Above- and below-ground diversity in restored peatlands: is there a link?

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Summary

Diversity of micro-organisms in peatlands is important from a functional point of view, and is greatly reduced by harvesting activities that remove the top layer of peatland where vegetation, microbes, fresh organic matter and seed banks are found. Restoration has proven to allow a good vegetation recovery: in some sites a particularly healthy moss cover dominated by *Sphagnum* species suggests a good potential for organic matter accumulation. Nevertheless, it was shown that the recovery of microbial activities was delayed in comparison with vegetation reestablishment following restoration, mainly as a consequence of the poor quality of the carbon in highly decomposed peat. Whereas aboveground diversity is well documented in restored sites, only scarce information has been gathered concerning micro-organisms. We used a multivariate approach to determine how surface vegetation and environmental conditions interact with structure and composition of microbial communities underneath it. We chose Phospholipid Fatty acids (PLFAs) as indicators of structural diversity. The preliminary results suggest that the structure of microbial communities is very heterogeneous under all types of vegetation communities, but that restoration seems to allow the recovery of a seasonal shift in the community composition.

Key index words: Monitoring of ecological restoration, microbial community structure, PLFAs, ombrotrophic peatlands, functional diversity.

Introduction

In North America, the post-vacuumed sites harvested for horticultural purposes remain dysfunctional, devoid of vegetation many years after abandonment or invaded by trees and shrubs, mostly as a consequence of drainage, compaction, frost-heaving and wind erosion (Price *et al.*, 1998, Campbell *et al.*, 2002, Poulin *et al.*, 2005), Consequently, active restoration measures are necessary to reinitiate colonisation by typical peat moss species: this is why a method called *Sphagnum* moss transfer has been developed for these highly disturbed sites (Rochefort *et al.*, 2003). The long-term objective of this kind of large scale restoration is to produce a self-sustainable functional ecosystem.

In natural ombrotrophic peatlands, one of the key functions is organic matter accumulation, which is the result of primary productivity processes exceeding decomposition processes. If vegetation and primary productivity recovery have been studied in details following restoration, understanding of decomposition processes remain fragmentary. Previous studies have shown that harvesting activities impact the microbial communities and that following restoration, the recovery the microbial compartment appears to be delayed in comparison with that of vegetation mainly as a consequence of the poor quality of the organic matter (Andersen *et al.*, 2006).

In UK, it was demonstrated that decomposition potentials varied substantially in relation to the degree of decomposition of peat, the physicochemical environment and the vegetation composition in spontaneously regenerated peatland, and that microbial communities could be an important indicator of the regeneration status of cut-over peatlands (Artz *et al.*, 2006). One of the objectives of this research is to study how changes in aboveground plant diversity and physicochemical conditions in the soils following *Sphagnum* moss transfer affect the composition of the microbial communities. Our research reports for the first time the status of belowground structural diversity in the context of monitoring active peatland restoration.

Materials and Methods

Study site

The sampling took place in the Bois-des-Bel Ecological Field Station (47° 58' N, 69° 26' W), near Rivière-du-Loup, Québec, Canada. A portion of 11 ha of the site was drained and vacuum-harvested from 1973 to 1980, when it was abandoned. Mining, oxidation and wind erosion led to the loss of a 65 cm peat layer in this area (Lavoie *et al.*, 2001) and exposed more decomposed *Sphagnum* peat to the surface. An 8 ha section of the site was restored in 1999 and a 2 ha area remained non restored for comparison purposes (Rochefort *et al.*, 2003) The natural peatland (47° 47' N 69° 28' W) that served as a donor site for the restoration was chosen as a reference site. It contains the same plant species, is devoid of any disturbances and is geographically close enough to limit climatic variability.

Sampling

All sampling sites were randomly selected in three areas to cover 5 different vegetation classes. The first class corresponds to the disturbed conditions (bare peat) found only in the non restored area (NR). Three of the classes were found in the restored area: *Polytricum strictum* carpets (POL), *Sphagnum spp.* dominated communities (SPH), and a mix of *Sphagnum* spp. and ericaceous shrubs (ERI). Finally, natural hummocks were sampled in the natural peatland (NAT). Samples of approximately 10 grams were taken at the same sampling sites early (June 19th 2006) and late (October 18th 2006) in the 6th growing season following restoration. At each sampling site, a trench was excavated with a shovel until water table was reached. Under the POL and NR communities, samples were taken in old peat above the water table (depth B) and below the water table (depth C). Under ERI and SPH communities, depth B and depth C samples were collected identically; however, we also sampled the new decaying vegetation above the water table (depth A). Finally in the natural section, we collected depths A and C only, because there is no old peat above the water table (B). A total of 36 samples were taken at each sampling date (see Fig. 1 for details).

Structure of microbial communities – PLFAs

The PLFAs were extracted and analysed in the Université de Poitiers (France). We used the method described in Bligh and Dyer (1959) and later modified by White *et al.* (1979) to isolate the fatty acids from the peat samples. Basically, we obtained the lipid fraction after a pre-extraction with $CHCl_3$:MeOH:Citrate (1:2:0.8) followed by an over night extraction in $CHCl_3$:citrate (1:1). Using silicic acid columns, the lipids were then fractionated into neutral, glyco- and phospholipids, and the latter were then methylated into Fatty Acid Methyl Esthers (FAMEs). The final step consisted of a derivatization with 4,4 dimethyloxazoline (DMOX), as proposed by Fay and Richli (1991). The FAME derivatives were then analysed with GC-MS and the resulting chromatograms were interpreted with the *X*calibur software.

Statistical analyses

Data analysis is still in progress and only preliminary results are available at the moment. We used Principal Component Analyses (PCA) to study how time of sampling, vegetation composition (percent cover of dominant species) and physicochemical soil conditions interact with the structure of the microbial community and its decomposition potential. For the microbial community structure, our matrix of data consists of distances obtained following Hellinger's transformation of the % mol PLFA g^{-1}. The R software was used for data transformations, and the CANOCO 4.5 software was used for the PCA.

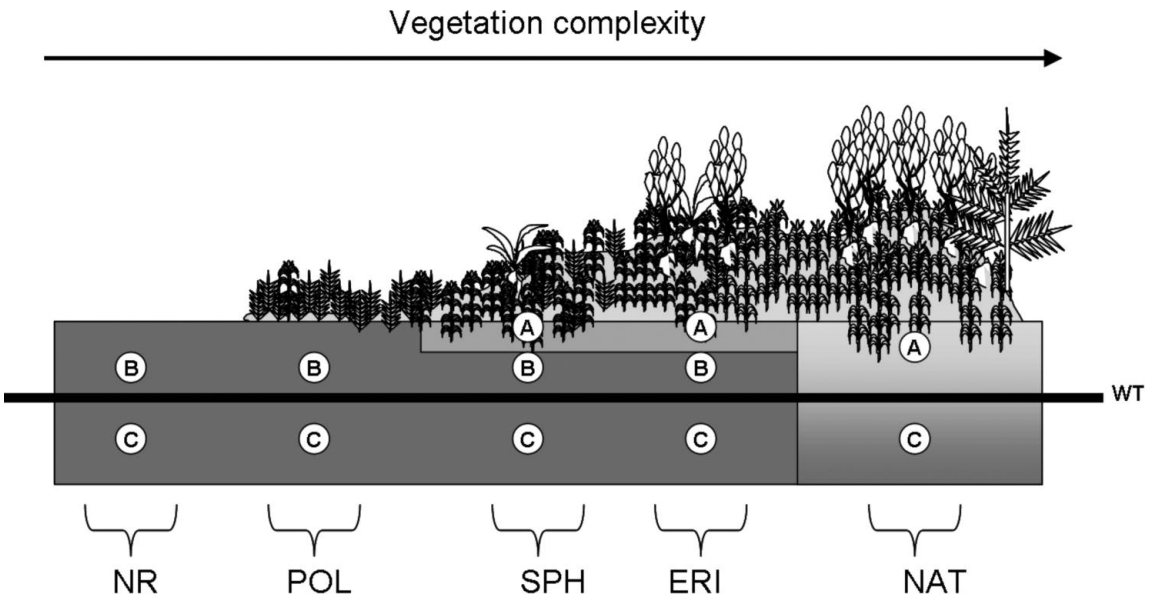

Figure 1. Schematic representation of the sampling design. Legend for the vegetation classes: SPH = *Sphagnum* dominated communities in the restored area; ERI = Ericaceous shrubs dominated communities in the restored area, POL = *Polytrichum strictum* carpet in the restored area; NAT = Natural hummock communities; NR = Bare peat found in Non-Restored conditions. Legend for the sampling depths: A = newly formed peat in aerated conditions, B = old peat in aerated conditions, C = old peat in water saturated conditions.

Results

Spatial heterogeneity of microbial communities

Even though the PCA (Fig. 2) expresses a large proportion of the variability on the first two axes (33.6% and 19.7% respectively for PC1 and PC2), there are no clear separation of the samples between the different classes of vegetation (Fig. 1a). It rather seems that even below the same vegetation community and the same depth, the PLFAs composition is highly variable. However, it is worth mentioning that the samples displaying the smallest biomass come from the non-restored sites (NR), the *Polytricum strictum* (POL) sites and one of the deeper ericaceous shrub site (ERIC): they are also those with the highest bulk density values and the more decomposed peat. Nevertheless, from the general disposition of the samples in the biplot, we can only infer that the diversity in PLFAs patterns can hardly be expressed solely using aboveground vegetation composition and simple physicochemistry variables as descriptors. The accessibility to various substrates of different quality as well as the nutrient availability might be needed to better predict changes in the structural composition of microbial communities. These results support the idea that the soil environment is highly heterogeneous even at a very small scale.

Despite such spatial variability, other studies were able to separate the communities found below *Sphagnum* or *Carex* types of vegetation (Borga *et al.*, 1994) and PLFA have been used as a monitoring tool to study wetlands of different successional stages (D'Angelo *et al.*, 2005). Our preliminary analysis did not demonstrate such a discriminative power, but we are confident that by regrouping PLFAs coming from similar functional groups of microbes and by furthering our analyses using RDA, we will be able to definitively test the hypothesis that the structure of the microbial community is related to aboveground vegetation composition.

Seasonal trends

When separating the samples from June and October in the biplot (Fig. 1b), a shift in the community composition over the season becomes more apparent. This seasonal transition has already been documented in the literature in natural ecosystems, and is mainly associated with changes in litter composition, access to water and nutrient pools and temperatures (Buyer and Drinkwater, 1997). Some samples do not follow this trend, again mainly from the non-restored (NR) or the *Polytricum strictum* (POL) communities, in other words from the less vegetated areas and more decomposed samples. The restoration, through input of vegetation and increased thickness of the *Sphagnum* carpet, might allow the recovery of transitions in the microbial community that are observed in reference systems and absent in degraded ones. This shift is likely due to a change in the proportion of some members of the communities.

Conclusion

The more detailed analysis of some key elements within the microbial communities and the separation of PLFAs into functional groups will allow us to understand better the temporal evolution of microbial communities under the different vegetation classes and its implications for the decomposition processes. The study of decomposition potential and real decomposition rates from the same samples will greatly complement this study and will be one of the next steps for a more comprehensive monitoring of ombrotrophic peatland functions following active restoration.

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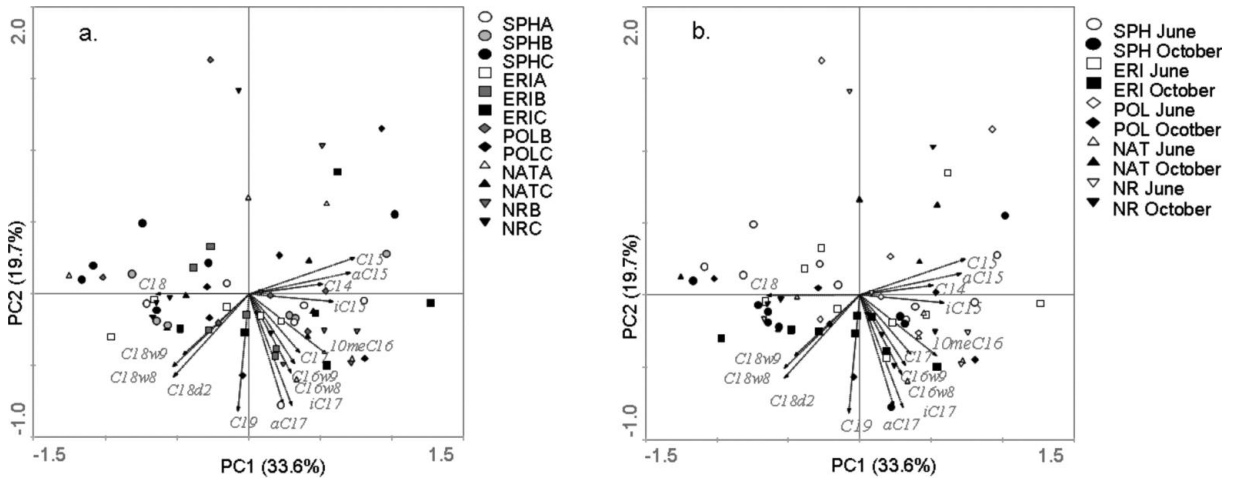

Figure 2. Principal Component Analysis (PCA) of PLFA composition (Hellinger's distances calculated from % mol PLFA g^{-1}) displaying a) the separation of the samples according to the vegetation classes and depths, and b) the separation of the samples according to the vegetation classes and the date of sampling. Legend for the vegetation classes: SPH = *Sphagnum* dominated communities in the restored area; ERI = Ericaceous shrubs dominated communities in the restored area, POL = *Polytrichum strictum* carpet in the restored area; NAT = Natural hummock communities; NR = Bare peat found in Non-Restored conditions. Legend for the sampling depths: A = newly formed peat in aerated conditions, B = old peat in aerated conditions, C = old peat in water saturated conditions.

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