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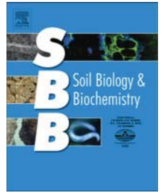

Changes in microbial community structure and function following Sphagnum peatland restoration

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ABSTRACT

This study examines the recovery of the microbial compartment following active restoration of a North American ombrotrophic peatland extracted for horticultural peat-based substrates and restored by the *Sphagnum* moss transfer method. We used phospholipid fatty acids (PLFAs) to portrait the microbial community structure and Community Level Physiological Profiles (CLPP) to describe the functional diversity of the microbial communities. Our results indicate that the PLFA profiles were different between the beginning and the end of the growing season, but that it was impossible to distinguish five different vegetation classes found along the disturbance-recovery gradient on the basis of the microbial community structure. The pH, the cover of mosses, *Ledum groenlandicum* and *Eriophorum vaginatum* var. *spissum* were the best environmental predictors for the PLFA composition. The newly formed peat found in aerobic conditions beneath restored *Sphagnum* carpets had the highest decomposition capacity, whereas the lowest rates were found in the surface samples of non-restored conditions or in the deepest horizons of the natural samples. A large proportion of the variation in the physiological profiles was explained with variables related to the vegetation cover, the physicochemical environment and the microbial structure of the community, which is very promising for future monitoring studies. Overall, this study demonstrates that the recovery of particular plant groups, namely mosses and shrubs in restored peatlands might be the driver of changes occurring in the structure of the microbial communities in restored peatlands.

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1. Introduction

The nature of the relation connecting functional diversity and ecosystem processes (Díaz and Cabido, 2001), and the link between aboveground plant diversity and belowground microbial diversity (Wardle et al., 2004) are two issues of high priority and interest in ecology. Addressing these issues is fundamental in enhancing our capacity to predict the responses of different ecosystems to climate change, improving our management of complex agro-ecosystems, and in upgrading our design of large-scale restoration projects where return to functionality is the primary purpose (Loreau et al., 2001; Wardle et al., 2004). A large proportion of our knowledge about these concepts stems from studies in grassland ecosystems or in artificial microcosms. Therefore, it is highly relevant to investigate these questions across diverse ecosystem types and processes, as it may help bring about a true synthesis of community and ecosystem ecology (Loreau et al., 2001).

Northern peatlands are critical ecosystems to study because these systems sequester approximately one-third of the terrestrial carbon of the planet (Turetsky et al., 2002). In Canada, they cover approximately 17 Mha, and the vast majority of Canadian peatlands are still in pristine condition. Nevertheless, some discrepancies exist between their geographic situation and their utilization. For example, in some regions of Eastern Canada, horticultural extraction of peat is a major economical activity, but the efforts for conservation are rather limited, which threatens the regional

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biodiversity. Moreover, when peat extracting activities cease, the sites remain disturbed and dysfunctional. For instance, since the vegetation cover is low in these sites and photosynthetic rates are limited, consequently cutover sites remain net CO_2 source to the atmosphere (Waddington et al., 2002).

Several cutover peatlands in Eastern Canada have been restored using the "*Sphagnum* moss transfer" method (Quinty and Rochefort, 2003) that aims to return the disturbed system to a functional state. Briefly, the vegetation on the top 5 cm of a natural peatlands is mechanically collected and spread over the site to be restored and then protected with straw mulch to enhance moss regeneration. One of the specific goals was to re-establish typical bog species. Following restoration, monitoring post-restoration projects have already revealed increases in biodiversity of plants, arthropods, amphibians and birds in comparison with cutover sites.

Restoration has proven to allow the recovery of a particularly healthy moss cover dominated by *Sphagnum* species (Isselin-Nondedeu et al., 2007); however, vegetation alone cannot insure the persistence of the restored ecosystem. Indeed, from a functional point of view, restoration could only be considered successful if remains of this vegetation could eventually reach the anaerobic horizon, the catotelm, without being decomposed totally by micro-organisms in the aerobic layer, the acrotelm. It has been demonstrated that microbial biomass recovery was delayed in comparison with vegetation re-establishment following restoration, mainly due to the poor quality of the carbon composing the highly decomposed peat forming the substrate over which the new acrotelm slowly forms (Andersen et al., 2006). It was also shown that methane consumption and production patterns varied between the beginning and the end of the growing season (Andersen, 2006). Nevertheless, there is little information concerning fungal and bacterial communities, even though the recovery of major functional groups of the microbial communities likely plays a determinant role in the return of pre-disturbance peatland functions. As plant biomass accumulates, the thicker vegetation layer containing fresh organic matter might stimulate decomposition by microbial communities (Glatzel et al., 2004). Most studies looking at decomposition in peatland focus on mass loss (Francez, 1995; Bragazza et al., 2007) or global activity and CO_2 fluxes (Waddington et al., 2002). The decomposition potential of the microbial community is mostly unknown in restored peatlands and needs to be quantified.

To fill this gap in our understanding of the processes characteristic of restored peatlands, we determined how surface vegetation and environmental conditions interact with the structure of microbial communities at the beginning and the end of a growing season. We performed the study in a gradient of increasing *Sphagnum* thickness characterized by five different vegetation classes, going from bare peat to natural hummock plant communities. We analysed phospholipid fatty acids (PLFAs) to estimate the proportion of key functional groups of micro-organisms (Zelles, 1999) in the peat. This method has been used in a large variety of systems (review by Kaur et al., 2005) including peatlands (Sundh et al., 1997; Jaatinen et al., 2007). The decomposition potential was evaluated with Community Level Physiological Profiles (CLPP) generated by Biolog EcoPlates™. This kind of physiological profiling has been used in many types of soils in the past (e.g., Garland and Mills, 1991). It has been less frequently and only more recently used with peat (Rinnan et al., 2008).

More precisely, we addressed the following hypotheses: 1) There are differences between the beginning and the end of one growing season, between the vegetation classes and between the different types of peat (old or newly formed) within the vegetation classes in a) the PLFAs profiles and b) the kinetics and patterns of carbon utilization of the microbial community; 2) The environmental conditions and the vegetation composition are significantly influencing the microbial community structure; and 3) Following restoration, the changes in microbial community structure, environmental conditions, and vegetation composition found along the gradient impact the decomposition potential of peat.

This study will complement the monitoring program following restoration by the *Sphagnum* moss transfer method with relevant information concerning processes related to the carbon accumulation function. In addition, our results will increase our knowledge on the nature of the relationship between aboveground plant diversity, belowground microbial community structure, and microbial functions in peatlands.

2. Materials and methods

2.1. Study site and sampling design

The sampling took place in the Bois-des-Bel Ecological Field Station (47° 58′ N, 69° 26′ W), in Québec, Canada. It contains a restored area as well as a non-restored section enclosed in a natural, relatively forested ombrotrophic peatland. The upper 65 cm of peat have been removed by vacuum-drawn tractors, and once extraction ceased, peat eroded away by wind and oxidation (Waddington and McNeil, 2002). The peat column left on the field was close to 2 m thick, and the upper 80 cm consisted mainly of *Sphagnum* peat (Lavoie et al., 2001). As a reference site, we sampled in the nearby natural peatland that corresponds well to the ecosystem targeted by restoration, as it served as a donor site of plant material for the restoration (47° 78′ N, 69° 47′ W). The vegetation mosaic in this site includes *Picea mariana* and *Larix laricina* as dominant tree species, a *Sphagnum* carpet (*Sphagnum fuscum*, *Sphagnum magellanicum*, *Sphagnum rubellum*, and *Sphagnum capillifolium*) and dense ericaceous shrubs: *Kalmia angustifolia, Ledum groenlandicum, Chamaedaphne calyculata* and *Vaccinium angustifolium*.

Samples were taken in the same locations early (June 19th, 2006) and late (October 18th, 2006) in the 6th growing season following restoration (Fig. 1). Three sampling locations were randomly selected among five classes of *Sphagnum* accumulation that also corresponded to different vegetation assemblages. These will be

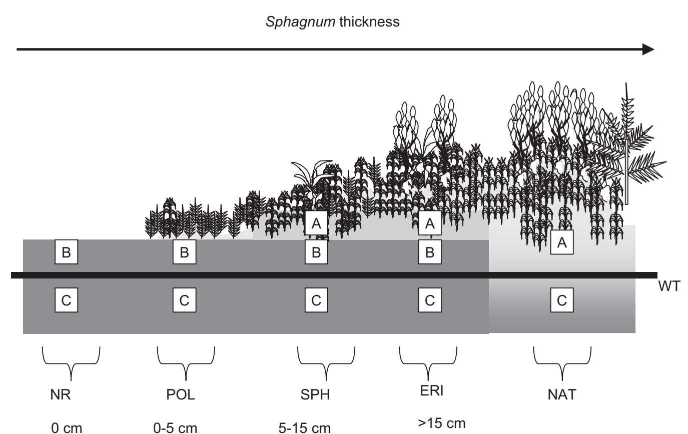

Fig. 1. Schematic representation of the gradient of increasing *Sphagnum* thickness. The names of the vegetation classes correspond to the characteristics associated with each thickness: 0 cm = bare peat found in the non-restored area (NR), <5 cm = *Polytrichum strictum* carpets (POL), 5–15 cm = *Sphagnum* carpets (SPH), >15 cm = mixed communities of *Sphagnum* and ericaceous shrubs (ERI) and finally, deep (>30 cm) hummock communities found in the natural area (NAT). In each sampling location, the samples were collected as indicated for the different substrates: New accumulating vegetation in aerobic conditions (A), old peat in aerobic conditions (B) and old peat in anaerobic conditions (C).

referred to as “vegetation classes”. The first class corresponded to the non-restored conditions with only bare peat (no accumulation) and no vegetation cover (NR). The natural hummock habitats (more than 30 cm deep) corresponded to the undisturbed conditions (NAT). The three intermediate classes were selected in the restored area using a GIS map with information about carpet thickness. The *Sphagnum* thickness < 5 cm were associated to *Polytrichum strictum* dominated communities (POL), the *Sphagnum* thickness of 5–15 cm to *Sphagnum* dominated communities (SPH), and the *Sphagnum* thickness > 15 cm to *Sphagnum* + Ericaceous communities (ERI).

At each sampling location, a trench was excavated with a shovel until the water table was reached. Two peat samples of about 10 g were put into sterile glass vials, (one sample for community composition and the other for decomposition potential). Another peat sample of 250 g was taken for physicochemical analyses (pH, EC, and bulk density). Three replicate samples were taken as follow: 1) Decaying *Sphagnum* in the aerobic peat horizon (H1–H2 on von Post scale) (A) was sampled in NAT, SPH and ERI; 2) Decomposed peat (H4 on von Post scale) exposed by harvesting and still found in the aerobic peat horizon (B) was sampled in NR, POL, SPH and ERI; and 3) peat in the anaerobic peat horizon (H4–H5 on von Post scale) (C) was sampled under all communities (Fig. 1). These three types of samples (A, B, C) are referred to as “peat types”. Since no significant peat accumulation was observed under POL and NR, A was not sampled under these communities; and as there is no old peat above the water table in NAT conditions, B did not exist under this community. A total of 36 peat samples were therefore collected per date of sampling. Samples for PLFA analysis were kept frozen until extraction. Samples for EcoPlate® were kept cool and analysed within five days.

We assessed the percent cover of all plant species in a 25 × 25 cm quadrat at each sampling point before the trench was excavated. In addition to all the species, the cumulative covers of all *Sphagnum* and all ericaceous shrubs were estimated visually. In order to reduce the data set, we eliminated the plant species that covered less than 10% of the sampling areas or that were present in less than 10% of the sites (Table 1). At time of sampling, we also noted the water table depth (WT) and the amount of *Sphagnum* that had accumulated since restoration, which was defined as *Sphagnum* thickness (ST). In the Natural section, ST corresponded to the depth to the H4 horizon H4 on the von Post scale. Each sample was later assigned a value of *Distance to surface* (DS = actual depth of sample from the surface) and *Distance to water* (DW = WT-depth of sample). We also measured the pH, electrical conductivity (EC) of the peat, and the bulk density for each peat sample (Table 2).

Table 2
Physico-chemical properties (mean ± SE) of the different types of peat (A = new accumulating vegetation in aerobic conditions, B = old peat in aerobic conditions, and C = old peat in anaerobic conditions) collected in the five classes of the gradient of increasing *Sphagnum* thickness (NR = bare peat, POL = *Polytrichum strictum* carpets, SPH = *Sphagnum* carpets, ERI = Ericaceous + *Sphagnum* carpets, and NAT = natural hummocks). EC = Electrical conductivity, BD = Bulk density, Distance to surface = Sphagnum thickness + sample depth and Distance to water = water table depth − depth of sample. $n = 3$ for each class × type of peat combination.

Date	Vegetation class	Type of peat	pH	EC	BD	DS[a]	DW[b]
				μS cm^{-1}	g cm^{-3}	cm	cm
JUNE	NR	*B*	4.0 (0.3)	51 (5)	0.067 (0.015)	0	47 (3)
		C	4.0 (0.3)	51 (5)	0.066 (0.012)	47 (3)	0
	POL	*B*	4.1 (0.1)	73 (9)	0.077 (0.014)	0	9 (4)
		C	4.0 (0.1)	78 (15)	0.066 (0.010)	9 (4)	0
	SPH	*A*	4.3 (0.3)	113 (35)	0.022 (0.004)	6 (1)	12 (1)
		B	4.3 (0.2)	77 (16)	0.067 (0.012)	11 (1)	7 (1)
		C	4.3 (0.2)	77 (16)	0.067 (0.012)	18 (2)	0
	ERI	*A*	4.0 (0.1)	84 (9)	0.021 (0.005)	8 (1)	10 (1)
		B	4.0 (0.0)	74 (7)	0.075 (0.004)	13 (1)	5 (2)
		C	4.0 (0.0)	74 (7)	0.075 (0.004)	18 (2)	0
	NAT	*A*	3.7 (0.3)	48 (17)	0.023 (0.005)	18 (2)	17 (2)
		C	3.7 (0.1)	59 (14)	0.033 (0.004)	35 (4)	0
OCTOBER	NR	*B*	4.0 (0.3)	61 (9)	0.068 (0.012)	0	37 (5)
		C	4.1 (0.2)	121 (25)	0.090 (0.007)	37 (5)	0
	POL	*B*	4.2 (0.1)	73 (12)	0.088 (0.016)	0	6 (1)
		C	4.1 (0.1)	73 (20)	0.086 (0.004)	6(1)	0
	SPH	*A*	4.3 (0.2)	69 (15)	0.032 (0.003)	8 (1)	5 (1)
		B	4.3 (0.2)	79 (11)	0.077 (0.013)	10 (1)	2 (1)
		C	4.4 (0.2)	114 (7)	0.058 (0.005)	12 (2)	0
	ERI	*A*	4.1 (0.0)	53 (4)	0.025 (0.005)	11(2)	6 (2)
		B	4.0 (0.1)	83 (25)	0.075 (0.010)	14 (1)	3 (1)
		C	4.0 (0.1)	99 (18)	0.078 (0.008)	18 (2)	0
	NAT	*A*	3.9 (0.1)	56 (24)	0.022 (0.003)	18 (2)	22 (2)
		C	3.6 (0.1)	81 (17)	0.051 (0.005)	40 (4)	0

[a] DS = Carpet thickness + |sample depth|.
[b] DW = |Water table depth| − |sample depth|.

2.2. Structure of microbial communities – PLFAs

Phospholipid fatty acids (PLFAs) were extracted from the peat samples following White et al. (1979). Briefly, peat samples (3 g) were shaken for 2 h in a buffer solution of $CHCl_3$:MeOH:Citrate (1:2:0.8). Then, equal volumes of $CHCl_3$ and Citrate were added, and the two phases separated overnight. The chloroform phases were reduced by evaporation. The lipids were then split into neutral, glyco- and phospholipids with silicic acid columns by eluting chloroform, acetone, and methanol, respectively. The phospholipids were then transesterified into Fatty Acid Methyl Esters

Table 1
Vegetation composition (mean % cover, SE in brackets) of the different classes found in the *Sphgnum* thickness gradient (NR = bare peat, POL = *Polytrichum strictum* carpets, SPH = *Sphagnum* carpets, ERI = Ericaceous + *Sphagnum* carpets, and NAT = natural hummocks). Only the species with a cover > 10% are presented.

Vegetation composition	June					October				
	NR	POL	SPH	ERI	NAT	NR	POL	SPH	ERI	NAT
Mosses										
Sphagnum spp.[a]	0	18 (16)	100 (1)	100 (0)	100 (0)	0	49 (25)	100 (1)	99 (1)	100 (0)
Polytrichum strictum	0	53 (13)	5 (1)	7 (4)	1 (1)	0	75 (9)	16 (2)	7 (4)	1 (1)
Shrubs										
Chamaedaphneae calyculata	0	17 (8)	0	27 (12)	13 (2)	0	2 (2)	2 (2)	30 (3)	10 (3)
Kalmia angustifolia	0	0	0	3 (3)	33 (9)	0	0	0	2 (1)	10 (10)
Rhododendron groenlandicum	0	20 (5)	0	9 (3)	30 (6)	0	7 (4)	0	5 (3)	14 (4)
Other										
Eriophorum vaginatum spissum	0	4 (3)	3 (2)	1 (1)	0	0	14 (9)	4 (1)	2 (1)	0
Vaccinium oxycoccos	0	3 (2)	1 (1)	2 (1)	0	0	7 (4)	1 (1)	2 (1)	0
Bare peat	100	3(3)	0	0	0	100	0	0	0	0

[a] *S. rubellum* and *S. fuscum*.

(FAMEs). The final step consisted of a derivatization with 4,4 dimethyloxazoline (DMOX) (Zhang et al., 1988). The DMOX derivatives were analysed by means of GC–MS (Trace GC Thermo Finnigan coupled to a Thermo Finnigan Automass).

We used the PLFAs i15:0, a15:0, i17:0 and a17:0 as markers of bacteria (Frostegård and Bååth, 1996); 18:2ω6,9 as maker of fungi (Bardgett et al., 1996; Frostegård and Bååth, 1996); 10Me16:0 and 10Me18:0 as markers of actinobacteria and sulfate-reducing bacteria (Kroppenstedt, 1985), as well as 18:1ω8 and 16:1ω8 as markers of methanotrophs (Dedysh et al., 1998) (Table 3). Other PLFAs detected in the samples were not specific to one particular functional group. Therefore, they were not used in the comparisons. We calculated total microbial PLFAs (Frostegård and Bååth, 1996) as well as F:B ratios and monosaturated-to-saturated ratios, as they are important features of the microbial community structure (Bossio and Scow, 1998).

The individual PLFAs, expressed as %mole of total, were subject to a Hellinger's transformation (Legendre and Gallagher, 2001) before the analyses. As a first step, we used a principal component analysis (PCA) to explore the variability of the PLFAs composition. We then used a redundancy analysis (RDA) to test the hypothesis 1a (PLFAs profiles vary between dates, vegetation classes and between depths within vegetation classes). As observations are not strictly independent due to spatial autocorrelation, we coded the different levels of structure (season, vegetation class, and type of peat) using dummy variables and used them as a constraining matrix in the RDA, as suggested in Lepš and Šmilauer (2003) for hierarchical (nested) designs. The effect of each level was tested using Monte Carlo permutation tests restricted using a split-plot design. For instance, when testing the effect of season, whole vegetation classes were permuted within season. Then, when testing the effect of vegetation classes, whole peat types were permuted within vegetation classes, but also within blocks defined by season (covariables). Finally, to test the effect of peat types, the permutations were randomly performed within the vegetation classes used as covariables.

We then performed a forward selection (using the "manual selection of environmental variables" option in Canoco) to test which variables among the vegetation composition (percent cover of plant species) and environmental conditions (distance to water, distance to surface, pH, EC and bulk density) had a significant influence on the PLFAs composition (hypothesis 2). We performed a first selection for the two dates together and then for each date separately. The selection procedure was stopped when the factor to be added was not significant anymore. The resulting models constructed with the significant variables only were used in a redundancy analysis to assess the proportion of the variation that they explained.

2.3. Function of microbial communities – Biolog EcoPlates^{TM}

The Community Level Physiological Profiles (CLPP) measurements were made at the Northern Forestry Centre, Canadian Forest Service (Edmonton, AB, Canada). All the manipulations were done under sterile conditions. One gram of fresh peat was mixed with sterile water and chopped into small fragments to create a homogeneous suspension. To achieve similar turbidity and density of peat particles in all samples, the suspension was added to sterile water in test tubes until an optical density of 0.40 ± 0.01 was reached. The aqueous suspensions obtained from each of the 36 samples were inoculated in Biolog EcoPlates™ (Biolog Inc., Hayward, CA, USA) using an automatic 100 μl dispenser. Each EcoPlate™ is a triplicate combination of 33 wells containing various carbon compounds (carbohydrates, carboxylic acids, amino acids, amines, polymers and miscellaneous) and including a control (water). A tetrazolium dye is combined to each substrate and

Table 3
PLFA composition (as various sum and ratios of %PLFAs ± SE) in the samples collected in June and October under the five classes found along the gradient (NR = bare peat, POL = *Polytrichum strictum* carpets, SPH = *Sphagnum* carpets, ERI = Ericaceous + *Sphagnum* carpets, and NAT = natural hummocks) and in three different types of peat (A = new decaying organic matter aerobic, B = old peat aerobic, C = old peat anaerobic).

			Bacteria	Methanotrophs	Actinobacteria/sulfate reducers	Fungi	Total mic. PLFAs[a]	F:B[b]	Mono:Sat[c]
			i15:0, a15:0, i17:0, a17:0	16:1w8, 18:1w8	10Me16:0, 10Me18:0	18:2w6	μg g^{-1} fresh peat		
June	NR	B	5.50 (1.62)	2.33 (0.32)	1.11 (0.368)	0.98 (0.24)	0.37 (0.16)	0.21 (0.04)	0.13 (0.00)
		C	1.51 (0.25)	1.49 (1.21)	0.30 (0.25)	1.82 (1.48)	0.90 (0.73)	0.10 (0.00)	0.14 (0.08)
	POL	B	5.57 (1.28)	1.80 (0.95)	0.60 (0.35)	0.64 (0.35)	0.26 (0.13)	0.30 (0.07)	0.10 (0.03)
		C	5.65(3.34)	1.84 (0.40)	0.80 (0.30)	0.31 (0.31)	0.41 (0.34)	0.20 (0.07)	0.11 (0.04)
	SPH	A	3.81 (0.73)	2.89 (0.79)	0.74 (0.20)	0.42 (0.22)	0.27 (0.13)	0.17 (0.03)	0.14 (0.04)
		B	5.07 (2.73)	1.12 (0.58)	1.02 (0.40)	1.39 (1.18)	2.48 (2.33)	0.22 (0.07)	0.11 (0.02)
		C	1.21 (0.12)	2.15 (0.65)	0.34 (0.13)	2.51 (1.26)	1.47 (1.09)	0.10 (0.01)	0.12 (0.01)
	ERI	A	2.59 (0.67)	2.60 (0.26)	0.60 (0.11)	2.02 (0.23)	1.65 (0.88)	0.14 (0.02)	0.17 (0.03)
		B	2.86 (1.63)	2.46 (0.28)	0.69 (0.35)	1.67 (0.29)	0.22 (0.09)	0.14 (0.03)	0.15 (0.01)
		C	6.44 (1.63)	1.80 (0.28)	1.30 (0.35)	0.29 (0.29)	0.13 (0.09)	0.22 (0.03)	0.10 (0.01)
	NAT	A	8.16 (1.51)	2.33 (0.31)	0.99 (0.53)	1.04 (0.69)	1.60 (1.12)	0.26 (0.04)	0.13 (0.02)
		C	3.52 (0.33)	2.03 (0.27)	0.84 (0.37)	1.71 (0.81)	0.40 (0.15)	0.19 (0.04)	0.11 (0.01)
October	NR	B	4.35 (0.99)	2.62 (0.51)	0.97 (0.08)	0.51 (0.26)	1.04 (0.47)	0.18 (0.05)	0.12 (0.02)
		C	2.79 (0.79)	2.38 (0.60)	0.69 (0.23)	1.74 (0.67)	1.21 (0.67)	0.19 (0.04)	0.19 (0.09)
	POL	B	5.50 (3.51)	3.71 (0.88)	0.73 (0.29)	1.86 (0.73)	9.59 (8.03)	0.20 (0.09)	0.17 (0.03)
		C	6.84 (3.87)	3.34 (0.26)	0.96 (0.28)	1.60 (0.41)	0.62 (0.22)	0.26 (0.08)	0.19 (0.07)
	SPH	A	3.87 (1.18)	4.08 (1.08)	0.73 (0.25)	2.32 (0.74)	2.70 (0.49)	0.17 (0.04)	0.20 (0.02)
		B	2.66 (1.61)	3.58 (0.96)	0.72 (0.32)	2.34 (0.74)	3.65 (1.92)	0.15 (0.02)	0.30 (0.09)
		C	2.83 (1.29)	5.19 (2.15)	0.69 (0.36)	1.17 (0.70)	18.53 (13.61)	0.12 (0.03)	0.15 (0.05)
	ERI	A	2.41 (0.69)	3.98 (1.74)	0.54 (0.32)	1.25 (0.33)	28.54 (22.76)	0.14 (0.02)	0.87 (0.60)
		B	3.17 (0.22)	2.77 (1.13)	0.76 (0.34)	2.18 (0.43)	2.02 (1.70)	0.16 (0.01)	0.24 (0.10)
		C	4.82 (1.47)	3.44 (0.41)	0.68 (0.20)	2.952 (0.38)	2.13 (0.58)	0.21 (0.03)	0.33 (0.12)
	NAT	A	3.13 (1.19)	2.53 (0.94)	0.51 (0.37)	2.21 (1.26)	3.79 (3.62)	0.15 (0.04)	0.16 (0.02)
		C	4.30 (1.43)	4.88 (1.89)	0.55 (0.06)	1.62 (0.476)	29.33 (1931)	0.21 (0.04)	0.33 (0.11)

[a] Frostegård and Bååth, 1996.
[b] Bardgett et al., 1996.
[c] Sundh et al., 1997.

liberated by microbial breakdown of the individual carbon compound. The color development in the wells of the EcoPlates™ was measured as the absorbance at 590 µm using the Biolog MicroLog™ 3E system and software. We took measurements every 24 h for five days in June and six days in October (Garland and Mills, 1991). Between measurements, the plates were incubated in the dark at 22 °C.

We followed the temporal evolution of color development using Principal Response Curves (PRC) (van den Brink and ter Braak, 1999; Lepš and Šmilauer, 2003). This technique allows studying the relative difference and evolution over time between a given treatment and a reference. It is based on a RDA that uses the interaction term between time and treatment (in this case, each of the vegetation class × type of peat combination) as constraining variables, except for the reference term, that is omitted in the constraining matrix. We analysed the two dates separately. For each date, we used NATA as a reference for all the aerobic conditions, namely NRB, POLB, SPH-A, SPH-B, ERI-A and ERI-B. We used NAT-C as a reference for anaerobic conditions, NRC, POLC, SPHC and ERIC. The effect of time over treatment can then be tested using Monte Carlo permutations (Lepš and Šmilauer, 2003). To our knowledge, this approach is unique among the studies using CLPP and fulfills the suggestions made by Preston-Mafham et al. (2002). We tested the hypothesis, 1b (decomposition potential varies between dates of sampling, vegetation classes and peat types) using the same approach described earlier for hypothesis 1a. The response variables were the color development data after 96 h.

The last hypothesis, stating that microbial structure, vegetation composition and environmental conditions would have a significant influence on the decomposition potential, was tested using the same approach as for hypothesis 2, i.e., using forward selection and redundancy analyses. Nevertheless, in this case, the constraining matrix included not only the percent cover of plants and the site characteristics, but also different ratios and sums of PLFAs (Table 3). The analysis was again performed first on the two dates together and then on each date separately. In addition, following the forward selection and the RDA with the significant variables, we ran a variation partitioning to discriminate the influence of each type of variable (microbial, vegetation, or soil characteristics) using partial RDAs (Lepš and Šmilauer, 2003). The significant variables of one type were used as constraining variables while the significant variables of the other two types were used as covariables, which allowed us to estimate the proportion of variation explained by each of the three types separately.

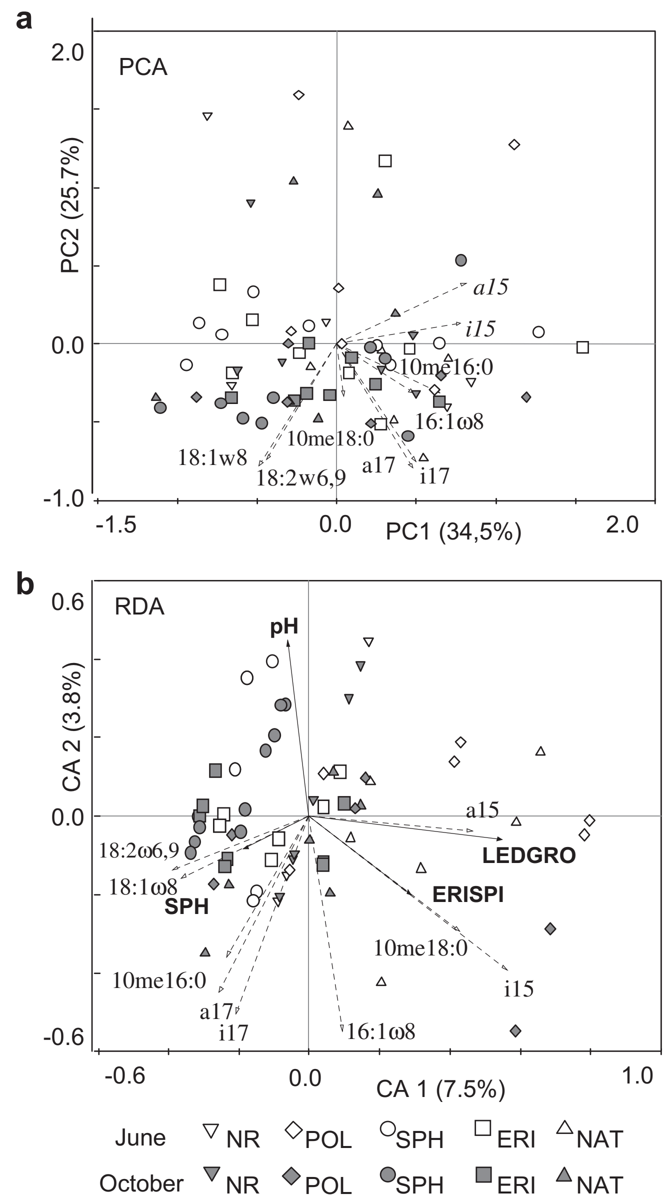

Fig. 2. Ordination diagrams (scaling focuses on inter-sample distances) for the a) Principal Component Analysis (PCA) and b) Redundancy Analysis (RDA) presenting sample scores, species scores (individual PLFAs, indicated by dashed arrows) and environmental variables scores (solid arrows) on the first two axes. Analyses were performed on the matrix containing Hellinger's transformed %mole of total PLFA data. June = white symbols, October = grey symbols. NR = Non-Restored, bare peat; POL = *Polytrichum strictum* carpets, SPH = *Sphagnum* carpets, ERI = Ericaceous + *Sphagnum* carpets, and NAT = natural hummocks. LEDGRO = % cover of *Ledum groenlandicum*, SPH = cover of *Sphagnum* species, ERISPI = % cover of *Eriophorum vaginatum spissum*.

3. Results

3.1. Microbial structure along the Sphagnum thickness gradient – PLFA

While the time of sampling in the season (June or October) significantly contributed to explain the patterns in PLFA composition (5%, $F = 3.598$, $p = 0.006$), the vegetation classes (within-time) and the peat types (A, B, C, within vegetation classes) did not ($F = 0.936$, $p = 0.542$; $F = 0.285$; $p = 0.997$, respectively). Overall, it was not possible to explain the composition of the PLFAs in the samples by the combination of the date of sampling, vegetation class on the sampling site, and type of peat ($F = 1.105$, $p = 0.287$). No clear segregation appeared in the PLFAs profiles of the samples in relation with the *Sphagnum* thickness classes (Fig. 2a).

The pH of the peat along with the percent cover of *L. groenlandicum,* total cover of *Sphagnum*, and *Eriophorum vaginatum* var. *spissum* explained a small but significant proportion of the variability in the PLFA patterns when the two dates were analysed together (13.4%, $F = 2.519$, $p = 0.002$; Fig. 2b). The fatty acids 18:2ω6,9 (fungi) and 18:1ω8 (methanotrophs) seemed to be positively influenced by an increased cover of mosses and were more abundant in the samples collected in the fall, particularly in the ERI, POL, and natural NAT classes. Conversely, the fatty acid a15:0 (bacteria) appeared to be more abundant where *L. groenlandicum* had a greater cover. Finally, i15:0 (bacteria) and 10Me18:0 (actinobacteria) tended to have a more elevated proportion under *E. vaginatum* var. *spissum*, which was mostly present in the *P. strictum* (POL) class. In Fig. 2a and b, Non-Restored (NR) samples and Natural (NAT) samples were more scattered than

Sphagnum + Ericaceous (ERI) and *Sphagnum* (SPH) samples, which were more clustered. We repeated the analysis for each sampling time separately to see if any within-time variation in the PLFA composition could be explained by the constraining matrix, but that was not the case, as none of the environmental variables was able to discriminate the classes or peat types.

3.2. Decomposition potential along the Sphagnum thickness gradient – CLPP

In June and in October, the color development in the EcoPlates™ evolved differently over time for the different vegetation classes and types of peat in the aerobic peat horizon (peat types A and B),

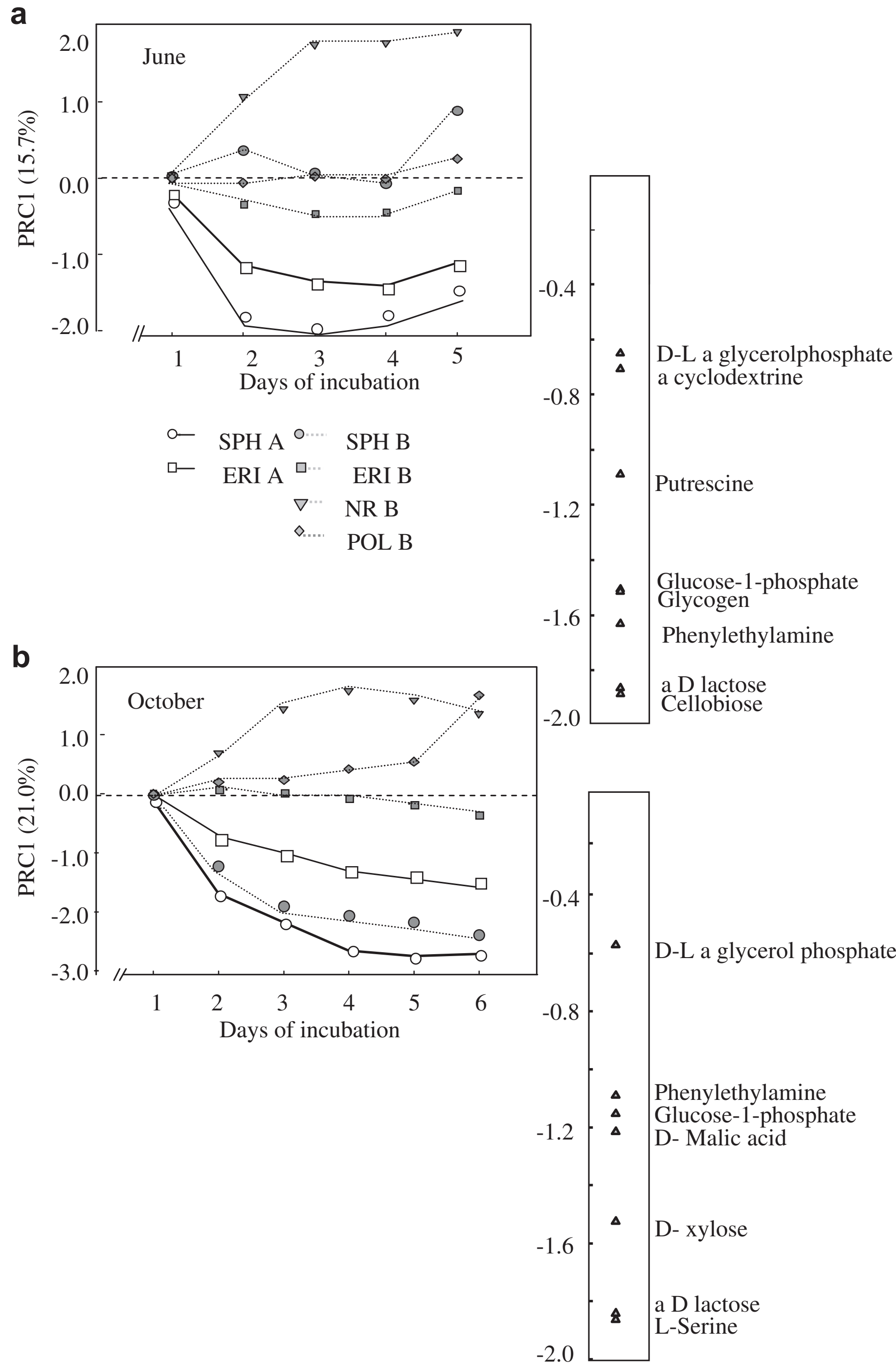

Fig. 3. Principal Response Curves (PRC) based on the color development data of the Biolog EcoPlates™ a) in June 7 years post-restoration and b) in October 7 years post-restoration. On the left side, the curves represent the relative difference between a treatment and the natural surface samples (NATA) corresponding to the zero line. On the right side, the vertical axis displays the canonical coefficient of the most significant carbon compounds. A highly negative value indicates a temporal pattern that is opposite to those of the curves, whereas a value closer to zero indicates a temporal pattern unrelated to those of the curves. The proportion of variability explained by the first response curves was 15.7% in June (F = 26.299, p = 0.001), and 21.0% in October (F = 45.829, p = 0.001). Legend: NR = non-restored; POL = *Polytrichum strictum* carpets; SPH = *Sphagnum* carpets; ERI = Ericaceous + *Sphagnum* carpets; and NAT = natural hummocks; A = new decaying organic matter aerobic, B = old peat aerobic.

but not in the anaerobic peat horizon (peat types C) This relates to shapes and the orientation of the curves in the Principal Response Curves (PRC) presented in Fig. 3. The response curves moving away from the zero line in the direction of the carbon compounds situated on the species axis were those for which the color development was stronger than the reference (NATA). The highest color development, hence highest potential activity, occurred in SPH-A and ERI-A samples in June and in SPH-A, SPH-B and ERI-A in October. A color development similar to that of the reference samples occurred in POLB, SPH-B and ERI-B in June and ERI-B in October. The NRB (June and October) samples displayed a very limited color development, as shown by their response curves moving away from the reference and in the opposite direction from the carbon compounds. The difference in color development generally increased with time. Analyses of the 96 h data further confirmed that the decomposition potential differed between the dates of sampling, the vegetation classes, their interaction term, as well as the types of peat within the vegetation classes. Nevertheless, season had the most important influence, given its higher mean square value (Table 4).

When the two dates were analysed together, the vegetation composition, the microbial structure, and the physicochemical variables explained a significant portion of the variability in the decomposition potential (Table 5). In total, the selected variables explained 25.7% of the variability ($F = 6.214$, $p = 0.001$). The decomposition potential decreased from June to October for most samples except the NAT ones (Fig. 4a). In both seasons, the SPH and ERI samples had the highest potential for decomposition, while the NR and NAT samples had the lowest. Complete *Sphagnum* cover is associated with greater decomposition of carbon compounds, whereas non-vegetated areas (bare peat), high proportion of bacteria and high bulk density are related to lower decomposition potential.

When the two dates were separated, the variables selected by the forward selection procedure explained up to 47.2% ($F = 5.298$, $p = 0.001$) and 42.2% ($F = 4.686$, $p = 0.001$) in June and October, respectively (Table 5). In June, the SPH-A and ERI-A samples had the highest decomposition potential, whereas the NR and NAT-C samples had the lowest (Fig. 4b). The POL samples had a variable decomposition potential, resulting in a heterogeneous distribution (Fig. 4b). The decomposition potential was higher under complete cover by *Sphagnum*, when the electrical conductivity was elevated, and when the total microbial PLFAs and the ratio of monosaturated: saturated PLFAs were high. The decomposition potential was low under higher cover of *L. groenlandicum* and when the proportion of bacterial PLFAs was elevated. In October, SPH samples had the highest decomposition potential (Fig. 4c). The ERI and NATA samples had intermediate values. NR, POL and NAT-C had the lowest color development. The decomposition potential increased with increasing *Sphagnum* thickness, but also with pH. It was low when the proportion of bacterial PLFAs was more elevated or when the bulk density increased.

Table 4
Result of variance decomposition for the color development after 96 h in the EcoPlates™ using RDA followed by Monte Carlo permutations ($n = 999$) restricted following Lepš and Šmilauer (2003) for hierarchical (nested) design. The constraining variables were the two sampling seasons (June and October), the five vegetation classes (non-restored; *Polytrichum strictum* carpets; *Sphagnum* carpets Ericaceous + *Sphagnum* carpets; and natural hummocks) and the types of peat (A = new decaying organic matter aerobic, B = old peat aerobic, C = old peat anaerobic). Mean square value is the variance explained corrected by the degree of freedom.

Variable	DF	% expl.	R^2adj	F	*p*
Season	1	8.1	8.10	18.949	0.001
Vegetation classes	8	6.9	0.86	3.844	0.006
Type of Peat	14	10.5	0.75	12.254	0.001
Residuals	48	74.5	1.55	n.a	n.a
Total	71	100.0	1.41	n.a	n.a

Table 5
Eigenvalues, F values and *P* values obtained from the partial RDA testing the influence of PLFAs composition, vegetation cover and environmental conditions over the color development after 96 h in the EcoPlates™. A forward selection based on Monte Carlo permutation ($n = 999$) kept only the significant variables in the models. a) for both dates together b) for June only and c) for October only. F and *p* values were estimated using Monte Carlo permutations. For each partial model, the other significant variables were used as covariables. Functional groups of PLFAs refer to the sums and ratios presented in Table 3. Abbreviations: BD = Bulk Density, DS = Distance to Surface, DW = Distance to water, mono:sat = monsaturated PLFA-to-unsaturated PLFA ratios, F:B = fungi-to-bacteria PLFA ratios. Cha cal = *Chamaedaphne calyculata;* Pol str = *Polytrichum strictum*, Rho gro = *Ledum groenlandicum*, Eri spi = *Eriophorum vaginatum spissum*, Vac oxy = *Vaccinium oxycoccos, Sphagnum* = % cover of all Sphagnum species, Shrubs = % cover of all Ericaceous shrubs.

Variables included in the model	Eigenvalue	% expl.	F	*p*
JUNE AND OCTOBER				
Bacteria, methanotrophs, actinobacteria., tot. mic. PLFAs	0.061	7.6	4.095	0.001
EC, BD	0.042	5.3	5.309	0.002
Cha cal., Pol str, Sphagnum, shrubs, peat	0.143	16.1	7.641	0.001
All the above	0.257	25.7	6.214	0.001
JUNE				
Bacteria, methanotrophs, actinobacteria, fungi, F:B, mono:sat, tot. mic. PLFAs	0.188	26.3	4.52	0.001
pH, EC, DS, DW, BD	0.121	18.6	4.079	0.001
Rho gro., *Sphagnum*, shrubs	0.076	12.6	4.244	0.001
All the above	0.472	47.2	5.298	0.001
OCTOBER				
Bacteria, methanotrophs, actinobacteria, fungi, mono:sat	0.153	20.9	4.572	0.001
pH, DS, DW, BD	0.082	12.4	3.188	0.001
Vac oxy, Eri spi, Pol str, Sph., shrubs	0.100	14.7	3.114	0.001
All the above	0.422	42.2	4.686	0.001

4. Discussion

4.1. Microbial community structure – PLFA

It was impossible to distinguish the five vegetation classes or the different types of peat on the basis of their PLFAs patterns, thereby refuting our first hypothesis. The only significant changes were found between the two times of sampling in the season (June vs. October), but that represented a very small proportion of the variability. This contrasts with another study in peatlands (Sundh et al., 1997) that found that PLFAs profiles varied among peatland habitats, but not over the growing season.

A recent study demonstrated that the bacterial community associated with *Sphagnum* spp. showed a high degree of host specificity (Opelt et al., 2007). The peat sampled in this study was uniformly composed of *S. fuscum* and *S. rubellum* remains, which might therefore correspond to a relatively homogeneous bacterial community. On the other hand, some of the dominating plants were significantly related to the PLFAs composition. For instance, a15:0 (bacteria) increased with the cover of *L. groenlandicum*, while i15:0 (bacteria) and 10Me16:0 (actinobacteria) increased with the cover of *E. vaginatum* var. *spissum*. This could be due to the improved development of the rhizosphere under these two plant species that stimulates bacterial growth.

The augmentation in the proportion of biomarkers generally attributed to type II methanotrophs (18:1ω8) and to fungi (18:2ω6,9) was positively associated with the augmentation of the total *Sphagnum* cover and negatively associated with large surfaces of

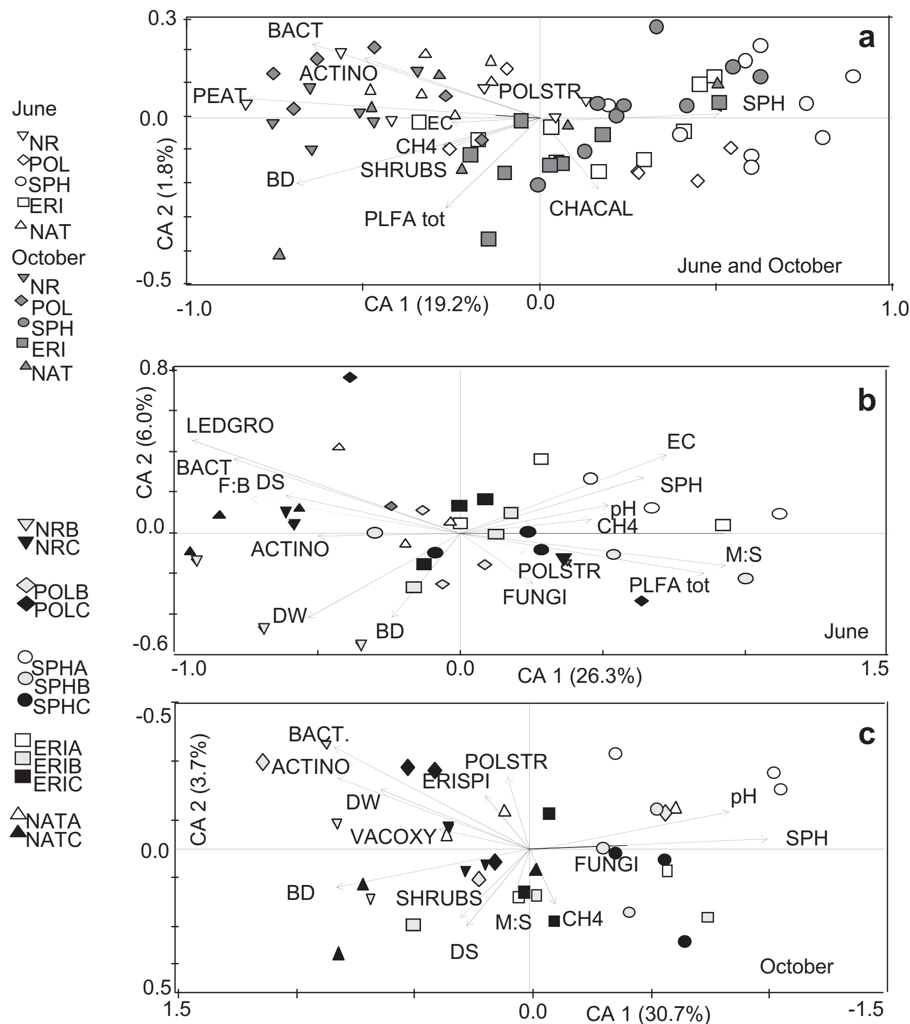

Fig. 4. Ordination diagrams (scaling focuses on inter-sample distances) presenting sample scores and environmental variables scores (solid arrows) on the first two axes of the redundancy analyses (RDA) using a) all samples b) June samples only c) October samples only. Analyses were performed on the colour development data after 96 h. NR = non-restored; POL = *Polytrichum strictum* carpets; SPH = *Sphagnum* carpets; ERI = Ericaceous + *Sphagnum* carpets; and NAT = natural hummocks; A = new decaying organic matter aerobic, B = old peat aerobic, C = old peat anaerobic. Legend for the environmental variables: BD = Bulk Density, DS = Distance to Surface, DW = Distance to water, EC = Electrical Conductivity, SPH = % cover of all *Sphagnum* species, ERISPI = % cover of *Eriophorum vaginatum spissum*, LEDGRO = % cover of *Ledum groenlandicum*, POLSTR = % cover of *Polytrichum strictum*, VACOXY = % cover of *Vaccinium oxycoccos*, PLFA tot = % microbial PLFA, CH4 = %PLFA Methanotrophs, Actino. = %PLFA Actinobacteria.

bare peat. Many other authors mention the presence of type II methanotrophs beneath *Sphagnum* cushions (e.g., Dedysh et al., 1998), which is confirmed by our results. The methanotrophic community found beneath *Sphagnum*/*Eriophorum* communities is different than that colonizing the soil under *Calluna vulgaris* communities, and the former is dominated by *Methylocystis* spp. (Chen et al., 2008), which are type II methanotrophs (Sundh et al., 1995). Fungi exist in large numbers and in a wide variety beneath *Sphagnum* and in close association with ericaceous shrubs (Thormann, 2006; Artz et al., 2007); in bogs, fungi predominate in lawns and hummocks (Jaatinen et al., 2007). Nevertheless, it is impossible to distinguish fungal groups using PLFAs. In fact, we detected the same proportion of fungal PLFA in all our samples, but they could be extracted from different functional groups or different morphological states. One also needs to be cautious when interpreting the relation between mosses and fungi, since the fatty acid 18:2ω6,9 can also be found in some mosses (Dembitsky and Rezanka, 1995) and in some cyanobacteria (Lechevalier and Lechevalier, 1988). Methanogens are another important microbial group found in natural peatlands that are susceptible to vary in relation with vegetation and environmental conditions, but this group is impossible to identify using simple PLFAs extractions (Kaur et al., 2005).

The effect of pH on PLFA patterns had been highlighted by other studies, where it was demonstrated that similar microbial communities were induced in different soils at the same pH, and that pH had a profound effect on the microbial community composition (Bååth and Anderson, 2003). In this study, the range of variation of pH among the samples was very small, which explains why the proportion of variation explained by this variable was also limited. Bogs display an overall poor quality of organic matter, subtle abiotic differences among sites, and saturated conditions that might modify or limit trophic interactions in the decomposer system relative to other ecosystem types (Fisk et al., 2003). On the other hand, previous studies have shown differences in nutrient status (N, P, K) among post-extracted, restored, and natural bogs (Andersen et al., 2006). Hence, we might have obtained a better discriminating power by measuring variables relating to the nutrient status as well as to the quality of the substrate (Jaatinen et al., 2007), both of which are important in structuring the microbial communities.

Sundh et al. (1997) suggested that changes over the growing season in the in situ microbial activity in a discrete peat section were perhaps primarily determined not by changes in biomass but rather by changes in the prevailing environmental conditions (e.g., supply of oxygen and other electron acceptors, temperature, and organic and inorganic nutrients). In our case, the fatty acids associated with methanotrophic bacteria (18:1ω8) and fungi (18:2ω6,9) were proportionally more abundant in October, which suggests that environmental conditions more suitable for these micro-organisms were found in the end of the growing season. A greater consumption of methane in October than in June was observed at the same site in a previous study (Andersen, 2006), which supports this idea. Nonetheless, in order to discriminate between temporal variability and seasonal pattern, it would be necessary to characterize the microbial community of peatlands of different regeneration stages at various moments during the growing season, but also for more than one year, to account for inter-annual variability.

4.2. Community Level Physiological Profiles – CLPP

Whereas no differences were found in the PLFA structure among the five vegetation classes of the gradient, there were clear distinctions in terms of carbon utilization patterns. This might reflect the importance of the physiological status of the bacterial community and/or the impact of functional diversity in the decomposition processes in peatlands, which are not distinguishable on the basis of fatty acid portraits.

During extracting activities, the layer where the most active microbial communities exist has been removed, and the exposed peat in cutover site is old and highly decomposed. In POL and NR, where vegetation has not yet fully recovered, the diminution of activity from June to October was greater than in the other classes. Without a healthy and thick moss cover, NR and POL are more prone to desiccation. Other studies have shown that intact peatlands with favourable microclimatic conditions have higher rates of organic matter decomposition than extracted peatlands (Basiliko et al., 2007; Watts et al., 2008). More importantly, the lower quality of the carbon, the greater acid-insoluble fraction, and higher bulk density of subsurface peat from deeper layers has been suggested to restrict the development of an active microbial biomass (Fisk et al., 2003; Andersen et al., 2006). All this creates harsh conditions that could force micro-organisms to enter a starvation-survival state (Morita, 1990) involving mechanisms such as reduction of cell-size or formation of spores and other inactive states. This could explain why samples with a higher proportion of bacteria do not have a greater decomposition potential; however, this hypothesis needs to be further investigated and tested in laboratory. In all cases, it appears that the types of carbon compounds and/or the short incubation period needed for color development in EcoPlate™ is not optimal for the micro-organisms of these layers, which translates into a slower and less intense color development in a smaller number of wells.

The lower activity found in the deeper anaerobic peat horizons of the natural area was also expected. Decreases in the microbial biomass (C and N) and in the production of CO_2 with depth have been reported in natural and restored areas (Francez et al., 2000; Andersen et al., 2006; Basiliko et al., 2007). Recent studies looking at diversity of fungi in peatlands suggested that the fungal assemblages undergo a successional shift as the quality of the peat changes (Artz et al., 2007), and that the quality and dynamics of C sources in the litter are likely to be important regulators of the microbial community structure (Thormann et al., 2004; Artz et al., 2006; Trinder et al., 2008). In deeper layers (associated with high values of bulk density and distance to surface, DS), the fungal community might be dominated by slow-growing fungi that have more affinities for complex carbon compounds – but that do not develop in the EcoPlates™. One of the major drawbacks of using Biolog EcoPlate™ is the biased selection toward fast-growing bacteria and fungi that may not represent the true diversity of the whole soil (Preston-Mafham et al., 2002). Other methods evaluating microbial functional diversity, such as Micro-Resp™ or multiple SIR (substrate-induced respiration), might have offered a different perspective and would definitely be relevant to use in the future.

The presence of ericaceous shrubs can also reduce the microbial activity through the inhibitory activity of phenolic allelochemicals found in ericaceous humus (Mallik, 2003) or through an increased competition for nutrients. Phenolic polymers excreted by ericaceous plants form protein–phenol complexes and induce a further reduction of available nitrogen in the already nutrient-stressed habitat. All these effects might cause a decrease in the activity of micro-organisms in the presence of a well developed ericaceous rooting mat. The intermediate position of samples taken under communities with ericaceous shrubs (ERI and some POL) in the PRC and RDA graphs (Figs. 3 and 4) support this hypothesis.

Interestingly, the samples taken below *Sphagnum* carpets – devoid of ericaceous shrubs – displayed a high decomposition potential (color development). It has been demonstrated that both microfungi (Thormann et al., 2002; Rice et al., 2006) and prokaryotes (Kulichevskaya et al., 2007) were capable of decomposing *Sphagnum* tissues and their metabolites. Our results suggest that some of the micro-organisms found under restored *Sphagnum* carpets have the potential to decompose a wide variety of substrates, like carbohydrates (e.g., glycogen, cellobiose, D-xylose), carboxylic acids (e.g., D-malic acid), or amines (e.g., putrescine). It seems that following restoration, the combination of fresh organic matter (low bulk density + presence of mosses) and the absence of ericaceous shrubs create conditions favourable for the development of an active microbial biomass that can decompose the newly accumulating organic matter. In a study comparing how typical pioneer species alter the functional response of the microbial community of a previously cutover peatland, it was found that new carbon inputs from plants colonizing an abandoned cutover peatland may support communities of micro-organisms that have functionally distinct roles in carbon turnover (Yan et al., 2008). Our results concur with this conclusion, adding that the effect is not only visible with pioneer species, but also with later successional species, such as *Sphagnum* species.

Seasonal variability in carbon utilization patterns has not been studied in restored peatlands before. Our results suggest that microbial activity is reduced in October compared with June, which is consistent with other studies investigating *in situ* CO_2 production (Petrone et al., 2001; Basiliko et al., 2007) or following incubation of peat samples (Andersen, 2006). It is generally agreed that temperature has a strong control over respiration by microbes: in October, as lower air and soil temperature might reduce the potential enzymatic activity of the microbial community; however, other seasonal phenomenon that have not been estimated in this study, such as litter fall, nutrient uptake or release by plants, etc., might modify the potential activity and the patterns of carbon utilization through changes in the microbial structure.

4.3. Conclusion: implications for monitoring of peatland restoration

Our results suggest that simple PLFAs extraction might not be the best tool to monitor overall changes in microbial community structure following ecological restoration in peatlands. Among these, the broad distribution of some PLFAs and the impossibility to distinguish among functional groups of fungi are likely to reduce the proportion of the variation in the PLFA profiles that can be explained by environmental variables. We suggest that if PLFAs were to be used in monitoring programs following the restoration of vacuum-milled peatlands, they should be complemented by

molecular approaches (Anderson and Cairney, 2004) to separate the different functional groups among fungi.

On the other hand, we obtained interesting results with the Biolog EcoPlate™ method. In terms of monitoring, decomposition potential evaluated by carbon utilization patterns relates to the organic matter accumulation capacity of the restored peatland, which is one of the key functions targeted by restoration. We were able to discriminate samples from different *Sphagnum* thicknesses and peat types using two complementary statistical approaches. We explained a significant proportion of the changes in carbon utilization with a small number of environmental variables (vegetation, physicochemistry, and microbial structure). Our results suggest that the decomposition capacity is lowest in abandoned peat fields and that it increases as the *Sphagnum* carpet thickens, but that the growth of ericaceous shrubs reduces this potential toward natural values. Nevertheless, *in situ* measures of actual rates of decomposition in relation with quality and quantity of organic matter input from vegetation assemblages will be necessary to further our understanding of organic matter accumulation and C sequestering potential in restored peatlands. In particular, the influence of ericaceous shrubs on *Sphagnum* growth and decomposition deserves more attention.

Both the structure and the decomposition potential of the microbial community were significantly affected by the sampling time in the season, but it is impossible at this point to determine whether this is due to normal temporal variability in environmental conditions or to a pattern relating to a particular seasonal cycle (litter input, vegetation phenology, or temperature). In a context involving long-term monitoring, temporal variability should be taken into account in the sampling design. Multiple-year studies in peatlands of various restoration ages seem to be a promising avenue to explore. We emphasize that the different aspects of microbial communities should always be considered and used together in long-term monitoring programs following restoration when the aim is to assess the functional status of a given site, because micro-organisms have a significant yet often underestimated role in ecosystem stability and sustainability.

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