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Moss Regeneration for Fen Restoration: Field and Greenhouse Experiments

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Abstract

Fen bryophytes are an important component of natural fens and should be included in fen restoration projects. The goal of this study was to examine the regeneration capabilities of nine bryophytes common to moderate-rich and poor fens in North America. A greenhouse experiment was carried out to examine the limitations and optima for the regeneration of fen bryophytes under different light and water regimes. A field experiment tested these same bryophytes in the presence of three potential nurse-plants. In the greenhouse experiment, the presence of shade increased regeneration success for eight out of nine species. A high water level was ideal for the regeneration of the majority of species tested. In the field experiment, *Sphagnum* species had the highest regeneration, and all species had higher regeneration under a dense canopy of herbaceous plants. Fen bryophytes show good potential for use in restoration projects because the tested bryophytes regenerated well from fragments.

Key words: brown mosses, moderate-rich fen, peatland, plant reintroduction, poor fen, *Sphagnum* mosses.

Introduction

Fen restoration is a good example of the subjectivity inherent in restoration (Higgs 2003). Vascular plants have largely been given priority (Pfadenhauer & Grootjans 1999; Cooper & MacDonald 2000; Kotowski et al. 2001; Lamers et al. 2002), even though bryophytes are an equally important element of fen vegetation (Mitsch & Gosselink 2000). Incorporating bryophytes in fen restoration projects will increase species richness and vertical diversity, creating a vegetation structure closer to undisturbed fens. Additionally, bryophytes are important to the ecosystem functioning of fens. They play an important role in water balance, energy flow, nutrient cycling, and the creation and modification of habitats occupied by other organisms (Longton 1984). Bryophytes have also been shown to produce more biomass and decompose more slowly than vascular plants in fen systems, contributing greatly to carbon storage (Vitt 2000). Fen bryophytes play an important role in the species composition and function of fen systems and deserve more attention in fen restoration research.

Many articles have been published on the regeneration capacities of *Sphagnum* mosses common to bogs (Rochefort et al. 1995; Campeau & Rochefort 1996; Bugnon et al. 1997; Buttler et al. 1998); however, few articles have been published on the regeneration capacities of fen bryophytes. Two studies (Poschlod & Schrag 1990; Li & Vitt 1994) recognized that fen bryophytes were capable of vegetative reproduction. However, these articles do not examine the effect of water level or the presence of a protective cover on the regeneration of mosses, factors that are crucial to the success of bryophyte regeneration in the context of restoration (Rochefort et al. 2003). Mälson and Rydin (2007) examined regeneration capabilities of rich fen bryophytes for fen restoration and found that a protective cover increased recolonization success and that even small changes in hydrology had an effect on biomass growth. Currently, no information exists on the regeneration of bryophytes for the restoration of poorer types of fen.

The microclimatic conditions of fen restoration sites can vary greatly depending on the prior land use and the amount of time since abandonment. On one extreme, restoration sites can be several hundred hectares of bare peat, as is the case for cutaway peatlands (Sliva & Pfadenhauer 1999; Cobbaert et al. 2004). The bare peat surface of an abandoned peatland is an extremely harsh environment, where water levels and temperature fluctuate greatly (Price et al. 2003). However, if these cutaway peatlands have been abandoned for several years, they are often spontaneously colonized by pioneer vegetation (Famous et al. 1991; Salonen et al. 1992), creating a more stable microclimate. On the other extreme, fen restoration is also carried out on former agricultural land, where reintroduced mosses will have to compete with a dense herbaceous layer (Kotowski et al. 2001; Lamers et al. 2002). The ideal reintroduction time minimizes the mortality due to adverse abiotic factors while minimizing mortality due to competition with spontaneously established species (Prach et al. 2001).

Microclimatic conditions have had a big impact on the success of moss regeneration in bog restoration. The protection of a straw mulch layer is essential to the regeneration of *Sphagnum* mosses (Rochefort et al. 2003).

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Moreover, the presence of the pioneer moss, *Polytrichum strictum*, which stabilizes the substrate and the microclimate, significantly improves the regeneration success of *Sphagnum* bryophytes (Groeneveld et al. 2007). The improved establishment of one plant through the presence of a nurse-plant, which is usually a pioneer species, has been observed in a variety of harsh environments (Bruno et al. 2003). Because cutaway peatlands with minerotrophic residual peat are quickly colonized by spontaneous vegetation (Graf et al. 2008), the nurse-plant effect could have a considerable impact on reintroduced vegetation.

In this study, we examined the regeneration capabilities of nine fen bryophytes common to North American poor and moderate-rich fens. Our goals were to examine the environmental conditions that enable vegetative regeneration and to study the effect of microclimate on the bryophyte regeneration. Greenhouse and field experiments were carried out in order to respond to the following questions:

(1) What are the optimum and limiting conditions (water level and shading) for the regeneration of nine bryophytes common to North American poor and moderate-rich fens?
(2) Are there differences in the abilities of these species to regenerate vegetatively?
(3) What effect do nurse-plants have on the regeneration success of the tested bryophytes?

Methods

Study Species

The nine fen bryophytes chosen are common in moderate-rich and poor fens of boreal North America and represent different realized niches. *Tomenthypnum nitens* (Hedw.) Loeske and *Sphagnum centrale* C. Jens. in Arnell & C. Jens. are found in the dry areas (hummocks) of fens (Andrus 1986; Gignac et al. 1991). *Polytrichum strictum* Brid., *Dicranum polysetum* Sw., and *Pleurozium schreberi* (Brid.) Mitt are common to hummocks or dry parts of both fens and bogs (Gauthier 1980; Gignac et al. 1991). *Dicranum polysetum* is found in similar abundance as *D. undulatum* Schrad. ex Brid. in peatlands of Eastern Canada, and because of their similar structure, only *D. polysetum* was tested (Rochefort, unpublished data; Poulin et al. 1999). *Sphagnum fallax* (Klinggr.) Klinggr. is common in wet parts (lawns and hollows) of poor fens and moderate-rich fens (Andrus 1986). *Warnstorfia exannulata* (Schimp. in B.S.G.) Loeske inhabits the wettest areas of poor fens (Vitt & Chee 1990). *Aulacomnium palustre* (Hedw.) Schwaegr. and *S. warnstorfii* Russ. are present over a wide range of pH and hydrological conditions (Gignac et al. 1991).

Greenhouse Experiment

The regeneration capabilities of the above-described bryophytes were assessed in a factorial greenhouse experiment testing four water levels (at 0, −10, −20, and −40 cm), both with and without shade over a 6-month period. Shade was created using shade nets that blocked 50% of the light (Industries Harnois, St.-Thomas-de-Joliette, Québec, Canada). Fifty percent shade corresponds to the average total vegetation cover of abandoned fens in North America (see also Table 3; Graf et al. 2008). The experimental design was a complete randomized block design with four blocks. Each plastic container (61 × 47 × 50.8 cm) was divided into nine subplots, and one small portion was left bare for taking abiotic measurements. Water level was controlled via plastic cylinder (6 × 3 × 51 cm) inserted into the peat, which was perforated with small holes. One-centimeter holes were drilled into the outside wall of the chamber at a specified height to allow the containers to drain to the appropriate water level after watering.

Bryophytes were collected from natural fens 3 weeks prior to experimental setup and were kept at 4°C. Moss species were randomly assigned to subplots of each container, and 25 fragments, each 3 cm in length (including the capitula for Sphagna), were evenly distributed. Each container was watered 20 mm/week (spread evenly over three waterings per week), which corresponds to the average weekly precipitation during the vegetation season in southern Québec. We used distilled water supplemented with a modified Rudolf solution (Faubert & Rochefort 2002) 5-fold diluted to simulate field conditions (rainwater). The temperature was set to 20°C for the 14-hour photoperiod and 15°C at night. The relative humidity was 80% and was adjusted to 50% after 2 months to control cyanobacteria development. Artificial light was supplemented when the natural light was below 300 watts/m^2.

To assess the water availability, the soil water potential (at −2 cm) and volumetric water content were measured weekly for each container. Soil water potential was measured using a tensiometer (Soil Measurement Systems, Tucson, AZ, U.S.A.) and water content using a WET sensor (Model 1.2 Delta-T Devices Ltd., Cambridge, U.K.) connected to a moisture meter type HH2 (Model 3.0, Delta-T Devices Ltd.). The temperature of two containers from each block, one with a shade net and the other without, was measured hourly for 60 days during the experiment using StowAway data loggers (Onset Computer Corporation, Pocasset, MA, U.S.A.). The relative humidity of the air 1 cm from the surface of the same containers was measured using a humidity and temperature meter Model 4465CF (Extech Instruments, Melrose, MA, U.S.A.) on three occasions during the experiment.

Peat samples were taken from each block (samples from each container were pooled). The samples were analyzed for pH, electrical conductivity, and concentrations of sodium (Na), iron (Fe), calcium (Ca), magnesium (Mg), total phosphorus (P), and nitrogen ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$). An Acumet Model 10 probe was used to measure pH (Fisher Scientific, Pittsburgh, PA, U.S.A.). Electrical conductivity was measured with an Orion Model 122 conductivity meter (Thermo Electron Corporation, Waltham, MA, U.S.A.), adjusted to 20°C, and corrected for hydrogen ions (Sjörs 1952). These measures were carried out using a 4:1 mixture of bidistilled water and peat. The P was extracted using the Bray 1 method (Bray & Kurtz 1945), and the extract was analyzed using flow injection analysis (Bogren & Hofer 2001). An inductively coupled argon plasma spectrophotometer (ICP-OES Optima 4300DV; Perkin Elmer, Waltham, MA, U.S.A.) was used to determine Na, Fe, Ca, and Mg concentrations (Mehlich 1984). The N content was determined following the Kjeldahl method (Bremner & Mulvaney 1982). The peat chemistry (Table 1) is characteristic of poor fen peat (Vitt & Chee 1990) and is representative of residual minerotrophic peat from cutaway peatlands in North America (Wind-Mulder & Vitt 2000; Graf et al. in press). The pH and conductivity were tested again at the end of the experiment and had not significantly changed.

Regeneration was estimated by assessing the percent living cover of each moss species after 6 months. For the acrocarpous mosses, all living bryophytes were the result of new regeneration because the fragment of the main stem served as the foundation of the new growth but rapidly died. However, the fragments of *Sphagnum* species and the pleurocarpous bryophytes could continue to grow. Therefore, it was difficult to distinguish new growth. Due to the inherent differences in the morphological growing habits of the bryophytes, each moss species was analyzed separately using analysis of variance (ANOVA) and calculated by the generalized linear model (GLM) procedure of SAS and a priori polynomial contrasts (SAS Statistical System software, version 9.1; SAS Institute, Inc., Cary, NC, U.S.A.).

Field Experiment

The field experiment was carried out over 2 years (2005 and 2006) on a cutaway peatland in southern Québec (lat 47° 45′N, long 69° 30′W). This site is part of a large complex of ombrotrophic bogs interspersed with *Alnus* swamps (Gauthier & Grandtner 1975) and has been classified as a low boreal peatland (National Wetlands Working Group 1988). The regional climate is characterized by cold winters and warm summers with January and July mean temperatures of −13 and 18°C, respectively. The mean annual precipitation is 963 mm, of which 72% falls as rain (Environment Canada 2002). The peat characteristics (same methodology as above) of the residual minerotrophic peat layer can be seen in Table 1.

The field experiment was a randomized block, split-plot design with nurse-plant treatments as the main factor and the bryophytes species as the subplot factor. The nurse-plant treatments were as follows: (1) *Scirpus cyperinus* (L.) Kunth; (2) *Equisetum arvense* L.; (3) *Polytrichum strictum*; (4) straw mulch cover; and (5) control. The first three treatments are plants that frequently spontaneously colonize cutaway minerotrophic peatlands in Canada (Graf et al. 2008). These plants additionally represent three distinct vegetation structures: *S. cyperinus* exhibits a large, tussock-forming structure, *E. arvense* is a small, early-successional plant, and *P. strictum* is a pioneer moss species. The nurse-plant treatments were repeated five times for a total of 25 plots, measuring 5 × 6 m with a 2-m buffer between plots.

The nurse-plant treatments were established prior to experiment start (2004). The experimental areas were scraped and leveled to homogenize the surface and remove any vegetation. In June, monocultures of *S. cyperinus*, *E. arvense*, and *P. strictum* were established. Mature *Scirpus* tussocks (circa 1.5 m high) were transplanted to the designated plots from on-site colonies. *Equisetum* was transplanted using rhizomes also collected on site. *Polytrichum strictum* plots were created by introducing moss fragments in a 1:5 donor to recipient ratio. The *P. strictum* plots were covered with straw to improve their regeneration (Groeneveld & Rochefort 2005), and all nurse-plant plots were lightly fertilized with rock phosphate (15 g/m^2) to aid establishment (Rochefort et al. 2003).

The following year, fragments of the study species, excluding *P. strictum*, were introduced in a 1:10 donor to recipient ratio onto eight subplots of 1.5 × 1.5 m. The subplots were located in the center of the main plots with a buffer zone of at least 1 m to the edge of the main plot to ensure similar treatments. The soil water potential was measured on 10 plots at −2 cm every 2 weeks during the growing season of 2005. Temperature and volumetric water content were measured from late June to mid-August 2005 (same instruments as above).

The regeneration of each moss was assessed by estimating the percent cover of each moss (using two 25 × 25–cm quadrats per subplot) at the end of each growing season. At the same time, the percent cover of the nurse-plant treatments, spontaneous vegetation, and total vegetation present was assessed (16 quadrats of 50 × 50 cm per main plot). This information was used to assess the success of each nurse-plant's establishment. Three outliers, main

Table 1. The means (± SE) of chemical properties of the peat from the greenhouse and field regeneration experiments show that the peat used for the experiments was a type of poor fen peat.

	Ca (mg/g)	*Mg (mg/g)*	*Fe (mg/g)*	*Na (mg/g)*	*P (mg/g)*	*N-NO$_3^-$ (mg/g)*	*N-NH$_4^+$ (mg/g)*	*Conductivity (μS/cm)*	*pH*
Greenhouse	3.8 (± 0.1)	1.15 (± 0.02)	0.5 (± 0.03)	0.82 (± 0.01)	44.4 (± 0.9)	3.4 (± 0.4)	148.7 (± 7)	30.0 (± 4.8)	4.64 (± 0.02)
Field	5.6 (± 0.4)	1.16 (± 0.2)	ND	0.31 (± 0.08)	28.0 (± 9.0)	ND	ND	23.9 (± 2.5)	4.97 (± 0.07)

ND, data not available.

plots that had an exceptionally low or high percent cover (> or < M ± SD) of the nurse-plant or spontaneous vegetation, were eliminated from the analyses. The regeneration (% cover) of the bryophytes was compared among moss species and among nurse-plant treatment. For the field experiment, the regeneration of the moss species could be compared because the mosses were reintroduced using the same donor to recipient ratio, not a specific number of fragments, and because fewer factors were being tested. The analysis was carried out using the MIXED (recommended for split-plot designs) and least significant difference (LSD) procedures of SAS (SAS Statistical System software, version 9.1).

Additionally, a regression analysis was carried out in order to detect a possible relationship between the regeneration of the introduced bryophytes and the vegetation cover. The average cover of all introduced bryophytes was compared with both the cover of the total vascular plants and the cover of each nurse-plant by itself. The average cover of all introduced mosses was used because of the great variation between the regeneration of bryophyte species. For this analysis, the outliers were included because the variation between treatments was accounted for. The soil water potential and the volumetric water content for each main treatment were compared using an ANOVA and protected LSD procedure of the SAS version 9.1.

Results

Greenhouse Experiment

All bryophytes were capable of regenerating vegetatively; however, some had more specific requirement than others. All species, except *Polytrichum strictum*, showed significantly higher regeneration under shade (Table 2; Fig. 1). Most species, except *P. strictum*, had the highest cover for the wettest treatments (Table 2). Water levels did not significantly affect the regeneration success of two species, *Warnstorfia exannulata* and *Sphagnum centrale* (Table 2; Fig. 1).

Although almost all species had a higher regeneration cover under shade, *Pleurozium schreberi* and *Warnstorfia exannulata* strictly required shade for regeneration; their covers were close to 0 for all treatments in full light. *Aulacomnium palustre* also showed a much higher percent cover for the shaded, wet treatments (0 and −10 cm water levels). However, unlike *P. schreberi* and *W. exannulata*, *A. palustre* did successfully regenerate in full-light conditions, even if the percentages were lower (Fig. 1). Two species, *Dicranum polysetum* and *Tomenthypnum nitens*, were capable of regenerating in a variety of conditions but, at the end of 6 months, had relatively low covers, especially for dry treatments.

The *Sphagnum* species were the most successful in regeneration; each had covers close to 100% for the shaded treatments with water levels at 0 and −10 cm (Fig. 1). Even in full-light conditions, *S. centrale* had an exceptionally high cover (42%) even for the harshest treatment (full light with −40 cm water level).

The temperatures of the treatments in full light were higher than those under shade nets. Fifty percent of the time, the daily maximum temperature was equal to or greater than 27°C for the shaded treatments compared to 31°C for the full-light treatments. There was also a clear difference between the air humidity underneath shade nets (72% ± 3) and full light (65% ± 3). The higher regeneration for the wetter treatments was indeed due to greater water availability. The water potentials (± SE) were −4.3 (± 0.3), −8.6 (± 0.5), −16.4 (± 0.7), and −33.7 (± 0.9) for the water levels 0, −10, −20, and −40 cm, respectively. Volumetric water content showed no difference in the 0 and −10 cm water levels (both were 74%). Probably, the difference was smaller than measurement errors. The −20 cm corresponded to 65% volumetric water content and −40 cm to 44%.

Field Experiment

Nurse-Plant Establishment. After two growing seasons, *Scirpus* showed the highest percent cover (circa 50%) followed by *Equisetum* and *Polytrichum* (20 and 9%, respectively; Table 3). The control plots experienced the highest invasion by spontaneous vegetation (Table 3). The *Equisetum* and *Polytrichum* treatments showed comparable covers for spontaneous vegetation, whereas the *Scirpus* and straw treatments were less colonized by spontaneous vegetation. The spontaneous vegetation was dominated by *Euthamia graminifolia* (L.) Nuttall, *Agrostis scabra* Willd., *Epilobium angustifolium* L., and *Betula populifolia* Marsh.

Moss Regeneration. After one growing season, the moss covers were modest (2%); however, the bryophytes grew considerably during the second season bringing the average cover to 8%. After two growing seasons, there was significantly higher moss regeneration under the canopy of *Scirpus* than other treatments (Fig. 2A). There was no difference in moss cover among the straw, control, *Polytrichum*, or *Equisetum* treatments.

The difference between the percent covers of the different moss species was highly significant after two growing seasons (Fig. 2B). *Sphagnum warnstorfii* and *Tomenthypnum nitens* had the highest cover (circa 15%; Fig. 2B). *Sphagnum centrale*, *Dicranum polysetum*, and *Aulacomnium palustre* were slightly less successful with cover of circa 10%. *Sphagnum fallax* had a relatively low cover (circa 5%), and two species, *Pleurozium schreberi* and *Warnstorfia exannulata*, had extremely low covers of 2 and 3%, respectively (Fig. 2B).

A regression analysis was carried out in order to see whether the higher moss regeneration under a *Scirpus* canopy was due to simply higher vascular plant cover or specifically the structure of *Scirpus*. There was no relationship ($r^2 = 0.05$) between the cover of the introduced bryophytes and the total cover of vegetation, which included the nurse-plants and the spontaneous vegetation. However, when we examined the relationship between the introduced moss species and the percent cover of *Scirpus*

Table 2. ANOVAs and a priori polynomial contrasts compared the regeneration success (% cover) of treatments from a factorial design, which tested the effects of shade (no shade and 50% shade) and four water levels (WL), 0, −10, −20, and −40 cm, for nine fen bryophytes in a greenhouse experiment.

		Aulacomnium palustre		*Polytrichum strictum*		*Dicranum polysetum (log (x + 1))*		*Tomenthypnum nitens*		*Pleurozium schreberi (log (x + 1))*	
Source	*df*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Blocks	3										
WL	3	8.82	0.0006	7.55	**0.001**	18.61	**<0.0001**	5.22	**0.008**	10.10	**0.0003**
Shade	1	29.34	<0.0001	0.69	0.42	7.73	**0.01**	14.45	**0.001**	14.19	**0.001**
WL × shade	3	5.59	**0.006**	2.37	0.10	0.70	0.56	2.32	0.10	2.82	0.06
Error	21										
Total	31										
Contrasts											
Linear effect (WL)	1	14.62	**0.001**	0.00	**0.99**	55.72	**<0.0001**	15.65	**0.0007**	24.03	**<0.0001**
Quadratic effect (WL)	1	0.00	0.99	20.16	0.13	0.09	0.77	0.02	0.90	1.21	0.28
Cubic effect (WL)	1	11.84	**0.002**	2.48	0.44	0.01	0.94	0.00	0.97	5.05	0.04
Linear effect (WL) × shade	1	8.98	**0.007**	4.60	0.87	0.77	0.39	6.91	0.02	6.96	0.02
Quadratic effect (WL) × shade	1	1.24	0.27	0.03	0.13	0.52	0.48	0.01	0.93	0.07	0.79
Cubic effect (WL) × shade	1	6.55	0.02	2.48		0.81	0.38	0.03	0.87	1.44	0.24

		Warnstorfia exannulata		*Sphagnum warnstorfii (log (x + 1))*		*S. fallax (log (x + 1))*		*S. centrale*	
Source	*df*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Blocks	3								
WL	3	2.89	0.06	25.86	**<0.0001**	23.16	**<0.0001**	2.32	0.10
Shade	1	30.60	**<0.0001**	12.83	**0.0018**	18.22	**0.0003**	11.12	**0.003**
WL × shade	3	2.79	0.07	0.96	0.43	0.72	0.55	0.62	0.61
Error	21								
Total	31								
Contrasts									
Linear effect (WL)	1	0.50	0.49	73.91	**<0.0001**	59.43	**<0.0001**	2.07	0.16
Quadratic effect (WL)	1	7.23	0.014	2.67	0.12	6.58	**0.018**	3.92	0.06
Cubic effect (WL)	1	0.93	0.35	1.02	0.33	3.47	0.08	0.97	0.34
Linear effect (WL) × shade	1	0.01	0.94	0.01	0.92	0.38	0.55	0.16	0.69
Quadratic effect (WL) × shade	1	3.16	0.09	0.30	0.59	0.02	0.88	0.01	0.93
Cubic effect (WL) × shade	1	5.20	0.03	2.57	0.12	1.78	0.20	1.70	0.21

Significant *p* values (<0.05) are in bold.

cover alone, the correlation was much stronger ($r^2 = 0.50$). The other nurse-plant treatments, *Equisetum* and *Polytrichum,* showed no relationship between their covers and the moss covers ($r^2 = 0.0004$ and $r^2 = 0.004$, respectively).

Environmental Variables. The overall low regeneration rates of the bryophytes are likely due to the harsh conditions of the first growing season. The soil water potential dipped during a dry period in August 2005 from −50 to −170 mbar. Price and Whitehead (2001) found that even short periods of conditions where the water potential is below −100 mbar result in poor *Sphagnum* establishment. The volumetric water content also showed a difference between the average June reading of 63% (± 0.01) and the average mid-August reading of 36% (± 0.009). There was no significant difference in the soil water potential or the volumetric water content among the nurse-plant treatments. There was, however, a marked difference in the temperatures measured for each treatment. The control plot showed the highest daily maximum temperatures. Most of the time, the control plots were 5°C warmer than straw and *Equisetum* treatments and 10°C warmer than *Scirpus* and *Polytrichum* treatments. Although the cover of *Polytrichum* was not as high as the *Scirpus* cover (Table 3), straw mulch was added during the *Polytrichum* establishment, greatly increasing the protective cover of this treatment.

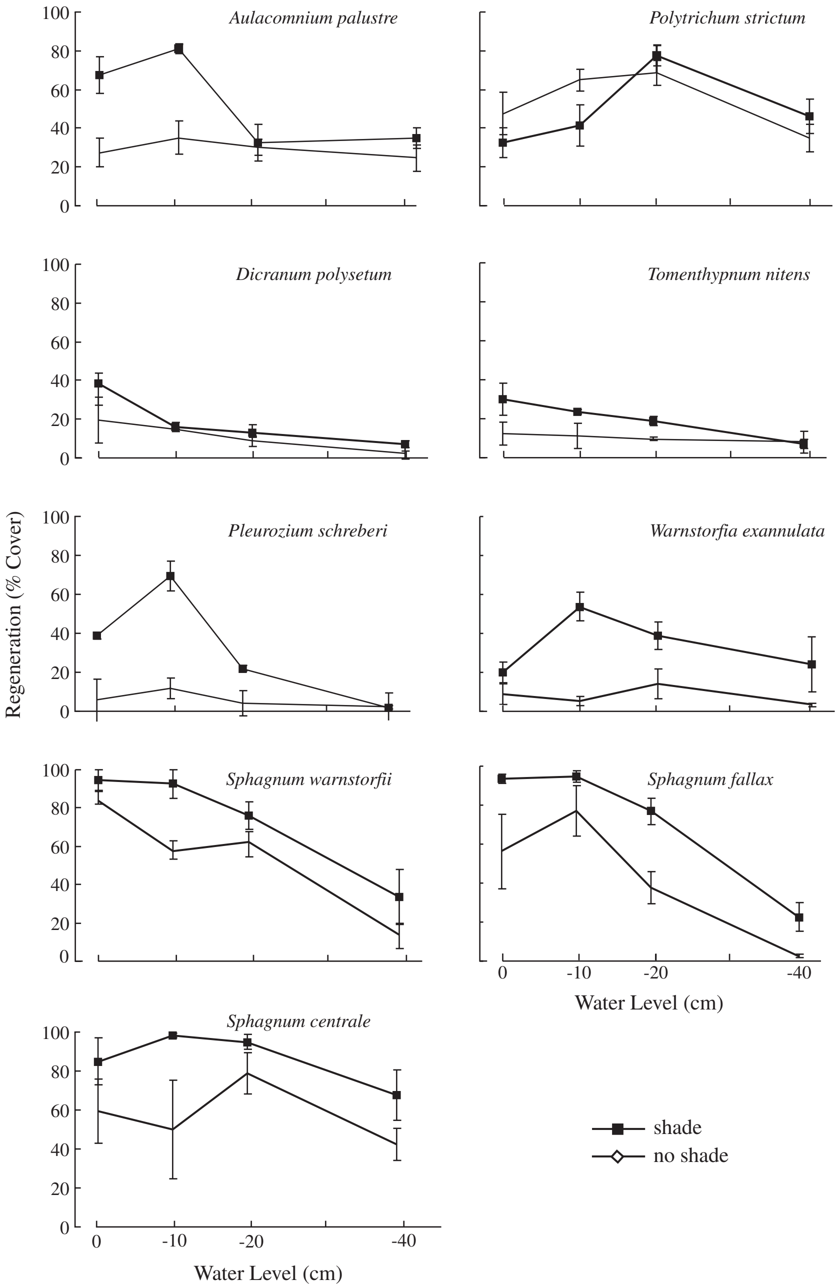

Figure 1. The regeneration (% cover) of the nine fen bryophytes tested in a greenhouse experiment. The factorial design tested four water levels in full-light and shade conditions (50% shade).

Discussion

The Effect of Shading

This experiment showed that all bryophytes (with the exception of *Polytrichum strictum*) had significantly higher regeneration under dense shade either through shade nets in the greenhouse experiment or under large herbaceous plants (*Scirpus*) in the field. The ability of the moss species to regenerate better under shade is not solely due to photoinhibition (Murray et al. 1993) but also to a moderate microclimate and moister substrate conditions. The presence of a protective cover has been shown to improve the moisture content of the substrate (Groeneveld et al. 2007).

The regression analysis showed that the presence and density of *Scirpus* were strongly related to successful moss regeneration. One confounding factor is that, due to *Scirpus*' large tussocks, the introduced mosses were applied in a greater density to the areas between tussocks on these plots. However, we believe that the higher regeneration is indeed due to the microclimate created by *Scirpus* because the difference between treatments was only detected after the second growing season. If the higher fragment density

Table 3. Percent covers of the nurse-plant treatments, spontaneous, and total vascular plant for first and second growing season.

	First Growing Season			Second Growing Season		
Nurse-Plant Treatments	*Nurse-Plant % (± SE)*	*Spontaneous Vegetation % (± SE)*	*Total Vascular Plant % (± SE)*	*Nurse-Plant % (± SE)*	*Spontaneous Vegetation % (± SE)*	*Total Vascular Plant % (± SE)*
Control	N/A	12 (± 3)	12 (± 3)	N/A	48 (± 3)	48 (± 8)
Equisetum	5 (± 2)	21 (± 5)	23 (± 5)	23 (± 2)	32 (± 3)	54 (± 6)
Polytrichum	6 (± 1)	7 (± 2)	12 (± 2)	9 (± 0.7)[a]	36 (± 6)	47 (± 9)
Scirpus	16 (± 4)	5 (± 1)	19 (± 2)	48 (± 2)[b]	18 (± 2)	64 (± 7)
Straw	N/A	8 (± 4)	8 (± 4)	N/A	20 (± 3)[c]	20 (± 3)

The total vascular plant is the nurse-plant and the spontaneous vegetation cover (not including the reintroduced bryophytes), which is not entirely the sum of the two due to superimposition. The outliers have been removed from the values for second growing season. N/A, not applicable.

[a] Mean before the outliers were removed is 16 (± 2).

[b] Mean before the outliers were removed is 42 (± 2).

[c] Mean before the outliers were removed is 27 (± 3).

had created a bias, it would have been evident after the first growing season. Similarly, in calcareous grasslands, the water-holding capacity of herbaceous litter allowed for higher growth of bryophytes (Rincon 1988). Shade improved regeneration (except for *P. strictum*) even for the wettest greenhouse treatments, where water was not a limiting factor. Perhaps this is an indication that air humidity is more important to moss growth than substrate humidity.

Apart from a higher regeneration of fen bryophytes under the *Scirpus* canopy, there was no difference in the bryophytes' regeneration among other nurse-plant treatments. It is odd that the control treatment showed similar regeneration rates as the other treatments, considering the temperatures were much higher. This could be explained by spontaneous revegetation. The temperatures were measured early in the first experimental season when there was little spontaneous regeneration. However, by the end of the second year, the total vascular plants' cover on plots where no nurse-plants were reintroduced was similar to the other treatments where nurse-plants had been reintroduced. Therefore, the conditions of the control plots were similar to the other treatments during the second growing season. On the other hand, the low daily maximum temperature measured on the *Polytrichum* plots should have translated to higher moss regeneration. In similar studies for bog restoration, *Polytrichum* indeed improved moss regeneration (Groeneveld et al. 2007). It seems that the tall, dense structure of *Scirpus* creates a more humid microclimate than the small *Polytrichum* moss. Possibly, relative humidity would have been a better parameter to characterize the microclimate for moss regeneration than temperature.

Regeneration in Relation to Water Availability

This study confirmed that optimal water content for moss growth is generally lower than saturation values, as was also observed by Busby and Whitfield (1977). In the greenhouse experiment, the highest regeneration for bryophytes was often observed at a water level of -10 cm (water potential of -8.6 and volumetric water content of 74%). *Sphagnum* species, for example, are subject to cyanobacteria contamination when constantly saturated (L. Rochefort 2007, Senior Chair holder of the Industrial Research Chair in Peatland Management, personal observations), as we observed in our greenhouse experiment. In the field, lengthy flooding inhibited the growth of bryophytes mainly due to physical disturbance, such as erosion and sedimentation (Quinty & Rochefort 2000). Therefore, fen restoration sites where the water level is just below the surface should show the highest moss regeneration, at least for nonaquatic bryophytes.

Regeneration Capabilities of Tested Species

In both the greenhouse and the field experiments, the *Sphagnum* species were among the most successful species in regenerating. *Sphagnum* mosses are better competitors and generally more productive than most nonsphagnous species when relative humidity at the air–peat surface is not limiting (Vitt 1990; Gignac 1992).

Polytrichum strictum showed different regeneration preferences than other tested mosses. This comes as no surprise because it is one of the most "developed" bryophytes with a water-conducting system that allows it to direct water under dry conditions (Bayfield 1973). Its leaves are also sun leaves, adapted for photosynthesis under drier conditions and greater light intensities than other bryophytes (Clayton-Greene et al. 1985).

Pleurozium schreberi had minimal regeneration success in the field and in all full-light greenhouse treatments, even though it inhabits dry areas and is an aggressive competitor in forest environments (Frego 1994). *Pleurozium schreberi* has a narrow fundamental niche and prefers shaded areas (Busby & Whitfield 1977; Mulligan & Gignac 2001). This study showed that shade is indeed crucial for regeneration of this species. Because this species is dominant in boreal forest, this could prove an important consideration for forest restoration after clear-cutting.

Conclusions

If the emphasis of fen restoration is the return of the peat-accumulating function, *Sphagnum* species that tolerate

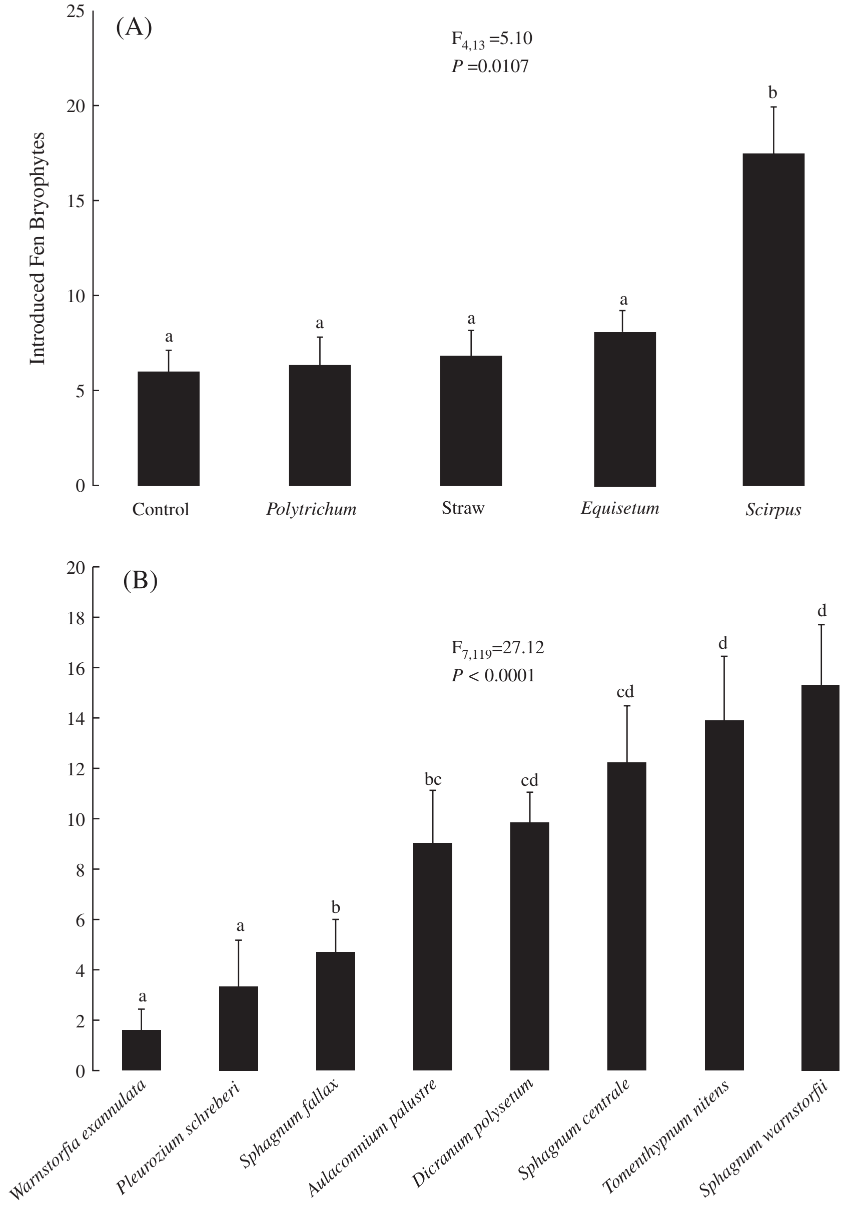

Figure 2. The regeneration of eight fen bryophytes in a field experiment is shown. An ANOVA, using the GLM procedure of SAS and a priori polynomial contrasts, showed no significant interaction between the nurse-plant treatments (main plots) and the species (subplot) allowing the data to be summarized with two graphs. (A) The moss regeneration of all introduced moss species for five treatments (canopy of three nurse-plants, straw, and control) is shown. (B) Additionally, the average percent cover of each bryophyte for all treatments confounded is shown after two growing seasons.

slightly minerotrophic conditions should be favored to jump-start succession toward a bog (Wind-Mulder & Vitt 2000). *Sphagnum* species are considered the keystone of bog restoration due to their ability to alter chemistry and hydrology of their environment as well as the great capacity to accumulate peat (Rochefort 2000). Some studies have suggested that even nonsphagnous bryophytes, such as *Tomenthypnum nitens*, *Drepanocladus revolvens*, and *Campylium stellatum*, also have the ability to acidify their environment and likely influence peatland succession (Glime et al. 1982; Karlin & Bliss 1984). If fen bryophytes are capable of altering their environment should they be considered the keystone species of fen restoration? A great amount of research has been carried out on the functional role of bryophytes in bogs (Clymo & Hayward 1982); however, little is known about their function in fens. More research on the functional roles of vascular plants and bryophytes in fen systems would enable fen restoration projects to focus on a few keystone vegetation groups.

Implications for Practice

This study demonstrates that fen bryophytes show good potential for use in fen restoration projects because all tested bryophytes were capable of vegetative regeneration. However, marked differences between the regeneration of the tested species were observed. Specifically, the following conditions improved regeneration:

- Most species showed the best regeneration with a water level just at or under the surface (0 to −10 cm that corresponds to a soil water potential between −4.3 and −8.6 and volumetric water content of 74%) in a controlled environment. All species, except *Polytrichum strictum*, had higher regeneration success under shade.
- The *Sphagnum* species showed the highest regeneration in both the field and the greenhouse experiments.
- The regeneration success of the bryophytes would benefit from the canopy of tall herbaceous plants, which create a protected microclimate. Therefore, restoration strategies, which include the reintroduction of large, tussock-forming vascular plants, such as plants from the Cyperaceae family, would complement the reintroduction of fen bryophytes.

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LITERATURE CITED

Andrus, R. 1986. Some aspects of *Sphagnum* ecology. Canadian Journal of Botany **64:**416–426.

Bayfield, N. G. 1973. Notes on water relations of *Polytrichum commune* Hedw. Journal of Bryology **7:**607–617.

Bogren, K., and S. Hofer. 2001. Determination of orthophosphate in Bray or Mehlich extracts of soils by flow injection analysis. Zellweger analytics Lachat instruments. Methods manual. Quick-Chem method 12–115–01–1-A. Zellweger Analytics, Milwaukee, Wisconsin.

Bray, R. L., and L. T. Kurtz. 1945. Determination of total organic and available forms of phosphorus in soils. Soil Science **59:**39–45.

Bremner, J. M., and C. S. Mulvaney. 1982. Nitrogen-total. Pages 595–624 in A. L. Page, R. H. Miller, and D. R. Keeney, editors. Methods of soil analysis. Part 2—chemical and microbiological properties. 2nd ed. Series of agronomy, vol. 9. American Society of Agronomy, Soil Science Society of America Publisher, Madison, Wisconsin.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution **18:**119–125.

Bugnon, J.-L., L. Rochefort, and J. Price. 1997. Field experiments of *Sphagnum* reintroduction on a dry abandoned peatland in Eastern Canada. Wetlands **17:**513–517.

Busby, J. R., and W. A. Whitfield. 1977. Water potential, water content and net assimilation of some boreal forest bryophytes. Canadian Journal of Botany **56:**1551–1558.

Buttler, A., P. Grosvernier, and Y. Matthey. 1998. Development of *Sphagnum fallax* diaspores on bare peat with implications for the restoration of cut-over bogs. Journal of Applied Ecology **35:**800–810.

Campeau, S., and L. Rochefort. 1996. *Sphagnum* regeneration on bare peat surfaces: field and greenhouse experiments. Journal of Applied Ecology **33:**599–608.

Clayton-Greene, K. A., N. J. Collins, T. G. A. Green, and M. C. F. Proctor. 1985. Surface wax, structure and function in leaves of Polytrichaceae. Journal of Bryology **13:**549–562.

Clymo, R. S., and P. M. Hayward. 1982. The ecology of *Sphagnum*. Pages 229–289 in A. J. E. Smith, editor. Bryophyte ecology. Chapman and Hall, London, United Kingdom.

Cobbaert, D., L. Rochefort, and J. S. Price. 2004. Experimental restoration of a fen plant community after peat mining. Applied Vegetation Science **7:**209–220.

Cooper, D. J., and L. H. MacDonald. 2000. Restoring the vegetation of mined peatlands in the Southern Rocky Mountains of Colorado, USA. Restoration Ecology **8:**103–111.

Environment Canada. 2002. Canadian climate norms 1971-2000. Atmospheric Environment Service, Ottawa, Ontario, Canada.

Famous, N. C., M. Spencer, and H. Nilsson. 1991. Revegetation patterns in harvested peatlands in Central and Eastern North America. Pages 48–66 in D. N. Grubich, and T. J. Malterer, editors. Proceedings of the International Peat Symposium. Peat and peatlands: the resource and its utilization. Duluth, Minnesota. International Peat Society, Jyväskylä, Finland.

Faubert, P., and L. Rochefort. 2002. Response of peatland bryophytes to burial by wind-dispersed peat. The Bryologist **105:**96–104 (see Erratum in The Bryologist **105:**299).

Frego, K. A. 1994. Factors influencing the local distribution of four bryophyte species on mature upland black spruce forests. Ph.D. dissertation. University of Toronto, Toronto, Ontario, Canada.

Gauthier, R. 1980. La végétation des tourbières et les sphaignes du parc des Laurentides, Québec. Études écologiques 3. Laboratoire d'écologie forestière, Université Laval, Québec, Canada.

Gauthier, R., and M. M. Grandtner. 1975. Étude phytosociologique des tourbières du bas Saint-Laurent, Québec. Naturaliste Canadien **102:** 109–153.

Gignac, L. D. 1992. Niche structure, resource partitioning, and species interactions of mire bryophyte relative to climatic and ecological gradients in Western Canada. The Bryologist **95:**406–418.

Gignac, L. D., D. H. Vitt, S. C. Zoltai, and S. E. Bayley. 1991. Bryophyte response to surfaces along climatic, chemical and physical gradient of Western Canada. Nova Hedwigia **53:**27–71.

Glime, J. M., R. G. Wetzel, and B. J. Kennedy. 1982. The effects of bryophytes on the succession from alkaline marsh to Sphagnum bog. American Midland Naturalist **108:**209–223.

Graf, M. D., L. Rochefort, and M. Poulin. 2008. The spontaneous regeneration of abandoned fens of Canada and Minnesota, USA. Wetlands **28:**28–39.

Groeneveld, E. V. G., A. Massé, and L. Rochefort. 2007. *Polytrichum strictum* as a nurse-plant to facilitate *Sphagnum* and boreal vascular plant establishment. Restoration Ecology **15:**709–719.

Groeneveld, E. V. G., and L. Rochefort. 2005. *Polytrichum strictum* as a solution to frost heaving in disturbed ecosystems: a case study with milled peatlands. Restoration Ecology **13:**74–82.

Higgs, E. 2003. Nature by design: people, natural processes and ecological restoration. MIT Press, Cambridge, Massachusetts.

Karlin, E. F., and L. C. Bliss. 1984. Variation in substrate chemistry along a microtopographical and water chemistry gradients in peatlands. Canadian Journal of Botany **62:**142–153.

Kotowski, W., J. Van Andel, R. van Diggelen, and J. Hogendoorf. 2001. Response of fen plant species to groundwater level and light intensity. Plant Ecology **155:**147–156.

Lamers, L., A. Smolders, and J. Roelofs. 2002. Restoration of fens in the Netherlands. Hydrobiologia **478:**107–130.

Li, Y., and D. H. Vitt. 1994. The dynamics of moss establishment: temporal responses to nutrient gradients. The Bryologist **97:**357–364.

Longton, R. E.. 1984. The role of bryophytes in terrestrial ecosystems. Journal of the Hattori Botanical Laboratory **55:**147–163.

Mälson, K., and H. Rydin. 2007. The regeneration capabilities of bryophytes for rich fen restoration. Biological Conservation **135:**435–442.

Mehlich, A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. Communications in Soil Science and Plant Analysis **15:**1409–1416.

Mitsch, W. J., and J. G. Gosselink. 2000. Wetlands. 3rd ed. Wiley, New York.

Mulligan, R. C., and L. D. Gignac. 2001. Bryophyte community structure in a boreal poor fen: reciprocal transplants. Canadian Journal of Botany **79:**404–411.

Murray, K. J., J. D. Tenhunen, and R. S. Nowak. 1993. Photoinhibition as a control on photosynthesis and production of *Sphagnum* mosses. Oecologia **96:**200–207.

National Wetlands Working Group. 1988. Wetlands of Canada. Polyscience Publications, Montréal, Canada.

Pfadenhauer, J., and A. Grootjans. 1999. Wetland restoration in Central Europe: aims and methods. Applied Vegetation Science **2:**95–106.

Poschlod, P., and H. Schrag. 1990. Regeneration vegetativer Teilchen von "Braunmoosen." Telma **20:**291–300.

Poulin, M., L. Rochefort, and A. Desrochers. 1999. Conservation of bog plant species assemblages: assessing the role of natural remnants in mined sites. Applied Vegetation Science **2:**169–180.

Prach, K., B. Sandor, P. Pysek, R. van Diggelen, and G. Wiegleb. 2001. The role of spontaneous vegetation succession in ecosystem restoration: a perspective. Applied Vegetation Science **4:**111–114.

Price, J. S., A. L. Heathwaite, and A. J. Baird. 2003. Hydrological processes in abandoned and restored peatlands: an overview of management approaches. Wetlands Ecology and Management **11:**65–83.

Price, J. S., and G. Whitehead. 2001. Developing hydrological thresholds for *Sphagnum* recolonization on an abandoned cutover bog. Wetlands **21:**32–42.

Quinty, F., and L. Rochefort. 2000. Bare peat substrate instability in peatlands restoration: problems and solutions. Pages 751–756 in L. Rochefort and J.-Y. Daigle, editors. Sustaining our peatlands, Proceedings of the 11th International Peat Congress, vol. II. Québec, Canada, 6–12 August 2000. International Peat Society, Edmonton, Alberta, Canada.

Rincon, E. 1988. The effect of herbaceous litter on bryophyte growth. Journal of Bryology **15:**209–217.

Rochefort, L. 2000. *Sphagnum*—a keystone genus in habitat restoration. The Bryologist **103:**503–508.

Rochefort, L., R. Gauthier, and D. Lequéré. 1995. *Sphagnum* regeneration—toward an optimization of bog restoration. Pages 423–434 in B. D. Wheeler, S. C. Shaw, W. J. Fojt, and R. A. Robertson, editors. Restoration of temperate wetlands. John Wiley & Son, Chichester, Great Britain.

Rochefort, L., F. Quinty, S. Campeau, K. W. Johnson, and T. J. Malterer. 2003. North American approach to the restoration of *Sphagnum* dominated peatlands. Wetlands Ecology and Management **11:**3–20.

Salonen, V., A. Penttinen, and S. Aila. 1992. Plant colonization of a bare peat surface: population changes and spatial patterns. Journal of Vegetation Science **3:**118.

Sjörs, H. 1952. On the relation between vegetation and electrolytes in north Swedish mire waters. Oikos **2:**241–258.

Sliva, J., and J. Pfadenhauer. 1999. Restoration of cut-over raised bogs in southern Germany—a comparison of methods. Applied Vegetation Science **2:**137–148.

Vitt, D. H. 1990. Growth and production dynamics of boreal bryophytes over climatic, chemical and topographical gradients. Botanical Journal of the Linnean Society **104:**35–59.

Vitt, D. H. 2000. Peatlands: ecosystems dominated by bryophytes. Pages 312–343 in A. J. Shaw and B. Goffinet, editors. Bryophyte biology. Cambridge University Press, Cambridge, United Kingdom.

Vitt, D. H., and W.-L. Chee. 1990. The relationship of vegetation to surface water chemistry and peat chemistry in fens of Alberta, Canada. Vegetatio **89:**87–106.

Wind-Mulder, H. L., and D. H. Vitt. 2000. Comparison of water and peat chemistries of a post-harvested and undisturbed peatland with relevance to restoration. Wetlands **20:**616–628.