

# Organic matter amendment enhances establishment of reintroduced bryophytes and lichens in borrow pits located in boreal forest highlands

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Borrow pits, gravel and sand extraction sites, are commonly reclaimed by reintroducing vascular plants. Even after vegetation rehabilitation, recovery is slow and likely only dominated by a few plant species, often atypical of the surrounding landscape. This study aims to evaluate the short term effect of different restoration treatments for establishing diaspores of bryophytes and lichens in disturbed boreal habitats. We tested different treatments: organic matter addition (peat), straw mulch, and nitrogen and phosphorous fertilisation. We found that amending with peat was the most effective treatment in establishing bryophyte and lichen using diaspores, but only if no mulch was applied. Applying mulch had a detrimental effect on the diaspores, whereas nitrogen and phosphorous fertilisation appeared to have no effect. The lack of a fertiliser response, however, may be due to the negative effect of applying mulch, since all plots treated with fertiliser and diaspores also included the mulch treatment.

## Introduction

Ecological restoration is defined as “the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (SERI 2004). Sometimes, natural succession is the driving force in moving a degraded ecosystem toward a functional and resilient restored ecosystem (Bradshaw 1997). Indeed, some authors argue that natural colonisation can be advantageous for restoring xeric and poor sites (Prach and Pysek 2001, Rehounkova and Prach 2008), especially when cost is a concern. Most research-

ers agree, however, that some active restoration is necessary to initiate the succession process (Walker and Walker 1991), especially when the environmental conditions of the disturbed site are extremely harsh (Prach and Hobbs 2008). Reintroducing vascular plants — indigenous or otherwise — through seeding, hydroseeding, or planting is among the most popular rehabilitation techniques used to restore disturbed areas characterised by poor or dry conditions, such as roadsides (Tyser *et al.* 1998, Tormo *et al.* 2007), dunes (Dejong and Klinkhamer 1988, Roze and Lemauviel 2004), grasslands (Foster *et al.* 2007),

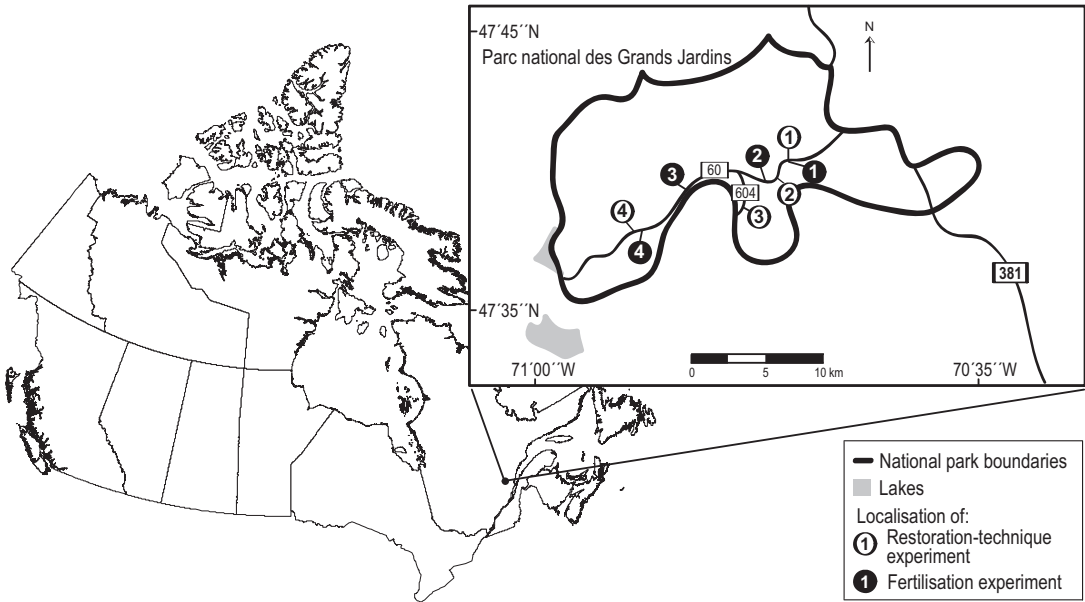
abandoned mines (Smyth 1997, Holl 2002, Reid and Naeth 2005), and some campsites (Zabinski *et al.* 2002, Cole 2007). According to these studies, rehabilitation techniques based on reintroducing vascular plants lead to a stabilisation of the substrate and help facilitate the establishment of indigenous species. Borrow pits — gravel and sand extraction sites associated with road construction — in northern Canada are also mainly rehabilitated by reintroducing vascular plants (Labbé and Fortin 1993, Houle and Babeux 1994, Rausch and Kershaw 2007). Several years after these revegetation efforts, however, the process of succession is slow and these sites can remain dominated by few species, often atypical of the surrounding landscape (Densmore and Holmes 1987, Matesanz *et al.* 2006).

Optimizing the success of revegetation efforts is important because the pace of road construction is intensifying in northern ecosystems in response to growing economy surrounding natural resource extraction including timber, minerals, tar sands and high flow rivers for hydroelectricity production. Borrow pits located in the boreal forest are also rehabilitated by seeding vascular plants, although bryophytes and lichens are among the dominant lifeforms of the original ecosystem (Boudreault *et al.* 2002). For this reason, efforts should be undertaken to facilitate the establishment of these lifeforms in reclaimed sites. Moreover, in disturbed habitats in the boreal forest, bryophytes and lichens are among the main primary colonisers (Hugron *et al.* 2011). Their effect on vascular plant establishment can be positive or negative. In some instances bryophyte and lichen mats can reduce the emergence of vascular plants seedlings (Zamfir 2000, Houle and Filion 2003), although they can also improve conditions necessary for their growth (Steijlen *et al.* 1995, Houle and Filion 2003). Nevertheless, in harsh environmental conditions, facilitation, rather than competition, between lifeforms is usually expected (Callaway and Walker 1997) and, therefore, reintroducing bryophytes and lichens species could be a good option for promoting borrow pit restoration by facilitating the establishment of vascular plants.

Without revegetation efforts, borrow pits often remain unvegetated, or sparsely vegetated, for many decades following extraction

activities (Harper and Kershaw 1996, Hugron *et al.* 2011). Natural recovery is limited because their substrate is predominantly composed of sand and gravel which is poor and unstable (Brady and Weil 2004). Reintroducing diaspores of pioneer species of bryophytes and lichens immediately after abandonment could improve site characteristics, thereby improving conditions for establishment and growth. Mosses can facilitate the establishment or growth of vascular plants by stabilising the substrate (Leach 1931, Groeneveld *et al.* 2007), increasing soil organic matter (Bardgett and Walker 2004), and improving microclimatic conditions that promote the germination and survival of seedlings (Bell and Bliss 1980, Delach and Kimmerer 2002). The reintroduction of bryophytes is a successful restoration technique for other ecosystems dominated by nonvascular plants such as peatlands (Rochefort 2000, Graf and Rochefort 2008) and alvars (Campeau 2009). Lichens of the genus *Stereocaulon* are able to fix atmospheric nitrogen and may significantly increase the quantity of available nitrogen in borrow pits (Gunther 1989). Some treatments can be applied to increase the survival and growth of reintroduced mosses and lichens. Among those, adding a layer of straw mulch which maintains higher relative humidity at the soil-air interface was shown to improve the survival of *Sphagnum* diaspores (Price *et al.* 1998). Mulching can also reduce wind erosion (Chambers *et al.* 1990) and promote the germination of the vascular seed bank (Cook *et al.* 2006). The addition of organic matter can improve the nutrient and water retention capacity of the soil (Reid and Naeth 2005) and increase soil carbon and nitrogen content (Curtis and Claassen 2009). Fertilisation (N and P) can improve the nutrient status of the soil, thereby accelerating the plant successional process (Kidd *et al.* 2006). It was demonstrated that phosphorous can promote the establishment and growth of bryophytes (Chapin and Chapin 1980, McKendrick 1987, Sottocornola *et al.* 2007).

We evaluated the success of different techniques using bryophytes and lichens in restoring borrow pits located in the boreal forest of Quebec, Canada. All treatments were tested for the purpose of rapidly initiating the successional processes following borrow pits abandonment.



**Fig. 1.** Location of the Parc des Grands Jardins and of the eight borrow pits where restoration experiments were installed.

Our study aims to evaluate the effectiveness of adding mulch, organic matter (peat) and fertiliser (N and P) in establishing diaspores of bryophytes and lichens.

establishment of spruce boreal forest and lichen woodlands which are found in the region at their southernmost distribution (Payette *et al.* 2000).

## Material and methods

### Study site

The study area is located in the highlands (mean altitude of 800–900 m a.s.l.) of the boreal forest, 120 km north-east of Quebec City (Quebec, Canada) within the boundary of the Parc National des Grands Jardins (PGJ; 47°38'N, 70°42'W). The study site is located in the transition zone between the mixed-conifer and hardwood and the boreal forests (Bergeron 1996). The region is characterised by a boreal climate with cold temperatures (average annual of 0 °C), due to its altitude and relatively low (1000 mm/year) precipitations. From 2008 to 2010, the average number of days with snow on soil was 160 per year (data from the Meteorological station of Parc national des Grands Jardins). This climate, coupled with a high turnover of natural and anthropogenic disturbances such as fire, logging and spruce budworm outbreaks, favors the

### Experimental design

We designed two experiments to test the effectiveness of different restoration techniques and fertilisers regimes on establishing a productive plant community on several borrow pits. The first experiment (hereafter called the Restoration-Technique Experiment) included three factors: (1) spreading diaspores of bryophyte and lichen, (2) mulching, and (3) peat amendment, with two levels for each factor (either with or without). We set up the treatments as a factorial experiment in a complete randomised block design, replicated in four different borrow pits (blocks) of the PGJ (see Fig. 1) for a total of 32 experimental units. The plots (1 × 1 m) were spaced at least 1 m apart within each borrow pit. The spreading of diaspores consisted of reintroducing a mixture of three bryophyte and two lichen species that were identified as the main primary colonisers of borrow pits by a previous study on natural colonisation of borrow pits of the PGJ (Hugron *et al.* 2011). The bryophytes selected were: *Polytri-*

*chum piliferum*, *Niphotrichum canescens* subsp. *canescens* (syn. *Racomitrium canescens*) and *Ceratodon purpureus*. The lichens comprised *Stereocaulon paschale* and *Trapeliopsis granulosa*. The species were hand collected in nearby abandoned borrow pits of the PGJ. Diaspores consisted of moss stems and lichens thalus that were separated one from another to facilitate spreading. Diaspores from all five species were mixed together and reintroduced simultaneously at a ratio of 1:5 (1 m<sup>2</sup> of collected plant material was spread on a 5 m<sup>2</sup> area which corresponded to a cover of approximately 75%). The spread mosses and lichens then were covered with long fiber oat straw mulch at a rate of 300 g m<sup>-2</sup> which offered a complete coverage of the plot, but still allowed some light to pass through. The mulch was autoclaved before applying to destroy the seeds of undesired species. Commercial blond peat was applied in a uniform layer of 2 cm that was incorporated into the surface layer of sand with a rake.

The second experiment (hereafter called the Fertilisation Experiment) was designed to test the effect of fertilisers on revegetation of borrow pits. The first factor consisted of four restoration methods (four levels) which were a subset of those described for the Restoration-Technique Experiment: (1) no treatment, (2) mulching only, (3) diaspore and mulching, and (4) peat amendment, diaspores and mulching. Nitrogen was added as slow-release polymer-coated urea (44-0-0) and phosphorous as slow-release phosphate rock (0-13-0) at the rates of 1 g N m<sup>-2</sup> and 3 g P<sub>2</sub>O<sub>5</sub> m<sup>-2</sup>, respectively. The experiment was designed as a factorial experiment with three factors tested: restoration methods (with four levels), nitrogen fertilisation (two levels: with and without) and phosphorous fertilisation (two levels: with and without) in a complete randomised block design, replicated in four different borrow pits (blocks) of the PGJ (see Fig. 1), for a total of 64 experimental units (1 × 1 m plots). The plots (1 × 1 m) were spaced at least two meters apart within each borrow pit. The fertilisers were applied manually in May 2008 and again in June 2010.

We set up experiments in eight abandoned borrow pits exhibiting very low vegetation cover. The substrate of all the borrow pits consisted of fluvio-glacial deposits dominated by

sand or gravel with organic matter nearly absent (mean ± SD = 1.5% ± 1.5%, measured by loss on ignition). For one single experiment, the blocks (borrow pits) were located at least one kilometer apart and the maximum distance between two borrow pits was 16 km (Fig. 1). The plots were all installed on a flat surface to avoid erosion. The surface of each plot was refreshed with a rake before applying the different treatments to simulate recent abandonment. A fish net (mesh size of 2 cm) was applied on all plots treated with mulching, diaspores and/or peat to protect against the effect of wind. All Restoration-Technique and Fertilisation Experiments plots were installed during the last week of May 2008.

## Monitoring

A vegetation survey was performed after three growing seasons (in mid-October 2010) in all the plots to evaluate the success of the treatments applied. The percent cover of all species was visually estimated in all experimental units in six randomly positioned sub-plots measuring 25 × 25 cm (1% increments between 1% and 10% cover and 90% and 100%, and 5% increments between 10% and 90% cover). Sub-plots were positioned in each experimental unit to minimise the edge effect (at least 10 cm away from the edge). If a portion of a sub-plot was disturbed (by animal tracks for example), it was not surveyed.

Data loggers (HOBO Pro V2, Onset Computer Corporation) were installed in each experimental unit of one block of the Restoration-Technique Experiment in order to measure the climatic effect of the mulching treatment. The data loggers recorded the temperature and the relative humidity at intervals of 30 minutes from 2 July to 15 September 2008.

## Statistical analyses

Mean coverage for each experimental unit (from sub-plots values) for the Restoration-Technique and Fertilisation Experiments was analysed with ANOVA for a factorial randomised complete-block design using the MIXED procedure of SAS (SAS Statistical System software ver. 9.2,

SAS Institute Inc., Cary, U.S.A.). Effect of treatments on bryophyte and lichen establishment data (% cover) was tested for total vegetation cover as well as for particular species or life-forms (bryophytes, lichens) separately. Variance was modeled with the GROUP statement of the function REPEATED to ensure homogeneity of variance and degrees of freedom were adjusted accordingly. We selected the best model using the Akaike Information Criterion (AIC). To evaluate significant interactions, post-hoc least-square means (LS means) were used to determine the significant differences among treatments. The normality assumptions were verified for all the analyses with the Shapiro-Wilk and Kolmogorov-Smirnov tests. In the Restoration-Technique Experiment, the mulch was lost due to wind in one of the experimental units. Therefore, the data from this plot were not included in the analyses.

## Results

### Restoration-Technique Experiment

Reintroducing diaspores of bryophytes and lichens had a positive impact on the revegetation of the borrow pits, but the magnitude of the positive effect was influenced both by mulching and peat addition. The most abundant species were bryophytes and lichens reintroduced from diaspores. *Niphotrichum canescens* was the most abundant with a mean cover of 30% when reintroduced, followed by the lichen *S. paschale* (12%). The mean cover of the three other reintroduced species remained low (< 2%). Apart from the five reintroduced species, we also identified three other species of bryophytes, five other species of lichens and 17 species of vascular plants that naturally colonised or were reintroduced along with the spread diaspores. The mean cover of these species, however, was very low (on average below 1%) and, for this reason, no statistical analyses were performed to measure the influence of treatments on their natural colonisation.

Mulching tended to have a significantly negative effect on cover, particularly for lichens (Table 1 and Fig 2c). Mean lichen cover was more than six times higher with no mulch (mean

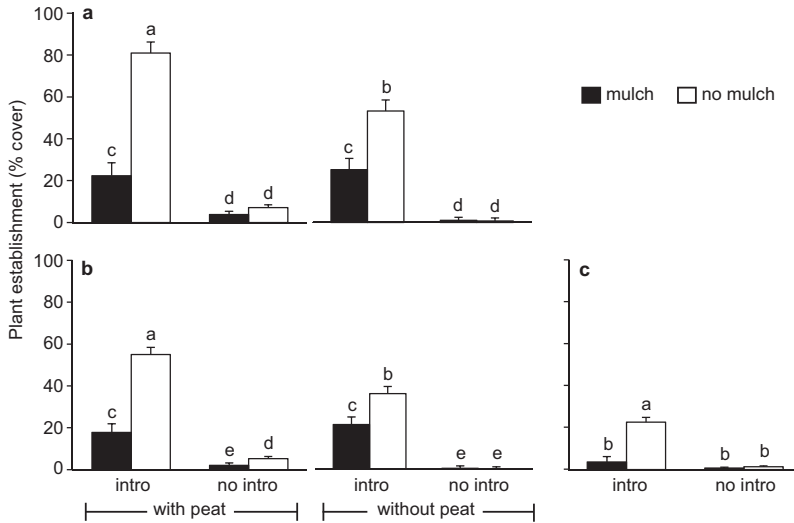
$\pm$  SE = 22.6%  $\pm$  2.3%) as compared with that in plots treated with mulch (mean  $\pm$  SE = 3.7%  $\pm$  2.5%). This trend was mainly associated with the species *S. paschale*, which accounted for more than 80% of the lichen cover when reintroduced. The presence of mulch also had a detrimental effect on total vegetation and bryophytes, but this effect was influenced by substrate amendment with organic matter (Table 1). For total diaspore cover, organic matter favoured the establishment of diaspores, but only if no mulch was applied (Fig. 2a). When peat was added to the substrate, total diaspore cover was four times higher in plots without mulch as compared with that in plots with mulch (mean  $\pm$  SE = 80.9%  $\pm$  5.3% vs. 22.4%  $\pm$  6.1%). This difference was reduced by half for plots without substrate amendment (mean  $\pm$  SE = 52.9%  $\pm$  5.3% vs. 25.2%  $\pm$  5.3%). *Niphotrichum canescens*, which accounted for about 90% of the bryophyte cover, was the most responsive species to peat amendment when it was reintroduced without mulch (Table 1). *Polytrichum piliferum* also responded positively to peat addition, whether it was reintroduced from diaspores or not (Table 1). Nevertheless, since *P. piliferum* cover was relatively low (mean  $\pm$  SE = 3.5%  $\pm$  0.8% with peat as compared with its virtual absence without peat), its net effect on total cover was negligible. The natural colonisation of *P. piliferum* is probably responsible for the significantly higher bryophyte cover observed in plots where the only treatment applied was peat amendment (Fig. 2b). Despite very low natural colonisation of vascular plants, field observations suggest that the presence of straw mulch may promote their establishment. Analyses performed on the cover of vascular plants confirmed that mulching had a positive effect on their establishment ( $F_{1,20} = 10.77$ ,  $p = 0.004$ ; results not shown).

### Fertilisation Experiment

Fertilisation, either with nitrogen or phosphorous, had no effect on plant establishment (Table 1). Significant differences in cover were found between the restoration methods only: where plots with reintroduced diaspores exhibited higher cover (Fig. 3 and Table 1).







**Fig. 2.** Effect of the reintroduction of diaspores (intro vs. no intro), mulching (mulch vs no mulch) and peat addition (peat vs. no peat) on the plant establishment (percent cover) for (a) total vegetation, (b) bryophytes and (c) lichens. Values are mean ± SE ( $n = 4$ ). For the treatment with diaspore introduction, organic matter and mulch,  $n = 3$  because the straw was blown away from one plot and was not used for statistical analysis. Different letters indicate significant differences among treatments (LS means,  $p < 0.05$ ). For total vegetation and bryophytes, the triple interaction was significant (see Table 1). For lichens, only the interaction intro × mulch was significant (Table 1), consequently, the means with and without peat were pooled.

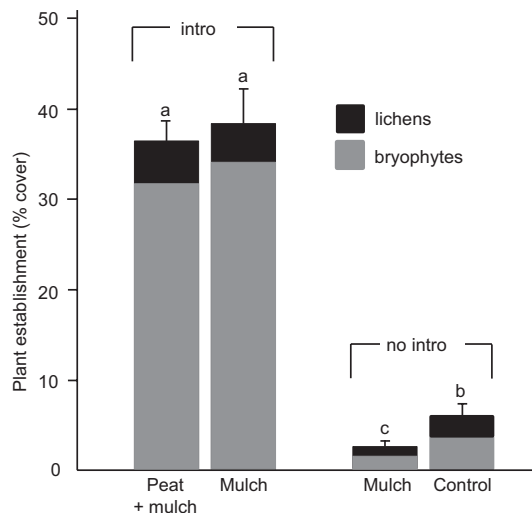
### Microclimate at air-soil interface

Plots treated with mulch experienced more humid conditions at the air-soil interface throughout the growing season, in terms of daily minimum and average relative humidity (RH; Fig. 4). Moreover, maximum daily temperatures were two degrees cooler below straw mulch (data not shown). Note, however, that the 2008 (first) growing season was very humid: from 2 July to 15 September, there were only thirteen days during which the relative humidity in the plots was below 40% for at least six consecutive hours. The temperature during those dry periods was on average 25.5 °C and lasted for no more than 10 hours. During those periods, the mulch protection helped maintain relative humidity about 7.6% higher than in the plots without mulch (data not shown).

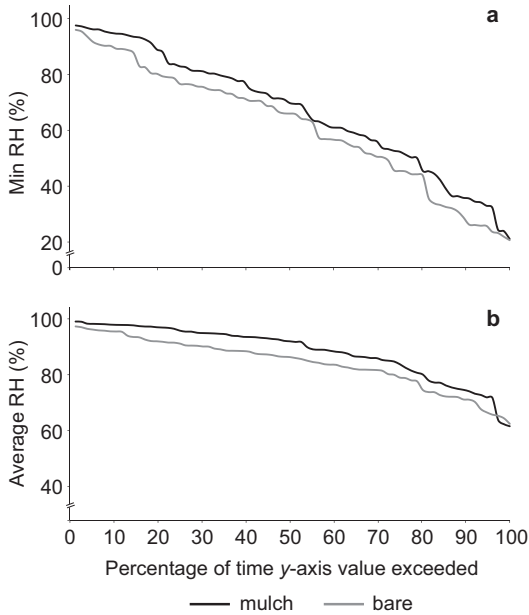
## Discussion

### Reintroduction of diaspores

Among the five species reintroduced, *Niphotri-*



**Fig. 3.** Effect of the methods of restoration on the plant establishment (% cover) for the Fertilisation Experiment. The methods used are control (bare ground with no amendment), mulching only, reintroduction (intro) of diaspores with mulch, and reintroduction of diaspores with mulch and peat. Values are means ± SE ( $n = 4$ ). Different letters indicate significant differences among treatments for total vegetation (LS means,  $p < 0.05$ ).



**Fig. 4.** Duration curves for (a) minimal daily relative humidity (%) and (b) average daily relative humidity (%). Data are from 2 July to 15 September 2008. This graph indicates that the average daily relative humidity (ADRH) was higher than 70% during 50% of the growing season in plots with mulch while the ADRH was superior to 66% during 50% of the growing season in plots without mulch.

*chum canescens* showed the best results in terms of survival and establishment. This species naturally thrives in xeric and poor environments like glacial forelands (Viereck 1966, Burga 1999, Raffl *et al.* 2006), volcan pumice (del Moral and Lacher 2005), borrow pits (Hugron *et al.* 2011), alvar grasslands (Zamfir 2000) and even rooftops (Anderson *et al.* 2010). In addition to its low nutrient needs and drought resistance, this species is easily cultivated (Fletcher 2005) for green roofs (Anderson *et al.* 2010), and various horticultural practices like Japanese gardens and green walls (Glime 2007). Moreover, this species likely established well because it generally exhibits fast growth rates. For example, the species was grown in a greenhouse from shredded fragments and produced a dense carpet (95% cover) in only 1.5 years (Y. Kim pers. comm.).

The lichen *Stereocaulon paschale* exhibited good survival without mulch, but no sign of expansion was observed after three growing seasons. These results are consistent with the fact

that lichens are organisms that are able to survive in situations of extreme dehydration (Sancho *et al.* 2007), but grow extremely slowly (Baron 1999). For example, the growth rate of a dimorphic lichen *Cladina stellaris* in subarctic Québec was documented to be between 1.9 and 5.6 mm per year (Boudreau and Payette 2004). Reintroducing diaspores of bryophyte *Politrichum piliferum* in the PGJ proved unsuccessful because most of the reintroduced diaspores died. However, natural colonisation of the species occurred in plots where organic matter was added. This species is the most frequent and abundant plant species in borrow pits of the region (Hugron *et al.* 2011) which shows its capacity to support the prevailing conditions in abandoned borrow pits. In both experiments, *Ceratodon purpureus* and *Trapeliopsis granulosa* exhibited poor survival rates and consequently are not recommended for restoring abandoned borrow pits.

## Mulching

At xeric and exposed sites, the plant successional process often starts with bryophytes and lichens that colonise “safe sites” where the humidity is higher and the velocity of the wind is lower (Cutler *et al.* 2008). Consequently, mulching is often recommended when rehabilitating land disturbances because it “mimics” those safe sites thereby reducing erosion (Petersen *et al.* 2004), preventing the occurrence of extreme temperatures (Blanco-Garcia and Lindig-Cisneros 2005) and increasing humidity at the air–soil interface (Price *et al.* 1998). These conditions promote germination of the seed bank and protect reintroduced diaspores. In our study, however, we observed a detrimental effect of the straw cover on the establishment of the reintroduced diaspores (Fig. 2). Indeed, much of the vegetation that established in plot with mulch was found between straw fibers. This observation can be partially explained by the abnormally humid growing season of 2008 which affected the survival of the diaspores. Indeed, the potential positive effect of the straw mulch in improving the relative humidity at the air–soil interface seemed to be overwhelmed by its shading effect, reducing the quantity of light reaching the plants.



Lichens are particularly sensitive to a reduction in the quantity of light (Cornelissen *et al.* 2001) because their photosynthetic capacity is lower than vascular plants on a per weight basis (Baron 1999). The net effect of mulching resulted in reduced survival rates of diaspores, especially lichens.

### Peat amendment

Harper and Kershaw (1997) suggested that the integrity of the organic horizon is one of the most critical factors affecting soil development and succession processes. They also asserted that in cases like borrow pits, where the organic horizon is completely removed, the complete recovery of the ecosystem can take several centuries. We, therefore, hypothesised that the addition of peat to the soil would improve diaspore establishment and growth. Results after three growing seasons showed that peat amendment improved the bryophyte and lichen establishment. The combination of treatments that exhibited the highest mean bryophyte and lichen cover was the reintroduction of diaspores with organic matter addition (mean  $\pm$  SE = 80.9%  $\pm$  5.3%). The improved water holding capacity of the amended soil likely explains the higher cover (Borgegård 1990, Harper and Kershaw 1997, Reid and Naeth 2005). Peat also has the ability to increase soil cation exchange capacity (CEC), which improves its nutrient retention capacity (Brady and Weil 2004).

### Fertilisation

Applying fertilisation commonly improves plant establishment or plant growth associated with land rehabilitation, particularly for vascular plants (Houle and Babeux 1994, Clemente *et al.* 2004, Reid and Naeth 2005, Pouliot *et al.* 2009). Many studies also have shown that adding nitrogen and phosphorous can improve the establishment and growth of mosses (McKendrick 1987, Gordon *et al.* 2001, Pouliot *et al.* 2009), especially in the family *Polytrichaceae* (Chapin and Chapin 1980, Sottocornola *et al.* 2007). Consequently, we expected that this treat-

ment would improve the establishment of the reintroduced moss diaspores, especially of *P. piliferum*. Instead we found no significant effect of nitrogen and phosphorous addition. The results may be in part due to the high precipitation in summer 2008 resulting in a rapid leaching of the nutrients provided by the initial dose, even though fertilisers added were “slow-release”. Moreover, the doses applied were low, e.g., for nitrogen less than 1/10 of the quantities commonly applied in forest fertilization (Weetman *et al.* 1987, Lepistö and Saura 1998, Turkington *et al.* 1998). Alternatively, since the diaspores were always reintroduced with mulch in the Fertilisation Experiment, it is possible that a potential positive effect of fertilisers was overwhelmed by the detrimental effect of the straw on the reintroduced diaspores.

### Conclusion

In summary, our study demonstrated that bryophytes and lichens reintroduced from diaspores can establish within three growing seasons on sandy substrate and promote the revegetation of abandoned borrow pits. Applying straw mulch had a detrimental effect on the reintroduced diaspores, while adding peat promoted their establishment if no mulch was applied. The results after three years suggest that nitrogen and phosphorous fertilisation do not promote the establishment of diaspores of bryophytes and lichens. The lack of a fertiliser response, however, may be due to the negative effect of applying mulch, since all plots treated with fertiliser and diaspores also included the mulch treatment. In these trials, peat was chosen to test the effect of adding organic matter as compared with that fertilisers. More trials, however, should be performed with other types of soil amendments for improving the fertility of the soils, such as municipal compost or sewage sludge. Moreover, trials including a wider range of fertiliser regimes would also be interesting. Long-term effects of these treatments on vascular plant establishment should be monitored to determine whether the reintroduction of diaspores of pioneer species of bryophytes and lichens can accelerate the successional processes as hypothesised.

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