Facteurs influençant la régénération des mousses de fen dans un contexte de restauration de tourbière

Thèse

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Résumé

La restauration de tourbière vers des fens est relativement nouveau au Canada. La première tentative de restauration à l'échelle écosystémique d'un fen utilisant la technique de transfert de tapis muscinal s'est résulté par un échec de l'établissement de la couche muscinale (bryophytes). La couche muscinale typique des fens (composée de mousses vraies de fen, souvent appelée mousses brunes) est une composante importante des fens naturels. La littérature scientifique sur le sujet de la restauration de la couche muscinale de fen est pauvre, ou difficilement applicable dans des conditions nord-américaines. Le but de ce projet est d'acquérir certaines connaissances écophysiologiques sur les facteurs influençant la régénération des mousses de fen. Les facteurs choisis proviennent de différentes étapes de la restauration écologique des tourbières ainsi que de la biologie et l'écologie des mousses. Les quatre espèces de mousses utilisées sont communément trouvées dans les fens naturels du Canada : *Aulacomnium palustre*, *Campylium stellatum*, *Scorpidium cossonii* et *Tomentypnum nitens*. Les traitements testés sont : a) effet de la distance, par rapport à l'apex, des fragments des mousses sur leur régénération; b) la fragmentation mécanique; c) la fertilisation phosphatée; d) le chaulage; e) une expérience combinant fragmentation, fertilisation ainsi que différentes communautés de mousse testées sur le terrain; et f) l'effet de *A. palustre* comme plante compagne.

Les principaux résultats sont : a) la régénération des fragments diminue en dessous de 2 ou 3 cm à partir de l'apex; b) la fragmentation augmente le nombre d'innovations quand il y a suffisamment d'eau (conditions de croissance humides); c) la fertilisation phosphatée a un effet positif sur la régénération des mousses lorsque maintenues en environnement contrôlé humide; d) le chaulage n'affecte positivement que l'espèce *C. stellatum*; e) *A. palustre* se régénère mieux sur le terrain que les autre mousses f) *A. palustre* n'apparaît pas être une bonne plante compagne pour *C. stellatum* et *S. cossonii*. Ces résultats visent à améliorer notre compréhension de la niche de régénération de mousses vraies de fen. Ici, nous établissons les connaissances de base pour une éventuelle restauration des fens à l'échelle de l'écosystème.

Abstract

Peatland fen restoration is relatively new in Canada. The first attempt at an ecosystem scale fen restoration project with the moss layer transfer technique was unsuccessful in establishing the moss layer (bryophytes). The typical fen moss layer (composed mainly of fen true moss, often called brown moss) is an important part of natural fen ecosystem. The scientific literature about fen restoration regarding the moss layer is either poor or inadequate for restoration in the North Americans conditions. The goal of this project is to develop some base ecophysiological knowledges on factors influencing the regeneration of fen true mosses. The chosen factors come from studying different steps of peatland restoration techniques, and the biology and ecology of fen true mosses. The four species chosen for this project are all commonly found across natural fens in Canada: *Aulacomnium palustre*, *Campylium stellatum*, *Scorpidium cossonii* and *Tomentypnum nitens*. The tested treatments are: a) the effect of a moss fragment position on the stem relative to the apex on their regeneration; b) mechanical fragmentation; c) phosphate fertilization; d) liming; e) an experiment combining fragmentation, fertilization and different moss communities tested on the field; and f) the effects of *A. palustre* as a nursing plant.

The main results are: a) regeneration decreases 2 or 3 centimeters below the apex; b) fragmentation increased the number of innovations for all species when enough water is available (moist growth conditions); c) phosphate fertilization has a positive effect on the regeneration in a controlled moist environment; d) liming only had a positive impacts on *C. stellatum*; e) *A. palustre* regenerate better in the field then the other species; f) *A. palustre* was not proven to be an effective nursing plant for *C. stellatum* and *S. cossonii*. These results aim at improving the understanding of fen true moss regeneration niche on bare peat surfaces. Here, we set the baselines for large scale and long-term fen regeneration attempts.

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Introduction

1. Problem Statement

Restoration of fen peatland ecosystems dominated by a layer of true moss (minerotrophic peatlands) is recent in North America. During the process of peat extraction, *Sphagnum* peat is extracted through large vacuums drawn by tractors, processed and used in horticultural products. The process of peat extraction can sometimes expose the sedge peat layer, a less commercially viable form of peat which is often found underneath the *Sphagnum* peat (Rochefort et al., 2016). When the sedge peat layer is reached, peat extraction usually ends. Without any intervention, it can be 20, 50 or even 120 years before vegetation recolonizes extracted sites (Quinty & Rochefort, 2003; Poulin et al., 2005; Graf et al., 2008). During this time, the post-extracted peatland will release CO_2 through the decomposition of the residual peat layer (Waddington et al., 2010). It is common for a post-extracted peatland to require human intervention for plant species to return and to reinitiate the peat accumulation process (Quinty & Rochefort, 2003; Graf et al., 2008).

Since its creation in 1992, the Peatland Ecology Research Group (PERG) has researched methods and management techniques for the responsible use of peatlands in North America. One of the main results of this research was the creation of the first peatland restoration guide in 1997, subsequently follow by a second edition published in 2003 (Quinty & Rochefort, 2003). One of the main goals of the guide is the restoration of vacuum extracted peatlands to facilitate the return of plant communities capable of peat accumulation. Bogs (ombrotrophic peatlands) are the most common type of peatland in Canada (Tarnocai et al., 2011) and usually the initial state of the pre-harvested peatlands. Through the years, a method called the Moss Layer Transfer Technique (MLTT) has been developed that successfully reintroduces both vascular plants and bryophytes typical of bogs onto post extracted peatland. This method has brought back active peat accumulation on restored sites (Waddington et al., 2010; Strack & Zuback, 2013; Strack et al., 2016; Nugent et al, 2018). However, in the case of fen (minerotrophic peatland) restoration, few attempts have been made in North America (Cooper & MacDonald, 2000; Graf & Rochefort, 2008; Rochefort et al., 2016). Therefore, we do not know if a similar approach of moss dominated vegetation transfer can yield the same result in a minerotrophic peatland. The reason for restoring post-extracted peatlands ecosystem towards fens rather than a bog is that the residual sedge peat layer has a higher concentration of minerals, a higher pH, and can sometimes be reconnected to the surrounding groundwater. Those are factors that do not occur in bogs but rather in fens. The cutover sites with a residual fen peat are more similar to natural fens and thus have better starting conditions for a restoration towards fen ecosystems (Graf, 2008).

In the first fen ecosystem scale restoration project of a post-extracted peatland using the MLTT with machinery, successfully reintroduced vascular plants typical of fens (Rochefort et al., 2016). However, this MLTT approach did not enable the establishment of a bryophyte layer typical of fens with the same degree achieved as with *Sphagnum* peatlands. One hypothesis of this research is that by using the MLTT method for restoration of bogs but changing the *Sphagnum* dominated vegetation transferred by the vegetation found in fens, it should be possible to restore a post-extracted degraded peatland to a functional fen. However, result show that fen true mosses, the main component of the bryophyte layer of fens, are reluctant to regenerate in the field. This is consistent with other studies, underlining the difficulty of reintroducing fen true mosses on post-extracted peatlands (Cobbaert et al., 2004; Graf & Rochefort, 2010; Lamers et al., 2015; Gauthier et al., 2018). Bérubé (2017) found that fen true mosses are an important part of fen ecosystems, hence the need to adapt the existing MLTT technique for a better return of the bryophyte layer in restored fens.

The goal of this project is to gather what we already know about fen true moss ecology, tease out what is useful for ecosystem restoration, and when needed, design experiments to test promising factors that could potentially enhance the regeneration of fen true mosses.

1.1 State of Knowledge About Fen Mosses in the Context of Ecological Restoration

The restoration of fens is a recent endeavor in North America. In contrast, there exists a long tradition of studies in European countries (Lamers et al., 2002; Bonn et al., 2016; Michalska-Hejduk et al., 2017). However, most of the knowledge gained from European fen restoration projects does not easily apply in the North American context. The two main reasons are: 1) the peatland ecosystem restoration challenges in Europe are mostly related to eutrophication, grazing, and land conversion to agriculture which are different than those in North America such as dealing with frost heaving, snowmelt spring floods and winter-related constraints, and; 2) the goal for peatland restoration in Europe is often a return of biodiversity rather than the return of a peat accumulation function through establishment of a moss layer. Relatively few European studies provide sufficient information regarding fen true moss species, focusing instead on vascular plants (Bérubé, 2017). Most of the knowledge about fen true mosses ecology comes from experiments done in either controlled environments or on small-scale plots (e.g. Mälson & Rydin, 2007).

The following studies contain information about the moss biology and ecology used in this project as well as information on fen and other moss ecosystem rehabilitation projects. We are specifically

looking for factors that could have an inhibitory or positive effect on moss regeneration. Some of those themes can overlap, but that is because they use different ways to answer the same question.

1.1.1 Water Table

The most studied factor across all projects was the impact of the water table on fen true moss regeneration (Cobbaert et al., 2004; Mälson & Rydin, 2007; Graf & Rochefort, 2010; Rochefort et al., 2015; Borkenhagen & Cooper, 2016; Priede et al., 2016) and its importance on the presence of fen true mosses in a given environment (Gignac, 1992; Bauer et al., 2007). The main conclusion of these articles is that a stable water table near the surface of the peat is beneficial to almost all fen mosses. Another key factor to take into consideration is the different communities of mosses created by minor changes in the peatland topography. Peatlands tend to form many microsites in which abiotic conditions vary slightly. A difference of merely 10 cm in the water table between two different microsites in any given peatland can be enough to create a different community of mosses. Mosses such as *Aulacomnium. palustre*, which is classified as a hummock species, tends to grow better when the water table is around 30 cm below the surface (Bauer et al., 2007; Borkenhagen & Cooper, 2016) but can very well regenerate at higher water tables (Graf & Rochefort, 2010). Species such as *Campylium stellatum* and *Scorpidium. cossonii*, which are found in wet carpets, prefer environments with a water table depth close to the surface (Mälson & Rydin, 2007; Borkenhagen & Cooper, 2016). Probably because of a preference for a lower water table *A. palustre* and *Tomentypnum.* nitens have been found to tolerate desiccation better than *C. stellatum* (Manukjanová et al., 2014). Post-extracted and restored peatlands tend to have water table levels that fluctuate more than in undisturbed peatlands. A study evaluating *Sphagnum* species found that species from hummock communities tolerated fluctuating water tables more than then species of other communities (Chirino et al., 2006). Selecting species which tolerate a fluctuating water table, but which is found in fens could be a good option to start fen restoration. If we can extrapolate information found in Chirino et al. (2006), hummock species might prove useful.

1.1.2 pH

The pH of fen peat is more neutral than in bogs. Fen pH tends to be neutral with values between 5.5 and 7.5 (Payette, 2001). The development of a peatland in North America usually started as a marsh-fen that accumulated peat, became more acidic, and *Sphagnum* species gradually took over and continued the acidification process (Udd et al., 2016) and developed into a bog (Kuhry et al., 1993). Because of that, some pH values of *Sphagnum* poor fen peat can overlap bog peat due to the ongoing process of succession. As successional stratification continues, the peatland becomes less alkaline, mostly because of the decrease in Ca^{2+} and Mg^{2+} cations inputs, due to being disconnected

from the surrounding groundwater. In post-extracted peatlands where the pH is higher, the concentration in nutrients doesn't significantly change (Andersen et al., 2011).

The vacuum extraction of peatlands also has an effect over the resulting residual peat layer pH (Wind-Mulder et al., 1996; Andersen et al., 2011) resulting in a pH higher than that of a natural bog and lower then a natural fen. In natural fens, a higher pH increases the occurrence and growth of fen true mosses especially with a pH close to 6 or 7 (Rochefort & Vitt, 1988; Gignac, 1992; Stechova et al., 2008), but they can usually be found across a wide range of pH (Gignac, 1992). Soil pH could be a driving factor for choosing which species can regenerate in the field (Rochefort & Vitt, 1988; Priede et al., 2016).

Artificially raising the pH through liming could potentially increase the number of innovations of fen true mosses (Vicherová et al., 2015). Fen peat contains more magnesium (Mg) and calcium (Ca) then bog peat (Maimer et al., 1992; Udd et al., 2016). Adding dolomitic lime would raise the pH to a more neutral level and add mineral such as Ca and Mg. Fens moss communities are influenced by the amount of Ca, with some mosses being more common in peatlands with high concentrations of Ca (Mettrop et al., 2018). The hypothesis is that creating conditions similar to those of natural fens, we can enhance fen true moss regeneration in peatland restoration projects. One study has found an increase in moss regeneration after the addition of lime (Mälson & Rydin, 2007). Perhaps this beneficial effect can also lead to an increase in moss cover. But it seems that liming peat does not necessarily increase nutrients available to the plant (Emond, 2013). It is possible that the beneficial effect of lime is not due to an indirect increase in nutrients. A study found an increase in plant available nitrogen following liming (van Diggelen et al., 2015) but only half a year after the application, and afterwards the amount of nutrients returned to pre-treatment levels.

1.1.3 Mulching/Shade

The importance of mulching, usually composed of straw, spread over the reintroduced moss material, in peatland restoration has already been demonstrated (Price et al., 1998). Fen true mosses, just like *Sphagnum* moss, are poikilohydric plants, meaning their water content is similar to the humidity of their surroundings in most situations (Proctor, 1984). Most bryophytes lack any protection against high light intensity and therefore tend to avoid environment with a direct exposure (Glime, 2017b). Because bryophytes optimal growth is reached in low light, photosynthesis is usually limited by the ambient humidity rather than light or temperature (Ingerpuu et al., 2005; Glime, 2017b). Adding straw mulch can create microenvironments that reduce evapotranspiration and ultimately increase moss regeneration. Also, mulch can usually be found

close to post-extracted peatlands making it a cheap and effective way to increase moss colonization without transporting materials from outside the region.

The benefits of straw mulch include intercepting rainfall, reducing direct radiation and heat at the soil level during the day, keeping the soil warmer during the night, limiting water evaporation, and increasing soil moisture. All this creates a more favorable environment for the moss and results in an increase in moss colonization in peatland restoration (Price et al., 1998). The type of protective cover does matter, it need to create shade (Rochefort & Bastien, 1998; Graf & Rochefort, 2010) and be affordable. Other substrates can be used which have the same beneficial effect as the mulch, such as an *Agrinet* (white shading screen looking like a gauze band) (Mälson & Rydin, 2007). Natural cover made by vascular plant canopy can also be beneficial (Graf & Rochefort, 2010; Borkenhagen & Cooper, 2016) or at least not detrimental to fen true mosses growth (Bauer et al., 2007). Hence the importance of providing cover for fen true mosses when the environment has fluctuating humidity levels.

Although, a negative correlation was found with the presence of vascular plants and moss growth (Kotowski et al., 2006; Hejcman et al., 2010) mostly through competition for space or light. It seems that, to a certain degree, vascular plants can serve as a sort of nursing plant which could provide a suitable habitat for the moss to grow, probably by creating shade for the moss. But at a certain threshold the vascular cover can become detrimental to moss growth through competition. Hejcman et al. (2010) found that bryophyte biomass is reduced under a vascular cover until the threshold of 400 g/m^2 of vascular plant dry biomass. Kotowski et al. (2006) found that removing some of the vascular plants led to an increase in bryophyte cover. Vascular plants are an integral part of natural fen species compositions and studying the effects on fen true mosses is necessary.

1.1.4 Fertilization

Phosphate fertilization is already used in most peatland restoration projects in Canada. Its use in fen restoration has been documented as having a positive effect on fen true mosses establishment (Rochefort et al., 2016). Plants growing on bare peat are phosphate limited due to its low presence in the residual peat layer (Wind-Mulder et al., 1996). However, there are few studies about the effect of phosphate fertilizers on fen true mosses. Phosphate fertilization has been linked to an increase in fen true moss cover when added to moss reintroduction in the field (Kotowski et al., 2006; Sottocornola et al., 2007; Rochefort et al., 2016). Most vascular plant species are excluded from post-extracted peatland surfaces by nutrient limitations (Quinty & Rochefort, 2003). Applying phosphate fertilizer could increase the vascular plant cover to the detriment of bryophytes cover through competition (Hejcman et al., 2010). In Europe, where the threat of eutrophication is a major

concern since most peatlands have been converted to cropland, they have found that the addition of phosphate and nitrogen can have a negative effect on fen true mosses (Bergamini & Pauli, 2001; Andersen et al., 2016). Potassium has been linked to an increase in desiccation resilience in mosses (Brown & Buck, 1979) and to a replacement of fen true moss by species that are calcifuges such as *Sphagnum* (Hájek et al., 2015). Since there was no evidence of potassium limitations in our peatland experimental sites, this factor has not been taken into consideration for these first trials.

The effect of nitrogen fertilization on true mosses is less known. Nitrogen fertilization has a positive effects on some species (Cusell et al., 2014) negative for others (Bergamini & Pauli, 2001; Andersen et al., 2016), or can be simply ineffective (Li & Vitt, 1994). It is considered that plant growth in post-extracted peatlands is not limited by nitrogen (Wind-Mulder et al., 1996). Further research is necessary to determine if fen true mosses can benefit from the addition of nitrogen fertilizer.

1.1.5 Fragmentation

Mosses are capable of vegetative reproduction through totipotency. This means they don't exclusively depend on sexual reproduction for the propagation of the species (Glime, 2017a). Many species have specialized asexual reproduction organs called gemmae that are ready to grow into mature mosses once a suitable environment is found (Glime, 2017a). This method of reproduction allows moss to mature much faster than through sexual reproduction. However, this specialized organ does not disperse very far (Laaka-Lindberg et al., 2003).The active spreading of moss material on post-extracted peatlands resolves the problem of dispersion and allows the colonization by mosses. Once the mosses are well established, they can continue to spread by asexual or sexual reproduction.

Bryophytes can also regenerate from unspecialized cells coming from all parts of the individual (i.e. leaves, shoot, branches) (Rochefort & Lode, 2006). A part of moss capable of regeneration (including gemmae) is called a propagule. There are inhibiting factors in place within either the moss or the soil around the moss that limits the number of new stems made by a single propagule (Glime, 2017a). Fragmenting the moss increases the number of new propagules, which will disperse onto a larger surface. There is potentially a tradeoff between the number of propagules and establishment success. Perhaps by limiting the number of propagules, the moss increases its chance to survive and grow while many propagules would result in smaller mosses and thus have less chance of survival. So even if fragmentation does increase the number of innovations, it might not result in a better establishment on the field. Further research is necessary to evaluate the right degree of fragmentation to maximize brown moss establishment in fen restoration.

Mosses do not have the same amount of nutrients in the first few centimeters of the apex, as in the lower part of the plant (Maimer et al., 1992). Moss capacity to regenerate from fragments diminished as the fragments are taken from the lower part (Campeau & Rochefort, 1996). This is because mosses, in contrast to vascular plants, grow from the top and die from the bottom (Proctor, 1984). As a result, many laboratory experiments using fen true mosses tend to keep only the first apical centimeters to reduce variability (Maimer et al., 1992; Li & Vitt, 1994; Mälson & Rydin, 2007; Vicherová et al., 2015). A study that measured the regeneration of *Sphagnum* species by the distance from which fragments were taken from the apex (Campeau & Rochefort, 1996) lead to the conclusion that the harvesting depth should be kept to a maximum of 10 to 15 cm. Such a study has not been made with fen true mosses. During the restoration of the Bic-Saint Fabien fen in 2009 (Rochefort et al., 2016), the harvesting depth was kept to a maximum of 10 cm, but this depth might not be suited for fen true mosses.

1.1.6 Facilitation and Competition

For this project, facilitation refers to the beneficial effect of the presence of one or more plants on the growth, recruitment or, in this case, regeneration of another species. Competition refers to the detrimental effect of a plant on another by competing for limited resources such as light or nutrients. As described in section 1.1.3, shade can be a favorable factor for the regeneration of fen true mosses. Shade provided by other plants can have a beneficial effect (Ingerpuu et al., 2005; Glime, 2017b) as it increases humidity near the soil and indirectly increases the window of time in which the moss can perform photosynthesis. The idea of measuring the interaction between the vascular cover and bryophyte cover in fens is recent (Graf & Rochefort, 2010; Hejcman et al., 2010). Since these plants populate two different layers of the environment, the interaction could shape the different communities found in fens. An increase in moss cover can be related to the increase in the vascular plant cover (Ingerpuu et al., 2005; Graf & Rochefort, 2010), but the opposite has also been found (Bergamini & Pauli, 2001; Kotowski et al., 2006; Hejcman et al., 2010; Udd et al., 2016). Depending on the density, it seems that the vascular plant cover can act both as a nursing plant or a competitor (Pouliot et al., 2011).

Moss species can also create competition or facilitation among one another. The possible facilitation effect that fen true mosses have among other fen true mosses has not been well studied. But finding a fen true moss that fills the same role as *Polytrichum strictum* as a nurse plant in bogs restoration (Groeneveld et al., 2007) could be useful for fen restoration. Mosses that inhabit the same ecological niche will not always exclude one another through competition (Mälson & Rydin,

2009). It might be possible that the heterogenous communities of mosses found are the result of one species facilitating the recruitment of another.

1.2 Goal and Experiments

The goal of this project is to study factors influencing the regeneration of fen true moss with the goal that some of the knowledge gained will be useful in peatland restoration. Four fen true moss species commonly found in fens across North America were chosen for this experiment: *Aulacomnium palustre*, *Campylium stellatum*, *Scorpidium cossonii* and *Tomentypnum nitens*, and their regeneration capacity under different factors were evaluated. The studied factors on the moss regeneration and establishment are: A) the regeneration potential of fragments in relation to their distance below the apex; B) the effect of mechanical fragmentation on fen true moss regeneration; C) the effect of enhanced nutrient availability through phosphate fertilization or liming on fen true moss regeneration; and D) the effect of *A. palustre* as a nursing plant for *C. stellatum* and *S. cossonii*.

Chapter 2: Materials and Methods

2.1 Factors Influencing the Regeneration of Fen True Mosses in Growth Chambers

The fen true moss species used in all experiments were: *Tomentypnum nitens* (Hedw.) Loeske, *Campylium stellatum* (Hedw.) Lange & C.E.O. Jensen, *Scorpidium cossonii* (Schimp.) Hedenäs and *Aulacomnium palustre* (Hedw.) Schwägr. These mosses are commonly found in natural fens across the northern hemisphere. They represent two types of communities: 1) mosses that commonly live in depressions or wet lawns (*C. stellatum* and *S. cossonii*); and 2) mosses that commonly live on hummocks or drier lawns (*A. palustre* and *T. nitens*). The moss gametophores used in these experiments were harvested in the Bic–Saint-Fabien natural peatland adjacent to the Parc national du Bic (Bas-Saint-Laurent region, Québec) with permission from the park. Mosses were sorted to keep only the desired species. However, some fragments of other unwanted species (other species than the 4 listed above or species from the other community) were involuntarily found in the experimental material. Consequently, the petri dish in which the experiment took place were regularly checked and "weeded" meaning that each stem corresponding from a different species than the one targeted was removed. The first set of experiments was performed between early March and late June 2016 in growth chambers. The field experiment, described in section 2.2, used the treatments which showed the best results in term of fen true moss regeneration from the first set of experiment.

2.1.1 Preparation, Growth, Monitoring and Analysis

All factors tested by incubating fen true moss in petri dish had their number of innovations counted. An innovation, in this study, correspond to a new stem of at least 1 mm in length. Each experiment was implemented as a random block experiment. The blocked variable was the mass of un-altered mosses fully saturated with water. Before placement in petri dishes, the mosses were weighed at maximum water content by being submerged in water then drained of any excess by resting on a flat surface. All experiments were performed in plastic petri dishes (diameter 14.2 cm) except for the distance below the apex experiment which used smaller petri dishes (diameter 8.75 cm). Commercial ombrotrophic peat (pH 4.7) was used for the base of all experiments, except for the fertilizing experiment, which used a more minerotrophic peat harvested from the Saint-Henri field site (pH 5.6). Petri dishes were prepared by covering the surface of petri dishes with about 0.5 cm of peat that was saturated with water. Deionized water was used for all experiments. Afterwards, the mosses were added as well as more water if the peat was deemed unsaturated. The mosses were then spread out over the entire surface and the petri dishes were sealed with Parafilm (Beemis NA, Neenah, WI, USA). The number of mosses per petri dish varied with species and experiments due

to the varying size of petri dishes and the various size of the gametophore (Table 1). Each treatment was replicated 10 times per species. The mosses used during the petri dish experiments of 2016 were harvested in summer 2015 or in summer 2016 and kept in a freezer at -4 °C to preserve the moss without any tissue damage (GRET, unpublished data, 2019).

The mosses received light from two fluorescent bulbs (160 watts) from 8:00 to 22:00 for 8 to 10 weeks, with a room temperature between 20 °C and 25 C. The first experiment lasted for ten weeks; later experiments lasted for 8 weeks. The petri dishes were rearranged randomly once every 2-3 weeks to reduce the effect from varying levels of light based on petri dish location. The petri dishes were kept moist with a few milliliters of deionized water if there was a leak, or if the peat was too dry. The Parafilm seal was replaced if broken.

Table 2.1: Number of moss fragments per petri dish per species per experiment for the growth chamber experiments.

	Number of fragments	
	Petri dish experiments	
Species	Distance below the apex	Other experiment
A. palustre	25	8
C. stellatum	25	8
S. cossonii	15	12
T. nitens	12	6

To evaluate the regenerative capacity of mosses, the number of innovations was counted using a dissecting microscope. An innovation is defined as a new stem produced by vegetative reproduction from a moss fragment (Fig. 2.1). Only innovations that were longer than 1 mm were counted. Each petri dish was inspected, and the innovations counted four weeks after the experiment begun of growth to evaluate the progression of the experiment. In the case of contamination by another species, innovations of the invasive species were ignored or removed, and any vascular plants inside the petri dish were removed.

Figure 2.1: New stems produced from laydown fragments by vegetative reproduction, also called innovations, of *Aulacomnium palustre* in a petri dish.

2.1.2 Distance Below the Apex Experiment.

The goal of this experiment was to measure the regenerative capacity of mosses with increasing distance below the apex. Individual moss stems used for this experiment had the same length for each species so that each treatment had the same number of fragments. The stems of *A. palustre* and *C. stellatum* were 4 cm long and *T. nitens* and *S. cossonii* stems were 5 cm long. Each stem was cut into 1 cm long fragments starting from the top (Fig. 2.2). The mosses *A. palustre* and *C. stellatum* had 4 treatments (0-1 cm, 1-2 cm, 2-3 cm, 3-4 cm) and the mosses *T. nitens* and *S. cossonii* had and additional treatment (4-5 cm). Each fragment was sorted according to their distance below the apex and placed in the same petri dish.

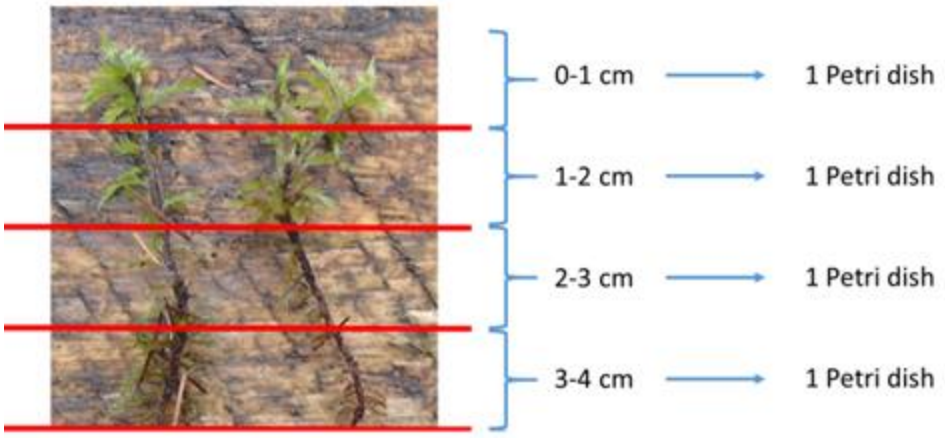

Figure 2.2: An illustration of cutting *Tomentypnum nitens* fragments for the distance below the apex experiment.

2.1.3 Mechanical Fragmentation Experiment

The goal of this experiment was to determine the effect of an increasing severity of fragmentation on the regeneration capacity of mosses. Three levels of fragmentation were used: no fragmentation, cut into 1 cm fragments, and grounded into 1 mm fragments. The cutting was done by hand using a cutting board with a ruler and a knife, and the grinding was done with a coffee grinder (DCG-20BKNC, Cuisinart, Ontario, Canada). Each group of mosses was weighed before the fragmentation.

2.1.4 Bone Meal Fertilization Experiment

The goal of this experiment was to determine the optimal dose of phosphate fertilizer for each species to increase regeneration. Seven treatments were tested: 0, 5, 10, 15, 20, 25 and 40 g/m^2. The last dose was chosen to see if it was possible to create phosphate toxicity for the mosses. The fertilizer used was bone meal (ENGRAIS NATUREL McINNES, Stanstead, Québec, Canada) (N total 2%, P_20_5 total 17%, Ca 25%, Org. mat. 25%). Each dose was spread evenly on the peat, covering the surface of the petri dish, then the mosses were added on top of the peat.

2.1.5 Liming Experiment

A dolomitic limestone ($MgCO_3$, $CaCO_3$) was used for this experiment (C-I-L, Premier Tech, Rivière-du-Loup, Québec, Canada): 162.5 g of lime was added to 10 L of peat saturated with water to increase the pH. Weekly pH measurements were made until the pH was stable for more than three weeks in a row. Two different pH levels were chosen, 4.7 and 6.6 (limed). The peat was spread in petri dishes, and the mosses added on top, then the dishes were sealed. The pH of 6.6 seems to represent the limit of the natural pH buffer within the peat.

To characterize the limed treatment, an analysis of the main nutrients within the peat was done. In the limed peat, the amount of calcium (Ca) had increased by 30%, the concentration of magnesium (Mg) had more than tripled, the total nitrogen (N) had significantly reduced in concentration, the available phosphorus (P) did not change but the standard error tripled, and potassium (K) had almost reduced by half (Table 2.2).

Table 2.2: Lime addition effect on the chemical elements found in the peat used in the liming experiment. Mean ± (SE) n=6.

		ppm					Total %
Peat	pH	P	Ca	Mg	K	N-NH4	N
Control	4.5	4.81 (0.41)	10441 (135)	1064 (13)	98 (9)	15.9 (0.4)	2.17 (0.02)
Limed	6.6	5.55 (1.41)	13496 (93)	3680 (29)	57 (4)	4.3 (0.7)	1.96 (0.02)

2.1.6 Statistical Analyses

To assess the effect of each treatment on all species, the data were analyzed with SAS software (SAS, Cary, NC, USA) with an ANOVA for a randomized block design using the MIXED procedure and a LSD to identify any differences in the number of innovations between each level of treatment. The normality and homogeneity of variance were tested, and the variance was changed with the GROUP statement using the function REPEATED. The mechanical fragmentation data were transformed using log10 to correct the homogeneity and the additivity. The weight of the biomass added in the petri dish for each experiment was measured and blocked, except for the distance below the apex experiment. The alpha value was set at $\alpha = 0.05$.

2.2 Testing Physical and Chemical Factors in the Field

2.2.1 Field Experiment

This experiment was put into place for two different plant communities (depression and hummock) for a total of 48 experimental units (EU; 2 fertilization x 2 fragmentation x 2 plant communities x 6 replicates). I used a factorial randomized blocked design, each having two levels of applications (fertilized and non-fertilized, fragmented and non-fragmented) to see any possible interaction between the treatments. There was a total of six blocks for three sites. A mix of fen true mosses *A. palustre* and *T. nitens* composed the hummock community, while *S. cossonii* and *C. stellatum* composed the depression community. The mosses used were harvested in the Bic–Saint-Fabien

peatland adjacent to the Parc national du Bic in Québec. Only the top layer (5 cm) of the moss carpet was harvested (no peat or dead moss). The mosses were mixed in a plastic container and stored for a month in a cool, shaded place near the location from which they were harvested. An industrial manure spreader and hand-held hedge trimmers were used to fragment the mosses. The fertilizer used was granular rock phosphate (0-13-0, P_2O_5 total 25%, P_2O_5 available 13%) at a dose of 15 g/m^2. The treatments were assigned randomly, and each 2 m x 2 m EU was separated from another EU by a 5 m buffer. Each EU that received the same treatment but contained different communities were separated by 1 m.

Mosses were introduced to the site by hand at a rate (square meter covered by the square meter harvested) of 2:9 for the hummock community and 4:13 for the depression community. The difference between the two communities comes from the fact that the hummock community was easier to harvest more living material per square meter. This created a difference in cover between the two communities for the same amount of surface harvested. Our goal was to have a high cover of mosses in each plot rather than mimicking the traditional restoration protocol which uses the surface harvested to calculate its application ratio (Quinty & Rochefort, 2003).The moss was spread on the peat, and then the plot, and a surrounding 1 m buffer, and covered with straw. A photo-degradable net (Eco Sodwrap Plastic Netting, Tamanet, USA) was laid on top of the straw to prevent loss of material.

The experiments took place in three rewetted cutover peatlands with an exposed minerotrophic peat layer (Table 2.3). Each site contained two blocks corresponding to two levels of soil moisture (relatively wet and dry environments): each site was levelled, and the vegetation growing on the site was removed. At Saint-Modeste, a drainage ditch was made to remove the excess of water. During the summer of 2016, the dry sector was colonized by *Equisetum arvense* this vegetation was removed by hedge trimmers before the experiment took place. At Saint-Henri, the driest sector had berms build around it to keep more water in the block.

Table 2.3: General information about the field sites. The mean rainfall was calculated with the historical data for the month of May through October as to represent the growing season. The pH represents the means of the 2 blocks.

Site	Saint-Henri	Bic Saint-Fabien	Saint-Modeste
GPS Position	46.70 N, -71.05 W	48.32 N, -68.83 W	47.83 N, -69.46 W
Years since last extraction	2	16	20
Mean rainfall 2016	115 mm	95 mm	95 mm
Mean rainfall 2017	99 mm	70 mm	70 mm
pH	5.37	5.91	5.40
Von Post	H4	H4	H4
Vegetation prior to the experiment	Bare peat	Mostly bare peat, *Equisetum arvense*	Trees, bushes and vascular plants

Sampling and Statistical Analyses

Each 2 m x 2 m plot was divided into 16 identical subplots, six of these were randomly chosen to gather data by the Point Intercept method. A density of 121 points for each 50 cm x 50 cm square was chosen to have a good representation of the moss carpet. I gathered data after 2 growing seasons (2017). Because the vascular cover and species differed across plots and blocks, I measured it as a co-variable that could influence bryophyte regeneration. Vascular cover was measured by estimating the vertical projection of each plant for each subplot before removing the straw. Each vascular species was identified. During the 2016 survey, the dry block at Bic Saint Fabien was reduced to 55 points because removing the straw also removed the mosses underneath it. The Point Intercept data was converted to a proportion by dividing the number of point associates to each species by the number of points in total.

I computed the mean cover of all species of a similar community for each treatment. To assess the effect of fertilization, fragmentation and the possible interaction between the two on the different communities, I used an ANOVA for a factorial randomized block design (2x2) with vascular plant cover as a co-variable using the MIXED procedure and a LSD to identify any significant difference between treatments with SAS software. For the analysis of the 2017 data, the dry block at the Bic Saint-Fabien site was removed from the analysis of the hummock community because so few mosses had grown that the possible effect of each treatment could not be measured.

2.2.2 Growth Chamber

To corroborate the field results about the effect of fertilization and fragmentation on the regeneration potential of fen true mosses, a follow-up petri dish experiment was done with the same conditions as the field experiment. The experiment was carried out with moss material kept in a freezer at -4 °C for 6 months within 10 replicated petri dishes. Treatments were the same as in the field with the exception that the fertilizer used was bone meal (dolomitic, 101% equivalent $CaCO_3$) instead of phosphate rock. The petri dishes were then placed in a growth chamber following the same condition as the petri dish experiment mentioned in section 2.1.4.

2.3 Nurse Plant

2.3.1 Conceptualization and Set Up

The goal of this experiment was to see if there was a change in the regeneration of the target species *Campylium stellatum* and *Scorpidium cossonii* when *Aulacomnium palustre* was used as a nurse plant. The three treatments were: 1) the effect of a pre-established *A. palustre* moss carpet, 2) the effect of the target species and nurse species being reintroduced simultaneously, and 3) a control target species reintroduced without the nurse plant. To assess the mechanism by which the nurse plant can affect the target species I included drought periods in this experiment to test the effect of the nurse plant on resistance to desiccation of the target species.

This experiment took place in a greenhouse located at the Université Laval. The average temperature during the day was 22-24 °C, and the relative humidity was kept between 50-60% for the entire experiment. I used eight plastic trays (22 cm x 44 cm) for each treatment. Each tray was filled with 4-5 cm of ombrotrophic peat (pH 4.7), the peat was then levelled and flattened. Each tray had holes pierced at the bottom to drain excess water. Under each pierced tray was another tray to collect the excess water and allow better control of the level of soil moisture. The mosses used were harvested in the Bic Saint-Fabien peatland located at Saint-Fabien-sur-Mer adjacent to the Parc national du Bic in Québec during the late summer of 2016. The mosses were stored in a freezer (-4 °C) for 8 months. Afterwards they were sorted out to have only the three main species, and ground using a kitchen blender (CombiMax 600, Braun, Frankfurt, Germany) to increase the number of innovations and facilitate spreading. The mosses were stored in a refrigerator for seven weeks (4 °C) and then a freezer for three weeks (-4 °C). Eight trays per treatment were chosen so that each bag of moss could cover approximately 70% of the surface when spread evenly.

The pre-established carpet treatment was made by spreading the *A. palustre* nurse moss evenly across the surface of a peat-filled tray and waiting ten weeks for the mosses to produce innovations. Each day, the level of soil moisture was evaluated visually and adjusted with a misting garden hose

using rainwater. For the implementation of the carpet, the soil moisture was kept high enough for the peat to remain saturated. Vascular plants were removed manually at least once per week during visual tray inspections. Any moss species that were found that did not correspond to the experimental mosses were left in the tray. To keep the surface soil moisture high, I used an Agryl net (Novagryl, Avintiv, Berry plastic, Waterloo, Canada) to block about 50% of the light to reduce evaporation. After five and ten weeks, the nurse moss cover was evaluated by dividing the tray in twelve equal subplots, randomly choosing five of those subplots and measuring the cover and the number of innovations in each subplot. Once the carpet was established, the target mosses were spread over the carpet. The implementation of the simultaneous reintroduces species treatment (target and nurse) started at the same time as the pre-established carpet treatment. To prepare this experiment, I mixed the target mosses with the nurse moss until the mixture became homogeneous then spread the mixture evenly over the tray. The control group was made by only spreading the target mosses on the tray.

After eight weeks, the percent cover of each species (target and nurse) and the number of innovations were measured, and the first simulated drought period was applied. This drought was severe, as I watered the tray only once each week with only enough water to keep the soils wet at the surface. I also removed the protective net during the drought. After two weeks, I watered the tray heavily so that most of the peat was fully saturated with water for two weeks. A second less severe drought was simulated by removing the bottom tray and watering the tray normally without collecting the excess water for four weeks and by removing the net. This less severe drought was followed by a four-week regrowth period. After this last regrowth period, the cover and number of innovations for each species was measured using the same method as described above.

Statistical Analyses

The data computed was the mean number of innovations of each species for a subplot and the estimated mean cover of the nurse plant and target plants by tray. The total cover of vegetative material was also estimated. The cover and number of innovations for species outside of the target species were also measured. To assess the effect of the two treatments (pre-established carpets and simultaneous growth) the SAS software was used to analyze the data with an ANOVA for a randomized block design using the MIXED procedure and an LSD to identify any differences in the number of innovations between each level of treatment.

2.3.2 Viability

The viability experiment was put in place to measure the effect of the delay between the two treatments on moss regeneration. This experiment took place in a growth chamber with similar

condition as the petri dish experiment in section 2.1. During the nurse plant experiment, half of the nurse mosses were stored for six weeks in a refrigerator (4 °C) and four weeks in a freezer (-4 °C), and the other half was used immediately. The mosses used in the viability experiment were the same mosses used in the nurse plant experiment. The dependent variable was the time of storage. The first group was placed at time 0, and the second group at time 10. The time 0 corresponds to beginning of the establishment of the nurse moss carpet and time 10 correspond to the beginning of the simultaneous reintroduction treatment. Approximately 200 g of moss saturated with water per treatment were prepared for this experiment. The treatment consisted of dividing 200 g of grounded moss per treatment in ten equal parts. The experiment took place in plastic petri dishes (diameter 14.20 cm) filled with 0.5 cm of moist peat (pH 4.7). Deionized water was added so the peat was saturated with water then the petri dish was sealed and stored in a growth chamber. The petri dishes received light from two fluorescent bulbs (160 watts) from 8:00 to 22:00 for 10 weeks, with the room temperature between 20°C and 25°C. After 10 weeks, when the simultaneous growth of the nurse plant experiment began, the other 200 g of mosses was put in a petri dish. At the same time, the innovations of the first treatment were counted with a dissecting microscope. The petri dishes were randomly rearranged once every 2-3 weeks to homogenize the treatment. The petri dishes were kept moist with a few ml of deionized water if there was a leak or if the peat was too dry, and the Parafilm seal was replaced if it was broken.

Statistical Analyses

SAS software was used to conduct an ANOVA comparing the mean number of innovations for each petri dish. Only the innovations that were longer than 1 mm were counted. In case of contamination by other species, the innovation was ignored, and any vascular plant inside the petri dish was removed.

Chapter 3: Results

3.1 Petri Dish Experiments

3.1.1 Distance Below the Apex

All species except *C. stellatum* significantly produced more innovations in the first two centimeters below the apex. Regeneration capacity decreased as fragments are taken from lower parts of the gametophore (Fig. 3.4). After the fourth or fifth centimeters, the fragment produced about half as many innovations as the fragments closer to the apex (Table 3.4).

Table 3.4: One-way ANOVA of moss species regeneration potential in relation of the fragment distance under the apex.

		A. palustre			*T.nitens*			*S. cossonii*			*C. stellatum*		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS	F	P>F	SS
Distance	4	30.37	**<.0001**	4377	5.48	**0.0011**	1102	4.86	**0.0024**	905	1.95	0.1384	8111
Error	45												
Total	49												

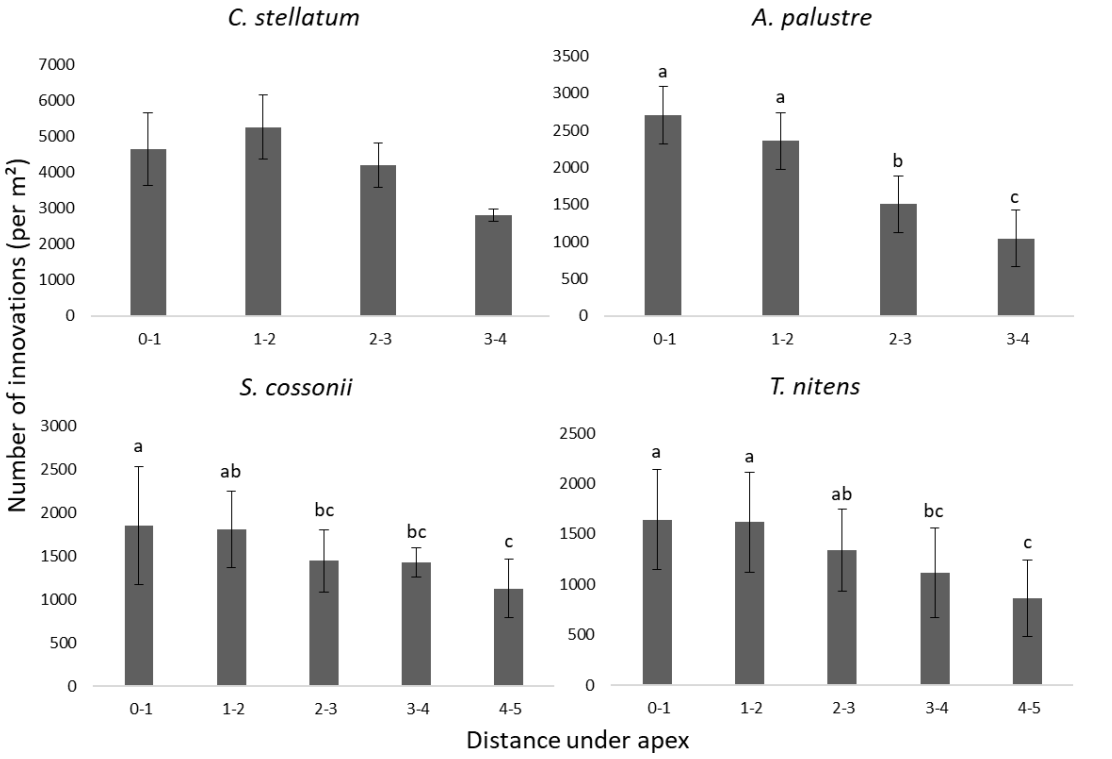

Figure 3.3: Difference between the mean (n= 10, α=0.05) number of innovations per m². Each bar represents fragment groups by 0-1, 1-2, 2-3, 3-4, or 4-5 cm below the apex. Note the different axis for each species. The different letters correspond to significant differences between treatments for the same species according to the LSD test.

3.1.2 Mechanical Fragmentation

The number of innovations produced by all species increased with the severity of fragmentation. The number of innovations produced increased greatly between 1 cm and 1 mm fragment size in

the petri dish experiment (Fig. 3.5). Results from the petri dish fragmentation experiment showed similar results for 2 species: *A. palustre* and *S. cossonii* (Fig. 3.10, Fig. 3.11). As the number of innovations increased, the mean variation of number of innovations also increased.

Table 3.5: One-way ANOVA of moss species regeneration potential in relation to the intensity of fragmentation in a randomized block design after a log transformation.

		A. palustre			*T.nitens*			*S. cossonii*			*C. stellatum*		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS	F	P>F	SS
Block	9	0.58		0.0608	1.27		0.1793	1.52		0.9375	1.19		0.1572
Fragmentation	2	86.33	**<.0001**	2.0260	136.00	**<.0001**	4.2541	38.04	**<.0001**	0.1686	48.93	**<.0001**	1.4422
Error	18												
Total	29												

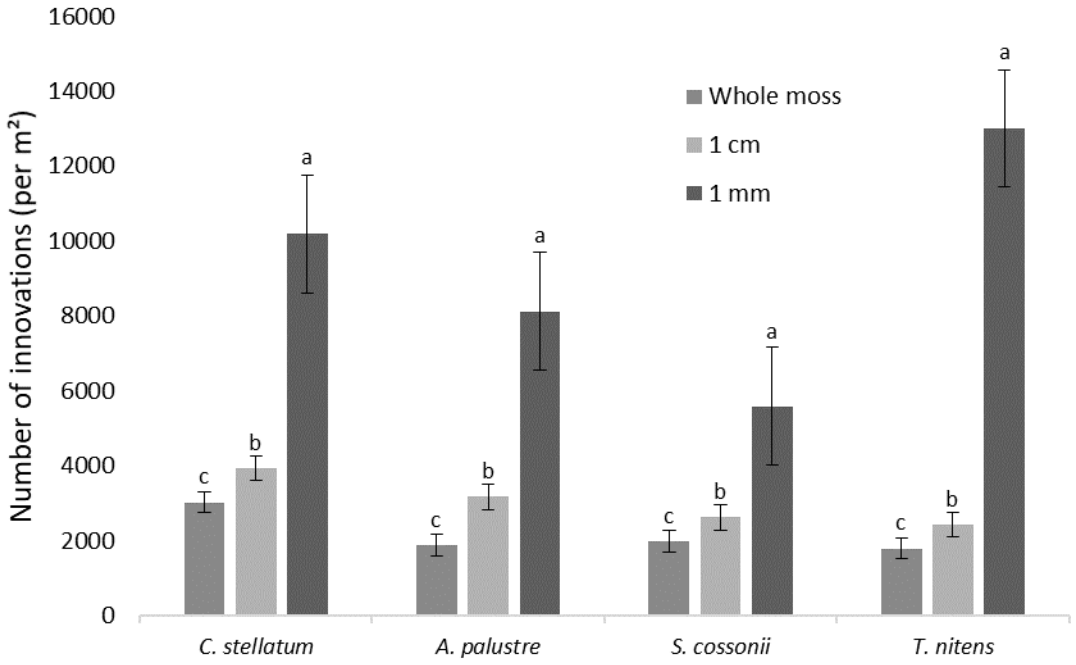

Figure 3.4: Effect of fragmentation on the number of innovations produced by each species in petri dishes. The data represent the means per m² ± SE, n=10, α=0.05. The mean amount of biomass added in each petri dish was: 0.52 g ± 0.13 for *Campylium stellatum*, 1.28 g ± 0.36 for *Aulacomnium palustre*, 1.01 g ± 0.22 for *Scorpidium cossonii* and 1.62 g ± 0.22 for *Tomentypnum nitens*. Different letters correspond to significant differences between treatments for the same species according to the LSD test.

3.1.3 Phosphate Fertilizer

The addition of phosphate fertilizer increased the number of innovations for 3 out of 4 species in the petri dish experiment (Fig. 3.6). There is no difference in number of innovations between the fertilization doses and there is no toxicity effect observe in the highest doses. However, none of the species reacted positively to the addition of fertilizer except when paired with fragmentation for the species *A. palustre* (Fig. 3.10, Fig. 3.11).

Table 3.6: One-way ANOVA of moss species regeneration potential in relation to the different doses of fertilizer in a randomized blocked design.

		A. palustre			*T. nitens*			*S. cossonii*			*C. stellatum*		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS	F	P>F	SS
Block	9	1.19		100	1.28		796	1.13		100	2.05		1832
Fertilization	6	11.58	**<.0001**	974	1.99	0.0827	829	9.09	**<.0001**	802	7.64	**<.0001**	491
Error	54												
Total	69												

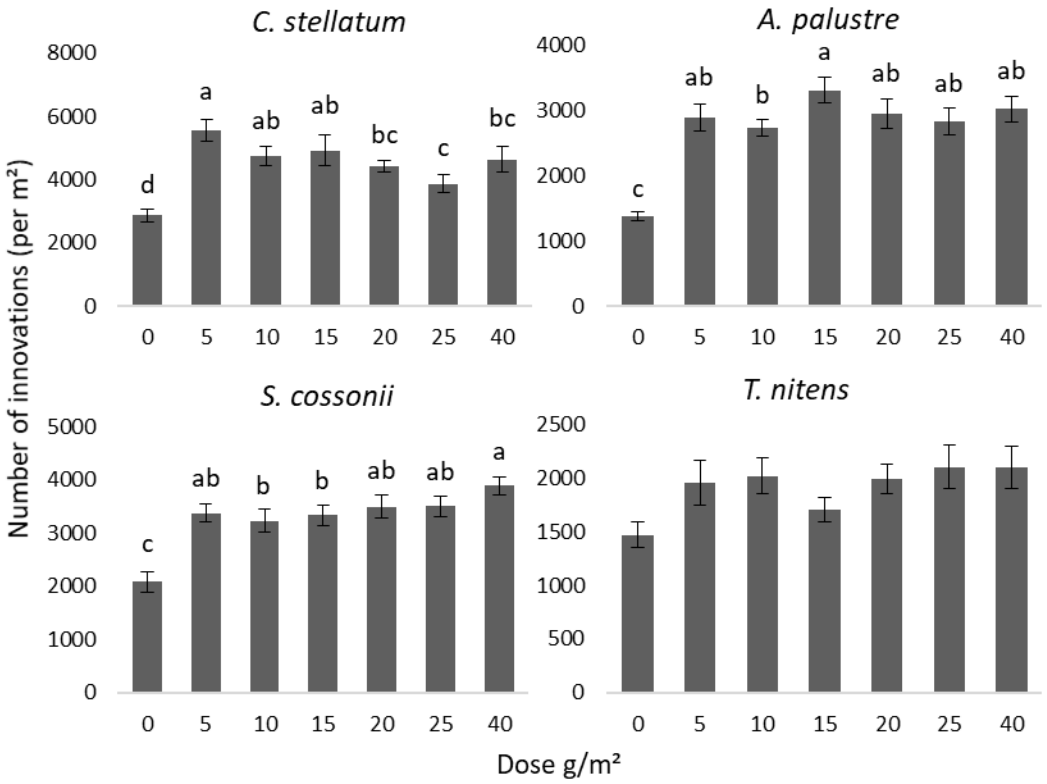

Figure 3.5: Effect of phosphate fertilization (N total 2%, P total 17%, Ca 25% and organic matter 25%) on the number of innovations produced by each species in petri dishes. The data represent the mean amount of innovation per m² ± SE, n=10, α=0.05. The amount of biomass added in each petri dish was: 0.35 g ± 0.08 for *Campylium stellatum*, 1.06 g ± 0.11 for *Aulacomnium palustre*, 1.01 g ± 0.18 for *Scorpidium cossonii* and 1.67 g ± 0.30 for *Tomentypnum nitens.* Note the different axis for each species. Different letters correspond to significant differences between treatments for the same species according to the LSD test.

3.1.4 Liming

Liming did not affect moss regeneration, except for *C. stellatum* which produce 50% more innovations following liming (Table 3.7). For all species, the variation of the mean number of innovations is smaller in limed treatment than in the control treatment (Fig. 3.7).

Table 3.7: One-way ANOVA of moss species regeneration potential in relation to the different doses of lime in a randomized blocked design.

		A. palustre			*T.nitens*			*S. cossonii*			*C. stellatum*		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS	F	P>F	SS
Block	9	0.35		1781	4.12		1309	1.75		2830	0.61		48131
Liming	1	2.44	0.1531	1394	0.11	0.7425	4	0.27	0.6172	48	10.72	**0.0096**	94688
Error	9												
Total	19												

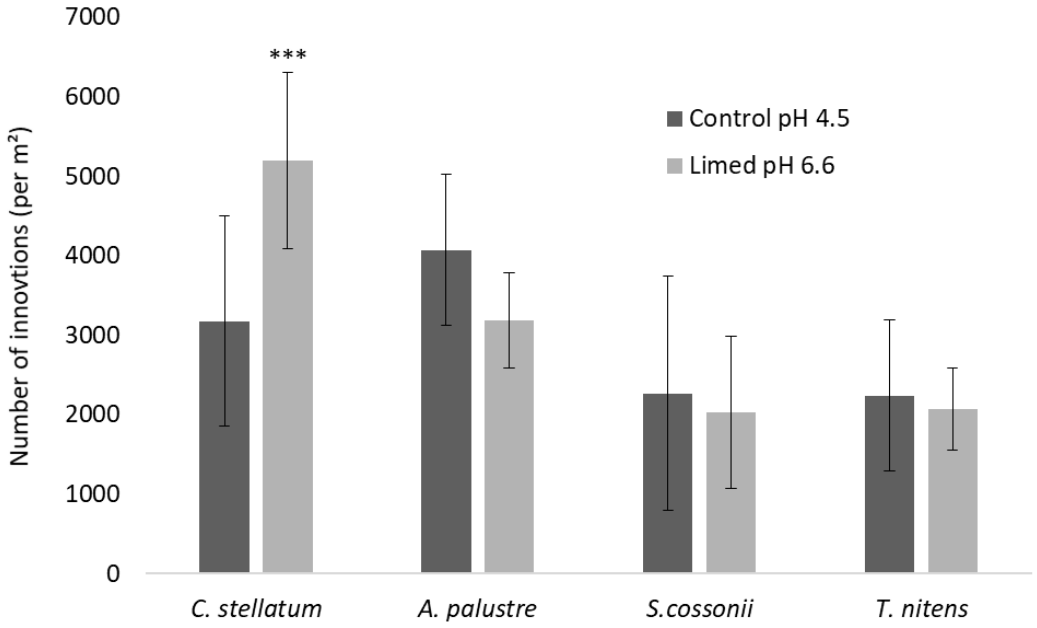

Figure 3.6: Mean number (n = 10) of innovations per species at two different pH levels (control and dolomite lime).Error bars show represent the SE, and significant differences, denoted by the *** symbol, were calculated according to the LSD test (α=0.05).The amount of biomass added in each petri dish was: 0.27 g ± 0.07 for *Campylium stellatum*, 0.93 g ± 0.14 for *Aulacomnium palustre*, 0.78 g ± 0.16 for *Scorpidium cossonii* and 1.42 g ± 0.43 for *Tomentypnum nitens*.

3.2 Field Experiment

3.2.1 Hummock Community

The moss *A. palustre* was significantly affected by both the fragmentation and fertilization and showed a negative interaction between the two (Table 3.8). Cover of *T. nitens* was not significantly affected by any treatment. When put together in the "target species" group, *A. palustre* and *T. nitens* did not show any significant difference among any of the treatments. The moss *A. palustre* had between 2 and 5 times more percent ground cover than *T. nitens* even though they were spread at roughly the same density (Fig 3.8). The vascular plant cover had a significant impact on the cover of *T. nitens* and was taken into account in the analysis as a co-variable.

When the same treatments were applied in a controlled environment, only *A. palustre* reacted positively to any treatments (Table 3.9). Fertilization had a positive impact on the number of

innovations but only when paired with fragmentation (Fig 3.9). The moss *T. nitens* did not react positively to any treatments.

Table 3.8: Two ways ANOVA of hummock community moss species in relation to the fragmentation treatment and the fertilization treatment. Pre-existing vascular plant cover of experimental field plots was used as a co-variable.

		A. palustre			*T. nitens*			target species		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS
Block	5	1.96		0.1027	2.35		0.0844	3.81		0.2090
Fragmentation	1	3.01	0.1030	0.0315	0.26	0.6205	0.0018	1.76	0.2051	0.0192
Fertilization	1	0.02	0.8908	0.0002	1.17	0.2957	0.0084	0.61	0.4479	0.0066
Frag x fert	1	5.64	**0.0313***	0.0590	0.26	0.6205	0.0018	3.50	0.0811	0.0384
Error	15									
Total	23									

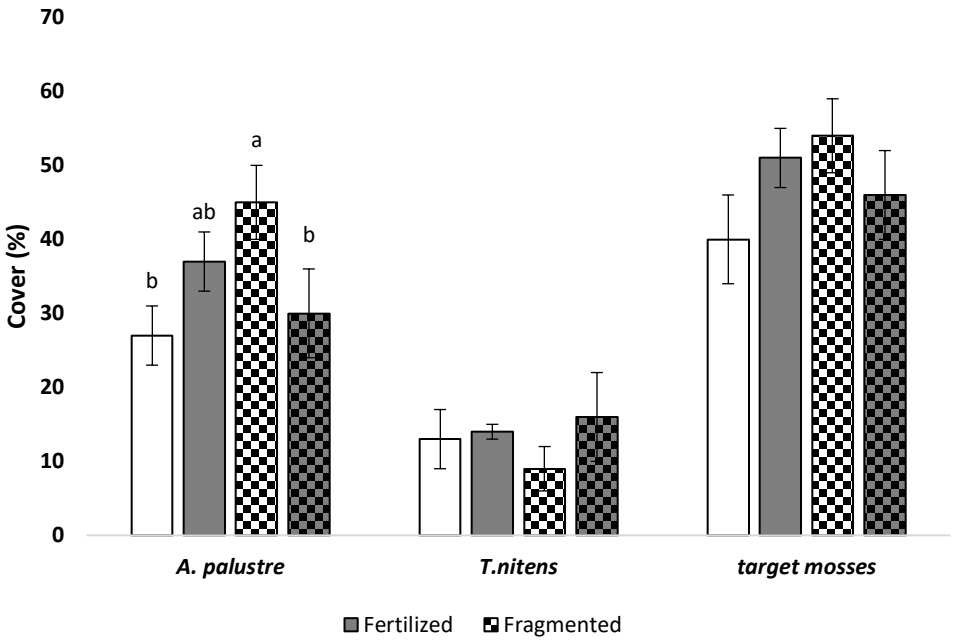

Figure 3.7: Effects of a combination of fragmentation and fertilization (0-13-0, P total 25%) on the cover of two mosses *Aulacomnium palustre* and *Tomentypnum nitens*. The target mosses represent the addition of both *Aulacomnium palustre* and *Tomentypnum nitens*. The data shown represent the mean cover per plot ± SE, n=6, α=0.05. Different letters correspond to significant differences between treatments for the same group according to the LSD test.

Table 3.9: One-way ANOVA of moss species from the hummock community regeneration potential in relation to the fragmentation treatment and the fertilization treatment in a controlled environment.

		A. palustre			*T. nitens*			target species		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS
Fragmentation	1	94.18	**<.0001**	42112	0.50	0.4854	129	112.83	**<.0001**	37565
Fertilization	1	4.34	**0.0447**	1938	3.89	0.0566	1014	0.45	0.5090	148
Frag x fert	1	0.00	1.0000	0	3.55	0.0680	925	0.47	0.4989	155
Error	34									
Total	38									

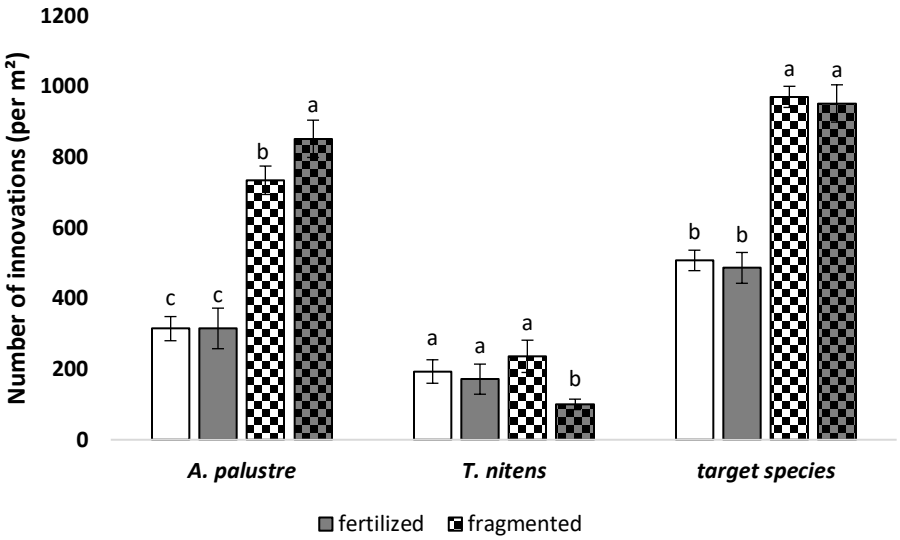

Figure 3.8: Effects of a combination of fragmentation and fertilization (0-13-0, P total 25%) on the number of innovations produced by each species in petri dishes. The target mosses represent the addition of both *Campylium stellatum* and *Scorpidium cossonii*. The biomass introduced had a ratio near 1:1. Each petri dish had a means of 10.19 g ± 0.11 of biomass. The data represents the means per m^2 ± SE, n=10, α=0.05. Different letters correspond to significant differences between treatments for the same group according to the LSD test.

3.2.2 Depression Community

The cover of *S. cossonii* was not significantly affected by any treatment (Table 3.9); however, overall, the percent cover was 5% or less. *C. stellatum* had almost 5 times as much cover as *S. cossonii* (Fig. 3.9). Fertilization did not significantly affect moss ground cover. No interaction between the two treatments was observed. Vascular plants did not have any effect on the ground cover of the mosses.

When the same treatments were applied in a controlled environment to results are similar for *C. stellatum* (Table 3.11). The moss *S. cossonii* did react positively to fragmentation and fertilization (Fig 3.11).

Table 3.10: Two ways ANOVA on the regeneration potential of moss species from depression communities in relation to a fragmentation treatment and a fertilization treatment tested in the field.

		S. cossonii			*C. stellatum*			target species		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS
Block	5	1.93		0.0088	13.11		0.2737	10.16		0.3829
Fragmentation	1	2.41	0.1413	0.0022	8.08	**0.0123**	0.0337	6.93	**0.0188**	0.0522
Fertilization	1	0.55	0.4692	0.0005	0.57	0.4601	0.0024	0.57	0.4636	0.0042
Frag x fert	1	0.00	0.9471	0.0002	0.10	0.7564	0.0004	0.08	0.7817	0.0006
Error	15									
Total	23									

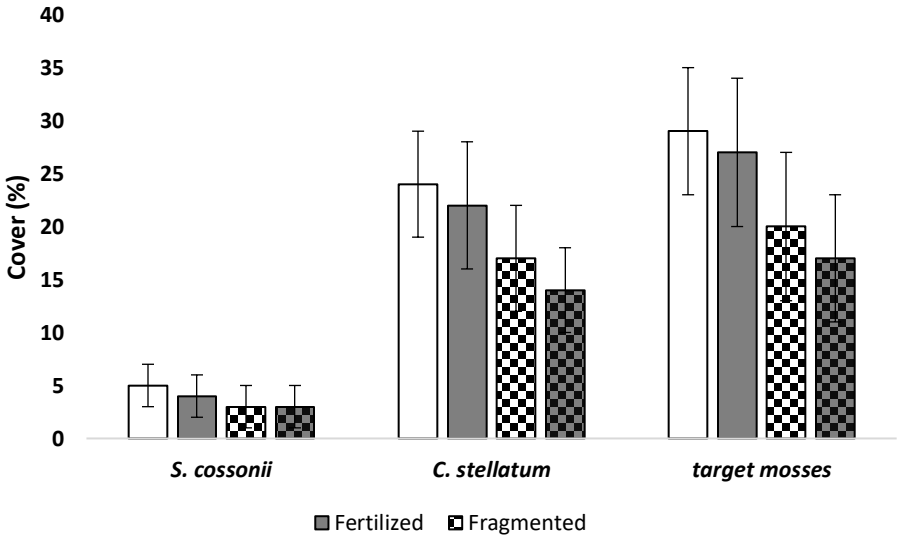

Figure 3.9: Effect of a combination of fragmentation and fertilization (0-13-0, P total 25%) on the cover of two mosses *Campylium stellatum* and *Scorpidium cossonii.* The target mosses represent the addition of both *Scorpidium cossonii* and *Campylium stellatum*. The data represent the mean ± SE, n=6 α=0.05. Different letters correspond to significant differences between treatments for the same species according to the LSD test.

Table 3.11: One-way ANOVA on the regeneration potential of moss species from depression communities in relation to a fragmentation treatment and a fertilization treatment in a controlled growth environment.

		S. cossonii			*C. stellatum*			target species		
Variation source	df	F	P>F	SS	F	P>F	SS	F	P>F	SS
Fragmentation	1	29.53	**<.0001**	4624	0.34	0.5628	655	0.81	0.3758	1798
Fertilization	1	9.44	**0.0042**	1478	1.08	0.3054	2077	3.16	0.0843	7061
Frag x fert	1	0.89	0.3531	138	1.61	0.2136	3082	0.86	0.3612	1913
Error	35									
Total	37									

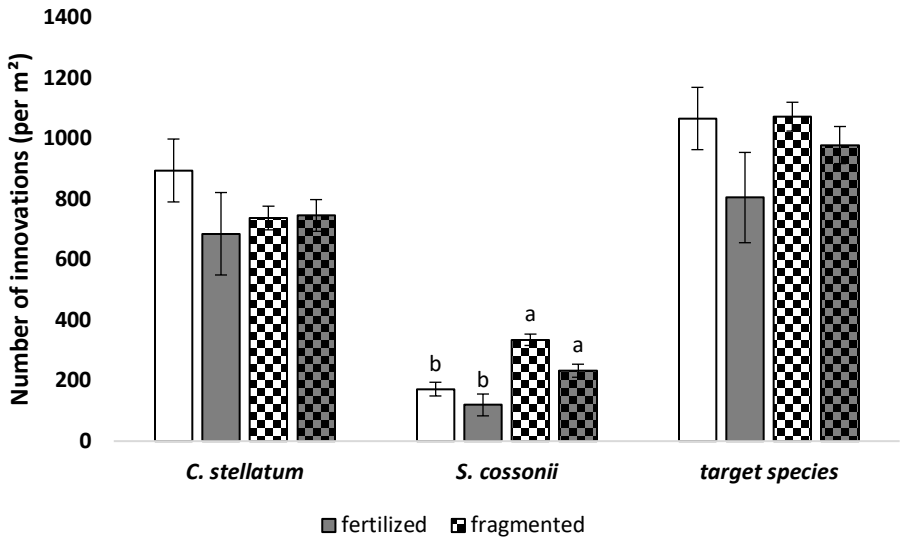

Figure 3.10: Effects of a combination of fragmentation and fertilization (0-13-0, P total 25%) on the number of innovations produced by each species in petri dishes. The target mosses represent the addition of both *Aulacomnium palustre* and *Tomentypnum nitens*. The biomass introduced is near a 1:1 ratio for each species. The data represents the means per m^2 ± SE, n=10, α=0.05. The average biomass added to each petri was 10.27 g ± 0.10 for both treatments that had fragmentation and 9.15 g ± 0.08 for the mosses which were not fragmented. Different letters correspond to significant differences between treatments for the same group according to the LSD test**.**

3.3 Nurse Plant

3.3.1 Greenhouse Experiment

There were no significant differences in the number of innovations in the target species, *S. cossonii* or *C. stellatum*, caused by the presence of the *A. palustre* nurse mosses (Table 3.11); indeed reintroducing the target species directly on a bare peat substrate led to an establishment rate as good as reintroduced in the presence of *A. palustre*. (Fig 3.11).

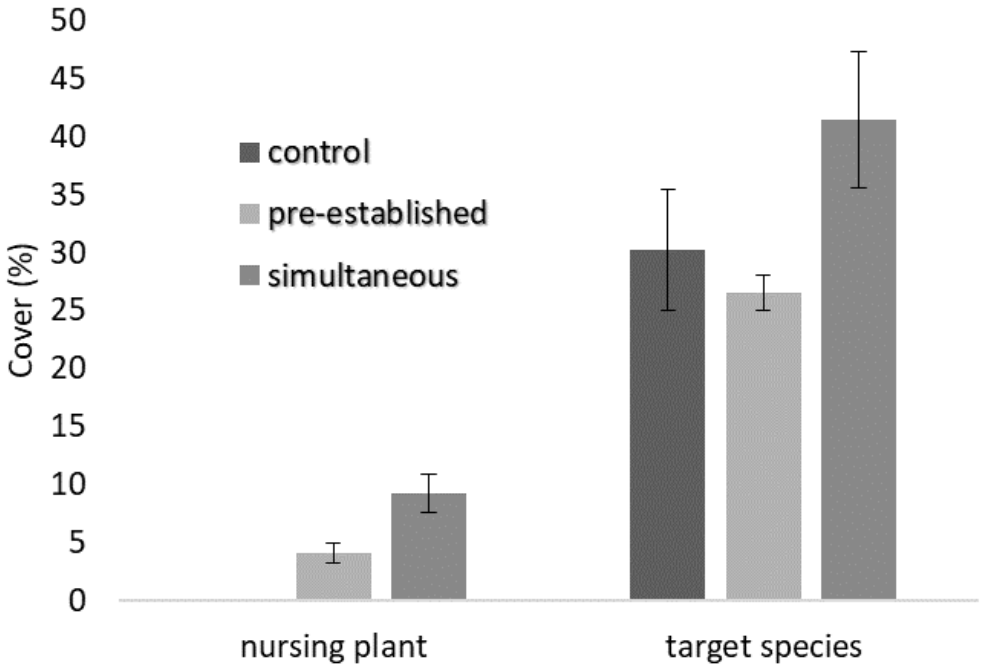

Figure 3.11: Effect of *Aulacomnium palustre* as a nurse plant on the percent ground cover of the target species *Scorpidium cossonii* and *Campylium stellatum*. The data represents the mean cover per tray ± SE, n=8, α=0.05. Different letters correspond to significant differences between treatments for the same species according to the LSD test.

Table 3.12: One-way ANOVA on the regeneration potential of moss species from depression community in relation to the nursing plant treatment.

		Target species		
Variation source	df	F	P>F	SS
Treatment	2	2.83	0.0813	975
Error	21			
Total	23			

Chapter 4: Discussion

A) The regeneration potential of fragments in relation to their distance below the apex

Our first hypothesis was that the further away the plant tissue is from the apex, the less would be its regeneration potential as found with *Sphagnum* mosses (Campeau & Rochefort, 1996). Our hypothesis is mostly correct with 3 out of 4 mosses showing such a pattern whereby the regeneration potential decreased with increasing distance below the apex. The results obtained here are in accordance with what is known about moss biology. Mosses grow from the top and progressively dies from the bottom as parts of the moss get progressively cut off from sunlight (Rochefort & Lode, 2006). The results are also in accordance with the fact that the main nutrients (N-P-K) are more concentrated in the apex of the gametophore (Maimer et al., 1992) making it the most vital part of the moss. Fen true mosses, however, are pleurocarpeous, meaning that they do not necessarily grow vertically and straight (Faubert, 2012). As such, the position of any fragment relatively from the apex is not necessarily a good indicator of the amount of sunlight it receives. Some gametophore might receive more sunlight than other because it grew horizontally. Since the variable measured was length, some bias might have been introduced since 2 different gametophores might have the same length, but one grew straight and the other grew creeping along the ground. The growth form of *Campylium stellatum* is usually to grow upward in carpets, so we did find it surprising that it was that species that did not show differential regeneration between the apex and up to 4 cm down the stem.

B) Effect of mechanical fragmentation

Our first hypothesis was that physical damage caused by the heavy machinery could explain the unsuccessful return of the moss layer during the restoration of the Bic-Saint-Fabien peatland (Rochefort et al., 2016); however, results indicate that the mechanical fragmentation was the most promising treatments for the future of fen restoration. In a controlled environment, all species increased in numbers of innovations following fragmentation. Under the more fluctuating microclimate conditions of the field, some species did not regenerate as well. By fragmenting the moss, we reduced their capacity to retain water by increasing the surface of contact between the moss and the surrounding air, thus increasing evaporation. In the field experiment, only *Aulacomnium palustre* reacted positively to the mechanical treatment while all species reacted positively in a controlled environment to fragmentation. However, the petri dish experiment measured the number of innovations while the field experiment measured percent ground cover. Those 2 variables are correlated but are not the same.

One of the reasons that could explain why fragmented mosses produce more innovations is that fragmentation increases the number of propagules. There are a few inhibitors within the moss which impede the growth of many innovations from the parent gametophore (Glime, 2017a). By fragmenting the mosses, we might have somehow reduced the effect of this inhibitor and allowed each fragment to produce innovations. This could also explain the difference in size of innovation between the fragmented and non-fragmented moss. For future restoration projects, fragmented moss should be used if the moss material is mainly composed of hummock species, especially if represented mostly by *A. palustre*.

C) The effect of enhanced nutrient availability through phosphate fertilization or liming

My results showed that fertilized mosses in a controlled environment produced optimal results no matter the amount of fertilizer used (5, 10, 15, 20, 25 and 40 g/m^2). It should be possible to use a dose as small as 5 g/m^2 and get optimal moss regeneration in the field. Another study also found a positive correlation between P availability and fen true moss growth at 25 g/m^2 (Rochefort et al., 2016). In addition, a small dose reduces the chance of fertilizer run off and induced the growth of algae in nearby water systems (Quinty & Rochefort, 2003). It is noteworthy that the highest dose of fertilizer did not resulted in phosphate toxicity.

The field experiment only found one species that reacted positively to fertilization, *A. palustre*. The lack of moisture is most likely the reason for an absence of significant results since there were big differences in rainfall among each site. The different results between the field and petri dish experiment can also be explain by the different kind of fertilizer used. We could not use powdered phosphate fertilizer on the field since it would be blown away by the wind. Phosphate rock was used instead which is the standard fertilizer for peatland regeneration (Quinty & Rochefort, 2003). The main difference between the two fertilizers is the phosphate release rate. Because the rock phosphate is shaped like a small pebble, only 50% of the fertilizer is directly available, the other half is slowly released. The powder form releases the fertilizer sooner and allows for more uniform spreading. It is also possible that the surrounding plants absorbed the fertilizer faster than the moss, rending the effect of the fertilizer in the field less important. However, since a small dose can have an effect on the moss this is unlikely to be the cause.

Overall, my result contradicts the field results of Rochefort et al. (2016) who found an increase of 180% bryophyte cover on the field following fertilization with a dose of 25 g/m^2. The difference in vascular plants cover between our field experiment could explain the difference (Ingerpuu et al., 2005; Graf & Rochefort, 2010). It is possible that the results will only show itself after a period of time beyond what this study captured. The study of Andersen et al. (2016) found that the addition

of a N-based or P-based fertilizer inhibited the relative growth of fen true mosses and that the no fertilizer treatments produced the best results. The experiment had many differences with this one, such as the substrate was a mixture of peat and sand, the pH was raised to 6.4 by adding CaCO3, the water table was kept between 2 and 3 cm below the surface, there was no control of temperature, and only slight control of the light. The study concludes that the nutriments already found in either groundwater or rainwater can be enough for fen true moss growth. Since the water used in their experiment contained already between 0.02 and 0.03 mg P/L and the experiment lasted 9 months, the mosses already received fertilizer through the watering process. Since my petri dish used deionized water, the only nutrient came from the peat and the fertilizer which could explain why there was an effect in the petri dish. If rainwater or groundwater already contains enough nutrients for an optimal fen true moss growth, then adding more nutrients will not influence growth. But if your water is void of such nutrient then adding nutrient will help growth. This is perhaps why the field experiment did not have any effect, the rain and the surface runoff water already provided all nutrients needed for an optimal growth.

The effect of fertilization on moss growth seems to be indirect. Fertilizing post-extracted peatlands can inhibit fen true mosses propagation by promoting vascular plant growth which can lead to competition for space and sunlight (Kotowski et al., 2006; Hejcman et al., 2010; Cusell et al., 2014). But vascular plants have been proven to be beneficial to fen true moss in certain circumstances such as creating microenvironments (Ingerpuu et al., 2005). The positive effect of vascular plants found in the study of Ingerpuu et al. (2005) were species specific, meaning that the positive effect was only with specific combination of moss and vascular species.

Only 1 species, *C. stellatum* reacted positively to the addition of lime. My results contrast with those of Vicherová et al. (2015) who found that fen true moss can benefit from a pH of 6.3 to 7.1 and a high [Ca^{2+}]. But this higher concentration and pH were not the result of liming. Furthermore, the study did not use peat as a medium for the growth of the moss but instead were submerged in a liquid solution. Since our lime contains Ca and Mg in almost equal part, it is hard to know if any of those elements influenced the result. The nutrient analyses of the peat did not show any greater concentration N, P or K nutrient availability following the liming treatment (Table 2.2). The reason why only *C. stellatum* reacted to the addition of lime could be partially explained by its ecology. The concentration of Ca and Mg within the moss are both affected by the species identity and the availability of the nutrients (Hájek et al., 2014). It is possible that Mg and Ca were a limiting factor for *C. stellatum* since it is a calcicole species and is naturally found in environments with relative high concentrations of those elements. The mosses *A. palustre* and *Tomentypnum nitens* only

tolerated the presence of Ca and Mg respectively since they do not have a high concentration of those elements within the moss (Hájek et al., 2014). The peat used in this experiment had nutrient values similar to a natural fen (Andersen et al., 2011). After the liming the main nutrient concentrations are more similar to a bog than a natural fen (Andersen et al., 2011). The fact that liming made the peat poorer in nutrients was found in another study (Emond, 2013). Another study (Mälson & Rydin, 2007) that experimented with liming found a positive effect on the survival and ground cover growth of fen true mosses including two species present in this study (*C. stellatum* and *Scorpidium cossonii*).

The experiment of Mälson and Rydin (2007) was factorial (moss cover x lime x species) and a few interactions were found. Lime had a positive interaction with the protective cover, furthermore, each species reacted differently to the addition of lime. Although the different reaction of species is true in both experiments. The liming in the Rochefort et al. (2016) study also did not have an effect on the moss cover after a dose of 15 g/m^2 of phosphate rock was applied and raised the pH from 4.4 to 5.4. The lime application was made 2 years after the initial experiment. In conclusion, it seems that the addition of lime does not, by itself at least, increase the fen true moss cover in post extracted peatlands. The application of lime did not have a negative effect on the growth of fen true moss and should be studied more with different treatments to assess its possible uses for optimizing regeneration on restored peatland sites.

D) The usage of *A. palustre* as a nurse plant

Our hypothesis for this experiment was that there might be a facilitation mechanism among different species of moss which allowed some of them to colonize a microsite due to field observation that moss colonies are rarely composed of 1 species. The pre- or concomitant reintroduction of the nurse plant *A. palustre* had no effect on the cover of the target species (*C. stellatum* and *S. cossonii*). However, many biases were introduced to this experiment. From the conceptualization to the maintenance, many factors could have contributed to the absence of significant results.

The first element that should be changed, if the experiment is to be repeated, is the target species. The mosses *S. cossonii* and *C. stellatum* are not species that are found with *A. palustre* in natural settings. The nurse plant and the associated target species simply did not have the same ecological niche (Gignac, 1992) and probably have different regeneration niches. For future experiments, I recommend switching to species that are commonly seen with *A. palustre* in natural fens, *T. nitens, Pleurozium shreberi,* and *Thuidium delicatulum* to name a few. I recommend keeping *A. palustre* as the potential nurse plant because my field experiment has shown it to be able to cover a vast

surface in a small period. Even though that does not necessarily make it a nurse plant, it could maybe play the same role as *Polytrichum strictum* for bog restoration and promote fen true moss growth by reducing the effect of frost heaving (Groeneveld et al., 2007). One of the main problems in peatland restoration is the effect of frost heaving (Quinty & Rochefort, 2003). In bog restoration this problem is solved by using *Polytrichum strictum* which acts as a nursing plant by reducing the harmful effect of frost heaving. The use of *A. palustre* to prevent frost heaving was not tested but could be a possible replacement for fen restoration. My experiments have showed that *A. palustre* spread relatively fast and does not impede the growth of other mosses.

Mosses were introduced in such a high cover that both the nurse and target mosses were spread on one another in the pre-established treatment. This high cover created an artificial competition for light and space. The moss on top had more access to light but less access to water than the moss underneath. Few bryophytes can tolerate low access to light (Glime, 2017b). A study has found a negative correlation between the density of shoot and the relative growth of *C. stellatum* and *S. cossonii* (Udd et al., 2016). Simply reducing the density of reintroduced moss could lead to different results.

Prior to setting up the experiment, the storage of the mosses was not optimal. Due to a mistake, the mosses that were intended to be used in the second half of the experiment were put in a refrigerator (4 °C) for about seven weeks instead of a freezer (-4 °C). A small experiment testing the viability of the material was put in place to test the difference in storage, both in length and in type on the regeneration of fen true moss. But due to a high amount of mold in the petri dish, caused by either the storage or a surplus of humidity, the results of this small experiment were deemed inconclusive.

The drought periods were also probably a cause of why no observation of nursing effect was made. The first one was so severe that cracks appeared on the peat. It was deemed too intense because this kind of drought is not representative of what happens in natural or restored peatlands except under highly unusual circumstances. It was also deemed too sudden, since the drought happened in a matter of days. As a result, the mosses did not have time to react to the change in water availability. A more gradual decline in the amount of water spread over a few weeks could have led to a more realistic depiction of a drought. This leads to a second, less severe, period of drought after a period of recovery. But even this second drought was deemed too severe. Bi-daily monitoring was necessary to keep the soil moisture high enough to affect the mosses physiology but low enough to be a source of stress. The difficulty to control the soil moisture could potentially lead to differences of stress among trays which could cause heterogeneity in its application. As a

result, both drought periods were too severe, not monitored well enough and were not depicting field moisture regimes during a drought. I still believe that the occurrence of drought could be one of the reasons uncovering an effect of the potential nurse plant on the target species.

Conclusion

One consistency among all experiments is that the number of innovations and cover of *Aulacomnium. palustre* is higher than *Tomentypnum. nitens* in both the field and the petri dish experiment. In the field experiment, *A. palustre* had 3 times more cover than *T.nitens* in the fragmented treatment. In the petri dish experiment, *A.* palustre as always more innovations than *T. nitens*. One big difference between the two species is that *A. palustre* reacted positively in the field experiment and the petri dish experiment to both the fertilization and fragmentation treatment as *T. nitens* only reacted to these treatments in the petri dish experiments.

Factors influencing the regeneration of fen true mosses

The treatment that produced the biggest increases in regeneration was the mechanical fragmentation treatment on *A.. palustre*. The other species only reacted positively to fragmentation in a controlled environment (constant air humidity) while *A. palustre* reacted positively in all experiment. Fertilization produced positive results for all species in a controlled environment, and only with *A. palustre* in the field experiment. However, the positive effect is negated when both fragmentation and fertilization is applied simultaneously to *A. palustre* showing a negative interaction between the two treatments. Liming affected *C. stellatum* positively while all the other species have not reacted to the addition of lime. Finally, the nursing plant experiment was inconclusive because too many factors in the end were not controlled. The main conclusion of the field experiment is that fen true mosses can regenerate in the field if favorable conditions are present (i.e. a high water table, protective cover raising the humidity at the air-substrate interface, etc,). Although it was not measured, it is clear with our field data that the most limiting factor was water availability. This is the case for many projects dealing with fen true mosses (Mälson & Rydin, 2007; Graf & Rochefort, 2010; Priede et al., 2016; Rochefort et al., 2016).

The unreliability of the nursing plant experiment data does not mean that the experiment was a waste of time, effort and money. Many of the faults leading to those conclusions resulted from poor planning in the control of all environmental factors, a lack of previous work dealing on the subject, and human error. One should look upon this experiment and see a framework to use as a starting point to build a better experiment. The idea of using *A. palustre* to prevent frost heaving is a promising idea.

This project was created as a first step toward a better understanding of fen true moss ecology. Eventually, this might lead to an adaptation of restoration technique to accommodate fen species. This project has reached its goal of learning more about fen true moss ecology. Other projects will be able to develop further the knowledge of fen true mosses in its applied uses on peatland restoration.

Limitations

Many bias and limitations are introduced during either the planning, implementing, or data collection of experiments.

Length of innovation within the petri dish: An observation made many times, but that was not measured, was the deformity observes on an innovation due to the shoot height. Many innovations within the petri dish were often twisted or bent because their length was longer than the petri dish height. It is unclear whether this phenomenon as affected the number of innovations in any way.

Mold and algae were a common problem during the experiments taking place in petri dishes. Many adjustments were made to limit the contamination, such as a higher monitoring of the humidity level. However, it is possible that early experiments had fewer innovations because of mold. The number of innovations may have been underestimated because mold was covering the surface of some of the petri dishes.

Liming: There are two major concerns that I must address to further analyze the results and to prevent future experiments from making the same mistake. First, is not taking in consideration the addition of mineral elements to the peat in the beginning of the experiment. We did not plan the possible effect of nutrients on moss regeneration and they were not properly monitored. Secondly, the delay between the end of the experiment and the peat chemical analysis was very long. The peat stayed many weeks inside containers within the growth chamber before being analyzed. When the time came to harvest a sample, the peat had stayed at 20-25ºC for many weeks which could have altered the chemical composition of the peat. The heat also dried the peat and could have enhance the decomposition rate. Liming should not be discarded as a possible amendment as its effect seems species specific and thus would have an effect on other moss species.

Field experiment: The implementation of this experiment began in early June and ended in late June. Bic–Saint-Fabien and Saint-Modeste were the last two sites to be implemented. This late implementation in the growth period caused these two sites to miss most of the rain of summer 2016. It is possible that the moss did not have a good early growth due to the lack of water available at each site. The year 2017 had a very dry month of July (Gouvernement du Canada, 2018b). With

some sites receiving as little as 14 mm of rain that month (Gouvernement du Canada, 2018a). It is possible that periodic drought during the year 2017 caused stress to the moss, especially the species belonging to the depression community. The difference in moss cover between the Saint Henri site, which had the highest moss cover, and the other sites is most likely due to a more even rain distribution and quantity. The main problem facing the interpretation of this experiment was the lack of water table data during the second year of the experiment. Due to time management, we used the average rainfall on each site to separate the dry from the wet site and used this information for interpretation.

The moss *A. palustre* consistently had higher percent ground cover than any other moss on the field. However, it did not always have the highest number of innovations in the petri dish experiment. The reasons why this species was the one who performed the best in establishment (highest percent ground cover) could be because it is more resistant to desiccation (Li & Vitt, 1994; Graf & Rochefort, 2010; Manukjanová et al., 2014), regenerate rapidly (Li & Vitt, 1994) and tolerate a vast spectrum of soil pH compared to other moss species (Gignac, 1992). My results suggest that moss from hummock communities are more suitable for fen restoration then moss from the depression community in terms of increasing percent ground cover. A similar conclusion was reached in bog restoration when it was found that *Sphagnum* species from the hummock community were better at colonizing bare peat in peatland restoration then their counterpart (Chirino et al., 2006). The species *A. palustre* should be a priority in future research to further study its possible use in fen restoration.

The moss *C. stellatum* had the second highest percent ground cover in the field, and the most innovation in nearly all petri dish experiments. *C. stellatum* can grow horizontally and cover more ground than *S. cossonii* which has a tendency to grow innovations vertically (Mälson & Rydin, 2009). This difference in growth patterns affected both species cover. My results are comparable to the study made by Drapeau Picard (2016) who successfully re-introduces a cover of *C. stellatum* comparable to what I have achieved in the field after 4 years. They installed the moss at the borders of pools which keep a lower and more constant water table.

Fragmentation should be used for hummock moss species especially if composed of *A. palustre*. This treatment could offer a low-cost option for an optimal regeneration potential. Furthermore, giving the negative interaction between fertilization and fragmentation, only one of those treatments should be applied at a time. Although fertilization also works with *A. palustre*, fragmentation had a stronger positive effect on both the amount of innovations and percent ground

cover. Fertilization could be used if the mosses are from the depression community. Fragmentation increased the amount of moisture an individual fragment can retain, making the moss who relies on a constant amount of water at disadvantage in an environment with fluctuating water table such as post-extracted peatlands. Although fertilization was not proven to have an effect in the field, it is also not harmful and could possibly have a positive effect given the right condition (high water table, mulch, etc.). The amount of fertilizer can be kept to a minimum to remove some of the costs associated with peatland restoration.

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