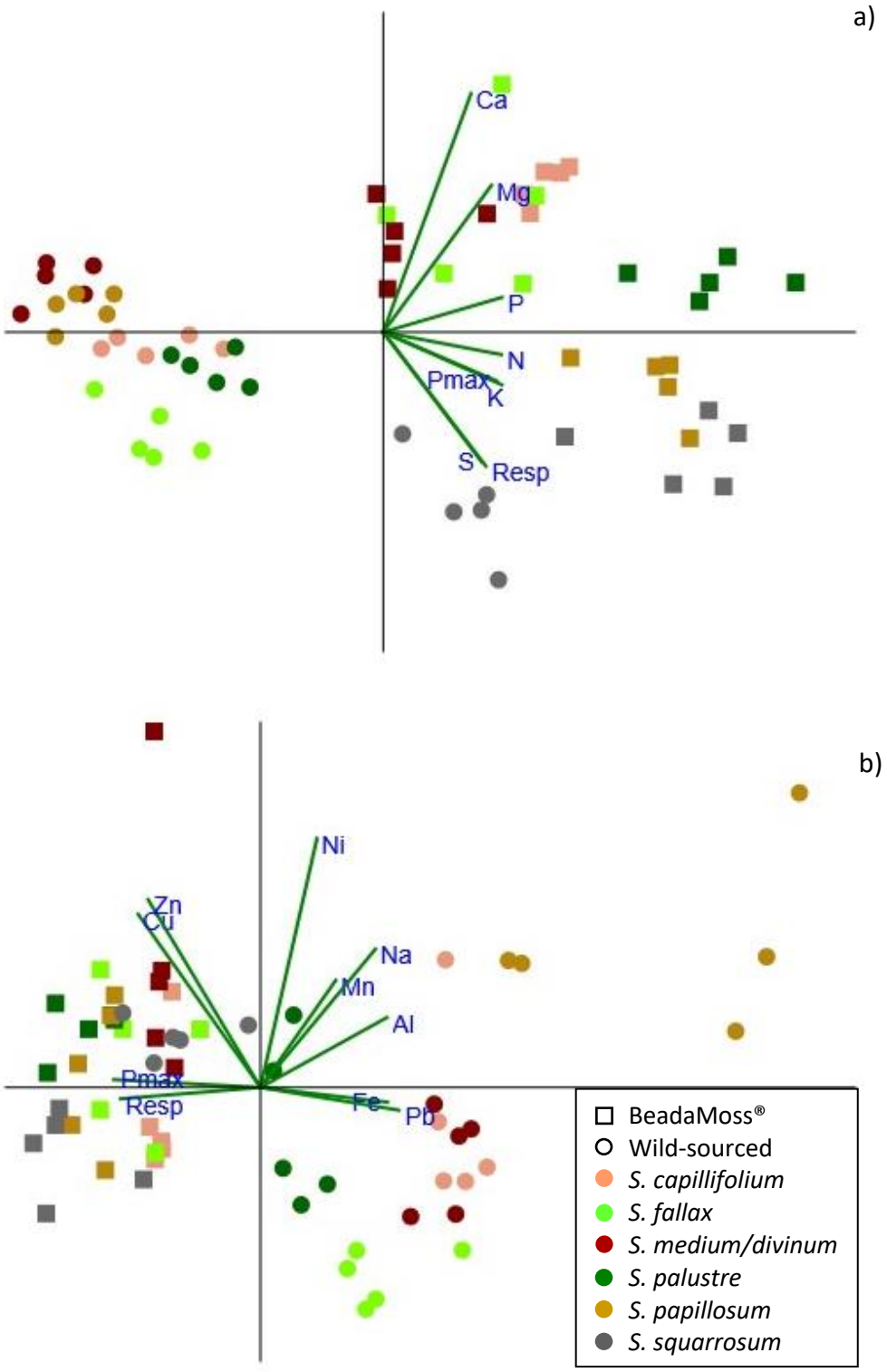


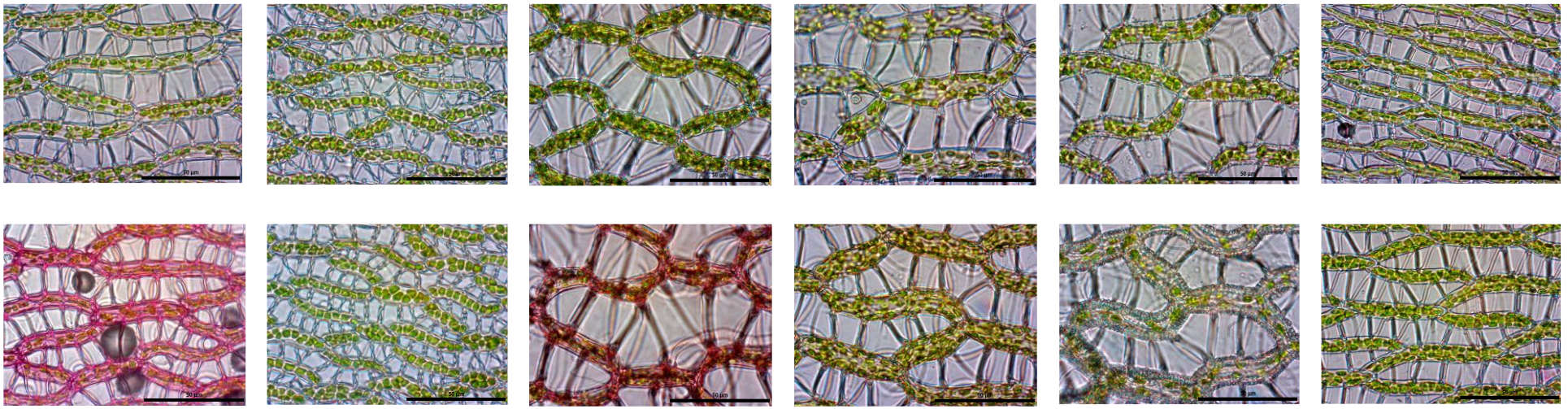
Figure 2.7. Principal component analysis (correlation matrix) of macronutrient (a) and micronutrient and trace element (b) content of *Sphagnum* samples with P_{max} and respiration by dry weight.

Table 2.6. Comparison between BeadaMoss® and wild-sourced *Sphagnum* samples of microscopic features: mean chlorocyst (cell) width (µm) and mean number of chloroplasts per chlorocyst; mean values (n = 9) ± SD; significant differences (ANOVA Tukey post-hoc HSD) between each pair are indicated: *p < 0.05; **p < 0.001.

Species	BeadaMoss® cell width (µm)	Wild cell width (µm)	BeadaMoss® chloroplast No.	Wild chloroplast No.
S. capillifolium	7.14 ± 0.91	6.65 ± 0.65	16.6 ± 2.7	10.6 ± 1.1 *
S. fallax	7.14 ± 0.83	6.96 ± 0.39	11.1 ± 2.7	7.1 ± 1.7
S. medium/divinum	9.86 ± 0.72	10.10 ± 1.36	16.7 ± 2.6	13.1 ± 2.9
S. palustre	12.37 ± 0.93	11.05 ± 1.24	24.5 ± 3.7	19.8 ± 4.3 *
S. papillosum	9.88 ± 0.59	9.84 ± 0.31	25.6 ± 3.9	12.2 ± 2.2 **
S. squarrosum	5.65 ± 0.59	7.30 ± 1.36 *	11.9 ± 2.4	15.1 ± 3.1

2.4 Discussion

This study found the maximum photosynthesis (P_{max}) and respiration rates measured on a range of *Sphagnum* species were broadly in line with the literature (Table 2.7), although rates were more variable across wild-sourced than BeadaMoss® species, and all were typically at the low end of the range for that of vascular plants. Light saturation was between 400 and 650 (BeadaMoss®), and 400 and 750 (wild-sourced) µmol m^{-2} s^{-1} (Haraguchi and Yamada [2011] stated 300 to 500 µmol m^{-2} s^{-1}; Rice *et al.* [2008] stated a maximum of 800 µmol m^{-2} s^{-1} and Loisel *et al.* [2012] reported 500 to 900 µmol m^{-2} s^{-1}). The wide range of P_{max} rates across samples in this study was likely due to the diversity of *Sphagnum* species and sources: BeadaMoss® species grown in BeadaMoss® company greenhouses (although original source material was from different sites); wild-sourced species as previously stated, each with a range of nutrient and shade regimes. It is notable that *S. medium/divinum* P_{max} rates were higher in shaded than open sites in a study by Bengtsson *et al*. (2016) (Table 2.7). This not only demonstrates the plasticity of this species in its adaptation to a range of environmental conditions, but highlights that a shaded environment is likely to promote moisture retention in *Sphagnum* due to reduced evaporative effects of wind and heat, which subsequently supports photosynthesis.



S. capillifolium *S. fallax* *S. medium/divinum* *S. palustre* *S. papillosum* *S. squarrosum*

Figure 2.8. Examples of BeadaMoss® (top of pair) and wild-sourced *Sphagnum* samples at 1000x magnification. Scale bar = 50 µm.

Table 2.7. Comparison of maximum photosynthesis (P_{max}) and respiration rates between samples in this study and values in the literature.

Authors	This study wild-sourced *Sphagnum*		This study BeadaMoss® *Sphagnum*		Rice *et al* ., 2008		Haraguchi and Yamada, 2011	Kangas *et al* ., 2014		Bengtsson *et al* ., 2016	Laine *et al* ., 2011		Reich *et al* ., 1997	
Sphagnum species	P_{max}	Resp	P_{max}	Resp	P_{max}	Resp	P_{max}	P_{max}	Resp	P_{max}	P_{max}	Resp	P_{max}	Resp
S. capillifolium (S)	6.97	-2.54	25.42	-4.97						15.78				
S. fallax (R)	16.14	-4.37	33.29	-5.42	13.90	-5.67	21.00			20.52				
S. girgensohnii (C)								42.49	-5.25	34.72				
S. medium/divinum (S) (open)	1.59	-1.17	21.15	-4.07	11.70	-4.63		35.04	-5.28	22.10				
S. medium/divinum (S) (heavy shade)										37.88				
S. palustre (C S)	15.23	-4.15	39.69	-5.88			20.00							
S. papillosum (S)	3.55	-1.91	36.85	-7.03			35.00			22.10				
S. riparum (R)								54.92	-10.39					
S. squarrosum (C)	25.32	-6.05	47.42	-8.15	15.40	-6.78								
S. tenellum (R)										37.88				
Wide range of *Sphagnum spp* .											22.44	-4.99		
Vascular leaves													21 to 289	-4.0 to -35.2

Leaf gas exchange sign convention used (i.e. CO_2 uptake positive and CO_2 emission negative); P_{max} and Respiration (nmol CO_2 g^{-1} s^{-1}); Bengtsson *et al* ., 2016 values estimated from graphs; C = competitive; R = ruderal; S = stress-tolerant (from Kangas *et al* ., 2014 [sensu Grime, 1977]); *S. magellanicum* reported assumed to be *S. medium/divinum* .

Literature sources (Rice *et al.,* 2008*;* Hájek *et al*., 2009; Bengtsson *et al*., 2016) suggest a rank in species photosynthesis rates, corresponding generally to the accepted phylogenetic order of growth rates and a gradient in production depending on light, moisture and nutrient levels (Kangas *et al*., 2014). Highly productive *Sphagna* tend to be competitive and ruderal (Grime, 1977) species of hollows (such as *S. squarrosum*, *S. fallax* and *S. fimbriatum*), thriving in shaded, high moisture and nutrient environments, adopting an open habit (Hájek *et al*., 2009), allowing more of the plant access to light for greater photosynthesis and thus faster growth rates (Krebs *et al*., 2016). These species also tend to be green, likely due to a high chlorophyll content (Hájek *et al*., 2009) with strong shoot growth and large capitula (Laing *et al*., 2014). *Sphagna* with lower productivity tend to be stress-adapted species (such as *S. capillifolium*, *S. medium/divinum* and *S. papillosum*), which grow in open, ombrotrophic bogs, subject to high light intensity, occasional drought, and low nutrient levels (Bonnett *et al*., 2010; Laine *et al*., 2011), where short, dense growth forms for greater acquisition and retention of moisture (Hájek *et al*., 2009; Loisel *et al*., 2012) and photoinhibition become more important to survival than the capacity for rapid growth (Rice *et al.*, 2008; Loisel *et al*., 2012). Other more generalist species (such as *S. palustre*, and some other loose hummock-forming species) thrive at points along this gradient. There are few *Sphagna* in unshaded, dry sites. Thus, photosynthesis rates are driven by moisture, light and nutrient levels, and different phylogenetic traits, as described above, give *Sphagnum* species competitive advantages to thrive in particular habitats (Hájek, 2014).

Samples of BeadaMoss® *Sphagnum* had a greater response to increasing light levels and greater P_{max} rates than those sourced from the wild, which is contrary to hypothesis 1) that there is no difference in the rates of photosynthesis and respiration between BeadaMoss® and naturally-occurring *Sphagnum*. This is perhaps because the BeadaMoss® samples had not yet developed photo-inhibitive adaptations to light stress in a shaded commercial greenhouse. However, in both BeadaMoss® and wild-sourced samples on a dry weight basis, the competitive, shade species, *S. squarrosum* had the highest rates of P_{max}, and respiration and the lowest density, and the stress-adapted species, *S. medium/divinum* had the lowest rates of P_{max} and respiration, and the highest

density throughout, demonstrating some parity in species behaviour, despite the tissue-culture process.

P_{max} and respiration rates were positively related throughout on a DW basis, most strongly in wild-sourced species suggesting this more mature, natural material may have reached an equilibrium, and reflecting the more even photosynthetic response of BeadaMoss® species to light. The ratio of P_{max} to respiration was consistently higher in BeadaMoss® than wild-sourced samples, despite generally higher BeadaMoss® respiration rates, which appears to show a greater capacity for net photosynthesis in BeadaMoss® plants. This is worth exploring as it may benefit the carbon balance of bogs being restored with tissue-cultured *Sphagnum*, at least in the early stages of establishment, although it is not clear whether the higher rates of photosynthesis in BeadaMoss® *Sphagnum* seen in this study will continue as the material is introduced to a natural environment. However, the material appears to outcompete wild-sourced *Sphagnum* in terms of ground cover in upland trials in Northern England (Crouch, 2018).

Hájek (2014) suggested the water content (WC) of 'well-hydrated' *Sphagnum* is 1500 to 3000% and both BeadaMoss® and wild-sourced samples in this study were within this range. Regimes for storage, hydration, acclimation and analysis of samples were standardised, but wild-sourced samples were at the lower end of the range, and 66% of the WC of BeadaMoss® samples. Perhaps the wild-sourced samples needed longer than a week to fully re-hydrate after sourcing during summer months. McNeil and Waddington (2003) suggest that *Sphagnum* photosynthesis rates may take 20 days to recover from the effects of drying. However, the loss of moisture by samples during analysis aligned approximately with species' water-retention strategies throughout, albeit more closely in wild-sourced species: drought-adapted species lost less moisture during sampling than hollow-adapted species.

Differences in P_{max} between BeadaMoss® and wild-sourced samples were particularly apparent in species from Section *Sphagnum*: *S. medium/divinum, S, palustre* and *S. papillosum*, where photosynthetic activity was noticeably lower in wild-sourced than BeadaMoss® samples. The difference between P_{max} rates of BeadaMoss® and wild-sourced samples of *S. capillifolium* was not so marked as other species. This species is

adapted to a range of peatland habitats and ecological niches, and has tolerance to shade (Bonnett *et al*., 2010). Wild-sourced, shade-tolerant species, *S. squarrosum, S. fallax* and *S. palustre*, had the highest P_{max} levels at the lowest light levels, on a dry weight basis, which concurs with findings by Rice *et al.* (2008) and Hájek *et al*. (2009).

The DW density of wild-sourced *Sphagnum* for each species studied was significantly greater than that of BeadaMoss® species, which were established under favourable light and moisture regimes, and were also in the early stages of rapid, linear growth (Laine *et al*., 2011). There was also a relationship between increasing density and declining P_{max} in wild-sourced *Sphagnum* samples but not in BeadaMoss® samples. In wild-sourced samples, there were two distinct groups by dry weight density*: S. fallax, S. palustre*, *S. squarrosum*, and *S. capillifolium, S. papillosum*, *S. medium/divinum*, showing obvious differences between shade-adapted, moisture-dependent species, and light-adapted, moisture-retaining species, which are only fully expressed in the natural environment.

BeadaMoss® *Sphagnum* species did not appear to be morphologically different to those sourced from the wild, although some characteristics, such as *S. papillosum* cell papillae, were less developed in BeadaMoss® samples, and there was less colour expression in BeadaMoss® samples (Figure 2.8). Chlorocyst size was not significantly different despite the relative immaturity of BeadaMoss® samples, apart from *S. squarrosum* species where those of wild-sourced samples were significantly larger than BeadaMoss® samples and the number of chloroplasts per cell was also greater. *S. squarrosum* samples were sourced from a nutrient-rich, shaded environment, beneficial to continued upward growth; greater cell size and number of chloroplasts were likely based on maturity and optimum growing conditions. In all other species, there was a significantly greater number of chloroplasts in BeadaMoss® than in wild-sourced samples, apart from *S. palustre*, where the difference was not statistically significant. This is consistent with BeadaMoss® plants being in the early stages of rapid growth (Laine *et al.*, 2011) but also not exposed to conditions of high light intensity and low moisture, and so they were perhaps acting more like shade plants (Marschall and Proctor, 2004). Therefore, hypothesis 2) that BeadaMoss® and wild-sourced *Sphagnum* are morphologically similar, and so are likely to have a similar photosynthetic capacity is only partially proved, as similar morphology did not lead to similar rates of photosynthesis. Additionally,

hypothesis 3) that BeadaMoss® *Sphagnum* is appropriate for use in peatland restoration projects to promote acrotelm development and CO_2 uptake, is supported in terms of adaptive and morphological traits tending to be similar in BeadaMoss® and wild-sourced *Sphagnum*, but not quite supported in terms of CO_2 uptake, as although BeadaMoss® materials in this study had higher net photosynthesis rates, performance after transfer to the field was not tested, although the potential is promising.

Chemical analysis of samples allowed further examination of difference in photosynthesis rates. Elements which support the processes of photosynthesis and growth can be separated into those required in large amounts for plant growth (macronutrients – principally N, P, and K, and also Ca, Mg and S) and those required in small amounts (micronutrients) (Barker and Pilbeam, 2007; Marschner, 2012). *Sphagnum* is reported to absorb nutrients rapidly and directly into plant tissue (Bragazza *et al*., 2004; Fritz *et al*., 2014) and store them efficiently, making them unavailable to others plants and thus engineering a low-nutrient environment (Malmer *et al*., 2003).

BeadaMoss® samples contained significantly higher levels of macronutrients, essential for plant photosynthesis and growth, than wild-sourced samples, and as CO_2 uptake and emission rates were also higher in BeadaMoss® *Sphagnum* it appears that hypothesis 4) BeadaMoss® and wild-sourced *Sphagnum* have a dissimilar chemical composition, likely to have a bearing on their photosynthesis and respiration rates, was supported. Higher levels of macronutrients in BeadaMoss® *Sphagnum* may be explained through standard horticultural processes of nutrient application associated with BeadaMoss® production. Conversely, wild-sourced *Sphagnum* had higher levels than BeadaMoss® of micronutrients, which are a smaller component of plant nutritional requirements but also support plant health. Amounts of micronutrients may have become diluted in BeadaMoss® plants due to their rapid growth rate. Higher levels of elements considered detrimental to plant health (such as Al, Fe and Pb) in some wild-sourced species may reflect the capability of bryophytes to absorb certain levels of pollution and act as bioindicators (Bragazza *et al*., 2004; Blagnytė and Paliulis, 2010) for example, metal pollution on Ruabon Moor (Pilkington *et al.*, 2005), the wild-source of *S. papillosum*.

The three main macronutrients associated with plant growth and photosynthesis, N, P and K, were strongly positively associated with P_{max} overall in this study, and reflected species' differences in macronutrient uptake and P_{max} rates. For example, wild-sourced species with the highest content of NPK were *S. squarrosum* and *S. palustre,* and those with the lowest content were *S. papillosum* and *S. medium/divinum*, which corresponded with P_{max} rates for those species.

In vascular plants, there appears to be a strong positive relationship between both net photosynthesis and dark respiration and leaf N content in a wide range of global biomes (Reich *et al*., 1997). Typically, mosses translocate nutrients into their tissues gradually over the growing season (Chapin *et al*., 1980) and the N:P and N:K ratios remain balanced (Sterner and Elser, 2002). Granath *et al*. (2012) and Mazziotta *et al*. (2019) found a positive relationship between N concentration and photosynthesis rates in *Sphagnum* (as in this study). However, *Sphagnum* growth reportedly improves with increasing levels of N until a 'critical concentration' is reached, at which there is no further promotion of plant growth despite increasing levels of N in tissues (Barker and Pilbeam, 2007). Saturated N levels limit further P- and K-accumulation, and so growth is P- and K-limited (Aerts *et al*., 1992; Lamers *et al*., 2000; Bragazza *et al*., 2004). Excess N is also leached into the environment, promoting the growth of vascular plants which may outcompete *Sphagnum* in the field (Lamers *et al*., 2000; Berandse *et al*., 2001; Bubier *et al*., 2007).

Wang and Moore (2014) reported averages of N = 9, P = 0.55, K = 7.5 mg g^{-1} DW content for 'hummock' *Sphagna* (*S. capillifolium* and *S. medium/divinum*) at the Mer Bleue ombrotrophic bog in Canada. Bragazza *et al*. (2004) had similar values of N = 8.2 and 9.2, P = 0.41 and 0.44, K = 4 and 4.44 mg g^{-1} DW for hummock and lawn species respectively in European mires, reported to have higher N deposition than Canadian mires. NPK levels of wild-sourced species in this study had higher N values but were otherwise similar to European values: N = 12.0 ± 2.13, P = 0.44 ± 0.24, K = 4.05 ± 1.87 mg g^{-1} DW. Wild-sourced *S. squarrosum*, however, had a higher NPK content (N = 20.9 ± 2.05, P = 1.83 ± 0.16, K = 13.6 ± 0.75 mg g^{-1} DW) and, as the sample was sourced from wet woodland, suggests that nutrients may continue to accumulate in fast-growing *Sphagnum* in conditions of shade (Ma *et al*., 2015) and optimum moisture (McNeil and Waddington, 2003).

All BeadaMoss® species had a high NPK content (N = 24.2 ± 4.41, P = 2.55 ± 0.49, K = 13.2 ± 2.79 mg g^{-1} DW). *Sphagnum* N concentration thresholds of 11 to 12 mg g^{-1} (Lamers *et al*., 2000; Bragazza *et al*., 2004), 15 mg g^{-1} (for *S. recurvum* - Section *Cuspidata*) (van der Heijden *et al*., 2000) and 20 mg g^{-1} (for a range of hummock and hollow species) (Berendse *et al*., 2001) have been reported. Nitrogen content in wild-sourced *S. squarrosum* in this study was at the top of the reported threshold range, with no apparent limitation of P or K, although those nutrients may have been supplied by the woodland habitat from which it was sourced. Nitrogen content in BeadaMoss® samples was well above the highest reported threshold – as high as 30 mg g^{-1} in some samples, with no evidence of toxicity or limitation of P or K.

Growth reduction is reported when *Sphagnum* experiences a considerable increase in N availability relative to conditions at the site from which it was obtained (Limpens and Berendse, 2003), implying that *Sphagnum* tolerance of N is site-adapted. Bragazza *et al*. (2004) found that, at elevated N levels the N:P ratio increased to 33.8 and 33.6 and N:K ratio increased to 3.7 and 4.0 for hummock and lawn species respectively. In this study, there were very high N:P and marginally high N:K ratios in *S. medium/divinum* (64.6 and 5.24) and *S. papillosum* (42.6 and 3.86 respectively) from open, ombrotrophic bogs compared to N:P and N:K ratios in *S. squarrosum* (11.4 and 1.54) and *S. palustre* (17.5 and 2.05 respectively) (from higher-nutrient, shaded environments) which are more aligned to ratios found in low nutrient sites by Wang and Moore (2014) and Bragazza *et al*. (2004). In wild-sourced *Sphagnum* in this study, the lowest P_{max} was found in *S. papillosum* and *S. medium/divinum*, and the highest in *S. squarrosum* and *S. palustre*. Although the plants with a high N:P ratio (*S. papillosum* and *S. medium/divinum*) appeared healthy and are likely adapted to these particular levels of nutrients, limitation of P by higher levels of N (as described above) may have reduced their photosynthetic capacity (Reich *et al*., 2009), although Granath *et al*. (2012) found that the negative effect of limited P was on production rather than photosynthetic rate.

This difference in stoichiometry between BeadaMoss® and wild-sourced plants may be due both to issues of maturity (Sterner and Elser, 2002), as levels of N may be naturally higher in young than in mature plants (Barker and Pilbeam, 2007), and site adaptation in

wild-sourced samples (Limpens and Berendse, 2003). Although BeadaMoss® plants were 'mature' in terms of development, they could be classed as 'young', being grown directly from tissue culture to the point at which they are available as BeadaHumok™ 'plugs' after only several months of growth. BeadaMoss® plants do not appear to be P- or K-limited (N:P = 9.61 ± 1.24 and N:K = 1.85 ± 0.09) suggesting that the right balance of nutrients is being applied in the horticultural process for these new plants. However, introduction of BeadaMoss® *Sphagnum*, grown under horticultural conditions, to a degraded site likely to be denuded of nutrients, may potentially cause an establishment 'shock' which could reduce its capacity for photosynthesis. The most important aspect of *Sphagnum* reintroduction is rapid establishment and growth, particularly lateral growth, to make an intact carpet as quickly as possible (Rochefort, 2000), which will progress restoration by keeping a cool, moist layer at the peat surface (Waddington and Warner, 2001), reducing evaporation and encouraging development of an acrotelm to promote peat accumulation (Rochefort *et al.*, 2003; Lucchese *et al*., 2010; Waddington *et al*., 2011; Worrall *et al*., 2011). This study on differences between BeadaMoss® and wild-sourced *Sphagnum* in terms of net photosynthesis, phylogenetic and adaptive traits, and nutrient assimilation has produced some promising results in terms of the suitability of BeadaMoss® materials for restoration and CO_2 uptake on degraded bogs, but more study is needed into performance of the materials in situ. However, studies by Crouch (2018) and Caporn *et al*. (2018) suggest successful growth in the field in both upland and lowland restoration trials.

2.5 Conclusions

BeadaMoss® *Sphagnum,* for the six species studied, had significantly higher P_{max} rates than equivalent wild-sourced species, little colour expression, an open habit, and higher numbers of chloroplasts than their wild counterparts, although capitula were smaller and more numerous. Higher P_{max} appeared to be associated with lower plant density, which may be related to light access to a greater proportion of the plants with more open habits, and phylogenetic differences in hollow-hummock forms. It may be that greater numbers of chloroplasts in BeadaMoss® *Sphagnum* will facilitate photosynthesis to drive rapid growth in early-stage plants, particularly in optimum conditions of moisture. P_{max} rates appeared to rise with higher nutrient concentrations in tissues of both BeadaMoss®

and wild-sourced samples. BeadaMoss® *Sphagnum* was grown with additional nutrients, but showed no sign of nutrient toxicity, or limited P or K, despite a N content approaching 30 mg g^{-1}, well above the highest reported concentration threshold in current literature. This could be related to BeadaMoss® *Sphagnum* being in the early stages of growth and more nutrient-demanding.

Overall, it appeared that nutrients had a beneficial effect on rates of photosynthesis, particularly apparent in BeadaMoss® *Sphagnum*, which is in the early stages of growth, and that plant adaptations to environmental stressors inhibited photosynthetic potential. There was a higher ratio of P_{max} to respiration in BeadaMoss® than in wild-sourced species, suggesting potential carbon balance benefits on application to the field. Further research is needed to assess whether the high rates of P_{max} (and anticipated high growth rates) seen in BeadaMoss® *Sphagnum* continue when plants are transferred to a field environment, and any influence of other features of the plants, such as the greater number of capitula or early nutrient application. The next chapter focuses on growth rates of a wider range of BeadaMoss® species and their potential efficacy in restoration settings, as individual species and in a commercially available mix.

Chapter 3 : Investigation of BeadaMoss® *Sphagnum* growth in a commercial mix of species

3.1 Introduction

Tissue-cultured *Sphagnum* produced by BeadaMoss® is being applied on peatland restoration sites to promote re-establishment of an acrotelm, necessary for ecohydrological function, as natural sources of *Sphagnum* are scarce (Caporn *et al*., 2018). (Source and preparation of material detailed in Sections 1.3.6 and 1.3.7). A multi-species mix is used with the intention that, on broad application, each species will thrive in the microhabitat in which it is most productive (Clymo and Hayward, 1982). There is wide evidence in the literature that *Sphagnum* species differ in their growth and production rates (e.g., Hájek *et al*., 2009; Laine *et al*., 2011; Loisel *et al*., 2012; Kangas *et al*., 2014; Laing *et al*., 2014; Bengtsson *et al*., 2016; Mazziotta *et al*., 2019) due to phylogenetic traits and adaptations to a range of peatland microhabitats (comparison of some key traits for UK *Sphagnum* species in Table 1.1). Micropropagated *Sphagnum* species, cultured concurrently, available at the same stage of development and not acclimated to the natural environment, offer a unique opportunity for studying species growth from an equivalent basis.

Individual *Sphagnum* species have adaptations to microhabitat that enable the genus to dominate the peatland environment (van Breemen, 1995) and growth and decomposition rates are thought to be positively related (Rochefort *et al*., 1990; Rydin and Jeglum, 2013; Bengtsson *et al*., 2018). *Sphagnum* species exist in an environmental continuum of moisture, nutrient and shade conditions (Mazziotta *et al*., 2019). *Sphagnum* thriving either aquatically, or semi-aquatically close to the water-table in hollows (e.g., *Sphagnum* from Sections *Cuspidata* and *Subsecunda* (Table 1.1) such as *S. cuspidatum, S. fallax, S. denticulatum* in this study) have low water-retention capacity (Thompson and Waddington, 2008; Bengtsson *et al*., 2016) and grow and degrade rapidly, particularly in conditions of optimum moisture (Grosvernier *et al*., 1997; Rydin and Jeglum, 2013; Laing *et al*., 2014). Those thriving higher above the water-table and often in open conditions, whether in lawns, carpets or hummocks (e.g., *Sphagnum* from Sections *Acutifolia* and *Sphagnum* such as *S. capillifolium, S. papillosum, S. medium/divinum* in this study) grow

and degrade slowly due to products of photosynthesis being invested more in stress-tolerance strategies for drought. These strategies may involve developing a dense growth habit and a more rigid structure for better retention of moisture and capillarity to the capitula (Turestky *et al*., 2008; Hájek *et al*., 2009; Loisel *et al*., 2012; Laing *et al*., 2014; Bengtsson *et al*., 2018). The hyaline cell pores size, connectivity and arrangement also determine the ability of some *Sphagnum* species to conserve water in drought conditions (McCarter and Price, 2014) by maintaining pressure in water-holding cells. The resulting slow degradation in drought-tolerant *Sphagnum* allows the accumulation of recalcitrant material necessary for peat formation in ombrotrophic conditions (Rochefort *et al*., 1990; Bengtsson *et al*., 2018). Moreover, the associated production of uronic acids generates the accumulated carbohydrates partially responsible for peatland carbon storage (Rydin and Jeglum, 2013; Bengtsson *et al*., 2018). *Sphagnum* species which typically grow in hollows or on hummocks, occupying different niches within the peatland environment (Johnson *et al*., 2014), respectively represent early and late stages of succession towards ombrotrophic bog (Fenton and Bergeron, 2007; Laine *et al*., 2011; Kangas *et al*., 2014). Species preference in restoration projects often depends on site conditions and the phase of restoration (Grosvenor *et al*., 1997; Quinty and Rochefort, 2003; Chirino *et al*., 2006) and required outcomes for biodiversity, carbon sequestration, or both (Alonso *et al*., 2012). Preferred species in commercial settings, e.g., in *Sphagnum* farming, depends on high productivity and product marketability (Gaudig *et al*., 2018; Kumar, 2018). Therefore, an informed choice of *Sphagnum* species application is key to successful outcomes.

The aims of this Chapter study were to explore whether phylogenetic traits in *Sphagnum* species were expressed in BeadaGel™, how establishment in different environments affected growth, and whether the commercial BeadaGel™ mix of 11 *Sphagnum* species was appropriate for restoration use in a range of peatland microhabitats and for other applications.

The objectives were to conduct systematic studies into BeadaGel™ growth in terms of innovations (new capitula - growth buds and shoots [Prager *et al*.,2012]), volume, mass and density of 11 species of tissue-cultured *Sphagnum* produced by the BeadaMoss® company in BeadaGel™ form, both individually and together in the commercially-

available BeadaGel™ 11-species mix. Growth and development would be tested in indoor and more natural conditions. The current proportion of each *Sphagnum* species within the commercial mix would be assessed, and the potential use of BeadaMoss® products, with the current mix of species, in a range of applications would be examined.

These objectives tested the hypotheses that:

1) BeadaGel™ *Sphagnum* will display the same phylogenetic traits as literature sources suggest for naturally-grown *Sphagnum*;
2) establishment either outdoors in Spring or indoors will deliver better growth and development outcomes than outdoors in Autumn;
3) the commercial mix with the current proportions of each *Sphagnum* species will prove appropriate for a range of applications in the field.

3.2 Methods

3.2.1 Sample preparation and analysis

The trial investigated growth of both the commercial mix of BeadaMoss® *Sphagnum* (as BeadaGel™) which incorporated 11 species in varying amounts, and each of the constituent species individually. BeadaGel™ consists of tissue-cultured *Sphagnum* suspended in a hydrocolloidal gel which can be applied onto a growing media or substrate surface, and full details of production and species selection are given in Sections 1.3.6 and 1.3.7. This is the same BeadaGel™ mix applied on Cadishead Moss for carbon greenhouse gas studies in Chapter 4, both on the newly established plots and the trial plots established in June 2014. This mix is grown on in BeadaMoss® greenhouses to produce BeadaHumok™ plug plants, which have been planted widely on both upland and lowland restoration trials and projects (Caporn *et al*., 2018).

Comparisons were made between establishment in indoor conditions ('indoor') and an outdoor ('outdoor') environment in Autumn (October 2015 start for both). Rain-watered horticultural peat substrate (30 ml) was added to pots of 50 mm diameter and 100 mm depth, which were drilled with two opposing 6 mm diameter holes at 15 mm from the

base (level with the substrate surface) to prevent inundation. Hydrocolloidal gel (150 ml) and 18 g of each species or the commercial species mix (drained of excess water but not dried) were thoroughly mixed to make BeadaGel™. BeadaGel™ (6 ml) was applied to each pot and spread as evenly as possible across the substrate surface, to make 10 replicates of each species and of the species mix (i.e., 120 pots indoors and 120 pots outdoors). Further trials (with 8 replicates, i.e., 96 pots) were conducted outdoors starting in March 2017 to determine whether this was a more favourable time of the year to establish BeadaGel™.

Outdoor samples were arranged randomly in a transparent, open container, base-drilled to prevent inundation, and covered with nylon mesh (which inhibited 47% of PAR) to reduce leaf/debris coverage and deter bird interference. Any debris falling onto samples was removed. Samples in indoor conditions (i.e., protected from the weather) were arranged randomly in trays, grown under natural lighting, supplemented with LED lamps during winter months, and repositioned weekly to even out sample access to light. Photosynthetically active radiation (PAR) recorded with a portable meter (SKP 215 - PAR 'Quantum' sensor, Skye Instruments Ltd) over 5 random mornings at 6 points around the samples was: Min = 40.3; Max = 665; Mean = 109 µmol m^{-2} s^{-1}. The maximum and minimum temperature was recorded daily (using a Brannan greenhouse digital thermometer) both indoors and outdoors. Mean minimum and maximum temperatures were 14.4 and 18.1°C indoors, and 6.7 and 16.1°C and 7.9 and 17.1°C outdoors, Autumn 2015 and Spring 2017 trials respectively. The pots were watered with rainwater as necessary to keep water levels close to the substrate surface.

Each sample volume was calculated at various times throughout growth and at harvest, from the mean height of the plants measured at 3 points above the substrate, and the percentage cover across the base area of the pot. In the early stages of growth, innovations (Figure 3.1) were photographed and counted (on a PC) to assess differences in establishment success between species grown indoors and outdoors in Autumn 2015, before plants began to develop recognisable capitula. This was at 2 months growth indoors and 6 months growth outdoors. Plants were harvested when the most productive samples had grown to the top of the pots, which was 8 months for those grown indoors, and 18 months and 15 months for those grown outdoors from Autumn

2015 and Spring 2017 respectively. Any substrate was carefully removed from samples at harvest. Samples were dried overnight at 105°C and dry-weighed. Calculations of DW density were made for each sample by dividing DW by volume at harvest (mg cm^{-3}).

3.2.2 Data management and analysis

Data was prepared through Microsoft Excel (2019) and analysed statistically using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Data were tested for normality using Shapiro Wilk tests, and found to be normally distributed. Two-way ANOVA with post-hoc Tukey HSD tested differences in number of innovations (new growth points), volume, productivity (DW mass) and DW density associated with establishment conditions and species. Four samples (one each of *S. capillifolium*, *S. denticulatum*, *S. fallax* and *S. fimbriatum*) were removed from volume, productivity and DW density measurements of those established in Autumn 2015 due to drainage failure of the pots which caused flooding, algal growth, and uncharacteristically minimal *Sphagnum* development. To check the efficacy of the BeadaGel™ mix compared to species grown individually, the equivalent percentage of each single species in the commercial mix was calculated to give the expected and actual proportion of species in the BeadaGel™ mix once grown.

3.3 Results

3.3.1 Innovations

There were fewer innovations in outdoor samples after 6 months than those in indoor conditions after 2 months (Figure 3.2) (one-way ANOVA test $F = 60.98$, $p < 0.001$, $df = 23$). Moreover, there were more innovations in indoor than outdoor samples of all species apart from *S. denticulatum*, statistically significant at $p < 0.001$ (on two-way ANOVA post-hoc Tukey HSD) in all but *S. denticulatum, S. medium/divinum, S. palustre, S. papillosum* and *S. subnitens*, which did not have significantly different numbers.

There was a statistically significant difference in the number of innovations between species established outdoors ($F = 19.54$, $p < 0.001$, $df = 11$): *S. fallax* (193 ± 25), *S.*

denticulatum (139 ± 22) and *S. fimbriatum* (125 ± 35) had the highest; *S. palustre* (57 ± 43), *S. subnitens* (36 ± 24) and *S. medium/divinum* (24 ± 13) had the lowest; the BeadaGel™ mix ranked 8th (80 ± 28) (Figures are mean ± SD). There was a greater statistically significant difference in numbers of innovations between species established indoors (F = 63.49, p < 0.001, df = 11): *S. capillifolium* (387 ± 55), *S. fallax* (291 ± 43) and *S. fimbriatum* (260 ± 42) had the highest; *S. palustre* (107 ± 46), *S. subnitens* (84± 12) and *S. medium/divinum* (54 ± 17) had the lowest; the BeadaGel™ mix ranked 7th (173 ± 31).

Figure 3.1. Example of 'innovations' - new capitula growth buds and shoots (*S. capillifolium*).

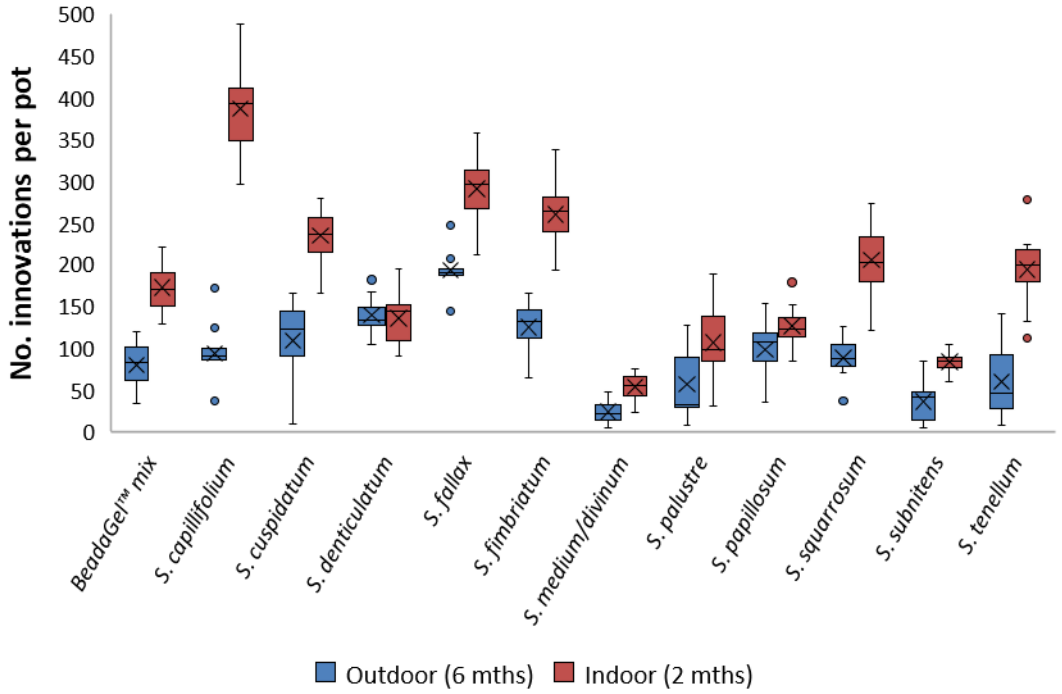


Figure 3.2. Early growth of samples (number of innovations = new growth points) established outdoors and indoors, in Autumn 2015, counted before development of capitula. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is inclusive.

3.3.2 Volume

BeadaGel™ applied to pots indoors in Autumn 2015 grew more rapidly than those grown outdoors, with a greater volume throughout, necessitating harvest at only 8 months. Those established outdoors in Autumn reached a greater volume at 12 months than those in Spring, as the Spring batch growth had plateaued over winter. Thereafter, growth in the Spring batch increased rapidly to harvesting at 15 months, whereas the Autumn batch growth plateaued over its second winter to a lower volume and later harvest (18 months) than the Spring batch (Figure 3.3). Indoors, *S. squarrosum* had the greatest volume and *S. tenellum* the least. In Autumn establishment outdoors, the greatest volume was in low-stress adapted species *S. cuspidatum*, *S. denticulatum*, *S. fallax*, *S. squarrosum*, and the least in high-stress adapted species, *S. capillifolium*, *S. medium/divinum*, *S. subnitens* and *S. tenellum*. In Spring establishment outdoors growth in volume was more even throughout species, although *S. squarrosum* had the greatest volume, and that of *S. medium/divinum* was noticeably lower than other species.

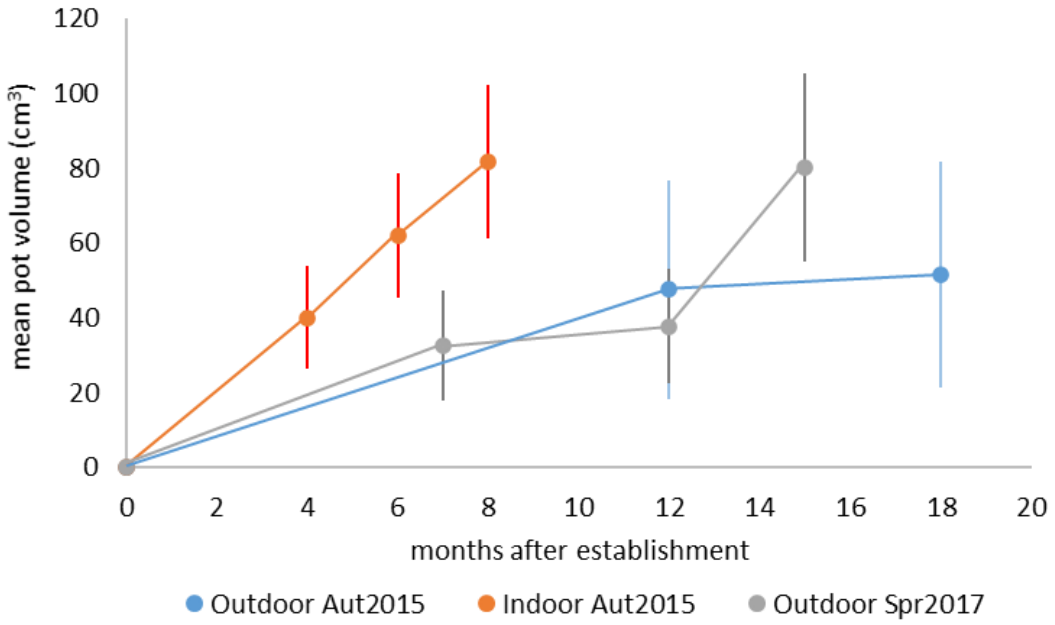


Figure 3.3. Volume of all samples measured at various points of growth until harvest (final point). Error bars ± SD.

3.3.3 Productivity (dry weight) and dry weight density

Samples grown indoors (harvested at 8 months) tended to have a lower DW (424.1 ± 128.7 mg [mean ± SD]) and a lower DW density (5.19 ± 0.98 mg cm^{-3}) (*n* = 120), than those established outdoors, particularly in spring 2017, although variability between samples was similar to the spring 2017 batch (coefficient of variation between sample means [CV] = 30.4% and 18.8% DW and DW density respectively). Those established outdoors in spring 2017 (harvested at 15 months) tended to have a greater DW (775.4 ± 196.3 mg) but a lower DW density (9.72 ± 1.53 mg cm^{-3}) (*n* = 96), and less variability between samples (CV = 25.3% and 15.7% respectively) than those established outdoors in autumn 2015 (harvested at 18 months) (DW: 599.5 ± 245.2 mg, CV = 40.9%; DW density: 13.96 ± 6.18 mg cm^{-3}, CV = 44.3%; *n* = 116) (Figure 3.4; full data in Appendix 1). If the primary growing period for mosses is considered to be May to September (Laine *et al.*, 2011), the autumn species outside had 5 months, and spring samples had 7 months of optimum growth period; autumn samples had two winter periods and spring samples only one.

The DW and DW density of species were statistically significantly different between growth environments as determined by one-way ANOVA ($F = 24.6$, $p < 0.001$, $df = 35$, and

F = 33.0, *p* < 0.001, *df* = 35 respectively). There were statistically significant differences between environments overall, and between environments for each species, as determined by post-hoc Tukey HSD (environment: *p* < 0.001 throughout; species as indicated on Figure 3.4).

There were statistically significant differences within each growth environment between species established in Autumn 2015 outdoors (*F* = 20.4 and 14.9 respectively, *p* < 0.001, *df* = 11) and indoors (*F* = 12.4 and 7.5 respectively, *p* < 0.001, *df* = 11), and in Spring 2017 outdoors (*F* = 7.5 and 6.6 respectively, *p* < 0.001, *df* = 11), as determined by one-way ANOVA (Species differences indicated on Figure 3.4)

Overall, there is little parity in rank order of species DW or DW density in growth environments, although a tendency in outside environments for *S. medium/divinum* to have a low DW and high density compared to other species (full data in Appendix 1).

Species established outside in Autumn with the greatest DW were *S. denticulatum*, *S. fallax* and *S. cuspidatum*, those with the lowest were *S. tenellum*, *S. subnitens* and *S. medium/divinum*, and BeadaGel™ mix ranked 8th. Species with the greatest DW density were *S. tenellum*, *S. capillifolium* and *S. medium/divinum*, those with the lowest were *S. squarrosum*, *S. cuspidatum* and *S. fallax*, and the BeadaGel™ mix ranked 5th.

Species established indoors with the greatest DW were *S. capillifolium, S. squarrosum* and *S. cuspidatum*, those with the lowest were *S. tenellum, S. papillosum* and *S. medium/divinum*, and the BeadaGel™ mix ranked 8th. Species with the greatest DW density were *S. capillifolium*, *S. tenellum* and *S. subnitens*, those with the lowest were S*. squarrosum*, *S. palustre* and *S. medium/divinum*, and the BeadaGel™ mix ranked 11th.

Species established outside in Spring with the greatest DW were *S. fallax* and *S. squarrosum*, followed by most other species of gradually reducing DW, with *S. capillifolium* and particularly *S. medium/divinum* ranking lowest (although DWs were greater than the lowest DWs in Autumn samples), and the BeadaGel™ mix ranked 10th.

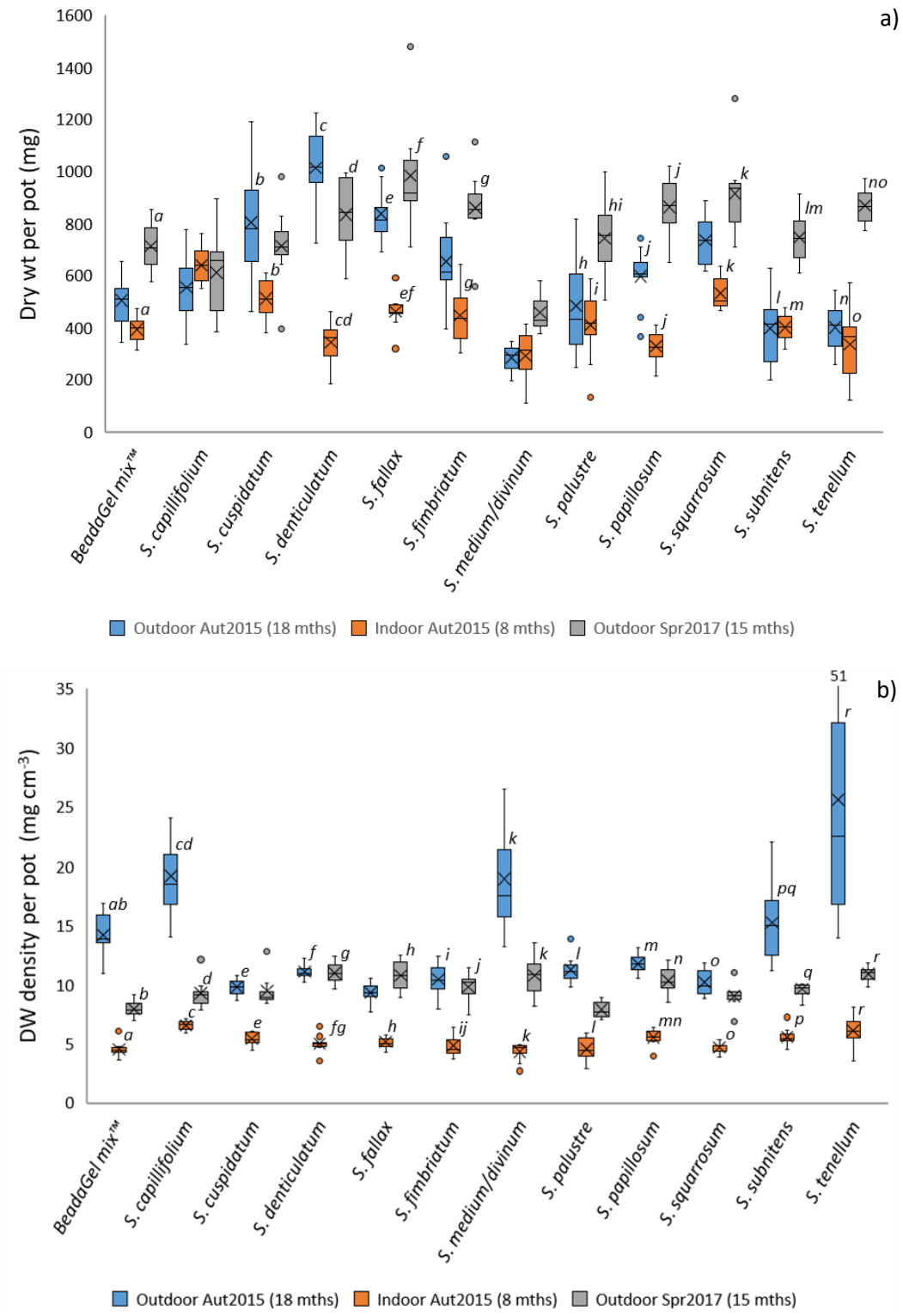


Figure 3.4. Dry weight (a) and dry weight (DW) density (b) of samples (at harvest), established outdoors in Autumn 2015 (at 18 months), indoors in Autumn 2015 (at 8 months) and outdoors in Spring 2017 (at 15 months). Statistically significant differences (two-way ANOVA post-hoc Tukey HSD $p < 0.05$) between environments for each species are indicated by a shared letter. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is inclusive.

Spring-established species with the greatest DW density were: *S. denticulatum*, *S. tenellum* and *S. medium/divinum*; those with the lowest were *S. capillifolium*, *S. squarrosum* and *S. palustre*; and the BeadaGel™ mix ranked 11th.

3.3.4 Expected species percentage in commercial BeadaGel™ mix

Compared to the standard percentage of each species used in the commercial BeadaGel™ mix (Table 3.1), growth varied depending on whether a species was established in spring or autumn outdoors, or established indoors, but *S. medium/divinum* DW was less than expected throughout, and *S. squarrosum* DW was consistently more than expected. *S. fallax* and *S. fimbriatum* DWs were much more than expected in an outdoor environment (less so indoors), *S. palustre, S. papillosum* and *S. tenellum* fared better than expected if established in spring rather than autumn in an outdoor environment, and *S. capillifolium* performed better than expected indoors rather than outdoors. The combined DW of species grown individually (each at the percentage present in the commercial BeadaGel™ mix) was greater than that of the commercial mix.

3.4 Discussion

This study of BeadaGel™ *Sphagnum* growth in indoor and outdoor environments produced dissimilar results in terms of establishment, productivity and phylogenetic expression, whereas *Sphagnum* species in natural settings appear to exist on a continuum of integrated environmental conditions in peatlands, coupled with certain adaptive physiological traits (Mazziotta *et al*., 2019). For example, in this study, growth of species established outdoors in Autumn most closely reflected generally accepted traits from the literature (Table 1.1). Species which are adapted to drought or open environments, such as *S. capillifolium*, *S. medium/divinum* and *S. tenellum*, had a greater density than species which are aquatic or semi-aquatic, such as *S. cuspidatum* and *S. fallax*, and species adapted to conditions between these extremes, such as *S. papillosum*, had intermediate values for density. This pattern was not the case for samples established outside in Spring or indoors. The commercial mix appeared likely to perform as designed in restoration projects, and some suggestions are made below for adaptations for early- and mature-stage restoration sites, and use in other applications.

Table 3.1. Expected and actual dry weight of samples established in autumn 2015 and spring 2017 outdoors and in autumn 2015 indoors, based on percentages of species within the commercial BeadaGel™ mix.

Species in commercial BeadaGel™ mix (%)	Outdoor Autumn 2015 - Harvest: 18 mths		Outdoor Spring 2017 - Harvest: 15 mths		Indoor Autumn 2015 - Harvest: 8 mths	
	Expected DW (mg)	Actual DW (mg) and % of expected	Expected DW (mg)	Actual DW (mg) and % of expected	Expected DW (mg)	Actual DW (mg) and % of expected
S. capillifolium (17.0%)	85.52	94.2 (110.2%)	121.11	103.8 (85.7%)	66.81	108.6 (162.6%)
S. cuspidatum (12.7%)	63.89	102.0 (159.7%)	90.47	90.6 (100.1%)	49.91	64.8 (129.9%)
S. denticulatum (1.3%)	6.54	12.2 (186.8%)	9.26	10.8 (116.9%)	5.11	4.5 (87.2%)
S. fallax (20.3%)	102.12	169.8 (166.3%)	144.62	199.6 (138.0%)	79.78	93.7 (117.5%)
S. fimbriatum (10.5%)	52.82	69.2 (131.0%)	74.80	90.3 (120.7%)	41.27	46.9 (113.6%)
S. medium/divinum (2.5%)	12.58	7.1 (56.4%)	17.81	11.4 (64.1%)	9.83	7.3 (74.1%)
S. palustre (16.6%)	83.51	80.2 (95.9%)	118.26	143.1 (121.0%)	65.24	68.0 (104.2%)
S. papillosum (9.4%)	47.29	56.0 (118.5%)	66.97	81.0 (121.0%)	36.94	30.8 (83.4%)
S. squarrosum (3.6%)	18.11	26.5 (146.3%)	25.65	32.9 (128.4%)	14.15	19.1 (135.2%)
S. subnitens (4.3%)	21.63	17.1 (78.9%)	30.63	32.1 (104.9%)	16.90	17.2 (101.8%)
S. tenellum (1.8%)	9.05	7.2 (79.6%)	12.82	15.6 (121.8%)	7.07	6.0 (85.4%)
BeadaGel™ Mix (100%)	503.05 (actual)		712.4 (actual)		393.01 (actual)	
All individual species		641.5 (127.5%)		811.3 (113.9%		466.9 (118.8%)

BeadaGel™ establishment indoors encouraged production of a greater number of innovations (growing points) than when grown outdoors, which subsequently led to rapid substrate cover and linear growth, perhaps through strand competition within each sample (Rydin and Jeglum, 2013; Bengtsson *et al*., 2016). In BeadaMoss® greenhouses, BeadaGel™ is grown into 'plug' plants (BeadaHumok™) which, after a period of 'hardening off' in cool greenhouses, have been used for wide-scale *Sphagnum* planting in upland and lowland peatland restoration projects in the UK (Caporn *et al*., 2018). More strands per plug perhaps makes them likely to be more resilient to water stress and to spread rapidly, as seen in long-term trials of BeadaHumok™ compared to clumps from wild sources (Crouch, 2018). It may also help to explain the greater rate of photosynthesis in BeadaMoss® compared to wild-sourced samples in Chapter 2.

The species producing the most growing points indoors were mostly highly-productive species that normally grow close to the water table (*S. cuspidatum*, *S. fallax*, *S.*

fimbriatum, *S. squarrosum*) (Gunnarsson, 2005; Laine *et al*., 2011; Kangas *et al*., 2014) but primarily *S. capillifolium*, which, although a hummock species, has adapted to a wide range of habitats by producing a tightly-packed hummock of small capitula (Bonnett *et al*., 2010). However, in outdoor conditions, *S. denticulatum*, *S. fallax* and *S. fimbriatum*, which are highly productive species in lowlands and uplands at high water levels (Grosvenier *et al*., 1997; Gunnarsson, 2005; Laine *et al*., 2011), produced the most growing points, which may be part of their strategy for rapid growth. *S palustre*, *S. subnitens* and *S. medium/divinum* produced the fewest innovations in both environments. These species are adapted to drier conditions or a wide habitat niche, putting more resources into structure than rapid growth (Loisel *et al*., 2012; Kangas *et al*., 2014; Bengtsson *et al*., 2016), and *S. palustre* and *S. medium/divinum* produce large, dense shoots, rather than many thin ones, for water-retention purposes (Loisel *et al*., 2012). *S. squarrosum*, a species of rapid growth with an open habit, also produces large capitula (Atherton *et al*., 2010; Laine *et al*., 2018), but in this case, it is an adaptation for maximising capitulum tissue area to photosynthesis in shade conditions (Mazziotta *et al*., 2019; Hajek *et al*., 2009; Rydin and Jeglum, 2013). This is shown by the comparatively average number of *S. squarrosum* innovations in the outdoor environment.

Establishment in Spring or Autumn in outdoor conditions probably made very little difference in terms of long-term growth overall. Samples established in Autumn had greater volume overall at 12 months than those established in Spring, but at harvest the Spring batch (at 15 months) had greater dry weight (and less variability between species) than the Autumn batch (at 18 months). Over time there would probably be no discernible difference in biomass based on time of establishment as long as there was sufficient moisture to support species at optimum productivity (Bengtsson *et al*., 2016), but any periods of drought in spring and summer, which have been a feature in recent years, would be obviously detrimental in the early stages of growth.

Variation in seasonal establishment outdoors appeared to make a difference to success for individual species. Establishment in Autumn favoured aquatic and semi-aquatic species (e.g., *S. cuspidatum* and *S. denticulatum*), which have high productivity in conditions of optimum moisture (Laine *et al*., 2011; Rydin and Jeglum, 2013). These species had greater productivity and lower density than more drought-tolerant species

(e.g., *S. medium/divinum* and *S. subnitens*). Of note was that productivity of *S. tenellum* was low, but DW density was particularly high, perhaps demonstrating that species' strategy for colonising bare wet peat as an early pioneer. All species established outdoors in Autumn that were comparable to those studied by Bengtsson *et al*. (2016) (Table 3.2), apart from *S. papillosum*, had a higher dry weight density, which perhaps indicates that BeadaMoss™ *Sphagnum* established in Autumn months may develop a good resilience to desiccation in the field. It is of particular note that the BeadaGel™ mix established outdoors in Autumn also had a greater density than most individual species. There was lower density in all the selected species established indoors than those in natural settings (Table 3.2), probably due to rapid linear growth, although the two species with greatest density indoors were *S. capillifolium*, a hummock-former and *S. tenellum*, an early pioneer on open, bare peat, following phylogenetic traits for desiccation avoidance. Samples of species established outdoors in Spring which are normally found at water-table level (*S. cuspidatum* and *S. fallax*) had a greater density than in those studied by Bengtsson *et al*. (2016) (Table 3.2), perhaps due to a lower moisture content than in natural settings although samples received regular watering, and the remaining species had a comparably lower density than the literature source, again probably due to greater linear growth in favourable conditions for those species. Spring establishment produced a mixed ranking; *S. squarrosum* and *S. palustre* had the greatest volume and lowest density. Both of these species have a more open habit, which results in greater photosynthetic capacity to promote a rapid growth rate (Hájek *et al*., 2009; Rydin and Jeglum, 2013; Ma *et al*., 2015; Krebs *et al*., 2016).

S. medium/divinum had consistently low productivity and high density throughout, which is typical for drought-adapted species, which invest more resources into decay resistance and structure for capillarity (Grosvernier *et al*., 1997; Laing *et al*., 2014; Bengtsson *et al*., 2018) and a dense growth form (Mazziotta *et al*., 2019) to avoid desiccation. However, across all species, density was greatest overall in samples established outdoors in Autumn 2015 which had two winter seasons and one growing season. Density was lower in samples established outdoors in Spring 2017 (than in Autumn 2015) with one winter season and two growing seasons, and those established indoors with no winter season had the lowest density overall. As samples were regularly watered and not allowed to dry out, those with the greatest access to light and warmth are likely to have developed

linear growth rather than density, but phylogenetic growth traits were still expressed, as for *S. medium/divinum*.

The samples established indoors grew rapidly, and the generally accepted inverse traits of productivity and density in different *Sphagnum* species from natural settings reported in the literature (Laing *et al*., 2014; Mazziotta *et al*., 2019) were not as evident in these immature samples grown concurrently in conditions of warmth, adequate light and moisture, as in those grown outdoors. This agrees with the findings of Bengtsson *et al*. (2016), that *Sphagnum* species' productivity is related to both phylogeny and environmental factors, and phylogeny is under-expressed in optimum growth conditions. Therefore, hypothesis 1) that BeadaGel™ *Sphagnum* would display the same phylogenetic traits as literature sources suggest for naturally-grown *Sphagnum* was supported, but was only clearly expressed when samples were grown in a more natural setting outdoors. Species that normally thrive above the water-table grew more slowly indoors than aquatic and semi-aquatic species, but density was also low, perhaps due to harvest in the early stages of development, when linear growth is most dominant (Laine *et al*., 2011). The exception was *S. capillifolium*, which developed more innovations than other species, and then a high DW and the highest density, which reflects the phylogenetic fitness of the species (Bonnett *et al*., 2010). Overall, hypothesis 2) that establishment either outdoors in Spring or indoors would deliver better growth and development outcomes than outdoors in Autumn was supported in terms of volume and productivity (dry weight) for outdoor establishment in Spring rather than Autumn, and for rapid development indoors.

When growth of individual species was compared to that expected in the BeadaGel™ mix, several species were more or less productive than expected which, depending on the anticipated outcomes, may be unwelcome in the field. A mixed culture may also introduce competition between species (Robroek *et al*., 2007; Ma *et al*., 2015). Slower-growing species may be shaded out, or an uneven hummock may form initially, which is more vulnerable to desiccation - the latter being the poorest long-term outcome. Longer strands of faster-growing species may suffer greater moisture loss and reduced photosynthesis (Rydin and Jeglum, 2013) which would even out the canopy of a mixed-species carpet. However, Robroek *et al*. (2007) suggest that capitula of *Sphagnum* grown in mixtures may have a lower water content than those grown singly, so the mixed carpet

may grow more slowly than with single species. This appears to be the case in this study, where productivity of the BeadaGel™ commercial mix was less than that of single species.

Table 3.2. Comparison between this study and a literature source studying a range of *Sphagnum* species, of dry weight density (non-decomposed) values for equivalent species. Values are mean dry weight density (mg cm^{-3}) ± SE. General descriptions from Laine *et al*. (2018).

Species	General Habit / niche	Bengtsson *et al* ., 2016	This study		
			Est. Outdoors Autumn 2015	Est. Indoors Autumn 2015	Est. Outdoors Spring 2017
S. capillifolium	Hummock open/shade	12.9 ± 0.92	20.2 ± 1.11	6.5 ± 0.20	8.9 ± 0.27
S. cuspidatum	Carpet open/pool	7.8 ± 0.62	9.8 ± 1.27	5.4 ± 0.20	9.5 ± 0.51
S. fallax	lawn/carpet open/shade/flush	6.4 ± 0.65	9.2 ± 0.33	5.2 ± 0.27	10.6 ± 0.52
S. medium/divinum	hummock/lawn/carpet open/shade	13.7 ± 1.33	19.6 ± 1.72	4.3 ± 0.30	10.8 ± 0.68
S. papillosum	hummock/lawn/carpet open	13.4 ± 1.63	11.6 ± 0.29	5.7 ± 0.17	10.3 ± 0.42
S. tenellum	lawn/small patches open	18.5 ± 1.15	24.4 ± 4.47	6.2 ± 0.50	10.9 ± 0.23

BeadaGel™ application in restoration settings

From results in this study, some species, such as *S. squarrosum* may become dominant in the BeadaGel™ mix, and as a species of shaded, often nutrient-rich environments (Atherton *et al*., 2010; Laine *et al*., 2018) *S. squarrosum* is perhaps not particularly useful for restoration projects, especially where the ultimate aspiration may be an ombrotrophic bog, where this species would not perform well (Harley *et al*., 1989). The mix may deliver a lower-than-expected abundance of *S. medium/divinum*, being slower to establish, and perhaps its presence could be improved by increasing the initial percentage and reducing or removing others (e.g., *S. squarrosum*) in the mix. An alternative is to remove the slower-growing species, such as *S. medium/divinum*, from the mix, as they may reduce overall effectiveness of faster-growing species, and introduce these at a later date as individual species, although this may involve extra effort and expense. However, *S.*

squarrosum does colonise in areas of recovering lowland bogs, for example, on the study site of Cadishead and Little Woolden Mosses in Chapter 4, in relic ditches and flooded areas dominated by *Juncus effusus* (personal observation) and, as a species of high productivity and nutrient uptake, could be useful in restoration towards ombrotrophic conditions. Additionally, the slower-growing species have a greater density, and are more likely to thrive in drier conditions.

Restoration projects which rely only on rewetting and natural recovery of peatland plants tend to result in poor biodiversity, limited CO_2 uptake by colonising aquatic and semi-aquatic *Sphagna*, and reduced resilience to environmental change, and so introducing a mix of *Sphagnum* species is highly beneficial for improved outcomes (Robroek *et al*., 2009). Much depends on the environment, as water table depth and shading effects can cause unexpected competition between species (Hayward and Clymo, 1983) and fast-growing species may not thrive in dry years (Bengtsson *et al*., 2018). Therefore, it is essential to have species in the mix which will are able to avoid desiccation in hummock microclimates and periods of drought, and *Sphagnum* introduction along with, or within existing vascular plants can also aid establishment (Pouliot *et al*., 2011). However, the 'stress-gradient hypothesis' suggests that, in peatlands, as the water table decreases, positive self-association becomes more critical to survival, and larger aggregates of a species will be more successful than smaller aggregates as they are able to create their own microclimate to avoid desiccation (Robroek *et al*., 2009). Rapid establishment is, therefore, essential and any species deemed unnecessary or unlikely to be resilient in a broad range of peatland environments, should not be included in the mix.

In the outdoor environment, the productivity and density of the BeadaGel™ mix compared with individual species varied depending on the time of establishment, reflecting the dominance of different species within the mix when growing conditions were optimal for them. The broad range of species in the current BeadaGel™ mix, caveated with the discussion above, appears to be a good choice to maximise *Sphagnum* coverage on large sites in the early stages of restoration, where hydrological conditions are still unpredictable, and so hypothesis 3) that the commercial mix with the current proportions of each *Sphagnum* species would prove appropriate for a range of applications in the field, appears sound. It may be that Autumn application is preferable

for early-stage restoration projects (as pioneer species dominated on Autumn establishment), where re-wetting is the key management strategy and a high volume of hollow-adapted species is needed. This could be followed up with a Spring application (as drought-adapted species performed better on Spring establishment) when restoration sites are more mature, some hollow-adapted species are already established, and the focus is on establishing peat-accumulating, drought-adapted species (Grosvernier *et al*., 1997; Kangas *et al*., 2014). It may also be that more targeted applications with specific aquatic/semi-aquatic or drought-adapted species are more effective options at each stage, or in hollow or hummock micro-topographies, although financial and labour costs may be higher.

BeadaMoss® is a viable source of *Sphagnum* as harvesting from protected sites is prohibited, and an ethical alterative to sourcing material from wild sources outside the UK (Caporn *et al*., 2018). It appears to be more productive than culture from clumps of wild-sourced *Sphagnum* (Crouch, 2018) which would also be an inherently slow process when only very small amounts of harvesting may be permitted within sites. BeadaMoss® materials have been trialled in English uplands and lowlands in a variety of settings (Caporn *et al*., 2018), and subsequently planted on a large scale in restoration work. Moors for the Future Partnership have ongoing long-term trials of BeadaMoss® products in a variety of forms and species mixtures, and reported that in their 2019/20 work season one million *Sphagnum* plugs had been planted within 1000 hectares of blanket bog, which had been previously revegetated to stabilise the peat from erosion (MFFP, 2020). Lancashire Wildlife Trust's Lancashire Peatlands Initiative have hosted trials and planted thousands (pers. comm. LWT) of BeadaMoss® *Sphagnum*-mix plugs in lowland sites in the Greater Manchester area, both the 11-species commercial mix and a new 'chunky' (hummock-forming) mix of 5 species, designed for use on drier sites, in areas of introduced and naturally-occurring *Eriophorum* species.

Genetic diversity in bryophytes, as with all species, is vital for resilience to environmental change (Rowntree *et al*., 2011). Tissue-culture micropropagation from small amounts of wild-sourced material and subsequent wide application could result in large colonies of *Sphagnum* with low genetic diversity, making them less resilient to potential environmental stochastic events such as those associated with anthropogenic climate

change. However, inoculation with a BeadaMoss® *Sphagnum* mix for restoration allows climatic change to select resilient species. Clonal material is also taken from a number of locations within a region and these clones are changed in the species mixes, so increasing the genetic diversity. Moreover, Stenøien and Såstad (1999) found little genetic variation intercontinentally, and other mechanisms for genetic fitness, such as 'genetic drift and mutation rate' (Rowntree *et al*., 2011) may be foremost in promoting genetic diversity. Concerns over a trade-off between the benefits of potentially rapid, large-scale cover with BeadaMoss® materials, for which there is currently no alternative choice where *Sphagnum* is naturally scarce and protected, and large areas of *Sphagnum* being vulnerable to climate change due to genetic homogeneity, could be ameliorated with a follow-up inoculation with small amounts of wild-sourced *Sphagnum* (with permission) so that interbreeding can occur. Research into the genetic diversity of BeadaMoss® products leaving the greenhouse, and subsequently after acclimation in restoration settings, would be helpful.

BeadaGel™ application in commercial settings

Sphagnum farming is a relatively new area of research, as part of a suite of possibilities for agricultural conversion to paludiculture on peatlands to reduce carbon losses (Wichtmann *et al*., 2016). Possible uses of farmed *Sphagnum* are as a constituent in peat-free growing media (Pouliot *et al*., 2015), in which it needs to retain some structure on drying so that fragments allow both aeration and retention of moisture in the product (Kumar, 2018). Hollow-adapted species appear to lose structure on drying (Laine *et al*., 2011; Bengtsson *et al*., 2016), which may make the material friable, difficult to handle, and clump together when wet, so probably not suitable for use in growing media (Kumar, 2018). Of the drought-adapted species in this study, those which are both highly productive and with high DW density are *S. capillifolium*, *S. palustre* and *S. papillosum*, although *S. capillifolium* is likely to prove too small to be useful in growing media. Both *S. palustre* and *S. papillosum* have already been used in long-term trials of *Sphagnum* farming outside the UK (Temmink *et al*., 2017; Gaudig *et al*., 2018a), although species selection is still under debate (Gaudig *et al*., 2018b).

EU-funded trials of both *Sphagnum* farming (*Sphagnum* Farming UK, 2018) and carbon capture (through the 'Care Peat' initiative: Care Peat, 2020) are ongoing at lowland sites in Greater Manchester, North Lancashire and Leicestershire, using BeadaMoss® plug plants and BeadaGel™. Results from *Sphagnum* introduction in restoration settings have either not been collected or not yet been made feely available, the *Sphagnum* Farming UK results are not yet published, and the Care-Peat project is at an early stage.

3.5 Conclusions

This study examined, for the first time, growth in indoor and outdoor environments of a wide range of micropropagated *Sphagnum* species, from an equivalent developmental and treatment base (in 'BeadaGel™' form), both as single species and in a commercially available mix. There was no consistent parity between the ranking of photosynthesis rates (by dry weight as nmol CO_2 g^{-1} s^{-1}) of the six *Sphagnum* species (both BeadaMoss® and wild-sourced) studied in Chapter 2 and the ranking of productivity rates (as dry weight) of the same six BeadaGel™ species grown in the range of conditions in this chapter. However, *S. medium/divinum* (a species which can adapt to open, drier conditions) consistently had the lowest rate of photosynthesis and lowest productivity, and *S. squarrosum* (a nutrient-tolerant species adapted to conditions of shade and high moisture) consistently had the highest rate of photosynthesis and second highest productivity in all growth scenarios. This demonstrates some relationship in BeadaMoss® *Sphagnum* between photosynthesis and growth rates of species adapted to habitat extremes of light, moisture and nutrient input.

BeadaGel™ *Sphagnum* productivity was greater indoors and with Spring establishment outdoors. However, samples established outdoors in Autumn had the greatest dry weight density, which could allow greater resilience in restoration field conditions and therefore better overall outcomes. The number of *Sphagnum* innovations (early growth points) was much higher, and they developed more rapidly, in indoor than outdoor growing conditions. This may be a strategy for high productivity in aquatic and semi-aquatic species, and promotion of a dense growth habit for hummock species, but potentially helps promote productivity in products from BeadaMoss® greenhouses.

The current BeadaGel™ mix, containing species adapted to a range of peatland environmental niches, appears to be highly suitable for broad application where rapid cover is needed in early peatland restoration projects, with conditions of hydrological instability and topographical variability. The species mix could be adapted to suit requirements in more mature restoration projects, and also for commercial applications to reduce current damage to peatlands from harvesting wild-sourced *Sphagnum*. BeadaMoss® produced with particular concentrations of individual species are not likely to deliver those proportions of species exactly in the eventual product, either grown on in the greenhouse as BeadaHumok™ plugs and hardened off for planting, or applied directly in the field in BeadaGel™ form. It should be accepted that such a high degree of certainty is not possible due to constraints of mechanisation and using natural materials.

There are opportunities for research into the genetic diversity of *Sphagnum* species in BeadaMoss® greenhouses, and after introduction into the field, to allay worries over its resilience to the effects of climate change on restoration sites, such as a change in rainfalls patterns and longer periods of drought causing loss of species where products have been applied on a wide scale. Research on the performance of BeadaMoss® products already applied in restoration and commercial settings needs to be conducted and made available as a matter of urgency, to improve the profile and commercial success of the products and to aid management decisions on peatland restoration with *Sphagnum*. The next chapter studies the carbon greenhouse gas budget of a degraded lowland bog, where the commercial BeadaGel™ mix studied in this chapter has been used as part of restoration management.

Chapter 4: Carbon greenhouse gas fluxes on a degraded lowland peatland using micropropagated *Sphagnum* moss in the restoration process

4.1 Introduction

Greenhouse gases (GHGs) have been monitored on peatlands for several decades using a range of equipment, such as eddy covariance towers, closed and open chamber systems, syringe extraction with gas chromatograph analysis, and instantaneous monitoring with portable infra-red gas analysers (e.g., PP Systems EGM) and more recently, the Los Gatos GHG analyser (described below) (see Table 4.9. in Discussion). Experiments also vary widely spatially, temporally, in study selection of peatland-type and condition, and in quality (Haddaway *et al*., 2014; Davidson *et al*., 2016).

Recently, work has been published outlining methods of using vegetation cover as a proxy for measuring GHG fluxes (Dias *et al*., 2010; Couwenberg *et al*., 2011) as a more financially viable evaluation for carbon-offsetting schemes than long-term GHG monitoring. It is extremely useful for large areas through drone or satellite imagery, although it does require strong evidence from robust ground-truthing. This is an attractive scheme, and already utilized in Germany through the 'MoorFutures' scheme associated with Greifswald Mire Centre (MoorFutures, 2019). In the UK, an emissions inventory for UK peatlands is under development, based on peat land-use and condition (Evans *et al*., 2017), although significant gaps in category reporting require further flux measurement data. However, peatlands are complex systems and sites can vary considerably depending on their past and current use, geological origin, restoration management, hydrological stability, and climatic variability (Hall *et al*., 1995; Krüger *et al*., 2015; Waddington *et al*., 2015; Renou-Wilson *et al*., 2019). Even sites which, judging by vegetation cover (e.g., with *Molinia caerulea*) are likely sources of GHGs, can actually be sinks (Jacotot *et al*., 2019).

The primary aim of lowland peatland restoration, however, is to develop a *Sphagnum*-dominated acrotelm of bog vegetation that stabilizes the water-table and protects

current peat stocks in the short-term, and promotes peat-accumulation and carbon storage in the long term (Waddington and Warner, 2001; Quinty and Rochefort, 2003; Lindsay, 2010; Lucchese *et al*., 2010; Waddington *et al*., 2011; Worrall *et al*., 2011). Therefore, regular monitoring of GHG fluxes in situ (Waddington *et al*., 2002) and associated environmental variables, with a consistent approach, such as that devised by Evans *et al*. (2016), is likely to be helpful in determining the potential for CO_2 uptake or emission on a degraded site, and inform restoration management for best outcomes.

To gain a better understanding of how carbon cycling in the ecological system interacts with the atmosphere (graphically described in Figure 4.1) it is necessary to understand and to quantify all the component parts of gaseous carbon exchange (Bussell *et al*., 2010). Measurements of Net Ecosystem Respiration (NER), Gross Primary Productivity (GPP), Net Ecosystem Exchange (NEE – the balance between NER and GPP) and methane flux, at least, should help to clarify the environmental variables driving changes in gaseous exchange, such as water table depth (WTD) (Holden, 2005; Lazcano *et al*., 2020), temperature (Lafleur *et al*., 2005; Carter *et al*., 2012; van Winden *et al*., 2012), redox potential (Tokarz and Urban, 2015), photosynthetically active radiation (PAR) (Frolking *et* al., 1998; Loisel *et al*., 2012), or plant and microbial dynamics (Jassey *et al*., 2013; Lazcano *et al*., 2020).

CO_2 is taken out of the atmosphere (GPP) through plant photosynthesis, and CO_2 is emitted (NER) from soils to the atmosphere through activities in vegetation roots (autotrophic) and soil micro-organisms (heterotrophic) (Bond-Lamberty *et al*., 2004; Lafleur *et al*., 2005). NER appears to be mainly influenced by soil temperature and moisture (Danevčič *et al*., 2010; Wang *et al*., 2014), although autotrophs and heterotrophs have different responses to these influences (Casals *et al*., 2011) and soil moisture may have little impact on autotrophic respiration (Lafleur *et al*., 2005).

CH_4 is produced in waterlogged, anaerobic soils through complex relationships between archaeal methanogens, soil and vegetation (Schimel, 1995; Turetsky *et al*., 2014) and emitted though flux, ebullition or wetland plants, some of which are conduits for CH_4 through aerenchymatous tissues, with larger plants such as *Eriophorum angustifolium* transporting larger amounts (Schimel, 1995; Greenup *et al*., 2000). CH_4 production

fluctuates depending on the availability of carbon, vegetation type and WTD (Schimel, 1995) and some research found that it does not appear to be directly influenced by photosynthesis (Greenup *et al*., 2000; Davidson *et al*., 2016). A WTD below 10 cm is reported to reduce CH_4 production, promote oxidation and prohibit release (Danevčič *et al*., 2010), although Evans *et al*. (2016) report a critical threshold of 25 cm for CH_4 release, which increases as the water table rises, and Davidson *et al*. (2016) report 40 cm and highlight the prominence of vegetation transport. It is also possible that the presence of *Sphagnum* mosses may reduce CH_4 flux due to the presence of methanotrophs which oxidise methane for use by the plant, although this may be temperature or WTD dependent (Kip *et al*., 2010; Larmola *et al*., 2010; van Winden *et al*., 2012).

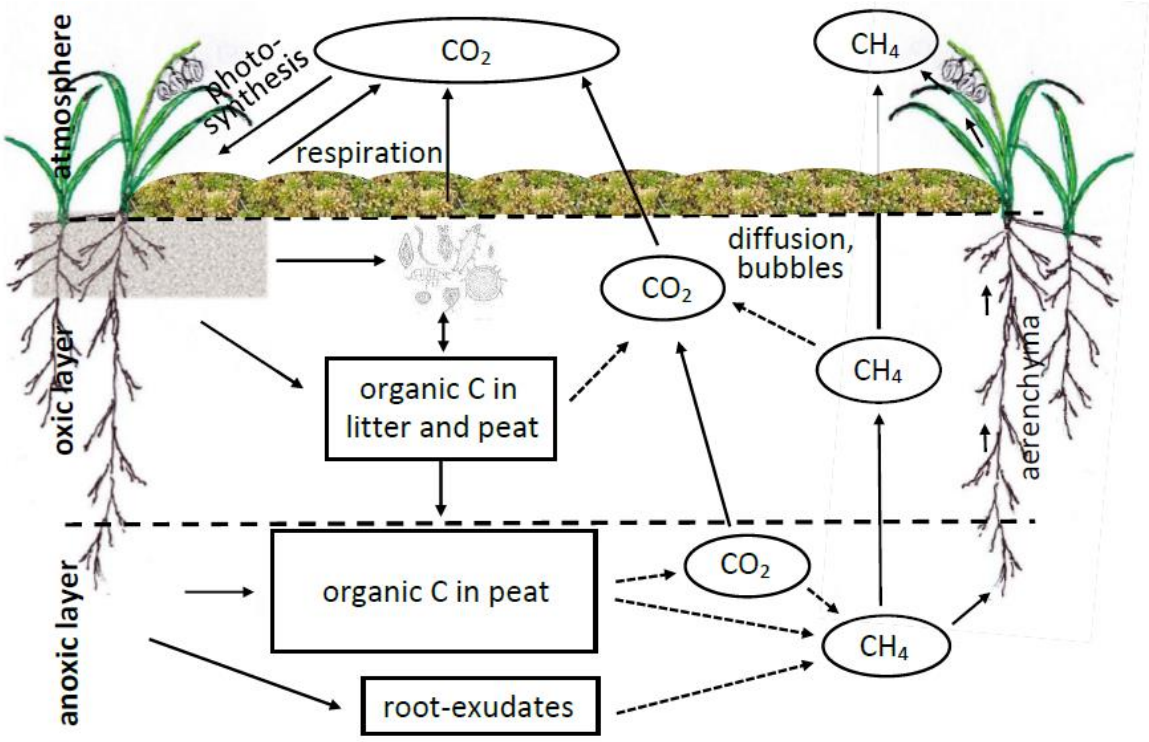


Figure 4.1. Peatland carbon flow between oxic layer, anoxic layer, and atmosphere; dashed arrows show microbial processes; faint arrow = reduced flow. Adapted and redrawn from Rydin and Jeglum, 2013.

Damaged, drained peatlands are a source of GHGs to the atmosphere (Waddington *et al*., 2002; Bonn *et al*., 2016; Evans *et al*., 2016), and restoration efforts are partly driven by the need to reduce emissions to mitigate climate change (Waddington and Warner, 2001). But it is particularly important to include CH_4 measurements when considering climate benefits of peatland restoration (Beetz *et al*., 2013), as the Global Warming Potential (GWP) of CH_4 is estimated to be 28 times higher than CO_2 (Myhre *et al*., 2013). CH_4 flux rates rise when re-wetting a damaged peatland - a key restoration technique (Glatzel *et al*., 2004; Haddaway *et al*., 2014; Evans *et al*., 2016) and wetland plants such as *E. angustifolium*, considered valuable nurse plants for key peat-building *Sphagnum* mosses (Rochefort, 2000; Pouliot *et al.*, 2011) also act as conduits for CH_4. Therefore, understanding, monitoring and managing these diverse factors is essential (Bussell *et al*., 2010). N_2O is also a potent GHG, but on a low-nutrient peatland site, not converted to agriculture, fluxes are generally very low or undetectable (Wilson *et al*., 2013; Beyer and Höper, 2015; Renou-Wilson *et al*., 2019) and were not explored in this study. Fluvial carbon losses, such as dissolved and particulate organic carbon (DOC) and particulate organic carbon (POC) can be significant in peatlands subject to drainage (Evans *et al*., 2016) and upland erosion (Evans *et al*., 2017), neither of which appeared to be significant factors on this study site and were also beyond the scope of this study.

The aims of this Chapter study were to ascertain whether the chosen restoration methods of rewetting and mixed planting of *Eriophorum angustifolium* with tissue-cultured *Sphagnum* moss at a degraded lowland bog were delivering a carbon greenhouse gas (CGHG) sink or source, whether maturity or type of vegetation were key factors, and to develop a greater understanding of the drivers of gaseous carbon flow at this site to inform site management for future beneficial outcomes.

The objectives were to monitor CGHG flux (CO_2 and CH_4) in areas of established *E. angustifolium* with and without *Sphagnum*, and in bare peat, over a period of two years, with accompanying monitoring of environmental variables, water table depth (WTD), and plant growth. [There were no areas with *Sphagnum* only to provide further control plots. Earlier trials of planting *Sphagnum* alone on this site on bare peat, even with mulch, were either not successful or plots were soon colonised by *E. angustifolium*; *Sphagnum* is often only successful when introduced with a nurse crop in a restoration setting (Quinty and

Rochefort, 2003; Pouliot *et al.*, 2011).] The unusually dry and warm summer in year 2 of the study also presented an opportunity to explore the impact of a changing climate on gaseous carbon flow dynamics within this restoring peatland.

These objectives tested the hypotheses that:

1) restoration of the site will result in a CGHG uptake compared to bare peat;
2) CGHG uptake will be greater with maturity of vegetation;
3) greater volumes of *E. angustifolium* will result in greater emission of methane;
4) the presence of *Sphagnum* moss will reduce the magnitude of methane emission;
5) periods of drought will have a deleterious effect on site CO_2 uptake.

4.2 Methods

4.2.1 Study site

Field trials were conducted on Cadishead Moss (53°27'10.8"N, 2°27'11.5"W), an 8 ha Site of Biological Importance (SBI) 10 km WSW of Manchester, UK (see Sections 1.4.1 , 1.4.2 and Appendix 6 for broader site descriptions) at a level elevation of 23 m asl (Figure 4.2). The site is adjacent to peat-extracted Little Woolden Moss, and is a fragment of the once-extensive Chat Moss lowland bog complex, which has been mostly urbanised or drained for agriculture or peat extraction so that only around 1% of the original complex remains as functioning peatland or under restoration management.

Cadishead Moss was originally ditch-drained and block-cut for peat, with some areas mechanically scraped (see Appendix 6), and after abandonment it became colonised by trees and scrub. The Lancashire Wildlife Trust (LWT) acquired the site in 2009, cleared scrub and trees, and rewetted through a series of peat bunds and plastic piling, leaving some deep internal ditches remaining. There is now a good coverage of *Eriophorum angustifolium* in wetter areas and *Molinia caerulea* in drier areas, with minimal areas of bare peat.

Figure 4.2. a) Location map and co-ordinates of Cadishead and Little Woolden Moss, close to the conurbation of Manchester, UK. Map from Multi-Agency Geographic Information for the Countryside (MAGIC) (https://magic.defra.gov.uk/MagicMap.aspx). b) Location of Cadishead Moss, and surrounding landscape matrix (blue dot marks the trial location). Map from EDINA Digimap Ordnance Survey Service https://digimap.edina.ac.uk/aerial

Weather data from Rostherne No2 weather station (NOAA, 2020), which is 11 km SE of Cadishead Moss (53°21'35.3"N, 2°22'49.8"W) shows annual rainfall was 807 mm yr^{-1} and mean air temperature was 4.9 °C in January, 17.3 °C in July and 10.4 °C annually over the study period from 1 September 2016 to 31 August 2018. The nearest Met Office data for Long Term Average (LTA) (1981 to 2010) rainfall (monthly mean) and air temperature

(monthly mean calculated by averaging mean maximum and minimum temperatures) was available from Woodford Meteorological Station (53°20'24.0"N, 2°09'14.4"W), 20 km SE of the site.

4.2.2 Field plots

Permanent collars were installed in areas of mature and immature *Eriophorum angustifolium*, with and without *Sphagnum*, and bare peat (i.e., five treatments) (Figures 4.3 and 4.4). Collars were cut from 300 mm internal diameter plastic waste pipe to 100 mm length and eased into the peat, leaving 40 - 50 mm standing above the surface. Tissue-cultured *Sphagnum* moss from the BeadaMoss® company, in the commercial BeadaGel™ mix (11 species of *Sphagnum* suspended in a hydrocolloidal gel) was applied at 3 litres m^{-2} in 1 m^2 areas with an immature, open sward of *Eriophorum angustifolium* (with collars in situ) to allow the gel to reach the peat surface. Preliminary measurements were made after several months, and regular measurements after one year, so that decomposition by any vegetation and roots damaged by collar placement did not influence GHG flux results (Rowson *et al*., 2013).

Six trial plots were established in October 2015 (Plots 4 - 9, termed 'immature' plots) in areas of sparse *E. angustifolium* growth. Each plot contained one collar of *E. angustifolium* (IEA), one collar in a 1 m x 1 m area of *E. angustifolium* plus new *Sphagnum* BeadaGel™ application (IEAS), and one collar of bare peat (Bare). BeadaGel™ was slow to establish during the early stages of the trial in 'immature' plots with sparse *E. angustifolium* cover. Straw mulch was initially used on plots established for an earlier growth trial in June 2014 ('mature plots – see below) to aid *Sphagnum* establishment (none remained at the start of this study), but may have interfered with results if applied at this stage in this trial. Hence, a decision was made to reapply BeadaGel™ at the same rate, 6 months after the start of GHG measurements, within the collar area only, and to provide removable environmental protection (to simulate straw mulch) in the form of greenhouse shading mesh, which remained in place until the end of the trial.

Three further plots (Plots 1, 2 and 3, termed 'mature' plots) were set up at the same time on areas established in June 2014 for a previous study, to allow monitoring at a more

advanced stage of BeadaGel™ growth in a tall, dense *E. angustifolium* sward. Each plot contained two collars in a 1 m x 1 m area of *E. angustifolium* plus *Sphagnum* (MEAS) and one collar of mature *E. angustifolium* (MEA) nearby. There was no bare peat in areas of mature *E. angustifolium*, so bare peat collars are only associated with immature vegetation plots. A table of the number of collars and type of plots is given in Table 4.1 and a diagrammatic illustration of plot set-up is given in Figure 4.3. All vascular plants were removed from bare plots (throughout the trial), and all vegetation other than *E. angustifolium* removed from vegetated plots. This was done when plants were tiny seedlings to minimise plot disturbance.

A plot dipwell was inserted close to each cluster of collars, within 1.5 m of each collar, to monitor water table levels, and measurements were recorded manually every week where possible, or a minimum of fortnightly. Dipwells were made from 1.5 m lengths of 40 mm diameter PVC pipe, and inserted 1 m into the ground (leaving 50 cm above the surface). Holes were drilled spirally every 10 cm along the length of the below-ground section, which was sealed at the base and covered with fine mesh material to prevent ingress of peat particles. Two small holes were drilled 10 cm below the top to prevent a vacuum within the pipe, and the top capped with flexible plastic to keep out rain and debris. Three measurements from the ground level to the top of the pipe were averaged and subtracted from the distance between the water table and the top of the pipe to calculate the level of the water table below the ground surface.

4.2.3 Gas flux and environmental monitoring

Carbon greenhouse gases (CO_2 and CH_4) were measured using an LGR™ 'Ultraportable Greenhouse Gas Analyser (CH_4,CO_2,H_2O)' (manufactured by Los Gatos Research, San Jose, California, USA), which has < 2 ppb precision for CH_4 and < 300 ppb precision for CO_2 (at a 1 second measurement rate). A closed chamber system was created using a clear (Perspex) chamber whereby changes in gas uptake or emission due to plant and soil photosynthesis and respiration could be measured in real-time. A Perspex extension was used for taller vegetation, with a partially inflated rubber tyre attached to create a good seal between the collar and the chamber and extension (Figure 4.5) similar to methods used on automated chambers at Mer Bleue bog (Lai *et al*., 2012).

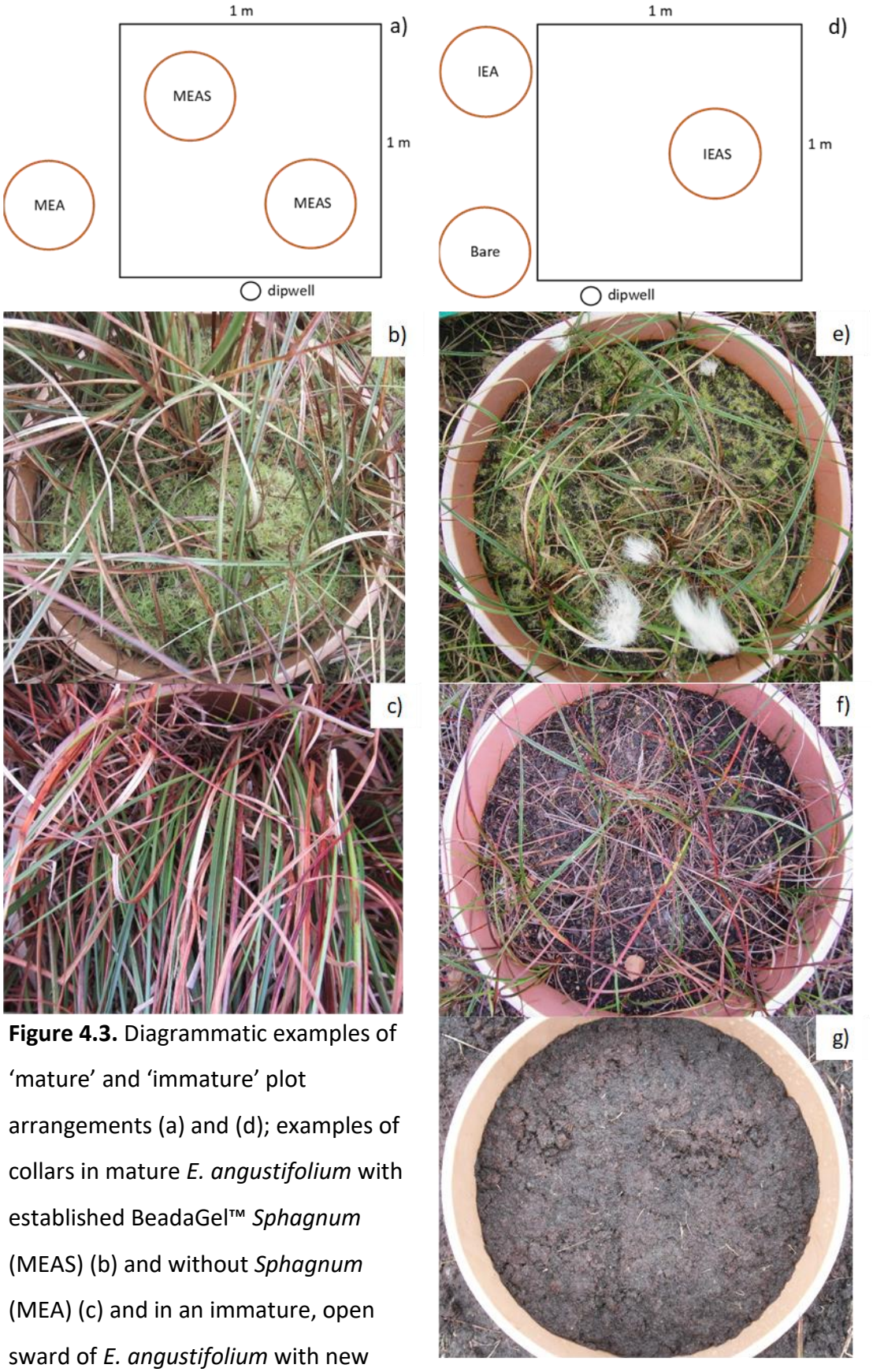


Figure 4.3. Diagrammatic examples of 'mature' and 'immature' plot arrangements (a) and (d); examples of collars in mature *E. angustifolium* with established BeadaGel™ *Sphagnum* (MEAS) (b) and without *Sphagnum* (MEA) (c) and in an immature, open sward of *E. angustifolium* with new BeadaGel™ *Sphagnum* application (IEAS) (e) and without *Sphagnum* (IEAS) (f), and in Bare peat (g).

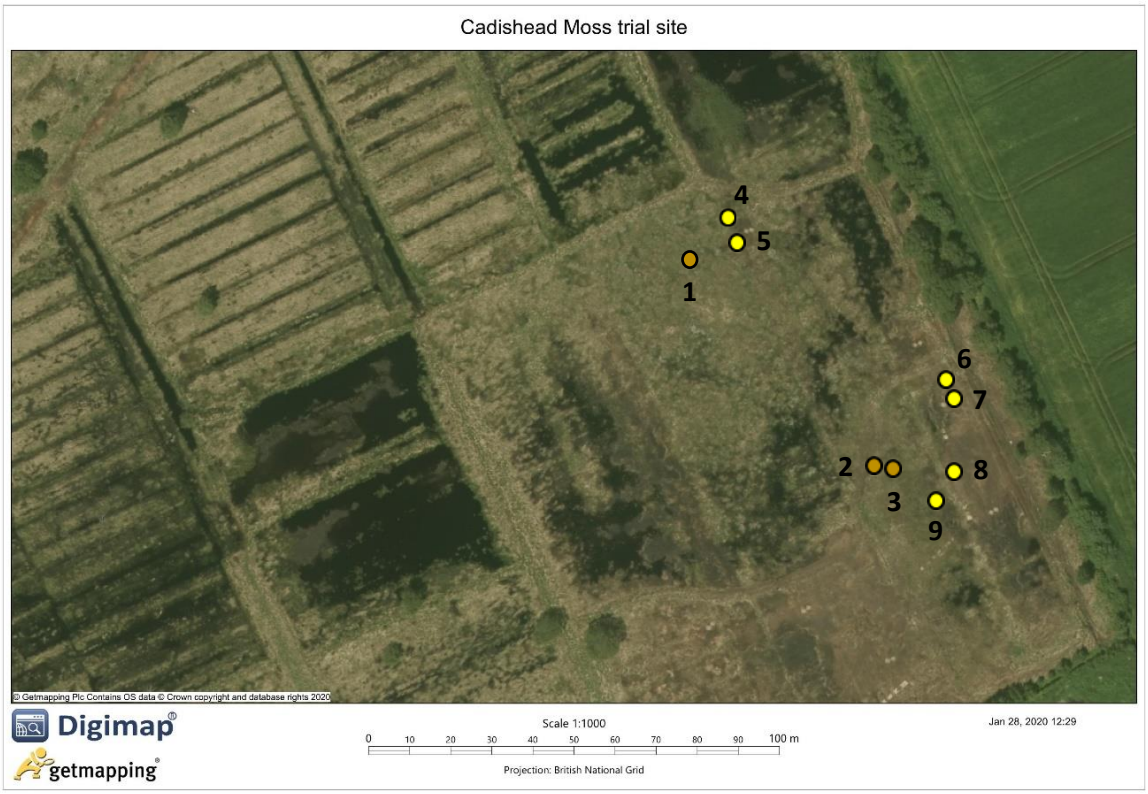


Figure 4.4. Detailed aerial photograph of trial site area on Cadishead Moss, showing remains of old drainage system and existing baulks, new bunds and inundated areas. Mature plots: orange dots; immature plots: yellow dots. Plots 1 - 5 and 9 in areas of increasingly mature *Eriophorum angustifolium* cover, with existing areas of immature growth; plots 6, 7 and 8 on bare peat area with patches of sparse *E. angustifolium* cover, close to the eastern edge of the site. Using: EDINA Digimap Ordnance Survey Service https://digimap.edina.ac.uk/aerial

Table 4.1. Number of collars and type of field plots.

No of replicates	Bare peat (control)	*E. angustifolium*	*E. angustifolium* plus BeadaGel™
Jun 2014 plots 'Mature'	0	3	6
Oct 2015 plots 'Immature'	6	6	6

Figure 4.5. Closed-chamber system with Los Gatos GHG analyser, showing transparent chamber with extension for tall vegetation.

Gases were directed around the chamber via a ring of tubing, pierced at 1 cm intervals and blocked with silicone gel half-way along to prevent gas cycling in the tube. Each end of the tubing ring was attached via more tubing through the chamber to the Los Gatos analyser (LG) inlet and outlet ports. A small (9v) fan in the chamber ensured good gas circulation (tested prior to use). The gas temperature was continuously recorded by the LG.

Carbon greenhouse gas (CGHG) fluxes were recorded from each collar fortnightly during the growing season and monthly during winter for two full years between September 2016 and August 2018, resulting in 33 monitoring visits. All the collars were monitored on each visit, in a random order. PAR (photosynthetically active radiation, measured in µmol m^{-2} s^{-1}), peat temperature at 5 cm depth and water table depth were recorded during measurements, as recommended by Alm *et al*. (2007). A soil temperature probe (Delta-T Devices Ltd) and PAR meter (Skye Instruments Ltd) were attached to a GP1 Delta-T logger, recording measurements at 10 s intervals.

Dark and light measurements were each taken over a 2 minute period (Davidson *et al*., 2016) with the chamber firstly obscured with a blackout cloth, then aerated before a measurement with the chamber uncovered, to obtain measurements of CO_2 respiration (NER) and net ecosystem exchange (NEE) and CH_4 flux.

4.2.4 Volume of vegetation and LG chamber space within collars

The volume of space within the LG chamber and extension (headspace) were known constants. The volume of space within the collar was variable due to changes in the volume of vegetation and swelling/shrinkage of the peat. The depth from top of collar to peat substrate or *Sphagnum* hummock surface was measured at six internal peripheral positions after each gas measurement, and the volume within the collar calculated and added to the headspace volume for flux calculations. If the *Sphagnum* hummock surface was above the collar top, the distance between the hummock top and the collar top were measured after each gas measurement at four internal peripheral positions (midway between centre and edge of collar) and at the highest point (usually central), to obtain a mean height above the collar, giving an internal collar volume which was negative. The volume of *E. angustifolium* (measured monthly) was subtracted from the headspace when calculating fluxes. *E. angustifolium* and *Sphagnum* measurements were also used to assess plant competition and any influence of the changing volume of vegetation on CGHG fluxes over time.

The volume of *E. angustifolium* within collars was calculated by monthly measurements of the number and length of plants (longest leaf – as outlined by Davidson *et al*., 2016). If there was dense growth (i.e., more than about 40 plants), the number of plants was counted and 10% of representative plants at random peripheral and central positions were measured as a mean. Seasonally, *E. angustifolium* plants from site (not within collars) were cut, measured (longest leaf), and the volume of each plant found via water displacement. A scatter graph was plotted of length against volume for use with calculations of plant volume in each collar, with a separate graph for flowering/seeding plants (Spring, Autumn and Winter R^2 = 0.7 to 0.8; Summer R^2 = 0.54 [non-flowering] to 0.6 [flowering plants]).

Sphagnum volume within collars was calculated by measuring depth to the substrate (which was firm) with a narrow, blunt-ended rod through the hummock at nine positions to obtain mean height. Volume was estimated using a cylinder equation with the height and estimated percentage area cover within each collar. Care was taken to create as little damage to the *Sphagnum* as possible when taking measurements.

4.2.5 CGHG measured flux data management

CGHG flux measurements were downloaded from the LG onto Excel spreadsheets and fitted to an Excel linear regression model to obtain gradient, R^2 and *p*-values for each 2-minute measurement. Graphs were used to visualise and remove erroneous start or end measurements, as recommended by Evans *et al*. (2016). The maximum number of observations (seconds) retained per measurement was 124 and the minimum number was 60, apart from two instances where a dramatic change in PAR during measurement reduced a representative measurement to less than 60 observations. Erroneous periods of measurement could be due to test error or, particularly in summer light levels, low CO_2 availability in the chamber due to high uptake by plants, causing a reduction in slope. Thresholds of $R^2 > 0.7$ and $p < 0.05$ were used to accept measurements, similar to protocols used by Evans *et al*. (2016). If neither of these conditions were met the measurement was discarded, but as CO_2 and CH_4 gases were measured in tandem, if one measurement met the criteria a system integrity failure was deemed unlikely and both gas measurements were retained. Only 1% of all flux measurements were discarded.

Fluxes were calculated using the following equation [eqn 1] adapted from Dossa *et al*. (2015):

$$Flux = \frac{\Delta CO2}{t} * \frac{PV}{RT} * \frac{1}{As} * \left(\frac{44*60*60}{1000}\right) \quad [1]$$

[Flux = g CO_2 (or CH_4) m^{-2} h^{-1}; P (atm) = atmospheric pressure; V (m^3) = chamber volume; R (L atm mol^{-1} K) = universal gas constant; T (K) = gas temperature in Kelvin; A_s (m^2) = surface area within collar; 44 g mol^{-1} = molecular weight of CO_2 (or 16 g mol^{-1} = molecular weight of CH_4)].

Measurements from the dark and light chambers give Net Ecosystem Respiration (NER) and Net Ecosystem Exchange (NEE) respectively. Gross Primary Productivity (GPP) was calculated [eqn 2] and values used for further analysis of flux data.

$$NEE = GPP + NER \quad [2]$$

The micrometeorological sign convention was adopted, whereby negative fluxes indicate removal from the atmosphere and positive fluxes indicate addition to the atmosphere.

Methane fluxes were calculated from measurements in the dark. CO_2 equivalents of CH_4 were calculated as GWP_{100} x 28 (Myhre *et al*., 2013) as adopted in the 1997 Kyoto Protocol, when calculating CGHG budgets in g CO_{2e} m^{-2} yr^{-1}.

4.2.6 Measured data statistical analysis

Collars are within five treatments groups: Mature *Eriophorum angustifolium* plus *Sphagnum* (MEAS), Immature *E. angustifolium* plus *Sphagnum* (IEAS), Mature *E. angustifolium* only (MEA), Immature *E. angustifolium* only (IEA) and Bare peat (Bare). Measured vegetation and flux data from all treatment groups were tested for normality using Shapiro Wilk tests, and found to be not normally distributed. A non-parametric test for repeated measures (Friedman's test) was used to determine any statistically significant difference between groups and post hoc analysis of flux data with Wilcoxon signed-rank tests was conducted with a Bonferroni correction applied. Data were analysed statistically using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp and also through the data analysis tools in Microsoft Excel 2019.

4.2.7 CGHG flux data modelling

Hourly air temperature (°C), rainfall (mm) and total solar radiation (W m^{-2}) datasets for the full period of measurement (1 September 2016 to 31 August 2018) were provided by the Whitworth Meteorological Observatory (Centre for Atmospheric Science, 2020) at the University of Manchester, 14.8 km ENE of the study site. Conversion of W m^{-2} to

Photosynthetically Active Radiation (PAR) µmol m^{-2} s^{-1}, assuming PAR (400 to 700 nm) is 45% of total solar radiation, is: 1 W m^{-2} ≈ 2.1 µmol m^{-2} s^{-1} (Biggs, 1984).

A linear regression between peat temperature (PT) and air temperature (AT) data from micrometeorological equipment on nearby Little Woolden Moss (LWM) was applied to Whitworth Observatory AT data to provide integrated PT data for the period of CGHG measurement. Water table depth (WTD) data, measured weekly to fortnightly throughout the study period, was infilled, assuming linear changes between measurement points, to provide an estimated hourly dataset (Alm *et al*., 2007; Renou-Wilson *et al*., 2019).

The measured CGHG flux data was plotted against measured environmental variables of PT, WTD and PAR for each collar and the linear regression R^2 values ranked to determine which variables best explained the measured values of NER, GPP and methane fluxes (Appendix 2) (i.e., primary, secondary and tertiary drivers). Non-linear regression (exponential) equations were fitted between each measured flux and primary driver to create each primary model [eqn 3]. Data from this model were subtracted from measured flux data, to leave residual data. Linear regression equations from residual data were fitted with the secondary driver (and subsequently with the tertiary driver, where used) [eqn 4] and added iteratively to the primary model to create a final model [eqn 5], checking for goodness of fit with measured flux data at each stage. Each equation was applied to hourly environmental data to provide an integrated model for gaseous fluxes from each treatment collar. Modelled values for GPP were altered to zero when PAR was zero. NEE was calculated from NER and GPP [eqn 2]. All data was manipulated through data analysis tools in Microsoft Excel (2019).

$$y_1 = abx_1 \quad [3]$$

$$y_2 = mx_2 + c \quad [4]$$

$$f_m = abx_1 + [(f - abx_1)\, x_2 + c] \quad [5]$$

Where:

f and f_m = measured and modelled flux (g CO_2 m^{-2} h^{-1})
x = independent environmental variable [PT (°C) or WTD (cm) or PAR (µmol m^{-2} s^{-1})]
y_1: exponential regression ($f:x_1$)
y_2: linear regression ($residual:x_2$)

a = co-efficient (or y-intercept)
b = exponent
c = co-efficient (or y-intercept)
m = line gradient

4.2.8 Modelled annual CGHG flux analysis

An annual CGHG flux value for each collar was calculated from the sum of each modelled flux dataset for each collar for each full year of study (September to August). Annual flux measurements for each treatment group (MEAS, MEA, IEAS, IEA and Bare) were calculated as the mean of annual data from each monitoring collar within treatments. CO_2 equivalents of CH_4 were calculated by multiplying methane values with a Global Warming Potential $(GWP)_{100}$ of 28 and adding to NEE (balance of NER and GPP) values to give a CGHG budget in g CO_{2e} m^{-2} y^{-1}. Data were analysed statistically using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Data were tested for normality using Shapiro Wilk tests, and found to be normally distributed. Two-way ANOVA with post-hoc Tukey HSD tested differences between treatment groups and also between years within treatments groups.

4.3 Results

4.3.1 Weather data

Monthly rainfall (Figure 4.6a) ranged from 24.1 to 93.7 mm in year 1 (September 2016 to August 2017) and from 14.3 to 124.2 mm in year 2 (September 2017 to August 2018). Monthly mean temperatures (Figure 4.6b) ranged from 5.6 ± 2.6 to 16.9 ± 3.0 °C in year 1 and from 3.5 ± 2.9 to 19.2 ± 3.6 °C in year 2. Monthly PAR ranged from 117.3 ± 113.0 to 599.9 ± 536.4 µmol m^{-2} s^{-1} in year 1 and from 113.2 ± 106.3 to 730.2 ± 580.5 µmol m^{-2} s^{-1} in year 2. Overall, rainfall, air temperature and PAR across the study period were more variable in year 2 than year 1, and there was disparity in the values on a seasonal basis (Table 4.2). Seasons are here defined by calendar months: Spring: March, April, May; Summer: June, July August; Autumn: September, October, November; Winter: December, January, February. Both years showed differences from the Met Office sourced long-term average (LTA) for the area; notably, there were slightly higher measured temperature values than the LTA values throughout. Despite higher-than-average rainfall in the

preceding three seasons, the combination of high PAR and air temperature and low rainfall in May, June, July and August of year 2 contributed to a prolonged drought which caused a dramatic drop in WTD in the 2nd summer (Figure 4.6a) to -48 ± 5.9 and -54 ± 6.9 cm (lowest mean value) in mature and immature plots respectively, 2.1 and 1.7 times (respectively) below the lowest points in the 1st summer.

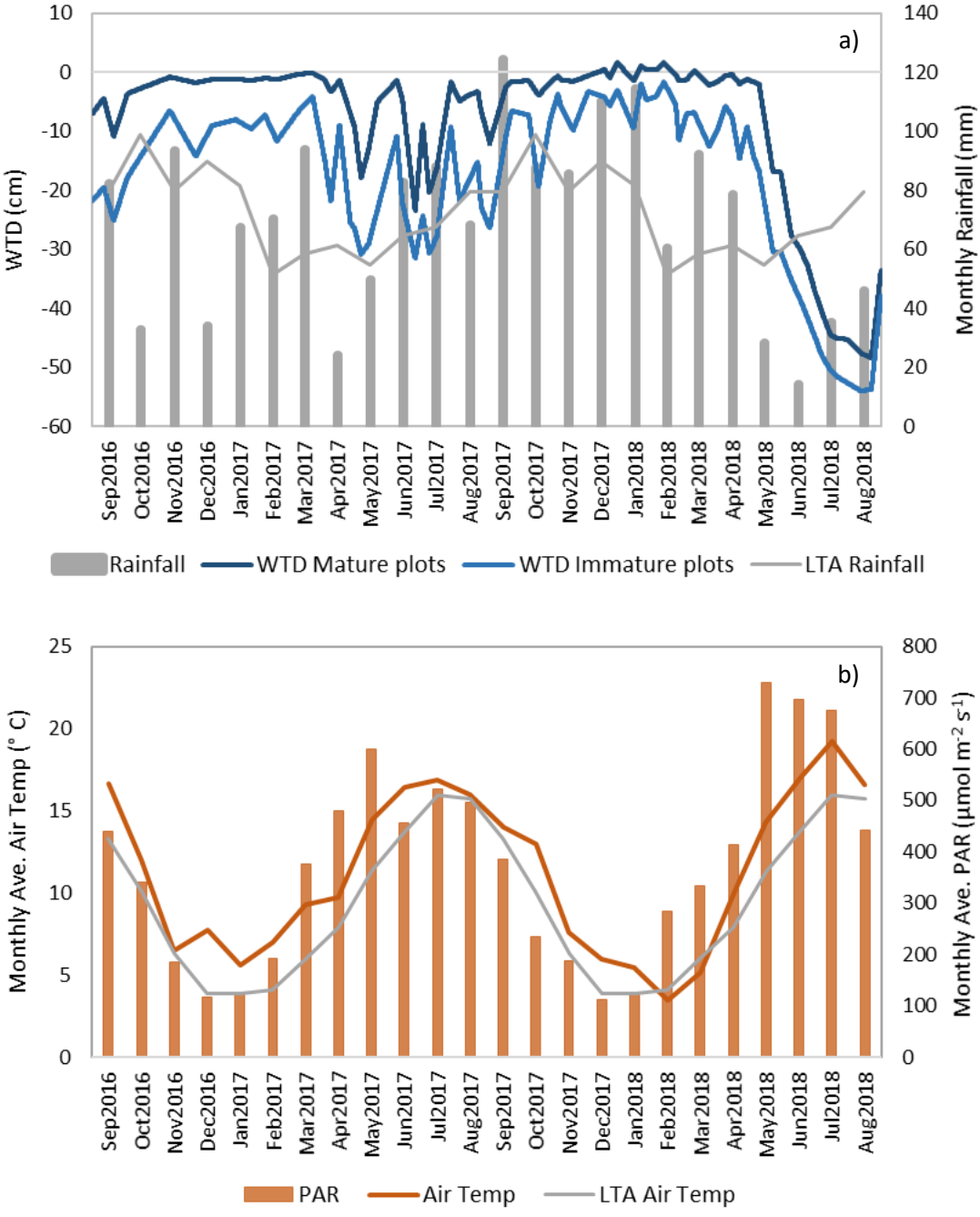


Figure 4.6. a) Monthly rainfall, long term average (LTA) rainfall, and corresponding mean water table depth (WTD) below the surface, measured across treatment plots grouped by vegetation maturity; b) Monthly average PAR (mean of hourly daylight data) and air temperature (mean of hourly data); study period September 2016 to end-August 2018.

Table 4.2. Seasonal environmental variables and WTD, with LTA data for comparison. Mean values ± SD.

Season / Year	RF (mm)	LTA RF (mm)	WTD (M) (cm)	WTD (I) (cm)	AT (°C)	LTA AT (°C)	PAR ($\mu mol\ m^{-2}\ s^{-1}$)
Autumn 2016	208.9	258.3	-3.7 ± 2.7	-14.9 ± 5.5	11.7 ± 5.0	9.9	158 ± 271
Winter 2016/7	171.9	222.8	-1.3 ± 0.2	-9.7 ± 1.7	6.8 ± 2.9	3.9	55 ± 120
Spring 2017	167.4	174.8	-4.6 ± 5.0	-16.7 ± 8.6	11.2 ± 4.2	8.4	302 ± 434
Summer 2017	239.0	211.2	-8.7 ± 6.2	-21.2 ± 6.0	16.4 ± 3.3	15.1	339 ± 438
Autumn 2017	297.4	258.3	-2.9 ± 2.7	-10.9 ± 5.8	11.5 ± 4.0	9.9	132 ± 236
Winter 2017/8	285.3	222.8	0.1 ± 0.8	-4.8 ± 2.1	5.0 ± 3.0	3.9	67 ± 150
Spring 2018	199.0	174.8	-3.8 ± 5.4	-13.8 ± 7.6	9.8 ± 5.4	8.4	306 ± 452
Summer 2018	95.8	211.2	-39.2 ± 8.0	-46.4 ± 7.0	17.6 ± 3.8	15.1	428 ± 526

RF = seasonal amount of rainfall; LTA = long-term average (1981 - 2010); WTD = water table depth below the surface; M = mature plots; I = immature plots; AT = monthly mean air temperature. PAR includes night hours. RF, AT and PAR data for study period sourced from Whitworth Observatory at the University of Manchester (http://www.cas.manchester.ac.uk/restools/whitworth/); LTA RF and AT sourced from the Met Office (https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcqrqyr80)

4.3.2 Vegetation data

The volume of *E. angustifolium* in both MEAS and IEAS collars (Mature and Immature *E. angustifolium* with *Sphagnum*, respectively) (Figure 4.7a and b) increased rapidly from April and reduced from October, with a greater volume in the 2nd than in the 1st winter. The MEAS *E. angustifolium* summer peak volume was similar in years 1 and 2, but less than the end-summer volume at the start of the project, whereas the IEAS *E. angustifolium* summer volume increased over the project period. *Sphagnum* volume in both MEAS and IEAS collars levelled off from October/November each year. In MEAS collars there was an increase in *Sphagnum* volume during Spring and Summer in year 1 but a gradual decrease in year 2, whereas in IEAS collars *Sphagnum* volume increased

rapidly from late Spring in year 1 (re-application in April) to mid-Autumn, and continued to increase in volume overall in year 2, although at a reduced rate during the summer drought.

The volume of *E. angustifolium* in both MEA and IEA collars (Mature and Immature *E. angustifolium*-only, respectively) (Figure 4.7 c) changed similarly over the study period. Growth increased from April and reduced from October (more quickly in IEA collars), but levelled off during the year 2 Summer drought (reducing slightly in IEA collars).

There was a statistically significant difference in the volume of *E. angustifolium* between MEAS and MEA plots: $\chi^2(2) = 16.860$, $p < 0.001$, $df = 1$ as determined by a Friedman Test. The volume of *E. angustifolium* was greater in MEA than in MEAS plots (Table 4.7). Moreover, in MEAS plots, *E. angustifolium* volume reduced slightly in year 2, whereas in MEA plots volume increased by 23.5%. There was a statistically significant difference in the volume of *E. angustifolium* between IEAS and IEA plots: $\chi^2(2) = 20.211$, $p < 0.001$, $df = 1$ as determined by a Friedman Test. The volume of *E. angustifolium* was increasingly greater in IEAS than in IEA plots (Table 4.7); by 21.2% in year 1 and by 42.3% in year 2.

Sphagnum volume (zero values in IEAS year 1 removed) increased from year 1 to year 2 (Table 4.7), in MEAS plots by 37.8% and in IEAS plots by 314%. However, during the year 2 summer drought there was obvious drying of vegetation (Figure 4.8a and b). *Sphagnum* became bleached and the action of taking measurements on collars containing mature *Sphagnum* (which had grown above the top of the collar) isolated material within the collar from the surrounding *Sphagnum* carpet, creating a dry edge effect. This was less pronounced in collars of immature vegetation, as *Sphagnum* was shorter than the collar top and also benefitted from mesh shading. Peat shrank in Bare treatments, and in some collars of immature *E. angustifolium*, creating a gap between peat and collar (Figure 4.8c and d).

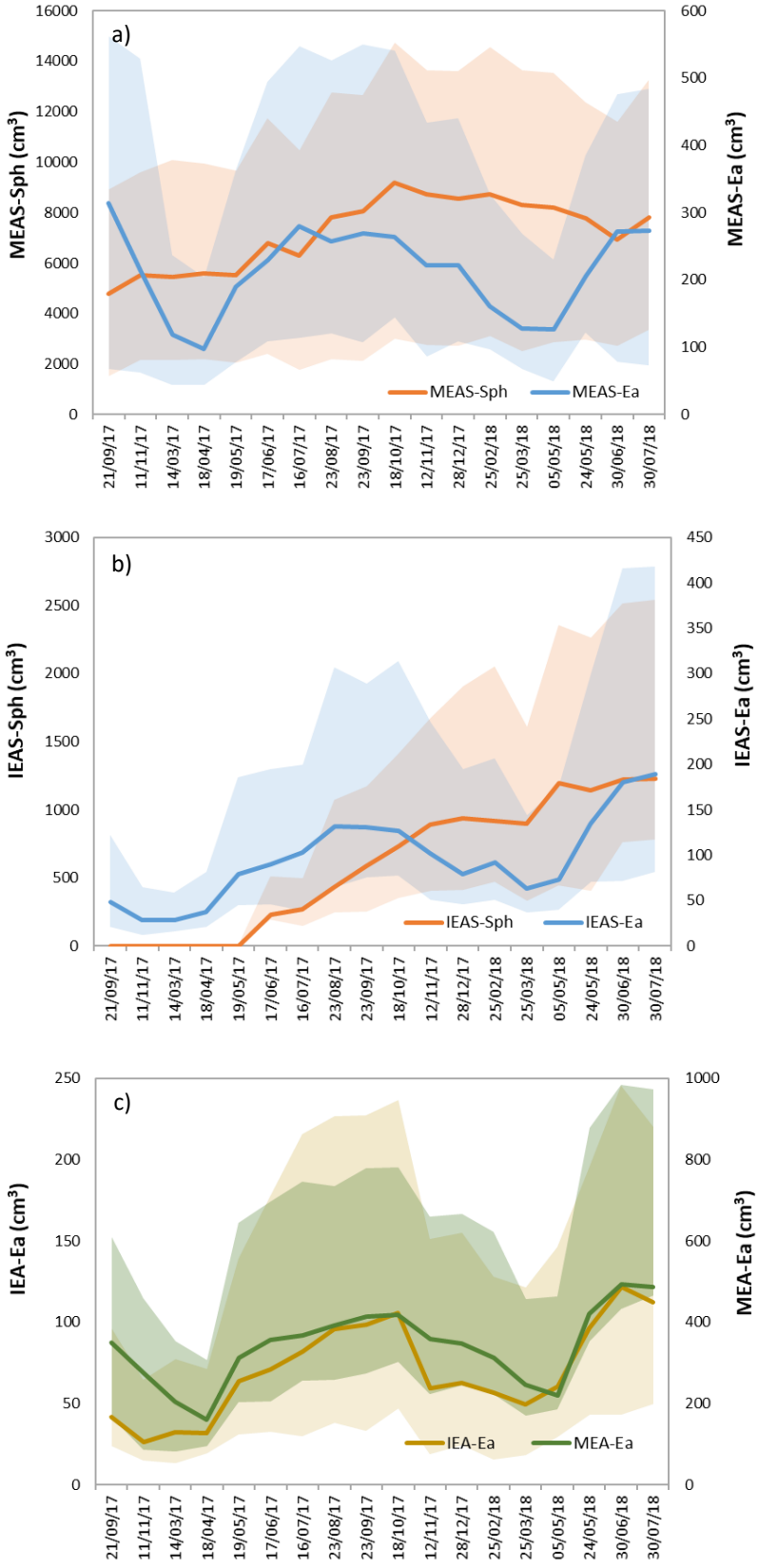


Figure 4.7. Change in collar vegetation volume (mean values per treatment; shading indicates min/max range) over the study period; a) and b) *E. angustifolium* with *Sphagnum*, MEAS and IEAS respectively (note, *Sphagnum* re-applied to IEAS collars in April 2017); c) *E. angustifolium* MEA and IEA.

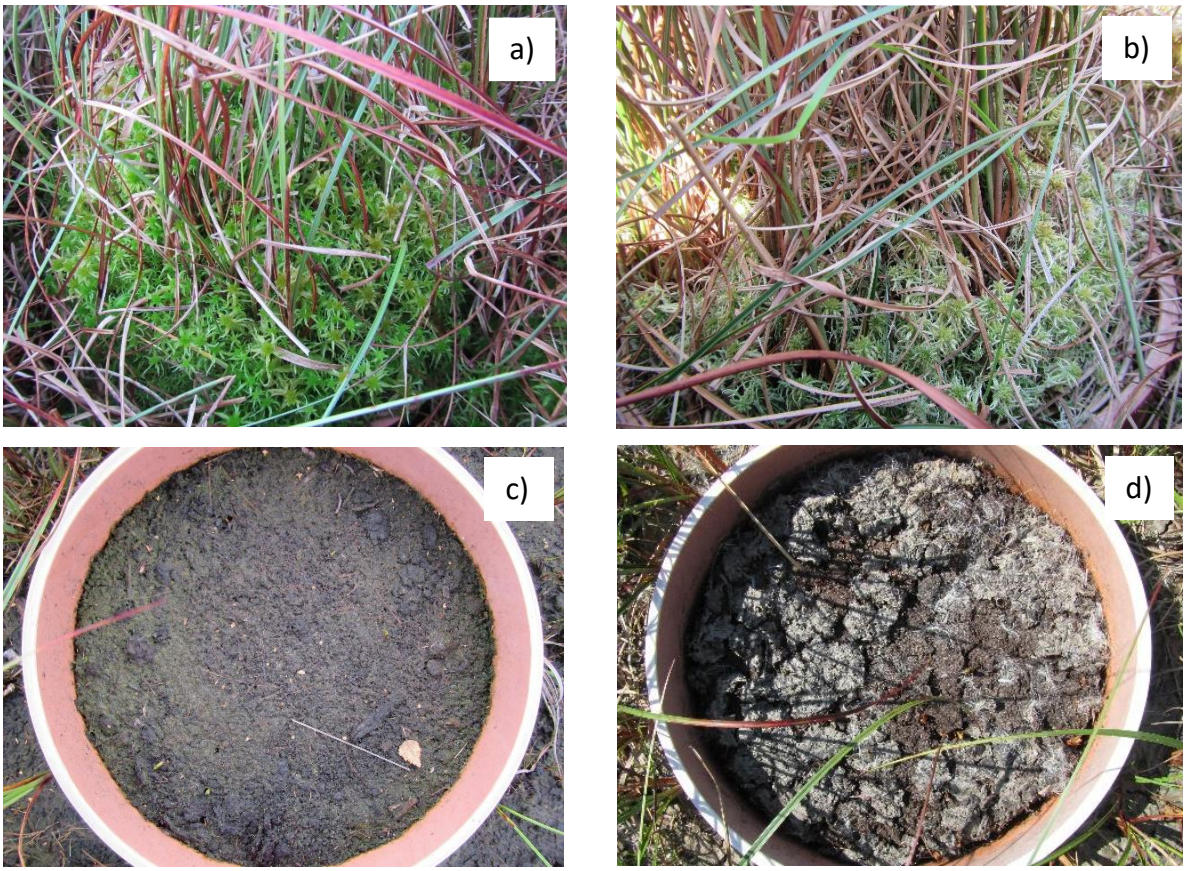


Figure 4.8. Examples of changes in condition of vegetated and bare collars between Autumn 2017 (left) and July 2018 (right).

4.3.3 Measured flux data

4.3.3.i Overview

Following the protocols described in section 4.2.3, the closed chamber system and the Los Gatos analyser delivered results with a good degree of accuracy (94% above the 0.7 R^2 threshold criteria), with very few (1%) discarded measurements. Reporting of flux measurements is by replicates of chamber measurements for each treatment across the study period or for each study year (September to August). Measured fluxes of CO_2 and CH_4 (Figures 4.9 to 4.12) followed the seasonal pattern of rising towards higher summer temperatures and plant and microbial activity, and falling towards lower winter temperatures, plant senescence and reduced microbial activity. The fluxes from mature vegetation, particularly MEA, were greater and more variable than those from immature vegetation. Bare plots had some GPP (Figure 4.10) due to algal growth and patches of

acrocarpous mosses, but NER was the strongest flux, resulting in an emission of CO_2 for much of the year, and methane fluxes (Figure 4.12) were very low throughout.

As data for measured flux measurements were not normally distributed, statistical differences were determined by Friedman Tests, and post hoc analyses with Wilcoxon signed-rank tests, conducted with a Bonferroni correction applied, resulting in a significance level set at $p < 0.0083$ throughout.

4.3.3.ii CO_2 fluxes

There was a statistically significant difference in measured NER fluxes between different treatments ($\chi^2[4, n = 99] = 287.141$, $p < 0.001$). Post hoc tests showed statistically significant differences in NER fluxes between MEAS and MEA treatments ($Z = -6.949$, $p < 0.001$) (MEA CO_2 emission > MEAS) whereas differences between IEAS and IEA were not significant. Differences between Bare and all vegetated plots were statistically significant at $p < 0.001$ (vegetated plot CO_2 emissions > Bare).

There was a statistically significant difference in measured GPP fluxes between different treatments ($\chi^2[4, n = 99] = 257.130$, $p < 0.001$). Post hoc tests showed statistically significant differences in GPP fluxes between MEAS and MEA treatments ($Z = -6.070$, $p < 0.001$) (MEA CO_2 uptake > MEAS), and between IEAS and IEA treatments ($Z = -5.993$, $p < 0.001$) (IEAS CO_2 uptake > IEA). Differences between Bare and all vegetated plots were statistically significant at $p < 0.001$ (vegetated plots CO_2 uptake > Bare).

There was a statistically significant difference in measured NEE fluxes between different treatments ($\chi^2[4, n = 99] = 167.064$, $p < 0.001$). Post hoc tests showed statistically significant differences in NEE fluxes between MEAS and MEA treatments ($Z = -4.904$, $p < 0.001$) (MEA CO_2 uptake > MEAS), and between IEAS and IEA treatments ($Z = -7.123$, $p < 0.001$) (IEAS CO_2 uptake > IEA). Differences between Bare and all vegetated plots were statistically significant at $p < 0.001$ (Bare plots overall CO_2 emission; vegetated plots overall CO_2 uptake).

4.3.3.iii CH_4 flux

There was a statistically significant difference in measured methane fluxes between different treatments (χ^2[4, n = 99] = 223.055, p < 0.001). Post hoc tests showed statistically significant differences in methane fluxes between MEAS and MEA treatments (Z = -2.841, p = 0.004) (MEA methane emission > MEAS) whereas differences between IEAS and IEA were not significant. Differences between Bare and all vegetated plots were statistically significant at p < 0.001 (vegetated plots methane emission > Bare).

4.3.3.iv Seasonal fluxes

The pattern of CO_2 emission by NER across treatments was MEA > MEAS > IEAS >< IEA > Bare in each season (Table 4.3). Uptake of CO_2 by GPP (and generally by NEE) followed a similar pattern (although IEAS > IEA), until year 2 spring and summer, when IEAS > MEAS. Methane emissions were highest in MEA plots and lowest in Bare plots. All other treatments had similar levels of methane emission, but values were low throughout.

In each season there was generally an increase from year 1 to year 2 in CO_2 emissions by NER (not MEAS in summer). There was also an increase in uptake of CO_2 by GPP from year 1 to year 2 until the summer season, when the uptake in year 2 was less than year 1. This resulted in reduced CO_2 uptake by NEE in both spring and summer of year 2 compared to year 1 in MEAS, MEA and IEA (IEAS summer only) and an increased CO_2 emission in Bare plots in year 2 spring and summer.

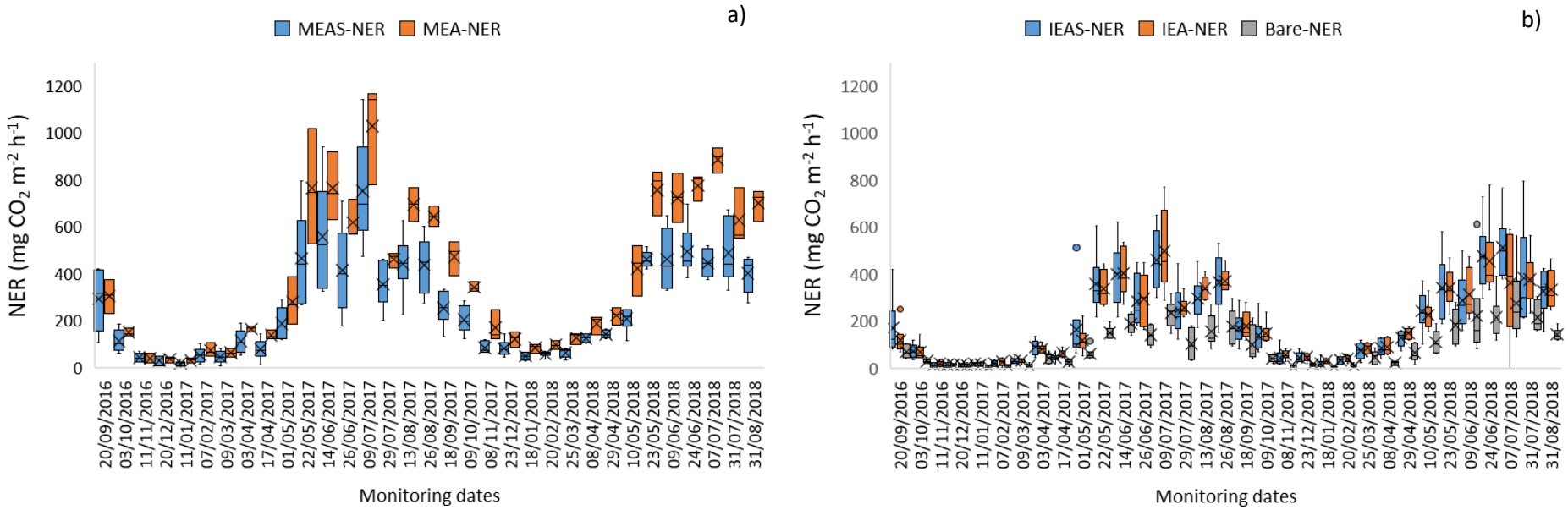


Figure 4.9. Measured NER flux values for each measurement visit in (a) mature and (b) immature plots. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is exclusive.

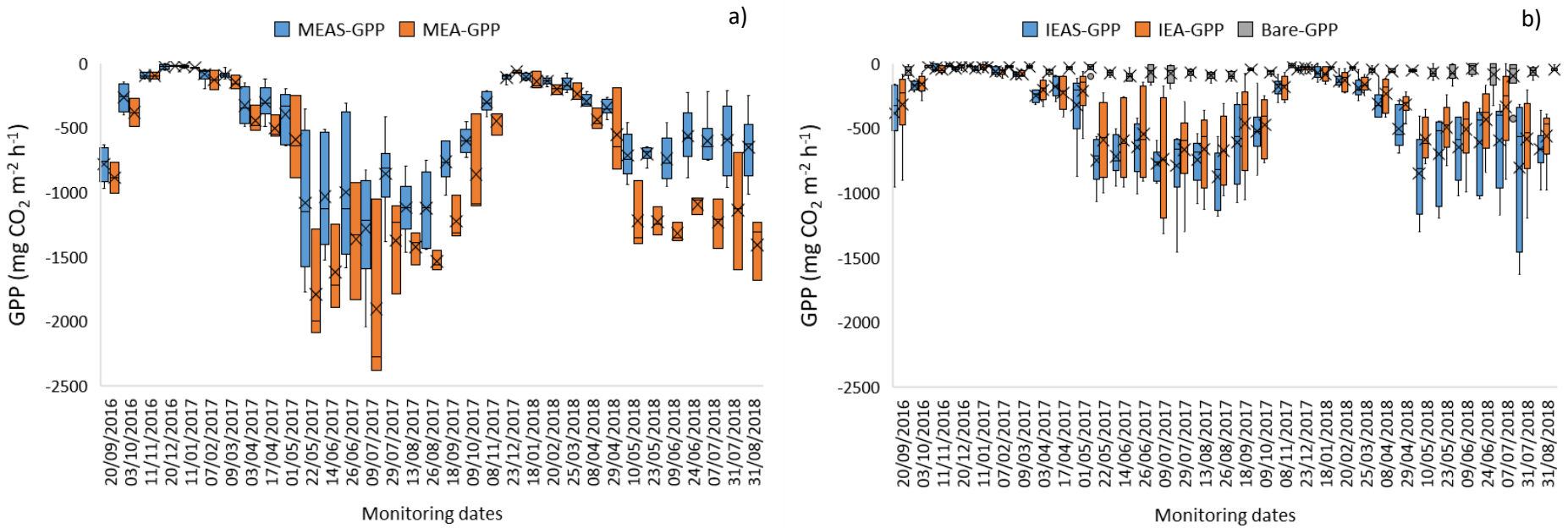


Figure 4.10. Measured GPP flux values for each measurement visit in (a) mature and (b) immature plots. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is exclusive.

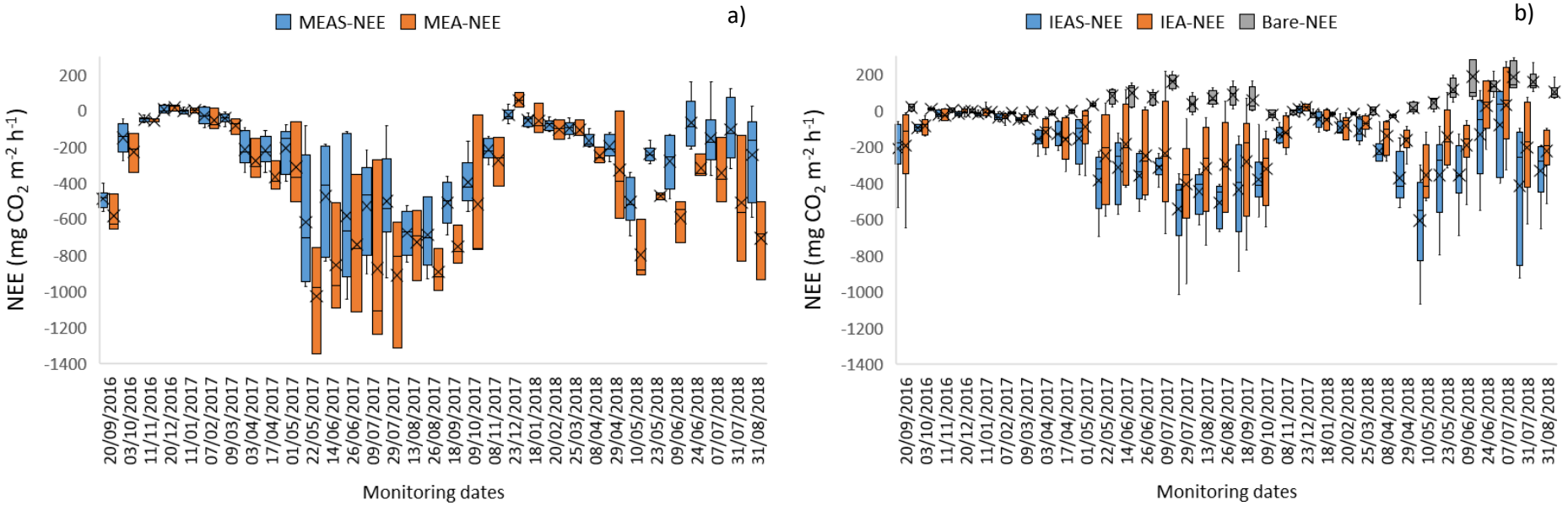


Figure 4.11. Measured NEE flux values for each measurement visit in (a) mature and (b) immature plots. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is exclusive.

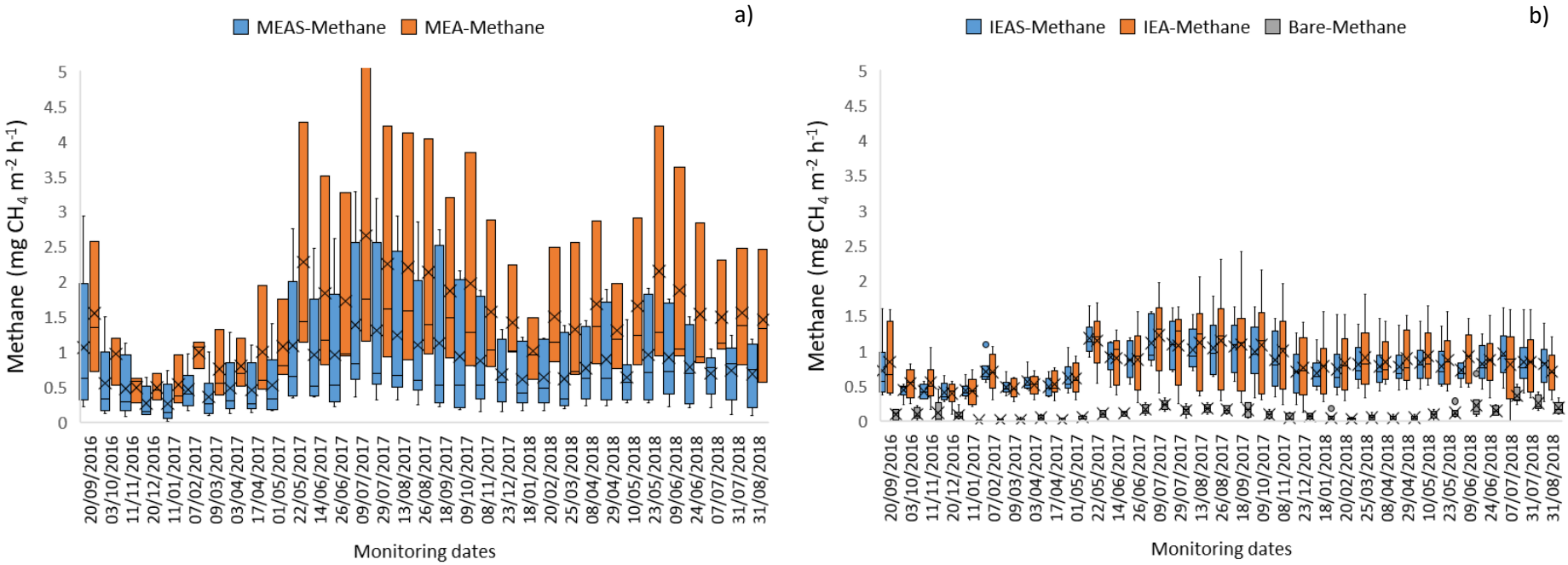


Figure 4.12. Measured methane flux values for each measurement visit in (a) mature and (b) immature plots. In box plots, crosses indicate the mean value, lines indicate the median, and interquartile range is exclusive.

Table 4.3. Seasonal averages in NER, GPP, NEE and methane fluxes in each treatment. MEAS, IEAS, IEA, Bare plots: Autumn and Winter *n* = 19 (years 1 & 2), Spring *n* = 30 (years 1 & 2), Summer *n* = 36 (year 1) *n* = 30 (year 2). MEA plots: Autumn and Winter *n* = 9 (years 1 & 2). Spring *n* = 15 (years 1 & 2), Summer *n* = 18 (year 1) *n* = 15 (year 2).

NER ($mg\ CO_2\ m^{-2}\ h^{-1}$) Season / Year	MEAS	MEA	IEAS	IEA	Bare
Autumn Y1	149.6 ± 131.9	164.1 ± 122.0	95.4 ± 99.1	69.3 ± 60.2	40.5 ± 29.4
Winter Y1	34.3 ± 26.5	47.0 ± 26.0	17.7 ± 9.1	18.8 ± 11.4	8.4 ± 5.9
Spring Y1	178.0 ± 179.7	282.3 ± 279.2	138.3 ± 152.5	127.1 ± 118.4	60.0 ± 53.3
Summer Y1	493.4 ± 209.1	703.1 ± 203.8	342.4 ± 134.3	361.7 ± 123.8	166.9 ± 69.6
Autumn Y2	181.3 ± 90.0	328.8 ± 140.8	118.3 ± 80.8	128.0 ± 68.5	55.2 ± 58.8
Winter Y2	63.0 ± 25.6	100.4 ± 27.4	35.4 ± 20.0	39.7 ± 14.5	10.8 ± 6.4
Spring Y2	200.5 ± 143.4	343.5 ± 245.4	175.1 ± 128.0	179.4 ± 108.1	86.1 ± 71.8
Summer Y2	458.7 ± 105.5	743.8 ± 114.5	398.1 ± 165.2	381.3 ± 128.4	215.2 ± 122.5
GPP ($mg\ CO_2\ m^{-2}\ h^{-1}$) Season / Year	MEAS	MEA	IEAS	IEA	Bare
Autumn Y1	-378.3 ± 315.5	-453.1 ± 357.4	-194.5 ± 219.1	-171.9 ± 204.2	-30.6 ± 28.6
Winter Y1	-43.1 ± 44.7	-59.1 ± 64.1	-43.7 ± 23.4	-35.5 ± 30.5	-18.0 ± 6.0
Spring Y1	-438.6 ± 425.9	-695.6 ± 626.9	-315.0 ± 276.2	-260.7 ± 239.9	-42.1 ± 28.0
Summer Y1	-1068.8 ± 389.9	-1537.6 ± 396.1	-757.4 ± 218.2	-646.1 ± 332.1	-81.6 ± 47.3
Autumn Y2	-554.7 ± 226.2	-844.4 ± 408.1	-436.1 ± 294.4	-371.6 ± 277.0	-41.8 ± 26.1
Winter Y2	-115.0 ± 30.5	-134.2 ± 71.3	-80.9 ± 55.9	-79.9 ± 66.1	-31.9 ± 12.3
Spring Y2	-442.3 ± 244.2	-734.6 ± 460.7	-510.2 ± 327.1	-356.6 ± 205.1	-59.4 ± 34.6
Summer Y2	-628.9 ± 226.7	-1238.6 ± 243.8	-662.2 ± 344.1	-482.5 ± 278.6	-63.2 ± 91.7
NEE ($mg\ CO_2\ m^{-2}\ h^{-1}$) Season / Year	MEAS	MEA	IEAS	IEA	Bare
Autumn Y1	-228.7 ± 201.0	-289.0 ± 244.6	-109.6 ± 123.2	-102.5 ± 151.9	7.7 ± 14.0
Winter Y1	-8.8 ± 32.7	-12.2 ± 45.0	-26.0 ± 17.0	-16.7 ± 26.5	-9.6 ± 8.5
Spring Y1	-260.6 ± 255.3	-413.3 ± 366.1	-176.7 ± 151.2	-133.6 ± 151.0	15.9 ± 41.8
Summer Y1	-575.5 ± 266.1	-834.4 ± 296.0	-415.0 ± 169.2	-284.4 ± 264.3	85.3 ± 58.7
Autumn Y2	-373.4 ± 161.9	-515.6 ± 311.1	-317.8 ± 227.0	-243.5 ± 226.5	7.3 ± 53.9
Winter Y2	-52.0 ± 37.9	-33.7 ± 90.3	-47.4 ± 56.8	-40.2 ± 62.4	-21.1 ± 10.9
Spring Y2	-241.8 ± 157.8	-391.0 ± 277.1	-335.0 ± 240.5	-177.2 ± 144.2	26.7 ± 59.3
Summer Y2	-170.2 ± 186.2	-494.8 ± 234.6	-264.1 ± 270.1	-113.9 ± 216.8	152.0 ± 105.1
Methane ($mg\ CH_4\ m^{-2}\ h^{-1}$) Season / Year	MEAS	MEA	IEAS	IEA	Bare
Autumn Y1	0.70 ± 0.73	1.01 ± 0.69	0.53 ± 0.30	0.64 ± 0.37	0.11 ± 0.11
Winter Y1	0.33 ± 0.27	0.67 ± 0.33	0.52 ± 0.20	0.51 ± 0.25	0.04 ± 0.05
Spring Y1	0.59 ± 0.59	1.18 ± 0.98	0.67 ± 0.32	0.65 ± 0.34	0.05 ± 0.04
Summer Y1	1.16 ± 0.98	2.13 ± 1.45	1.01 ± 0.32	1.06 ± 0.52	0.17 ± 0.07
Autumn Y2	0.98 ± 0.89	1.80 ± 1.17	0.97 ± 0.41	1.06 ± 0.64	0.10 ± 0.08
Winter Y2	0.65 ± 0.43	1.31 ± 0.64	0.71 ± 0.32	0.79 ± 0.42	0.05 ± 0.05
Spring Y2	0.78 ± 0.56	1.62 ± 1.05	0.80 ± 0.30	0.89 ± 0.39	0.07 ± 0.06
Summer Y2	0.77 ± 0.45	1.58 ± 0.92	0.83 ± 0.32	0.86 ± 0.37	0.24 ± 0.14

4.3.4 Measured flux data and environmental variables

Relationships between flux values and environmental variables were tested using the regression function in Microsoft Excel 2019. Relationships between primarily peat temperature at 5 cm depth (PT) and secondly water table depth (WTD) appeared to explain most of the variability in NER and methane fluxes, and PT followed by PAR then WTD explained variability in GPP. R^2 values of linear regression for each treatment are given in Appendix 2. Relationships between GPP and PT for amalgamated vegetation plots followed a 3rd order polynomial curve, particularly in immature plots (Figure 4.13a and b). GPP appeared to increase until the peat temperature was 16 - 17 °C, then reduce above this temperature, particularly in immature vegetation where there were notably higher peat temperatures (maximum 23.5 °C) than in plots of mature vegetation (maximum 19.3 °C). Methane flux increased with greater volumes of *E. angustifolium* (Figure 4.13c) but there was no apparent relationship between volume of *Sphagnum* and magnitude of any flux.

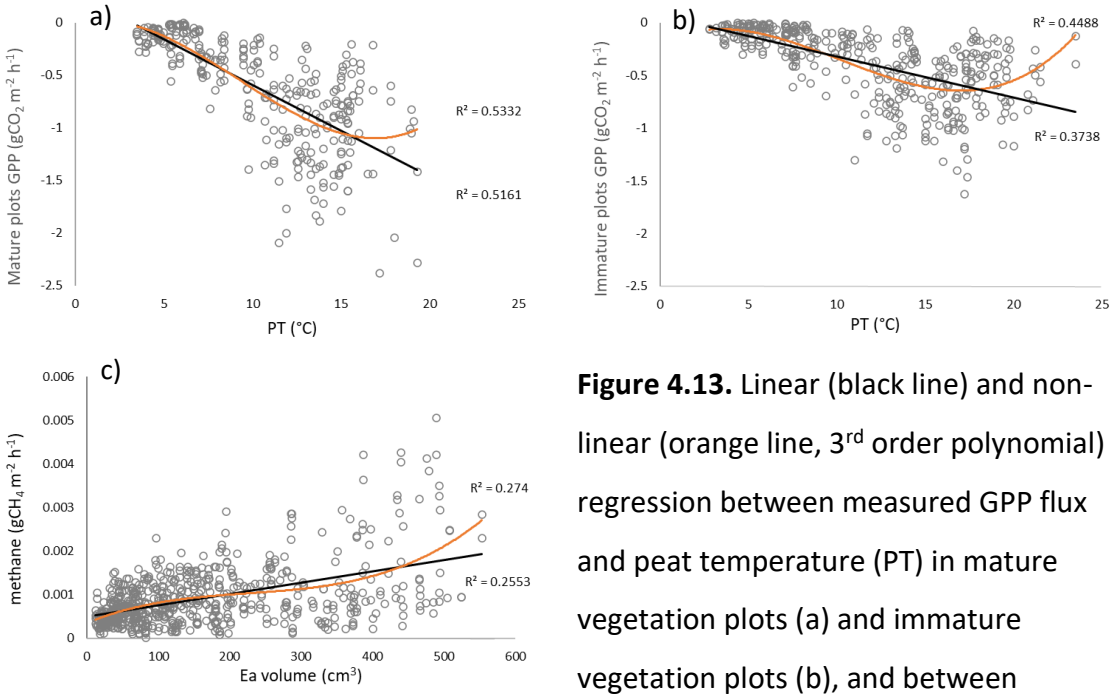


Figure 4.13. Linear (black line) and non-linear (orange line, 3rd order polynomial) regression between measured GPP flux and peat temperature (PT) in mature vegetation plots (a) and immature vegetation plots (b), and between methane flux and *E. angustifolium* (Ea) volume (c), with associated R^2 values.

4.3.5 Modelled flux data

The drivers for measured fluxes of both NER and methane were best explained by PT then WTD, and those for GPP fluxes were best explained by PT, then PAR, then WTD (R^2 values of linear regression used to test relationships prior to modelling are shown in Appendix 2). Linear regression with measured and modelled flux values (Appendix 3) showed that this modelling process appeared sufficiently robust to apply to a large dataset of environmental variables to generate yearly estimates of each gaseous carbon flux. However, NER and methane data provided a better fit than GPP, and data from Bare plots (shown separately in Appendix 3 as the values were much lower than for vegetated plots), had greater variability.

Modelled and measured values (Figures 4.14 to 4.17) appeared to follow similar trends. Winter flux values in year 1 appeared to be greater than measured values, although values were small, but generally the remainder of measured fluxes sat within the range of modelled values. There was a high variability in measured values of methane fluxes, which the model smoothed to follow the measured mean, although year 1 winter modelled values still appeared a little higher than measured values.

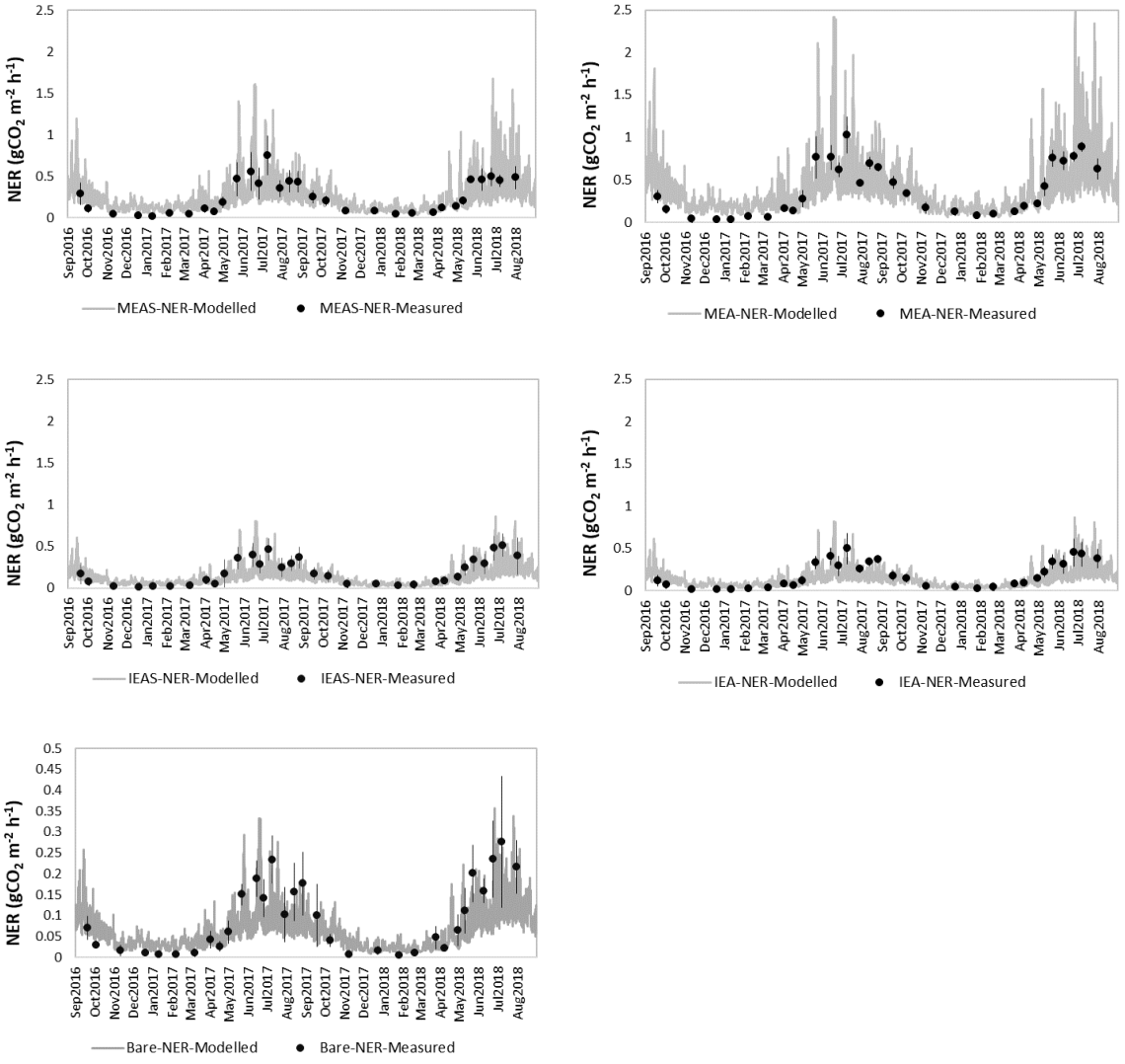


Figure 4.14. Modelled and measured Net Ecosystem Respiration (NER) CO_2 fluxes in each treatment. Shading shows modelled hourly data, points show mean measurements, and error bars show measured standard deviation from the mean. Data for Bare plots displayed with actual range for greater clarity.

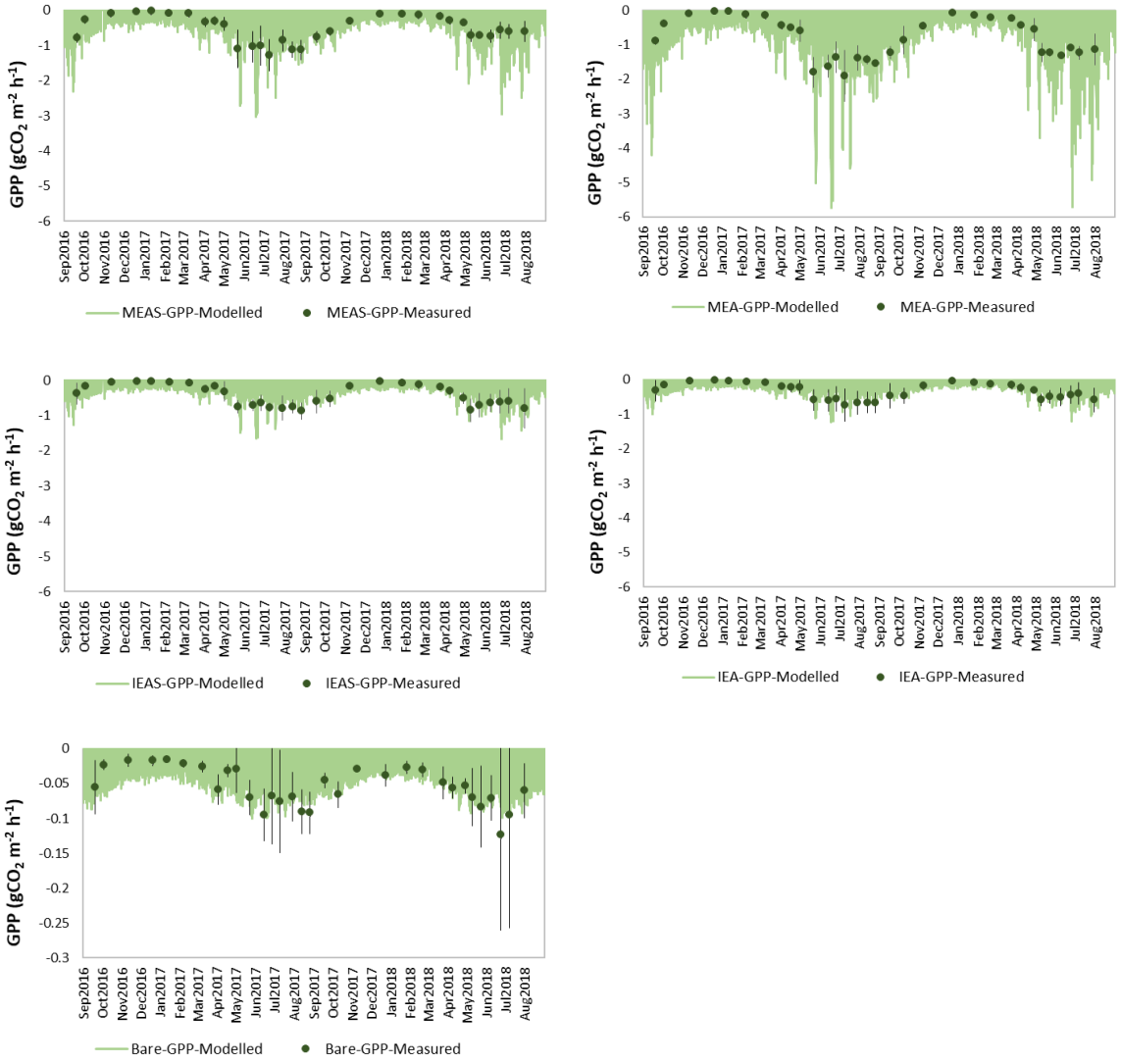


Figure 4.15. Modelled and measured Gross Primary Productivity (GPP) CO_2 fluxes in each treatment. Shading shows modelled hourly data, points show mean measurements, and error bars show measured standard deviation from the mean. Measured GPP data are daytime values. Modelled data includes zero-values for GPP during the night. Data for Bare plots displayed with actual range for greater clarity.

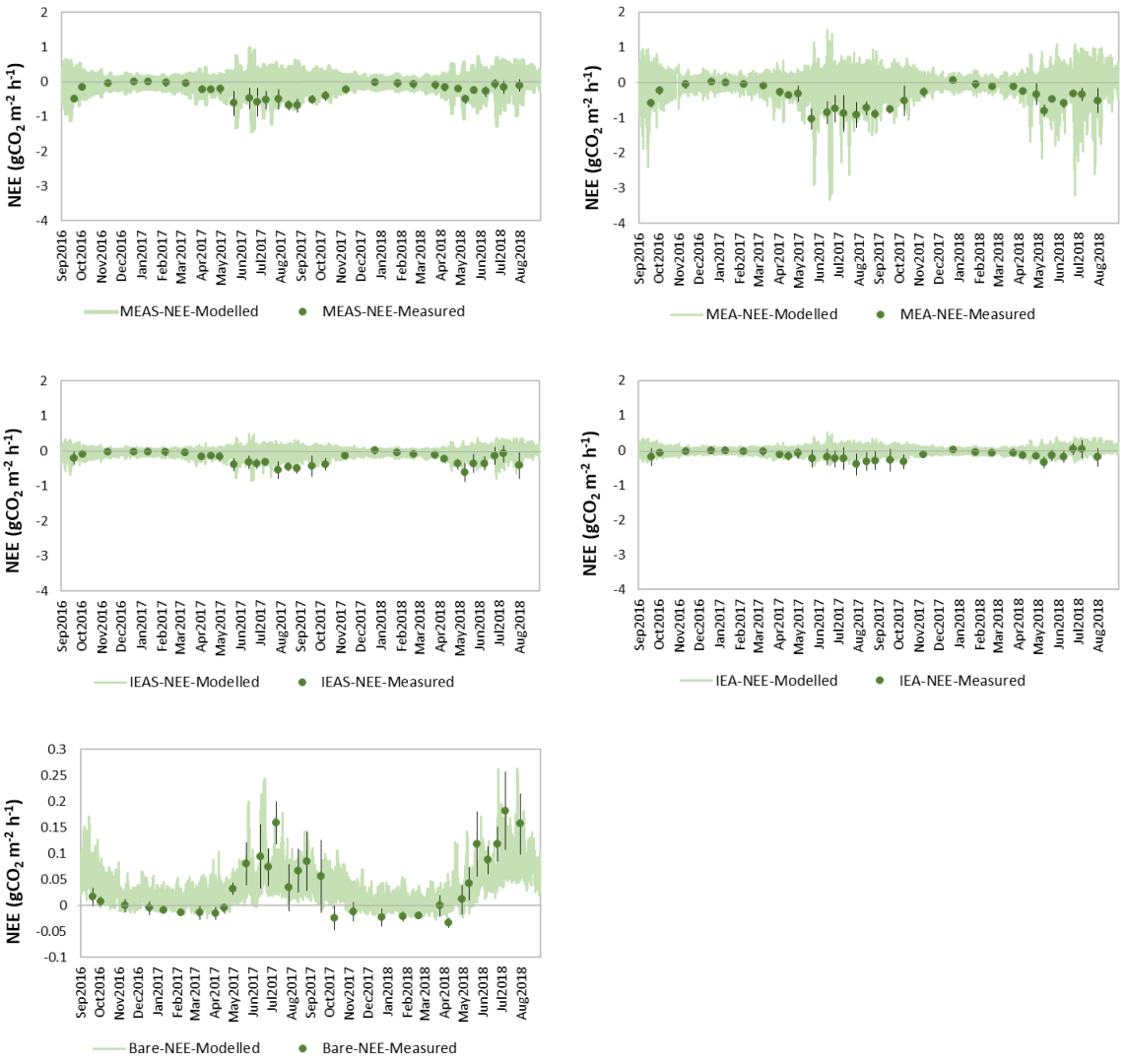


Figure 4.16. Modelled and measured Net Ecosystem Exchange (NEE) CO_2 fluxes in each treatment. Shading shows modelled hourly data, points show mean measurements, and error bars show measured standard deviation from the mean. Measured NEE data are daytime values. Modelled data includes zero-values for GPP during the night, hence graphs show both positive and negative values. Data for Bare plots displayed with actual range for greater clarity.

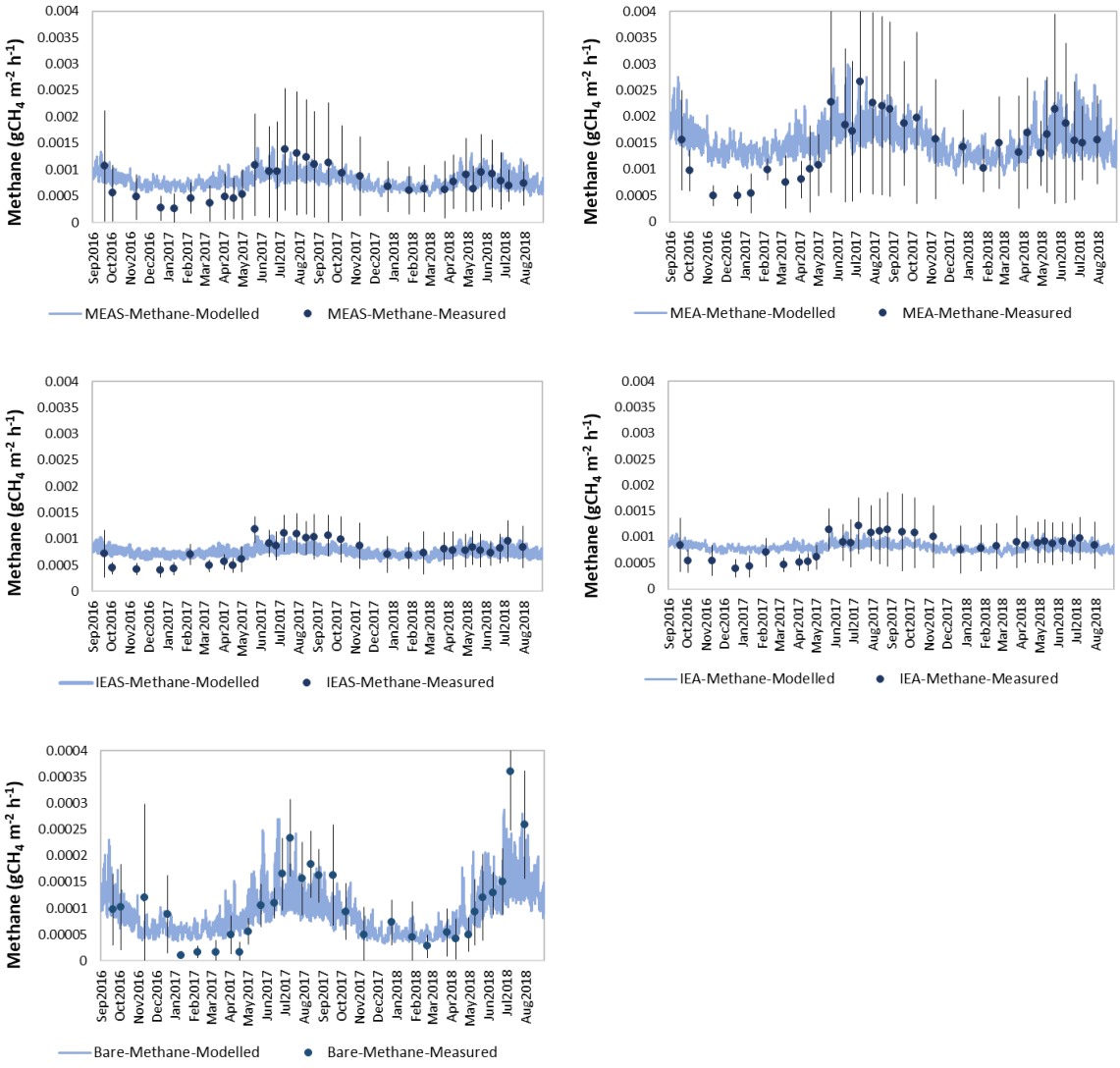


Figure 4.17. Modelled and measured methane fluxes in each treatment. Shading shows modelled hourly data, points show mean measurements, and error bars show measured standard deviation from the mean. Data for Bare plots displayed with actual range for greater clarity.

4.3.7 Modelled CGHG budgets

When methane emission fluxes were converted to CO_{2e} and added to NEE to give an overall annual carbon greenhouse gas (CGHG) budget (Table 4.4), NEE values were considerably reduced in vegetated plots and IEA plots became a CGHG source rather than sink. As methane emission from Bare plots was minimal (11-12% of that from vegetated plots), the addition of CH_4-CO_{2e} made little difference to the CGHG budget of Bare plots, but they remained a greater source of CGHG than vegetated plots throughout.

Annual NER in vegetated plots was similar in both study years (Table 4.4), but GPP was lower in year 2 leading to a corresponding reduction in the year 2 CGHG uptake in MEAS, MEA and IEAS plots, and increase in CGHG emissions in IEA and Bare plots. CGHG uptake was larger in plots of mature vegetation without *Sphagnum* (MEA > MEAS) and larger in plots of immature vegetation with *Sphagnum* (IEAS > IEA) in both years, however uptake was similar in plots with *Sphagnum* in year 1 (MEAS ≈ IEAS) but greater in plots with immature vegetation in year 2 (IEAS > MEAS).

As data for modelled flux values were normally distributed, statistical differences were tested with two-way ANOVA and post-hoc Tukey HSD. The difference in the CGHG budget between treatment groups was statistically significant in both year 1 ($F = 6.594$, $p = 0.001$, $df = 4$) and year 2 ($F = 3.638$, $p = 0.020$, $df = 4$). However, post-hoc Tukey HSD tests showed that differences were only significant between Bare plots and each of MEAS, MEA and IEAS ($p < 0.01$) in year 1, and Bare plots and IEAS ($p < 0.05$) in year 2, and differences between years within treatment groups were not statistically significant. This was likely due to the wide range of data from collars in each treatment (Appendix 4), demonstrated by high values for standard deviation in Table 4.4. However, the data appeared to represent an overall progression towards a CGHG sink function in this peatland restoration site (Appendix 5), with high CGHG emission from bare peat, a net CGHG loss becoming a net gain as vegetation colonised and matured, but also a reduction in CGHG sink during drier conditions in the second study year.

Table 4.4. Plot composition and characteristics with associated modelled annual sum flux measurements. MEAS = mature vegetation (*E. angustifolium* with *Sphagnum*); MEA = mature vegetation (*E. angustifolium* only); IEAS = immature vegetation (*E. angustifolium* with *Sphagnum*); IEA = immature vegetation (*E. angustifolium* only); WTD = water table depth (cm); NER = net ecosystem respiration; GPP = gross primary productivity; NEE = net ecosystem exchange; CO_2 equivalents of CH_4 were calculated by multiplying by Global Warming Potential $(GWP)_{100}$ of 28 and added to NEE values to give a carbon greenhouse gas (CGHG) budget in g CO_{2e} m^{-2} yr^{-1} (final column); Values reported as mean ± SD.

Plot type	Year	WTD (cm)	*E. angustifolium* (cm^3)	*Sphagnum* (cm^3)	NER (gCO_2 m^{-2} yr^{-1})	GPP (gCO_2 m^{-2} yr^{-1})	NEE (gCO_2 m^{-2} yr^{-1})	Methane (gCH_4 m^{-2} yr^{-1})	Methane (gCH_4-CO_{2e} m^{-2} yr^{-1})	CGHG budget (gCO_{2e} m^{-2} yr^{-1})
MEAS	1	2.04 ± 3.97	216.77 ± 126.44	5968.39 ± 2567.12	2098.86 ± 586.57	-2647.75 ± 628.53	-548.89 ± 335.18	7.14 ± 5.88	199.94 ± 164.62	-348.95 ± 279.64
	2	9.30 ± 14.05	214.27 ± 106.25	8223.62 ± 2851.15	2070.02 ± 551.70	-2331.27 ± 619.96	-261.25 ± 346.50	6.68 ± 5.28	187.10 ± 147.71	-74.15 ± 301.38
MEA	1	2.04 ± 3.97	300.90 ± 132.83	0	3211.11 ± 543.39	-4114.78 ± 1013.23	-903.67 ± 488.22	13.71 ± 9.39	383.94 ± 262.98	-519.73 ± 501.99
	2	9.30 ± 14.05	371.73 ± 114.66	0	3199.34 ± 534.29	-3771.39 ± 1070.04	-572.05 ± 535.74	12.89 ± 8.61	360.80 ± 241.07	-211.25 ± 542.68
IEAS	1	10.10 ± 8.76	64.80 ± 47.79	310.59 ± 157.31	1053.04 ± 411.40	-1570.72 ± 520.12	-517.68 ± 231.77	6.66 ± 1.95	186.60 ± 54.73	-331.07 ± 202.75
	2	18.69 ± 14.92	117.19 ± 70.20	975.72 ± 429.50	1081.61 ± 424.69	-1513.53 ± 496.59	-431.92 ± 206.50	6.50 ± 1.85	181.87 ± 51.81	-250.06 ± 178.36
IEA	1	10.10 ± 8.76	53.45 ± 40.93	0	1143.54 ± 297.26	-1329.66 ± 728.44	-186.12 ± 490.84	7.17 ± 3.34	200.67 ± 93.38	14.54 ± 421.91
	2	18.69 ± 14.92	82.37 ± 50.78	0	1148.70 ± 302.53	-1260.54 ± 670.82	-111.84 ± 440.82	6.97 ± 3.18	195.15 ± 88.92	83.30 ± 381.17
Bare	1	10.10 ± 8.76	0	0	537.37 ± 76.45	-222.01 ± 47.53	315.36 ± 75.34	0.77 ± 0.22	21.48 ± 6.17	336.84 ± 73.44
	2	18.69 ± 14.92	0	0	544.43 ± 100.77	-220.71 ± 47.06	323.72 ± 87.58	0.77 ± 0.23	21.65 ± 6.35	345.37 ± 84.22

4.4 Discussion

This chapter study showed, in a year of expected seasonal fluctuations (year 1), a clear progression from a net carbon greenhouse gas (CGHG) source to a net CGHG sink in a peatland restoration site. There was continual CGHG emission from bare peat, a reduction in emissions on initial colonisation with bog vegetation (in this case, *Eriophorum angustifolium*), to increasing uptake on mature colonisation and the introduction of *Sphagnum* mosses. However, in a year with variable weather patterns and a long period of summer drought (year 2), the CGHG uptake was very small, and areas of bare peat and sparse vegetation, particularly with no *Sphagnum* cover, became increasing sources of CGHG. This could suggest that the site has minimal resilience to anticipated climate change in the UK such as the increased frequency of hot summers (Lowe *et al*., 2018; Met office, 2019). However, although drought in the second year of this study may have limited the potentially positive influence of increasing vegetation cover on this developing restoration site, the vegetated plots remained, in the main, small CGHG sinks and, crucially, the avoided CGHG losses of not restoring the site (i.e., leaving it bare) were large, so supporting hypothesis 1), that restoration of the site will result in a CGHG uptake compared to bare peat. This highlights the urgent nature of restoring degraded peatlands for best outcomes in terms of climate change mitigation targets (Nugent *et al*., 2019).

Flux modelling was undertaken so that differences between day and night fluxes could be incorporated for an assessment of the overall CGHG budget, and modelled yearly CGHG fluxes were within the range of those in published literature (Table 4.5). However, there are some caveats to this study. The high standard deviation on flux measurements may be due to differences in microtopography at collar locations, which were not assessed. A dipwell in each collar, or preferably peat moisture measurements on each monitoring visit, may have helped to explain the wide variation in fluxes in each treatment. Moreover, the lack of continuous environmental monitoring was detrimental to the modelling process, as hourly measurements, although sourced from Whitworth Observatory less than 15 km away, were at height from a city environment. Additionally, hourly PT was extrapolated for modelling from micrometeorological measurements on a

nearby bare peat site from the year after the study period, which may not closely resemble conditions on the vegetated study site. This will have reduced precision. However, PT measurement was felt to be more useful for modelling, being more closely related to peatland environmental processes driving NER and CH_4 emission, and PT was directly monitored during flux measurements.

A further study caveat is that the use of permanent collars, even at 5 cm depth, isolated the material within, causing it to shrink away from the collar during the drought period; it may not be a problem on well-hydrated sites. This issue is recognised (Komulainen *et al*., 1999). Larger collars could reduce edge effects but make working practices more cumbersome, and portable collars (instead of permanent collars) would perhaps damage vascular plants on each application. There appear to be no current workable alternatives to permanent collars for a study that requires fixed GHG monitoring points.

One of the aims of this study was to ascertain the influence of the type and maturity of vegetation on CGHG fluxes, but altered weather patterns between the two years of study influenced volume and condition of both *E. angustifolium* and *Sphagnum,* and thus their capacity for CGHG uptake. *E. angustifolium* growth and senescence followed expected seasonal trends in the first year of study, but growth stalled in the second summer in all plots but IEAS, indicating that the drought may have caused early senescence in the vascular plants (Bubier *et al*., 2003), and reduced their ability to photosynthesise. The year 2 summer drought also reduced *Sphagnum* volume in mature plots and caused severe surface desiccation, reducing photosynthetic potential (McNeil and Waddington, 2003; Bortoluzzi *et al*., 2006; Rydin and Jeglum, 2013; Helfter *et al*., 2014). Additionally, reduced PAR in the Spring of year 2 (probably cloudier conditions) may have limited photosynthesis (Lafleur *et al*., 2003; Loisel *et al*., 2007) and contributed to lower levels of GPP for the year (Nijp *et al*., 2015). This resulted in an overall reduction in CO_2 uptake (through GPP) in the second year. However, *Sphagnum* in immature plots grew in volume throughout the study period (there may have been greater GPP in *Sphagnum* in the early stages of growth [see Chapter 3]), and was given some environmental protection by being lower in the *E. angustifolium* sward than *Sphagnum* within mature *E. angustifolium*. IEAS plots were also covered with mesh shading to support *Sphagnum* establishment and early growth. This may have helped retain soil moisture and reduce evapotranspiration for a

healthy layer of *Sphagnum*, but also appeared to favour continued *E. angustifolium* growth and development in these plots during the drought period. This method was used to replicate straw-mulching, but is not likely to be employed in large-scale restoration works.

More generally, the volume of *E. angustifolium* was less in plots with *Sphagnum* than without in mature vegetation, which may be related to reduction in exposure of *E. angustifolium* leaves to sunlight as the *Sphagnum* grew and increasingly covered them, or a greater capacity of *Sphagnum* to harvest nutrients for growth (Malmer *et al*., 2003; Bragazza *et al*., 2004; Fritz *et al*., 2014). Immature *Sphagnum* had not reached a height at which it competed with *E. angustifolium* for light. The WTD was higher in plots with mature vegetation than those with immature vegetation and bare peat (particularly on the drier edge of the site). Wilson *et al*. (2013) also found this, and potential explanations are that evaporation is reduced through increased shade from dense *E. angustifolium* (Price *et al*., 2003), or that a higher WTD (retained through bunding, creating basins) supports proliferation of *E. angustifolium* (Rochefort *et al*., 2016).

CO_2 uptake through GPP was greater in plots of mature *E. angustifolium* only, which had the greatest volume of *E. angustifolium* overall, suggesting that hypothesis 2) CGHG uptake will be greater with maturity of vegetation was supported. But the overall picture was more complex. NER emission was also highest in these plots, and greater than those with immature vegetation or bare peat, and rates of NER and GPP were closely related. This accords with studies suggesting NER is higher in vegetated than bare plots and is related to litterfall, temperature and rainfall (Bortoluzzi *et al*., 2006; Evans *et al*., 2016; Jordan *et al*., 2016), but the most important factor may be inputs of carbon products from photosynthesis (Bond-Lamberty *et al*., 2004). However, greater NER emission was also related to warmer, drier conditions, which concurs with most literature sources (e.g., Danevčič *et al*., 2010; Wang *et al*., 2014), particularly for bare peat (Bortoluzzi *et al*., 2016) although this can alter depending on the plant assemblage in a heterogeneous peatland system (Juszczak *et al*., 2012). The NER increase in bare plots each year could be related to the surrounding encroachment of vegetation, and root growth within the column of bare peat inside the collar, even though surface vascular plant growth was removed, but is more likely due to greater microbial decomposition in dryer, warmer

conditions (Juszczak *et al*., 2013). Algae and acrocarpous bryophytes (no *Sphagnum* growth) were not removed, however, as this is a natural progression of bare peat cover, and this may have influenced carbon cycling in these plots.

Methane emissions (as $gCH_4\text{-}CO_{2e}\ m^{-2}\ yr^{-1}$) considerably reduced CGHG uptake in each vegetated treatment, and highlighted the importance of including methane measurements in GHG studies (Bussell *et al*., 2010; Haddaway *et al*., 2014). However, methane fluxes were generally mid-range to low in all treatments compared to some studies on re-wetted sites using instantaneous measurements (e.g. Davidson *et al*., 2016; Beyer and Höper, 2015; Evans *et al*., 2016, Table 4.5), which is perhaps to be expected in a site where microbial communities are still recovering from the effects of long-term drainage during peat-cutting and subsequent evapotranspiration from scrub cover (Andersen *et al*., 2013; Juottonen *et al*., 2015; Nugent *et al*., 2018) and perhaps a reduction in substrate nutrient availability (Basiliko *et al*., 2007) prior to restoration 10 years ago. It might be expected that methane fluxes in this study would increase as the site matures with more plant growth, plant litter and a higher, more stable water table, leading to greater availability of labile carbon (Glatzel *et al*., 2004; Lafleur *et al*., 2005; Urbanová *et al*., 2011), and measured seasonal results in this study prior to the year 2 summer drought supported that trend. However, overall methane fluxes in the year with drought were lower throughout vegetated plots compared to the previous wet year, in accordance with the accepted view that methane flux declines (CH_4 oxidises) in dry sites (Danevčič *et al*. 2010; Turetsky *et al*., 2014; Abdalla *et al*., 2016), although it is not clear whether aerenchymatous plant senescence also influenced the results. However, MEA plots had greater volumes of *E. angustifolium* and approximately twice the methane emission of other vegetated plots, supporting hypothesis 3), that greater volumes of *E. angustifolium* will result in greater emission of methane. There was no particular relationship between high WTD and high methane emissions in vegetated plots overall, contrary to empirical evidence (Glatzel *et al*., 2004; Danevčič *et al*., 2010; Urbanová *et al*., 2011; Evans *et al*., 2016).

Methane flux was higher in vegetated plots without *Sphagnum*, even in immature vegetation where *E. angustifolium* volume was higher in plots with *Sphagnum* than without, supporting hypothesis 4), that the presence of *Sphagnum* moss will reduce the

magnitude of methane emission. Flux from bare plots was 9.9% that of vegetated plots overall. These results concur with those from Bortoluzzi *et al*. (2006) who reported the rank of highest to lowest fluxes to be *Eriophorum*-dominated, *Sphagnum*-dominated, then bare plots, and Couwenberg *et al*. (2011) who reported a strong relationship between methane flux and the density of aerenchymatous leaves. Methane flux, then, appears to be related to the amount of *E angustifolium*, but is also reduced when *Sphagnum* is present, suggesting some methanotrophic consumption of methane in the *Sphagnum* layer (Kip *et al*., 2010; Larmola *et al*., 2010; van Winden *et al*., 2012; Nugent *et al.*, 2018).

In common with Leppälä *et al*. (2011), this study found that reduction in CGHG uptake in a dry year compared to the previous wet year was driven more by changes in GPP than NER. However, plant dynamics were a complex factor. Greater volumes of *E. angustifolium* appeared to be related to greater CGHG uptake. This is partly contrary to findings of Kivimäki *et al*. (2008), that stands of mixed sedges and *Sphagnum* sequestered more carbon than those of sedges alone due to lower NER, but the maturity of the vegetation is a factor in this study. Wilson *et al*. (2013) found greater NEE in plots with sedges when compared to those with *Sphagnum* only. Moreover, Tuittila *et al*. (1999) suggest that a restored site colonised with mature *Eriophorum* (*E. vaginatum*) was a carbon sink resilient to interannual changes in weather. However, other studies found that NEE was greater in re-wetted *Sphagnum*-dominated than sedge-dominated sites (Beyer and Höper, 2015; Evans *et al*., 2016; Renou-Wilson *et al*., 2019) due to a lower NER to GPP ratio, and the presence of *Sphagnum* may reduce NER by retaining moisture (Waddington and Warner, 2001). In this study, an open sward of immature *E. angustifolium* was a CGHG source, but with a layer of *Sphagnum* it became a CGHG sink, which was also greater than plots with mature vegetation during the drought in the second summer. This was due to lower CGHG emission through NER and higher uptake through GPP in plots with *Sphagnum* than without, suggesting both an effect of increased moisture retention (Waddington and Warner, 2001) as well as more plant material. This may indicate that establishment of a layer of *Sphagnum* is more crucial in immature than in mature vegetation, in terms of CGHG uptake, and so efforts to create a beneficial microclimate at the peat surface (e.g., mulch, nurse planting, etc) should be one of the

fundamental processes for peatland restoration (Quinty and Rochefort, 2003; Groeneveld *et al*., 2007; Waddington *et al*., 2010; Pouliot *et al*., 2011).

Large standard deviation in the flux data (noted by Bortoluzzi *et al*. [2006] in their study, and seen in data from other sources, Table 4.5), demonstrates the heterogeneity of the site, and the complex nature of associations between carbon cycling in degraded peatlands under restoration measures and fluctuations in environmental factors, and that using vegetation type and density as a proxy for carbon balance measurements may not capture the CGHG state of individual sites sufficiently. However, chamber-based measurements are time-consuming and have their own limitations, as highlighted above. This study conducted a fairly small number of measurements (33 over two years) and only during the day, although they were conducted throughout each year. Nevertheless, snapshot measurements can give a good indication of the site trajectory in terms of CGHG emission or uptake, and compare magnitude of fluxes between types of site management, particularly if conducted over several years to capture changes in the site vegetation assemblage and a range of environmental conditions. Associations and partnerships with local academic institutions would allow use of specialist equipment across several projects to make best use of resources.

This study found the *E. angustifolium*-dominated area of Cadishead Moss with and without *Sphagnum* introduction to be an overall net CGHG sink, despite the inclusion of methane emissions, particularly in year 1 and minimally in year 2, which included a summer drought. The mean CGHG of vegetated monitoring points (so, assuming equal distribution) was -264.39 ± 368.95 g CO_{2e} m^{-2} yr^{-1} in year 1 and -99.01 ± 339.59 g CO_{2e} m^{-2} yr^{-1} in year 2. This supported hypothesis 5), that periods of drought will have a deleterious effect on site CO_2 uptake, although the overall uptake, albeit small, in year 2 shows the site had some resilience to drought. The yearly CGHG emission from bare peat (mean of 341.10 ± 75.47 g CO_{2e} m^{-2} yr^{-1}) shows the benefits of restoration in terms of avoided CGHG losses (Worrall *et al*., 2011; Renou-Wilson *et al*., 2019). The CO_{2e} sink strength in vegetated plots in year 1 was greater than some other similar restored bogs (e.g., those studied by Drewer *et al*., 2010; Beyer and Höper, 2015 and Renou-Wilson *et al*., 2019). The findings from this study are contrary to those of Evans *et al*. (2016) at the nearby Astley Moss rewetted cut-over bog, where measurements were taken on a

generally inundated part of that site, and very high (43.7 g CH_4 m^{-2} yr^{-1}) methane emissions pushed the NEE sink of -336 g CO_2 m^{-2} yr^{-1} (revised to -41 g CO_2 m^{-2} yr^{-1} across the entire site based on vegetation assemblage) into a site CGHG source. More data is needed on lowland bogs under restoration to further refine the inventory of greenhouse gas emission factors for UK peatlands (Evans *et al*., 2017), and this study can contribute to that.

It could be argued that the wider range of fluxes from areas of *E. angustifolium*-only in this study indicate that addition of *Sphagnum* may reduce CGHG uptake in mature vegetation but is less likely to result in CGHG emission, with the added benefit of reduced decomposition (Section 1.2.4) and so more efficient peat accumulation than with *E. angustifolium* only. This study site is not yet in equilibrium, has a widely fluctuating WTD, and has changed over the study period in terms of vegetation cover and density. Few studies, although assessing GHG fluxes in relation to type of vegetation, (Wilson *et al*., 2013; Strack and Zubak, 2013; Renou-Wilson *et al*., 2019) and over time (Waddington *et al*., 2010) fully address the question of the dynamic nature of vegetation on fluxes in a single peatland system under restoration measures over time, although there are some good, recent examples (e.g., Nugent *et al*., 2018; Nugent *et al*., 2019). This broader, integrated approach is worth exploring in more depth, particularly when funding for restoration work may depend on evidence for change over short time-scales.

As climate change continues to affect weather patterns in the UK, and summer drought may become more common (Lowe *et al*., 2018; Met Office, 2019), it is likely that the CGHG sink function in degraded peatlands, even those undergoing restoration, will reduce and emissions will increase unless greater resilience, particularly in terms of maintaining water table levels, can be engineered.

4.5 Conclusions

This study examined whether the restoration methods at the chosen site, of rewetting, allowing colonisation with *E. angustifolium*, and actively introducing BeadaGel™ *Sphagnum*, delivered benefits in terms of CGHG uptake, whether the maturity of the

vegetation was a factor in the magnitude of CGHG fluxes, and how resilient the site was likely to be in the face of climate change.

Avoided CGHG losses, particularly with more mature vegetation and the introduction of *Sphagnum* in immature vegetation, were considerable. Therefore, turning CGHG losses into gains appears to be achievable in a short time frame, and is accelerated with *Sphagnum* introduction in the early stages of restoration. The presence of *Sphagnum* and changing weather patterns had considerable influence on the magnitude of fluxes. Methane emission in mature plots with *Sphagnum* was half that of plots without, and less in immature plots with *Sphagnum*, despite a comparatively greater volume of *E. angustifolium* than in those without. Although methane fluxes (when converted to CH_4-CO_{2e} with a Global Warming Potential $[GWP]_{100}$ of 28) contributed to reduce overall CGHG uptake benefits, it was not sufficient to turn the site into a CGHG source, and demonstrated the capacity of *Sphagnum* presence in reducing methane emission. However, a greater volume of mature *E. angustifolium* alone sequestered more CGHG in both study years than when accompanied by *Sphagnum*, despite greater methane emissions. Nonetheless, the presence of *Sphagnum* was less likely to result in CGHG emission. Moreover, introduction of *Sphagnum* improved CGHG uptake when *E. angustifolium* was sparse, in both a wet and dry year, and uptake was higher in these plots than all others in the dry year.

During periods of drought CGHG uptake continued in vegetated areas of the site while CGHG emissions from bare areas increased. Therefore, rehabilitation of this degraded peatland through rewetting and revegetation has improved its resilience to anticipated climate change scenarios of increased periods of drought, particularly if *Sphagnum* is introduced, albeit with added protection, in the early stages of restoration. However, the level of historic degradation on this site may be a continuing factor limiting the effectiveness of restoration measures to put the site on the road to recovering functions of peat accumulation and carbon sequestration. The next chapter examines the quality of the peat at CGHG monitoring points for a greater understanding of the remaining legacy of degradation which may still influence the CO_2 uptake on the site, and what may be done to remedy it.

Table 4.5. Examples of the variety of carbon GHG flux measurements from the literature

Authors	Study type and location	Study focus / plant cover	Measurement method	CO_2 fluxes in literature Converted to g CO_2 m^{-2} yr^{-1}	CH_4 fluxes in literature Converted to g CH_4 m^{-2} yr^{-1}
This study	Degraded, domestic-cut, rewetted lowland bog, Manchester UK	Mature *E. angustifolium* with *Sphagnum*	CCS: Los Gatos UGGA; 2-year period	NER: 2070.02 ± 551.70 to 2098.86 ± 586.57 NEE: -261.25 ± 346.50 to -548.89 ± 335.18	6.68 ± 5.28 to 7.14 ± 5.88
This study		Mature *E. angustifolium* only		NER: 3199.34 ± 534.29 to 3211.11 ± 543.39 NEE: -572.05 ± 535.74 to -903.67 ± 488.22	12.89 ± 8.61 to 13.71 ± 9.39
This study		Immature *E. angustifolium* with *Sphagnum*		NER: 1053.04 ± 411.40 to 1081.61 ± 424.69 NEE: -431.92 ± 206.50 to -517.68 ± 231.77	6.50 ± 1.85 to 6.66 ± 1.95
This study		Immature *E. angustifolium* only		NER: 1143.54 ± 297.26 to 1148.70 ± 302.53 NEE: -111.84 ± 440.82 to -186.12 ± 490.84	6.97 ± 3.18 to 7.17 ± 3.34
This study		Bare peat		NER: 537.37 ± 76.45 to 544.43 ± 100.77 NEE: 315.36 ± 75.34 to 323.72 ± 87.58	0.77 ± 0.22 to 0.77 ± 0.23
Greenup *et al* ., 2000	Ombrotrophic peatland, UK	*E. vaginatum*- and *S. papillosum* -dominated	Flow-through chamber, CG, 40-hour period		26.3 to 29.8
Greenup *et al* ., 2000		*S. papillosum* lawn-dominated			3.50 to 4.38
Bortoluzzi *et al* ., 2006	Recovering ex-cut-over bog, Jura Mountains 867 m asl, France	Bare peat	CO2 - CCS with portable IRGA; 1-3 weekly, 2-year period; CH4 - syringe and GC; 4-weekly, 2-year period (not during snow cover)	NER: 69.7 to 113 NEE: 69.7 to 113	0.27 to 0.80
Bortoluzzi *et al* ., 2006		*Eriophorum*- dominated		NER: 444 to 785 NEE: −249 to −620	2.00 to 5.20
Bortoluzzi *et al* ., 2006		*Sphagnum* -dominated		NER: 682 to 1247 NEE: -345 to -678	0.67 to 3.60
Lafleur *et al* ., 2003	Mer Bleue ombrotrophic bog, Canada	*Sphagnum* cover with small shrubs	EC, 4-year period	NEE: −278 ± 49 to −37 ± 49	
Lund *et al* ., 2007	Eccentric bog, southern Sweden	Low density tree cover	EC, 1-year period	NEE: −78.6 ± 20.0	

Authors	Study type and location	Study focus / plant cover	Measurement method	CO_2 fluxes in literature Converted to g CO_2 m^{-2} yr^{-1}	CH_4 fluxes in literature Converted to g CH_4 m^{-2} yr^{-1}
Danevčič *et al* ., 2010	Slovenia	Undrained bog forest	CCS, syringe and GC; weekly, 15-month period	NER: 1218	0.31
Danevčič *et al* ., 2010		Drained bog forest		NER: 1787	-0.28
Danevčič *et al* ., 2010		Drained fen grassland		NER: 1778	0.31
Drewer *et al* ., 2010	Auchencorth Moss 267 m asl, Scotland	Bog	CH_4: CCS, syringe and GC bi-monthly to monthly; CO_2: EC; 3-year period	NEE: −118 to −183	0.2 to 0.5
Drewer *et al* ., 2010	Lompolojänkkä, Finland	Fen	CH_4: CCS, syringe and GC bi-monthly to monthly; CO_2: EC; 2-year period	NEE: −5.48 to −52.9	17 to 23
Carter *et al* ., 2012	Northern Europe	Peatlands	CCS, syringe and GC	NER (soil): 847 to 2097	1.23 to 9.19
Salm *et al* ., 2012	Estonia	Natural peatland	CCS, syringe and GC; 1 year period	NER: 553	11.4
Salm *et al* ., 2012		Drained peatland		NER: 704	3.2
Salm *et al* ., 2012		Abandoned mining peatland		NER: 1043	0.009
Salm *et al* ., 2012		Active mining peatland		NER: 638	0.016
Wilson *et al* ., 2013	Post-milled site, re-wetted, Bellacorick, Co. Mayo, Ireland.	*E. angustifolium* microsite	CCS, EGM CO_2 analyser, 2 - 4 weekly; CH_4: syringe and GC, monthly; 3-year period	NEE: −553 ± 319 to −2152 ± 590	6.83 ± 1.81 to 7.20 ± 1.87
Wilson *et al* ., 2013		*Juncus-Sphagnum* microsite		NEE: −158 ± 142 to −774 ± 170	9.07 ± 0.27 to 13.1 ± 0.21
Wilson *et al* ., 2013		*Sphagnum* microsite		NEE: −50.9 ± 180 to −542 ± 253	10.9 ± 0.80 to 16.4 ± 0.40
Wilson *et al* ., 2013		Bare peat microsite		NEE: 137 ± 13.2 to 299 ± 75.9	0.15 to 0.15

Authors	Study type and location	Study focus / plant cover	Measurement method	CO_2 fluxes in literature Converted to g CO_2 m^{-2} yr^{-1}	CH_4 fluxes in literature Converted to g CH_4 m^{-2} yr^{-1}
Helfter *et al* ., 2014	near-pristine ombrotrophic moorland, Auchencorth Moss 267 m asl, Scotland	Hummock (sedge/grass) and hollows (*Sphagnum*)	EC, 11-year period	NEE: −19.1 to −498	
Hommeltenberg *et al* ., 2014	Southern Germany	Natural bog-pine site	EC	NEE: −227 ± 73.3	7.06 ± 0.45
McVeigh *et al* ., 2014	Atlantic blanket bog, 150 m asl, Glencar, SW Ireland	Vascular plants, bryophytes, pools	EC, 10-year period	NER: 810 to 898 g C-CO_2 m^{-2} yr^{-1} NEE: −118 to −290 g C-CO_2 m^{-2} yr^{-1}	
Turetsky *et al* ., 2014	Global	Temperate peatlands	Lit synthesis		39.7
Turetsky *et al* ., 2014		Bogs			35.1
Turetsky *et al* ., 2014		Disurbed sites, drying			1.61
Turetsky *et al* ., 2014		Disturbed sites, rewetting			30.2
Beyer and Höper, 2015	Re-wetted, former peat-mining site, Lower Saxony, Germany	*Molinia caerulea* -dominated	CCS, Licor portable GHG analyser, 4-weekly, 27 months	NER: 3221 ± 436(SE) NEE: −122 ± 156(SE)	0.11 ± 0.04(SE)
Beyer and Höper, 2015		*Eriophorum* -dominated		NER: 3499 ± 359(SE) NEE: −315 ± 424(SE)	24.3 ± 2.3(SE)
Beyer and Höper, 2015		*Sphagnum* -dominated		NER: 1842 ± 301(SE) NEE: −348 ± 82.5(SE)	31.1 ± 0.9(SE)
Beyer and Höper, 2015		*Sphagnum* cultivation		NER: 1658 ± 139(SE) NEE: −362 ± 73.7(SE)	3.2 ± 0.8(SE)
Levy and Gray, 2015	semi-natural peatbog, Forsinard 120-438 m asl, Scotland	Blanket bog with pools	CO_2: EC; CH_4: CCS, syringe and GC; 6-year period	NER: 1690 NEE: −418	5.77
Abdalla *et al* ., 2016	Northern hemisphere	Northern peatlands	Lit synthesis		16.0 ± 28.0

Authors	Study type and location	Study focus / plant cover	Measurement method	CO_2 fluxes in literature Converted to g CO_2 m^{-2} yr^{-1}	CH_4 fluxes in literature Converted to g CH_4 m^{-2} yr^{-1}
Davidson *et al* ., 2016	Arctic tundra, northern Alaska	Wet sedge	CCS, Los Gatos UGGA; 3 month-period		19.6 ± 23.6
Davidson *et al* ., 2016		Tussock sedge			5.37 ± 8.88
Davidson *et al* ., 2016		Moss			2.92 ± 3.15
Davidson *et al* ., 2016		Dry gramminoid			1.17 ± 3.85
Davidson *et al* ., 2016		Moss-shrub			0.70 ± 1.75
Davidson *et al* ., 2016		Moss-lichen			0.00 ± 0.23
Evans *et al* ., 2016	Peat-milled site, Manchester UK	Active mining peatland		NER: 506 NEE: 506	0.24
Evans *et al* ., 2016	Re-wetted, domestic-cut site, Manchester, UK	*Molinia caeulea* -dominated	CCS, Los Gatos UGGA, 2 - 4 weekly, 22-month period	NER: 3241 NEE: −1397	42.1
Evans *et al* ., 2016		*Sphagnum* -dominated		NER: 1104 NEE: −216	46.8
Evans *et al* ., 2016		*Molinia-Eriophorum* -dominated		NER: 2031 NEE: 389	42.4
Evans *et al* ., 2016	Re-wetted peat-milled site, Thorne Moors, UK	*E. vaginatum* -dominated	CCS, Los Gatos UGGA, 2 - 4 weekly, 16-month period	NER: 3652 NEE: 818	15.87
Evans *et al* ., 2016	Abandoned peat-milled site, Thorne Moors, UK	Bare peat		NER: 524 NEE: 524	-0.05
Hambley *et al* ., 2019	de-forested and re-wetted peatbogs, Forsinard Flows 180-196 m asl, Scotland	16 yr ex-forestry peatland	EC, 1-year period	NEE: −260	
Hambley *et al* ., 2019		10 yr ex-forestry peatland		NEE: +293	

Authors	Study type and location	Study focus / plant cover	Measurement method	CO_2 fluxes in literature Converted to g CO_2 m^{-2} yr^{-1}	CH_4 fluxes in literature Converted to g CH_4 m^{-2} yr^{-1}
Jacotot et al., 2019	Acidic fen, central France	*Molinia caerulea* - dominated;	EC; 2-year period	NEE (mean): −291 to -1235	−0.19 to 0.19
Renou-Wilson *et al* ., 2019	Post-milled, drained, Blackwater, Irish Midlands	Bare peat	CCS, EGM CO_2 analyser, 2 - 4 weekly; CH_4: syringe and GC, monthly; 4-year period	NEE: 554 ± 40.3	0 ± 0
Renou-Wilson *et al* ., 2019	Post-milled, re-wetted, Blackwater, Irish Midlands	Reed microsite		NEE: −136 ± 840	8.93 to 12.0
Renou-Wilson *et al* ., 2019		Sedge microsite (*C. rostrata, E. angustifolium*)		NEE: 330 ± 249	5.60 to 6.00
Renou-Wilson *et al* ., 2019	Drained, domestic-cut site, Moyarwood, West Ireland	*Sphagnum* , low shrubs, ditches	CCS, EGM CO_2 analyser, 2 - 4 weekly; CH_4: syringe and GC, monthly; 4-year period	NEE: 502 ± 88.0	1.03 ± 0.65
Renou-Wilson *et al* ., 2019	Re-wetted, domestic-cut site, Moyarwood, West Ireland	*Sphagnum* , low shrubs, ditches		NEE: −180 ± 249	26.3 ± 6.67

CCS = Closed chamber system; EC = eddy covarience tower; GC = gas chromatography; NER = Net Ecosystem Repiration; NEE = Net Ecosystem Exchange; Micrometerological sign convention used whereby positive values indicate gas flux emission from the ecosystem to the atmosphere and negative values indicate gas flux uptake into the ecosystem from the atmosphere; ranges indicate lowest to highest yearly flux across study periods; mean values ± standard deviation unless otherwise indicated.

Chapter 5: Analysis of surface peat chemistry and peat cores at carbon GHG flux trial plots

5.1 Introduction

Assessment of the quality of peat at the surface and in underlying layers on a degraded peatland can provide an insight into the degree of damage sustained and the likelihood of successful restoration, as well as an understanding of GHG flux drivers. A degraded peat surface can be hostile to establishment of non-vascular plants (Renou-Wilson *et al*., 2019), particularly *Sphagnum* mosses (Quinty and Rochefort, 2003), due to peat shrinkage and compaction, reducing hydrological conductivity and making moisture unavailable at the surface (Price *et al*., 2003), but also due to chemical changes (Wind-Mulder *et al*., 1996) as decomposition also reduces nutrient availability to support both plants and microbial communities (Krüger *et al*., 2015) and these effects can linger in the early stages of peatland restoration (Andersen *et al*., 2006). Therefore, peat substrate quality and oxidation on a degraded peatland (in terms of carbon and mineral content, pH, density and porosity) can influence the magnitude of autotrophic and heterotrophic respiration and plant photosynthesis, and thus gaseous GHG flux rates (Andersen *et al*., 2006). Moreover, peat quality can be highly variable depending on remaining depth and past drainage, use or mining regimes (Basiliko *et al*., 2007; Lindsay and Clough, 2016; Zając *et al*., 2018) and degree of water table draw-down (Macrae *et al*., 2012), making site comparisons difficult. Additionally, this site is close to the large conurbation of Greater Manchester, so may not only have legacy effects from the Industrial Revolution (Fletcher and Ryan, 2018), but also current effects from air pollution and surrounding land usage (apis, 2020).

Empirical critical loads for pollutants on this type of site ('Degraded raised bogs still capable of natural regeneration H7120') are compared with Manchester Mosslands (3-year mean for 2016-18) (apis, 2020) in Table 5.1 and show high critical loads for nutrient nitrogen, ammonia and acidity. This is likely to be detrimental to lichens and bryophytes through competition from increased growth of nitrogen-demanding scrub (Krupa, 2003), and potential toxicity through greater mobilisation of Al^{3+} ions (apis, 2020). NOx and SO_2 do not appear to be of current concern, and concentrations of these factors show a

consistently falling trend since 2012 (apis, 2020), indicating current critical load exceedances are probably from agricultural sources.

Table 5.1. Comparison between Critical Loads of air pollutants determined for H7120 habitats and averages recorded on the Manchester Mosses SAC (apis, 2020).

Air Pollutant	Habitat Empirical Critial Loads (annual mean)	Set for	Manchester Mosses Average
Nitrogen Deposition kg N ha^{-1} yr^{-1}	5 - 10	All vegetation	19.7
Acid Deposition: Nitrogen | Sulphur keq ha^{-1} yr^{-1}	0.45 | 0.25	All vegetation	1.4 | 0.3
Ammonia Concentration µg NH_3 m^{-3}	1	Lichens and Bryophytes	2.13
NOx Concentration µg NOx m^{-3}	30	All vegetation	20.68
SO_2 Concentration µg SO_2 m^{-3}	10	Lichens	2.15

Cadishead Moss has been subject to degradation and change through drainage and repeated harvesting for peat, and then during the restoration process (see Appendix 6). The area containing the field plots was block-cut for peat and then mechanically scraped, and subsequently bunded during restoration, creating a contrasting area to much of the site, where *Molinia caerulea* and *Calluna vulgaris* strips between shallow ditches edged mostly with *Sphagnum fimbriatum, S. palustre* and *E. angustifolium* remain. Some other scraped areas on site are generally wet basins surrounded with bunds and colonised with *E. angustifolium, S. cuspidatum and S. fimbriatum*. More bunding work to raise water levels has recently been completed, intending to effect reduction in *Molinia caerulea* cover.

The aims of this study were to assess the potential influence of peat quality, in terms of elements and characteristics, on carbon greenhouse gas (CGHG) fluxes monitored at the

site (Chapter 4), and site recovery. The objectives were to analyse peat at the surface (physically and chemically) and along 1 m peat cores, and determine the peat depth at each CGHG flux trial plot. The hypothesis was that long-term degradation of the site had resulted in poor-quality surface and sub-surface peats, which continued to create obstacles to restoration and reduced capacity for CGHG uptake.

5.2 Methods

5.2.1 Surface peat chemical analysis

Before the start of GHG flux field monitoring, 3 samples were collected to a few centimetres depth (24th June 2016) from around each trial plot and homogenised to make up one replicate composite sample per plot (*n* = 9) (plot locations at Figure 5.1 and also see Chapter 4). Fresh well-mixed peat samples (5 gm of each) were added to 25 ml DI water, stirred regularly, and electrical conductivity (Jenway 4510 analyser) measured in the order of sample preparation. Samples were re-stirred and pH measured (Jenway 3510 analyser), leaving the probe in the solution for 30 seconds. Further fresh-peat samples were prepared for extraction of ammonium and nitrate using 1% KCl (as recommended by Allen, 1989) for ion chromatography (IC) (Thermo Scientific Dionex AS analyser) and extraction of elements (Ca, Fe, K, Mg and P) using 0.1M EDTA (as recommended by Lo and Yang, 1999) for inductively coupled plasma - optical emission spectrometry (ICP-OES) (Thermo Scientific iCAP 6000 Series ICP Spectrometer). Extractable values were seen as more useful determinants of element bioavailability in the peat than total values (Rosenburgh, 2015). Peat samples (mean weight of 2.5316 ± 0.0235 g) were put into 100 ml conical flasks, including one blank sample for each extraction, with 25 ml of the appropriate extraction solution, and the flasks agitated on an orbital shaker for 30 minutes. Samples were filtered through Sartorius™ Minisart™ Plus Syringe Filters (0.2 µm) into tubes for analysis (1 ml for IC and a minimum of 10 ml for ICP-OES), discarding the first 5 ml of filtrate to remove any filter contaminants.

The remainder of the fresh samples were weighed, then oven dried overnight at 105°C and weighed again to find the mass difference to give sample moisture content. Samples were removed for dry analysis (see below) and the remainder re-weighed and placed in a

muffle furnace at 550°C for 3 hours to find the mass difference (loss-on-ignition) from which organic matter and mineral fractions were estimated. Total C and N content were analysed (using a LECO FP628 elemental analyser) using 0.1513 ± 0.0006 g (mean sample weight) of dry, ground peat placed into tared aluminium foil cups twisted into capsules, with five calibration capsules prepared in the same way using EDTA LECO calibration 502-092 (mean weight 0.1508 ± 0.0005 g).

Additionally, surface peat samples were also taken with a small metal cylinder from trial plots (1 per plot) on 8th September 2019 at 0 - 10 cm depth to assess the degree of decomposition using the Von Post scale, whereby organic soils are squeezed through the fingers and the colour and viscosity of the exudate is assessed on a scale from H1 (undecomposed) to H10 (fully decomposed) (Stanek and Silc, 1977). The degree of humification, particularly if the peat depth is known, may help to determine the quality of peat remaining (e.g., *Sphagnum* ['white'] peat or fen peat) and the likely restoration potential (Lindsay and Clough, 2016).

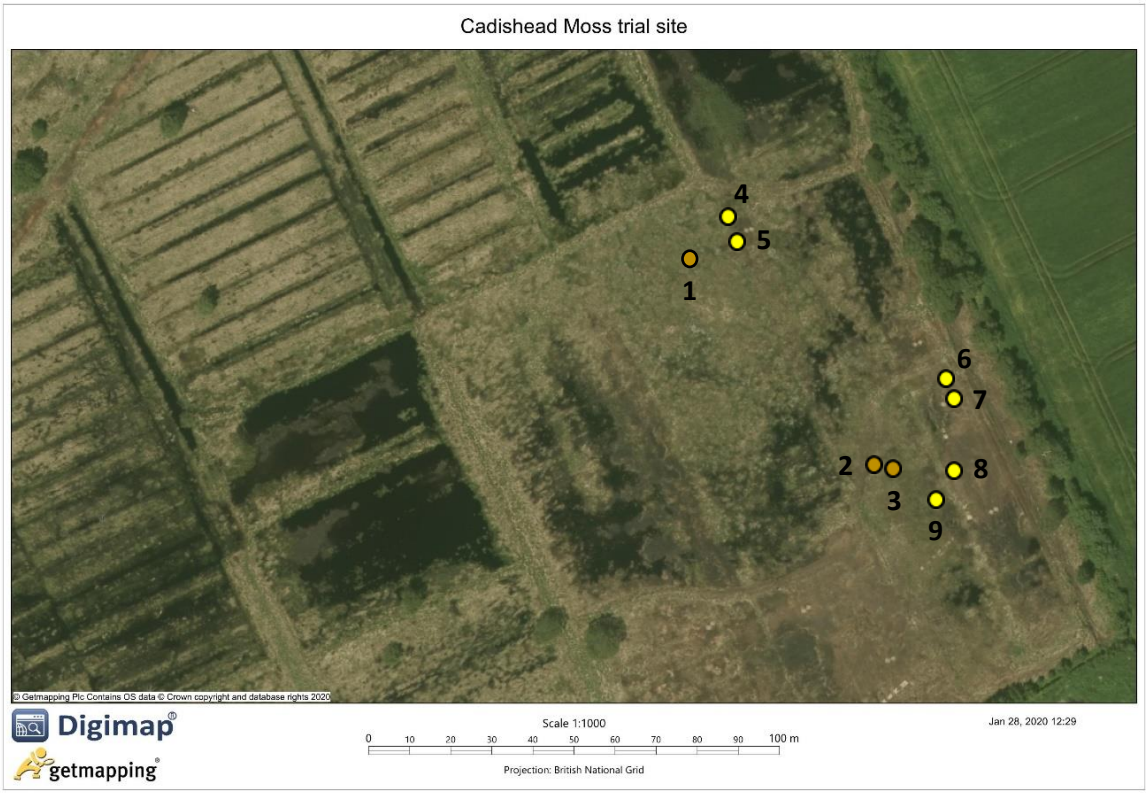


Figure 5.1. Detailed aerial photograph of trial site area on Cadishead Moss, showing location of trial plots. Mature plots: orange dots; immature plots: yellow dots. Using: EDINA Digimap Ordnance Survey Service https://digimap.edina.ac.uk/aerial

5.2.2 Peat core analysis and peat depth measurements

Nine peat cores (one from each CGHG flux trial plot) (Figure 5.1 and also see Chapter 4) were harvested with a Russian corer on 11th to 14th January 2019, on the edge of each *Sphagnum* application area (examples at Figure 5.2). Each core was separated into 5 cm segments and bagged on site immediately after coring. Bags were cold-stored before starting analysis 3 days later. A sample cube (mean size of 10.9 ± 2.0 cm^3) was cut from each segment with a sharp knife to prevent compression, measured and weighed into a crucible, dried at 105°C overnight (about 18 hours), re-weighed and muffle-furnaced at 550°C for 3 hours to calculate loss-on-ignition. Fresh samples were analysed for electrical conductivity and pH and the remainder dried, ground, and analysed for %C and %N using the LECO analyser, as above, using 0.1510 ± 0.0006 g (mean ± SD) of peat material and 0.1511 ± 0.0007 g of EDTA LECO calibration 502-092. Peat depth was measured on 5th February 2019 in the *Sphagnum* area on each trial plot, using 5 mm diameter threaded rods.

5.2.3 Water table depth measurements

Methods for monitoring the water table depth (WTD) are given in section 4.2.2.

5.2.4 Data analysis

Data was grouped by mature (plots 1, 2 and 3) and immature (plots 4 to 9) vegetation treatment plots (as for CGHG study plots, Chapter 4; location: Figure 5.1). Data from surface (0 - 5 cm depth) of peat core samples analysed post-trial were used for comparison with data from surface peat samples analysed pre-trial to assess any changes over the period of study. There were two single spikes in mineral content at 35 - 40 cm in both 'mature' plot 1 (12.2%) and 'immature' plot 6 (19.9%), assumed to be due to either a processing error, or a relic of peat harvesting and/or restoration. These data were removed from analysis of mineral content so that background means could be assessed. Data were prepared through Microsoft Excel (2019) and analysed statistically using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Data were tested for

normality using Shapiro Wilk tests. Pre- and post-trial surface peat chemistry data were found to be both normally and non-normally distributed, and Mann-Whitney U tests were used to test for differences across all samples and between and within samples from mature and immature plots. Peat core data was found to be non-normally distributed and Kruskal-Wallis H tested differences in data along cores taken from mature and immature plots.

5.3 Results

5.3.1 Surface peat chemistry

Extractable ammonium, nitrate and macronutrient content of surface peat was only sampled pre-trial (data in Appendix 7). Ammonium and nitrate levels were highly variable across plots, with a notably high nitrate value on 'mature' plot 1 (wet with dense vegetation) and a high ammonium value on 'immature' plot 7 (dry, edge of site with sparse vegetation). The levels of total N and extractable Ca were similar throughout plots, with variability in other elements, particularly K.

There were statistically significant differences in values of a range of peat characteristics across all plots pre- and post-trial as determined by Mann-Whitney U tests: both pH and conductivity ($U = 81$, $p < 0.001$), both % organic and % mineral matter ($U = 80$, $p < 0.001$), and %C ($U = 67$, $p = 0.019$) ($n = 18$ throughout). Differences in values of FW:DW ratio, % moisture, %N and C:N ratio were not significant. Statistically significant differences in values pre- and post-trial within mature ($n = 6$) and immature ($n = 12$) plots, as determined by Mann-Whitney U tests, are indicated in Table 5.2. Observing the data (Table 5.2; full data in Appendix 8), post-trial pH and electrical conductivity were lower throughout compared to pre-trial. The FW:DW ratio and the moisture content of samples was higher pre-trial and post-trial in mature than in immature plots, but the FW:DW ratio and the moisture content had decreased in mature plots and increased in immature plots over the period. The organic content increased and mineral content decreased in both mature and immature plots over the trial period, with a greater change in immature plots so that organic content was greater, and mineral content was lower in immature plots than mature plots post-trial. The %C had reduced post-trial throughout, but %N had

decreased in mature plots and increased in immature plots post-trial to produce a corresponding increase in C:N ratio in mature plots and decrease in immature plots.

Table 5.2. Comparison of surface peat characteristics pre-trial (unshaded) and post-trial (shaded) on GHG flux trial plots, grouped by vegetation maturity (see Chapter 4). Values: mean ± SD; significant differences between each pair, determined by Mann-Whitney U tests, are indicated: *$p < 0.05$.

Surface peat characteristics	Mature		Immature	
pH	4.73 ± 0.13	4.23 ± 0.17	4.93 ± 0.22 *	4.05 ± 0.28 *
Conductivity (µS)	43.40 ± 2.86	29.37 ± 5.70	46.48 ± 5.09 *	31.05 ± 4.54 *
FW:DW ratio	8.42 ± 0.77	7.82 ± 1.65	6.66 ± 1.11	6.84 ± 1.09
Moisture content (%)	88.05 ± 1.13	86.88 ± 2.72	84.66 ± 2.37	85.04 ± 2.58
Organic content (%)	96.87 ± 0.87	97.96 ± 0.76	95.11 ± 1.91 *	98.38 ± 0.38 *
Mineral content (%)	3.13 ± 0.87	2.04 ± 0.76	4.89 ± 1.91 *	1.62 ± 0.38 *
%N	1.44 ± 0.07	1.31 ± 0.16	1.39 ± 0.08	1.47 ± 0.33
%C	50.98 ± 0.67	49.54 ± 0.02	50.72 ± 1.11	49.10 ± 1.30
C:N ratio	35.50 ± 1.73	38.20 ± 4.87	36.58 ± 2.69	34.34 ± 5.68

Unshaded = pre-trial (24 June 2016); Shaded = post-trial (14 January 2019).

5.3.2 Peat cores and peat depth

Peat cores from CGHG trial plots were grouped for analysis by vegetation maturity (as in Chapter 4) (see Figure 5.1 and Appendix 6). Cores were visibly different (examples in Figure 5.2) in terms of peat colour, texture and vegetation content. Cores were not examined for vegetation content, but most cores had obvious sedge-graminoid material in lower sections. All cores had dark and coarse or open-textured (oxidised) peat at the surface, to a depth of between ~10 and ~ 28 cm, containing coarse stems and roots of *E. angustifolium*. In most cores there was friable peat at the surface, to a depth of between

~ 3 and ~24 cm. Peat colour then changed to varying striated mixtures of black/orange, orange/black, with varying openness of texture, and fine *E. angustifolium* roots throughout.

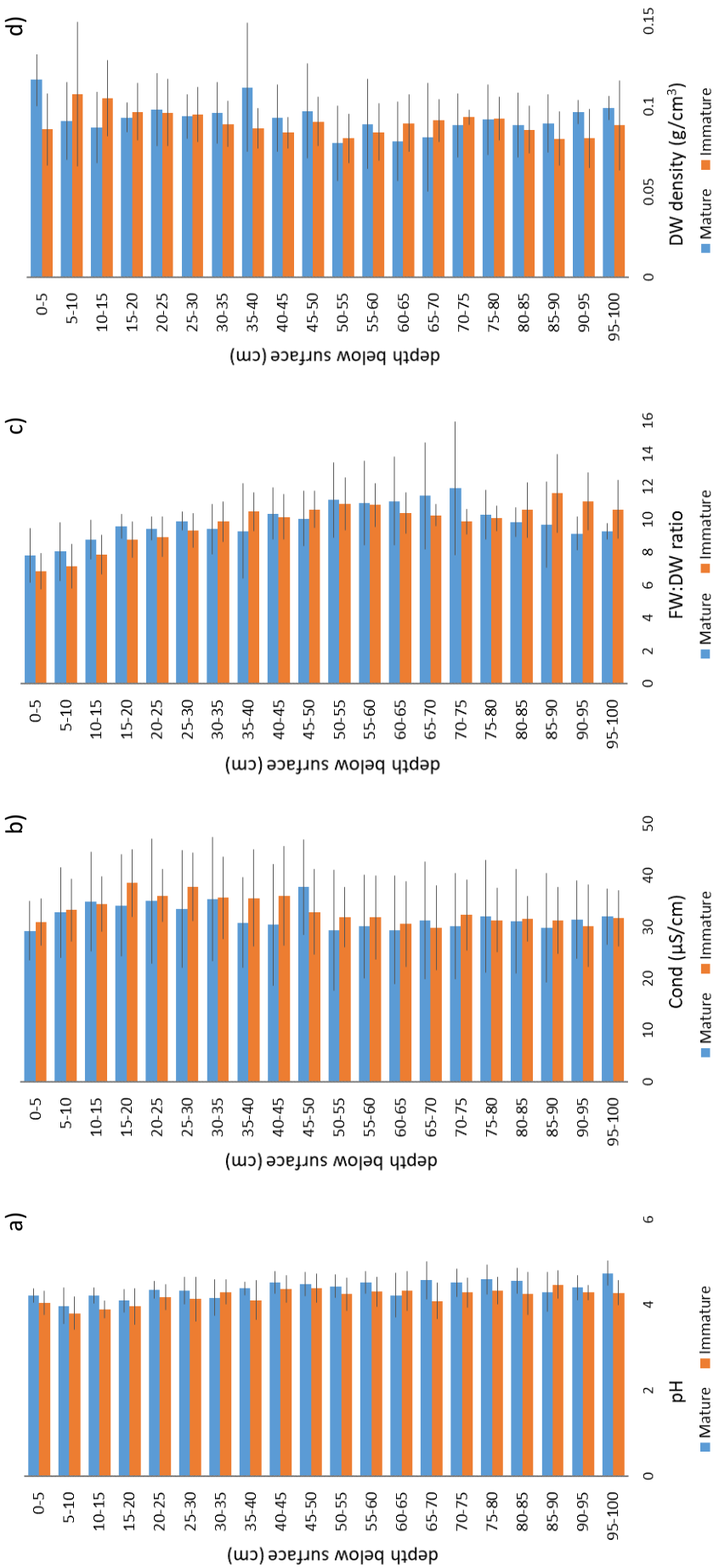
A Kruskal-Wallis H test showed that there was no statistically significant difference in measurements (collated sample values from 0 - 100 cm depth) of EC, FW:DW ratio, FW or DW density, %N, %C, or C:N ratio between mature and immature plots. However, there was a statistically significant difference in pH (H = 9.30, p = 0.02) and % mineral and organic contents (H = 22.25, $p < 0.001$ for both) (df = 1 throughout), although these values (Figure 5.3 and Appendix 8) were still within a narrow range and what might be expected in an acidic, organic soil.

Looking at general trends in the peat cores (Figure 5.3), pH was generally slightly higher and conductivity slightly lower along the cores in the mature than immature plots. FW:DW ratio was lowest at the surface of cores particularly those from immature plots, increasing gradually down the core depth to approximately 55 - 60 cm depth, after which the ratio remained approximately the same along the remainder of the cores, although greater in immature cores in the deepest layers. DW density was mixed throughout although mature cores at the surface were surprisingly dense, and there was a greater density in immature cores at 5 to 15 cm below the surface. The percentage of mineral content was greater in cores from mature plots at most levels, and conversely the percentage of organic content was greater in cores from immature plots (not shown). The percentage of nitrogen was greater in cores from the immature plots in the top 15 cm (and C:N ratio conversely greater in cores from mature plots), and then similar between types until the deepest layers, where %N and C:N ratio varied between core types. The mean C:N ratio across all samples was 44.9 ± 7.69, CV = 17.2%. The differences appear to be driven more by changes in %N (mean 1.15 ± 0.23, CV = 19.9%) than in %C (mean 50.1 ± 3.28, CV = 6.6%).

The WTD (Figure 5.4) was consistently deeper below the surface in plots of immature than in mature vegetation, with a dramatic drop in summer 2018 across all plots, as discussed in Chapter 4. The mean WTD below the surface at the time of coring (January) was -2.0 ± 2.6 cm in mature plots and -8.7 ± 1.4 cm in immature plots.

Figure 5.2. Examples of peat cores taken from CGHG flux trial plots (Chapter 4). Top of core at left of each picture.

Peat depth was more than 2 m in all plots apart from plot 4, where depth was 1.96 m (Appendix 8). Mean peat depth was 2.29 ± 0.23 m. Surface peat decomposition was assessed at Von Post scale H4 - 5, although assessment was difficult as plot 1 was flooded, plot 6 had very little water content, and plot 8 contained obvious sedge/grass material.


a)
pH
Mature
Immature
depth below surface (cm)
b)
Cond (µS/cm)
Mature
Immature
depth below surface (cm)
c)
FW:DW ratio
Mature
Immature
depth below surface (cm)
d)
DW density (g/cm3)
Mature
Immature
depth below surface (cm)

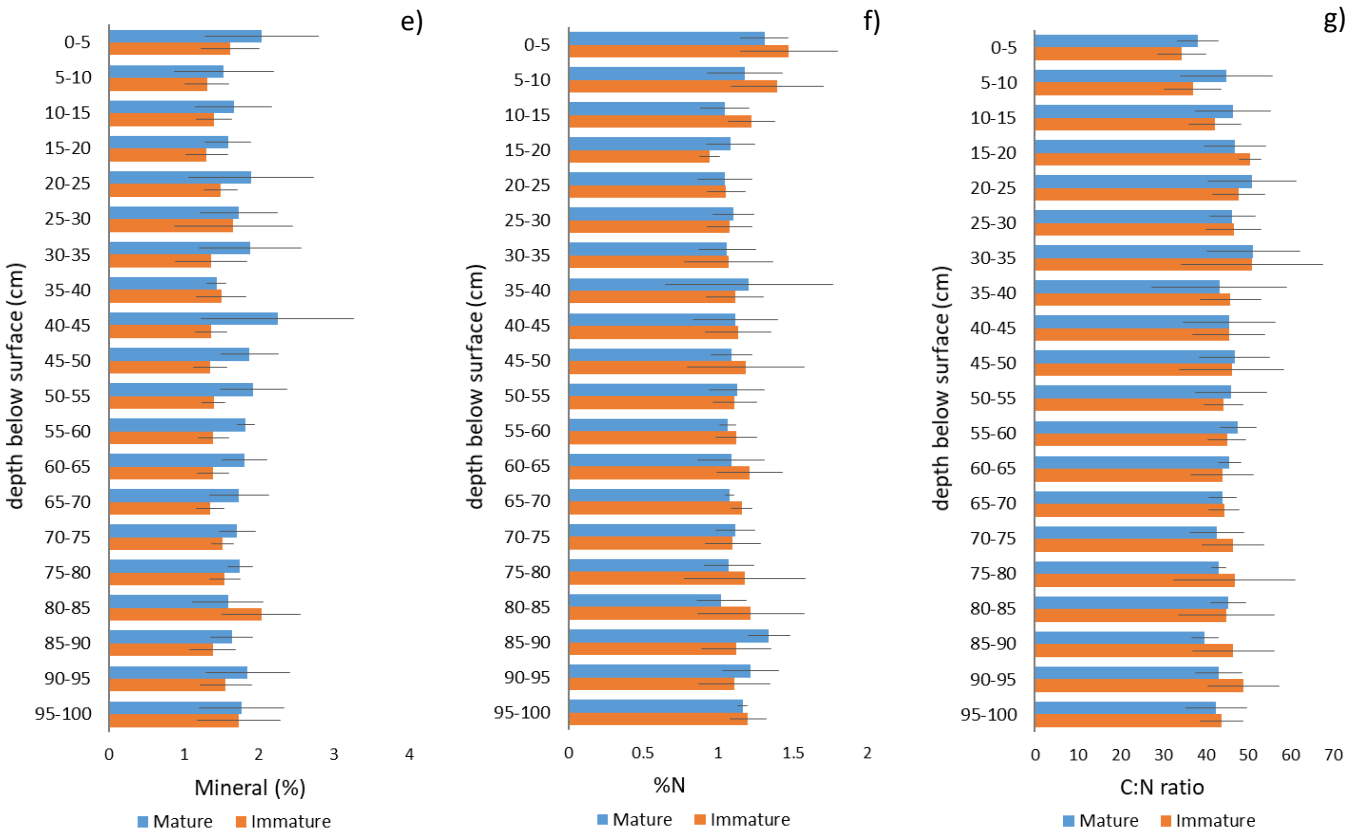


Figure 5.3. pH (a), Conductivity (b), Fresh weight:Dry weight (FW:DW) ratio (c), DW density (d), % mineral matter (e), % Nitrogen (f), C:N ratio (g) along peat cores from surface to 1 m depth in 5 cm increments, harvested from CGHG plots (see Chapter 4) of 'mature' and 'immature' vegetation. Mature plots *n* = 3, immature plots *n* = 6. Values = mean ± SD error bars.

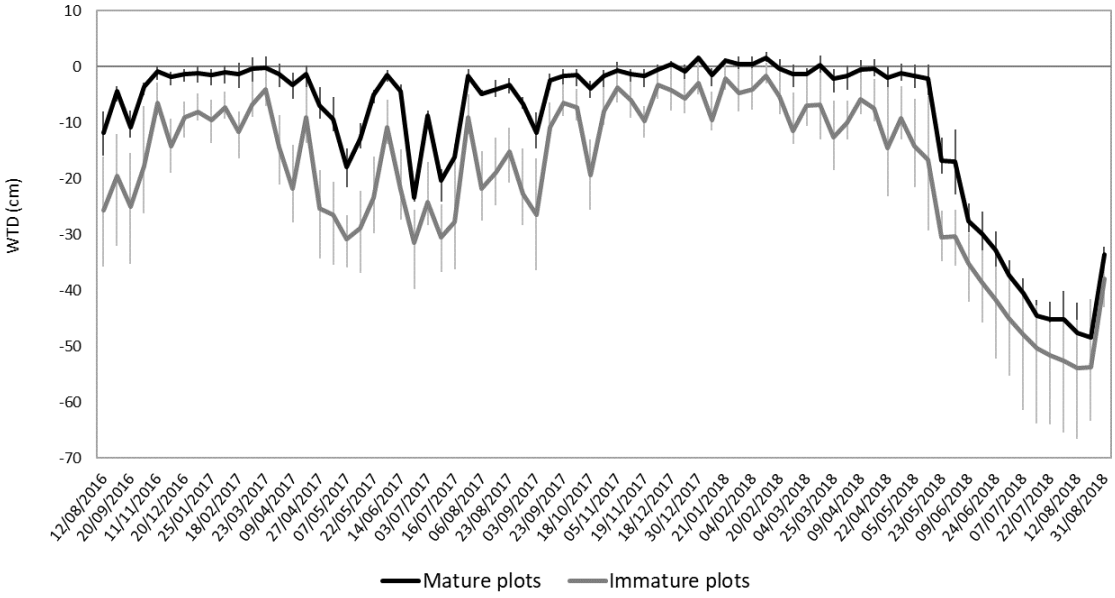


Figure 5.4. Mean water table depth (WTD) measured at CGHG flux monitoring plots (grouped by 'mature' and 'immature' vegetation, Chapter 4) from August 2016 to end of two-year trial period, August 2018. Error bars show minimum and maximum values.

5.4 Discussion

Cadishead Moss is a degraded peatland site that has undergone restoration re-wetting measures, is almost completely covered in vegetation, and is now recognised as a 'Site of Biological Importance'. This should not signify that the underlying peat has recovered to anything approaching the same functionality as that of natural peatlands. Greater understanding of peat quality and characteristics, which have not been assessed on this site before, are useful to understand the current level of site condition and inform future management decisions which could accelerate site rehabilitation and resilience, particularly in the face of future climate scenarios.

The quality of surface peats on this site retain many of the characteristics of peat-extracted sites. It is difficult to draw direct comparisons between studies in the literature due to the wide range of site variables such as climate, underlying geology, remaining peat depth, mining techniques and duration, and restoration techniques and time-spans which influence peat chemistry and hydrological capacity (Basiliko *et al*, 2007; Zając *et al*., 2018), and also how these disturbed systems influence the highly complex nature of

microbial processes driving their carbon balance (Andersen *et al*., 2013), but some inferences may be drawn from the results of this study.

The elements P (non-detectable [ND] to 0. 003 mg g^{-1}) and K (ND to 0.052 mg g^{-1}) in surface peat were variable across plots but appeared to be quite low compared to a study on semi-natural bogs (Table 5.3) where P and K are usually conserved primarily in the upper peat layers, and so low levels of P and K on this site are indicative of entire surface peat removal (Andersen *et al*., 2006). However, it was difficult to find studies with comparable methods and values for extractable rather than total elements. Levels of Mg were also low (ND to 1.13 mg g^{-1}). Basiliko *et al*. (2007) suggest that peat harvesting not only removes macronutrients, but also cations such as Mg which support enzyme activity and metabolism of elements, such as N and P. These in turn support microbial activity and CO_2 production, drivers of the carbon cycle which regulates CGHG flux. The level of total N appears to be within the range for other disturbed bogs in the literature at 1.41 %, perhaps sustained by high levels of atmospheric nitrogen deposition (Table 5.1), making both the N:P and N:K ratios rather high. Higher nitrogen levels may promote growth of invasive species over that of more nitrogen-sensitive plants such as bryophytes (Phoenix *et al*., 2012).

However, both ammonium and nitrate appear low (apart from Plot 1) compared to other studies. A relatively high pH (for peat bogs) and dry conditions, as on much of this site, generally allow greater growth and activity of nitrifying bacteria. But nitrogen consumption by plants may be high (Glatzel *et al*., 2008) and, if coupled with low bacterial numbers and activity, would result in low ammonium and nitrate levels in surface peat (Wind-Mulder *et al*., 1992). High nitrate levels in the location of Plot 1, which had a more consistently high water-table likely to reduce the number of nitrifying bacteria, may be due to localised nutrient input at the time of sampling.

The Quinty and Rochefort (2003) guide to peatland restoration using the moss layer technique advocated adding phosphorus, with caution, to promote plant growth in the P-limited environment of a bare peat site, although Taylor *et al*. (2019) found there to be 'trade-offs between benefits and harms' related to adding inorganic fertilizer to restoration sites. Phosphorus addition apparently encourages linear *Sphagnum* growth

but not density (Fritz *et al*., 2012), and *Sphagnum* etiolation due to nutrient inputs can cause vulnerability and loss of growth in periods of drought (Aerts *et al*., 2001). Nutrient (lime and fertilizer) application is part of the restoration process on UK uplands, to promote vascular plant growth and prevent further erosion (Uplands Management Group, 2017). But Lancashire Wildlife Trust (LWT) personnel are opposed to adding broad-application nutrients to this lowland site, which would likely promote unwanted scrub development. However, BeadaMoss® products currently used in restoration efforts on Cadishead Moss provide only localised nutrients, which may help counteract the low-nutrient status of the degraded peat surface enough to promote *Sphagnum* development.

The moisture content of the peat is similar pre-trial (summer 2016) and post-trial (winter 2018) which perhaps indicates increased peat compaction and reduced permeability (Price *et al*., 2003) caused by the summer 2018 drought. Electrical conductivity (not corrected for pH) and pH were both slightly lower post-trial, and well within the thresholds of < 100 µS and < 5 respectively throughout for restoration to an ombrotrophic bog (Quinty and Rochefort, 2003). Mineral content was generally lower post-trial and organic content was higher, perhaps reflecting the increase in vascular vegetation cover, mineral nutrient uptake and resulting decomposition of material over the course of the trial. Post-trial %C was lower than pre-trial throughout, which could perhaps be due to improved microbial activity with a greater organic input and aerobic conditions, particularly during the recent summer drought, resulting in marginally increased CGHG emission in most plots in the second year. The %C content is still within the range for natural bogs in the literature (Table 5.3).

Table 5.3. Comparison of peat characteristics, total N and C, and extractable P, K, Ca, Mg, NH^{-}_{4} and NO^{-}_{3} between a range of literature sources and this study.

Author	Site type	Sampling depth	Location	pH	EC (µS)	Density (g cm^{-3})	N (%)	P (%)	K (%)	Ca (mg/g)	Mg (mg/g)	NH^{+}_{4} (mg/kg)	NO^{-}_{3} (mg/kg)	C (%)	C/N	Organic (%)	Mineral (%)
Andersen *et al* ., 2006	Natural	oxic layer above WTD	Bois-des-Bel field station, Québec, Canada	3.8	70		0.48	0.047	0.077	1.3	1	2.2	<0.001				
	Restored (majority vegetated)			4.46	170		0.65	0.02	0.024	2.8	2.1	2.7	<0.001				
	Cutover (majority non-vegetated)			4.14	85		0.57	0.017	0.024	2.9	2.5	1.3	<0.001				
Andersen *et al* ., 2011	Bog	0 - 60 cm [one bog area studied 0 - 5.5 m]	Québec, Canada No. of sites: Bog 8, Exploited 93, Restored 6, Fen 14	2.9 - 3.2	9 - 40		0.58	0.023 - 0.045	0.009 - 0.083	0.10 - 0.90	0.39 - 0.98						
	Exploited			2.9 - 5.0	11 - 182		1.20 - 1.29	0.003 - 0.026	0.012 - 0.016	0.89 - 4.75	0.20 - 1.69						
	Restored			4.5	185		1.23	0.026	0.034	6.89	3.06						
	Fen			6.2	240		1.76	0.065	0.18	15.75	1.26						
Macrae *et al* ., 2012	Natural Bog	0 - 10 cm	St-Charles-de-Bellechasse, Québec, Canada			0.05 - 0.06	0.89 - 1.19	0.03 - 0.06					2 - 7	47 - 51	43 - 53	96 - 97	
	Drained Bog					0.07 - 0.09	1.09 - 1.92	0.04 - 0.05					13 - 15	48	25 - 44	92 - 95	
	Natural Poor Fen					0.04 - 0.05	1.31 - 1.76	0.04 - 0.05					4 - 5	46 - 49	28 - 35	93 - 94	
	Drained Poor Fen					0.07 - 0.08	1.52 - 3.08	0.05 - 0.06					14 - 16	47 - 49	16 - 31	91 - 92	
Kruger *et al* ., 2015	Natural	0 - 5 cm	Ahlen-Falkenberger peatland, N Germany			0.03	1.06							46	52		1.61
	Drained - extensive grassland					0.22	2.29							47	25		8.65

Author	Site type	Sampling depth	Location	pH	EC (µS)	Density (g cm^{-3})	N (%)	P (%)	K (%)	Ca (mg/g)	Mg (mg/g)	NH^{+}_{4} (mg/kg)	NO^{-}_{3} (mg/kg)	C (%)	C/N	Organic (%)	Mineral (%)
Evans *et al.*, 2016	Re-wetted bog	0 - 50 cm	Range of peatlands, UK	2.66		0.14	1.47							47.1	32	95	5.4
	Extracted bog			2.96		0.24	1.43							50	35	93	7.5
	Low-nutrient Fen			5.48 - 7.54		0.17 - 0.37	2.03 - 2.59							32 - 45	16 - 18	48 - 87	13 - 52
Zając *et al* ., 2018	Bog - recovering 30 years post-extraction	0 - 10 cm	Bór za Lasem, S Poland	3.29	134	0.22	1.65	0.0018				78	1.51	56	34	89	10.9
Renou-Wilson *et al* ., 2019	Milled-recovering 30 yrs (Phragmites peat)			4.9	350	0.14 - 0.19	2.14							52.4	24.5		
	Drained-recovering 30 yrs (Sphagnum peat)			4.4	102	0.08 - 0.13	1.32							51.5	39		
This study	Bog - recovering 10 years post-extraction	0 - 5 cm	Cadishead Moss, UK	4.86	46	0.097	1.41	0.0001	0.0016	3.3	0.6	3.6	1.72	51	36	96	4.3

Missing values in literature sources were either not assessed or were not comparable with this study due to experimental differences. Mean values to two sig. fig. only where applicable are included (i.e. SE or SD values are omitted). Ranges of values are across several sites (e.g. Evans *et al* . (2016) for low-nutrient poor fens), or wthin sites as provided in the literature. EC = electrical conductivity; Density = dry weight density; this study: density - post-trial; all other values pre-trial.

From a visual inspection of colour and texture, the peat cores appeared to contain *Sphagnum* and sedge material, in common with Swindles *et al*. (2016) who signified the orange layers of cores were *Sphagnum*/sedge peat. Characteristics and elements of peat cores in this study align quite well with those corresponding to lower profiles of raised bogs and upper profiles of poor fens (Table 5.4) (Rydin and Jeglum, 2013), although due to the variability of sites and levels of disturbance on degraded bogs (Basiliko *et al*., 2007; Zając *et al*., 2018; Renou-Wilson *et al*., 2019), this is likely to be an over-simplification.

The water table was generally lower in plots clustered near the edge of the site (6,7 and 8, see Appendix 6), close to what used to be a boundary ditch before infilling works by LWT, and where *E. angustifolium* continues to be sparser than in other plots at the time of writing. Low WTD is of particular concern, as the influence of nitrogen deposition is increased when concentrated in solution (Pearce and Van der Wal, 2008) and there may be cumulative N-load effects (Sheppard *et al*., 2014), compromising bryophyte growth and making scrub development more likely on dry peatland sites with high atmospheric nitrogen deposition.

The lowest WT drawdown during summer 2017 and particularly during the summer 2018 drought was in plot 4 which had, notably, the only peat depth less than 2 m. Lindsay and Clough (2016) suggest that there is both seepage of water into mineral layers in base peats, higher surface evaporation in shallower peat layers, and that restoration to ombrotrophic bog conditions is unlikely on degraded sites with a peat layer less than 2 m deep, which is likely to be remnant fen peat.

Significant proportions of the top 50 cm of the cores were black, oxidised, rough-textured peat, showing evidence of the compaction and hydrological instability typical of damaged peatlands where the acrotelm has been removed (Price *et al*., 2003; Lindsay and Clough, 2016). Moreover, all bare plots, and some plots with immature vegetation, cracked during the summer drought, which is a likely sign of humification, and no doubt allowed greater evaporation down the peat profile (Lindsay and Clough, 2016). Variability in character and composition throughout the peat core layers could be evidence of disturbance from previous peat cutting and subsequent restoration works, an erratic

water table level currently, or artefacts of changes during initial fen peat formation. Because this area of the site has been previously scraped with none of even the original block-cut surface remaining, rehabilitation to ecohydrological function, particularly in the short term, is questionable (Price *et al*., 2003). The chapter hypothesis appears to be supported, that long-term degradation of the site had resulted in poor-quality surface and sub-surface peats, which continued to create obstacles to restoration and reduced capacity for CGHG uptake, as the quality of the peat continues to contribute to poor hydrological control.

Table 5.4. Adapted from Rydin and Jeglum (2013) p 101; Data from National Wetlands Working Group, Canada, 1988. Boxed sections show similar values to this study.

Depth (cm)	von Post humification	Peat type	Ash (%)	Total elements (%)					
				Ca	Mg	Fe	N	P	K
Raised bog with concentric patterns									
0 - 50	2	*Sphagnum-Carex*	1.8	0.12	0.02	0.03	1.35	0.06	0.02
50 - 100	4	*Carex-Sphagnum*	1.1	0.20	0.03	0.04	0.81	0.03	0.01
150 - 200	6		1.5	0.36	0.01	0.04	0.98	0.002	0.01
310 - 350	2	Brown moss	2.2	0.61	0.21	0.09	1.77	0.04	0.01
Basin fen									
0 - 20	2	*Sphagnum-Carex*	3.3	0.24	0.04	0.14	1.48	0.07	0.06
70 - 100	7	-	9.0	0.84	0.04	0.33	2.59	0.06	0.08
135 - 175	5-6	-	6.0	1.11	0.04	0.30	2.36	0.04	0.06
Peat margin swamp									
0 - 50	3	*Sphagnum* -wood-*Pleurozium*	7.9	2.16	0.13	0.22	1.63	0.04	0.04
201 - 215	4		7.2	2.42	0.04	1.31	1.13	0.05	0.78
This study									
0 - 5	4 - 5	*Sphagnum* - graminoid	4.3	0.33	0.06	0.11	1.41	0.00*	0.00**

Surface peat samples for this study taken on 24 June 2016 prior to field gaseous carbon flux trials; mean of 9 plots; Von Post humification assessed 14 January 2019, post-trial. * 0.000099 ** 0.0016 **Note: this study - Ca, Mg, Fe, P, K are extractable, not total values.**

There was a lower C:N ratio between 0 and 15 cm depth, particularly in immature plots, and the C:N ratio along peat core profiles was variable between plots, with differences being driven more by changes in %N than %C. This suggests episodes of mineralization under aerobic conditions (Macrae *et al*., 2012) but the C:N ratio also reflects the typically nutrient-poor environment (Renou-Wilson *et al*., 2019), and N values are within the range of literature sources for a re-wetted bog. The high organic content throughout all cores is

typical for a peat soil, and the mineral content is also typically low although a little more variable, apart from very high values in two cores at 35 - 40 cm, which is perhaps due to past disturbance.

Density along peat cores was surprisingly similar considering the visual differences, knowledge of long-term disturbance on the site and the high variability in WTD, although the highest density was in peat just below the surface in plots with poorer vegetation cover. Higher density at the surface was expected throughout due to peat shrinkage, although the peat is perhaps already highly decomposed, being at the base of the original catotelm (Andersen *et al*., 2006), and values were within the range of other damaged bogs under restoration measures (Table 5.3). Zauft *et al*. (2010) found that, over a range of mire types, there is a strong relationship between increasing peat %C content and declining peat density, but this study found only a very weak, insignificant relationship (R^2 = 0.020) which is probably due to the comparably small variation in conditions on the same site in this study.

5.5 Conclusions

The aims of this study were to assess how site recovery, and CGHG flux in particular, may have been influenced by peat quality. Perhaps unsurprisingly, analysis of the peat surface and deeper layers provided evidence of compaction and humification due to repeated disturbance and drainage, which is likely to have reduced nutrient availability to support both microbial communities and plant growth, and confirms that the study site is still in the early stages of recovery from long-term peat extraction, despite broad colonisation with specialist bog plants.

Although the organic content of the surface peat layer had increased over the trial period, improving conditions for vascular plant growth and microbial activity, difficulties with hydrological control, particularly coupled with the possibility of frequent dry summers in the future, ensures retention of an oxic surface layer of peat, less able to retain moisture, thereby probably creating a positive feedback loop of hydrological instability. A regularly low WTD may also promote cumulative nitrogen loads on the site, detrimental to peatland plant biodiversity. Unfortunately, the scope of peat analysis in this study was

limited to a very broad overview pre- and post-trial. Chemical analysis of the changing environment on this degraded site over time, particularly with integrated studies of surface peats, plants and water, could be used to quantify restoration progress (Andersen *et al*., 2010), and would allow greater understanding of the nutrient cycling and microbial activity underpinning its capacity for CGHG sequestration. However, particular focus should be concentrated on improving hydrological control on the site to maintain an optimum WTD for long-term recovery.

Chapter 6: Study Synthesis

Micropropagated *Sphagnum* (BeadaMoss®) was a central theme of this thesis, which explored its capacity for photosynthesis, growth and influence on carbon greenhouse gas (CGHG) fluxes when introduced as part of restoration measures on a degraded lowland bog. *Sphagnum* is a key species in lowland bog development (van Breemen, 1995; Rochefort, 2000), and re-introduction is seen as essential for re-establishing an acrotelm in degraded bogs (Rochefort *et al*., 2003), which both protects carbon stocks in the peat body and creates a cool, moist layer at the surface to resist decomposition, reduce ecosystem respiration and promote peat accumulation (Waddington and Warner, 2001; Price *et al*., 2003; Lucchese *et al*., 2010). As *Sphagnum*-dominated peatlands are scarce in the UK, harvesting of *Sphagnum* from natural sources for any purpose is prohibited and so BeadaMoss® *Sphagnum* has been developed for wide-scale use in restoration projects (Caporn *et al*., 2018). The overall aims of this thesis were to discover if BeadaMoss® *Sphagnum* is likely to have the same properties as that from natural settings in terms of carbon assimilation and growth, to support degraded peatland recovery and resilience. If the UK is to reach its ambitious climate mitigation targets of net zero GHG emissions by 2050 (Committee on Climate Change, 2019) more attention needs paying to soils, which are one of the largest emitters of CGHG (Oertel *et al*., 2016; Melillo *et al*., 2017), and peatlands are estimated to hold 25% of global soil carbon (Rydin and Jeglum, 2013). In the UK, much of the peatland resource is currently degraded or under agriculture, and lowland peatlands are now a large-scale carbon source (Evans *et al*., 2016; Committee on Climate Change, 2019). Therefore, restoration could provide a key contribution to climate change mitigation (Waddington and Warner, 2001; Bain *et al.,* 2011; Alonso *et al*., 2012; Joosten *et al*., 2012). Data is still needed from UK peatlands to guide allocation of resources to the most essential peatland restoration work for climate change mitigation (Evans *et al*., 2017).

Maximum photosynthesis (P_{max}) rates of BeadaMoss® *Sphagnum* (Chapter 2) were higher than those of wild-sourced *Sphagnum*, both in this study and generally in comparison with the wider literature (Rice *et al*., 2008; Haraguchi and Yamada, 2011; Bengtsson *et al*., 2016) for each of the six species studied (*S. capillifolium*, *S. fallax*, *S. medium/divinum*, *S.*

palustre, *S. papillosum* and *S. squarrosum*). BeadaMoss® *Sphagnum* respiration rates were also higher, but the ratio of P_{max} to respiration was higher in BeadaMoss® than wild-sourced samples (5.58 and 3.41 respectively) meaning that, overall, the CO_2 uptake of BeadaMoss® was greater than that of wild-sourced *Sphagnum* suggesting that productivity would also be higher, although there are adaptive trade-offs related to shade and moisture in natural settings (Bengtsson *et al*., 2016).

There were negative relationships between density and P_{max} across all *Sphagnum* species and sources. However, wild-sourced species more closely followed expected traits of dense growth, low productivity for stress-tolerant species growing in open habitats, and low density, high productivity for ruderal and competitive species growing in habitats with higher nutrient inputs, shade or near the water-table (Rice *et al*., 2008; Laine *et al*., 2011; Kangas *et al*., 2014; Mazziotta *et al*., 2019). BeadaMoss® species, having been grown in commercial greenhouses, did not have a clear rank of P_{max} related to expected growth traits, but species with the highest and lowest photosynthesis rates, *S. squarrosum* and *S. medium/divinum* respectively, were the same as those from natural sources. These species were also found to have high and low productivity respectively in Chapter 3 growth trials. Moreover, BeadaMoss® and wild-sourced samples were morphologically similar in terms of chlorocyst (cells containing chloroplasts) structure and size, although BeadaMoss® samples showed signs of immaturity.

Growth trials of BeadaGel™ (Chapter 3) showed that samples grown indoors grew rapidly with low DW density on harvesting and only loosely followed phylogenetic tendencies of each species (which then, presumably, are only fully expressed in natural settings), similarly to BeadaMoss® samples in trials of photosynthesis rates. Particularly noticeable was the greater number of innovations (growth points [Prager *et al*., 2012]) on samples established indoors than outdoors, and the variation between species. This likely leads to rapid development of BeadaHumok™ (*Sphagnum* plugs grown on from BeadaGel™ application, see section 1.3.6) in BeadaMoss® greenhouses, and BeadaMoss® *Sphagnum* used for photosynthesis measurements also had more capitula than wild-sourced *Sphagnum*. It would be useful to know if this is an establishment factor particular to BeadaGel™ *Sphagnum* in the field, to favour its use over wild-sourced propagules. Species established outside showed comparative production rates that might be expected

from their phylogenetic growth form as described above. Samples established in Spring were generally more productive than those established in Autumn, but this was perhaps only a short-term early boost in productivity and over time growth rates may have evened out due to seasonal variations, but much depends on sufficient moisture availability in the early stages of growth (McNeil and Waddington, 2003).

There were positive relationships between macronutrients N, P and K and P_{max} across all *Sphagnum* studied. However, there appeared to be P- and K-limitation through saturated N levels, which limit P- and K-accumulation (Aerts *et al*., 1992; Lamers *et al*., 2000; Bragazza *et al*., 2004) of some wild-sourced species, particularly *S. medium/divinum* and *S. papillosum* sourced from open (drier), ombrotrophic conditions, and photosynthesis rates were low in these samples. Nitrogen content of BeadaMoss® *Sphagnum*, as high as 30 mg g^{-1} in some samples, was far higher than the critical thresholds suggested in the literature of 11 to 12 mg g^{-1} (Lamers *et al*., 2000; Bragazza *et al*., 2004), 15 mg g^{-1} (van der Heijden *et al*., 2000) and 20 mg g^{-1} (Berendse *et al*., 2001), and did not limit P or K or produce toxicity, suggesting these were young, nutrient-demanding plants in the early stages of linear growth (Laine *et al*., 2011).

The Cadishead Moss study site (and probably many others in the UK) has higher nitrogen and ammonia inputs than recommended critical loads for this habitat type, but retains the legacy of peat extraction in the surface peat in being P- and K- limited. This may have inhibited natural colonisation and establishment of *Sphagnum* in these areas of the site (Sundberg and Rydin, 2002). BeadaMoss® *Sphagnum*, with inherently higher nutrient content, is potentially more likely to establish and thrive than wild-sourced propagules in this environment, although the low levels of other nutrients for growth could be a constraint for newly establishing *Sphagnum*, or could promote an establishment 'shock', reducing capacity for photosynthesis. However, it has established and grown on this site, although it required initial support through mulch/mesh to retain moisture as recommended by Rochefort *et al.* (2003). BeadaMoss® *Sphagnum* growth also appears to be more successful in the field than that from wild-sourced propagules or clumps in the English uplands (Crouch, 2018) and successful growth has been reported, particularly in lowland restoration sites with BeadaGel™ and BeadaHumok™ within *E. angustifolium* protection (Caporn *et al*., 2018). Higher CO_2 uptake in BeadaMoss® *Sphagnum* may

continue into the field and make a useful contribution to *Sphagnum* proliferation and the site CGHG budget. Further trials of samples over time in the field, particularly in lowlands where rainfall is lower and moisture levels at the peat surface more variable, would determine whether a good capacity for CO_2 uptake, or another growth factor, such as the high number of growth points, promote success of BeadaMoss® *Sphagnum*.

Rebuilding a functional acrotelm is a primary goal in peatland restoration (Quinty and Rochefort, 2003; Tomassen *et al*., 2010; Waddington *et al*., 2011), to develop resilience to stochastic events such as the summer drought during this study, and promote peat accumulation (Price *et al*., 2003; Lucchese *et al*., 2010*;* Lindsay and Clough, 2016). This presents great challenges on degraded fragments of peatland where damage to peat structure and its capacity to hold water hampers hydrological conditions sufficient to support the establishment and growth of *Sphagnum* mosses, keystone species for functional peatlands (van Breemen, 1995; Rochefort, 2000).

This study found the *E. angustifolium*-dominated area of Cadishead Moss with and without *Sphagnum* introduction to be an overall CGHG sink, particularly in the first year of study, with typical weather patterns for the area, but much less so in the second year, when there was reduced PAR in spring and a summer drought. The mean CGHG uptake for all vegetated monitoring points, assuming equal distribution, was -264.39 ± 368.95 g CO_{2e} m^{-2} yr^{-1} in year 1 and -99.01 ± 339.59 g CO_{2e} m^{-2} yr^{-1} in year 2. CGHG emission from bare peat monitoring points was similar in each year with an overall mean of 341.10 ± 75.47 g CO_{2e} m^{-2} yr^{-1}.

E. angustifolium on the area studied on Cadishead Moss was emerging from a layer of catotelmic peat of poor quality and a depth close to the 2 m limit for restoration to bog (Lindsay and Clough, 2016). There was a highly fluctuating WTD, despite bunding, which maintains compaction and humification in the upper peat layers. This is liable to encourage scrub proliferation and prevent *Sphagnum* establishment, particularly with a future scenario of regularly hot, dry summers, and an acrotelm is unlikely to develop (Figure 6.1) without further intervention. Analysis of peat prior to, and during restoration should give realistic indications of restoration potential and progress and how to direct efforts, and could be based on evaluation of peat in local semi-natural and harvested sites

to establish baseline values and targets (Andersen *et al*., 2006). As a minimum, moisture, density, pH and electrical conductivity (EC) would give an indication of peat quality, and N, P, K and %C values would be helpful in showing recovery of microbial and plant nutrient cycling.

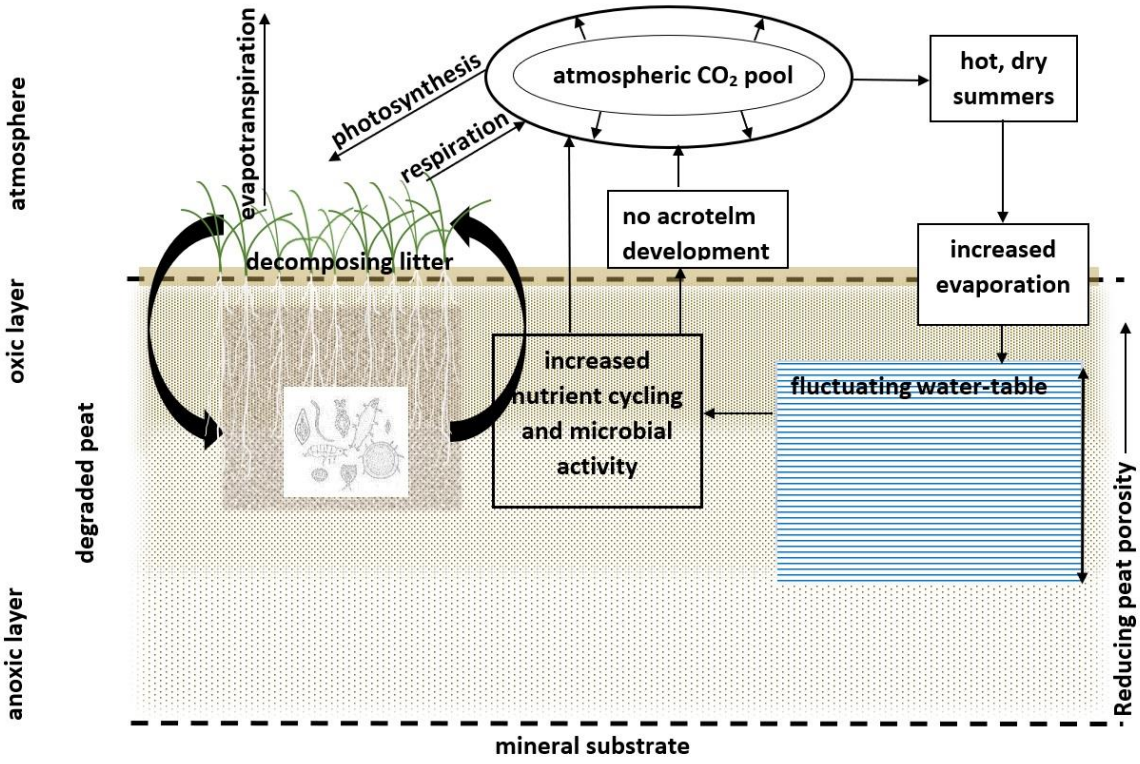


Figure 6.1. A potential climate-change scenario for a degraded peatland, showing a positive feedback loop of hydrological instability.

Fluxes in plots of mature *E. angustifolium*-only were more 'dynamic' than those with *Sphagnum*. NER (with accumulation of dry litter, not measured, perhaps contributing to this), CO_2 uptake and methane emissions were all higher. Strack *et al*. (2016) also found positive relationships between vascular plant cover and photosynthesis on recovering bogs, and Bortoluzzi *et al*. (2006) found *E. angustifolium* promoted greater methane efflux. Methane emission was greatest in plots with a dense sward of mature *E. angustifolium*, irrespective of water table depth and, when converted to CO_2 equivalents, made a significant contribution to CGHG emissions. Mature plots with *Sphagnum* had a lower volume of *E. angustifolium*, perhaps because *Sphagnum* scavenges nutrients more efficiently than vascular plants (Heijmans *et al*., 2002; Malmer *et al*., 2003; Bragazza *et al*., 2004; Fritz *et al*., 2014), and a lower exchange of gases, particularly methane. So, the

addition of *Sphagnum* may result in reduced CGHG uptake in mature vegetation, but is not likely to result in increased CGHG emission. Additionally, immature vegetation plots with a layer of *Sphagnum* covering the peat surface assimilated more CO_2 (Gunnarsson, 2005) as seen in the drier 2nd year of this study, and more so than in mature plots, and may have helped support CO_2 uptake in accompanying *E. angustifolium* while reducing methane release. There was drying of etiolated *Sphagnum* and early vascular plant senescence in mature plots in year 2, and correspondingly reduced capacity for photosynthesis.

Early *Sphagnum* establishment initially failed in some plots with only sparse *E. angustifolium* protection, and repeat application was only successful once a surface mesh cover was employed to substitute for straw mulch, so retention of moisture at the surface appears more important than nurse plants (Grosvernier *et al.*, 1997) to establish a layer of *Sphagnum*. This layer needs to be intact (Waddington *et al*., 2011) so as to eventually outcompete vascular plants, reduce decomposition and promote development of a functioning acrotelm. Mulching (Rochefort *et al*., 2003), and potentially, irrigation (Schumann and Joosten, 2008), may be necessary to support *Sphagnum* through the early stages of growth until an acrotelm is deep enough to be self-sustaining and the current peat body (the catotelm) is constantly saturated. Indeed, an experimental project (*Sphagnum* Farming UK) using BeadaMoss® *Sphagnum palustre* to produce *Sphagnum* as a peat replacement, using both protective covers/mulch and irrigation has produced intact, deep carpets of *Sphagnum* in less than 2 years (data not yet published). Establishing a *Sphagnum* layer, then, appears to be the most important factor in supporting lowland peatland restoration, resilience and CGHG uptake, particularly early in restoration process, and retaining moisture at the surface appears to be more beneficial than a high, consistent WTD, which would develop over time as a new acrotelm establishes. Hydrological stability will support plant growth and may allow time for microbial communities to develop, but may also increase methane production (Glatzel *et al*., 2004; Urbanová *et al*., 2011) although the development of a *Sphagnum* layer and a methanogenic microbial community may limit methane emission (Kip *et al*., 2010; van Winden *et al*., 2012).

Degraded peatlands are complex systems due to their diverse location, formation and subsequent use and restoration management (Basiliko *et al*., 2007; Alonso *et al*., 2012; Krüger *et al*., 2015; Waddington *et al*., 2015; Renou-Wilson *et al*., 2019), and their slow repair of essential ecohydrological function (Worrall *et al*., 2011) means that short-term studies can make only minor contributions to management decisions which will have long-term implications for the sites and their climate change mitigation potential (Taylor *et al*., 2019). In this respect, Cadishead and the adjacent Little Woolden Moss would make an ideal site for a long-term field station, having both block-cut and mechanically harvested peat areas together on Cadishead Moss, and the adjacent Little Woolden Moss being an industrially milled site with varying depths of peat remaining (from a few centimetres to perhaps 2 metres or more). The sites are within easy reach of major universities in the region with long-term peatland interests and expertise, and Lancashire Wildlife Trust as landowners readily embrace academic input to their restoration management plans.

The commercial BeadaMoss® mix of 11 *Sphagnum* species was developed for broad application, on the assumption that each species would find a niche in the range of microtopography within a peatland landscape, whether that be hollows, hummocks or within shaded and higher nutrient environments of developing vascular vegetation. There are suggestions in Chapter 3 on potential changes to the currently available BeadaMoss® species mix, and best application times, to optimise productivity in the field at various stages of restoration. More studies are needed into the interaction between vascular plant and *Sphagnum* growth in restoration settings as although *Sphagnum* may deny nutrients to vascular plants, vascular plants which could nurse *Sphagnum* establishment, such as *E. angustifolium,* may compete with *Sphagnum* for light (Pouliot *et al*., 2011), *Sphagnum* may become etiolated and more vulnerable to desiccation (Aerts *et al*., 2001; Rydin and Jeglum, 2013), as in the mature plots in this study, which is likely to reduce *Sphagnum* photosynthesis rates below levels for continued development (Hájek *et al*., 2009). On this study site, management to encourage *E. angustifolium* growth appears to deliver benefits in terms of gaseous carbon uptake, as also found by Tuittila *et al*. (1999) and Wilson *et al*. (2013). However, Evans *et al*. (2016) caution against allowing *E. angustifolium* to become too dominant due to the potential of greater methane efflux. Moreover, they suggest research is needed to establish the optimum water table depth

for carbon benefits where *E. angustifolium* is used to support *Sphagnum* establishment, a recommended restoration technique (Price *et al*., 2003). Overall, establishment of *Sphagnum* appears key to establishing a functional acrotelm to re-establish peat-accumulation processes (Rochefort, 2000), and BeadaMoss® materials have grown on this site and have the potential to proliferate rapidly, and thus promote a more reliable site CGHG sink in the long-term.

Restoration projects such as Lancashire Wildlife Trust's Little Woolden Moss (Figure 6.2), adjacent to the Cadishead Moss study site, clearly demonstrate benefits of restoration in terms of biodiversity and cultural ecosystem services, but it would also be good to know the system is reliably sequestering carbon from the atmosphere and that methane release from dominant *Eriophorum spp*. is not contributing hugely to climate warming, even in the short-term. Studies such as in this thesis, that demonstrate CGHG uptake and emission of different vegetation types and bare peat can help site managers quantify CGHG values of their restoration management, and give confidence to funders off-setting their carbon emissions or supporting 'green' initiatives, particularly if a range of ecosystem services benefits are delivered in one system.

Recommendations for restoration management on Chat Moss degraded bog sites are:

1) creating a *Sphagnum*-dominated, intact acrotelm should be the overriding restoration aim to promote recovery and long-term resilience to future climate change scenarios;
2) early objectives should be concentrated on closing the vegetation cover to reduce evaporation and avoid CGHG losses from bare peat; *E. angustifolium* rapidly proliferates laterally and methane emissions do not negate climate benefits of good CO_2 uptake;
3) early *Sphagnum* introduction appears to have particular benefits in improving resilience to climate change, providing cool, moist conditions and a full plant cover on the peat surface for greater CO_2 uptake, as seen in this study;
4) maintaining a stable WTD but particularly keeping moisture at the surface is key to promote conditions necessary for *Sphagnum*-dominated acrotelm development;

5) mulching the *Sphagnum* layer and potentially providing irrigation, particularly during periods of drought, are likely to be necessary;
6) BeadaMoss® *Sphagnum* is a good component to have in the restoration toolkit, and the benefits of easy management, availability and potentially rapid establishment justify the initial outlay;
7) establishment of permanent monitoring points and regular collection of measurement data is essential to ensure restoration is on the correct trajectory, and should include, as a minimum, monthly WTD, annual plant composition and cover, *Sphagnum* and litter depth, underlying peat quality (at least, moisture, density, pH and EC).

Figure 6.2. Little Woolden Moss, east end of the site; bare peat on 9th May 2013 (top) and abundant *Eriophorum spp.* cover with *Sphagnum cuspidatum*-filled pools on 22nd April 2019 (bottom). Images: A Keightley.

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Appendices

Appendix 1. Mean dry weight (DW) and DW density of BeadaGel™ samples in all trials

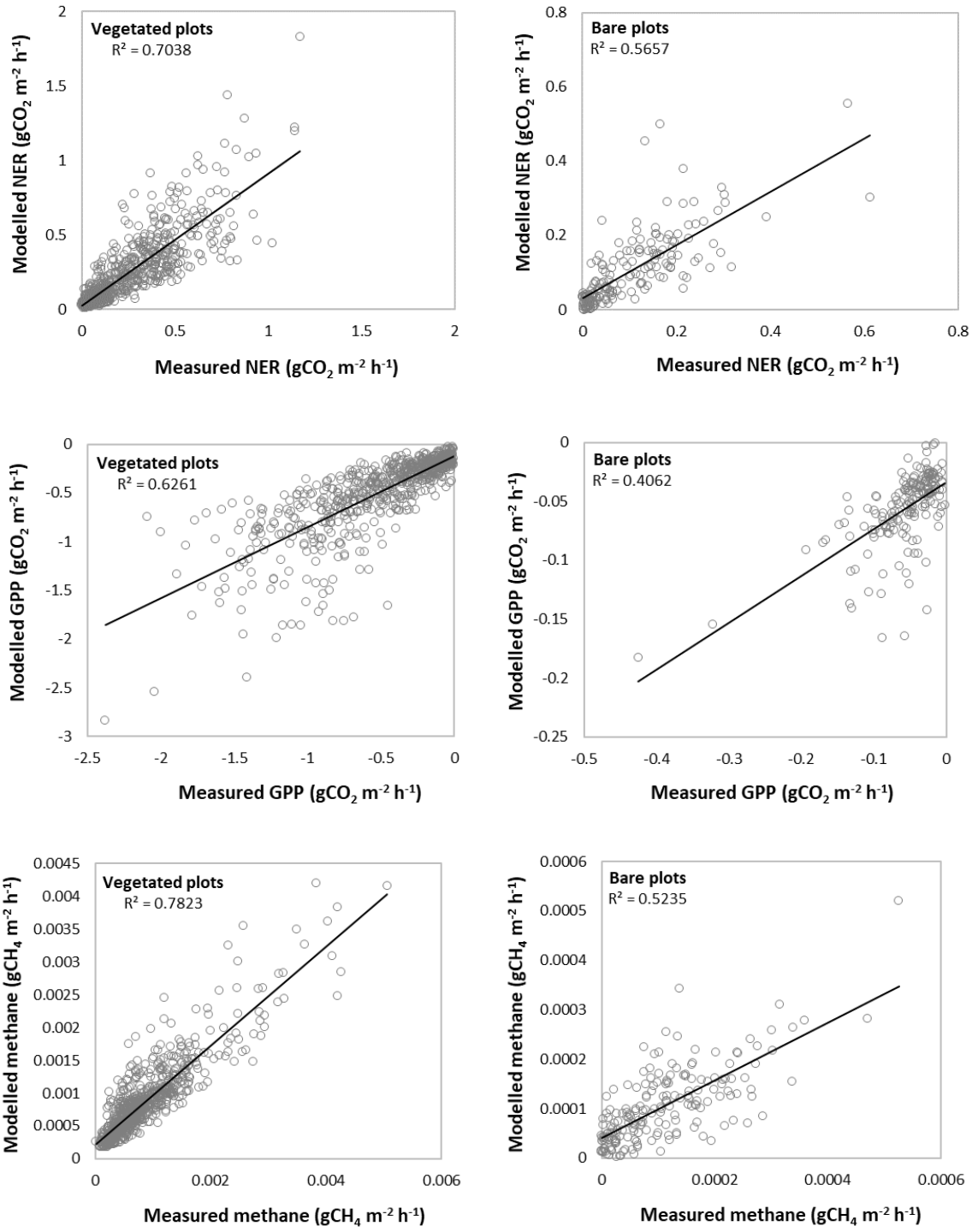
Species	Phylogenetic habit	Light/shade	Outdoors Autumn 2015		Indoors Autumn 2015		Outdoors Spring 2017	
			DW (mg)	DW density (mg cm^{-3})	DW (mg)	DW density (mg cm^{-3})	DW (mg)	DW density (mg cm^{-3})
BeadaGel™ mix			503.1 ± 101.3	14.2 ± 2.01	393.0 ± 50.0	4.5 ± 0.63	712.4 ± 98.0	7.9 ± 0.72
S. capillifolium	dense hummock	open-shaded	554.4 ± 132.8	19.2 ± 3.39	638.9 ± 73.0	6.6 ± 0.44	610.7 ± 175.0	9.3 ± 1.3
S. cuspidatum	lawn, aquatic, semi-aquatic	open	803.2 ± 220.7	9.8 ± 0.71	510.4 ± 79.7	5.4 ± 0.56	713.4 ± 165.1	9.5 ± 1.5
S. denticulatum	carpet, aquatic, semi-aquatic	open	1013.2 ± 159.7	11.1 ± 0.63	342.9 ± 83.2	5.0 ± 0.73	833.0 ± 157.4	11.0 ± 0.95
S. fallax	lawn, carpet	open-shaded	836.5 ± 104.1	9.3 ± 0.82	461.7 ± 67.6	5.2 ± 0.61	983.1 ± 231.1	10.8 ± 1.4
S. fimbriatum	soft hummock, loose carpet	open-shaded	653.4 ± 203.9	10.5 ± 1.42	446.6 ± 108.8	4.8 ± 0.88	859.6 ± 156.4	9.8 ± 1.3
S. medium/divinum	low hummock, lawn, carpet	open, semi-shaded	283.7 ± 50.7	18.9 ± 4.54	291.4 ± 107.4	4.3 ± 0.76	456.9 ± 73.6	10.8 ± 1.9
S. palustre	cushion, mat, untidy	open-shaded	482.9 ± 210.6	11.3 ± 1.13	409.4 ± 136.6	4.6 ± 1.0	743.1 ± 156.0	7.9 ± 0.70
S. papillosum	hummock, lawn, carpet	open	595.9 ± 115.3	11.7 ± 0.79	327.8 ± 62.0	5.6 ± 0.71	862.0 ± 122.8	10.3 ± 1.2
S. squarrosum	untidy, small cushion, mat, single shoots	shaded	735.9 ± 98.9	10.2 ± 1.08	531.3 ± 65.7	4.7 ± 0.47	915.0 ± 179.1	9.0 ± 1.1
S. subnitens	mod. dense cushion, sm. hummock	open-shaded	396.9 ± 144.4	15.2 ± 3.41	400.3 ± 52.3	5.6 ± 0.76	747.1 ± 99.0	9.6 ± 0.95
S. tenellum	small flat patches, low cushion, single shoots	open	400.6 ± 92.0	25.6 ± 11.7	335.7 ± 135.4	6.1 ± 1.3	868.1 ± 78.3	10.9 ± 0.64

Phylogenetic habit: from Atherton *et al* . (2010) and Laine *et al.* (2018). Values: mean ± SD

Appendix 2. R^2 values of linear regression to test relationships between measured environmental variables and fluxes prior to modelling data.

Plot	NER		GPP			METHANE	
	PT	WTD	PT	PAR	WTD	PT	WTD
MEAS	0.73 ± 0.04	0.42 ± 0.19	0.60 ± 0.14	0.31 ± 0.07	0.13 ± 0.09	0.34 ± 0.08	0.11 ± 0.16
MEA	0.75 ± 0.03	0.46 ± 0.10	0.69 ± 0.06	0.33 ± 0.06	0.22 ± 0.01	0.42 ± 0.05	0.07 ± 0.02
IEAS	0.73 ± 0.07	0.63 ± 0.09	0.61 ± 0.11	0.40 ± 0.06	0.30 ± 0.12	0.26 ± 0.06	0.09 ± 0.09
IEA	0.69 ± 0.08	0.57 ± 0.09	0.53 ± 0.21	0.40 ± 0.08	0.25 ± 0.09	0.20 ± 0.08	0.06 ± 0.04
Bare	0.63 ± 0.09	0.59 ± 0.20	0.23 ± 0.21	0.17 ± 0.09	0.15 ± 0.15	0.41 ± 0.17	0.39 ± 0.21

MEAS = mature vegetation (*E. angustifolium* with *Sphagnum*); MEA = mature vegetation (*E. angustifolium* only); IEAS = immature vegetation *(E. angustifolium* with *Sphagnum*); IEA = immature vegetation (*E. angustifolium* only); NER = net ecosystem respiration; GPP = gross primary productivity; NEE = net ecosystem exchange; PT = peat tempeature; WTD = water table depth (cm); PAR = photosynthestically active radiation (µmol m^{-2} s^{-1}). Reported as collated mean collar values ± SD.



Appendix 3. Measured Net Ecosystem Respiration (NER), Gross Primary Productivity (GPP) and methane flux values plotted against modelled flux values showing linear trendline and R^2 values (p-value < 0.001 throughout); collated vegetated plot data, and bare plot data.

Appendix 4. Individual collar composition and characteristics with associated CGHG flux measurements

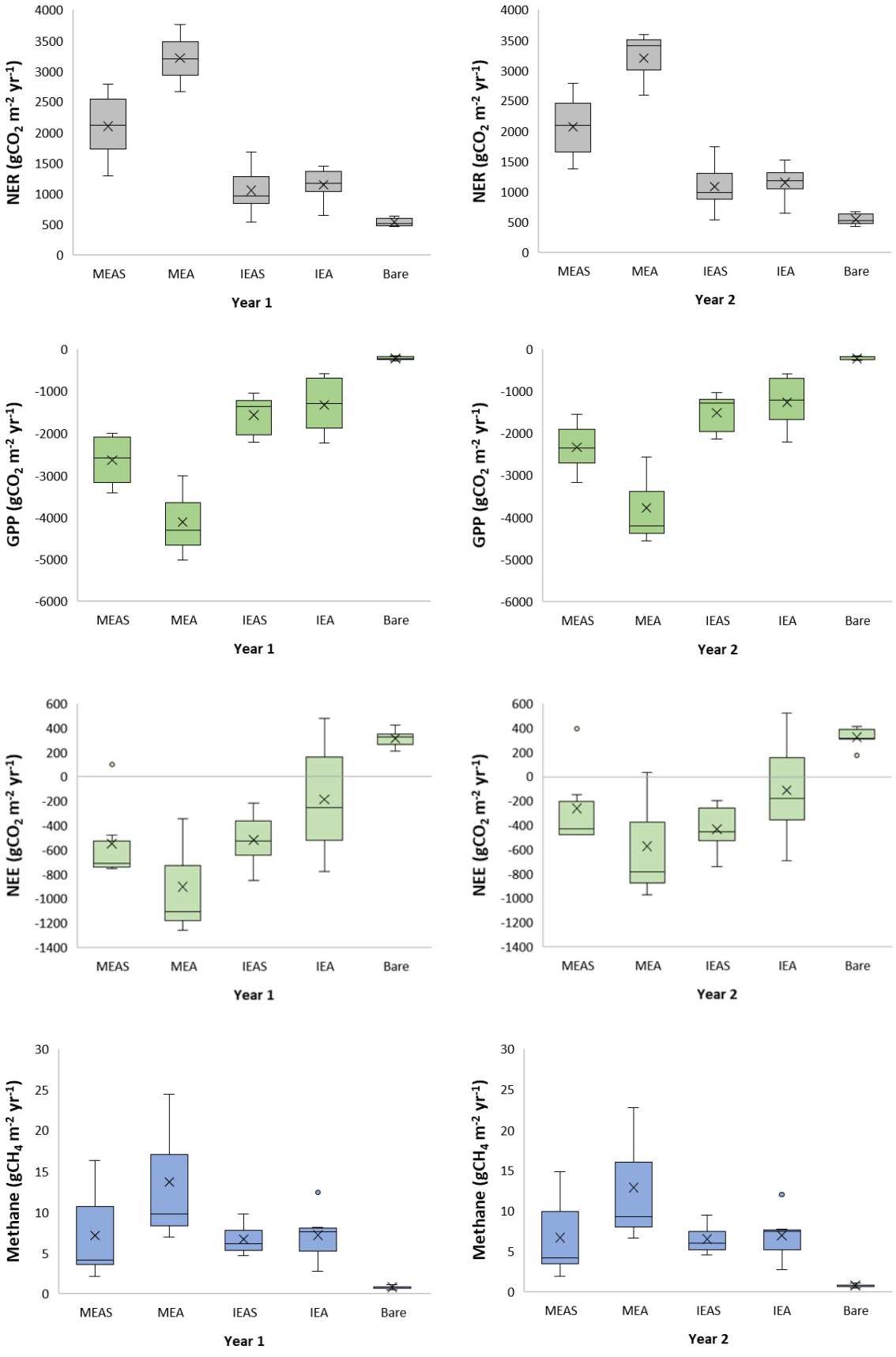
Collar ID	Plot type	Year	WTD (cm)	*E. angustifolium* (cm^3)	*Sphagnum* (cm^3)	NER ($gCO_2\ m^{-2}\ yr^{-1}$)	GPP ($gCO_2\ m^{-2}\ yr^{-1}$)	NEE ($gCO_2\ m^{-2}\ yr^{-1}$)	Methane ($gCH_4\ m^{-2}\ yr^{-1}$)	Methane (gCH_4-$CO_{2eq}\ m^{-2}\ yr^{-1}$)	CGHG budget ($gCO_{2e}\ m^{-2}\ yr^{-1}$)
1A1	MEAS	1	0.48 ± 3.26	250.77 ± 100.25	6011.50 ± 2751.89	2133.03	-2885.49	-752.46	12.69	355.28	-397.18
		2	7.48 ± 13.11	279.31 ± 76.55	10406.03 ± 929.14	2239.36	-2719.68	-480.32	11.78	329.75	-150.57
1A2	MEAS	1	0.48 ± 3.26	359.13 ± 121.42	5529.93 ± 2516.15	2685.89	-3425.04	-739.15	16.34	457.52	-281.63
		2	7.48 ± 13.11	300.15 ± 94.44	9800.98 ± 1620.06	2789.60	-3172.25	-382.64	14.80	414.27	31.62
1C	MEA	1	0.48 ± 3.26	398.77 ± 99.56	0	3204.52	-4311.09	-1106.56	24.44	684.18	-422.38
		2	7.48 ± 13.11	413.05 ± 105.86	0	3412.00	-4194.50	-782.51	22.72	636.04	-146.46
2A1	MEAS	1	2.03 ± 3.89	78.98 ± 28.88	1471.65 ± 820.24	1286.37	-2031.88	-745.52	4.47	125.11	-620.40
		2	9.11 ± 14.00	120.71 ± 48.58	2818.68 ± 333.12	1373.79	-1852.40	-478.61	4.52	126.61	-352.00
2A2	MEAS	1	2.03 ± 3.89	154.83 ± 67.04	2682.90 ± 1549.74	1605.01	-2284.55	-679.54	3.75	104.89	-574.65
		2	9.11 ± 14.00	187.67 ± 64.95	6782.01 ± 387.76	1556.11	-2031.04	-474.93	3.79	106.20	-368.73
2C	MEA	1	2.03 ± 3.89	161.01 ± 73.07	0	2671.04	-3017.76	-346.72	9.76	273.17	-73.55
		2	9.11 ± 14.00	287.62 ± 100.70	0	2591.47	-2554.49	36.97	9.26	259.16	296.13
3A1	MEAS	1	3.61 ± 4.31	164.02 ± 86.45	5928.97 ± 3229.04	2098.07	-1996.25	101.82	2.06	57.73	159.55
		2	11.31 ± 14.91	104.30 ± 25.43	9752.68 ± 1050.41	1934.86	-1539.19	395.67	1.87	52.40	448.07
3A2	MEAS	1	3.61 ± 4.31	292.91 ± 94.27	5375.49 ± 2811.72	2784.79	-3263.29	-478.50	3.54	99.10	-379.40
		2	11.31 ± 14.91	293.52 ± 90.41	9781.35 ± 564.62	2526.40	-2673.06	-146.67	3.33	93.36	-53.31
3C	MEA	1	3.61 ± 4.31	342.92 ± 85.51	0	3757.75	-5015.48	-1257.73	6.95	194.46	-1063.27
		2	11.31 ± 14.91	414.53 ± 96.05	0	3594.57	-4565.17	-970.60	6.69	187.19	-783.41

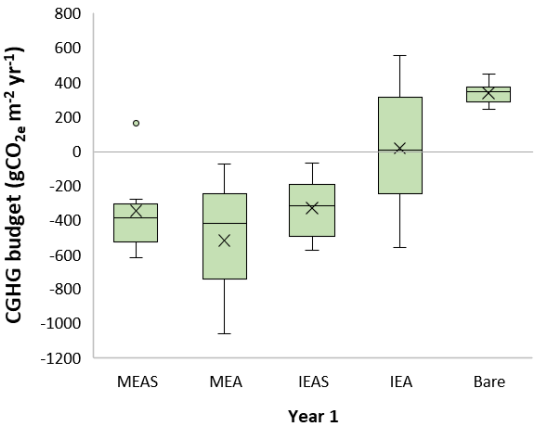
Collar ID	Plot type	Year	WTD (cm)	*E. angustifolium* (cm^3)	*Sphagnum* (cm^3)	NER ($gCO_2\ m^{-2}\ yr^{-1}$)	GPP ($gCO_2\ m^{-2}\ yr^{-1}$)	NEE ($gCO_2\ m^{-2}\ yr^{-1}$)	Methane ($gCH_4\ m^{-2}\ yr^{-1}$)	Methane (gCH_4-$CO_{2eq}\ m^{-2}\ yr^{-1}$)	CGHG budget ($gCO_{2e}\ m^{-2}\ yr^{-1}$)
4A	IEAS	1	9.63 ± 7.11	68.85 ± 38.82	270.57 ± 41.15	806.17	-1486.23	-680.06	4.68	131.05	-549.01
		2	20.99 ± 17.37	69.47 ± 22.41	849.27 ± 180.41	845.00	-1383.71	-538.71	4.53	126.84	-411.87
4B	Bare	1	9.63 ± 7.11	0	0	508.58	-255.27	253.32	0.79	22.15	275.47
		2	20.99 ± 17.37	0	0	563.34	-248.00	315.34	0.83	23.34	338.67
4C	IEA	1	9.63 ± 7.11	48.18 ± 31.71	0	1288.79	-1785.06	-496.26	8.08	226.28	-269.98
		2	20.99 ± 17.37	100.55 ± 44.55	0	1251.70	-1609.54	-357.83	7.76	217.28	-140.55
5A	IEAS	1	9.18 ± 8.27	71.72 ± 51.05	348.48 ± 31.51	1370.56	-2218.52	-847.96	9.76	273.38	-574.58
		2	18.61 ± 15.11	154.87 ± 63.78	1404.65 ± 330.43	1398.86	-2137.20	-738.34	9.47	265.11	-473.23
5B	Bare	1	9.18 ± 8.27	0	0	462.72	-251.37	211.36	1.08	30.13	241.48
		2	18.61 ± 15.11	0	0	431.61	-256.90	174.71	1.09	30.59	205.30
5C	IEA	1	9.18 ± 8.27	94.31 ± 63.09	0	1388.82	-1919.75	-530.93	12.44	348.38	-182.54
		2	18.61 ± 15.11	150.43 ± 36.54	0	1342.71	-1692.19	-349.48	12.00	336.07	-13.41
6A	IEAS	1	13.23 ± 11.10	42.97 ± 18.60	217.71 ± 85.91	989.29	-1205.60	-216.31	5.16	144.38	-71.93
		2	21.73 ± 14.70	84.80 ± 35.44	483.41 ± 224.53	993.07	-1193.45	-200.38	5.14	143.81	-56.57
6B	Bare	1	13.23 ± 11.10	0	0	634.16	-211.88	422.28	0.83	23.17	445.45
		2	21.73 ± 14.70	0	0	659.18	-245.76	413.42	0.85	23.75	437.17
6C	IEA	1	13.23 ± 11.10	35.21 ± 8.51	0	1024.59	-801.55	223.04	4.48	125.46	348.51
		2	21.73 ± 14.70	52.34 ± 25.46	0	1030.24	-815.79	214.45	4.46	125.02	339.47

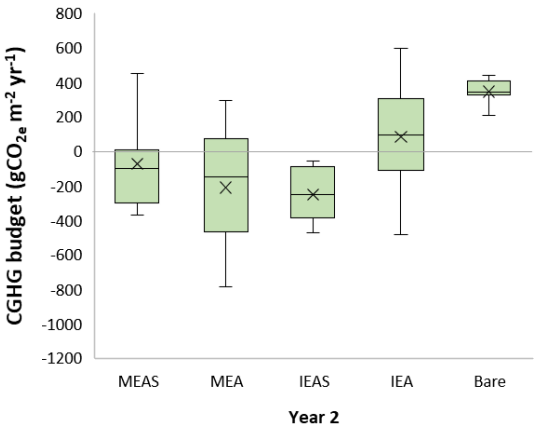
Collar ID	Plot type	Year	WTD (cm)	*E. angustifolium* (cm^3)	*Sphagnum* (cm^3)	NER ($gCO_2\ m^{-2}\ yr^{-1}$)	GPP ($gCO_2\ m^{-2}\ yr^{-1}$)	NEE ($gCO_2\ m^{-2}\ yr^{-1}$)	Methane ($gCH_4\ m^{-2}\ yr^{-1}$)	Methane (gCH_4-$CO_{2eq}\ m^{-2}\ yr^{-1}$)	CGHG budget ($gCO_{2e}\ m^{-2}\ yr^{-1}$)
7A	IEAS	1	11.02 ± 9.01	61.02 ± 38.62	302.94 ± 194.14	937.34	-1249.93	-312.60	5.62	157.42	-155.18
		2	17.92 ± 12.87	108.39 ± 46.07	1037.20 ± 234.76	983.30	-1193.95	-210.65	5.50	154.08	-56.57
7B	Bare	1	11.02 ± 9.01	0	0	520.52	-174.04	346.47	0.88	24.56	371.04
		2	17.92 ± 12.87	0	0	478.49	-157.85	320.64	0.84	23.58	344.22
7C	IEA	1	11.02 ± 9.01	27.44 ± 8.07	0	1057.78	-579.40	478.38	2.77	77.63	556.01
		2	17.92 ± 12.87	32.30 ± 12.91	0	1099.49	-578.07	521.43	2.75	77.02	598.45
8A	IEAS	1	11.30 ± 9.75	31.33 ± 18.48	269.08 ± 63.99	532.51	-1048.01	-515.50	6.62	185.30	-330.20
		2	19.02 ± 13.25	75.22 ± 30.50	693.04 ± 250.03	533.86	-1033.27	-499.41	6.58	184.33	-315.08
8B	Bare	1	11.30 ± 9.75	0	0	468.81	-160.95	307.87	0.47	13.08	320.94
		2	19.02 ± 13.25	0	0	469.09	-162.53	306.56	0.46	13.00	319.57
8C	IEA	1	11.30 ± 9.75	31.68 ± 16.97	0	649.50	-662.32	-12.82	7.50	209.91	197.09
		2	19.02 ± 13.25	49.79 ± 16.04	0	645.54	-653.38	-7.84	7.43	208.14	200.30
9A	IEAS	1	6.27 ± 6.32	112.88 ± 65.97	454.75 ± 327.32	1682.38	-2216.02	-533.64	8.15	228.10	-305.54
		2	13.88 ± 14.97	210.39 ± 80.38	1386.78 ± 356.83	1735.56	-2139.59	-404.03	7.75	217.02	-187.00
9B	Bare	1	6.27 ± 6.32	0	0	629.40	-278.54	350.86	0.56	15.79	366.65
		2	13.88 ± 14.97	0	0	664.84	-253.20	411.64	0.56	15.64	427.28
9C	IEA	1	6.27 ± 6.32	83.87 ± 32.66	0	1451.73	-2229.90	-778.17	7.73	216.35	-561.82
		2	13.88 ± 14.97	108.79 ± 35.46	0	1522.51	-2214.29	-691.78	7.41	207.35	-484.43

MEAS = mature vegetation (*E. angustifolium* with *Sphagnum*); MEA = mature vegetation (*E. angustifolium* only); IEAS = immature vegetation (*E. angustifolium* with *Sphagnum*); IEA = immature vegetation (*E. angustifolium* only); WTD = water table depth (cm); NER = net ecosystem respiration; GPP = gross primary productivity; NEE = net ecosystem exchange; CO_2 equivalents of CH_4 were calculated by multiplying by Global Warming Potential $(GWP)_{100}$ of 28 and added to NEE values to give a carbon greenhouse gas (CGHG) budget in $gCO_{2e}\ m^{-2}\ y^{-1}$ (final column). Values reported as mean ± SD.

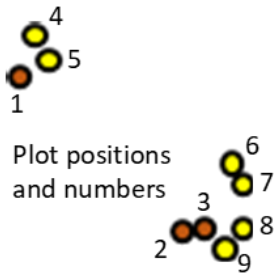
Appendix 5. Modelled yearly fluxes (NER, GPP, NEE, Methane, CGHG budget: combined NEE and CH_4-CO_{2e}) comparing treatment types in each study year. Box plots show collated collar data; crosses indicate the mean value, lines indicate the median, and interquartile range is inclusive.




800
600
400
200
0
-200
-400
-600
-800
-1000
-1200
CGHG budget (gCO2e m-2 yr-1)
MEAS
MEA
IEAS
IEA
Bare
Year 1


800
600
400
200
0
-200
-400
-600
-800
-1000
-1200
CGHG budget (gCO2e m-2 yr-1)
MEAS
MEA
IEAS
IEA
Bare
Year 2

Appendix 6. Google Earth maps of Cadishead Moss over 20 years showing changes on site and immediate surroundings. Mature plots: orange dots; immature plots: yellow dots.



Maps dated:

a) 1 January 2000 site still hand-cut; general scrub cover
b) 1 January 2005 plots area mechanically scraped and extracted
c) 2 June 2009 mechanical scraping extended; scrub elsewhere
d) 22 July 2012 restoration re-wetting; plots area dry
e) 6 April 2013 widespread scrub removal; plots area bunded
f) 24 March 2017 edge re-enforcement bunding to aid re-wetting
g) 19 April 2018 further bunding works, not affecting plots area

Map interpretation aided by Paul Thomas, Natural England.


6/2009
Cadishead and Little Woolden Moss
c)
© 2019 Infoterra Ltd & Bluesky
Google Earth
1945
Imagery Date: 6/2/2009 53°27'16.18" N 2°27'22.48" W elev 26 m eye alt 695 m


7/2012
Cadishead and Little Woolden Moss
d)
Image © 2019 DigitalGlobe
© 2019 Infoterra Ltd & Bluesky
Google Earth
1945
Imagery Date: 7/22/2012 53°27'15.92" N 2°27'23.69" W elev 26 m eye alt 695 m


4/2013
Cadishead and Little Woolden Moss
e)
Image © 2019 DigitalGlobe
Google Earth
1945
Imagery Date: 4/6/2013 53°27'15.82" N 2°27'21.63" W elev 26 m eye alt 695 m


3/2017
Cadishead and Little Woolden Moss
f)
Google Earth
1945
Imagery Date: 3/24/2017 53°27'08.97" N 2°27'16.66" W elev 23 m eye alt 695 m


Cadishead and Little Woolden Moss
g)
© 2018 Google
Google Earth
1945
Imagery Date: 4/19/2018 53°27'12.23" N 2°27'28.48" W elev 24 m eye alt 695 m

Appendix 7. Total nitrogen and extractable ammonium, nitrate (mg kg^{-1}) and macronutrient (mg g^{-1}) content of surface peat samples on each carbon GHG flux trial plot analysed pre-trial (plots 1 – 3, 'mature' plots; plots 4 – 9 'immature' plots, Chapter 4).

Plot	NH^{+}_{4}	NO^{-}_{3}	N	P	K	Ca	Mg
1	5.78	13.28	13.73	0.0000	0.0292	4.06	1.05
2	0.25	0.05	14.23	0.0010	0.0000	3.28	1.13
3	0.45	0.08	15.19	0.0007	0.0063	3.41	0.48
4	4.00	0.28	15.08	0.0022	0.0231	2.65	0.24
5	1.30	0.04	14.07	0.0010	0.0000	3.31	0.55
6	6.34	0.33	13.76	0.0003	0.0010	2.34	0.75
7	10.02	0.72	12.66	0.0000	0.0250	3.13	0.60
8	3.85	0.58	13.89	0.0008	0.0043	2.60	0.16
9	0.00	0.09	14.00	0.0029	0.0519	4.96	0.00

Appendix 8. Comparison of surface peat characteristics pre- and post-trial, including peat depth, on each carbon GHG flux trial plot (plots 1 – 3, 'mature' plots; plots 4 – 9 'immature' plots, Chapter 4)**.**

Plot	1	2	3	4	5	6	7	8	9
Peat depth (m)	2.77	2.09	2.12	1.96	2.77	2.28	2.18	2.26	2.27
pH	4.65	4.67	4.88	4.73	4.63	4.96	4.96	5.02	5.25
	4.13	4.13	4.42	3.78	3.67	4.17	4.01	4.32	4.32
Conductivity (µS)	41.8	46.7	41.7	52.4	47.3	47.6	39.4	41.5	50.7
	35.1	29.3	23.7	33.8	37.6	31.0	31.5	27.8	24.6
Fresh:Dry Weight ratio	9.0	8.7	7.6	6.5	8.5	6.0	6.1	5.4	7.4
	6.3	9.6	7.6	8.0	7.9	5.2	5.9	6.9	7.2
Moisture content (%)	88.9	88.5	86.8	84.5	88.2	83.5	83.6	81.6	86.5
	84.1	89.6	86.8	87.5	87.3	80.9	83.0	85.5	86.0
Organic content (%)	97.6	97.0	95.9	92.3	96.7	93.7	96.7	94.5	96.8
	97.1	98.4	98.4	98.5	97.8	98.3	98.9	98.7	98.2
Mineral content (%)	2.36	2.95	4.08	7.72	3.35	6.31	3.25	5.52	3.20
	2.91	1.59	1.61	1.54	2.22	1.67	1.11	1.35	1.80
% N	1.37	1.42	1.52	1.51	1.41	1.38	1.27	1.39	1.40
	1.44	1.36	1.13	1.38	1.15	2.10	1.36	1.49	1.36
% C	51.26	50.22	51.47	49.39	49.46	50.72	51.83	50.95	51.97
	49.55	49.52	49.54	48.11	47.29	50.58	48.65	49.61	50.36

Unshaded = pre-trial (24 June 2016); Shaded = post-trial (14 January 2019) and peat depth (5 February 2019). Plots 1 - 3 'mature' plots; plots 4 - 9 'immature' plots (see Chapter 4).