Sphagnum mosses cultivated in outdoor nurseries yield efficient plant material for peatland restoration

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SUMMARY

Sphagnum mosses are often reintroduced for peatland restoration or needed for the initiation of cultivation basins for *Sphagnum* farming. Finding *Sphagnum* dominated peatland where plant collection is permitted can be challenging and hampers peatland restoration in some regions. Theoretically, starting from small initial collections in natural areas, *Sphagnum* could be multiplied at *Sphagnum* cultivation sites and then be used as donor plant material for restoration. However, it is uncertain whether cultivated *Sphagnum* possesses the same regeneration capacity as moss fragments originating from natural peatlands. In this study we compared the establishment of *Sphagnum* mosses and peatland plant diversity on experimental plots that were revegetated with cultivated *Sphagnum* and *Sphagnum* originating from natural peatland. We found that reintroducing cultivated *Sphagnum* carpets of thickness > 5 cm and carpets collected from natural peatlands resulted in the same *Sphagnum* establishment. The cover of vascular plants and the diversity of peatland plants were similar in plots restored using cultivated *Sphagnum* and plots that were revegetated with plant material collected from natural peatland. If the cultivated plant material is to be used for restoration purposes, the donor site for initiating the *Sphagnum* cultivation site should contain a high peatland plant diversity.

KEY WORDS: diaspores, donor site, paludiculture, seeding plant material, *Sphagnum* farming

INTRODUCTION

In the past 25 years, there has been a growing amount of research concerning bog restoration (Chimner *et al.* 2017) and *Sphagnum* cultivation, also called *Sphagnum* farming (Gaudig *et al.* 2018). This increasing interest in the reintroduction of *Sphagnum* mosses translates into an increasing demand for *Sphagnum* donor plant material. In countries like Canada, where peatlands are widespread and abundant, collecting mosses from a natural peatland (donor site) is generally permitted. After plant collection, the *Sphagnum* remaining at donor sites is expected to recover within ten years (Guêné-Nanchen *et al.* 2018). However, in countries where peatlands are scarce and where *Sphagnum* species are rare or legally protected, finding a donor site is more challenging depending on the protection status (Caporn *et al.* 2018).

To cope with shortage of donor propagules, one research avenue is to micropropagate *Sphagnum* tissues in the laboratory (details of the method not disclosed; Caporn *et al.* 2018) or by axenic *in vitro* cultivation (Beike *et al.* 2015). This type of cultivation technique requires only very small quantities of source material and rapidly produces propagules that are free of potential diseases and other plant diaspores. Establishment success in the field is variable (ranging from almost no survival to 99 %) depending on the form of micropropagated propagule used and the site conditions (Caporn *et al.* 2018). Another avenue is to cultivate *Sphagnum* outdoors in *Sphagnum* cultivation sites (Gaudig *et al.* 2014, Pouliot *et al.* 2015, Gaudig *et al.* 2017, Hoshi 2017, Gaudig *et al.* 2018). Given the initial availability of *Sphagnum* fragments to establish cultivation, outdoor *Sphagnum* farming is possible and has the potential to rapidly increase the *Sphagnum* biomass, especially if water inputs and outputs are controlled (Pouliot *et al.* 2015, Gaudig *et al.* 2017). In Germany, cultivated *Sphagnum* has been used as the donor plant material for setting up new cultivation basins and expanding the cultivation area of a *Sphagnum* farming site with optimised hydrology (Greta Gaudig, personal communication). To date, however, outdoor cultivated *Sphagnum* has never been rigorously tested as a donor plant material for restoration purposes. The goal of the study reported here was to answer the following questions:

1) Does cultivated plant material possess the same regeneration potential as material collected from a natural peatland?
2) At what stage is cultivated plant material ready to be harvested?
3) Does the use of cultivated plant material reduce

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plant diversity in the restored sites, compared to the use of donor plant material collected from a natural peatland?

We expected cultivated plant material to possess the same regeneration potential as plant material collected from a natural peatland, as soon as the cultivated *Sphagnum* carpet reached the same thickness as the layer of material collected from the natural peatland (~ 10 cm); and that the diversity of plots revegetated with cultivated plant material would be similar to that of plots revegetated with *Sphagnum* carpets collected from a natural peatland.

METHODS

Study site

The experiment was carried out on a vacuum-extracted peatland in eastern Canada (New Brunswick; 47° 49' N, 64° 38' W). This region is subject to the Atlantic maritime climate, which is relatively cool and humid. During the three years of the experiment, average air temperature was 5.1 °C and average annual precipitation was 1298 mm (Environment Canada 2018). The experimental plots (= experimental units) were located on a rewetted portion of the peatland that was adjacent to a restored sector. The residual peat was ~ 45 cm deep with a von Post humification of H5, pH 3.3 and electrical conductivity 113 µS cm^{-1}. Water table depth was not monitored during the experiment, but repeated observations prior to implementation of the experiment had shown that the hydrology of the experimental site was representative for sites that are used for peatland restoration after peat extraction.

Experimental design

The experiment was set up in a complete randomised block design (to minimise the effect of a gentle slope across the experimental site), replicated six times. The area of each experimental unit (EU) was 6 m^2 and there were 36 EUs in total. Donor plant material, composed of *Sphagnum* mosses along with seeds and rhizomes of other peatland plants, was harvested from six different donor sectors within the same peatland complex. Five of the donor sectors were cultivation basins of different ages (see Table 1 for characteristics of the cultivated donor material and Pouliot *et al.* (2015) for a detailed description of the cultivation basins), while the sixth was an undisturbed portion of the peatland (NAT; Table 1). Donor vegetation (including vascular plants and true mosses) was harvested by hand down to the residual peat surface in the *Sphagnum* cultivation basins or, in the natural peatland, to a thickness corresponding to the maximum thickness of moss carpet collected from the cultivation basins (about 13 cm in B8; Table 1). An area defined by a 0.5 m^2 quadrat was harvested for each EU (6 m^2), resulting in an introduction ratio of 1:12, as recommended to practitioners for restoration (Quinty & Rochefort 2003). The plant material was spread evenly across the EU by hand, covered with wheat straw (3000 kg ha^{-1}), then anchored to the ground with a net.

Monitoring

Establishment of the vegetation was evaluated three growing seasons after reintroduction of the plant material. Cover of non-vascular plants was evaluated in ten systematically distributed quadrats (25 × 25 cm) *per* EU, while cover of vascular plants was evaluated in two (1 × 1 m) quadrats. All plant species were identified to the lowest taxonomic level possible and cover (%) was recorded.

Statistical analyses

One-way ANOVAs were performed to evaluate the effect of donor material origin on plant establishment. The variables analysed were: mean cover *per* EU of *Sphagnum* and vascular plants, and plant α-diversity (total number of species). The MIXED procedure of the SAS software package (SAS Statistical System Software, v. 9.4, SAS Institute Inc., Cary, NC, USA) was used. Homogeneity and normality of variances was ensured by modelling the variance with the GROUP statement of the REPEATED function and degrees of freedom were adjusted accordingly. If significant differences were found between reintroduced plant material origins (alpha = 0.01), a Dunnett test was performed to compare plant material originating from the cultivation basin with that from the natural site (considered as a control).

RESULTS

After three growing seasons, the establishment of plant material originating from the older cultivation basins B6 and B8 was similar to the establishment of plant material collected from the natural portion of the peatland. The moss carpet in these two cultivation basins exhibited characteristics that were close to those of moss carpets in natural peatlands (*i.e.* thickness $\geq$ 5 cm and completely covered by *Sphagnum* capitula; Table 1). On the other hand, the establishment of plant material originating from the younger basins B2, B3 and B4 was 4–12 times lower than for plant material collected from natural peatland ($F = 81.5$; $p < 0.0001$; Figure 1). The moss

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Table 1. Main characteristics of the reintroduced plant material (mean values ± SE). For dominant species, the data presented are mean % cover ± SE.

	B2	**B3**	**B4**	**B6**	**B8**	**NAT**
Origin of donor material	*Sphagnum* cultivation basins[1]					Natural peatland
Number of years of cultivation	2	3	4	6	8	NA
Moss carpet characteristics						
Sphagnum thickness (cm)	< 1	< 1	1 ± 0	4.7 ± 1.0	13.0 ± 1.8	12.8 ± 1.9
Sphagnum cover (% ± SE)	56 ± 22	20 ± 15	77 ± 5	91 ± 8	95 ± 7	99 ± 3
Dominant species[2]	RUB (35 ± 21) FLA (16 ± 13) Lei ano (3 ± 2) Pol str (2 ± 1)	RUB (12 ± 13) FLA (6 ± 4) Lei ano (4 ± 4) Pol str (2 ± 2)	RUB (58 ± 21) FLA (10 ± 9) MAG (8 ± 12) Lei ano (7 ± 3) Pol str (1 ± 1)	RUB (63 ± 23) FUS (14 ± 7) FLA (14 ± 22) Pol str (3 ± 1)	RUB (72 ± 16) MAG (17 ± 13) Pol str (5 ± 5)	RUB (62 ± 34) FUS (32 ± 31) Pol str (2 ± 1)
Alpha diversity (number of species)	7	8	9	6	5	9
Vascular plants						
Vascular plant cover (%)	9 ± 7	6 ± 2	3 ± 3	23 ± 11	20 ± 9	39 ± 14
Dominant species[3]	Kal ang (5 ± 5) Dro rot (2 ± 1)	Dro rot (3 ± 2)	Dro rot (1 ± 1)	Eri vag (11 ± 9) Eri ang (9 ± 6) Vac oxy (2 ± 1) Rho gro (1 ± 1)	Eri ang (16 ± 7) Vac oxy (3 ± 1) Cha cal (1 ± 1)	Emp nig (14 ± 9) Cha cal (10 ± 6) Mai tri (8 ± 4) Vac oxy (3 ± 2)
Alpha diversity (number of species)	12	11	8	12	6	13

[1] The *Sphagnum* cultivation basins were located in a block-cut peatland and were established 2–8 years prior to the start of the experiment described in this article. The plant donor material that was used to initiate the cultivations was collected in the naturally revegetated trenches adjacent to the cultivation basins. A complete description of the steps performed to implement the cultivation basins, as well as information about growing conditions, can be found in Pouliot *et al.* (2015).

[2] FLA: *Sphagnum flavicomans* (Cadot) Warnst.; FUS: *Sphagnum fuscum* (Schimp.) Klinggr.; Lei ano: *Leiomylia anomala* Engel & Graggins; MAG: *Sphagnum magellanicum* Brid.; Pol str: *Polytrichum strictum* Menzies *ex.* Brid.; RUB: *Spagnum rubellum* Wilson. Bryophyte nomenclature follows Faubert (2012, 2013).

[3] Cha cal: *Chamaedaphne calyculata* (L.) Moench; Dro rot: *Drosera rotundifolia* L.; Emp nig: *Empetrum nigrum* L.; Kal ang: *Kalmia angustifolia* L.; Eri ang: *Eriophorum angustifolium* Honck.; Eri vag: *Eriophorum vaginatum* L.; Mai tri: *Maianthemum trifolium* (L.) Sloboda; Rho gro: *Rhododendron groenlandicum* (Oeder) Kron & Judd; Vac oxy: *Vaccinium oxycoccos* L. Vascular plant nomenclature follows Brouillet (2010+).

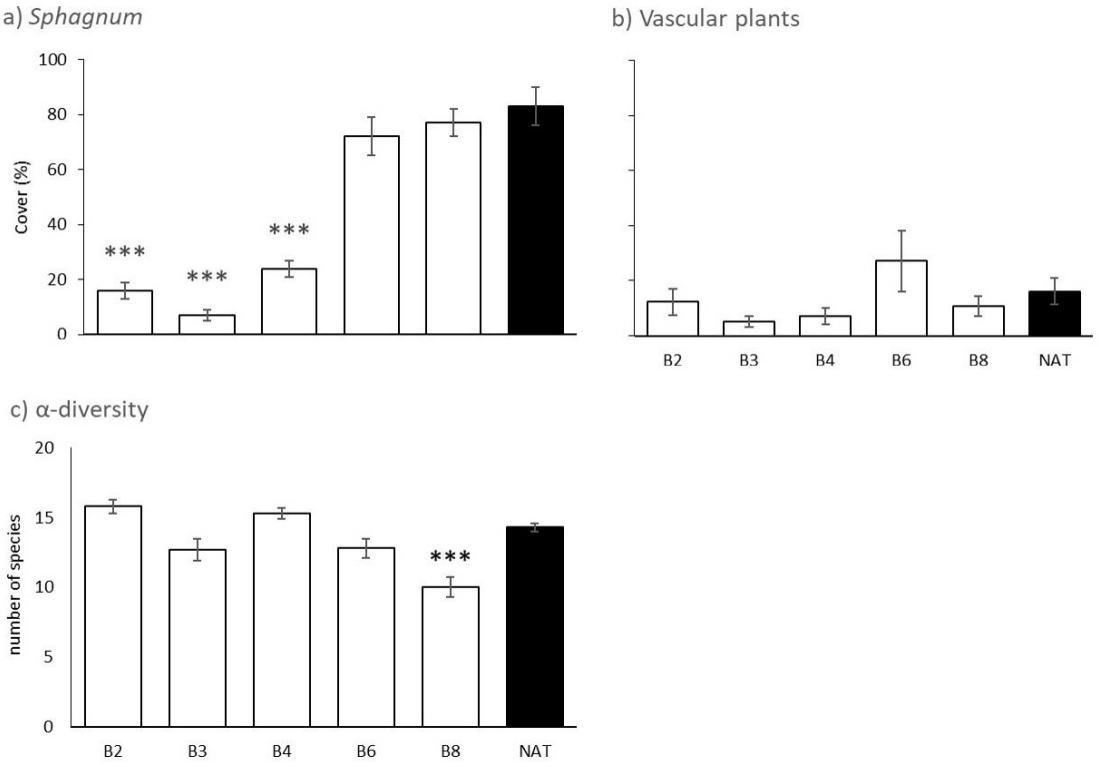

Figure 1. Mean (± SE) plant cover (a and b) and α-diversity (c) in EUs revegetated with plant material originating from six different sectors of the same peatland complex. B2, B3, B4, B6 and B8 were cultivated *Sphagnum* mosses while NAT was plant reintroduction material collected in a natural peatland. Significant differences (Dunnett test) in cover between the cultivated reintroduction material (B2–B8) and the control treatment (NAT) are indicated by stars: *** = $p < 0.0001$.

carpets in these three cultivation basins were characterised by an incomplete cover of *Sphagnum* capitula and thickness ≤ 1 cm (Table 1). No matter which *Sphagnum* species was dominant in the donor site, the dominant *Sphagnum* species in the EUs were *Sphagnum rubellum* and *Sphagnum fuscum*.

Even if the vascular plant cover in the cultivation basins was lower than in the natural peatland (Table 1), all EUs revegetated with cultivated *Sphagnum* had similar vascular plant cover to EUs revegetated with plant material originating from natural peatland, which was 16 ± 5 % (mean ± SE; F = 3.5; p = 0.04; Figure 1). The most abundant vascular plants observed in the EUs were *Eriophorum vaginatum* (7 ± 5 %) and *Vaccinium oxycoccos* (3 ± 2 %). Plant alpha diversity in EUs revegetated with plant material harvested from *Sphagnum* cultivation basins was generally similar that in EUs revegetated with plant material collected from the natural peatland, *i.e.* 14 ± 1 species (Figure 1). The only treatment exhibiting a significantly lower diversity (F = 23.1; $p < 0.0001$) was the one revegetated with plant material originating from B8. All species identified in the EUs were peatland plants.

DISCUSSION

Our study shows that cultivated *Sphagnum* can be used as a donor plant material for restoration of *Sphagnum* peatland. *Sphagnum* cover and lawn thickness are found to be better indicators of readiness for harvest than the age of the cultivated carpet, as pointed out by Gaudig *et al.* (2017). In order to reach the same plant establishment as that resulting from the introduction of *Sphagnum* collected in natural peatlands, we determine that the carpet of cultivated *Sphagnum* must be fully covered by *Sphagnum* capitula and be at least 5 cm thick. This is only half the 10 cm thickness usually recommended for peatland restoration (Quinty & Rochefort 2003); however, this recommendation is not based on a biological threshold, but rather on the technical constraint of working with heavy machinery. The surprisingly high establishment success of restoring with a *Sphagnum* carpet only 5 cm thick can be partly explained by the manual plant reintroduction resulting in smaller losses of plant material than occurs during the mechanical reintroduction used for large scale restoration projects. In this particular *Sphagnum* cultivation site,

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where water input was solely through precipitation, six to eight years of cultivation were needed for the newly formed *Sphagnum* carpet to develop the required characteristics, but less time may be necessary in cultivation sites with optimised hydrology. Indeed, recent results from *Sphagnum* cultivation sites in Germany and Canada equipped with automated irrigation/drainage devices have shown that a dense 5 cm thick *Sphagnum* carpet can be generated in 3–4 years (Gaudig & Krebs 2017, Gaudig *et al.* 2017, Hugron *et al.* 2017).

Our study also shows that using cultivated *Sphagnum* material can result in a plant diversity similar to that resulting from the use of plant material originating from a natural peatland, and that the reintroduced plant community is composed exclusively of peatland plants. However, if the diversity of the donor site is lower than that of regional natural peatlands (*e.g.* B8; Table 1), the restored site is likely to exhibit lower diversity, highlighting the importance of choosing a diverse donor site for the *Sphagnum* farming site if it is intended that the plant material will used for restoration purposes. The lower plant diversity in B8 compared to the other donor sites probably arises mainly from the collection and reintroduction methods used to initiate the cultivation basin eight years before the start of this experiment. In this particular case, the plant material was collected without shredding the plants, resulting in un-fragmented plant material rather than the fragmented plant material used for the other cultivation basins. The plant material was also spread by hand, instead of mechanically with a manure spreader, probably resulting in a targeted reintroduction of bryophytes because whole ericaceous shrubs and tussock-forming plants were discarded. Nevertheless, even if a diverse donor site is chosen, one should not expect a complete recovery of diversity because of the potential presence of recalcitrant plants, as demonstrated in other ecosystems (Koch 2007). This topic should be investigated further in peatland restoration as, in the current study, we identified *Maianthemum trifolium* and *Larix laricina* as species found in the natural peatland donor site that had not established in the EUs after three years (results not shown). These species were also absent from the cultivation basins used as donor sites.

Cultivation of *Sphagnum* mosses as plant material for reintroduction can thus be considered as an interesting reclamation option for degraded peatlands, not only for the production of undecomposed fibres for use in horticultural growing media (Blievernicht *et al.* 2012, Jobin *et al.* 2014), but also for the establishment of diaspore nurseries for future restoration projects. Establishing diaspore nurseries could be of particular interest in countries where donor sites for ecological restoration are scarce because of lack of natural peatlands or legal protection. Establishing nurseries in those countries would also have the beneficial side effect of reducing restoration costs because the purchase of *Sphagnum* diaspores can be one of the most expensive elements of *Sphagnum* reintroduction projects (Wichmann *et al.* 2017). Even in countries where natural peatlands are sufficiently abundant, establishing local nurseries near future restoration sites could be beneficial as the restoration cost is known to increase significantly with transportation distance (Quinty & Rochefort 2003). Because the introduction ratio for the establishment of *Sphagnum* cultivation sites is usually 1:10, meaning that 1 m^2 of donor plant material can be spread on 10 m^2 of the area to be cultivated, only a small quantity of donor plant material collected from a natural peatland would be needed to establish a nursery. Theoretically, the area of the nursery could be increased ten-fold each time the cultivated *Sphagnum* carpet reached a thickness of 5 cm, possibly every 3–5 years according to previous trials (Gaudig & Krebs 2017, Gaudig *et al.* 2017, Hugron *et al.* 2017), so that *Sphagnum* collected from only 100 m^2 of natural peatland could be multiplied to create a cultivated area of 1 ha after 6–10 years and 100 ha after 12–20 years.

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