Sphagnum re-introduction to degraded Peatland

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I would also like to dedicate this Thesis to my grandparents, without which it would have never been a possibility. Finally my thanks to Kimberley, for everything.

Declaration of originality

The results presented in this thesis are based on my own research within the School of Science and the Environment, the Manchester Metropolitan University. All assistance received from other individuals and organisations has been acknowledged, and full reference is made to all published and unpublished sources used.

This thesis has not previously been submitted for a degree at any other institution.

Table of Contents

List of Figures

1. Abstract

Healthy functioning peatlands are of global and national importance as active carbon sequestering ecosystems. The Southern Pennines contains some of the most severely degraded Blanket Bog in the UK through historic air pollution, overgrazing and poor management has led to an actively eroding system. Current landscape restoration is focused on surface stabilisation and increasing biodiversity, ultimately *Sphagnum* reintroduction, a keystone species in functioning bog. To stabilise eroded peat and provide a nurse crop of amenity grass cover, it has been necessary to directly apply additions of Lime and NPK Fertiliser to the highly acidic nutrient poor peat surface. There has been concern over the potential effects that this nutrient application could have on the growth of *Sphagnum* which is specific nutrient poor acidic soil. Although previous studies have found application of Fertiliser to be directly toxic to *Sphagnum* (particularly greenhouse experiments), there has been little consideration of the mitigating effect of the typical climatic effects and high precipitation faced in the field.

This project aims to investigate the effect of Lime and NPK Fertiliser effect on *Sphagnum* growth, and if this potentially negative effect is mitigated by field conditions.
Field trials were setup in the Southern Pennines. Trials were undertaken on two distinct *Sphagnum* communities in different stages of development: Young establishing and Mature established. Additions of Lime and NPK Fertiliser were applied alongside a Control of no addition. Growth measurement data was collected to provide a comparison of treatments. Greenhouse experiments were also trialled to replicate the field experiments under controlled conditions. Applications of Lime and NPK Fertiliser were applied separately and in combination alongside a control to test *Sphagnum* growth response to specific nutrient application. The trial was also split by watering treatment to attempt to replicate the field precipitation conditions. Half the trial was subjected to watering similar to Southern Pennines rainfall levels, the other treatment was watered at 'optimum' greenhouse levels.

Results from the greenhouse experiment showed there was a significant nutrient effect on *Sphagnum* growth. Fertiliser alone Lime and Fertiliser combined had a significant negative effect on growth and caused dieback of *Sphagnum*. This negative effect was mitigated by watering treatment. When watered at field precipitation levels it was found that the negative impact of Nutrient was reduced. There was a strong significant difference between the watering treatments. This result was replicated in the field, When Nutrient was applied to Young establishing *Sphagnum* there was no significant effect to *Sphagnum* area size (mm^2) or total count. When nutrient was added to mature established *Sphagnum* there was an immediate negative effect on *Sphagnum* height increment (mm^2), but this was short lived and followed by a period of recovery and re-growth.

It was concluded that impacts of Lime and NPK Fertiliser on *Sphagnum* may be less than originally thought. Where possible Nutrient addition directly applied to *Sphagnum* should be avoided since there is a potential direct toxic effect and the potential for increased competition form vascular plants. However *Sphagnum* communities do not appear to be at risk of irreparable damage from Lime and NPK Fertiliser application.

2. Introduction

Peatlands are the world's largest soil carbon pool; they support unique biological communities, have a high capacity to filter pollutants and play a major role in catchment water storage (Soro *et al.* 1999). The capacity to maintain these vital functions is being lost in many parts of the world as peatlands are being degraded through drought, erosion, fire, land conversion and other agents (Carroll *et al.* 2009). UK blanket bogs are a peatland of great international importance with an area about 10% of the world total. However, Tallis (1998) suggested that as much as about 14% (350,000 ha) of UK blanket bogs may be in an eroded condition. Recent appreciation of the global and local importance of peatlands has led to increasing efforts toward conservation, natural restoration of harvested/drained land and direct intervention to restore peatland structure and function (Gorham & Rochefort, 2003). Without some intervention, eroding peat surfaces in many areas will remain exposed and prone to progressive erosion and loss without ever naturally re-vegetating (Tallis, 1998).

In the UK, the most degraded peatlands are the blanket bogs of the southern Pennines, where fire, air pollution and heavy grazing have resulted in large areas of eroding bare peat surfaces (Holden *et al.* 2007). Efforts to restore the ecological functions of the degraded blanket bogs have focused on re-vegetation of grass and dwarf shrub. But there has also been a more recent interest to re-establish *Sphagnum* bog moss communities which are the original, dominant vegetation of these blanket bogs (Tallis, 1998), and the keystone species in terms of the structure and function of the ecosystem (Rochefort, 2000).

Since 2008 Manchester Metropolitan University have supplied pivotal research support for moorland managers and a biotechnology firm to develop *Sphagnum* restoration using a novel encapsulated *Sphagnum* bead, Beadamoss™. All trials reported here used propagated *Sphagnum* produced by Micropropagation Services (EM) Ltd. The moss was cultured from single fresh capitula of *Sphagnum* collected, from field sites, close to the intended sites of *Sphagnum* restoration. Capitula were surface sterilised and transferred to agar-based culture medium under sterile conditions using standard tissue culture methods (Murashige and Skoog 1962). After a further 9 weeks *Sphagnum* plants were prepared for transfer to outdoor or greenhouse growing-on conditions through the production of either *Sphagnum* liquid gel (BeadaGel™), *Sphagnum* plugs (BeadaHumok™) or solid gel beads (Beadamoss®).

This project aims to develop this research further by a combination of (a) new experiments to examine the influence of nutrient addition to mature and establishing *Sphagnum* communities and (b) monitoring of long-term research trials).

3. Literature review

3.1 Definition, extent and importance of Peatlands

The Ramsar Convention (1971) proposed a definition of peatlands as:

"ecosystems with a peat deposit that may currently support a vegetation that is peat-forming, may not, or may lack vegetation entirely. Peat is dead and partially decomposed plant remains that have accumulated in situ under waterlogged conditions" (JNCC, 2011).

The IUCN (2012) defines peatlands as areas of land with a naturally accumulated layer of dead plant material (peat) formed under water-logged conditions. In the UK, *Sphagnum* species are the most important species in formation and successful functioning of peatlands and there formation (Bragg *et al.* 2010) Peatlands are of international importance having been targeted as a priority action under international agreements (IUCN, 2011), occurring in over 175 countries and covering an estimated 3% of the worlds land area equivalent to 4 million km^2 (JNCC, 2011).

Wilson *et al* (2011) states that organic waterlogged deposits, or peat, cover 8% of the United Kingdom's land surface and 10.4% of Scotland's, with the UK possessing over 10 to 15% of the world blanket peat (Tallis, 1998). The UK itself contains an estimated 46,000-77,000 km^2 of peat covered land making it amongst the top 10 world nations in terms of total peatland area (IUCN, 2011). UK peatlands are currently of huge importance in both national greenhouse carbon budgets (Worral *et al.* 2003, Worral *et al.* 2009) and global carbon cycling (Freeman *et al*. 2001, Evans *et al.* 2008). Under healthy conditions peatlands are noted for their capacity for long term carbon storage (Lindsay, 2010). Active peatlands are waterlogged and generally cold meaning that plant decomposition is slow (Rydin & Jeglum, 2006) and inhibited from decaying fully by acidic and anaerobic conditions (Takeshi, 2008). Due to anaerobic conditions the rate of biomass accumulation is higher than the annual rate of decomposition leading to the formation and accumulation of peat (Hungren *et al.* 2013). Carbon is therefore removed from the atmosphere into plant tissue by photosynthesis and stored in the dead plant remains as an accumulation of peat (IUCN, 2011).

Healthy peatlands are of international importance as Carbon sinks (Yu *et al,* 2011). In pristine conditions peat forming bogs can accumulate plant material and actively sequester huge amounts of atmospheric Carbon (Freeman *et al.* 2001, Worral *et al*. 2004) with current estimates predicting that Britain's actively functioning bogs could absorb around 400,000 tonnes of carbon a year (Worral & Evans, 2010). Lindsay (2010) has reported that, in the UK, the typical Carbon content of peat is around 52% carbon by dry weight (JNCC, 2011).
It is estimated that northern peatlands store approximately a third of the world's soil carbon through long-term accumulation of atmospheric carbon dioxide as peat (Gorham 1991, Lucchese *et al*. 2010). Whilst British peatlands are the UK's largest carbon sink, with 3 billion tonnes of carbon stored; they contain more $C0_2$ than the combined forests of Britain (150 MT) and France (Worral, 2007).

Functioning peatlands offer a wide range of other ecosystem services. Bonn *et al*. (2010) provides a good review of current services provided by upland conservation. Functioning upland ecosystems provide services/benefits to wider society well beyond their boundaries (Beniston, 2000), such as water provision, flood mitigation, and climate regulation through carbon storage in peat (as mentioned above). Uplands are also often areas of outstanding natural beauty that provide significant cultural and recreational value (Bevan 2010, Curry 2010). Uplands also tend to be biodiversity hotspots due to topographic and climatic variations (Kerr & Packer, 1997); over 50% of nationally important British wildlife sites lie within the 12% of the country classed as uplands (Bonn *et al*. 2010).

3.2 Blanket Bog definition

UKBAP (2008) define Blanket Bog as a 'globally restricted peatland habitat confined to cool, wet, typically oceanic climates'. Blanket Bogs typically rely on atmospheric input and the term blanket 'bog' strictly applies only to blanket 'mire' which is exclusively rain-fed. With large quantities of water derived directly from precipitation they are predominantly rain fed systems rather than ground water due to their high altitude and impermeable underlying strata (Bragg *et al*. 2001). Blanket Bogs are described as being remarkable in that they are organic landforms built from living plants and their partially decayed remains (peat) (Coulson, 1978). They are distributed internationally, generally between 45° and 60° latitudes and at continent fringes. Being dependent on an oceanic climate, generally they occur at altitudes above 500 m and require an annual temperature of <15˚C, (Charman, 2002). They currently cover 7.5% (22,500 km^2) of the British Isles (Tallis, 1998).

3.3 Current state of UK peatlands

Damage to peatlands worldwide (Luchesse *et al.* 2010) and locally (Evans *et al.* 2006), has resulted in degraded peatlands changing from active carbon sequestering sinks to large persistent sources of atmospheric CO_2 (Petrone *et al*. 2001), due to increased soil decomposition and reduced vegetation productivity (Waddington *et al*. 2010). Britain's commitment to the climate change act of 2008 of reducing the UK's CO_2 by 2050 (COCC, 2008), means that Carbon budgets are of great importance in the UK's political landscape.

Peatlands contain more than half of the 10 billion tonnes of Carbon stored within UK soils (Birkin *et al.* 2011). The IUCN (2011) state that a loss of only loss 5% of UK peat would equate to the total annual UK greenhouse gas emission. British peatlands are the UK's largest carbon sink, with 3 billion tonnes of carbon stored; they contain more CO_2 than the combined forests of Britain (150MT) and France (Worral, 2007). It is currently estimated that degradation to UK peatlands accounts for almost 3.7 MT CO_2 emissions per year (Worral *et al*. 2001).

Only 18% of UK bogs are classified as having 'near natural' vegetation (IUCN, 2011). The EU habitats directive has recognised the need for peat forming bog habitat protection (Wilson *et al.* 2010) with 54% of the UK's Natura 2000 protected blanket bog being classified as in ‘unfavourable condition’ and only 19% of lowland raised bog reported as in ‘favourable condition’ (Williams, 2006). Natural England (2010) estimated that 98% of blanket bog peatlands and 100% of raised bog peatlands are subject to levels of Nitrogen deposition causing habitat damage in the UK.

3.4 Drivers of degradation in the British peatlands

Degradation to UK peatlands has occurred through a number of drivers and aggravation from negative anthropogenic impacts (Evans, 2005; Tallis, 1987). Peatlands are by nature easily disturbed by change affecting their natural functioning (Rochefort, 2000) and if exacerbated this can cause physical degradation and erosion of peat substrate (Lindsey, 2010).

Alterations in peatland stability can occur via both direct anthropogenic environmental pressure (often relating to land management) and external climatic issues (IUCN, 2014). Increased economic interest and anthropogenic pressure has resulted in considerable impact on the functioning of UK peatlands (Rea *et al.* 2011). Direct impacts of land management and agriculture along with indirect historical impacts of atmospheric nutrient deposition from the burning of fossil fuels all contribute to physical degradation of peat (Ramchunder *et al.* 2009). These drivers directly alter the hydrological and ecological functioning of peatland environments. Reductions in water table and removal/damage of keystone plant communities result in peat being directly exposed to physical damage and removal by erosion (Lindsay *et al*, 2014).

The main causes of degradation to British peatlands have been extensively covered by Lindsay (2010) and summarised by Parry *et al.* (2013). Here follows an outline of the major factors leading to degradation of UK peatland:

3.4.1 Pollution and air pollution legacy

The JNCC Peatland Assessment (2011), using the UK Air Pollution Information System (APIS), presents evidence that much of UK peatland is subject to critical load exceedance. Deposition of nitrogen (as ammonia or nitric acid from nitrogen oxides) is an ongoing problem, but during the last two centuries peatlands have also been subject to deposition of sulphuric acid rain from fossil fuel burning, soot particles and heavy metals from transport and industry (JNCC 2011).

Historic atmospheric pollution across the Southern Pennines has contributed to the loss of S*phagnum* and the acidification of peat (IUCN, 2011). Sulphate deposition has been linked to an increase of DOC production. Parry *et al* (2013) noted that blanket peatlands in the UK are located near heavily industrialised cities that have emitted large amounts of heavy metals, sulphur dioxide (SO_2) and nitrous oxides (NO_x) into the atmosphere. Peatland vegetation, particularly *Sphagnum* mosses, are sensitive to atmospheric pollution (Smart *et al*, 2010) and as a result atmospheric pollution is linked to the exposure of large areas of bare peat and the initiation of gullies in blanket peat areas, such as in the Peak District in the UK

(Phillips *et al*, 1981). Although the recent decline in atmospheric pollution has removed some of the direct impacts, the legacy and degradation caused by atmospheric pollution, still remains a challenge to many peatland managers (Lindsey *et al*. 2014).

3.4.2 Fire and Burning

Following wildfires, erosion or severe overgrazing, upland peat can be left completely without vegetation. The surface of bare peat can rapidly dry out and become hydrophobic, and the dry peat particles are susceptible to erosion, which can expose the underlying mineral substrate (JNCC, 2011). Burning on a bare peat surface can destroy the active acrotelm which can take over 50 years to recolonise (Lindsay *et al,* 2014). Burnt bogs often exhibit altered vegetation composition, this is often associated with long term carbon loss due to damaged acrotelm (Lindsay *et al*, 2014). Lindsay (2014) state that burning as a restoration method contains a high intrinsic risk due to peat mobilisation and potential loss via POC.

3.4.3 Grazing and agricultural use

Wilson *et al.* (2011) stated that whilst the UK possesses 10–15% of the world's blanket peat (Tallis, 1998) it has also been subject to some of the most intensive drainage activity which peaked in the 1960–1970s in response to heavy government subsidies (Baldock, 1984). Grazing and trampling are to some effect supportive of blanket peat development (Simmons, 2003). However, at unsustainable levels grazing can have an adverse effect on peatland (Lindsey *et al*. 2014) due to its susceptibility to erosion (Parry, 2013).

The most direct ecosystem impact is physical damage, as grazing can initiate and exacerbate erosion (Ellis and Tallis, 2001) and result in degradation of vegetation communities leading to an irreversible switch to species poor alternatives (Elkington et al, 2001). As well as changing the character of semi-natural vegetation, land management can also establish completely artificial vegetation on peatlands. Drainage, and cultivation or harrowing followed by reseeding and applications of fertiliser and lime, can create agriculturally improved grasslands dominated by sown forage species such as perennial rye-grass or white clover (MG7). In lowland peatlands, increased drainage and intensity of agricultural use enables cultivation for cereals, field vegetables, or root crops. This also leaves the peat surface bare for periods of the year. (JNCC, 2011) and a vegetation change towards more vascular vegetation species (Ward et al., 2007). Damage to acrotelm and S*phagnum* sensitivity can result in a loss of peat forming species.

However, the presence of grazing animals also prevents the colonisation of successional vegetation species such as birch. Whilst trying to restore grassland to *Sphagnum* may need grazing, but only 1 stage (Lindsay *et al*, 2014).

3.4.4 Commercial Peat Extraction

Birkin *et al.* (2011) explains how this degradation can lead to a negative feedback loop. Damage leading to reduced water table and peat drying promotes anaerobic conditions encouraging decomposition of organic material meaning direct release of greenhouse gases. Affected peatland has been converted from an active carbon sink to directly emitting source. Water level reduction also results in changing vegetation communities and succession to non-peat forming systems (Ramachunde *et al*. 2009). Removal of acrotelm leads to non-functioning, non-active Bog (Lindsay *et al*, 2014). The lack of function leads to the peat land becoming an active $C0^2$ source as further degradation of peat through erosion unlocks $C0^2$.

3.5 Restoration of UK peatlands in the Southern Pennines

The current aim in the Southern Pennines is to actively restore upland areas to try to return them back to a pre-damaged state and to restore their functions for a number of ecosystems services roles, such as reinstating active peat accumulation and decrease the amount of erosion. This role is currently being under taken by the Moors for the Future Partnership funded by national and European funding agencies.

The ultimate endpoint of any blanket bog restoration is the creation of a fully functioning peat accumulating acrotelm layer (Hinde, 2008) and to reinitiate self-regulatory mechanisms that lead to functioning peat accumulating ecosystem, by re-establishment of typical peatland flora and fauna, this is ultimately achieved by the reintroduction of *Sphagnum* mosses which are a key species in peat accumulation (Sottocornola, 2007).

Recent restoration processes mainly focused on the re-vegetation of large areas of nutrient-poor, acidic bare peat. The first phase of restoration is identifying drivers of degradation and ensuring further erosion is halted, methods often include stock exclusion and managing visitor pressures (footpath management etc.) (Buckler *et al.* 2013). The next phases of restoration focus upon the stabilisation of the peat surface and the creation of suitable habitat for native plant species.
This is attempted by altering the extremely acidic peat by the application of Lime to the site which increases the pH of the soil to tolerable levels for plant establishment. Amenity grass seed is then applied followed by yearly applications of NPK fertiliser to provide nutrients for the seed establishment (Caporn *et al,* 2007). In many cases applications of heather brash or geo-jute are applied to stabilise the peat and create a suitable micro-climate to encourage seed/plant development. The seed introduced to the moors, which is a mix of amenity grasses (*Lolium, Festuca, Agrosta* etc.), does not represent the desired endpoint of a healthy functioning blanket bog, in relation to its vegetation make-up, but works in the short term to stabilise peat and reduce the amount lost through erosion processes (Buckler *et al*. 2008). The aim of the application of this grass is that it will act as a nurse crop for more specialised bog vegetation (e.g. E*riophorum Spp.*, *Empetrum* etc.) without prolonged addition this grass cover will dieback leaving the bog species to dominate (Anderson *et al*. 1997)

When a healthy functioning bog environment is created it is hoped to introduce *Sphagnum*, to initiate peat accumulation (Caporn *et al*. 2007). The presence of S*phagnum* is of the utmost importance in healthy ombrotrophic peat forming bog systems as it is a keystone species in terms of ecosystems function (Van Breemen, 1995). In northern peat forming systems, S*phagnum* often forms the majority of the biomass (Malmer *et al*. 2003) and often the greatest share of the primary production (Malmer, 1993). *Sphagnum* usually produces the largest proportion of peat as the litter is more resistant to decay than that of vascular plants (Johnson & Damman 1993). Therefore the final stage of restoration involves the addition of *Sphagnum* propagules to create an active blanket bog with a functioning acrotelm. This is arguably the most important stage of restoration, but it also provides the largest gap in current academic and practical knowledge in the field.

3.6 *Sphagnum* Ecology and restoration

3.6.1 *Sphagnum* Ecology

Due to its high rate of biomass production and slow rate of decay (Clymo & Hayward, 1982) *Sphagnum* is an active peat forming species and makes up the majority of peat deposits (Rydin *et al.* 1999. Limpens *et al,* 2003), and can be described as keystone species in active bogs (Rydin & Jeglum)
Ombotrophic peatlands provide harsh conditions that *Sphagnum* is well adapted to live in (Carroll *et al*. 2008). The re-establishment of *Sphagnum* is therefore key in returning the habitats to functioning ecosystems (Hinde, 2008), as the net loss of peat can be prevented by actively forming a fully functioning peat-accumulating acrotelm layer (Caporn *et al*, 2011). A *Sphagnum* carpet present in a healthy bog system maintains a high water table due to its sponge like qualities and maintains a low pH level (Chirino *et al*, 2006). Hydrological conditions within an active bog are often regulated by *Sphagnum* (Rydin *et al*, 1999), and *Sphagnum* productivity is at its highest in high water availability conditions (Clymo, 1970). Price *et al*. (1997) and Schouwenaars (1988) suggest that the water table should be no less that 40cm for successful regeneration, although this is not necessarily true to blanket bog environments (Carroll *et al*. 2009). Tallis (1997) comments that in conservation terms a *Sphagnum* rich vegetated bog is the most desirable plant cover, mainly due to its ability to out-compete other plant species by the creation of acidic, anoxic environment (Clymo & Hayward, 1982) Functioning *Sphagnum* dominated bog reduces competition and keeps decomposition low maintaining the function of *Sphagnum*-rich bogs as sinks for carbon (Van Breemen, 1995).

3.6.2 *Sphagnum* Restoration Principles

Research has stated that *Sphagnum* is extremely good at regenerating (under appropriate conditions); it can reproduce vegetatively from almost any distinct part of the plant (including stem and leaf fragments) (Clymo, 1970. Bugnon *et al*. 1997). Regeneration can be achieved by scattering these fragments (diaspores) onto a peat surface (Rochefort, 2000).

Successful re-introduction of *Sphagnum* therefore relies on the spreading of diaspores, together with associated techniques to improve moisture conditions to prevent desiccation, along with the introduction of associated shelter plants and suitable growing conditions (Wheeler and Shaw 1995, Rochefort *et al.* 2003, Bugnon *et al*. 1997).

The return of *Sphagnum*, by natural recovery or managed re-introduction, is essential to restore degraded ombrotrophic peatlands to an active, functional state (Van Breemen, 1995; Rochefort, 2000). *Sphagnum* provides the form and function of raised and blanket bogs, with its widespread dominance required to confer a suite of ecosystem services (Lindsay, 2010: Rydin & Jeglum, 2013). The majority of research on *Sphagnum* restoration has been on lowland raised bogs following commercial exploitation for peat extraction, forestry plantation and agriculture (Anderson *et al*, 2010, 2013. Rochefort *et al,* 2001, 2008, 2009. Gaudig *et al.* 2005, 2014) and evidence from these trials shows the need for maintenance of a high water table and a stable peat surface for successful *Sphagnum* establishment (Quinty and Rochefort 2003). Similar requirements are likely for the restoration of *Sphagnum* to damaged blanket bogs, which have been degraded through the action of various drivers including over-grazing, accidental fire and air pollution (Anderson *et al* 2009). On blanket bog there are fewer published reports of successful *Sphagnum* application (Hinde, 2009) and whether there is an obligate requirement for a high water table is less certain since blanket bogs occur in areas of high precipitation and cloud cover (Rydin & Jeglum, 2013). An early example of restoration of *Sphagnum* to upland blanket bog was reported by Ferguson and Lee (1983) who transplanted *Sphagnum* into the degraded bog surface in 1979 in the Southern Pennines. These efforts met initially with only limited success but better results were seen in the longer term (Ferguson & Lee, 1983; Caporn *et al,* 2006, 2010) indicating the potential for *Sphagnum* restoration into these upland systems.

3.6.3 Propagation and culture of *Sphagnum*

To address the related challenges of provision of large volumes of *Sphagnum* for spreading without damaging high conservation value donor sites, the production of an alternative source of *Sphagnum* using standard tissue culture methods. Starting from tiny amounts of source materials, *Sphagnum* is cultured under laboratory conditions and is produced in large volumes in a variety of forms suited to application to different peatland surfaces in order to restore *Sphagnum* to cut-over raised bogs and degraded blanket bogs.

All trials reported here used propagated *Sphagnum* produced by Micropropagation Services (EM) Ltd. The moss was cultured from single fresh capitula of Sphagnum collected, from field sites, close to the intended sites of *Sphagnum* restoration. Capitula were surface sterilised and transferred to agar-based culture medium under sterile conditions using standard tissue culture methods (Murashige and Skoog 1962). Cultures were raised at 20 C under moderate lighting (50 µmol m-2 s-1 Photosynthetically active radiation, PAR) provided by cool white fluorescent lamps. After approximately 10 weeks, when plants were around 20 mm in length, they were sub-divided and transferred to fresh culture media at a temperature of 18 °C and irradiance of 100 µmol m^{-2} s^{-1}PAR. After a further 9 weeks *Sphagnum* plants were prepared for transfer to outdoor or greenhouse growing-on conditions through the production of either *Sphagnum* liquid gel (BeadaGel™), *Sphagnum* plugs (BeadaHumok™) or solid gel beads (Beadamoss®).

The product BeadaGel™ is a suspension of whole plants of 5 to 25 mm length in flowing, hydrocolloidal gel medium (BeadaGel™). In contrast, BeadaMoss® beads comprises numerous (typically 10 number) smaller *Sphagnum* plantlets/fragments sections following cutting to a size of approximately 5 mm length. Both of these products are typically transferred to field locations within 10 days of preparation. A further product, *Sphagnum* plugs (BeadaHumok™) is produced from micro-propagated Sphagnum on to peat compost-based plugs (40 mm x 60 mm). The *Sphagnum* plugs are grown on in glasshouse conditions at high humidity, under natural daylight and a range of temperatures depending on seasonal climate, to keep the plants moist they receive rain water. Transfer of *Sphagnum* plugs to the field is within 4 to 6 months.

4. Project aims and objectives

The focus of this project is to gain an insight into a number of factors affecting establishment and survival of micro-propagated *Sphagnum* on degraded peat lands. Throughout there will be an assessment of the current restoration methods used in the field so far and their successes along with a view to potentially finding the most appropriate re-establishment method. The ultimate aim is to increase understanding and to identify the factors that are aiding the success of current *Sphagnum* re-introduction procedure. This will inform current and future restoration policy.

One of the main aims of the project will focus upon the current practice of nutrient addition and its use in current peatland restoration. The aim is to shed light on the question of whether the addition of nutrient is a successful restoration technique considering its effect on establishing *Sphagnum*.

I) Investigating Lime and NPK Fertiliser application to *Sphagnum*: Field trials. Comparing growth of mature and establishing *Sphagnum* following Lime and Fertiliser Application

The addition of Lime and NPK Fertiliser is a common technique used in the restoration of degraded peatland. The aim is to increase soil pH and promote favourable conditions to allow the ultimate goal of re-introducing keystone species, specifically *Sphagnum*. However, previous research by our group (Hinde, 2008) found that additions of Lime and NPK Fertiliser can be damaging to establishing *Sphagnum* under greenhouse conditions.

Around 1-2 years after the original treatment, a re-application of Lime and NPK Fertiliser is used to maintain favourable soil conditions and enhance plant cover. The aim of this study was to investigate the potential negative effect of this re-application of Lime and Fertiliser on both newly establishing and mature communities of *Sphagnum* in the field.

In this research, Field trials on degraded peatlands at Holme Moss in the South Pennines used applications of Lime and Fertiliser to (a) young, establishing *Sphagnum* propagules and (b) to mature *Sphagnum* lawns. Effects were monitored by scoring survival and measurements of radial growth and height over an extended period.

II) Investigate the effect of Lime and Fertiliser treatments on young *Sphagnum* propagules. Greenhouse trials.

Greenhouse trials were used to examine if standard field applications of Lime and Fertiliser, known to benefit grass and heather growth and enhance favourable conditions, are damaging to Young establishing *Sphagnum* plants. The greenhouse trials provided the opportunity to control soil moisture levels and monitor individual growth of plants much more intensively than is possible in the field.

Sphagnum propagules in various forms (micro-propagated, cut sections collected from field) were grown on trays of peat under greenhouse conditions. Growth was be then measured over 6 months. Different treatments of Lime and Fertiliser (separate, combined and control) will be applied to the propagules standard field application rates, (ascertained from current restoration practice).

Watering was delivered at two different rates to investigate the potential mitigation of nutrient effect through watering treatment and possible leaching effect. Typical field rain levels were ascertained and applied as a treatment. Another treatment of 'optimal' greenhouse watering was also be applied. Previous Greenhouse studies have not focused on the possible effect of soil moisture levels as a factor affecting *Sphagnum* development and lime and Fertiliser.

Effects were monitored by measurements of radial growth and scoring of survival and final plant mass at the end of the experiment. In addition, Chlorophyll Fluorescence was used as a measure of growth success.

III) Monitoring of long-term *Sphagnum* restoration trials (See Appendix).

There have been numerous field based *Sphagnum* research projects alongside less academic field trials setup by various organisations. Much of this work has yet to be followed up due to studies being concluded or due to a lack of time and funds set aside for continued monitoring of existing trial sites. This contributes to gaps in the body of research in this particular field.

In earlier MSc and PhD projects (2008 to 2013) many *Sphagnum* trials plots were set up. Continued monitoring is required to gain the most from these trials, as it is now clear that gathering useful data can take several growth seasons. The objective was to repeat and continue monitoring. Data collection will be relevant to the site and condition of the plots. Where possible data was collected in relation to a range of treatment variables: species planted, date of planting, type of surface and surface treatment. Dependent on the condition of the plots, evidence collected may be more anecdotal and comment, where relevant, is made on factors contributing to *Sphagnum* survival. Pertinent observations were made and the collected information used to inform conclusions and discussion regarding successes of *Sphagnum* reintroduction on degraded peatland. Continued monitoring took place on existing experimental plots in the Southern Pennines and Cumbria.

The aim was to visit many of these trial plots to gain evidence of their continued survival and possible successes. Some of these plots represent the oldest existing plots that contain micro-propagated *Sphagnum*.

5. Investigating Lime and NPK Fertiliser application to *Sphagnum*: Field trials. Comparing growth of mature and establishing *Sphagnum* following Lime and NPK Fertiliser Application

5.1 Introduction

Historic pollution and degradation within the Southern Pennines of England have resulted in large areas of nutrient-poor, acidic bare peat (Tallis *et al*, 1997). Landscape restoration works have been undertaken in the Southern Pennines specifically by Moors for the Future Partnership (amongst others). The early phases of restoration focus upon increasing soil pH and peat stabilisation, particularly via the application of amenity grass seed (Buckler *et al*. 2013). Amenity grass seed is added alongside an application of Lime and NPK fertiliser, the aim being to increase the pH and provide beneficial promotion of growth (Caporn *et al*. 2007). The amenity seed establishes a root-mat to bind surface peat and reduce erosion (Dixon *et al,* 2013), and also provides a nurse crop to support the introduction of native plant including the re-introduction of *Sphagnum* (Buckler *et al.* 2013).

The aim of our research was to investigate how this Lime and NPK Fertiliser application could affect the establishment of both newly introduced *Sphagnum* propagules and any pre-existing *Sphagnum* communities in the restoration areas. There is a consensus that whilst Lime and NPK Fertiliser will allow nurse vegetation to be established, the nutrient availability and optimal pH (>4) could provide a competitive advantage to vascular plants, inhibiting *Sphagnum* growth (Lunt et al. 2010). Previous greenhouse trials have also shown that both Lime and, in particular NPK Fertiliser, can have a negative effect on *Sphagnum* growth (Boatman & Lark 1971, Granath *et al*. 2011, Sunderberg & Rydin 2002). However, it is unknown how *Sphagnum* is affected under the harsh climatic conditions in the field where the impact of Lime and NPK Fertiliser could be mitigated by environmental factors e.g. heavy rainfall, leaching, temperature etc.

To investigate nutrient application and its effects upon *Sphagnum* reintroduction, field trials were established in the Southern Pennines. The trials were set up upon areas of degraded blanket bog that had previously received treatment as part of Moors for the Future restoration efforts. The trails aimed to investigate the effect of Lime and NPK Fertiliser when applied to (a) young, establishing *Sphagnum* propagules and (b) to mature *Sphagnum* lawns. Representing the impact of current restoration methods used in the field at present.

5.2 Effect of Lime and NPK fertiliser on Young establishing *Sphagnum.*

5.2.1 Site Description: Holme Moss

Holme Moss is a high level (Altitude: 524 m.a.s.l) plateau of degraded blanket bog. Located in the Southern Pennines (OS grid reference SE 09377 04454) Holme Moss falls within the Northern boundaries of the Peak District National Park. Situated between the major conurbation centres of Manchester (30km ENE) and Sheffield (40km WNW), the site has been used for urban plumes studies (Beswick, 2003). The site has the focus for several studies and research projects. Holme Moss is home to a large transmitting station and associated infrastructure (Aqiva, 2017).The severity and extend of degradation have been well-noted (Anderson *et al.* 1997) with large areas of eroded blanket bog. Historically Holme Moss has been subject to a range of internal erosion pressures (Parry *et al.* 2013) notably direct fire damage (Tallis, 1987) and Atmospheric pollution (Beswick *et al*, 2003). The site is defined by severe type 1 gully erosion (Allot *et al.* 2009). Common features include exposed peat haggs or hummocks surrounded by an expanse of bare peat (Allot *et al.* 2009) and exposed underlying, bare mineral substrate (Tallis 1987, 1997). Species poor vegetation is prevalent and there is a notable absence of keystone blanket-bog species, specifically *Sphagnum* species. Holme Moss vegetation is primarily characterised by acid grassland, particularly where the blanket bog surface has eroded (Hinde, 2009). Areas of intact blanket bog vegetation remain and roughly resemble NVC community's M19 and M20a composed primarily of *Calluna* and *Eriophorum* species (Elkington *et al.* 2001); M20a is likely linked to previous history of burning and grazing at Holme Moss (Tallis, 1987). Holme Moss has undergone various restoration treatments; heather brash and amenity seed mix where applied to stabilise the peat surface (Buckler *et al*. 2013).

5.2.2 Methods

This trial was designed consisting of replicated fixed quadrats arranged within an existing experimental field site used in a previous study as part of Rosenburgh's PhD Thesis (2017). This previous trial setup consisted of replicate blocks that had been sown with *Sphagnum* bead species, provided by Micropropagation Services Ltd.
The original trial aimed to investigate the factors effecting *Sphagnum* reintroduction on degraded blanket bog. Replicate blocks consisted of 4m x 1m treatment strips (separated by a 0.5 m gap) were setup on differing substrates (re-vegetated, bare peat and intact Bog vegetation). Treatment blocks were sown with micro-propagated *Sphagnum* beads at a 400m^2. The plots were then monitored and the data included in Rosenburgh (2017) final Thesis.

A treatment block was identified from Rosenburgh (2017) experimental trials. The site was located on an area of re-vegetated bare peat from previous restoration programme on Holme Moss. Treated with applications of Lime and NPK Fertiliser and amenity grass seed mixture. Two treatment strips were chosen from the experimental block and a series of permanent quadrats were setup within the previous trials. The treatment strips had previously been sown with *Sphagnum Fallax* at a rate of 400 m^2. *Sphagnum Fallax* is identified as the most successful *Sphagnum* species established from Micropropagated beads with an establishment rate of 0.996% in species trials (Rosenburgh, 2017). *Sphagnum Fallax* is seen as suitable for reintroduction due to its pollution and low pH tolerance (Caporn *et al.* 2006. Smith, 2004).

Six permanent quadrats were placed at random within the two treatment strips on the Holme moss site. Quadrats were arranged in two experimental blocks of three replicates randomly spaced.
The quadrats were 0.5m x 0.5m in size, aligned north to south, Strong bamboo canes were used to mark the corners of each permanent plot. Quadrats were fully monitored to ascertain a base count of *Sphagnum* cover. Every viable *Sphagnum* capitulum was recorded to gain a full count of total *Sphagnum* growth within each quadrat. A secondary count was undertaken to ascertain a base count of total *Sphagnum* clumps. (Clumps are defined as an area of *Sphagnum* that could have originated from a bead and can represent both a single or a group of capitulum that don't appear independent of one another).Within each quadrat, the location of ten individual *Sphagnum* were randomly selected. These individual *Sphagnum* were marked and their longest and shortest axis were measured and recorded (mm) and marked and mapped for future monitoring.

Three of the fixed quadrats were chosen to be treated with an application of Lime and NPK fertiliser. Agricultural Lime and a slow release NPK fertilizer (NPK ratio 11/32.5/16.5) was sourced from Moors for the Future, and was the same material used in the Phase 4 of the restoration process at Holme Moss (MFTF, 2010). In field restoration Lime and NPK fertiliser is applied via helicopter spreading. Lime is applied at a rate of 1000kg / ha in the field; NPK fertiliser is applied at a rate of 365kg / ha. These levels were scaled down from the application per hectare currently used in the field and calculated to represent the appropriate application for a ¼ m^2 quadrat.

(NPK ratio 11/32.5/16.5) and agricultural Lime. Field application of Lime in the landscape restoration is 1000kg / ha; NPK fertiliser is applied at a rate of 365kg / ha. The application for our 0.5m x 0.5m quadrat was worked from the application per hectare currently used in field restoration, Lime at a density of 8.62g per ¼ m^2, Fertiliser at 3.136g per ¼ m^2.
The Lime and NPK Fertiliser was weighed out in the faculty laboratory on a calibrated balance scale. The grains were decanted into paper bags and transported to the field site. The Lime and Fertiliser was spread by hand onto the quadrats from above. A large meshed sieve was used to simulate the spreading of the grains via a helicopter hopper, and to gain an even spread across the quadrat.

5.2.3 Data Collection

Data collection took place over the course of a year. After the quadrat setup, a full base count of all *Sphagnum* within each quadrat was undertaken. As previously described both total *Sphagnum* capitulum and clumps was taken. This full count was repeated on every data collection trip. All quadrats were photographed and the exact location of all *Sphagnum* was 'mapped' on scaled graphs. Within each quadrat 10 individual *Sphagnum* were identified and permanently marked out (Schwarzer & Joshi, 2017). The longest and shortest axis of these individual was measured (mm) and recorded. A field lens was used to aid with the exact measurements. These marked individuals were repeatedly monitored at every data collection.

To monitor the effect of Lime and NPK fertiliser, grass density was recorded. Density was ascertained using the Touches per pin method (Scowcroft *et al.* 2007). A gridded quadrat was placed over the fixed quadrat and a thin cane was dropped at 8 locations on the grid. The number of grass individuals touching the cane was recorded. A survey of associated vegetation with in the quadrat was recorded. Throughout all monitoring and data collection, disturbance to the quadrats was kept to a minimum.

5.3 Effects of Lime and NPK fertiliser on established, mature *Sphagnum*

5.3.1 Site Description: Black Hill

Black Hill (Altitude: 582 m.a.s.l) is located adjacent to Holme Moss 2km to the NW (OS grid Reference SE 07814 04687). With its close proximity Holme Moss, Black Hill has endured the same internal erosion pressures; Wild fire, atmospheric pollution, grazing and trampling etc. (Tallis, 1987. Unlike Holme Moss, Black Hill is not directly assessable by road; it also has no human infrastructure like the Transmitting tower complex. Black Hill is dissected by the Pennine way trail and has suffered from erosion due to significant visitor numbers (Tallis, 1995); though Pearce-Higgins (1997) commented that recreational disturbance to surrounding blanket bog has fallen dramatically after the resurfacing of this section of the Pennine Way.

Topographically Black Hill is comparable to Holme Moss as a high-level plateau of degraded bog. Like Holme Moss, the site is defined by extensive erosion and species poor vegetation. Gullies and type 1 and 11 erosion (Allot *et al.* 2009) are widespread.

With the extensive degradation, Black Hill has subsequently undergone revegetation and restoration treatments. The vegetation matrix is primarily acid grassland with large swathes of amenity grass including *Lolium and Festuca* Species (Hinde, 2009). Areas of intact remain and again resemble the M19-M20a communitys (Elkington *et al.* 2001), and extensive gullies have revegetated both naturally and via restoration and contain sedges and *Eriophorum* species (Rosenburgh, 2017). Naturally, occurring *Sphagnum* establishment has been observed on Black Hill (Hinde *et al.* 2010).

5.3.2 Methods

This experiment involved the setup of 6 permanent fixed quadrats on an area of mature *Sphagnum*. The experimental site was identified on Black Hill. As previously mentioned, Black Hill has undergone extensive restoration work, specifically stabilisation techniques involving Lime and NPK fertiliser. A naturally revegetated gully was chosen. The gully was identified as being an area of mature *Sphagnum* that had undoubtable naturally established. The source could possibly be via airborne spores but most likely introduced amongst heather brash (used in surface stabilisation) harvested from *Sphagnum* rich donor site (Buckler *et al.* 2013). After a survey, the experiment site was found to have a 100% established *Sphagnum* cover containing the species most commonly found in the Southern Pennine area: *S. fallax*, *S.*

capillifolium, *S. cuspidatum*, *S. papillosum S. palustre* and *S. fimbriatum* (Carroll *et al.*, 2009). Other associated bog vegetation such as *Polytrichum* and *Eriophorum spp.* were noted.

A series of 6 fixed quadrats were placed randomly along the gully floor. The quadrats were marked out with strong canes, aligned north-west to south-west. The quadrats were a ¼ m^2, 0.5m x 0.5m in size, consistent with the previous experiment on Holme Moss.

Since the experiment was focused on evaluating the growth of established *Sphagnum;* a vertical growth measurement was deemed to be most appropriate (Fenton & Bergeron, 2007; Pouliot *et al.* 2011). The crank wire method commonly used in the field to measure vertical *Sphagnum* (Clymo, 1970) was selected. Changes in vertical height of *Sphagnum* is determined in relation to fixed stationary point, usually a 'cranked' wire (Clymo, 1970).
A 0.5m x 0.5m grid was laid across each fixed quadrat. 10 squares within each grid were randomly selected and locations numbered and marked. 10 peg with a large flat head was pushed into the *Sphagnum* lawn surface at each location in the quadrat. 10 pegs per quadrat, across 6 quadrats equalling a number of 60 sampling points. The locations relate to a grid quadrat that can be laid across each fixed quadrat to allow rapid identification of each sampling peg.

The sampling pegs were chosen with the crank wire method considered. The pegs were biodegradable 'plastic' material, used locally in restoration methods to secure geojute material into gully sides (Buckler *et al.* 2013). The pegs were shaped with a large flat head, rounded with one strait side. The peg was pushed flat into the *Sphagnum* surface. The head of the peg flush with the level of the top of *Sphagnum* capitulum heads, with the straight edge of the peg used as the sampling point on each peg.

Three of the fixed quadrats were chosen to be treated with an application of Lime and NPK fertiliser. As with the previous trails the same mixture of slow release NPK fertiliser (NPK ratio 11/32.5/16.5) and agricultural Lime. Field application of lime in the landscape restoration is 1000kg / ha; NPK fertiliser is applied at a rate of 365kg / ha. The application for our 0.5m x 0.5m quadrat was worked from the application per hectare currently used in field restoration, Lime at a density of 8.62g per ¼ m^2, Fertiliser at 3.136g per ¼ m^2.

As previously, the Lime and NPK fertiliser was weighed out in Faculty laboratories and transported to site in paper bags. The same application method using a large meshed sieve was employed to simulate spreading from above via a helicopter hopper, and to ensure an even coverage across the treatment quadrat.

The layout of the quadrats in the experimental site was not selected randomly. The quadrats were located within a mature *Sphagnum* lawn in gully floor. The trial site was a functioning channel actively transporting water after precipitation events. The untreated quadrats were placed at the highest point in the centre of the gully floor. The quadrats treated with Lime and NPK fertiliser were essentially arrange downstream of the gully head. The aim was to ensure that lime and NPK was not leached and transported via water flow to untreated quadrats, potentially affecting the experimental outcome.

5.3.3 Data Collection

Collection centred upon growth data from the sample pegs. The experimental site (gully floor) was chosen due to its 100% established *Sphagnum ssp.* cover. The experimental design was focused on assessing mature *Sphagnum*; opposed to establishing *Sphagnum* numbers. Therefore, a base count of *Sphagnum* numbers within each quadrat was not deemed necessary.

Growth of *Sphagnum* was measured using the placed pegs. The fixed pegs were monitored repeatedly throughout the experiment. The straight edge of the peg was used as the fixed point from which to measure growth (Clymo, 1970). A ruler was used to measure *Sphagnum* lineal growth (mm). The flat head of the pegs were pushed flat with the surface of the *Sphagnum* lawn, thus representing a value of 0mm upon setup. *Sphagnum* growing above the fixed point of the peg was measured as a +positive value (mm) or a -negative value (mm) if the *Sphagnum* was below the start 0mm point of the peg. The pegs were inserted on 02/07/13 and monitored at 02/08/13, 19/09/13, 01/11/2013, 04/06/14 and 19/08/14.

5.4 Field Trials: Results

Using SPPS statistical package, data was tested for normality using the Kolorov-Smirnov/Shapiro Wilkes tests. Appropriate statistical tests were then decided upon dependant on the normality of the selected data.

5.4.1 Effects of Lime and NPK fertiliser on established, mature *Sphagnum.* Black Hill

Sphagnum height (mm^2) was significantly reduced by Lime and NPK fertiliser treatment (*P*= <0.001), but the extent of inhibition changed over time. For example, at the first two time points growth was reduced by the treatment below the starting value while the control increased in length. By the last two dates there was much less effect of the treatment on the length. This was shown using a paired sample T-Test split by date, due to large variability in the data there was no real significant difference between dates, but there was a difference from the mean: Date 2 (*P*= 0.094) Mean -21.2000, Date 3 (*P*= 0.072) Mean -14.3333, Date 4 (*P*= 0.070) Mean -16.5333. There was no significant difference recorded at dates 5 and 6 (*P*= >0.400).

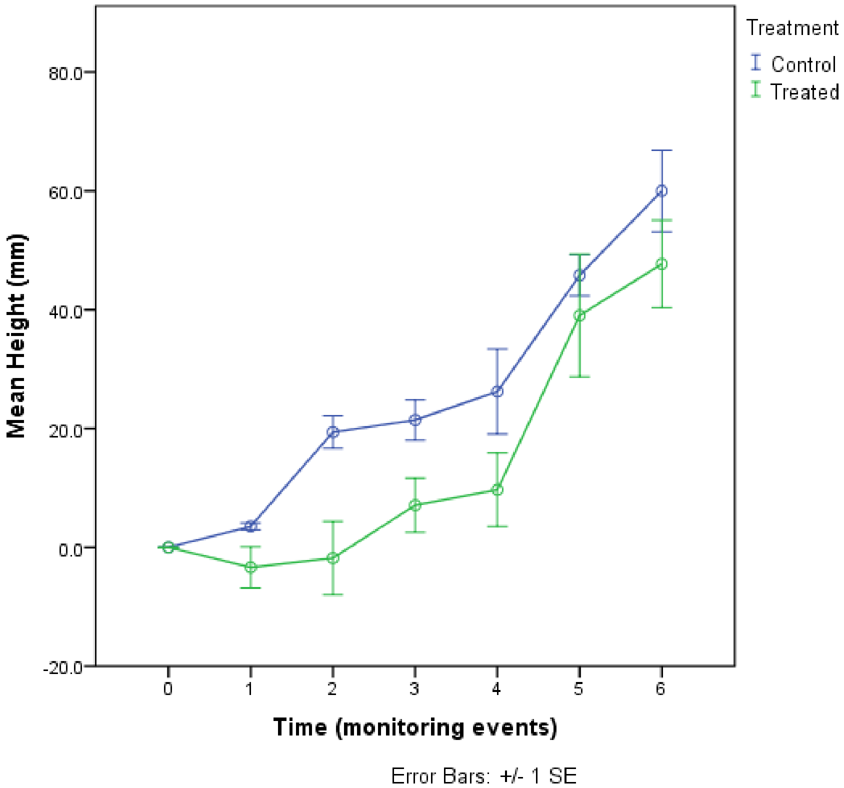


Fig 5.1 The effect of Lime and NPK Fertiliser treatment on height increment in mature *Sphagnum* on Black Hill. Overall difference between treatments analysed using a paired sample T-Test (*t*= 5.411, df= 20, *P*= <0.001).

5.4.2 Effect of Lime and NPK fertiliser on young, establishing *Sphagnum.* Holme Moss.

5.4.3 Total *Sphagnum* clump counts

ANOVA was used to analyse the effect of Lime and NPK Fertiliser addition on total *Sphagnum* numbers within treatment plots. There was no significant effect of NPK on Total *Sphagnum* numbers, although it is close to a significant difference (F= 3.417, P= 0.067). Graphical showed that there was a gradual reduction over time, so further ANOVA split by date were used. Test results showed a significant difference between *Sphagnum* count on nutrient treated and control at Date 1 (F= 7.250, P= <0.05), at Date 2 there was no significant effect between treatments (F= 0.000, P= 1.000) the final date 3 showed a strong treatment effect, with a significant difference in *Sphagnum* numbers on treated and control (F= 7.420, P= <0.05).

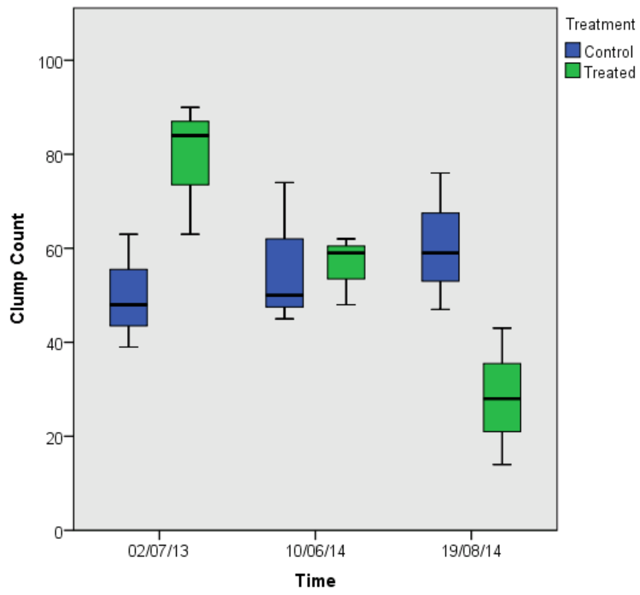


Fig 5.2 Comparison of Mean (± 1 SE) total *Sphagnum* plants recorded on experimental Holme Moss plots, after applications of Lime and NPK fertiliser using ANOVA (F= 3.417, P= 0.067). ANOVA split by date: Date 1, (F= 7.250, P= <0.05) Date 2, (F= 0.000, P= 1.000) Date 3, (F= 7.420, P= <0.05)

5.4.4 *Sphagnum* individuals Area (mm^2)

ANOVA was used to analyse the effect of Lime and NPK Fertiliser addition on Area (mm^2) of *Sphagnum* individuals within treatment plots. Test results showed no significant effect of NPK on *Sphagnum* Area (mm^2) (F= 3.417, P= 0.067). ANOVA split by date showed no significant difference in area (mm^2) between nutrient treated and control at date 1 although close (F= 6.205, P= 0.067). There was no significant effect of treatment at date 2 (F= 2.490.226, P= 0.190) or date 3 (F=.669, P= 0.459).

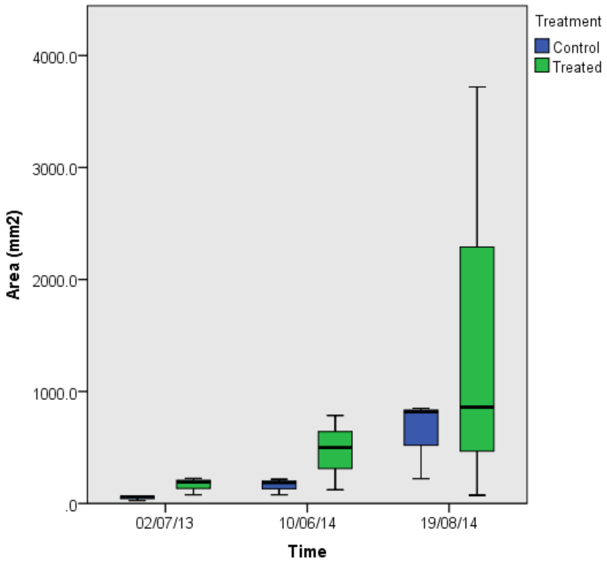


Fig 5.3 Comparison of Mean (± 1 SE) Area (mm^2) of *Sphagnum* recorded on experimental Holme Moss plots, after applications of Lime and NPK fertiliser using ANOVA (F= 2.463, P= 0.127). ANOVA split by date: Date 1 (F= 6.205, P= 0.067) Date 2 (F= 2.490.226, P= 0.190) Date 3 (F=.669, P= 0.459)

5.4.5 Effect of Lime and NPK Fertiliser addition. Vascular plant density.

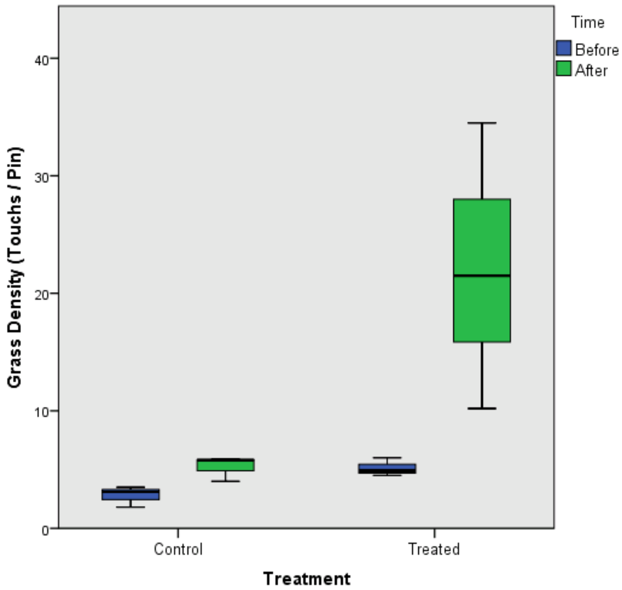


Fig. 5.4 Comparison of Vascular plant density on Holme Moss, before and after applications of Lime and NPK Fertiliser. Overall difference between treatments analysed using a paired

sample T-Test, (control) no nutrient addition (*t*= 2.719, df= 2, *P*= 0.113) and treated with nutrient (*t*= 4.363, df= 2, *P*= <0.05).

A paired T-Test was used to find any significant difference in grass density after applications of Lime and NPK Fertiliser. T-Test results showed that there was no significant difference between grass density on control plots were there was no nutrient application (*P*= 0.113). There was a significant difference between grass density before and after treatments of Lime and NPK Fertiliser *P*= <0.05) showing a significant interaction between grass density and nutrient addition.

5.5 Discussion

5.5.1 Young establishing *Sphagnum*. Holme Moss.

Overall, application of Lime and NPK Fertiliser in the field does not appear to have had as serious an impact on *Sphagnum* as anticipated. There was no significant nutrient effect on Young establishing *Sphagnum* area (mm^2) (*P*= 0.127) or total counts (*P*= 0.067)

As previously mentioned, much research has noted that increased nutrient availability can have negative impacts on *Sphagnum* development (Baker, 1990. Rydin, 1986. Granath, 2000). Previous experimental trials by Hinde (2008) in the faculty greenhouses at MMU, used field applications of nutrients, and found that additions of Lime and NPK Fertiliser had a strong significant negative effect on *Sphagnum* growth, with a significant reduction in *Sphagnum* growth on all treatments that had application of NPK Fertiliser. Until this experiment, this Lime and nutrient application to *Sphagnum* had been untested in the Southern Pennines.

There have been several field experiments that have noted negative nutrient effect on *Sphagnum*. Many have reported Nitrogen (N) having a negative relationship between *Sphagnum* growth (Limpens & Berendse, 2003, Press *et al*, 1986) and can actively depress *Sphagnum* height increment (Limpens *et al.* 2004). Fritz *et al.* (2012) found *Sphagnum* dominated bog to be highly susceptible to excess nitrogen and can cause a decline in photosynthetic rates.

Most of these trials take place on pristine, usually *Sphagnum* dominated peatlands, not comparable to the highly degraded peatland of our study in the Southern Pennines. Our study site is degraded and extremely acidic low pH (Tallis, 1983) with poor soil quality from historic atmospheric pollution (Caporn, 1997).

It is possible that the reason there was no significant nutrient effect on Young establishing *Sphagnum* area (mm^2) (P= 0.127) or total counts (P= 0.067), was that the *Sphagnum* in our trials was benefiting from the raised pH, a result of Lime addition. Although *Sphagnum* is adapted to low nutrient acidic environments (Clymo & Hayward, 1982), Rosenburgh (2015) suggested pH 3.5 as a threshold for negative effects on *Sphagnum* (Andrus, 1986), low pH can also mobilise toxic metal ion in the soil and cause a reduction in *Sphagnum* growth (Lee *et al.* 1993).

The addition of Lime and the subsequent raised pH could well be beneficial in the degraded Southern Pennines. Caporn *et al.* (2007) found that Lime addition in the Southern Pennines, raised pH and enabled plants to better make use of nutrients in fertiliser. Although no significant difference between treated and control plots on *Sphagnum* area (mm^2) this means nutrient addition did not have a negative effect on growth. Looking at the area (mm^2) split by date there was no significant difference between treatments on date 3 (P= 0.459) looking at the graphical output (Fig.5.4), it appears that there was an increase in area size on treated plots when compared to Control. This increase could have been due benefits of increased pH (Lamers *et* al, 2011) to the nutrient being made available (Caporn *et al*, 2007). It is important to remember that any potentially damaging effect of nutrient has potentially been diluted and leached away by heavy rainfall at the site (Beswick, 2003) and reduced to more usable levels.

Current research has shown that although N can be damaging to *Sphagnum*, at low/medium deposition N has no discernible effect (Lamers *et al*. 2000). At low deposition N can be limiting factor to *Sphagnum* growth, and with increased N *Sphagnum* can show positive growth response (Berendse *et al.* 2001).

The effect of Phosphorous (P) in NPK fertiliser should be considered, as it can alleviate negative impacts of N deposition (Limpens *et al*. 2004). Phosphorous is the most limiting nutrient for the establishment of *Sphagnum* (Boatman & Lark 1971, Sunderberg & Rydin 2002). Baker (1990) found that *Sphagnum* innovation and establishment in ombotrophic conditions was limited by high concentration of Phosphorous, with increasing P concentrations causing other elements such as nitrogen to also become limiting factors.

Another factor that could have influence the *Sphagnum* area (mm^2) increase (Fig.5.4- Date 3) when compared to the control, is that site at Holme Moss has already had previous treatments of lime and fertiliser. Amenity grass was artificially established at the side with Lime and NPK addition, so the soil chemistry could be a related factor. Hinde (2008) found that conductivity at the site was high but possibly reduced by the raising of pH from lime addition. pH readings at the site fell within the range of 4-5 so this could have positively influenced the growth of the *Sphagnum* Area (mm^2), but not significantly from the control plots that would have also been benefiting from the residual raised soil pH.

When looking at *Sphagnum* total count, again there was no significant effect of nutrient on the total count (P= 0.067), although it could be interpreted as nearing a significant interaction. Lime and NPK Fertiliser has not negatively affected the number of *Sphagnum* individuals when compared to the control with no nutrient addition. When the data was analysed by date, there was a significant reduction in *Sphagnum* total count on treated plots

(*P*= <0.05) this is evident from Fig 5.3. Again Lime and NPK has not been as damaging as possibly anticipated. The gradual reduction in *Sphagnum* numbers is more likely due to increased competition from vascular plants (Berendse *et al*. 2001) than from a direct toxic effect from nutrient addition.
Vascular plant density was significantly increased compared to the control when with Lime and Nutrient addition (*P*= <0.05). Competition from vascular plants can have a significant influence of *Sphagnum* counts Breemen *et al.* (2001) found that additions of fertiliser to bog communities reduced *Sphagnum* mass growth, because it increased the cover of vascular plants and acrocarpous moss which was found to outcompete *Sphagnum.* Rosenburgh (2015) noted increases in N can only be absorbed by *Sphagnum* to a certain limit (Aerts, 1990); above this threshold, N will become available to more vigorously growing species increasing competition with *Sphagnum* for light (Haultier *et al*. 2009) water availability (Fritz *et al*. 2014) and also with other mosses (Mitchell *et al.* 2002).

This competition effect rather than nutrient toxicity can also be linked to the increase in *Sphagnum* area (mm^2) (Fig.5.4- Date 3). Although the total *Sphagnum* count reduced (*P*= <0.05), there was no significant difference in area (mm^2) hinting at no nutrient effect. If there were a toxic nutrient effect then the area (mm^2) would not have seen an increase. Therefore, S*phagnum* in the treated plots is possibly benefiting from nutrient availability due to Lime addition increasing pH, but this nutrient increase is also leading to an increase in vascular plants (*P*= <0.05) that is leading to a reduction in *Sphagnum* numbers due to being outcompeted by vascular plants and other mosses.

It is also important to consider Vascular plant increase in relation to *Sphagnum* establishment, although it can potential limit *Sphagnum* total count (*P*= <0.05) at date 3, overall there no recorded significant difference in total *Sphagnum* count between treated and control plots (*P*= 0.067). It should be remembered that the increased nutrient effect is only supposed to be temporary (Buckler *et al*. 2013) and will disappear leading to a reduction in the amenity grass, and leaving behind more associated vegetation (Anderson *et al*. 2009). Whilst monitoring nutrient treated quadrats, it was noted that there was an increase in more associated moorland plants, particularly *Polytrichum strictum* and lichens not just the restoration amenity grass. Groeneveld *et al. (*2007) describes that *Polytrichum* carpet provides a favourable microclimate for *Sphagnum* moss. *Polytrichum* is also recommended for protecting *Sphagnum* on sites with harsh climatic conditions (Groeneveld & Rochefort, 2005). This increase in associated bog species fits with the restoration objectives in the southern Pennines, which aims to increase biodiversity (Buckler*et et al*. 2013).

Increased Vascular plants cover can be helpful to *Sphagnum* establishment, again may explain the increase in area (mm^2) (Fig 5.4- Date 3). Malmer *et al*. (1994) found that locations, abundance and distribution of *Sphagnum* within a Peatland, appear to be determined by the life-forms and architecture of vascular plants. It has been often shown that vascular plants accommodate the growth of Sphagnum (Fenton & Bergeron 2006, Tuittila *et al*. 2006). Silva *et al*. (1999) reported *Sphagnum* reintroduction had been found to only be efficient after vascular pioneer species were established. Vascular plants have been found to provide a stable microclimate for *Sphagnum* (Soro *et al.* 1999) and promote *Sphagnum* growth by providing both scaffolding and protection (Rydin & Jeglum, 2006). It has already been found that protective microclimate provided by vegetation and the soil stabilisation it provides have been beneficial to growth of *Sphagnum* propagules spread in the Southern Pennines (Hinde, 2008. Rosenburgh, 2015). Rosenburgh (2015) found *Sphagnum* application completely failed on areas of bare peat, where active erosion and harsh microclimatic conditions removed, buried or otherwise killed *Sphagnum* propagules applied in the field.

One reason that the young *Sphagnum* was not negatively affected and no significant difference was found between treated and control area (mm^2) (*P*= 0.127) or total counts (*P*= 0.067) could be down to species influence. The experiment was setup on pre-existing areas of *S. fallax*, purposely chosen, as it had been the most successful species in establishing in the previous trial (Rosenburgh 2015), this is possible inherent bias. *S. fallax* is much more tolerant of acidity, growing at pH levels down pH 3.5 (Carroll *et al,* 2008) and has been found to be productive even under less than favourable conditions (Clymo, 1971). To differentiate if there was any species effect this would have to be tested in the field across different *Sphagnum* species; although this is probably not necessary. When compared to the mature *Sphagnum* plots, which were setup or more floristically diverse site (With the 6 common southern Pennines species present), *Sphagnum* was affected by nutrient addition but also showed regrowth and recovery. Previous studies most recently Rosenburgh (2015), found *S. fallax* best suited species for reintroduction in the Southern Pennines restoration, as tolerant to the legacy effects of industrial pollution and desiccation. The fact that there was no significant nutrient effect to *S. fallax* just adds to this body of evidence.

5.5.2 Mature establishing *Sphagnum*. Black Hill

Lime and Fertiliser caused immediate damage to mature *Sphagnum* vertical growth, with a significant difference between treated and control (*P*= <0.001). Direct contact with nutrient produced a bleaching and hints at a direct toxic effect as shown in Fig 5.4. Mature *Sphagnum* there was an immediate treatment effect but this seemed to be short lived, possibly being mitigated by dilution through heavy rainfall and water movement (Lunt *et al*. 2010) which was evident in the area, which relates to the greenhouse results (Discussed later).
This direct toxic nutrient effect retarded *Sphagnum* height were there was direct contact. A 'burn effect' was recorded where rock nutrient landed, this direct burn effect has been noted *with* a bleached radius around grouse dung deposited *Sphagnum* hummocks (Hope *et al.* 2010). This negative nutrient effect fits more with the literature that shows nutrient, particularly N, can actively depress *Sphagnum* height increment (Limpens *et al.* 2004).

When we look at the graphical output Fig.5.1, it is clear that although there was a direct negative nutrient that depressed *Sphagnum* height increment (mm) the effect was short lived and there was regrowth and recovery. The strongest influence on recovery was probably heavy rainfall at the site (Beswick *et al*. 2003), reducing the nutrient levels via leaching action (Lunt *et al*. 2010).

Fig 5.5. Direct effect of Lime and NPK Fertiliser on mature *Sphagnum*. Note localised bleaching effect where pelleted nutrient has landed.

The regrowth of *Sphagnum* on the treated site was possibly facilitated by the direct dieback of *Sphagnum* opening up gaps and micro-topography for re-colonisation by other *Sphagnum* to grow into (Rydin, 1986). Although sensitive to the negative effects of trampling (Pellerin *et al*. 2007) and grazing (Rawes, 1983), in *Sphagnum* dominated lawns (like the Black Hill site), disturbance can open gaps for colonization by opportunistic *Sphagnum* to grow into (Rydin, 1986, Drobyshev, 1999) and accentuate micro-topography of bogs (Pouliot, 2011).

Young *Sphagnum* may be more vulnerable to the effects of Lime and Fertiliser due to being at an earlier stage of development and therefore susceptible to competition. This could explain the recovery of the mature *Sphagnum* on Black Hill, the site was more established, a moist gully floor that 100% *Sphagnum* spp. cover. Amenity plants find establishing harder in *Sphagnum* dominated areas as *Sphagnum* modifies it its local environment increasing water level and creating acidic conditions (Chirino *et al*, 2006). And its ability to out-compete other plant species by the creation of acidic, anaerobic environment (Clymo, 1983. Clymo & Hayward, 1982) that reduces competition and keeps decomposition low maintaining the function of *Sphagnum*-rich bogs as sinks for carbon (Van Breemen, 1995). Rosenburgh (2015) increased *Sphagnum* presence can modify hydrology and create the formation of a positive feedback loop (Van Breeman, 1995).
Considering the ability of mature *Sphagnum* to recover from nutrient application (fig.5.1) and its ability to resist vascular plant competition, it is probable that Mature *Sphagnum* is at low risk from Lime and NPK fertiliser addition. Looking at Fig.5.1, *Sphagnum has* recovered over time until almost caught up with relative control height (mm), (date 6). If the plots were monitored now, I would predict no significant difference in *Sphagnum* height increment (mm), with treated *Sphagnum* showing full recovery and no lasting effect of Lime and NPK Fertiliser application.

6. Investigate the effect of Lime and NPK Fertiliser treatments on young *Sphagnum* propagules. Greenhouse trials.

6.1 Introduction

Indoor experiments were instigated to examine the potential growth response of establishing *Sphagnum* following applications of Lime and NPK fertiliser. The main aim of the trial being to measure the growth response of *Sphagnum* under the controlled conditions of a greenhouse. Greenhouse trials were used to examine if standard field applications of lime and NPK fertiliser, known to benefit grass and heather growth and enhance favourable conditions, are damaging to young establishing *Sphagnum* plants. The greenhouse trials provided the opportunity to control soil moisture levels and monitor individual growth of plants much more intensively than is possible in the field. This greenhouse experiment is a repetition of field trials under controlled greenhouse conditions.

Studies have alluded applications of Lime and NPK fertiliser can be harmful to *Sphagnum* (Fritz *et al*, 2011. Diggelen *et al*. 2015). Previous experimental trials by others and myself within Manchester Metropolitan University (Hinde, 2008) have found Lime and NPK fertiliser to be harmful to *Sphagnum* propagules under optimal greenhouse conditions.
This trial was designed to use the controlled conditions provided by the greenhouse to reproduce more associated field conditions that *Sphagnum* in the field is exposed to. Linking the greenhouse experiment to the trials setup in the field previously in this thesis, should provide realistic comparison to inform discussion surrounding Lime and NPK fertiliser application to *Sphagnum*.

This trial was designed to investigate the effect of different treatments of Lime and NPK fertiliser to *Sphagnum* propagules. To explore specific effect, differing combinations of nutrient were added to replicate trays of *Sphagnum* propagules; Lime, NPK fertiliser, Lime & NPK combined and a control of no added treatment.

Sphagnum propagule type included both micro-propagated *Sphagnum* beads and 'cut' *Sphagnum* fragments collected from field sites. This was perceived as more representative of current academic interest; as nutrient addition is currently underway areas in the field treated with micro-propagated beads as part of current restoration, but also areas of 'naturally' establishing *Sphagnum* generated from other sources (eg. Brash) addition. These *Sphagnum* propagules were grown on trays of peat and growth measured over 6 months.

A combined watering treatment was applied with two separate treatments split between the experimental trays. Watering was delivered at two different rates to investigate the potential mitigation of nutrient effect through possible leaching effect. The aim being one treatment was watered at the perceived standard Greenhouse level, whilst the other received 'field' watering input. Typical field rain levels were ascertained from metrological records taken from the Southern Pennines (Beswick *et al*. 2003). Previous greenhouse studies have not focused on the possible effect of soil moisture levels as a factor affecting *Sphagnum* development and Lime and Fertiliser.

Experimental treatment was monitored by measurements of radial growth and scoring of survival of *Sphagnum* plants at the end of the experiment. In addition, Chlorophyll Fluorescence was also used to measure growth response.

6.2 Aims and objectives

The greenhouse experiment was setup to investigate the growth of *Sphagnum* under optimal greenhouse conditions. Whilst measuring the growth response of *Sphagnum* propagules to nutrient addition in relation to simulated field conditions. The specific aims of this study:

i. Compare the growth response of *Sphagnum* after differing treatments of Lime and NPK fertiliser: applied singularly, in combination and as a control of no added treatment.

ii. Investigate *Sphagnum* propagule growth: consider the growth of micro-propagated bead material and cut *Sphagnum* established from the field.

iii. Compare *Sphagnum* propagule growth response in relation to nutrient application.

iv. Explore the effect of standard watering compared to ‘field levels’ of watering: is growth response of *Sphagnum* in relation to nutrient mitigated by leaching process.

6.3 Methods

6.3.1 Greenhouse setup

The experimental design of this study included two watering treatments (standard and ‘field’), four applications of nutrient addition (Lime, NPK fertiliser, combined Lime & NPK and Control) and two *Sphagnum* propagule types (micro-propagated beads and cut *Sphagnum* gathered from the field) for a total of 18 treatment combinations The four nutrient treatments each had 3 replicates trays per treatment. This was replicated for comparative watering treatment resulting in a total of 32 trays. Each tray contained both propagule types beads at a density of 12 per tray and cut stems at a density of 8 pieces per tray. These trays were established in a structured block within the MMU Faculty greenhouses.

16 cm x 21 cm horticultural seed trays sourced from a commercial supplier were filled with peat to a depth of roughly 5cm in each container. Peat was compressed to a uniform density to approximate a natural peat surface. Peat was collected by hand using a trowel, from an eroded area on Holme Moss, site of previous field trials.

Sphagnum propagules were added to every tray. Each tray was divided in half and bead and cut propagules added respectively. Micro-propagated beads were added to one half of the tray at a rate of 12 beads per tray. Tweezers were used to position beads in 3 uniform rows of 4 beads. Cut *Sphagnum* was added to the remaining half of the trays at a rate of 8 pieces per tray. This *Sphagnum* was harvested from the Holme Moss study site and transported back to faculty laboratories. *Sphagnum* was thoroughly washed in de-ionised water and kept in cold storage until ready for processing. This *Sphagnum* was cut down to 2cm pieces of stem material with the capitulum head removed.

The trays were regularly watered using a hand mister filled with deionised water, approximately 5ml per tray. Transparent plastic sheeting was used to minimise evapotranspiration and desiccation, and horticultural shade netting used to reduce overexposure to sunlight during summer months. Supplemental lighting using standard horticultural vapour lamps (150 µmol m-2 s-1) was provided to extend daylight hours. Greenhouse ventilation system was programmed to maintain mean temperature ranged between 15^0c & 30^0c. *Sphagnum* propagules were left for a period of 4 weeks to allow establishment before any treatments were applied.

6.3.2 Nutrient addition

4 different nutrient treatments were setup: lime, NPK fertiliser, combined Lime & NPK and Control (no application). Agricultural Lime and a slow release NPK fertilizer (NPK ratio 11/32.5/16.5) was sourced from Moors for the Future (same material used in field trials). In field restoration, Lime and NPK fertiliser is applied via helicopter spreading. Lime is applied at a rate of 1000kg / ha in the field; NPK fertiliser is applied at a rate of 365kg / ha. These levels were scaled down from the application per hectare currently used in the field and calculated to represent the appropriate application for a 16 cm x 21 cm horticultural seed tray. 3.36g of NPK and 1.226g of Lime per tray.
The Lime and NPK Fertiliser was weighed out in the faculty laboratory on a calibrated balance scale. The grains were decanted into paper bags and transported to the greenhouse. The Lime and Fertiliser was spread by hand onto the trays from above. A large meshed sieve was used to gain an even spread across the tray. 3 trays prepared per treatment to allow for a suitable level of replication.

6.3.3 Watering treatment

The nutrient trial applications were replicated so that a watering treatment could be applied to the trays. Two watering treatments were setup. The first was the standard greenhouse watering: The 16 trays used were sealed with no outflow point for passage of water. Watering undertaken using a hand mister; de-ionised water was applied directly to *Sphagnum* and peat surface, around 5ml de-ionised water per tray (Pouliet *et al.* 2010). Trays were kept covered with transparent plastic sheeting for optimal moisture retention.

The second treatment, described as ‘field’ watering input. 16 trays of the replicated nutrient treatment were setup. A series of nested trays with perforated bottoms allowed water to flow out of the tray when watered, representing the movement of water through peat layers in the field (Johnson, 1998). Water was collected in solid containers beneath trays and analysed for Ph and conductivity.

Average yearly rainfall levels (mm) from the Holme Moss study site (2415 mm per yr) were ascertained from weather station records (Beswick *et al.* 2003). Average levels were worked from the records and averaged out to a monthly rainfall (mm). A fine nozzle watering can was used to replicate rainfall from above. This treatment (mm) of deionised water was applied twice a week at the rate of 3985ml per tray. These trays were also covered with transparent plastic sheeting to avoid desiccation.

6.4 Data Collection

Data collection took place over the course of the experiment, the treatment phase of which lasted a year. The monitoring dates were 18/04/14, 09/07/14 and 04/12/2014. Monitoring took place involved physically measuring the size of *Sphagnum* propagules. Due to the differing nature of the growth of the propagules this was done in 2 ways. Due to the radial growth of bead propagules, beads were measured along the Horizontal, Vertical and Diagonal axis (mm) using callipers and a ruler. Cut *Sphagnum* propagules grew in a linear fashion, as such single length measurement was recorded, again using a ruler. All data was logged and entered in into an excel spread sheet for further analysis.
As well as manual size measurements, Chlorophyll fluorescence was employed to measure growth response of *Sphagnum.* Chlorophyll fluorescence is light energy absorbed and re-emitted by chlorophyll and other associated photosynthetic molecules (Misra *et al*. 2010). A portable Hansatech^{tm} Chlorophyll fluorometer was used in the greenhouse. 3 propagules per tray were randomly chosen. Hansatech^{tm} clips were used to dark adapt the *Sphagnum* for a standardised 10 seconds, and Chlorophyll fluorescence (Fv/Fm) measured (Murchie & Lawson, 2013). As well as this all experimental trails were photographed for visual reference and anecdotal comments about growth and success were recorded.

6.5 Results

6.5.1 Effects of Nutrient addition and watering treatment on Cut *Sphagnum*.

A 2-way ANOVA tested the effect of nutrient and watering on Cut *Sphagnum* length (mm). The ANOVA showed that watering treatments had a strong significant effect on *Sphagnum* length, with a strong significant difference between watering treatments (F= 14.863, P= <0.001).

Looking at the addition of nutrients, the test results showed that nutrient had a strong significant effect on *Sphagnum* growth (F= 22.395, P= <0.001), and a post-hoc Tukey test showed that *Sphagnum* lengths were significantly different from the Control (C) with additions of Fertiliser (F) (P= <0.001), and Lime and Fertiliser (L+F) (P= <0.001). F and L+F showed no significant difference (P= 0.296).There was also a Significant interaction between nutrient addition and watering treatments (F= 3.985, P= <0.05).

The data was also split into the 3 monitoring dates and analysed by using 2 way ANOVA to show treatment effect over time. Data presented with graphical output below. (It is important to add that were it appears to be a missing data point, this actually represents a 0 measurement due to total *Sphagnum* dieback). D = Standard watering, W = Field watering.

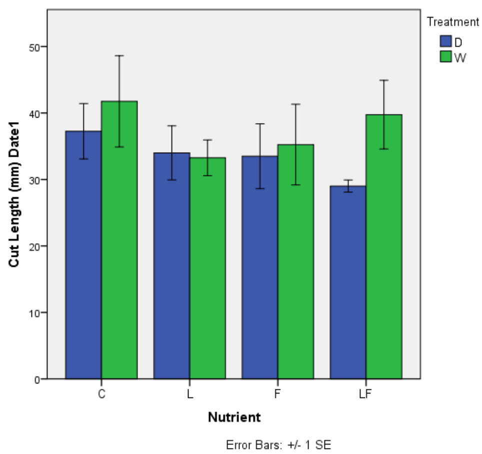


Fig. 6.1 Mean (± 1 SE) *Sphagnum* length (mm) with nutrient addition and watering treatments at date 1, the first monitoring following experimental setup. Two-Way ANOVA showed no significant treatment effect of nutrient addition (F= 0.688, P= 0.580) or watering treatment (F= 1.500, P= 0.223).

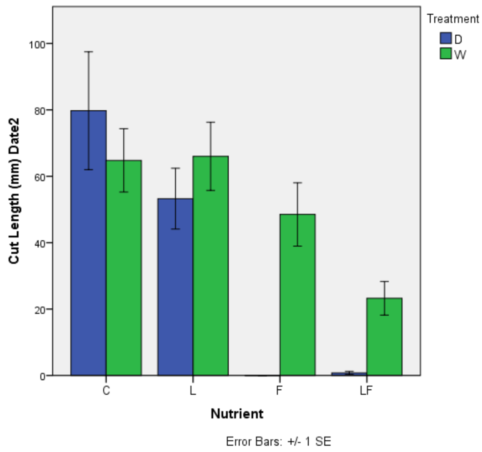


Fig. 6.2 Mean (± 1 SE) *Sphagnum* length (mm) with nutrient addition and watering treatments at monitoring date 2. Two-Way ANOVA showed a strong significant effect of nutrient on *Sphagnum* length (*F= 0*.688, *P*= <0.001), post-hoc Tukey test showed strong significant difference between F (*P*= <0.001) and L+F (*P*= <0.001), and the other treatments (C, L). There was a strong significant effect of watering treatment (*F= 0*.6383, *P*= <0.05).

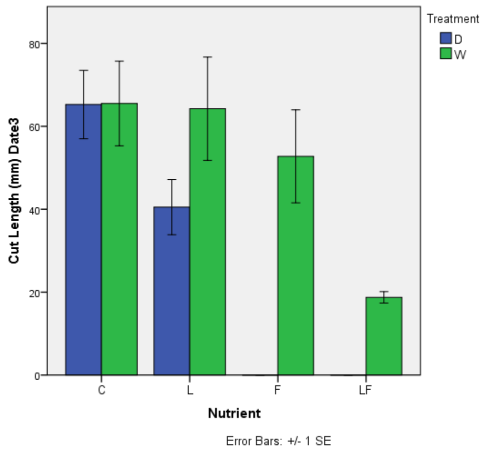


Fig. 6.3 Mean (± 1 SE) *Sphagnum* length (mm) with nutrient addition and watering treatments at the third monitoring date, representing the longest *Sphagnum* exposure to treatment. Two-Way ANOVA showed a strong significant effect of both water treatment (*F*= 18.256, *P*= <0.001) and Nutrient addition (*F*= 20.392, *P*= <0.001) on *Sphagnum* length, post-hoc Tukey test again showed strongly significant difference between F (*P*= <0.001) and L+F (*P*= <0.001), and the other treatments (C, L).

6.5.2 Effects of Nutrient addition and watering treatment on micro-propagated *Sphagnum* bead area.

A 2-way ANOVA showed that watering treatments had a strong significant effect on *Sphagnum* length, with a strong significant difference recorded between watering treatments (F= 32.453, P= <0.001).
Addition of nutrient showed a significant effect on *Sphagnum* growth (F= 1.583, P= <0.05). Results showed a strong significant interaction between nutrient addition and watering treatments (F= 7.695, P= <0.001).

The data was also split into the 3 dates the experiment was monitored and analysed by using 2 way ANOVA to show treatment effect over time. Data presented with graphical output below.

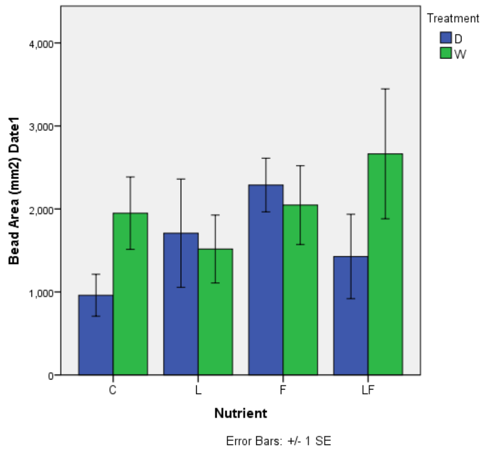


Fig. 6.4 Mean (± 1 SE) *Sphagnum* bead area (mm^2) with nutrient addition and watering treatments at date 1, the first monitoring following experimental setup. Two Way ANOVA no significant treatment effect of nutrient addition (F= .906, P= 0.453) or watering treatment (F= 1.569, P= 0.222).

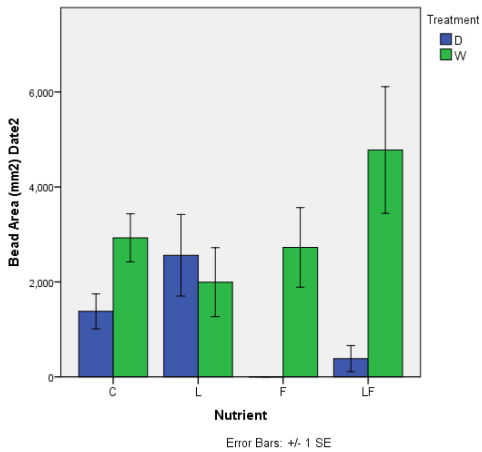


Fig. 6.5 Mean (± 1 SE) *Sphagnum* bead area (mm^2) with nutrient addition and watering treatments at monitoring date 2. Two-Way ANOVA showed a strong significant effect of watering treatment on *Sphagnum* growth (F= 15.576, P= <0.001). Although results showed

no significant difference between nutrient application (*F*= 1.023, *P*= 0.400), this is probably due to the large variance in the data. Test result showed a significant interaction between nutrient addition and watering treatments (*F*= 4.124, *P*= <0.05).

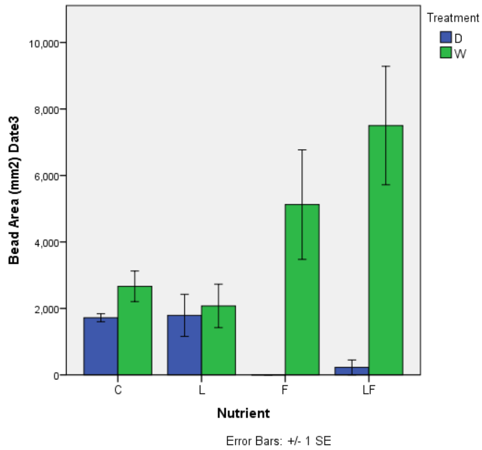


Fig. 6.6 Mean (± 1 SE) *Sphagnum* bead area (mm^2) with nutrient addition and watering treatments at the final monitoring date, representing the longest *Sphagnum* exposure to treatment. Two Way ANOVA showed that there was a strong significant effect of both water treatment (*F*= 15.576, *P*= <0.001) and Nutrient addition (*F*= 1.023, *P*= <0.001) on *Sphagnum* length. Test result showed a strong significant interaction between nutrient addition and watering treatments (*F*= 6.430, *P*= <0.001).

6.5.3. Results of Chlorophyll Fluorescence (Fv/Fm) of Cut *Sphagnum*.

2-way ANOVA tested the effect of nutrient and watering treatments on *Sphagnum* Chlorophyll Fluorescence (Fv/Fm). The ANOVA showed that watering treatments had a strongly significant effect on Fv/Fm, with a strong significant difference recorded between watering treatments (*F*= 49.948, *P*= <0.001). Test results also showed that nutrient had a strong significant effect on *Sphagnum* Chlorophyll Fluorescence (*F*= 24.202, *P*= <0.001), post hoc Tukey test showed that Fluorescence was significantly different from the Control (C) with additions of F (*P*= <0.001), and L+F (*P*= <0.001). F and L+F showed no significant difference (*P*= 1.000), between C and F there was no significant difference (*P*= 0.107). Test result showed a strong significant interaction between nutrient addition and watering treatments (*F*= 1.287, *P*= <0.001).
The data was also split into the 3 dates the experiment was monitored and analysed by using 2 way ANOVA to show treatment effect over time. Data presented with graphical output below.

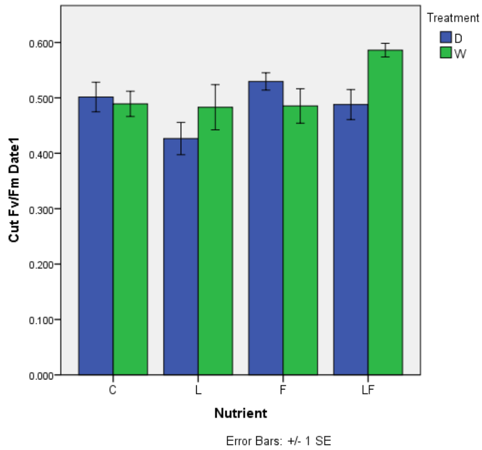


Fig. 6.7 Mean (± 1 SE) Fv/Fm of cut *Sphagnum* with nutrient addition and watering treatments at date 1, the first monitoring following experimental setup. Two-Way ANOVA showed a strong significant treatment effect of nutrient addition (*F= 3*.179, *P*= 0.028) but not watering treatment (*F*= 1.658, *P*= 0.201). Test result showed a strong significant interaction between nutrient addition and watering treatments (*F*= 2.861, *P*= <0.05).

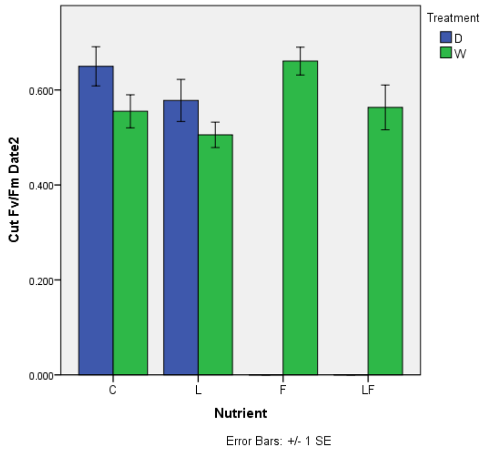


Fig. 6.8. Mean (± 1 SE) Fv/Fm of cut *Sphagnum* with nutrient addition and watering treatments at monitoring date 2. Two-Way ANOVA showed a strong significant effect of watering treatment on *Sphagnum* Fv/Fm (*F*= 128.644, *P*= <0.001). There was also a strong significant effect of nutrient addition (*F*=45.340, *P*=<0.001). Test result showed a strongly significant interaction between nutrient addition and watering treatments (*F*= 75.087, *P*= <0.001).

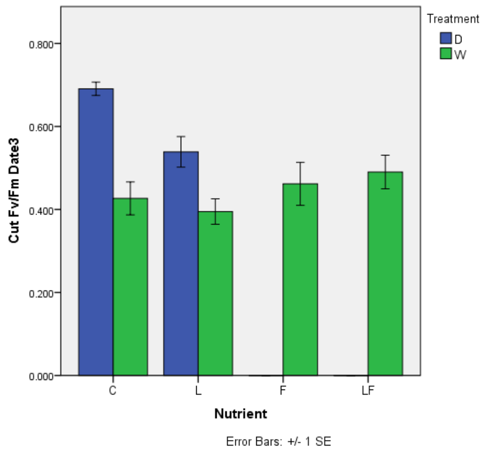


Fig. 6.9. Mean (± 1 SE) of cut *Sphagnum* Fv/Fm with nutrient addition and watering treatments at the third monitoring date, representing the longest *Sphagnum* exposure to treatment. Two-Way ANOVA showed a strong significant effect of both water treatment (*F*= 12.8.644, *P*= <0.001) and Nutrient addition (*F*= 45.340, *P*= <0.001) on *Sphagnum* Fluorescence, post-hoc Tukey test showed strongly significant difference between C and all other treatments, L and L+F (*P*= <0.001) and L (*P*= <0.05).

6.5.4 Results of Chlorophyll Fluorescence (Fv/Fm) of micro-propagated *Sphagnum* beads.

A 2-way ANOVA tested the effect of Nutrient and watering treatments on *Sphagnum* Chlorophyll Fluorescence (Fv/Fm). The ANOVA showed that watering treatments had a strongly significant effect on Fv/Fm, with a strong significant difference recorded between watering treatments (*F*= 5.551, *P*= <0.05). Test results also showed that nutrient had a strong significant effect on *Sphagnum* Chlorophyll Fluorescence (*F*= 2.433, *P*= <0.05), Test result showed a strong significant interaction between nutrient addition and watering treatments (*F*= 15.564, *P*= <0.001).

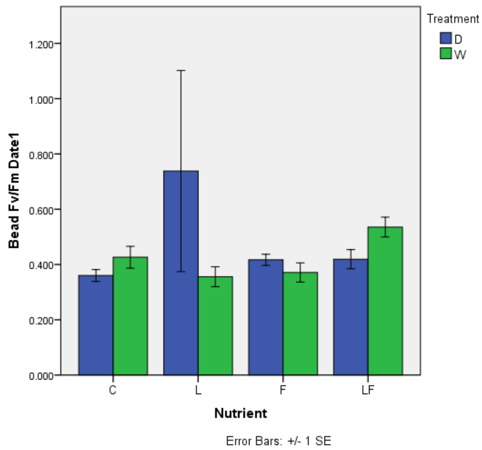


Fig. 6.10. Mean (± 1 SE) Fv/Fm of *Sphagnum* beads with nutrient addition and watering treatments at date 1, the first monitoring following experimental setup. Two-Way ANOVA showed no significant treatment effect of nutrient addition ($F= 0.628$, $P= 0.599$) or watering treatment ($F= 0.432$, $P= 0.513$). Test result showed no significant interaction between nutrient addition and watering treatments ($F= 1.441$, $P= 0.236$).

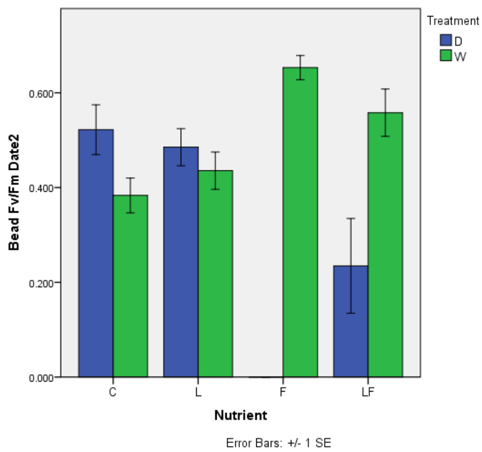


Fig. 6.11. Mean (± 1 SE) Fv/Fm of *Sphagnum* beads with nutrient addition and watering treatments at monitoring date 2. Two-Way ANOVA showed a strong significant effect of watering treatment on *Sphagnum* Fv/Fm ($F= 30.508$, $P= <0.001$). There was also a strong significant effect of nutrient addition ($F=3.016$, $P=<0.05$). Test result showed a strongly significant interaction between nutrient addition and watering treatments ($F= 26.087$, $P= <0.001$).

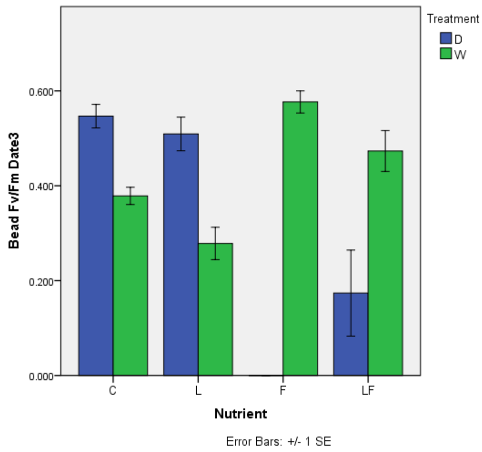


Fig. 6.12. Mean (± 1 SE) Fv/Fm of *Sphagnum* beads with nutrient addition and watering treatments at the third monitoring date, representing the longest *Sphagnum* exposure to treatment. Two-Way ANOVA showed a strong significant effect of both water treatment (*F*= 16.8.273, *P*= <0.001) and Nutrient addition (*F*= 6.837, *P*= <0.001) on *Sphagnum* Fv/Fm post-hoc Tukey test showed strongly significant difference between C and all other treatments, L and L+F (*P*= <0.001) and L (*P*= <0.05). Test result showed a strongly significant interaction between nutrient addition and watering treatments (*F*= 42.562, *P*= <0.001).

6.6 Discussion

6.6.1 Effect of Nutrient addition

Nutrient had a significant effect on cut *Sphagnum* growth (*P*= <0.001), nutrient addition, particularly Fertiliser (F) and Lime & Fertiliser (L+F) combined, significantly reduced cut *Sphagnum* growth. Post Hoc results showed no significant difference between Control (C) and Lime (L) (*P*= <0.296), but showed that Fertiliser (F) and Lime & Fertiliser (L+F) were significantly different to C and L (*P*= <0.001). This result was mirrored by the effect on bead *Sphagnum* area (mm^2) (*P*= <0.05) again with L and L+F having a negative effect on *Sphagnum* growth.

On all trial treatments, Lime did not appear to have a significant negative effect on *Sphagnum* growth. All *Sphagnum* in the trials was grown on peat collected from the Holme Moss (HM) site, used in field trials. As previously discussed, HM peat suffers from historic pollution (Tallis, 1987) and is very acid with a low pH (Buckler *et al,* 2013). It is likely that the *Sphagnum* grown on this peat is benefiting from the raised pH (Lamers *et al*, 2011), or at the least the raised pH out ways any negative direct effects the lime could potentially have. Caporn *et al.* (2007) found that Lime addition in the Southern Pennines, raised pH and enabled plants to better make use of nutrients for growth by mitigating acidic conditions. Previous greenhouse trials by Hinde (2008) showed no significant effect of Lime on *Sphagnum* growth, and this correlates with the results of this trial (*P*= <0.296). Although *Sphagnum* growth was not negatively affected by Lime addition, it didn't seem to provide any improved condition to aid

Sphagnum establishment, Lime was not significantly different to the control in any of the Post hoc tests (*P*= >0.999).

There are previous recordings of negative Lime effects on *Sphagnum* that should be considered, particularly in the field. Lime has been found to have a direct negative effect with *Sphagnum* growth reduced by direct toxicity of high concentration of calcium ions contained within Lime (Carrol *et al.* 2008). Lime addition has been found to affect *Sphagnum* in aquatic setting (Erikson *et al*, 1983), although long-term monitoring of Lime addition to *Sphagnum* communities concluded no significant negative effect (Bragg & Clymo, 1995). Furthermore, Rochefort *et al.* (1995) showed *Sphagnum* establishment benefited from a treatment of Lime addition. When comparing the results of this experiment to previous greenhouse work by Hinde (2008) and associated fieldwork of Rosenburgh (2015), it seems unlikely that Lime has a negative effect on the growth and establishment of *Sphagnum* at the usual field application rates.

The main significant effect on both bead and cut *Sphagnum*, was the treatment of Fertiliser (F) and Lime & Fertiliser (L+F) (*P*= <0.001), F and L+F addition significantly reduced *Sphagnum* growth in these trials.

Sphagnum is specialised to low nutrient levels (Jauhiainen *et al*. 1998) therefore, it is unsurprising that this relationship was found. There has been considerable research into the nutrient effects on *Sphagnum* growth particularly Nitrogen (Berendese *et al.* 2011. Press *et al.* 1986) and Phosphorous (Sunderburgh & Rydin, 2002. Limpens *et al*. 2004), much of which has already been discussed in in relation to our field experiments. Specific greenhouse study by Granath *et al.* (2011) on direct physiological growth responses of *Sphagnum* with N addition, found N addition reduced biomass, and as with our trial, length (mm^2) increment in *Sphagnum.* Previous work by Hinde (2008) again found Fertiliser to have a negative effect on *Sphagnum* growth. Unsurprisingly, high concentrations of Fertiliser added under optimal greenhouse conditions, lead to a negative growth effect on *Sphagnum*. As discussed earlier lime had no significant effect on *Sphagnum* growth, but the combined treatment of L+F was found to negatively affect both cut (*P*= 0.697) and bead (*P*= 0.995) *Sphagnum.* From the previous evidence, it would appear Lime was not responsible for the negative growth response, but the effect would be due to the application of Fertiliser. Although since the treatments were added together, their influence on *Sphagnum* growth cannot be separated, so it has to be said in the context of these results that Lime and Fertiliser application is inhibitory to *Sphagnum* establishment under the optimal greenhouse conditions. A potential explanation for this could again be down to the Holme Moss peat that was used for the experiment. As previously mentioned Holme Moss peat is degraded and historically polluted (Tallis, 1987), the Southern Pennine soil have been found to have high N content (Caporn & Emmett, 2009). This could have increased the N availability to *Sphagnum* past toxic thresholds (Limpens *et al*. 2004). Addition of Lime has been shown to increase peat pH and increase availability of nutrient aiding plant growth (Caporn *et al.* 2007), this could have added to potentially toxic availability of N. Despite the mitigating effect of Phosphorous (P) upon nutrient balance (Granath *et al* 2011), the N availability surpassed toxic thresholds.

6.6.2 Effect of Watering Treatment

Watering treatment had a strong effect on *Sphagnum* growth, there was a strong significant difference in S*phagnum* growth between watering treatments on both *Sphagnum* Cut length and Bead area (mm^2) (*P*= <0.001). This results is extremely important as it shows that watering levels can mitigate the nutrient effect on *Sphagnum* growth. With the experimental conditions being controlled and 'optimal' in a greenhouse trial, the results are often exaggerated or unrealistic (Granath, 2011). Optimal greenhouse experiments have proved unequivocally that Fertiliser can be damaging to *Sphagnum* growth (Hinde, 2008), but this result shows that the negative nutrient effect can be mitigated, and relates to the field trials results where no lasting nutrient effect was found.

This experiment was designed so that the standard greenhouse treatment would be kept regularly misted, and kept within closed trays meaning no wash through of nutrient, effectively a closed system. The 'field' watering treatment, consisted of heavy 'Field' additions of watering (worked from Southern Pennines precipitation data) and nested containers to allow runoff. Within every standard watering treatment, *Sphagnum* with an addition of F and L+F suffered near total direct toxicity and plant die-back (Fig.6.1 to Fig.6.12). As we saw on the Black Hill field trials, there was a toxic 'burn' effect, where nutrient was in direct contact with *Sphagnum*. In the standard watering treatment with the closed trays, all S*phagnum* within the trays was in direct contact with nutrient.

Within the 'field' watering treatment, *Sphagnum* within all nutrient additions experienced growth actively established. This can only be down the heavy water flow actively washing through the peat and diluting ions, reducing acidity and nutrient concentration. This active run-off of Lime and NPK Fertilizer must explain the success of *Sphagnum* growth on establishing areas in the field trials, although Fertiliser can be directly toxic to *Sphagnum* growth, the effect can be mitigated by harsh climatic conditions and high levels of rainfall, experienced in the Southern Pennines.

6.6.3 Chlorophyll Fluorescence (Fv/Fm)

Chlorophyll Fluorescence (Fv/Fm) showed a significant nutrient effect with F and L+F having a negative effect and lowering (Fv/Fm), this was recorded on both Cut and Bead *Sphagnum* (*P*= <0.05). Watering again had the strongest effect with a highly significant difference between watering treatments for Cut and Bead *Sphagnum* (*P*= <0.005).

This is unsurprising, as the previous results has shown that nutrient addition with standard greenhouse conditions caused direct *Sphagnum* dieback and direct negative effects of nitrogen can include lower photosynthesis; increased metabolic costs (Fritz, 2014). There is some ambiguity with this result though, as it has been found that increased N availability can stimulate the formation of Chlorophyll (Fritz *et al.* 2004). Arroniz- Crespo *et al* (2008) found high N loads received by upland *Sphagnum*, increased chlorophyll concentration, whilst reducing biomass. In our experiment the biomass was reduced via direct *Sphagnum* dieback. The toxicity of nutrient availability actively killed *Sphagnum* in the standard watering treatment; therefore, it was impossible to take readings as all *Sphagnum* in the treatment had been killed. It has been found that high N availability directly stimulates *Sphagnum* Chlorophyll production (Arroniz-Crespo *et al*. 2008. Granath *et al*. 2009) and related high Chlorophyll Fluorescence (Fv/Fm) can be an early *Sphagnum* stress indicator (Fritz *et al*, 2011). This could explain some of the higher readings in the early recording dates (Fig 6.7).

There was a theory that the Chlorophyll Fluorescence (Fv/Fm) may have been effected by the growth of algae on the standard watering treatment trays that had received nutrient addition. Many of the *Sphagnum* plants were actively dying, and as such should have given low Fv/Fm readings, but were covered in algal blooms flourishing in the moist nutrient rich treatment. It was thought that this healthy green algae covering the *Sphagnum* could give a false reading, but the ultimate dieback of *Sphagnum* meant this ultimately was not an issue. Both Rochefort *et al* (1995) and Hinde (2008) also found algal blooms on nutrient treated *Sphagnum* trials. Hinde (2008) noted the algae indicated the death of *Sphagnum* from fertiliser additions and that the algae was not detrimental to *Sphagnum* growth. This was also noted in greenhouse experiments under taken by Basilier (1980).

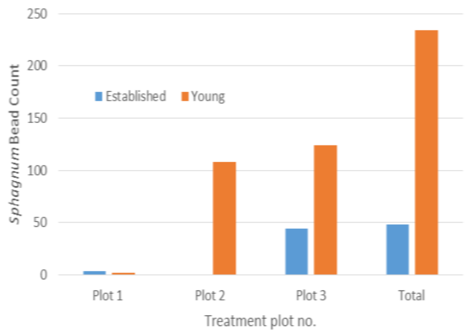
7. **Monitoring of long-term *Sphagnum* restoration trials**

7.1. **Monitoring of *Sphagnum* species trial plots planted onto cotton grass, Kinder summit 2011.**

7.1.1 Experimental Site: High, upland plateau of degraded blanket bog. Surface substrate of badly eroded peat. Heather brash spread to stabilise peat, area planted with young *Eriophorum spp.* as part of current restoration work. Large areas of mature established *Eriophorum* (Cotton Grass) and other associated dwarf shrubs recorded.

7.1.2 Experimental setup: Plots setup (24/08/11). 3 blocks of trials were set up on two differing substrates- Young plug planted Cotton grass and Mature established Cotton grass. Each block contained 7 treatment strips, sown with 6 species of micro-propagated 'bead' *Sphagnum* and a control. These blocks were replicated twice and then on the different substrate. 6 blocks in total.

7.1.3 Findings:



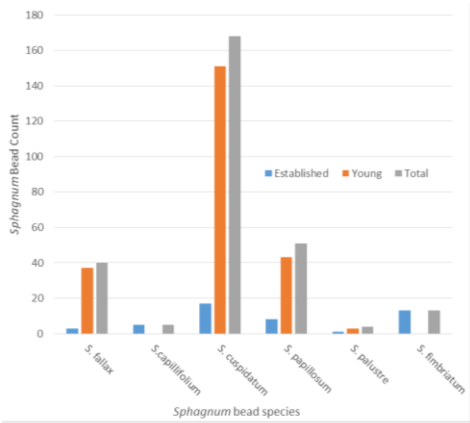


Fig 7.1 Graph showing total *Sphagnum* bead count established on mature and young Cotton grass

Fig. 7.2 Graph showing Total number of recorded *Sphagnum* beads per species, on Mature and Young Cotton grass

7.1.4 Observations: There has been successful *Sphagnum* establishment on both the trialled substrates (Young and established Cotton grass). Both the applied micro-propagated bead propagules and naturally established *Sphagnum* were recorded growing in both the Cotton grass plots. Potentially, Cotton grass could be the ideal environment to introduce *Sphagnum* into (Ferland & Rochefort, 1997. Joosten, 1992). This effect has been recorded at the Kinder site previously (Proctor *et al.* 2013).

There does not appear to be a species effect apparent from this trial. The site substrate and physical conditions seemingly exert more effect over *Sphagnum* growth response. Micro-topography seemed to be the main factor influencing the establishment of *Sphagnum* within these trial plots.

Cotton grass growth has been very successful in the area and there are now large tracts of restored cotton grass across the Southern Pennines (Maskill, 2013). This improves the biodiversity and quality on this degraded ecosystem. Cotton grass would appear to give *Sphagnum* a good environment to establish by potentially providing water regulation and act as a nurse crop amongst other mutually beneficial actions. With this in mind, I think that continued monitoring of these plots is extremely important in trying to ascertain their contribution to restoration efforts within the Southern Pennines.

7.2 ***Sphagnum*** **restoration trials using BeadaMoss™ at Wedholme Flow and Bolton Fell Moss.**

7.2.1 Experimental Site: Lowland raised bog sites located within the larger South Solway Mosses area. Both sites have recently undergone commercial peat extraction, and contain areas of cutover milled peat devoid of vegetation.

7.2.2 Experimental setup: Several experimental blocks consisting of *Sphagnum* treatment strips were set up. 7 strips in total. 6 strips sown with 6 species of micro-propagated 'bead' *Sphagnum* and 1 control, to trial species effect. Treatment blocks were set up in spring and a repeat in autumn order to examine any seasonal influences on establishment. Treatments were covered with a standard cover of heather brash. A control of no cover was trialled, as well as two alternative cover treatments: Biodegradable plastic film and a nurse crop of plug planted Cotton grass.

7.2.3 Findings: Within the limitation of available time, it was not possible to complete detailed quantitative survey of counts of growing *Sphagnum*, beads still present but with limited growth or developing beads. Instead, gross estimates of quality were given and observations made on the factors influencing *Sphagnum* growth.

7.2.4 Observations:
Bead Growth: *Sphagnum* plants grown from beads were evident across all brashed parts of the blocks at both sites highlighting the positive role of brash in both stabilisation and reducing the drying of the surface sites. Where the brash was intact or only a little disturbed, there was abundant evidence of hundreds of established Sphagnum bead plants with the plants being generally small and still in the earlier stages of development. These was an effect of brash type, coarse brash had been moved more by the wind and water, but where it remained it appeared to provide a better covering, leaving gaps for *Sphagnum* and light. It is also notable that some young *Sphagnum* plants looked as though they could have been introduced via the brash. Something that has been noted in other field trials (Buckler *et al.* 2013).

Cotton Grass addition: The plots of planted cotton grass have shown good growth, particularly in the wetter areas where they had spread to provide a thick cover. The *Sphagnum* growth in the plug-planted cotton grass areas showed the benefit of cotton grass cover in establishment of *Sphagnum* communities, either from beads application or natural re-colonisation. The success of *Sphagnum* planted in companion with Cotton grass has been recorded in other field trials

Influence of season: It is difficult to distinguish the effects of season from those of the different types of brash used as covering. There were many more beads still visible from the autumn planting, but the viability of these was not clear under the thick brash crust that had formed. The spring planted beads have also had an additional season to develop or decay. From other trials elsewhere, we expect spring planting to perform better, particularly if autumn planting experiences a harsh winter.

Protective cover: After investigation, for *Sphagnum* to establish on bare peat, the surface needs some form of covering to provide protection against the natural weather elements, to reduce wind blow erosion of peat and to lower the rate of drying of the peat surface. It is clear that planting *Sphagnum* onto bare peat is not viable.

7.3 **Featherbed Moss Plug PlugaMoss™ Plant Monitoring**

7.3.1 Experimental Site: High, upland plateau of degraded blanket bog. Area of intact bog vegetation overlaying dep peat deposit. Area influenced by deep eroded gullies with small 'pans' of un-vegetated bare peat substrate.

7.3.2 Experimental setup: Trialling an innovative new method of *Sphagnum* reintroduction in the field. *Sphagnum* 'Plugs'; micro-propagated *Sphagnum* strands cultivated into plug plants, were planted in the field to trail survival and potential growth success. 100 plugs were planted in the field; half were planted into bare peat pans, the others directly into gully sites arranged either north of south facing.

7.3.3 Findings:

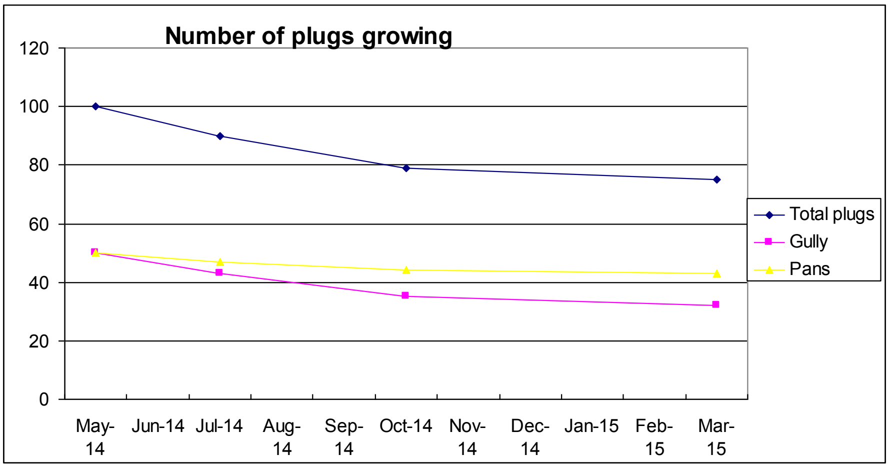


Fig 7.3 Graph showing survival of *Sphagnum*: total number of plugs, comparison between pans and gullies

7.3.4 Observations: Peat pan locations have shown the highest plug ‘survival’ %. But care should be taken with the term survival; it is more likely that the majority of ‘missing’ plugs are actually buried in-situ by peat sediment or washed out, distributed and subsequently buried in sediment. There is the possibility that the peat burial is not necessarily harmful, and could be potentially be beneficial, in some cases. The pans are also at danger of drying out and desiccation. It was noted that brash was particularly beneficial in protecting plugs from drying (this was only trialled on the peat pans). A small amount coarsely chopped brash has been observed as the most effective, providing reduced evapo-transpiration and a level of structural support. Fine chopped brash is less useful as it tends to form an impermeable crust.

The gullies have the highest number of ‘missing’ plugs; not surprising considering the variable water levels. The gullies have shown evidence of fast water flow, flooding and peat sediment deposition.

When planting the plugs attention needs to be paid to the water flow patterns within the specific gully, take care were possible to plant on the side where water flow appears to be slower to avoid erosion/washing out. Gullies probably provide a more hospitable environment for *Sphagnum* plugs to establish than bare peat pans due to vegetative mix and its associated benefits; previous experience has shown *Sphagnum* doesn’t thrive on bare peat. There have since been trials where Plugs have been planted directly into intact bog surface, this comparison should be of interest to find the most suitable substrate for plug planting

8. Final Discussion and Conclusions

The main body of this work focused upon the current practice of nutrient addition and its use in current peatland restoration in relation to *Sphagnum* growth. There has been concern that the practice of nutrient addition in the Southern Pennines could affect both mature already established *Sphagnum* communities and potentially affect the establishment of young *Sphagnum* propagules added as part of ongoing restoration efforts. There has been a strong body of work concerning the complex interaction of the potentially negative nutrient-*Sphagnum* interactions (Limpens & Berenese 2004; Fritz *et al.* 2011). This body of work included research carried out by Hinde (2008) in our Faculty greenhouses, which found applications of Fertiliser and Lime combined to have a significant negative effect on *Sphagnum* growth. From the results of our research it was found that under optimal controlled greenhouse conditions the Fertiliser addition negatively affects *Sphagnum* growth and establishment. Importantly the effect of this Fertiliser addition was found to be mitigated by field conditions. When tested in the greenhouse, simulated field rainfall and the ability for water run-off, reduced this negative Fertiliser effect on *Sphagnum* growth. By dilution of ions, reduction of acidity and active 'leaching' action, nutrient concentration was reduced below toxic threshold levels.

This effect was also found when trialled in the field; there was no significant lasting effect of Lime and NPK fertiliser on *Sphagnum* growth. When nutrient was applied to mature *Sphagnum* there was a direct toxic effect and there was some immediate height increment reduction (mm^2) but this effect was short lived and followed by a period of regrowth and recovery almost equal to the Control treatment *Sphagnum.*
Nutrient addition to young establishing *Sphagnum* in the field showed no effect of nutrient on *Sphagnum* growth, with no significant difference in growth found between *Sphagnum* in Control plots and those with additions of Lime and NPK Fertiliser. Although there was no significant effect of nutrient directly to *Sphagnum* growth, there was an increase in vascular plant density and the potential for increased *Sphagnum*- vascular plant competition. The effects of this vascular plant increase and potential competition are unclear, a steady reduction in total *Sphagnum* count was recorded on plots with nutrient application. When monitoring the plots it was noted that there were several incidences of *Sphagnum* being actively outcompeted and 'choked' by plant growth. Such competition has been found to be harmful to *Sphagnum* establishment (Van Breeman *et al.* 2001) the potential for shading is has been noted as a serious concern (Rosenburgh, 2015). If the competition is solely provided by the amenity grass spread to stabilise peat surface, then this should not provide a significant problem, as this is forecast to dieback as the Fertiliser addition wears off (Buckler *et al,* 2013). If nutrient encourages associated moorland plant increase, then this could be seen as a positive, with increased plant biodiversity (Anderson *et al.* 2009), and through potentially beneficial growth interactions e.g. microclimate (Soro *et al.* 2009), nurse crop (Tuitilla, 2006) and as 'scaffolding' aiding growth (Rydin & Jeglum, 2006).

In both the field and greenhouse experiments Lime did not have a detrimental effect on *Sphagnum* growth, only Fertiliser, and Lime and Fertiliser combined had a negative effect, and this was mitigated by field conditions and high precipitation (Beswick *et al*, 2003). It is undoubted that additions of Lime are beneficial to restoring the degraded and acidic peat of the Southern Pennines (Caporn, *et al*. 2007). There is evidence of direct negative Lime effect to *Sphagnum* (Carroll *et al*, 2008), but field applications have returned no lasting negative effects (Bragg & Clymo, 1995) and Hinde (2008) found Lime to provide no positive or inhibitory effect to *Sphagnum* growth.

It is worth noting that although Lime addition provided no negative impact to *Sphagnum* there is an inherent risk with Lime addition. *Sphagnum* (Species highlighted for re-introduction in the Southern Pennines) have an optimum pH of between pH 4-5. If Liming lead to pH being raised above this optimal threshold, *Sphagnum* communities would be affected, and lead to a change in species composition outcompeting specialised acid tolerant bog species (Arroniz-Crespo *et al*. 2008). This is unlikely as Liming in the Southern Pennines was only found to raise pH by round 0.5 (Caporn *et al*. 2007). Current restoration additions are relatively low, the Southern Pennines peat is very acidic and there is the potential for reduction by high precipitation (Beswick *et al*. 2003) and leaching/runoff action (Van Breeman, 1995).

From the results of this study it seems concerns regarding applications of Lime and NPK Fertiliser to establishing *Sphagnum* appear unfounded. *Sphagnum* has established in the field onto previously nutrient treated areas and matured into healthy functioning communities (Rosenburgh, 2015). Our field trials showed nutrient additions did not significantly affect Young establishing *Sphagnum*. Although optimal greenhouse experiments show the detrimental impact of nutrient effect to *Sphagnum* (Hinde, 2008), the harsh climatic conditions and high precipitation of the Southern Pennines has been under estimated. Although nutrient is added in the field at the same density found to be toxic to *Sphagnum* in the greenhouse, it does not remain at this level for long. Lime and Fertiliser applications are likely to be reduced, simply by being washed away or utilised by other higher plants (Caporn *et al*, 2007).

When considering the practical application of Lime and NPK in the field as part of restoration work, nutrient should be considered a low risk to *Sphagnum* establishment. Although the age and establishment phase of *Sphagnum* should be considered.

Mature *Sphagnum* is at lower risk to nutrient application, the results off our study showed a direct toxic effect but also a relatively quick recovery period of re-growth. Mature *Sphagnum* seemed less susceptible to increased competition from vascular plants that nutrient addition provides. Where possible nutrient addition should be avoided as it is not necessary for its development and growth and does cause harm. Although nutrient application is less likely to occur in the field, as an area containing the necessary conditions allowing *Sphagnum* to establish is obviously at a lower need of restoration. But with degraded peatland often being a patchwork mosaic of various vegetation, any remnant fragments of mature or naturally regenerated *Sphagnum* included in Nutrient addition are probably at a low risk of being irreparably damaged.

Young establishing *Sphagnum*, particularly propagules added as part of *Sphagnum* re-introduction work, should be consider more closely. Again there was found to be no significant effect of Nutrient on *Sphagnum* growth in the field, but the competition provided by increase in vascular plant density needs to be contemplated. Where possible Nutrient addition to Young *Sphagnum* propagules should be avoided, and if there is a need to increase peat stabilisation or provide a nurse crop, this should aim to be provided by associated bog plants. One example would be *Eriophorum* species (Rydin & Jeglum, 2013), the positive effect of which was noted as beneficial during the extended monitoring of existing *Sphagnum* trails. Again Young establishing *Sphagnum* propagules appear to be at relatively low risk from nutrient application.

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10. Appendix

Extended *Sphagnum* Monitoring Reports

10.1 Monitoring of *Sphagnum* Species trial plots planted onto cotton grass, Kinder summit 2011.

1. Site Description.

Location: Kinder summit, south east from trig point. (SK 08596 87634)

Kinder scout (Sk08426 89614) is a high upland plateau of blanket peatland (Allott *et al.* 2009). Located in the dark peak area of the Southern Pennines and strongly influenced by the underlying Millstone Grit and associated Geology (Natural England, 2014). Altitude at the summit is 625m (2050 feet).

The landscape is heavily degraded and Cloughs are a common feature in this landscape, formed by the incision and deep erosion of fast flowing streams (PDNPA, 2009). Erosion has been heavily exacerbated by human influences, particularly heavy grazing pressure (Anderson and Radford, 1994) and recreational use (Montgomery & Shimwell, 1985)

The upland plateau includes blanket bog and sub-alpine dwarf shrub habitats (Natural England, 2014). This heavily eroded area with vast expanses of bare peat support a dwarf shrub mosaic vegetation cover dominated by bilberry (*Vaccinium myrtilus*), crowberry (*Empetrum nigrum*), heather (*Calluna vulgaris*), cotton grass (*Eriophorum angustifolium* and *vaginatum*, the latter in wetter areas) (Montgomery & Shimwell, 1985). Cloudberry (*Rubus chamaemorus*) is also widespread in this area (Evans *et al* 2005).

1.1 Introduction

The Plots were setup and planted in August 2011 (24/08/11), and were monitored after a three-year growth period.

The plots consist of 3 replicates of *Sphagnum* species trial treatments on two different surfaces of cotton grass cover. The treatments consisted of 6 species of Micro-propagated *Sphagnum* beads and a control (of no *Sphagnum* addition), applied in treatment strips to areas of young and established Cotton grass.

The 6 *Sphagnum* species applied were *S. fallax*, *S. capillifolium*, *S. cuspidatum*, *S. papillosum,* 4 m x 1 m treatment strips separated by a 0.5 m gap between treatment strips *S. palustre* and *S. fimbriatum* and a control. These were applied in. Micro-propagated *Sphagnum* beads were applied at a rate of 400 m^2 (Rosenburgh, 2015). The trials were setup on 2 differing substrates and were replicated leaving a total of 3 blocks for each substrate trail. 6 blocks in total.

The young Cotton grass plots were bare ‘peat pan’ areas newly planted with cotton grass plug plants. The *Eriophorum* plug plants were micro-propagated from material collected from the site previously. This work had been undertaken as part of moors for the futures Phase 5 restoration of the site. The plug plants had been applied to diversify the sward and to bind the peat surface, whilst restoring the natural seed bank (Buckler *et al.* 2013). The plugs were planted at a density of around 1 plant per m^2 directly into areas of bare peat.

The comparison plots were setup onto established areas of previously planted cotton grass and the other associated species that comprise ‘restored’ areas. Plant sward was much thicker with more associated leaf litter.

It is also notable that there was evidence that the area had been treated with lime and NPK fertiliser by Moors for the future as part of the ongoing restoration efforts on Kinder Summit. Low pH and acidity can be a limiting factor to *Eriophorum spp.* and to a lesser effect lack of Potassium (K) availability (Richards *et al.* 1995).

Monitoring began on the young cotton grass plot and its two replicates. The blocks measured 10 m x 4 m^2, and consisted of seven 1 m x 4 m^2 plots containing six different species of *Sphagnum* beads and one control plot which was left clear with no *Sphagnum* beads being applied.
Monitoring on the plots comprised of methodically searching through the treatment plots counting any bead plant material found, and marking the location with a small cane. Individuals for each treatment were noted, and then added to make a total count for each plot. It is important to note that for *Sphagnum* individuals that appeared to be from bead material, the count was of capitulum heads therefore the numbers are representative of actual *Sphagnum* individuals.

1.2 Monitoring data

	Established	Young	Total
Plot 1	4	2	6
Plot 2	0	108	108
Plot 3	44	124	168
Total	48	234	282

Fig. 1 Total *Sphagnum* bead counts on Young and Established Cotton grass

	Established	Young	Total
S. fallax	3	37	40
S.capillifolium	5	0	5
S. cuspidatum	17	151	168
S. papillosum	8	43	51
S. palustre	1	3	4
S. fimbriatum	13	0	13
Total	48	234	182

Fig. 2 Total *Sphagnum* species bead counts on Young and Established Cotton grass

Fig. 3 Total *Sphagnum* bead counts on Young and Established Cotton grass

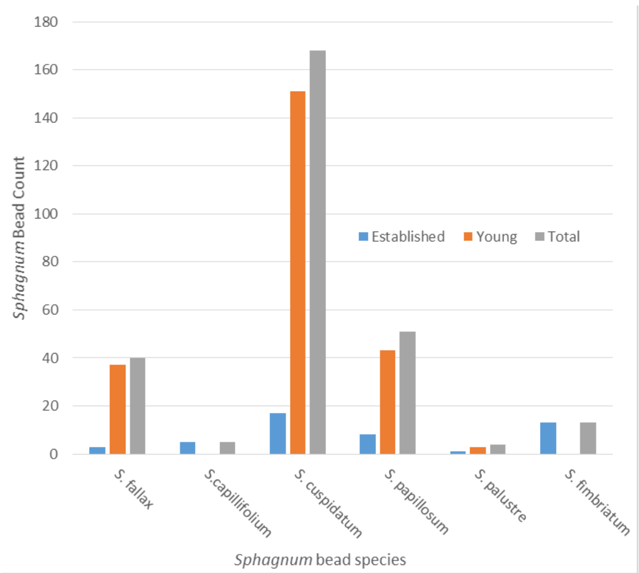


Fig. 4 Total *Sphagnum* species bead counts on Young and Established Cotton grass

1.3 Discussion: Young Cotton grass plots

The main focus of the trip was to revisit the plots after a two growth period since its setup. Initially the main issue was remarking the plots, which over the course of time have become increasingly difficult to locate. Being GPS marked, finding the plot location was fine but working out the plots layout was more difficult. Due to weathering, frost heave and potential physical movement (walkers, sheep etc.) many of the canes used to mark out the plots have been displaced, leaving the original layout hard to decipher. This was particularly notable on the established cotton grass plots.

Action should be taken to improve/remark the plots to ensure their viability for ongoing future monitoring.

The plots were located on a relatively flat 'peat pan' areas, low and relatively damp areas that at the time of bead application would have been newly treated with cotton grass plug plants. Around the plots the area consisted of newly restored areas of amenity grass, treated with lime and NPK fertiliser as part of Moors for the futures Phase 4 restoration. The area also contained several peat hummocks that had been treated with an application of brash, to aid peat surface stabilization (also a potential source of *Sphagnum* material). There were also several plastic piling blocked gullies, and therefore several areas of standing water.

Overall, the *Sphagnum* beads applied on these plots have shown reasonably good establishment on some treatments, but in others there appears to have been no apparent growth.

(Plot 2) The most obvious bead success has been on plot 2 (SK 08590 87561). This plot had a recorded 108 individuals established from bead material. 105 of these individuals were located within the *S. Cuspidatum* treatment. Only 3 other individuals were located within the plots, these were found within the *S. Palustre* treatment.

One explanation for this pattern on plot 2 could be that there is some kind of treatment effect, with certain *Sphagnum* spp. establishing more successfully than others on this surface. After looking at the location of the treatments within the plots I think it is likely that there is a more physical explanation for the distribution pattern of establishment (Pouliot *et al.* 2011).

As previously mentioned plot 2 had one of the highest counts of *Sphagnum* bead individuals, but only on one of the species treatments, the other treatment contained virtually no growth. The treatment that experienced the most successful growth was treated with *S.Cuspidatum*.
It is important to note that the treatment plot itself differed topographically to the other treatment strips within the plot. When compared to the other strips the plot was slightly raised above the level of the other treatments; as a result it varied much more in its surface type. The plot was noticeably more 'lumpy' containing small hummocks and several small niches and hollows within the surface. Diverse micro-topography, of this type has been found to be a positive factor in relation to *Sphagnum* establishment (Johnson *et* al, 2014. Price *et al*, 1990). The other plots within the treatment were based on the 'peat pan' type are mentioned earlier. These plots were typified by the fact that they were predominantly flat, particularly damp and covered only by a canopy of Cotton Grass.

The *S.Cusp* treatment strip differed from the others by being more floristically diverse. As mentioned, the other strips tended to only contain cotton grass on bare peat with no under growth. Being slightly raised by a few inches above the level of the other strips it doesn't appear to suffer from water inundation or movement and being more stable has allowed establishment of other species Lachance & Lavoire, 2004). The treatment contained more grass and some dwarf shrub spp. such as *Calluna*. There was noticeably more moss species such as *Hypnum* and even some *Polytrichum*. This supports the theory that the area was slightly drier than the other plots. It is probable that the treatment height has meant it has avoided the potential detrimental effects of water movements, allowing the development of a more diverse plant community. This plant community has more environment growth niches such as hollows etc. and the plant community could have potentially acted as a good nurse environment for the beads by providing cover and protection from evapo-transpiration (Bubier *et al*, 1992. Price *et al.* 1998).

As mentioned the other treatments within plot 2 showed virtually no growth, with only three other *Sphagnum* individuals found in the *S.Palustre* treatment. The cotton grass was the main cover within these plots, there appeared to be very little undergrowth; the surface substrate was bare peat. There also appeared to be evidence of water movement on these other treatment strips. There is the possibility that water moves across these plots regularly, and inundation events could have occurred on these treatments. The bare peat surface hints that the peat surface is more mobile on these treatments and there could be active peat deposition.

In these treatments, judging by the evidence of water movement and the mobile peat surface, it is likely that the beads have been buried under a layer of peat deposit. This could potentially account for the virtual absence of beads within these treatments when compared to the success of bead establishment on the first treatment, just a few metres away.
Thus far there is no evidence to support how beads in the field will be affected by this burial under peat. Green house trials by Micro-propagation service ltd. have shown that beads can survive burial and can grow up and through the peat surface (Micro-propagation services. *Per comms*.)

(Plot 3) When considering the bead establishment on plot 2 and the potential for a physical influence rather than a treatment effect, young cotton grass (YKG) plot 3 (SK 08611 87552) shows an establishment pattern that could back this theory up.

Unlike plot 2, YCG plot 3 shows a much more even and equal spread across the treatments. Treatments 1, 2 and 3 within the plot all had an average of 40 odd *Sphagnum* individuals. This distribution shows a more normalised establishment pattern as similar numbers of beads have established in each treatment.
But out of 7 treatments only three actually contained *Sphagnum* individuals. The other 4 treatments contained no *Sphagnum*, one of the treatments was a control but the other 3 treatments had applications of *Sphagnum* but no recorded individuals. Again, this could be explained by the location of the treatments, all three strips: *S.Fallax, S.Papillosum* and *S.Cuspidatum* were all located directly next to each other. All the consecutive treatments then contained no *Sphagnum* individuals.
There could have been some physical influence within these treatments and again there is the possibility of water movement in the area, or a possible flood event could have occurred.

There is also the potential damage occurring from of trampling (IUCN, 2017) on the strips as the area is on access land and grazed by sheep. It is less likely that trampling is a factor, but it is a possibility to consider.

(Plot 1) The first plot YCG plot 1 (SK 08644 87581) is confusing when compared to the other two replicates. Unlike the other plots where *Sphagnum* has varied between treatments within plots, with some treatments containing good numbers of established *Sphagnum* individuals and others having none (potentially due to physical influences), YCG plot 1 has experienced virtually no bead establishment. Only 2 *S.Fallax* individuals were found to have been established on this plot. There was no clear evidence as to why establishment on this plot has been virtually non-existent.

Overall, on the young cotton grass plots, *S.Cuspidatum* was the species that had the most established beads, with 151 individuals on two of the treatment plots. This isn't particularly what would be expected as *S.Cuspidatum* is the most aquatic of the species applied to the plots. However, considering how wet the plots are this could explain their abundance. Looking at the distribution of bead establishment it is more likely that water influence has been destructive or at least not positive. It is more likely that *S.Cuspidatum* has been the most successful on Young cotton grass plots simply due to where it was applied. The layout of the treatments was randomised, so *S.Cuspidatum's* placement and subsequent 'success' was probably down to luck.

1.4 Discussion: Established Cotton Grass plots

These plots, as with the others, needed to be completely re-marked. A lot more effort was needed to establish the actual layout of some of the plots. In some cases the canes had been completely lost and the layout was far from obvious. These plots would definitely benefit from some permanent marking.

These plots were located on a slightly raised area in comparison to the young plots. The area was hummocky mixed grass with predominant cotton grass cover. There was no evidence of excessive water movement, but the peat surface itself still appeared damp and suitable for *Sphagnum.*
The plots were of the same layout and treatment as the young plots but applied on to areas of more established cotton grass. The beads were applied to an area of cotton grass that was further along in its development than the young plots that were plug planted previously.

These established plots were much harder to ascertain a result. Bead establishment appears much lower than on the Young plots. On many of the treatments no beads were found and on others there were extremely low counts. Despite this, there was a lot of evidence of natural *Sphagnum* re-growth. The surrounding area had at some time been treated with applications of brash. It is more than likely that this 'natural' *Sphagnum* had introduced into the locale via this application of *Sphagnum* rich brash. Some of this *Sphagnum* has managed to re-colonise onto some of our plots. Unlike the Bead material this *Sphagnum* was counted as a whole clump rather than individual capitulum. These *Sphagnum '*clumps' were counted directly so it should be noted that they are of various sizes. No record of clump size or species composition was recorded (just the treatment in which the clump was found).

It should be noted that the density of grass cover on the established plots made it much more difficult to monitor properly. As previously mentioned, the Cotton grass is now well established and covers the area in a thick blanket. This made it difficult to find bead individual in the treatments, the 'natural' *Sphagnum* clumps on the other hand stood out due to their size. It is worth noting this could be a potential bias in the results and put down to human error whilst monitoring. Any count of beads on these established plots should be taken as a minimum count.

As mentioned the *Sphagnum* counts on the established plots were relatively low.

Plot 2 (SK 08593 87609) had no recorded *Sphagnum,* either bead or 'natural', on any of the treatments. It is unclear why this would be, as the area should provide a good environment for *Sphagnum* establishment being both damp and well covered. There is the possibility that the area, as a result of being slightly raised, could be dryer throughout the year and was only damp at the time due to previous rainfall. This would negatively affect *Sphagnum* establishment (Robroek *et al*, 2007. Grosvernier *et al.* 1997). There is also the possibility that the thick grass cover hindered the monitoring. Another explanation is that the plot was possibly remarked out wrong. The established plots were a lot harder to remark as many of the canes had completely disappeared; it took a lot of time and effort to re-lay the plots. There is the possibility that this plot was marked out wrong, and the wrong area of cotton grass was monitored. Although this still would not account for the absence of any *Sphagnum* from natural re-growth (Brash addition etc.).

Plot 1 (SK 08596 87634) of the established plots showed a low count however, both bead and 'natural' were recorded. Beads Individuals were found on three of the 6 *Sphagnum* treatments (*S.Fallax, S.Papillosum and S.Cuspidatum*) albeit in extremely low numbers. Only 1 individual was found in both *S.Cusp* and *S.Pap*. And only 2 individuals were found in the *S.Fall.* Interestingly, in The Young cotton grass Plot 3, these were also the only three *Sphagnum* species out of the six treatments that had recorded bead establishment. On the young plot this was potentially down to physical disturbance.

Again, on this established cotton grass, it is possible that there could be potentially more individuals that were just missed during the monitoring. The presence of beads within the plots shows their ability to establish within this area.

Some natural re-growth was recorded in 3 of the treatment areas, notably the control strip which contained up to 7 clumps of ‘natural’ *Sphagnum.* The re-colonisation of this area by *Sphagnum*, possibly from brash, shows the potential of the established areas of cotton grass as area to support healthy *Sphagnum* establishment as represented in current literature (Lavoie *et al,* 2004. Koyamo & tsuyuzaki, 2010).

Plot 3 (SK 08592 87581) produced a higher count of established beads. Overall 44 bead individuals were recorded in plot 3. It is worth noting that this is the only plot in which bead individuals were recorded in all 6 of the *Sphagnum* treatments and also in the control. At least on individual was found in every species treatment. *S.Cuspidatum, S.Fimbriatum* and *S.Papillosum* showed the highest bead establishment with 16, 13 and 7 individuals respectively. These treatment strips were located next to each other within the plot layout. *S.Fallax* and *S.palustre* had only one recorded individual on each treatment, but when considering their presence within the treatment and the higher numbers of beads in neighbouring treatments within the plot; it is likely that there would be other individuals within the plots that we missed. Natural re-grown *Sphagnum* clumps were also recorded in all but one of the treatment strips. Considering the large count of *Sphagnum* from both parent materials it highlights that plot 3 provides an excellent environment for *Sphagnum* growth.

1.5 Conclusions: what can be drawn from these plots?

Overall *Sphagnum* establishment on these plots has been positive. Beads have developed on most of the plots that they were applied to. There was strong evidence of natural *Sphagnum* establishment on many of the treatment plots, particularly the established cotton grass plots. This *Sphagnum* probably introduced via *Sphagnum* rich heather brash application (Maskill, 2013) used in to stabilise the peat surface as part of Moors for the futures Phase 3 restoration (Buckler *et al.* 2013)

On the Young Cotton grass plots it appeared that there might have been physical disturbance that could have adversely affected *Sphagnum* growth. As previously mentioned, the young plots may have been disturbed by water movement and burial by peat. It is unclear whether this disturbed peat will yield any *Sphagnum* growth.

It is not as clear why there was absence on the established plots, although there was a potential issue with locating one of the plots. In addition, the thicker undergrowth made it harder to locate *Sphagnum* individuals.

There does not appear to be a species effect apparent from this trial. The site substrate and physical conditions seemingly exert more effect over *Sphagnum* growth response. Micro-topography seemed to be the main factor influencing the establishment of *Sphagnum* within these trial plots.

There has been successful *Sphagnum* establishment on both the trailed substrates (Young and established Cotton grass). Both the applied Micro-propagated bead propagules and naturally established *Sphagnum* were recorded growing in both the cotton grass plots.
Potentially, Cotton grass could be the ideal environment to introduce *Sphagnum* into (Ferland & Rochefort, 1997. Joosten, 1992). The plots showed that cotton grass holds water quite well and the area is usually permanently damp, cotton grass is a good indicator of moist ground and creates a useful associated micro-relief (Grovsnier, 1995). Cotton grass litter causes increased irregularity of peat surface whilst actively impedes the flow of water (Holden *et al.* 2008). This effect has been recorded at the Kinder site previously (Proctor *et al.* 2013).
The grass itself acts as good cover and protects the young plants in there development. Cotton grass can also act as a matrix or 'scaffold' that can support *Sphagnum* and aid in Hummock development (Tuitilla *et al.* 2000). Once established Cotton grass litter can accumulate and provide a beneficial microclimate aiding *Sphagnum* growth (Wheeler *et al* 1995). Research by Sundberg & Rydin (2002) used Cotton-grass (*Eriophorum Spp*.) brash in field trials to investigate any influence on *Sphagnum* growth. It was found that it provided nutrient addition beneficial to *Sphagnum*, through decomposition/leaching of metabolites. It was also found to 'trap' and impede other litter from associated vegetation that provided limited nutrient input (Sundberg & Rydin, 2002).

Overall, the young cotton grass plots had the highest count of established beads, despite the potential disturbances. It is possible that the beads have not developed as well in the established plots, but as mentioned, this could have been down to difficulty in locating them within the dense grass. The natural re-growth of *Sphagnum* we experienced on the established plots certainly verifies the viability of this environment for *Sphagnum* introduction.

Cotton grass growth has been very successful in the area and there are now large tracts of restored cotton grass across the Southern Pennines (Maskill, 2013). This improves the biodiversity and quality on this degraded ecosystem. Cotton grass would appear to give *Sphagnum* a good environment to establish by potentially providing water regulation and act as a nurse crop amongst other mutually beneficial actions. With this in mind, I think that continued monitoring of these plots is extremely important in trying to ascertain their contribution to restoration efforts within the Southern Pennines.

10.2 *Sphagnum* restoration trials using BeadaMoss™ at Wedholme Flow and Bolton Fell Moss monitoring

1. Introduction: *Sphagnum* BeadaMoss™ planting trials

Large areas of cut-over lowland raised bogs within Natural England managed conservation areas in north Cumbria are undergoing restoration work involving management of the water table and re-vegetation using *Sphagnum* moss and other typical bog vegetation. This report describes restoration trials started in 2011, monitored in May 2013 that aimed to establish *Sphagnum* using BeadaMoss™ (Micropropagation Services (EM) Ltd) in small experimental plots on bare cut-over peat surfaces.

Sphagnum propagules in an encapsulated bead form 'BeadaMoss™' were spread on bare peat surfaces at Wedholme Flow and Bolton Fell Moss at spring and autumn dates in 2011 in order to examine any seasonal influences on establishment.

The first set of plots was set up on 12th May 2011 followed by another set on 20th September 2011. The beads were added at an approximate density of 67 ml (approx. 300-400 beads) per m^2 to the surface of bare peat in plots of 4 m x 1 m strips at Wedholme Flow and 6 m x 1 m strips at Bolton Fell Moss. A range of common *Sphagnum* species that are considered to be more pioneering species was used, 6 in total, of carpet and peat forming species *S. fallax*, *S. capillifolium*, *S. cuspidatum*, *S. papillosum S. palustre* and *S. fimbriatum* and a control of no added *Sphagnum*.

The standard procedure was to cover beads treatment strips straight away with chopped heather brash. As a control some areas were left as 'bare' with *Sphagnum* beads applied directly to bare peat and no cover added. Two alternatives to brash were also tested at each site:

(i) covering with biodegradable plastic film (approximately 1 m width) as used in outdoor vegetable seedling protection was tested in September 2011;

(ii) planting a nurse crop of cotton grass, *Eriophorum angustifolium* (approximately 1 m width) at a density of 4 plants per m^2. The cotton grass was planted in May 2011 and the bead planting within the established sedge took place in September 2011.

1.1 Field Sites and Descriptions

1.2 Wedholme Flow, Cumbria

Wedholme Flow is a lowland raised bog located in the South Solway Mosses National Nature Reserve in North Cumbria (Grid Reference: NY 220530). Wedholme Flow includes several existing Sites of Special Scientific Interest (SSSI) (English Nature, 1986) and is a proposed Special Area of Conservation (SAC) as part of the Natura 2000 network under the EC habitats directive. The site stands at an altitude of 13 m above sea level and receives annual rainfall of around 900 mm yr-1 (McMullen et al., 2000). Wedholme Flow covers an area of 780 ha, including 125 ha of original uncut *Sphagnum* rich raised bog surface. One of the largest intact areas in England, although large areas of the site have been damaged by peat extraction (Rockell et al., 2008). Peat extraction was partially halted in 1990 when English Nature purchased 186 ha of the site to regenerate cutover areas, while commercial peat extraction continued in the eastern section of Wedholme until 2002.

McMullen *et al*. (2000) and Milton *et al*. (2005) observed the plant communities within Wedholme Flow, noting that the northern and southern uncut areas possess active peat forming vegetation dominated by *Sphagnum*, providing 30-70% ground cover. The most commonly recorded species included *S.magellanicum, S.tenellum, S.papillosum, S.subnitens* and *S.capillifolium*. Accompanying vascular plants included *Calluna vulgaris, Erica tetralix, Eriophorum spp, Andromeda polifolia* alongside *Rhynchospora alba* and *Drosera rotundifolia.* It was noted that the southern part of uncut areas possess a much higher *Sphagnum* cover and is in much better condition than the northern area that is considered to be somewhat more degraded. The drier areas around the edges of uncut areas were dominated by *Calluna vulgaris*. The central and eastern parts of the site, the most recently cut-over and extracted, still contains areas of milled peat mostly devoid of vegetation.

1.3 Bolton fell, Cumbria

Bolton Fell Moss, close to the Scottish border in north Cumbria (Grid Reference: NY 486686) is an extensive area of remnant lowland raised bog, an extremely rare habitat and part of only 6000 ha left in the UK. It is the largest raised bog SSSI and the only SSSI of its hydrological type in East Cumbria (English Nature, 2001). The site stands at 110 m above sea level with peat deposits of up to 11 m depth (on uncut western side) overlaying carboniferous sandstone and Mudstone strata. Bolton Fell Moss is a designated Special Area of Conservation (SAC) and also classified as a Site of Community Interest (SCI) in 2009.

Commercial peat extraction began at Bolton fell in 1959 and was still underway at the time of this site visit in May 2013, but was expected to be finished in late 2013. Much of Bolton Fell Moss has been cut-over or milled leaving a bare peat surface. There are some areas where the original bog surface remains relatively intact, supporting good NVC M18 vegetation.

However, these areas are surrounded by old domestic cuttings, deep drains associated with extraction, railway lines and milling fields. These impact the hydrology; drying out the edges, allowing scrub and dense heather to become established (English Nature, 2001).

2. Wedholme Flow Observations May 1st 2013

2.1 General observations

By May 2013, up to two years from the original plantings at Wedholme Flow, the treated areas had undergone surface erosion and there was evidence of surface water movement. The south block (Spring) showed signs of a water channel along the middle of the block (west to east). In the north block (Spring) there was evidence of flooding from the adjacent ditch producing a partial covering of bedraggled strands of *S.Cuspidatum* washed in from with the ditch water.

2.2 Bead growth

2.2.1 Benefits of brash cover

(Quinty & Rochefort, 2000). Within the brash treated areas across the site there was evidence of new bead plants developing and also evidence of dormant but potentially viable beads. In contrast to other *Sphagnum* from the ditch or the brash, the plants grown from beads tended to appear as single or clusters of small capitula, rather than plants with long stems. From inspection of the bare plots across the site we found no visible, viable bead material on these surfaces.

Brash had been added to about half of all plots in 2011. In May the brash was very rough chopped and some was prepared quickly on site. In September the added brash was double chopped and much finer. On the May 2013 visit, the fine double chopped brash was mainly still in place from the planting date and it had formed a hard crust. It is unsure whether this was advantageous or not. Although it had moved little and provided surface protection, it had probably not allowed enough light to penetrate and lacked gaps for the *Sphagnum* to come through. Intact beads were found under these thick areas of 'thatched' brash and had only grown further in the gaps or near the edges of the plots where the brash was thinner. However, over the longer term it may be a benefit, as the stabilisation effect of brash (parry *et al,* 2014) and the moisture retention offered to establishing *Sphagnum* (Price *et al.* 1998. Quinty & Rochefort, 2000) have been well documented.

2.2.2 Benefits of cotton grass planting

The *Sphagnum* growth in the plug-planted cotton grass (*Eriophorum angustifolium*) areas showed the benefit of cotton grass cover in establishment of *Sphagnum* communities, either from beads application or natural re-colonisation. The success of *Sphagnum* planted in companion with cotton grass has been recorded in other field trials, and the benefit is represented in current literature (Ferland & Rochefort, 1997. Joosten, 1992. Tuitilla *et al.* 2000. Holden, 2008). Cotton grass plugs grew well in the moist sites, but not where it was either too wet (or suffered water movement erosion) or too dry. In sites that had seen the best establishment *Eriophorum angustifolium* had grown rapidly and colonised peat outside of its planting zone.

2.2.3 Influence of flooding

There were also only a few bead plants on the blocks where the site had suffered flooding from the adjacent ditch, removing the brash, and probably washing the beads away. Young *Sphagnum* was abundant in the cotton grass planted area on the north block. However, the heritage of the plant material was uncertain as the area had been inundated with flood water from the adjacent ditch bringing with it an input of *S.cuspidatum*. It is uncertain whether the *Sphagnum* growth was from beads or from the ditch. Quinty *et al.* (2000) observed that establishment of *Sphagnum* propagules exposed to flood events was less successful. But Rochefort *et al.* (2002) observed that flooding did not warrant any serious impediment to *Sphagnum* regeneration and contributed to an elongated period of moisture availability.
In the other blocks away from the ditch, bead plants were found growing well amongst the planted cotton grass plots. There also appeared to have been water movement on the south blocks, where a current had washed away some material on the bottom end of the plots.

2.2.4 Effect of plastic covering

The plastic covering did not appear to confer any advantage over the bare or brashed plots. Only the buried edge of the sheeting remained, showing the tattered remnants had been completely eroded by the weather and sunlight. It is unknown how long the covering actually lasted and therefore how much protection it afforded and for how long. Again there was no evidence of any bead plants or viable materials on these plots, leading us to believe that there was no positive benefit on *Sphagnum* bead establishment or development. Earlier reports indicated that it was potentially detrimental as on warm days high temperatures were observed under the clear plastic film.

3. Bolton Fell Observations May 2nd 2013

3.1 General observations

At Bolton Fell Moss the beads were applied to two distinct areas of bare peat. One area nearest to the peat factory works with ridge and furrow-like topography was called ‘Normal’ and the other site just north of the intact bog was called ‘Flattened’ as here the ground had been levelled and a peat bund added to contain the area to reduce flooding and surface runoff.

At the Normal site, there was evidence of much water movement and many of the plots in the central area of the site were not accessible due to flooding. Many canes had been moved or uprooted, due to either water movement, frost heave or possible high winds. Surfaces facing the prevailing wind were coated in peat. Some of the blocks were partially accessed but the water had moved the brash around. The blocks towards the south side of the Normal site were less damaged by flood water due to being slightly raised above the flood water level, and plant growth appeared to have benefited from the high water table supporting excellent cotton grass growth from the May 2011 planting.

The Flattened site was enclosed by a by a low peat bund and appeared to be better protected against flooding as fewer canes were dislodged. No areas were underwater although some areas, which were filled-in ditches, were very soft.

3.2 Beads growth

3.2.1 Limitation of survey

Within the limitation of available time (one very full day for 4 people) it was not possible to do a detailed quantitative survey of counts of growing *Sphagnum*, beads still present but with limited growth or developing beads. Some gross estimates of quantity present have been given.

3.2.2 Benefits of brash cover

The heather brash covering at Bolton was similar to that at Wedholme with separate applications of coarsely cut brash used in May 2011 and fine double chopped brash used in September. The finer cut brash had again stayed firmly in place but a similar hard crust had formed over the plots, and in some cases, this was even thicker on some of the blocks in comparison to Wedholme.
Where the brash was intact or only a little disturbed, there was abundant evidence of hundreds of established Sphagnum bead plants with the plants being generally small and still in the earlier stages of development. The hard brash crust that remained on the brash treatment from September 2011 was investigated by lifting up ‘plates’ of crust Under the crust there several small fragments of original bead as well as small Sphagnum bead plants. Many more beads and developing bead plants were found on the plots with fine chopped brash than on the coarse brash plots. The crust had evidently protected the beads and provided an environment allowing bead development, but it could be so thick that it prevented the bead plants from further growth, as they were unlikely to break through the crust and could have been smothered by it. Further observation will be needed to judge this. There were few established *Sphagnum* bead plants on these plots.

It was noticeable that bead plants, developing beads, and potentially viable beads were found in greater abundance than on the similar plots at Wedholme. Plants from beads were evident across most or all of the blocks surveyed in the Bolton Normal area and the Flattened area, but only within the protected brash plots and cotton grass plots, highlighting the importance of brash protection (Quinty, *et al.* 2000) and potential of cotton grass companion planting (Tuitilla *et al.* 2000). There was no evidence of bead plants growing on bare plots at the Bolton site. This would leads us to believe that some form of covering, or ‘nurse’ is necessary to reduce weather effects, provide stability and prevent loss from transpiration (Rosenburgh, 2015).

3.2.3 Benefits of cotton grass planting

The plots of planted cotton grass (*Eriophorum angustifolium*) have shown good growth in two years, particularly in the wetter areas where they had spread to provide a thick cover. However, cotton grass growth was much poorer and even absent in and around the flooded areas of the normal site where there was evidence of the plugs being washed out by wave action. Again, Cotton grass provided good protection for establishing *Sphagnum* bead plants and there were tens of plants growing.

3.2.4 Effects of plastic cover

Again, as at Wedholme, the plastic covering appears to have had no beneficial effect on bead development. The buried edge was the only remains of the sheeting and for such films to be useful a longer lived material would be needed.

3.2.5 Influence of flooding

Access was prevented to the central areas of the normal site as they were flooded and underwater. We were able to get to one of the blocks to partially monitor them, but half of every plot tailed off underwater and was inaccessible. The parts of the plots that we managed to look at had been badly disturbed by water movement, the brash had been partially washed away and there was little evidence of any beads or bead plant material.

3.2.5 Influence of season

It is difficult to distinguish the effects of season from those of the different types of brash used as covering. There were many more beads still visible from the autumn planting, but the viability of these was not clear under the thick brash crust that had formed. The spring planted beads have also had an additional season to develop or decay. From other trials elsewhere, we expect spring planting to perform better, particularly if autumn planting experiences a harsh winter.

4. Conclusions and Recommendations

From observations in May 2013 of the *Sphagnum* restoration plots set up in 2011 at Wedholme Flow and Bolton Fell Moss the following conclusions can be made:

4.1 Careful control of water table appears essential as both flooding and drying-out affected Sphagnum bead survival and establishment at each of the sites. The importance of water table as part of bog restoration is well known, but our trials emphasised the importance it could have on *Sphagnum* BeadaMoss™ establishment at the sites. Peat water content and surface level appear critical. Plots that appeared almost permanently damp (i.e. a few cm above water table) gave the best *Sphagnum* establishment and plots higher above the water table showed much lower levels of success. Avoiding surface flooding is essential especially for extended periods; even newly planted cotton grass did not survive continuous inundation. As well as water table, water movement needs to be controlled to avoid bead and brash material being washed away. With this in mind careful attention should be paid to site preparation and the use of bunds to control water height and flow.

4.2 After finding no established *Sphagnum* bead plants on sites that were not treated with some protective covering it is evident that for *Sphagnum* to establish on bare peat the surface needs some form of covering to provide protection against the natural weather elements, to reduce wind blow erosion of peat and to lower the rate of drying of the peat surface. It is clear that planting *Sphagnum* onto bare peat is not viable.

4.3 Surface coverings tested at both sites that proved beneficial were common cotton grass (*Eriophorum angustifolium*) – planted in advance of the *Sphagnum* – and heather brash. A cover of plastic film over experimental plots did not improve establishment.

4.4 The fragment size of heather brash covering should probably be somewhere between the two sizes tested here. The coarse brash was too big and likely to be blown away while the fine, double chopped brash was too thin and formed a hard crust. This crust provided some protection but did not offer sufficient gaps for light and *Sphagnum* growth.

4.5 It is recommended that a much larger area of cotton grass should be trialled as a 'nurse' plant in advance of *Sphagnum* planting. This could be planted at a lower density than on the experimental plots. The cotton grass trial planted in the trial plots was at a relatively high density and due to its success had spread into adjacent plots. Over a larger scale cotton grass could be planted at about one plug per 1-2 square metres. This planting density has been successful at several other sites including blanket bogs in the Southern Pennines. This planting should be adequate to give a full cover in around two growth seasons. This would mean delaying *Sphagnum* application, but our trials indicate it could provide improved *Sphagnum* cover in a shorter period of time when compared to heather brash. Planting cotton grass onto the bare peat areas also provides the benefit of improving the plant community since cotton grass is an associated species that has a part in an active peat forming bog and will encourage the growth of other bog vegetation.

10.3 Featherbed Moss Plug PlugaMoss™ Plant Monitoring

Since planting Plugs have been monitored twice: First on 25/07/2014 then on 24/03/2015

The first set of monitoring was qualitative mapping and counting plugs and using a descriptive to comment on the state of the plug.

Second set of monitoring mirrored this, but with additional size measurements also recorded. Size was measured using size rings: Unless stated (Marked on the spreadsheet using a *) all measurements were taken by placing a 50mm ring over the plug and giving a percentage cover. With larger plug growth a larger 90mm (marked with*) was used.

Plug Survival

100 plugs planted, split between gully and peat pan locations, and arranged either north of south facing.

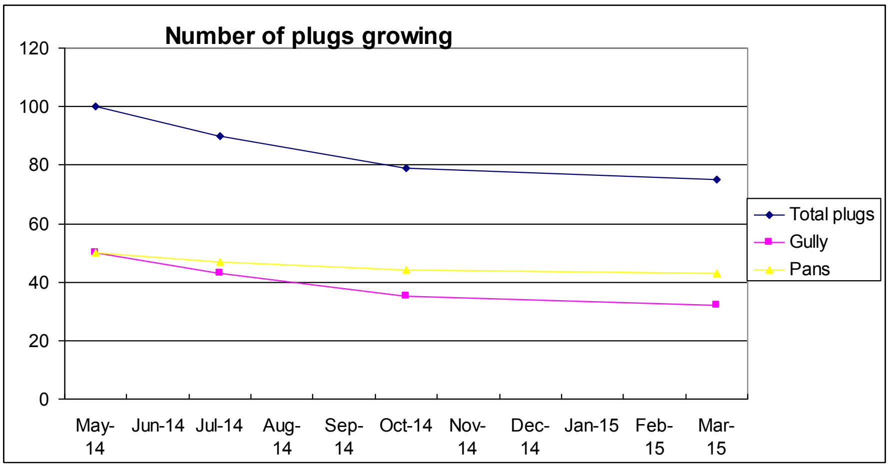
Plant date	25/07/2014	24/03/2015
total plugs= 100	total plugs= 99	total plugs= 75
gully=50	gully=49	gully= 32
pans=50	pans=50	pans=43

- First count: 99% plug survival rate with only 1 plug missing. 1 plug missing from gully site 2:
  a relatively mobile peat surface with evidence of water movement and flooding.

- Second Count: 75% of plugs still in-situ. Majority of unaccounted plugs occurred in the gully sites (18 plugs missing), these most likely having been buried or washed out by water movement.

- Only 7 plugs missing from peat pan sites. More stable locations than the gullies but vulnerable to flooding and drying /desiccation.

- All 7 missing plugs on the peat pan site where planted on the northern side of the plots (southern facing?)



Observations

Peat pan locations have shown the highest plug ‘survival’ %. But care should with the term survival: it is more likely that the majority of ‘missing’ plugs are actually buried in-situ by peat sediment or washed out, distributed and subsequently buried in sediment. There is the possibility that the peat burial is not necessarily harmful, and could be potentially be beneficial, in some cases.

The Peat Pan locations are in some ways more stable than the Gullies, for example there isn’t the same force of water movement. The pans sites do appear to be vulnerable to flooding, but it is more a case of standing water, possibly not overly harmful and tackled by planting the plugs higher along the edge of the pans.

The peat pans showed some good growth but there were still 7 plugs unaccounted for ‘’no *Sphagnum* visible’’. Of the ‘missing’ plugs (in the peat pans), all of them were northern distributed (Southern Facing?). It is possible that the plugs have become buried by peat; there was a suggestion that it could have been windblown, possibly explaining the northern distribution of missing plugs.

The pans are also at danger of drying out and desiccation. It was noted that brash was particularly beneficial in protecting plugs from drying (this was only trialled on the peat pans). A small amount coarsely chopped brash has been observed as the most effective, providing reduced evapo-transpiration and a level of structural support. Fine chopped brash is less useful as it tends to form an impermeable crust.

The Gullies have the highest number of ‘missing’ plugs; not surprising considering the variable water levels. The Gullies have shown evidence of fast water flow, flooding and peat sediment deposition.
When planting the plugs attention needs to be paid to the water flow patterns within the specific gully, take care were possible to plant on the side where water flow appears to be slower to avoid erosion/washing out.

Water cover and flooding present an issue, due to burial from mobile peat sediment; This can probably be avoided by planting the plugs higher up along the gully walls, again it comes down to each gully and its specific environment.

Plugs that had been planted within areas of vegetation, specifically cotton grass, within the gullies appeared to have benefited. Reducing erosion from water movement, shading and also acting as a supportive scaffold for growth.
Areas of vegetation are preferential to bare areas of peat as they are less likely to be affected by water movement disturbance or sediment deposition, whereas bare areas of peat are likely to lie within the waters course.

Gullies probably provide a more hospitable environment for *Sphagnum* plugs to establish than bare peat pans due to vegetative mix and its associated benefits; previous experience has shown *Sphagnum* doesn't thrive on bare peat. There have since been trials where Plugs have been planted directly into intact bog surface, this comparison should be of interest to find the most suitable substrate for plug Planting

An idea for a future further study would be to look at gully width and depth and correlate to plug survival, there could be a possible ideal gully type for Plugs e.g. wide and shallow?

Fig.1. Photos from Featherbed Moss 24 March 2015. Examples of locations and plugs after 10-11 months, following planting in May 2014. Top left – lower gully in which many plugs were not found, probably buried or washed away over winter. Top right – typical successful plug in one of the upper gullies, where it was well meshed in to the cotton grass. Bottom right – typical successful plug against the edge of the peat pan, showing remains of the valuable brash covering. Bottom left – peat pans where plugs were planted around the edges of the peat.