# *Sphagnum* restoration on degraded blanket and raised bogs in the UK using micropropagated source material: a review of progress

S.J.M Caporn[1], A.E. Rosenburgh[1,2], A.T. Keightley[1], S.L. Hinde[1,3], J.L. Riggs[1], M. Buckler[4] and N.A. Wright[5]

[1]School of Science and the Environment, Manchester Metropolitan University, UK
[2]Department of Biosciences, Durham University, UK
[3]National Trust, Dark Peak Estate, Hope, Derbyshire, UK
[4]Moors for the Future Partnership, Moorland Centre, Fieldhead, Hope Valley, Derbyshire, UK
[5]Micropropagation Services (EM) Ltd., East Leake, Leicestershire, UK

## SUMMARY

There is a growing demand for a supply of *Sphagnum* to re-introduce to degraded peatlands. However, available supplies of *Sphagnum* of the desired species are often limited. We describe the propagation of *Sphagnum* from vegetative material in sterile tissue culture and the introduction of juvenile mosses into the field. *Sphagnum* produced in the laboratory in three different forms (beads, gel and plugs) was introduced to different peatland surfaces on upland degraded blanket bog and lowland cut-over peatland in northern England. On degraded blanket bog, the establishment of mixed-species *Sphagnum* plugs was typically 99 % while the survival of beads was much lower, ranging from little above zero on bare eroding peat to a maximum of 12 % on stabilised peat surfaces. On lowland cut-over peatland, all trials took place on peat with an expanding cover of *Eriophorum angustifolium* and tested *Sphagnum* gel as well as beads and plugs. This work showed that survival and establishment of plugs was high (99 %) and greater than for beads. *Sphagnum* gel reached a cover of 95 % in two years. The vegetative micropropagation of *Sphagnum* offers an effective source of *Sphagnum* for re-introduction to degraded peatlands.

**KEY WORDS:** BeadaMoss®, bog restoration, peatland, *Sphagnum* micropropagation

## INTRODUCTION

*Sphagnum* delivers the form and function of raised and blanket bogs, and its widespread dominance provides a suite of peatland ecosystem services (Lindsay 2010, Rydin & Jeglum 2013). Degraded sites, where the *Sphagnum* cover has been lost due to human interventions such as harvesting, peat extraction, drainage, overgrazing, fire and air pollution are slow to recover without further human action (Quinty & Rochefort 2003). The return of *Sphagnum* moss, by natural recovery or managed re-introduction, is an essential factor for repair of degraded ombrotrophic peatlands (Van Breemen 1995, Rochefort 2000). The majority of published research on *Sphagnum* restoration has been conducted on lowland raised bogs following commercial peat extraction, forestry plantation and agriculture (e.g. Sliva & Pfadenhauer 1999, Rochefort *et al.* 2003, Haapalehto *et al.* 2011). Repair of lowland cut-over peatland to *Sphagnum*-dominated bog communities has been achieved after considerable human intervention at numerous locations in different parts of the world (Glatzel & Rochefort 2017). Evidence from these trials typically reveals requirements for maintenance of a high water table and some form of protection against desiccation, *e.g.* straw mulch or nurse plants, to facilitate successful *Sphagnum* establishment (Quinty & Rochefort 2003, Groeneveld *et al.* 2007). Similar requirements are likely for the restoration of *Sphagnum* cover on blanket bog which has been degraded through the action of various causes including overgrazing, accidental fire and air pollution (Anderson *et al.* 2009). However, in the case of blanket bog, there are fewer published reports of successful *Sphagnum* application (Hinde *et al.* 2010). It is also less certain whether there is an obligate requirement for a steady, high water table because blanket bog occurs in areas of high precipitation and cloud cover (Rydin & Jeglum 2013) so that moisture arriving from above may compensate for a poor supply of water from below. An early example of restoration of *Sphagnum* to upland blanket bog in the UK was reported by Ferguson & Lee (1983) who transplanted *Sphagnum* into the degraded bog surface of the English southern Pennines in 1979. Their efforts met with only limited


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success initially (attributed to the high levels of air pollution at the time); but better results were recorded from the original transplants after 25 years (Caporn *et al.* 2006), indicating good potential for successful *Sphagnum* restoration into these upland blanket bogs.

The recent successful methods of *Sphagnum* introduction to peatlands, whether for the purpose of restoration of damaged or degraded peatbog surfaces (Quinty & Rochefort 2003) or for *Sphagnum* farming, as demonstrated in Canada (Pouliot *et al.* 2015) and Germany (Gaudig *et al*. 2017) require a large supply of moss propagules (also known as diaspores) to be removed from a donor site and transported to the recipient location. In some parts of the world, particularly western Europe, there are insufficient donor sites to provide the required quantity of moss, since most of the *Sphagnum*-rich locations are in conservation areas and many of these are regulated under the EU Habitats Directive (92/43/EEC). Even where *Sphagnum* can be legally sourced without significant harm to donor sites, there are potential problems of accidental transfer of pest species and pathogens such as heather beetle (*Lochmaea suturalis*) (Scandrett & Gimingham 1991) and bulgy eye (*Cryptosporidium baileyi*) (Baines *et al.* 2014), as well as *Sphagnum* diseases such as the parasitic fungus *Lyophyllum palustre* (Limpens *et al.* 2003). Furthermore, the consequences of deliberately transferring *Sphagnum* provenances from one region to another are not understood.

Here, we report the production and field application of an alternative source of *Sphagnum* material using standard tissue culture propagation methods, which addresses some of the challenges and constraints outlined above. Starting with as little as a single capitulum from a known provenance, *Sphagnum* is cultured under laboratory conditions to produce a variety of propagule products in large volumes, each adapted for application to peatland in different conditions. Since 2008 we have conducted numerous independent trials involving application of propagated *Sphagnum* to both cut-over lowland raised bog and degraded upland blanket bog. This article reviews a representative selection of these trials to demonstrate the success, pitfalls and future potential of this approach.

## METHODS

### Propagation and culture of *Sphagnum*

All trials used micropropagated *Sphagnum* produced by Micropropagation Services (EM) Ltd. (Leicestershire, United Kingdom). Full details of culture methods are not presented since this is commercially sensitive information. For each species, 5–10 capitula were collected (with permission) from one population (within an area of 1 $m^2$), causing very little damage to the donor *Sphagnum* colonies. The culture procedure started with single fresh *Sphagnum* capitula which were surface-sterilised and transferred to agar-based culture medium under aseptic conditions using standard tissue culture methods (Murashige & Skoog 1962). Cultures were raised at 20 ºC under moderate lighting (50 µmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR), *i.e.* $\lambda = 400$–700 nm) provided by cool white fluorescent lamps. After approximately ten weeks, when plants were around 20 mm in length, they were sub-divided (capitula produce several new shoots) and transferred to fresh culture medium at temperature 18 °C and irradiance 100 µmol $m^{-2}$ $s^{-1}$ PAR. After a further nine 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®) (Figure 1). In this article, these products are often referred to as *Sphagnum* gel, *Sphagnum* plugs and *Sphagnum* beads, respectively. The *Sphagnum* gel is a suspension of whole plants of length 5–25 mm in flowing hydro-colloidal gelatinous medium. In contrast, the *Sphagnum* beads are composed of numerous (typically ten) smaller *Sphagnum* plantlets/fragments, following cutting to approximately 5 mm length, embedded in a more solid form of the same gel material. Both beads and gel were normally transferred to field locations within ten days of preparation. The *Sphagnum* plugs were produced by applying micropropagated *Sphagnum* gel to cylindrical peat blocks (36 mm diameter × 60 mm height) and growing on in a glasshouse under natural daylight at a range of temperatures depending on seasonal climate (minimum 10 ºC, maximum 35 ºC), misting with rainwater to keep the moss surface moist. Typically, *Sphagnum* plugs were transferred to the field within 4–6 months. The application rate of *Sphagnum* fresh biomass was lowest for beads (8.8 g $m^{-2}$), intermediate for gel (330 g $m^{-2}$) and highest for plugs (around 650 g $m^{-2}$).

In the research reported here, the *Sphagnum* beads were single-species except in Trial D, but the *Sphagnum* gel and plugs contained a mixture of the following eleven species (with proportions): *S. capillifolium* (Ehrh.) Hedw. (ssp. *capillifolium*) 8–15 %, *S. cuspidatum* Ehrh. ex Hoffm. 8–15 %, *S. denticulatum* Brid. 1–3 %, *S. fallax* (H.Klinggr.)


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(a) BeadaMoss®

(b) BeadaGel™

(c) BeadaHumok™

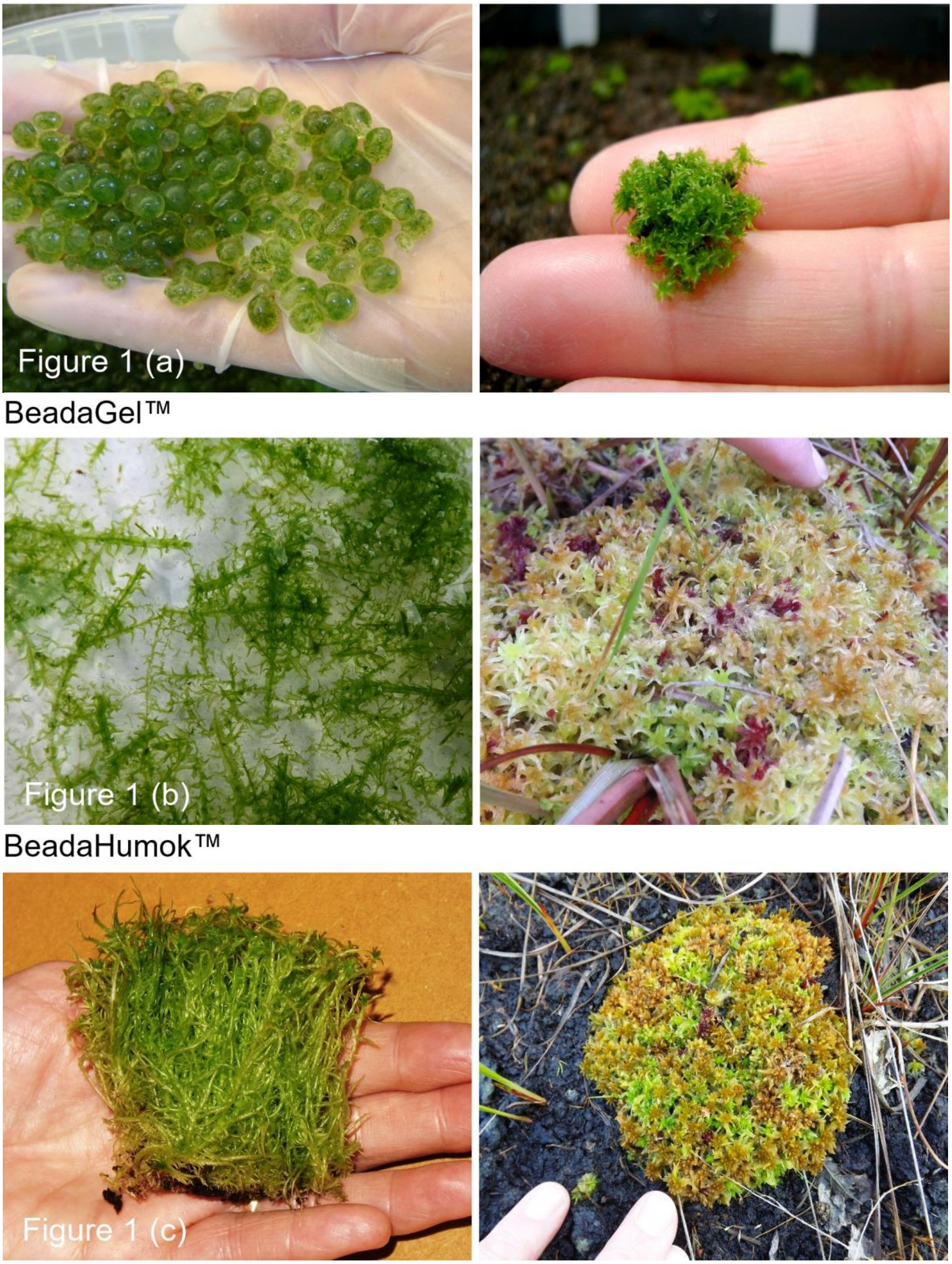


Figure 1. Micropropagated *Sphagnum* moss. (a) *Sphagnum* beads (BeadaMoss®), right-hand picture shows BeadaMoss® *Sphagnum* growth six weeks after planting in a glasshouse. (b) *Sphagnum* gel (BeadaGel™), right-hand picture shows BeadaGel™ 15 months after planting in the field (Cadishead, Manchester). (c) *Sphagnum* (BeadaHumok™), right-hand picture shows BeadaHumok™ 15 months after planting in the field (Cadishead, Manchester).


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H.Klinggr. 20–30 %, *S. fimbriatum* Wilson 8–15 %, *S. magellanicum* Brid. 1–3 %, *S. papillosum* Lindb. 8–15 %, *S. squarrosum* Crome 1–3 %, *S. palustre* L. 20–25 %, *S. tenellum* (Brid.) Pers. ex Brid 1–3 %, and *S. subnitens* Russow & Warnst. 5–10 %. These species are described by the British Bryological Society in Atherton *et al*. (2010) and authorities are given by Smith (2004). The *Sphagnum* was collected from northern England, *S. magellanicum* and *S. tenellum* from the county of Cumbria and the remaining species from the Peak District National Park in the county of Derbyshire.

**Field trials**

Upland field trials took place on degraded blanket bog around 35 km east of Manchester (northern England), at the Dark Peak Site of Special Scientific Interest (SSSI) within the Peak District National Park. The trials were located on Black Hill (53.5330 ºN, 1.8835 ºW; altitude 582 m a.s.l.) and on the adjacent Holme Moss, 2 km to the south east on the same peat-covered plateau. Mean annual rainfall recorded at Holme Moss weather station (University of Manchester, Beswick *et al.* 2003) over the period 1994–2001 was 2,416 mm. At an upland weather station 10 km to the south (data available for 2004–2013), Clay & Evans (2017) found that rainfall was distributed fairly evenly over the year and that, on average, the driest three months (February to April) received 75 % of the monthly average over the year. Vegetation also receives moisture directly from the frequent cloud cover, and this ‘occult precipitation’ is not fully included in measured rainfall. The long-term (2004–2013) average January, July and annual temperatures were 1.9 ºC, 13.2 ºC and 6.9 ºC (Clay & Evans 2017).

The Dark Peak SSSI and its surroundings have a long history of ecological change due to pressures exerted by air pollution (Ferguson & Lee 1983), overgrazing, fire, extreme weather and climate change. This is the most degraded area of blanket bog in the British Isles (Tallis 1987, 1998) and is now undergoing large-scale restoration led by the Moors for the Future Partnership (Buckler *et al*. 2013). The condition of the blanket bog and efforts to restore its plant cover are described elsewhere (Anderson *et al.* 2009, Buckler *et al.* 2013). The landscape used for the blanket bog research trials was typically a mosaic of four different surface types (Table 1, Figure 2a). The micropropagated *Sphagnum* was applied to the first three of these, *i.e*. bare peat, treated (re-vegetated) peat and native vegetation; erosion gullies being unsuitable for *Sphagnum* introduction. The water table was highly variable across this landscape, ranging from high on the Holme Moss cottongrass (*Eriophorum angustifolium*) dominated plateau site used in Trial C (described below) to very erratic or absent at many other sites where the peat layer was very thin (*e.g*. less than 10 cm) due to erosion of bare peat, which can extend down to the mineral bedrock. Water table levels were not recorded at the upland sites due to the difficulty of collecting representative data in such a variable landscape. However, features of the water table at nearby locations within this degraded peat landscape are discussed by Allott *et al*. (2009).

Table 1. The main classes of surface on upland blanket bog and lowland cut-over peatland, as defined for this study.

| Surface type | Description |
|---|---|
| Upland bare | Bare peat, following degradation of native vegetation and erosional loss of peat. |
| Upland treated | Areas treated with lime and fertiliser applications followed by seeding with a nurse crop (amenity grass mixtures and *Calluna vulgaris*; no *Sphagnum*) on bare peat or degraded vegetation (Buckler *et al*. 2013). |
| Upland native vegetation (‘vegetated’) | Elevated areas of vegetation including extensive areas of deep peat and smaller areas on peat hags, often dominated by the native species *Eriophorum angustifolium, Eriophorum vaginatum*, *Empetrum nigrum*, *Vaccinium myrtillus*, *Calluna vulgaris* and *Deschampsia flexuosa.* |
| Upland gully | Extensive gullies of bare peat eroded, in places, to the mineral substrate or naturally revegetated with native shrubs, sedges, grasses and bryophytes (these areas were not used for trials). |
| Lowland cut-over | Naturally regenerating *Eriophorum angustifolium* cover over previously bare peat following commercial peat extraction. |


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Lowland field trials took place on Cadishead Moss (53.4523 ºN, -2.4551 ºW; altitude 24 m a.s.l.), within the Manchester Mosslands raised bog complex 13.8 km west-south-west of Manchester city centre. Although usually upwind of the city, the area has received industrial air pollution from this region's industry as well as from north Cheshire, south Lancashire and the Liverpool conurbation to the west. Average annual rainfall for the years 2012–2015 at Astley Moss weather station, 3 km to the north, was 1011 mm. Rainfall was unevenly distributed over the year. The driest months were January, February, March and September (55–81 % of overall monthly average) and the wettest months were May, November and December (25 % to nearly 50 % wetter than average). Mean January, July and annual temperatures were 5.2 ºC, 16.4 ºC and 10.1 ºC, respectively. Therefore, this site is warmer with less rainfall (and cloud cover) than the upland location.

Cadishead Moss is an 8 ha peatland that was historically drained and hand-cut for peat and has been owned and managed by the local (Lancashire) Wildlife Trust since March 2009. It was subsequently peat-bunded and partially levelled for re-wetting purposes, but some internal drainage ditches remain. The trials were conducted on peat within open stands of young cottongrass (*E. angustifolium*) (Figure 2b). In recent years, water table levels (relative to peat surface) on the lowland study plots have ranged between -41.5 and +1.6 cm in summer, and between -13.4 cm and +2.1 cm in winter.

The trials of micropropagated *Sphagnum* at the upland and lowland sites started at different dates and ran for various periods (Table 2).

*Trial A:* Sphagnum *bead pilot trials on blanket bog*

The aim was to investigate the influence of a range of peat surface treatments and application dates on the survival and establishment of *Sphagnum* beads on degraded blanket bog. The set-up of experimental plots and early monitoring are described by Hinde *et al*. (2010), and only outlined here. The plots (0.5 × 0.5 m) were established on bare peat surfaces at Holme Moss and on treated surfaces at Black Hill (see Table 1). The surface treatment was lime, fertiliser and a mix of amenity grass seed as described by Buckler *et al* (2013). This treatment was applied two years prior to introduction of *Sphagnum*, to stabilise the eroding peat surface. The plots were marked out with gridded quadrats, within which *Sphagnum fallax* propagules were placed by hand using forceps at an overall density of 100 beads *per* plot (i.e. 400 beads m$^{-2}$). *S. fallax* was used because it was the first species produced in bead form, and the only one available at the time of the pilot study. Plots were set up either with or without a light covering of heather brash (50% cover of cut stems of *Calluna vulgaris* - there was no evidence that *Sphagnum* was introduced on the heather stems) to examine the role of this material in protecting the establishing beads. The plots/ quadrats (replicated three times) were set out in a block design in October 2008, and repeat

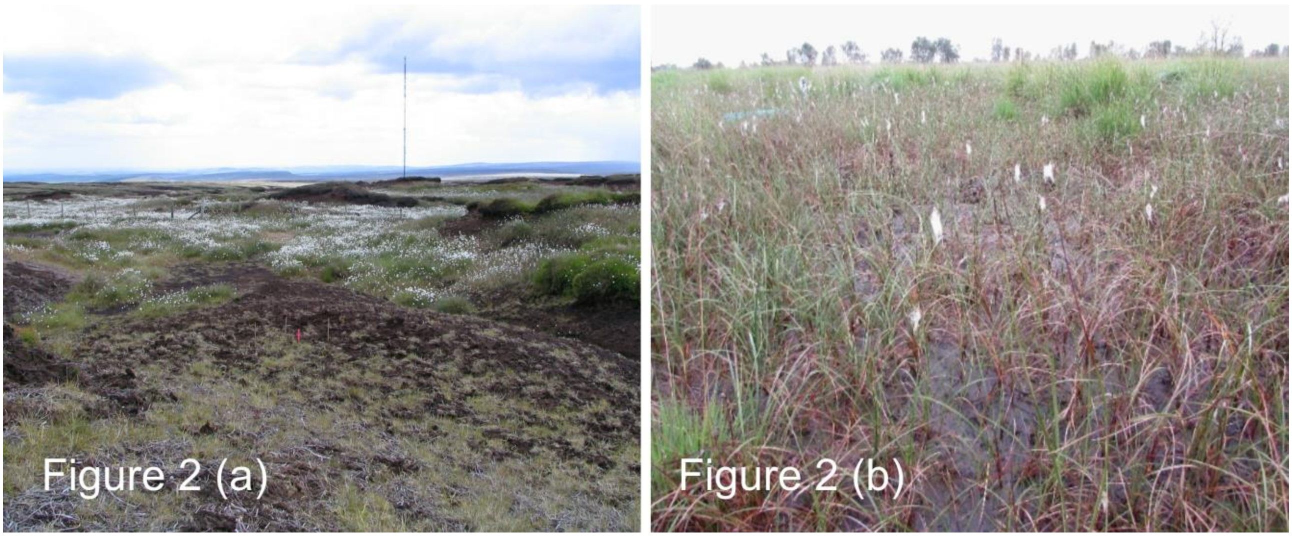


Figure 2. Landscapes typical of the upland and lowland degraded peatlands: (a) upland blanket bog landscape near Holme Moss in 2008 showing bare peat, peat with a thin cover of grasses following treatment with lime, fertiliser and grass seed, native plants (in this case mainly *E. vaginatum*) and deep gullies resulting from erosion; (b) lowland cut-over peatland at Cadishead in 2014 after re-wetting and spontaneous recovery of *E. angustifolium*.


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series were started in November 2008, March 2009 and May 2009. The results of monitoring the plots for surviving, living *Sphagnum* beads after 1–2 years have been reported (Hinde 2009, Hinde *et al*. 2010). However, at that stage it was not possible to assess the potential for development of the beads into mature plants. Therefore, the numbers of healthy established mature capitula *per* plot recorded during a later (June 2014) survey are reported here.

*Trial B:* Sphagnum *bead trials on blanket bog*

The aim was to investigate the growth of different species of *Sphagnum* beads under a wider range of conditions associated with different peatland substrates and times of year. Numerous field trials (Table 2) were established between November 2009 and August 2012 on degraded blanket bog at Holme Moss and Black Hill. Trials consisted of replicate blocks on different substrate types, broadly categorised as ‘bare’, ‘treated’ or ‘native vegetation’. Three replicate blocks were selected for their similar substrates and nearby locations. The normal experimental blocks consisted of seven 4 m × 1 m treatment strips, for six species and one control (to which no beads were added), with gaps of at least 0.5 m between strips. Each treatment strip was sown with beads of a single *Sphagnum* species, scattered by hand at a rate of 400 beads $m^{-2}$. The *Sphagnum* species used across the various trials were *S. capillifolium*, *S. cuspidatum*, *S. fallax*, *S. fimbriatum*, *S. palustre* and *S. papillosum*. All of these species are naturally present in the region (Carroll *et al.* 2009). In some cases, fewer species were used because some of the full set were not available. The trials were observed regularly and *Sphagnum* plants established from the beads were recorded after at least two years, when the treatment plots were searched thoroughly for all visible *Sphagnum* plants and their species were recorded. Further details are given by Rosenburgh (2015).

*Trial C:* Sphagnum *plug trials on blanket bog*

The aim was to examine the survival and growth of *Sphagnum* plugs (mixed species, as detailed in Methods) in sedge-dominated vegetation and bare peat on degraded blanket bog. Trials of plug establishment on Holme Moss were conducted in two different areas: (a) three plots of 36 *Sphagnum* plugs each were placed randomly on small areas of eroded bare peat (‘peat pans’) with open patches of young *E. angustifolium* (approximately 30 % cover) in August 2015; and (b) an area of vegetated blanket bog (see Table 1) on the Holme Moss plateau area, dominated by dense (100 % cover) mature *Eriophorum* spp. with no existing *Sphagnum*, was planted with 36 *Sphagnum* plugs in each of four 1 $m^2$ plots in August 2015. The initial area of each *Sphagnum* plug was 10.2 $cm^2$. A repeat application next to Area (b) plots occurred in October 2015 in order to compare summer and autumn application, and plug area measurements were recorded in June and November 2016.

*Trial D:* Sphagnum *beads, gel and plug trials on lowland cut-over peatland*

The aim was to compare the growth of different forms of *Sphagnum* (beads, gel and plugs, all of mixed species) on lowland cut-over peatland where the conservation target is to restore lowland raised bog habitat (Tables 1 and 2). The trial area already had a low-density sward of naturally regenerating *E. angustifolium* (Figure 2b).

In June 2014, two separate trial areas were established with gel (110 g *Sphagnum* mix added to 1 L gel, applied at 3 L $m^{-2}$) and plugs (30 plugs $m^{-2}$) only. All plots were mulched with straw at 300 g $m^{-2}$

Table 2. *Sphagnum* propagation materials, locations and dates of the field trials. Trials A–C were conducted in the Peak District National Park, and Trial D on the Manchester Mosslands (See Methods for further details). The terms ‘bare’, ‘treated’ and ‘vegetated’ are explained in Table 1.

| Trial | Propagule | Trade name | Habitat | Treatment | Start date |
|---|---|---|---|---|---|
| A | beads | BeadaMoss® | blanket bog | bare *vs*. treated | 2008–2009 |
| B | beads | BeadaMoss® | blanket bog | bare *vs*. vegetated *vs*. treated | 2009–2012 |
| C | plugs | BeadaHumok™ | blanket bog | bare *vs*. vegetated | 2015 |
| D | gel<br>beads<br>plugs | BeadaGel™<br>BeadaMoss®<br>BeadaHumok™ | lowland raised bog | straw / peat / no mulch<br>over existing vegetation | 2014 |


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and covered with thin plastic bird netting to retain the mulch during early establishment. *Sphagnum* growth was assessed after 4, 14 and 24 months by recording percentage cover of gel and area cover of plugs.

In December 2014, three further blocks were established in the same part of the site to examine the benefits of different protective coverings. This involved treatments with straw mulch (as in June 2014), light peat mulch (0.3 L $m^{-2}$) or no mulch, on three replicate plots incorporating 1 $m^2$ blocks with beads (400 $m^{-2}$), plus gel and plugs applied at the same rates as in June 2014. *Sphagnum* growth was assessed after 18 months by recording percentage cover of beads and gel, and area cover of plugs.

### Statistical analysis

Statistical analyses were performed using non-parametric tests in SPSS version 22 (IBM 2013) and Figures were drawn in R (R Core Team 2017) using ggplot2 (Wickham 2009). Statistical analyses are not reported for all of the trials due to high variability of the data compounded, in some cases, by the experimental design.

## RESULTS

### A: *Sphagnum* bead pilot trials on blanket bog

For the first replicated field trial of beads (set up in 2008–2009), monitoring of the number of *S. fallax* capitula in June 2014 revealed that establishment on treated, vegetated surfaces (Black Hill site) was significantly better than on untreated bare ground (Holme Moss site) (Mann Whitney U test, U = 406, n = 48, $p = 0.009$). There was no significant effect of sowing month overall (Kruskal-Wallis test, H = 2.28, n = 48, $p = 0.527$) and, although brash covering increased the bead establishment each month, this positive effect was only marginally statistically significant (Mann Whitney U test, U = 370, n = 48, $p = 0.071$). However, inter-plot variation was substantial, making it difficult to detect statistically significant patterns (Figure 3). For example, by June 2014, the most successful plots contained over 400 *Sphagnum* capitula on the treated, brashed site but only 36 on the bare peat, brashed site (Figures 4 a, b). On the worst plots at both sites, there were no established plants.

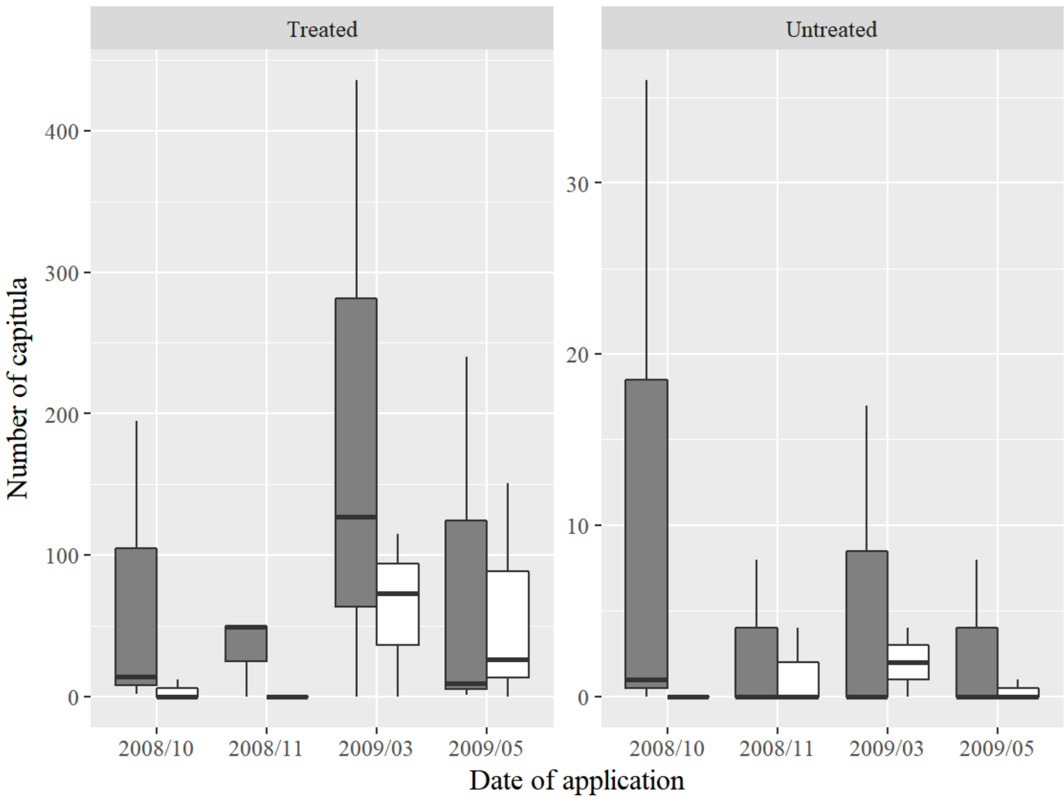


Figure 3. Number of *S. fallax* capitula recorded in June 2014 after application of *Sphagnum* beads in various months between October 2008 and May 2009, on either treated (with lime, fertiliser and grass seed, left-hand panel) or untreated (right-hand panel) peat surfaces (Trial A). In each application, half the plots were covered with heather brash (grey columns), whilst the other half remained uncovered (white columns). Note that the two panels have different y-axis scales.


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**B: *Sphagnum* bead trials on blanket bog**

The most successful *Sphagnum* bead application of this second period of trials was established in August 2010 on treated peat, where 12.2 % of *S. fallax* beads established successfully (Table 3) and subsequently grew to provide almost complete ground cover within a few years (Figure 4c). The next most successful trial was the application into *E. angustifolium* dominated vegetation in November 2009, where established beads developed into small *S. fallax* clumps around 10 cm across within seven years (Figure 4d); followed by April 2010, May 2011, September 2010 and finally August 2012. Growth of *Sphagnum* beads on bare peat was poor, with a successful establishment rate of 0.004 % across all trials plots. Vegetated and treated substrates proved more successful on average, with 0.30 % and 0.51 % surviving, respectively. There was no *Sphagnum* growth on any of the control plots (where no *Sphagnum* was applied) indicating that there were no other sources of *Sphagnum*. Despite the poor establishment of beads overall, there was an indication across all of these trials that *S. fallax* was the most successful species, with overall establishment of 1.0 %, followed by *S. cuspidatum* (0.17 %), *S. papillosum* (0.04 %), *S. palustre* (0.04 %), *S. fimbriatum* (0.018 %) and *S. capillifolium* which failed to grow. The overall

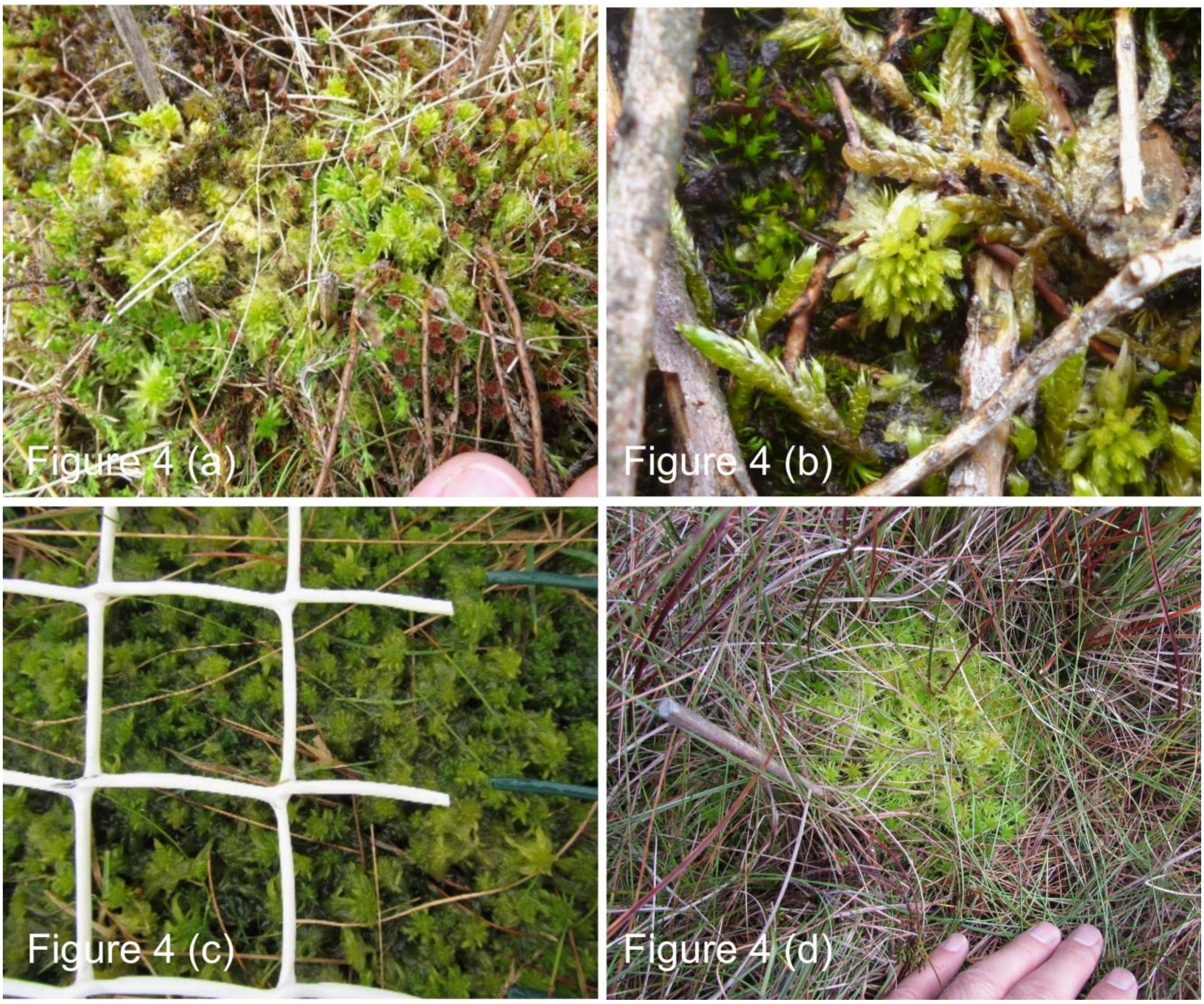


Figure 4. *S. fallax* growth from beads after application to various degraded peat surfaces on blanket bog: (a) dense growth on treated surface (lime, fertiliser and grass seed) with brash cover; (b) poor establishment on untreated bare peat with brash cover; (c) vigorous growth on treated (lime, fertiliser and grass seed) peat pan; (d) healthy *Sphagnum* colony arising from one or more beads in *E. angustifolium* dominated intact peatland with water table consistently close to the peat surface. All photographs were taken more than six years after the planting date (a and b: Trial A; c and d: Trial B).


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average *Sphagnum* bead establishment was 0.31 %. Statistical analyses are not reported due to the highly variable data compounded by the nested and irregular experimental design.

### C: *Sphagnum* plug trials on blanket bog

(a) Almost all of the mixed species *Sphagnum* plugs (see Methods) applied into the blanket bog in 2015 survived and showed fast rates of lateral growth. By June 2016, all but one of the plugs added into the Holme Moss peat pans had survived (99.1 %) and the mean plug size was $81.0 \pm 42$ cm$^2$; a mean increase of $796 \pm 408$ % on the original plug size (10.2 cm$^2$) (error terms are standard deviations). However, the plugs within the sedge patches grew better than those on bare peat, where there were signs of disturbance by birds and weather.

(b) Plugs in the dense *Eriophorum*-dominated vegetation on the more exposed plateau had 99.3 % survival, but they were more tightly packed with less lateral growth than those in the sheltered peat pans. By June 2016, the average area of plugs applied in August 2015 was $64.7 \pm 29.1$ cm$^2$; a mean increase of $635 \pm 286$ % on the original plug size. This was almost twice the growth of plugs applied two months later in October 2015, which had a mean area of $37.3 \pm 12.7$ cm$^2$ and mean increase in size of $367 \pm 125$ % (Figures 5 and 6). By November 2016, the better growth of the August compared with the October planting was even more evident. The species composition within plugs has not, so far, been assessed.

### D: *Sphagnum* beads, gel and plug trials on lowland cut-over peatland

*Sphagnum gel*

After 16 weeks, the *Sphagnum* gel application of the June 2014 trial had produced a mean *Sphagnum* cover of 56 %, which increased to a dense carpet (95 % cover) after two years (Figure 7). By this stage the *Sphagnum* mat had a mean thickness of 2.3 cm in the least-developed plot and 5.1 cm in the best plots, where it was associated with dense *E. angustifolium*. The healthy *Sphagnum* growth from gel was composed of several species (see Methods) as evidenced by the range of colours and growth forms (Figure 8a), although the species composition in the field was not analysed.

Table 3. Summary of % and (number) of successfully established *Sphagnum* beads across the field trials on degraded blanket bog (Trial B). Percentages were calculated on the basis of the number of *Sphagnum* beads initially applied. No data (-) indicates that the species was not used in the trial. Species abbreviations: *S.cap*: *S. capillifolium*; *S.cus*: *S. cuspidatum*; *S.fal*: *S. fallax*; *S.fim*: *S. fimbriatum*; *S.pal*: *S. palustre*; *S.pap*: *S. papillosum.*

| *Date* | *Substrate* | *S.cap* | *S.cus* | *S.fal* | *S.fim* | *S.pal* | *S.pap* |
|---|---|---|---|---|---|---|---|
| November 2009 | bare | - | 0 | 0.02 (1) | 0 | 0 | 0 |
| | vegetated | - | 3.75 (60) | 1.79 (86) | - | - | - |
| | treated | - | 0 | 0.02 (1) | 0.08 (4) | 0.29 (14) | 0.25 (12) |
| April 2010 | bare | - | 0 | 0 | 0.04 (2) | 0 | 0 |
| | vegetated | - | 0 | 0 | 0.06 (1) | 0.44 (7) | 0 |
| | treated | - | 0.04 (2) | 0 | 0 | 0.06 (3) | 0.06 (3) |
| August 2010 | bare | - | - | 0 | - | 0 | - |
| | treated | - | - | 12.19 (585) | - | 0 | - |
| September 2010 | bare | - | 0 | 0 | 0 | 0 | 0 |
| | vegetated | - | - | 0.06 (3) | - | 0.02 (1) | - |
| | treated | - | 0 | 0 | 0 | 0 | 0 |
| May 2011 | vegetated | - | - | 0 | - | 0 | - |
| | treated | 0 | 0 | 0.19 (9) | 0 | 0 | 0 |
| September 2012 | vegetated | 0 | - | 0 | 0 | 0 | - |
| | treated | 0 | - | 0 | 0 | - | - |


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*Sphagnum plugs*

The initial growth of plugs after application in June 2014 was unimpressive; it appeared to be hindered by straw mulch smothering the *Sphagnum*. However, this became less of a problem as the straw decomposed, and survival of the *Sphagnum* plugs was high (99 % after 14 months). Two years after application, the plugs had increased in size almost eight-fold, to a mean area of 76.5 $cm^2$ (Figures 7 and 8c). Observation indicated that *Sphagnum* became etiolated where *E. angustifolium* growth was most dense, and grew less well where plots were regularly inundated during the winter months.

*Influence of cover materials on establishment*

*Sphagnum* propagules (beads, gel or plugs) responded differently to the application of various cover materials following application to the peat

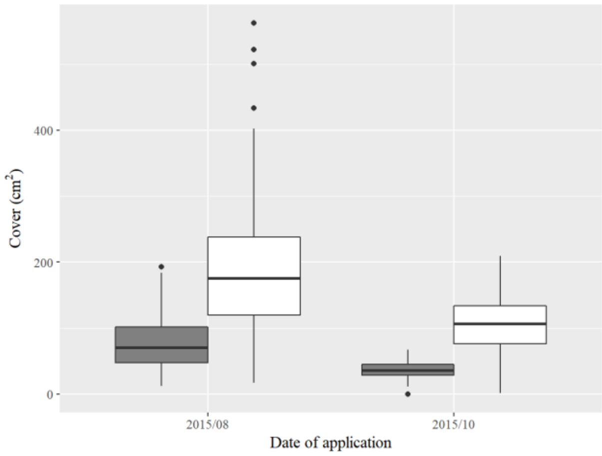


Figure 5. Cover ($cm^2$) of *Sphagnum* plugs planted into *E. angustifolium*-dominated blanket bog vegetation in August and October 2015, and later monitored in June (grey) and November (white) 2016 (Trial C). The original cover was 10.2 $cm^2$.

Figure 6. Example of a BeadaHumok™ mixed species plug growing amongst dense cottongrass (*E. angustifolium* and *E. vaginatum*) on blanket bog at Holme Moss after 24 months (Trial C).

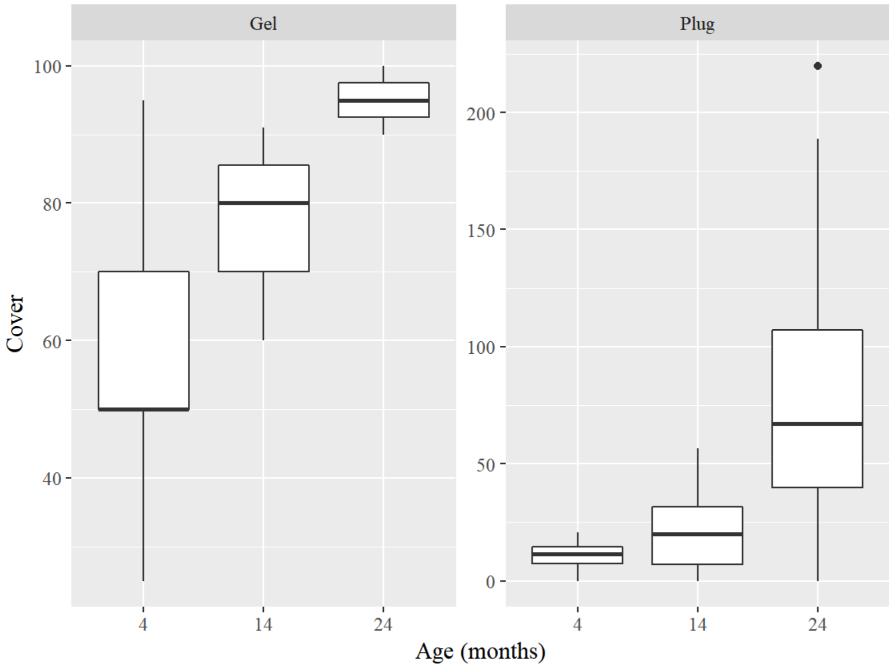


Figure 7. *Sphagnum* cover (% and $cm^2$, respectively) of plots of different ages, following application of BeadaGel™ (left) and BeadaHumok™ (right) in June 2014 to lowland peatland after site re-wetting and spontaneous growth of *E. angustifolium*, as shown in Figure 2b (Trial D).


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surface within open stands of *E. angustifolium*. There was a clear benefit to *Sphagnum* gel establishment from either a light peat layer or a straw mulch (Figure 9). In contrast, there was little benefit from either type of covering in the establishment of *Sphagnum* beads or plugs. The establishment of *Sphagnum* plugs was better within areas of dense *E. angustifolium*. In areas with low *E. angustifolium* cover, plugs tended to suffer bird disturbance (notably pulling apart and scattering, presumably insectivorous behaviour) and straw mulch smothered the plugs where the ground was inundated for long periods. There was also some loss from field vole (*Microtus agrestis*) activity (nesting or using as latrines) in areas of dense vegetation. The success of bead growth was highly variable across treatments and plots (so data are not shown), some showing low rates of cover growth and others achieving very high cover (Figure 8b); but overall, the rate of increase in cover for beads was slower than for gel or plugs. The effects of straw addition were mixed, since the straw mulch was advantageous to bead establishment when applied in areas of sparse *E. angustifolium* cover, but reduced light availability too much in areas of dense vegetation, particularly if the straw layer also became swollen during long periods of inundation.

## DISCUSSION

Micropropagated *Sphagnum* is an effective propagule and potentially available in large quantities, offering significant benefits for peatland restoration. There is very little damage to the habitat since the donor *Sphagnum* material is sampled in extremely small quantities. Another benefit is the opportunity to adjust the exact species composition. In restoration of the varied mire landscapes found on the degraded


Figure 8. Three forms of *Sphagnum* (multispecies) at Cadishead lowland cutover peatland restoration site (Trial D): (a) BeadaGel™ planted June 2014 amongst open *E. angustifolium*; (b) BeadaMoss® planted December 2014 amongst dense *E. angustifolium* with no further covering; (c) BeadaHumok™ development after planting with straw mulch amongst dense *E. angustifolium* in December 2014; Photos May–June 2016.


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upland blanket bog of northern England, where the different *Sphagnum* species occupy a variety of niches matching their ecological requirements (Rydin & Jeglum 2013), a wide range of *Sphagnum* species can be simultaneously introduced in a manufactured mixture allowing different species to establish in their preferred niches. For recent applications on upland and lowland peatlands in the UK, up to eleven species have been provided in the micropropagated *Sphagnum* materials. A further advantage is the generation of a ‘clean’ *Sphagnum* culture, free of potential disease.

Our field trials on degraded upland blanket bog and a lowland cut-over peatland in northern England have explored application methods, establishment and the growth potential of these novel *Sphagnum* propagules in three forms (beads, gel, plugs) over the last ten years. The degraded upland blanket bog where our earliest field trials took place proved to be a difficult test environment. We have learnt much about the transfer of micropropagated materials from favourable laboratory and greenhouse conditions to the field. Not surprisingly, the environmental requirements for successful *Sphagnum* establishment, particularly regarding moisture and protection, appear to be similar to those found by others introducing mature *Sphagnum* by translocation from established mires (*e.g.* Quinty & Rochefort 2003, Pouliot *et al*. 2015).

At the outset of the *Sphagnum* restoration trials on upland blanket bog, we believed that rain and occult precipitation to the hills of this high-rainfall region would compensate for a water table that was in most cases highly spatially variable or even absent (where the peat had eroded to the mineral bedrock) (Allott *et al.* 2009). Despite the high rainfall (1500–3000 mm year$^{-1}$) recorded at Holme Moss, plus additional occult precipitation (Beswick *et al*. 2003), the atmospheric moisture inputs are temporally unreliable and exposed surface peats dry rapidly during rain-free periods (*e.g.* two weeks in spring–summer), often to the point of becoming a fire risk (Albertson *et al*. 2010). Indeed, related research on Bleaklow Hill, a nearby degraded upland blanket bog frequently bathed in cloud-water, found that *Sphagnum* naturally occurred only where near-surface water flow was common in surface depressions or gullies (Rogers 2014). These results help to explain our observations of poor growth of beads on the upland sites where the elevated peat mounds or slopes of shallow peat frequently chosen for application were evidently not wet enough to support consistent establishment of *Sphagnum* beads. As a result, bead survival was low in many of our early trials on these surfaces (Trials A and B), at least within the timescale of these trials, often resulting in gaps in the results and limiting the value of subsequent statistical analysis (Trial B). The two best

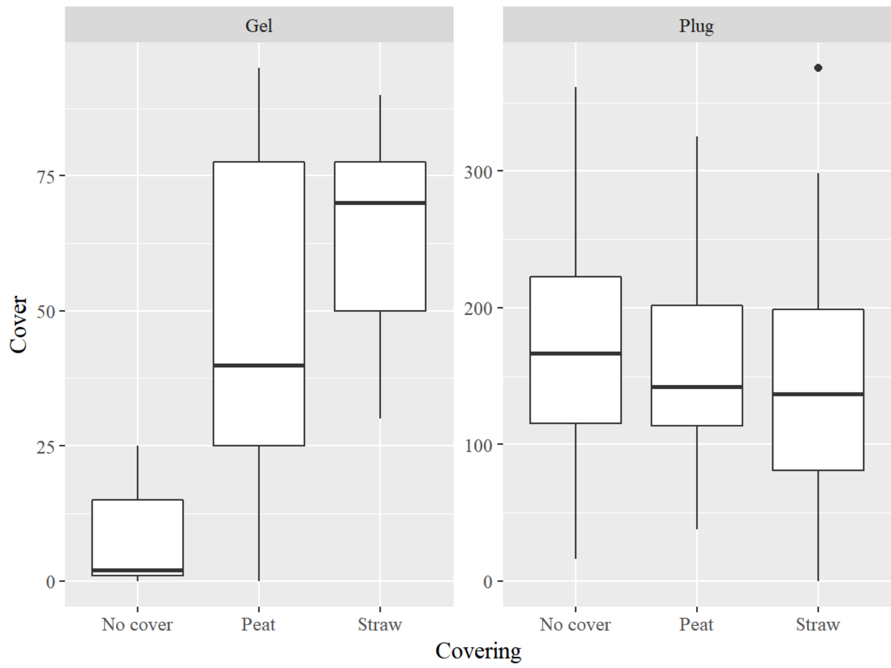


Figure 9. *Sphagnum* cover (% and cm$^2$, respectively) 18 months after application of BeadaGel™ (left) and BeadaHumok™ (right, initial plug size was 10.2 cm$^2$) comparing plots with peat, straw or no mulch (Trial D). The *Sphagnum* was planted in December 2014 on lowland peatland after site re-wetting and spontaneous growth of *E. angustifolium.*


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cases of establishment of beads in Trial B occurred at a frequently wetted peat pan site and in dense cottongrass (*E. angustifolium*) vegetation where we observed consistently high and stable moisture availability and shade. The requirement for high water table or continuous surface moisture to support *Sphagnum* introduction is without question in the literature (*e.g.* Quinty & Rochefort 2003) and the observations from our studies on this damp hilltop, despite the atmospheric moisture inputs, are consistent with this view.

The first trial (A), set up in 2008–9, showed that bare peat was not a good surface for bead establishment. Indeed, the unsuitability of eroding, bare peat for establishment of any vegetation without major intervention was shown in the 1980s and 1990s as part of the Moorland Management Project (Anderson *et al.* 1997). In our trials, even the application of *Calluna* brash to bare peat surfaces failed to sufficiently improve the conditions for survival of the juvenile *Sphagnum*. However, the establishment of *Sphagnum* from beads improved significantly at the treated site that had previously received the standard restoration treatment of a 'nurse' crop of young grass along with lime and fertiliser (Caporn *et al.* 2007, Buckler *et al*. 2013). On these treated, stabilised surfaces, adding *Calluna* brash marginally increased *Sphagnum* establishment in each month. In earlier research on moorland restoration in this region the standard recipe of lime, fertiliser and 'nurse' grass seed was required to provide a stable soil surface and protection for the subsequent establishment of desirable species either by deliberate sowing (*e.g. C. vulgaris*) or through natural colonisation (*e.g. Eriophorum* species). These additional benefits of *Calluna* brash addition are also well known in moorland restoration in England (Anderson *et al.* 2009, Buckler *et al.* 2013). Experimental trials on peatlands elsewhere have shown clearly the benefits for *Sphagnum* establishment of companion 'nurse' plant species such as *Polytrichum strictum* (Groeneveld *et al.* 2007) and a number of vascular plants that provide support and moderate the microclimate (Pouliot *et al.* 2011). Adding straw to protect the surface is a well-established technique in restoration of *Sphagnum* cover on cut-over peatlands in Canada (Quinty & Rochefort 2003) but may be ineffective on a wind-exposed upland conservation site in the UK. The best growth of *Sphagnum* beads at the upland site over the past decade of research occurred on a wet peat pan where an open sward of companion cottongrass (*E. angustifolium*) provided protection (Figure 10).

In Trial B, a wider range of blanket bog *Sphagnum* species was tested using *Sphagnum* beads containing single species. The results suggested that *S. fallax* established and survived best, but statistical evidence was lacking due to the highly variable data. *S. fallax* is a pioneer *Sphagnum* species that can succeed in a wide range of habitats (Atherton *et al*. 2010) and was recommended for use in restoration by Grosvernier *et al.* (1997). However, bogs dominated by this species are less favoured in conservation terms in the UK (JNCC 2009). A commonly observed feature of re-wetted cut-over lowland peatlands is that, without *Sphagnum* introductions, these sites often remain dominated for many years by simple communities of pool and lawn species, typically *S. cuspidatum* and *S. fallax* (Robroek *et al.* 2009). However, by introducing micropropagated *Sphagnum* mixtures comprising these fast-growing colonisers along with other higher-interest *Sphagnum* species, a productive and valuable community mix could be achieved.

*Sphagnum* plugs (Trial C and D) were very successful in both upland and lowland trials. Typically, a high proportion (> 95 %) of plugs established and survived. However, we found in other trials (not reported here) that they were vulnerable where the peat surface was mobile, leading to burial or loss of the underlying substrate, so careful selection of sites is essential. The advantage of *Sphagnum* plugs is most probably due to the larger plant mass being better able to withstand extreme fluctuations in environmental conditions (notably desiccation and waterlogging) and crowding by other vegetation. The size of *Sphagnum* plants in micropropagated material varies widely, from the

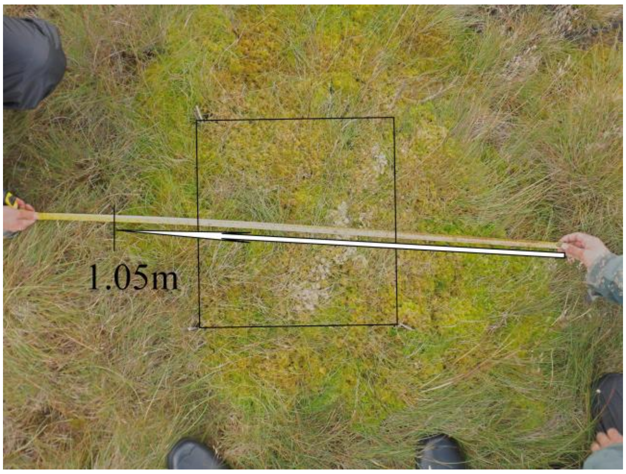


Figure 10. Example of excellent growth of *S. fallax* on upland blanket bog. Single *Sphagnum* beads were planted in each of the 100 grid squares of the 0.5 m × 0.5 m quadrat frame, into a wet peat pan with an open sward of *E. angustifolium*, in 2008. This photograph was taken six years later.


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1–5 mm moss fragments within beads to the fully developed (20–40 mm) plants in the gel and plugs. The larger and faster growing *Sphagnum* plugs can establish more quickly and cover the ground sooner than the juvenile plants within beads and gel. The benefits of introducing large rather than small *Sphagnum* samples into degraded peatlands was shown by Robroek *et al.* (2009). However, the greater financial costs of the larger *Sphagnum* material should be considered (see below). The success of *Sphagnum* plugs in restoration is evident from the current application of around 1.2 million plugs to 960 hectares of sedge and grass dominated communities in the blanket bogs of the English southern Pennines by the Moors for the Future Partnership.

The lowland trials commenced in 2014 after seven years of experience with the micropropagated materials in upland locations. The more favourable lowland climate conditions promoted generally better results. The lowland site is on average warmer and does not experience the harsh winds, frost and snowfall observed in the uplands. Of the three forms of micropropagated *Sphagnum*, plugs and gel were most able to establish and rapidly increase in cover. In the trials on the lowland Cadishead site, application and spreading of the *Sphagnum* propagules was confined to areas where cottongrass (*E. angustifolium*) cover was continuous. This companion vegetation proved to be a key component of *Sphagnum* restoration, while the benefits of other coverings (loose peat or straw) were mixed, depending on the *Sphagnum* product (beads, gel or plugs). Although lowland peatlands typically provide a less hostile climatic environment, other difficulties - notably flooding, disturbance by birds (pulling apart and scattering) and damage by small mammals (tunnelling under/using as latrine) - were found to impose greater constraints here than in the uplands.

**Costs and logistics in the production and application of micropropagated *Sphagnum***

The cost of *Sphagnum* materials produced by micropropagation depends on many factors, but an indication of prices in 2018 is given in Table 4. Production costs rise with increased investment of resources into the materials; *e.g*. *Sphagnum* plugs (grown-on for longer in the greenhouse) are more expensive than beads and gel. There is flexibility in choosing the density of application, depending on how quickly *Sphagnum* cover is required and the financial budget. The unit cost of micropropagated *Sphagnum* is reducing fast as production quantities rise; prices have fallen by 50 % in the last three years and are likely to fall further in the future.

*Cost-benefit considerations*

Of the three forms of micropropagated *Sphagnum*, plugs and gel were most successful to establish and increase cover over the surface. However, taking into account the amount of applied *Sphagnum* biomass in each product, the ease of application and, therefore, its cost, beads proved the most cost effective at approximately £0.16 *per* 1 % cover $cm^{-2}$, whereas gel costs £0.30 *per* 1 % cover and plugs £0.85 *per* 1 % cover $cm^{-2}$ (based on cover data from Trial D reported in Figure 9). This cost-benefit analysis probably over-estimates the cost of gel because of the high application rate which restrains its ability to spread and increase cover. It should be noted that the treatments were not normalised for the quantity of *Sphagnum* biomass within the different products tested (see Methods).

*Labour costs*

The cost of labour for application of the different products varies with topography, application density and method. Example costs for application on upland areas in the UK are: approximately £60 $ha^{-1}$ for beads

Table 4. Indicative costs (in GBP/pounds sterling) of micropropagated *Sphagnum* materials in 2018 and their usual methods of application (Micropropagation Services Ltd.).

| *Sphagnum* form | Cost *per* unit | Quantity ($ha^{-1}$) | Cost ($ha^{-1}$) | Application method |
|---|---|---|---|---|
| beads | £10 *per* litre | 35–200 litres | £350–£2,000 | by hand |
| gel | £10 *per* litre | 35–5,000 litres | £350–£50,000 | backpack or machine |
| plugs | £0.40–£0.50 *per* plug | 1,250–10,000 plugs | £500–£5,000 | by hand |


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at 35 L $ha^{-1}$, and £150 $ha^{-1}$ for plugs at 1,250 $ha^{-1}$. A full costing should also take into account delivery to the general locality as well as logistics for moving materials to the (often poorly accessible) sites. Transport of large volumes of *Sphagnum* propagules to various remote upland blanket bogs and poorly-accessible cut-over lowland peatlands has often been by helicopter (in the uplands) and soft-track motorised vehicles. Once at the field site, the mode of *Sphagnum* application may be relatively simple. Typically, beads are broadcast by hand, plugs inserted individually by hand, and gel applied from a backpack sprayer or similar device delivering small-volume 'blobs' (Figure 11). Recent technical advances have produced a '*Sphagnum* Application Machine for BeadaGel™', towed by a soft-track buggy, which was made for the MoorLife 2020 restoration project in the UK Southern Pennine hills (Figure 12). Machines for large-scale application of micropropagated *Sphagnum* materials are under development.

Figure 11. *Sphagnum* application to peat surface in 'nurse' vegetation using a backpack 'blobbing' machine at Cadishead (Lancashire Wildlife Trust).

Figure 12. Prototype planting machine for Beadagel™ being trialled by the MoorLife 2020 project (National Trust and Moors for the Future Partnership). Inset photo: grooves cut into surface vegetation to ensure that *Sphagnum* gel contacts the peat surface.

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Author for correspondence: Professor Simon J.M. Caporn, School of Science and the Environment, Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester M1 5GD, UK
Tel: 0161 247 3661; Email: S.J.M.Caporn@mmu.ac.uk


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