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Manure derived biochar can successfully replace phosphate rock amendment in peatland restoration

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ABSTRACT

Phosphate rock fertilization is commonly used in peatland restoration to promote the growth of *Polytrichum strictum*, a nurse plant which aids the establishment of *Sphagnum* mosses. The present study tested whether 1) phosphorus fertilization facilitates the germination of *P. strictum* spores and 2) biochar derived from local pig manure can replace imported phosphate rock currently used in peatland restoration. Various doses of biochar were compared to phosphate rock to test its effect directly on *P. strictum* stem regeneration (in Petri dishes in a growth chamber) and in a simulation of peatland restoration with the moss layer transfer technique (in mesocoms in a greenhouse). Phosphorus fertilization promoted the germination of *P. strictum* spores as well as vegetative stem development. Biochar can effectively replace phosphate rock in peatland restoration giving a new waste management option for rural regions with phosphorus surpluses. As more available phosphorus was present in biochar, an addition of only 3 –9 g m^{-2} of pig manure biochar is recommended during the peatland restoration process, which is less than the standard dose of phosphate rock (15 g m^{-2}).

1. Introduction

According to SER (2004), ecological restoration comprises all the processes which help the recovery of a degraded, damaged or destroyed ecosystem. Plants, especially *Sphagnum* mosses, poorly recolonize vacuum-milled peatlands without active human intervention, even many decades post-abandonment (Poulin et al., 2005), making peatland ecological restoration necessary. The main goal of peatland ecological restoration is to restore the long-term function of the carbon sink by promoting, as short-term goals: 1) the development of a moss carpet dominated by *Sphagnum*, which will allow the formation and the accumulation of peat and 2) the return of the diplothelmic hydrological layers (Graf et al., 2012; Rochefort, 2000; Sliva and Pfadenhauer, 1999; Vasander et al., 2003) which regulate the processes of decomposition and nutrient sequestration.

A restoration approach called the moss layer transfer method, developed in the 1990s, has successfully allowed the return of plant communities dominated by *Sphagnum* mosses in North American bogs (Poulin et al., 2012). This approach typically includes six steps: 1) site preparation to remove biological crusting and redistribute water, 2) harvesting of donor plant fragments, 3) spreading donor vegetation, 4) mulch application, 5) blocking drainage ditches and 6) phosphorus fertilization (Graf et al., 2012; Quinty and Rochefort, 2003; Rochefort and Lode, 2006).

The interest of phosphorus fertilization is to accelerate the establishment of *Polytrichum strictum* Brid., a pioneer species that can tolerate the harsh conditions found on bare peat surfaces. An important role played by *P. strictum* during the first 2–3 years post restoration is to reduce wind erosion and frost heaving, two important barriers to *Sphagnum* moss establishment (Groeneveld and Rochefort, 2005; Quinty and Rochefort, 2003). *P. strictum* stabilize the peat surface with its rhizoids and acts as a nurse plant by creating humid microclimates favorable to *Sphagnum* moss establishment on bare peat (Groeneveld et al., 2007). Once established, *Sphagnum* mosses eventually outcompete *P. strictum*, which gradually decreases in abundance (Rochefort et al., 2013). Depending on the circumstances, the growth of *Sphagnum* mosses can be enhanced by phosphorus fertilization (Aerts et al., 1992; Baker and

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Boatman, 1990; Li and Vitt, 1994; Limpens et al., 2004) or not (Ferland and Rochefort, 1997; Sottorcornola et al., 2007). However, until now it has not been possible to separate direct positive effects of fertilizer on *Sphagnum* moss growth from indirect positive effects via the promotion of nurse species such as *P. strictum* on restored peatlands.

The decision to fertilize or not depends on the balance between frost heaving risks (need for *P. strictum* as a nurse plant and soil stabilizer) versus the risk of invasion by non-peatland invasive species (Sottocornola et al., 2007). Where frost heaving risks outweigh those of invasion by undesirable species, a low dose of 15 g m^{-2} granular phosphate rock is recommended (Quinty and Rochefort, 2003). A higher dose would increase *P. strictum* cover (linear positive effect, Sottorcornola et al., 2007). However, a 30% *P. strictum* cover threshold should not be exceeded in restored peatlands because above this level it competes with *Sphagnum* moss rather than promoting its establishment (González et al., 2013). In addition, even if fertilization is known to increase growth of *Polytrichaceae* mosses after its establishment (Chapin and Chapin, 1980; Sottocornala et al., 2007), no studies indicate whether the phosphorus acts on spore germination or vegetative fragment regeneration for the specific bog species *P. strictum*.

Pig manure biochar could be an alternative fertilizer to phosphate rock during peatland restoration. Indeed, the content of available phosphorus in biochar is thought to be larger than in phosphate rock. However, no information is available to confirm this assumption. The biochar can be produced from several biomasses (wood, agricultural crop residues or animal manure) through a pyrolysis process. In this process, the biomass is heated at relatively high temperature (350 °C–750 °C) in an oxygen free environment, converting it into a carbonized solid fraction (biochar) and a gas fraction which subsequently can be partly converted by condensation into bio-oil (Bridgwater, 2003). Current applications of biochar include the amendment of agricultural soils for improving crops yield, the treatment of gaseous or liquid effluents, the use as bio-fuel and as a carbon sequestration material (Demirbas et al., 2006; Gaunt and Lehmann, 2008; Lehmann, 2007; McHenry, 2009; Navia and Crowley, 2010; Sun et al., 2014; Uzoma et al., 2011).

Two main advantages arise from the conversion of pig manure to biochar for use as fertilizer in peatland restoration. First, biochar produced from animal manure feedstock generally contains more phosphorus than biochar from lignocellulosic biomass (Ro et al., 2010). Therefore, less fertilizer is needed for an equivalent dose of phosphorus. Second, the conversion adds value to a by-product present in excess in some areas. Because of regulations limiting the amount of manure that can be spread, the supply of manure for use as fertilizer often exceeds demand in areas with a high concentration of swine production. Consequently, many farms are required to adopt new disposal practices. Converting the excess manure into biochar is a solution with environmental, technical and economic benefits for swine producers. Biochar is drier, lighter and more resistant to decomposition than the raw material from which it is derived, thereby facilitating the storage, management and long-range transport for use outside the animal production areas. However, the use of biochar as a fertilizer during peatland restoration has never been tested.

The first objective of the present study was to determine if the phosphorus acts on spores or fragments of *P. strictum* (in a growth chamber), the hypothesis being that it acts on both. The second objective was to test the effectiveness of biochar as a substitute for phosphate rock fertilization during peatland restoration. First, a preliminary study was carried out in order to estimate the available P in the biochar as compared to phosphorus rock. Second, two experiments were carried out to test the effects of biochar 1) on *P. strictum* in a growth chamber experiment and 2) on a small-scale peatland restoration experiment in a greenhouse. The hypothesis was that more available phosphorus would be present in biochar, and as a result, a lower dose could be used compared to phosphate rock to obtain the same effects on plants.

2. Material and methods

2.1. Germination of *P. strictum* spores

This experiment was conducted in Petri dishes and aimed to verify the effects of phosphorous fertilization on the germination of *P. strictum* spores. Six doses of phosphorus (phosphoric acid – H_3PO_4) were tested: 0, 0.14, 0.25, 1, 20 and 100 mg of P L^{-1}. The experiment was designed as a completely randomized design with five repetitions.

Capsules of *P. strictum* were collected in a peatland located in eastern Québec, Canada (47°49′N and 69°28′W). The spores of six capsules were mixed with 45 ml of water (for an approximate ratio of 367 000 spores/ml). Five drops (32 µl each) were placed in each Petri dish which had been filled beforehand with sterilized horticultural peat. Petri dishes were watered with a five-time diluted modified Rudolph solution (Campeau and Rochefort, 1996) to which was added the appropriate volume of H_3PO_4 to achieve the wanted concentrations of phosphorus. Petri dishes were then sealed with paraffin to minimize water loss. The treatment with 0.14 mg of P L^{-1} corresponded to the amount of phosphorus in the modified Rudolph solution. In the control without phosphorus, the stock solution normally used with KH_2PO_4 was changed for a solution with KOH to maintain the same amount of potassium. Petri dishes were placed in a growth chamber (photoperiod = 14 h) where the temperature was maintained at 25 °C during the day and 22 °C during the night. After 45 days of growth, the number of leafy gametophytes was recorded in each Petri dish.

2.2. Production of biochar from pig manure and evaluation of P availability for dose determination

The biochar used in this study was produced using a pyrolysis system with a feedstock consisting of the dried solid fraction of pig manure. The solid fraction came from a growing-finishing barn using an under slat separating system (perforated belt). This solid fraction was then dried with the SHOC^{MD} process, a bio-dryer that dries and sanitizes organic sludges to create a final product that is free of pathogens and offensive odors. This product was then pyrolized at 500 °C for 1.5 h.

Chemical characteristics of the biochar obtained from pig manure and of the phosphate rock were analyzed at the Research and Development Institute for the Agri-Environment (IRDA) laboratory (Quebec City, QC, Canada) (Table 1). Despite the phosphate rock contained close to five times more of total P than in biochar, the major portion is unavailable to the plants. In opposite, biochar contains a higher proportion of available P (close to six times more) than in phosphate rock. The obtained concentrations were used in order to choice the fertilization doses for the following experiments.

2.3. *P. strictum* fragment regeneration in Petri dishes

This experiment was carried out in Petri dishes and aimed to test a wide range of biochar doses on *P. strictum* fragments. It was designed as a completely randomized experiment and included a control treatment without phosphorous fertilization, three doses of phosphate rock including the reference dose typically used in restoration and six doses of biochar. Based on the results of the preliminary P availability tests, the biochar doses contained

Table 1
Characteristics of the resulting biochar from the pyrolysis of the solid fraction of pig manure and the phosphate rock and quantified difference between biochar and phosphate rocks.

Element[a]	Units	Biochar	Phosphate rock	Coefficient of enrichment by biochar
Total P	ppm	24 873	111 866	0.2
Available (Olsen P)	ppm	1631	280	5.8
Available (Bray method)	ppm	2312	403	5.7
Al	ppm	563	1975	0.3
B	ppm	143	70	2.0
Ba	ppm	28	37	0.8
Ca	ppm	42 718	303 746	0.1
Cd	ppm	0	16	0.0
Co	ppm	8	0	80.0
Cr	ppm	8	169	0.0
Cu	ppm	802	20	40.1
Fe	ppm	15 975	2548	6.3
K	ppm	33 302	2212	15.1
Mg	ppm	13 233	15 138	0.9
Mn	ppm	761	30	25.4
Mo	ppm	8	4	2.0
Na	ppm	8408	8089	1.0
Ni	ppm	13	15	0.9
Pb	ppm	1	2	0.5
S	ppm	10 648	21 524	0.5
Sr	ppm	141	1681	0.1
Zn	ppm	1299	166	7.8
Dry matter[b]	% w.b.	99.5	95.0	1.0
Ash	% w.b.	32.6	89.7	0.4
pH	–	10.4	8.2	1.3
C[c]	% d.b.	61.9	–	–
H[c]	% d.b.	1.2	–	–
N[c]	% d.b.	3.7	–	–

[a] The United States Environmental Protection Agency (USEPA) method (2012) using inductively coupled plasma optical emission spectrometry (ICP-OES) was used for the detection of major and minor elements.
[b] Dry matter was measured at 105 °C.
[c] Evaluated by dry combustion (Leco TruSpec, St. Joseph, MI, USA).

between 2 and 48% of the total phosphorous and between 50% and 1200% of the available phosphorus present in the reference dose of phosphate rock (Table 2). Each fertilization treatment was replicated five times.

P. strictum stems were collected in a peatland located in central Quebec, Canada (48°49′′N and 72°10′W). Stems were chopped into 1–2 cm long fragments, then spread on filter papers (pore size = 8 μm) placed in 14 cm diameter Petri dishes. Two grams of fragments were placed in each dish, covering about 20% of the surface. Fertilizer was sprinkled on top of the mosses. The filter paper was then saturated with distilled water and the Petri dish sealed with paraffin to minimize water loss. The Petri dishes were placed in a growth chamber with a 14 h photoperiod and a constant 22 °C temperature. After 11 weeks, the number of *P. strictum* stems was counted and algal proliferation was estimated using six classes: 0 = 0% of algae cover, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100%.

2.4. Peatland restoration in mesocosms

This experiment was conducted in mesocosms in greenhouses and aimed to simulate the steps of ecological restoration for cutover bogs. The reference dose of phosphate rock typically used in restoration was used as a control (PR-1), along with three doses of

Table 2
Description of fertilization treatments. PR = Phosphate rock, BC = Biochar. The x indicates the treatments used for a given experiment. The treatment highlighted in gray corresponds to the reference dose of phosphate rock typically used in peatland restoration.

Treatment	Dose of PR or BC (g/m^2)	Fraction of the reference dose for total P*	Fraction of the reference dose for available P**	**Experiment**: *P. strictum* regeneration on filter papers (Petri dishes)	**Experiment**: Peatland restoration in mesocosms (greenhouse)
Control	0	0 %	0%	x	x
PR-1/2	7.5	50 %	50%	x	
PR-1	**15**	**100 %**	**100%**	**x**	**x**
PR-3/2	22.5	150 %	150%	x	
BC-1/50	1.5	2 %	50%	x	
BC-1/25	3	4 %	100%	x	
BC-1/12	6	8 %	200%	x	
BC-1/8	9	12 %	300%	x	x
BC-1/4	18	24 %	600%	x	x
BC-1/3	27	36 %	900%	x	
BC-1/2	36	48 %	1200%		x

* 118 715 ppm of total phosphorus in phosphate rocks vs. 24 873 ppm in biochar (~5 times more in phosphate rocks, see Table 1).
** 280 ppm of available phosphorus in phosphate rocks vs. 1 631 pm in biochar (Olsen P method) and 403 ppm of available phosphorus in phosphate rocks vs. 2 312 ppm in biochar (Bray method, see Table 1).
In both cases, there are 6 times less available phophorus in phosphate rocks.

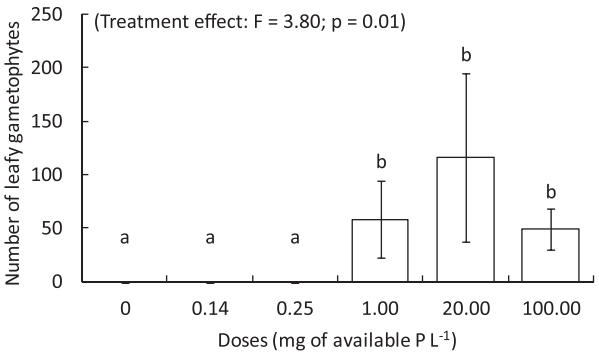

Fig. 1. Effect of phosphorus fertilization on the germination of *P. strictum* spores. The variable measured was the number of leafy gametophytes (mean ± SE) after 45 days in growth chambers. n = 5 for all treatments. Letters indicate significant differences following a protected LSD test.

biochar (BC-1/8, BC-1/4 and BC-1/2) and a treatment without fertilization (see Table 2 for more details). The experiment was set up as a complete randomized block design with four replications.

Plant material, composed mainly of *Sphagnum rubellum* Wilson along with *P. strictum*, was collected in a natural peatland near Quebec City, Canada (46°39′N and 71°19′W). As advised for peatland restoration, only the top 10 cm of the moss carpet was collected since this material has the highest regeneration capacity (Campeau et Rochefort, 1996; Quinty et Rochefort, 2003). The moss carpet was broken apart and fragments were uniformly spread in mesocosms on top of horticultural peat that had previously been rewetted (mean peat pH of 3.34 ± 0.02 SE). The spreading ratio was 1:10, which means than 1 m^2 of plant material collected in a donor peatland was spread over 10 m^2 of surface being restored. Fertilizers were sprinkled on top of the mosses, in one application at the beginning of the experiment. Water level was controlled independently for each mesocosm using a system of perforated pipes and drains which maintained the water table 15 −25 cm below the peat surface. Mesocosms were watered with rain water once every week, and mosses at the surface were kept humid by a misting system. During the first 50 days of the experiment, the environmental conditions were kept humid to facilitate germination of *P. strictum* spores (18 °C during the day and 14 °C during night; along with a constant relative humidity (RH) of 60%). After 50 days, conditions were modified to promote the growth of Sphagnum (22 °C/50% RH during the day and 18 °C/85% RH during the night). After 125 days, *Sphagnum* moss and *P. strictum* cover was visually evaluated for each experimental unit. Additionally, the number of *Sphagnum* capitulum and *P. strictum* stems was counted in five 25 cm^2 quadrats placed haphazardly in each mesocosm.

2.5. Chemical analyses in peat and plant tissues

Four times during the experiment (at 20, 41, 91 and 125 days), a composite sample of material was collected under the living moss carpet of each experimental unit of a given treatment. The samples were analyzed for total and available phosphorus concentrations as well as pH, allowing the detection of trends over time. In addition, at the end of the experiment (after 125 days), a composite sample of living moss tissues (*Sphagnum* and *P. strictum*) from each experimental unit was collected for each of the five treatments. Chemical analyses were run at the IRDA laboratory.

2.6. Statistical analyses

One way ANOVAs were performed to test if fertilizers had a significant effect on the regeneration of *P. strictum* fragments in terms of leafy gametophyte numbers (in the spore germination experiment) and new stems development (in the *P. strictum* fragment regeneration and mesocosm restoration experiments). In the *P. strictum* fragment experiment, analyses were also done on algal proliferation. Analyses were performed on the number of *Sphagnum* capitula and the cover of *Sphagnum* and *P. strictum* mosses. Following the ANOVA, a protected LSD was run for the number of leafy gametophytes in the spore germination experiment and for the number of new *P. strictum* stems and the algal proliferation in the *P. strictum* fragment experiment. For the restoration experiment, Dunnett's test was used with the reference dose of phosphate rock as the control (15 g m^{-2}; PR-1 in Table 2).

The GLM procedure in SAS software was used (SAS Statistical System software, v. 9.2, SAS Institute Inc., Cary, NC, USA). Significant probability levels were set to $\alpha = 0.05$ and all data were tested for homogeneity as well as for normality. The number of new *P. strictum* stems, new *Sphagnum* capitula and leafy gametophytes were square-root transformed prior to analysis.

3. Results

3.1. Germination of P. strictum spores

After 45 days, no germination was observed for the three lowest doses of P (0, 0.14 and 0.25 mg of available P L^{-1}) whereas germination occurred at the three highest doses (1, 20 and 100 mg of available P L^{-1}; Fig. 1). Due to the high variance observed within the same fertilization treatments, no significant differences were detected between the three highest doses. Thus, a minimal phosphorous dose located somewhere between 0.25 and 1 mg of available P L^{-1} appeared necessary to induce the germination of *P. strictum* spores; once this threshold is reached, increasing the dose will not improve the germination rate.

3.2. P. strictum fragment regeneration in Petri dishes

Regeneration of *P. strictum* fragments in terms of new stems was similar between all doses of phosphate rock, the four lower doses of biochar and the unfertilized control (Fig. 2A). However, the two highest doses of biochar (BC-1/3 and BC-1/4) significantly reduced the development of new stems by 2.4 times compared to the two lowest doses of biochar (BC-1/25 and BC-1/50) and the three doses of phosphate rock. Two high doses of biochar (BC-1/4 and BC-1/8) favored algal proliferation (more than 50% cover) whereas they were virtually absent in the control treatment and low in the two lower doses of biochar (BC-1/25 and BC-1/50). The three doses of phosphate rock as well as the midway dose of biochar (BC-1/12) induced an intermediate algae development (cover between 25 and 50%; Fig. 2B).

3.3. Peatland restoration in mesocosms

When compared in terms of *P. strictum* cover, none of the treatments tested were significantly different from the typical dose of phosphate rock used in restoration (Fig. 3A), meaning that any addition of P was beneficial compare to the control without fertilization. In addition, of the three biochar doses, only BC-1/4 resulted in a significantly higher number of new *P. strictum* stems (1.6 times higher; 16 340 ± 1153 vs. 10 100 ± 1258 stems m^{-2}) than the dose of phosphate rock typically used in restoration. In contrast, the development of new stems in the unfertilized control was significantly lower compared to the typical dose of phosphate rock used in restoration (3 times lower; 3240 ± 1957 vs. 10 100 ± 1258 stems m^{-2}; Fig. 3B).

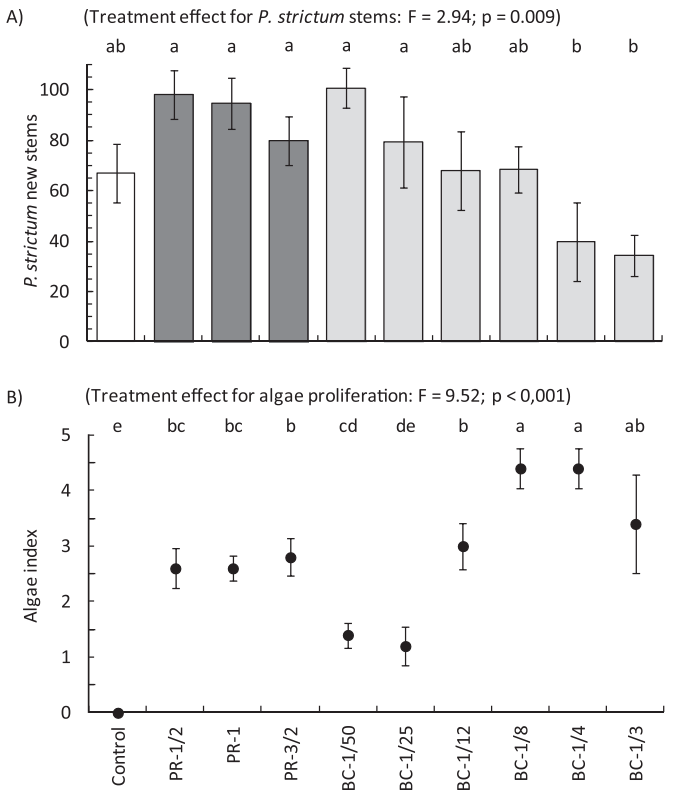

Fig. 2. Effect of fertilization on regeneration of *P. strictum* stems (A) and on algal proliferation (B) (mean ± SE) after 11 weeks on filter paper in Petri dishes placed in a growth chamber. PR = phosphate rock (in dark gray), BC = biochar (in light gray). The fraction after the treatment refers to the fraction of total P found in the treatment in comparison with the reference dose (PR-1, 15 g m^{-2}). Index for algal proliferation is as follows: 0 = 0% of algae cover, 1 = 1–5%, 2 = 6–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100%. n = 6 for all treatments. Capital letters (for the regeneration of *P. strictum* stems) and lower case letter (for algal proliferation) indicate a significant difference following a protected LSD test.

Sphagnum moss cover exhibited the same response as *P. strictum*: none of the treatments induced a significant change as compared to the typical dose of phosphate rock used in restoration (Fig. 3C). However, *Sphagnum* capitula tended to be smaller as the biochar dose increased (pers. obs.). Indeed, even if there was no difference in terms of cover, the number of *Sphagnum* capitula was 1.4 times higher for the BC-½ treatment compared to the reference dose of phosphate rock normally used in restoration (29 320 ± 1380 vs. 18 220 ± 1776; Fig. 3D). Moreover, a greening of *Sphagnum* mosses was observed as the dose of biochar increased, going from red that is normally observed for *S. rubellum* in the unfertilized control and the PR-1 treatments to yellow-green in the highest dose of biochar (Annex 1).

3.4. Chemical analyses in peat and plant tissues

Increasing the biochar dose increased the concentrations of Cu, Fe, K and Mg in moss tissues (Table 3). The concentrations of these elements were similar between PR-1 and the unfertilized control, but they were 1.5–2.9 times higher for the BC-1/2 treatment. Concentrations of Cu, Fe and K were higher in the biochar than in the phosphate rock, possibly indicating that plant uptake for these elements was higher as the element availability was higher. Such trends in nutrient concentrations may explain the color changes noted in *Sphagnum* mosses, although the statistical significance cannot be determined due to a lack of replication. Phosphorus tissue concentrations also increased with increasing biochar doses. However the trend over time is not clearly defined (Table 4). However, biochar or phosphate rock additions did not change the peat pH observed in the mesocosm experiment (Table 4).

4. Discussion

4.1. The effect of phosphorus on P. strictum regeneration

Available phosphorus often limits plant growth in natural bogs (Bedford et al., 1999; Bridgham et al., 1996) and its concentrations is even lower in cutover peatlands (Wind-Mulder and Vitt, 2000), suggesting phosphorus deficiency (Andersen et al., 2006). Consequently, phosphorus addition during the restoration process can accelerate the establishment of *P. strictum* (Ferland and Rochefort, 1997; Sottorcornola et al., 2007) a nurse species for Sphagnum moss establishment(Groeneveld et al., 2007; Groeneveld and Rochefort, 2005).

This study showed that phosphorus (in phosphate rock or in biochar) promotes the growth of *P. strictum* by increasing spore germination and enhancing vegetative spread. As spores are likely to accumulate on the moss surface year after year and can remain viable for a long time (During, 1979), the vegetation reintroduced during peatland restoration likely contains a sufficient quantity of spores whose germination could be stimulated by phosphorus fertilization. This stimulation is similar to that observed post-fire on peatlands. During a fire, nutrients immobilized in plant tissues are released resulting in a strongly increased cover of pioneer mosses (Benscoter and Vitt, 2008; Kuhry, 1994). These pioneer mosses facilitate *Sphagnum* establishment (Benscoter, 2006). In the decades following a fire or the restoration, *Sphagnum* mosses gradually outcompete the pioneer mosses and *P. strictum* colonies as the initial flush of nutrients is depleted.

The decreased development of new *P. strictum* stems following the two higher doses of biochar (18 and 27 g m^{-2}) could indicate fertilizer toxicity or nutrient deficiencies from nutrient antagonism. However, as the regeneration experiment was done on filter paper, the ability of peat to retain nutrients or mitigate contaminants was absent. In contrast, field experiments on restored peatlands using various doses of phosphate rock (up to 25 g m^{-2}), showed a linear increase in *P. strictum* regeneration with dose (Sottorcornola et al., 2007), indicating that on peat, toxic effects and nutritional deficiencies are less likely to be a problem. As more algae were present following higher doses of biochar, light competition between mosses and algae could also explain the lower development of new *P. strictum* stems.

4.2. Biochar as a replacement for phosphate rock during peatland restoration

This experiment confirmed the hypothesis that biochar derived from pig manure contains more available phosphorus than phosphate rock. In addition, biochar addition effects on phosphorus sorption are influenced by soil acidity (Wang et al., 2015; Xu et al., 2014), with a decreasing phosphorus sorption with an increasing of the acidity. As peat is a very acid soil, the phosphorus availability coming from the biochar addition is even more likely to increase. In both cases, less biochar than phosphate rock is needed to achieve the same response. Between 1.5 and 9 g m^{-2} of biochar had the same effect as 15 g m^{-2} of phosphate rock (the dose typically used during peatland restoration).

The success of a peatland restoration project is jeopardized when *P. strictum* exceeds 30% cover (González et al., 2013). In this situation *P. strictum* could outcompete rather than facilitate *Sphagnum* moss establishment. However, even at the highest dose of biochar, *P. strictum* cover never reached the 30% threshold and

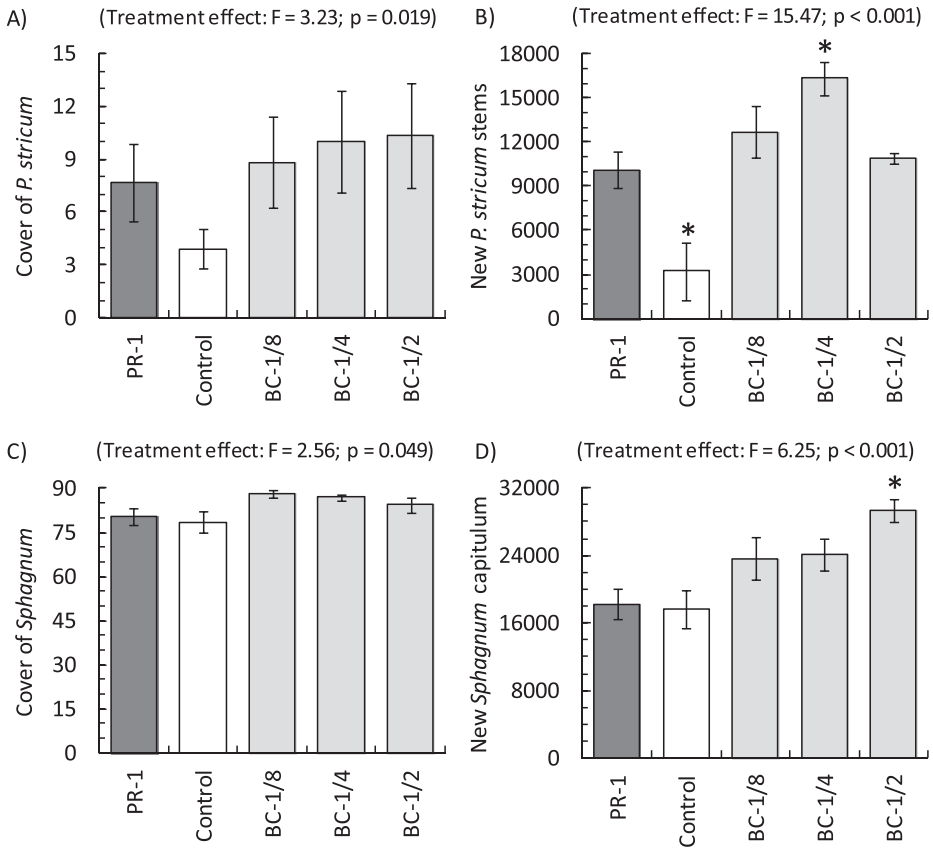

Fig. 3. Effect of fertilization on A) cover of *Polytrichum strictum*, B) regeneration of *P. strictum*, C) cover of *Sphagnum* mosses and D) regeneration of *Sphagnum* capitula after 125 days in mesocosms in greenhouse. All values are expressed as mean ± SE. PR = phosphate rock (in dark gray), BC = biochar (in light gray). The fraction after the treatment refers to the fraction of total P found in the treatment in comparison with the reference dose (PR-1, 15 g m^{-2}). n = 4 for all treatments. An asterisk indicates a significant difference following a Dunnett's test compared to the usual dose of phosphate rock used in restoration (PR-1).

the number of *P. strictum* stems and *Sphagnum* capitula increased. Therefore, even higher doses of biochar could be considered. More stems of *P. strictum* will further stabilize soils subject to frost heaving and create favorable microclimates for *Sphagnum* mosses (Groeneveld et al., 2007). The *Sphagnum* moss carpet is usually fluffier in restored peatland than in natural ones, making the return of a typical peatland hydrology difficult (McCarter and Price, 2012). The creation of denser carpets of *Sphagnum* mosses could thus improve the hydrology in restored peatlands by favoring the transfer of water through capillarity to adjacent *Sphagnum* stems.

4.3. Biochar as a fertilizer in peatland restoration: the pros and cons

The use of pig manure biochar as a fertilizer in ecological peatland restoration has three major positive points. First, less fertilizer is needed in comparison with the reference dose of phosphate rock. The amount of phosphorus added will thus be reduced, lessening possible environmental impacts. Second, the transformation of pig manure to biochar gives a new option for the management of this agricultural waste (Navia and Crowley, 2010) and the exportation of phosphorus in regions where agricultural lands have a phosphorus surplus. Contrary to imported phosphate rock (for example from Northern Africa), biochar can be produced locally, reducing the phosphorus surplus of farms surrounding the peatland to be restored. Third, the carbon in pig manure biochar is sequestered in a slowly decomposing peat matrix, reducing carbon emissions. For example, applying biochar instead of synthetic fertilizers to agricultural lands generates 2 to 5 times less CO_2 emissions (Gaunt and Lehmann, 2008).

The main downside to the use of biochar is that the chemical composition varies according to its inputs. The phosphorus and other nutrients content on biochar can vary considerably in function of the pig manure properties, the technology used for separation of the manure and the parameters used for the pyrolysis. It is therefore essential to perform chemical analyses prior to use and adjust the application rate accordingly. In addition, heavy metals in the manure are concentrated in the biochar. For example, in the solid fraction of pig manure, the contents of Cu and Zn were 438 and 557 ppm, respectively, the content go up to 802 and 1299 ppm in the biochar (IRDA, unpublished data). This is similar to result from wastewater sludge biochar (Hossain et al., 2011). Thus, biochar could be a material with a higher potential for toxicity and its repeated application may contribute to overload the soil and water of the zone and can bioaccumulate in plants. However, as a single low dose of fertilization is normally added during peatland restoration, toxic effects of biochar addition are not suspected. Despite that, four nutrients (Cu, Fe, K and Mg) showed an increase in plant tissue concentrations linked with increasing availability in biochar high doses. At toxic concentrations, these nutrients can disturb many aspects of plant metabolism such as pigment synthesis, membrane integrity, photosynthetic processes or enzymatic activity, resulting in growth and morphology perturbations (Balsberg and Pahlsson, 1989; Fernandes and Henriques, 1991; Folkeson and Andersson-Bringmark, 1988; Snowden and Wheeler, 1993; Wu et al., 1991). Few studies are available on nutrient toxicity for mosses or peatland species and the toxic thresholds for these nutrients are likely to vary between species (Folkeson, 1981; Snowden and Wheeler, 1993). Apart from the *Sphagnum* moss color difference possibly caused by a

Table 3
Concentrations of micro- and macro-nutrients (in ppm except for total C and total N) in **moss tissues** (*Sphagnum* and *Polytrichum strictum*) in the mesocosm peatland restoration experiment. Values represent a composite sample composed of material taken from each experimental unit of a given treatment.

Nutrient (ppm)[a]	Fertilization treatment				
	PR-1	Control	BC-1/8	BC-1/4	BC-1/2
Al	136	149	95	89	123
B	2	2	2	2	4
Ba	9	11	9	8	10
Ca	6912	1754	1826	1902	2545
Cd	0	0	0	0	0
Co	0	0	0	1	1
Cr	4	1	1	1	1
Cu	12	15	16	20	38
Fe	193	232	209	282	608
K	2826	2896	3577	4137	4987
Mg	803	714	772	910	1119
Mn	43	67	49	45	66
Mo	0	0	0	0	1
Na	1581	1231	1474	1418	1305
Ni	2	1	1	1	2
P	2800	483	919	1344	2087
Pb	3	4	3	3	3
S	1226	1126	1166	1041	1356
Sr	37	8	9	9	11
Zn	219	263	253	242	305
Available P[b] (Olsen)	184	63	161	306	505
Total C[c] (in mass %)	46	45	46	46	46
Total N[c] (in mass %)	1	1	1	1	1

[a] Micro- and macro nutrients were measured by the digestion method EPA-3050 (EPA, 1996) using an ICP-OES.
[b] Olsen P method (Olsen et al., 1954), with an automated spectrophotometer (Technicon).
[c] Evaluated by dry combustion (Leco TruSpec).

modification of pigment synthesis, no signs of growth retardation or any other toxic fertilizer effect was observed, suggesting that toxic thresholds were not reached even at the highest biochar dose. However, a better understanding of toxic thresholds for *Sphagnum* and other mosses is definitely needed.

Phosphorus leaching must also be considered. The amount of available phosphorus in biochar is higher than in phosphate rock. Consequently, it may be released more quickly than the phosphorus in phosphate rock, which is considered a slow-release fertilizer

Table 4
Concentration of total and available P (in ppm) and pH in **peat** under the moss carpet in the mesocosm peatland restoration experiment. Values were measured in a composite sample composed of material taken from each replicate of a given treatment. PR = phosphate rock, BC = Biochar, N.A. = not available.

Treatment	After X days	Total P	Available P (Olsen)	pH
PR-1	20	157	16	3.32
	41	177	27	3.21
	91	167	19	N.A.
	125	171	24	3.47
Control	20	152	14	3.31
	41	155	18	3.19
	91	168	17	N.A.
	125	161	15	3.46
BC-1/8	20	157	19	3.26
	41	152	18	3.27
	91	164	17	3.38
	125	163	14	3.36
BC-1/4	20	156	18	3.28
	41	155	25	3.33
	91	179	21	N.A.
	125	184	13	3.46
BC-1/2	20	199	30	3.40
	41	223	38	3.29
	91	221	39	N.A.
	125	183	19	3.45

(Nieminen and Jarva, 2000). For example, over the course of a 14 week experiment done in the IRDA laboratory, between 21 and 25% of the phosphorus in biochar was leached from small pots (7 cm diameter × 7 cm height) filled with peat and *Sphagnum* mosses (IRDA, pers. Comm.). However, the greater volume of peat in restored peatlands (as compared to pots) limits phosphorus lost through leaching and runoff. In a fertilization experiment on cloudberry in northeastern Quebec, added nutrients showed very limited dispersion (Hébert-Gentile et al., 2011), suggesting that most of the fertilizers were immobilized in peat. In addition, a restored cutover bog is a relatively closed system as the ditches have been blocked for rewetting, and the remaining water losses are mostly through evaporation. Also, phosphorus retention is higher in low-input sites such as peatlands and is primarily controlled by vegetation and microorganisms in a closed cycle (Kellogg and Bridgham, 2003). Finally, the total quantity of phosphorus added in peatland restoration (typical dose of phosphate rock = 8.5 kg of P/ha) is much lower than in traditional agriculture where annual P fertilization rates can be two to ten times higher for some annual crops (Cela et al., 2010; Farmaha et al., 2012; Lutcher et al., 2010; Mallarino et al., 2011; Sheng et al., 2012). In some cases, the incorporation of biochar in agricultural soils can decrease fertilizer runoff (Lehmann, 2007; Navia and Crowley, 2010). Despite these points, if biochar is applied in large scale restoration projects, monitoring phosphorus outflow is definitely recommended.

As biochar has a lighter ash-like consistence, it could be difficult to spread uniformly with the machinery generally used (a conical spreader behind a tractor, see Quinty and Rochefort, 2003). It may be necessary to mix the biochar with the plant fragments before their reintroduction. Additionally, tests are underway to increase the biochar fragment size by modifying the pyrolysis parameters (IRDA, pers. comm.), which will facilitate their spreading.

4.4. Conclusion: use of biochar during large scale peatland restoration

Results obtained in the present small scale studies are encouraging and indicate the relevance of testing biochar in large scale field restoration. The reduced growth of *P. strictum* and higher algal proliferation observed in Petri dishes at the higher biochar doses suggest that lower doses should be used for field restoration. Therefore between 3 and 9 g m^{-2} biochar is recommended. The dose must be adjusted according to the nutrient levels in the biochar. This dose is lower than the dose of phosphate rock typically used during peatland restoration (15 g m^{-2}). It corresponds to approximately 4–12% of the total amount of phosphorus and 100–300% of the available phosphorus present in the reference dose of phosphate rock. Field tests should also include the monitoring of various elements such as the phosphorus in water outflow and changes in acidity, conductivity, water retention or concentration of potential toxic elements that can be caused by biochar addition. However, as the proposed doses are really low, biochar should not modify these parameters. A comparison of the costs as well an environmental life-cycle assessment is needed to define the relative benefits of biochar versus phosphate rock. Clearly, the local production of biochar from a renewable resource versus the importation of non-renewable phosphate rock, the reduced phosphorus surplus of local farms and the lower application rates indicate that biochar is an excellent option for ecological peatland restoration.

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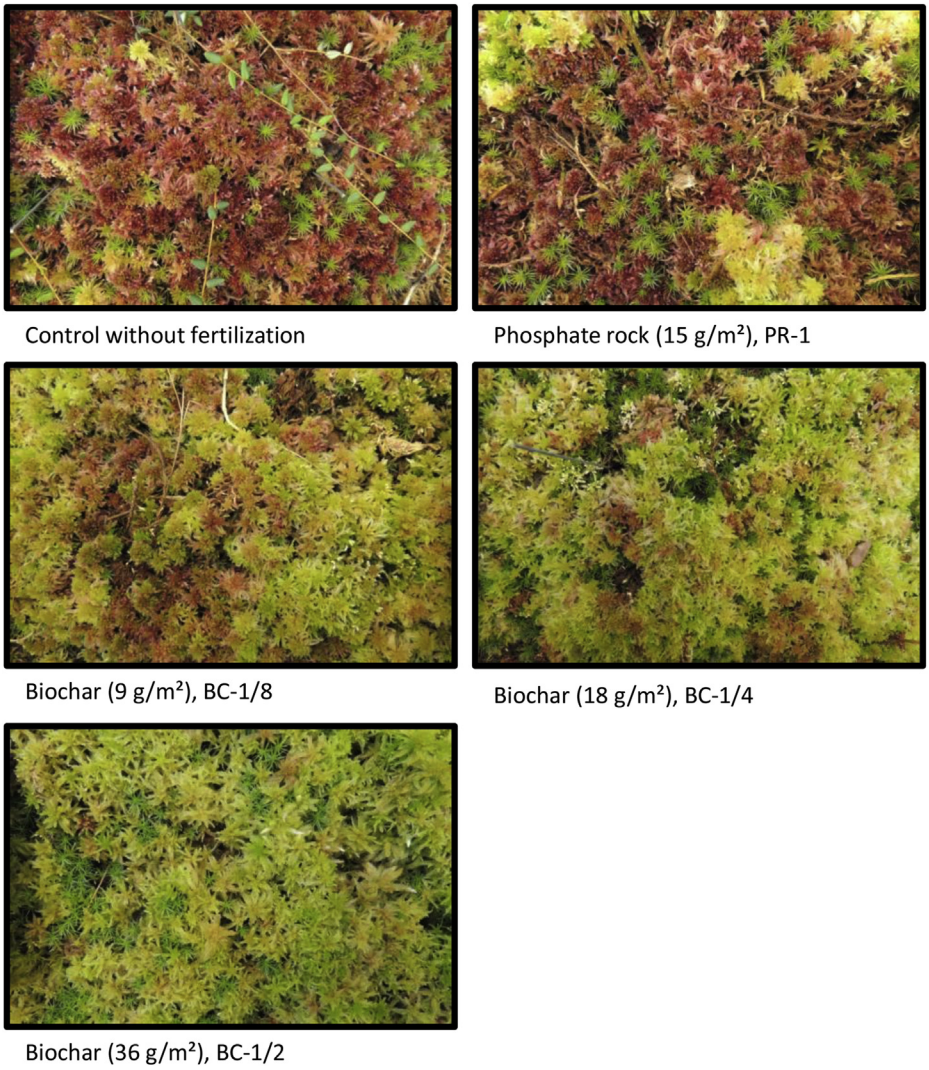
Annex 1. Color changes of *Sphagnum* mosses following fertilization treatments. See Table 3 for more details.



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