

Vacuum-extraction of peatlands disturbs bacterial population and microbial biomass carbon

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Abstract

Knowledge concerning the microbial characteristics of natural and post-vacuum extracted ombrotrophic peatlands, as well as peatlands under restoration is limited. In one experiment, microbial comparisons of paired neighboring natural and post-vacuum peatlands in eastern Québec (Canada) were studied to assess the effects of peat mining on microbial indicators and nitrogen (N) cycling. Microbial counts, microbial biomass carbon (MB-C) and N mineralization were examined over two growing seasons. Also, in a second experiment, bio-indicators of the microbial status (microbial counts, MB-C and the quotient of MB-C to total carbon) of one peatland harboring natural, restored and post-vacuum extracted treatments were assessed sporadically over 6 years. The first experiment revealed that peat mining decreased populations of total bacteria, hemicellulolytic and cellulolytic microorganisms and MB-C, but increased peat ammonium content and N mineralization. The bacterial population was found to be lower in the post-vacuum extracted treatment than in natural treatment (control) and under restoration treatment, whereas the actinomycete population was higher in the post-vacuum extracted and restoration treatments than the natural one. Over the 6-year-time course experiment, the MB-C, total C and their quotient revealed a gradual increase in the peatland under restoration, but they remained similar in the post-vacuum extracted peatland treatment. This supports the concept that the total bacterial population and MB-C may be used as an ecological indicator to monitor major site disturbance using paired natural restored peatland. Published by Elsevier Science B.V.

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1. Introduction

Ombrotrophic peatlands or bogs are acid wetlands (pH < 4.8) with a extremely low concentrations of mineral elements (Vitt and Chee, 1990). These conditions, combined with the absence of oxygen, low temperature and vegetation producing chemical inhibitors,

impede the decay of bog plants and allow the accumulation of partially decomposed organic matter, known as peat (Williams and Crawford, 1983; Groffman et al., 1996).

In North America, the vacuum method of mining peatlands results in bare sites. Description and pictures of how vacuum-extracted peatlands look like can be found in Crum (1988). In brief, the peat fields are approximately 200–400 m long by 30 m wide, and separated by drainage ditches. They are deeply entrenched during active peat extraction. Once extraction activity ceases, the ditches are blocked to help the re-wetting

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process of the residual peat. These sites are characterized by modifications of the microclimate and humidity of the ground (Price, 1996; Price et al., 1998) and the absence of peat-accumulating plant species (Lavoie and Rochefort, 1996). The harvest of these plants eliminates the production of the natural litter, i.e. about 80% of the annual plant production (Malmer, 1986) which is an important source of nutrients used for the growth of microorganisms. These disturbances can alter microbial populations and biomass, and nitrogen (N) mineralization that need to be understood if one aims to achieve sustainable peatlands restoration.

In the past, microorganisms have been used as bio-indicator measurement of human impact on agricultural (Smith and Paul, 1990; Granatstein and Bezdicsek, 1992; Babich et al., 1996) and peatland soils (Maltby, 1992). Today, microbial comparison of natural, post-vacuum extracted and restoration ombrotrophic peatlands are limited and bio-indicators are not available. Also, microbial processes are essential for nutrients transformation and cycling in peat, plant productivity and ecosystem functioning (Clymo, 1991; Maltby, 1992; Groffman et al., 1996). Nitrogen released by mineralization, a microbial process, is an important component of soil fertility and nitrification is an important indicator of N availability in ecosystems (Groffman et al., 1996). Therefore, differences in microbial processes can be reflected on the peat N content, but again, such bio-indicators are not available to assess N availability within the different peatland ecosystems.

A first objective of this study was to evaluate the change in magnitudes in microbial populations, microbial biomass carbon (MB-C) and N mineralization of natural and post-vacuum extracted peatlands. For this purpose, in 1993 and 1994, we examined paired natural and post-vacuum extracted peatlands, where microbial populations and MB-C were evaluated *in vitro* and *in situ*, along with N mineralization. A second objective was to find bio-indicators of the microbial status of one peatland harboring natural, restored and post-vacuum extracted treatments. In these peatland treatments, we quantified and identified bacteria, actinomycetes and fungi in 1995, and measured the MB-C, total C and their quotient in 1993, 1995 and 1998.

2. Materials and methods

2.1. Sites description

In 1993 and 1994, peat was sampled in seven sites located in Québec; Bas-St-Laurent area at Rivière Ouelle, Bois-des-Belles, Rivière du Loup, Saint-Modeste and Cacouna (for characteristics of these peatlands see Desrochers et al., 1998) and Lac-St-Jean area at Sainte-Marguerite and l'Ascension. Five natural peatlands and six post-vacuum extracted peatlands were sampled for a total of 54 peat cores (Table 1).

On average, the mean annual temperature of sampled sites is 2.6°C with total annual precipitation of

Table 1
Number of peat cores and site characteristics of the natural and post-mined peatland in the regions of Bas-St-Laurent and Lac-St-Jean

Peatland sites	Natural				Post-vacuum extracted			
	<i>n</i> ^a	Depth of oxic zone (cm)	Decomposition (H-value)	Bulk density (g cm ⁻³)	<i>n</i>	Depth of oxic zone (cm)	Decomposition (H-value)	Bulk density (g cm ⁻³)
Bas-St-Laurent								
Rivière Ouelle	6	29	1–5	0.063	6	30	6–7	0.128
Bois-des-Belles	3	32	2–5	0.080	3	14	3–4	0.119
Rivière-du-Loup, Saint-Modeste and Cacouna	6	12–27	1–5	0.090	6	7–21	4–6	0.109
Lac-St-Jean								
Ste-Marguerite	6	39	1–3	0.055	6	20	3–6	0.117
L'Ascension	6	40	1–3	0.062	6	40	3–4	0.074

^a Number of peat cores.

900 mm of which 69% occurs as rain. The mean temperature in July is 17.3°C. The vegetation of natural peatlands consisted mostly of *Sphagnum* species, the dominant ones being *S. fuscum* (Schimp.) Klinggr., *S. capillifolium* (Ehrh.) Hedw. and *S. magellanicum* Brid., ericaceous shrubs like *Chamaedaphne calyculata* (L.) Moench., *Kalmia angustifolia* L. and *Ledum groenlandicum* Retzius, some black spruces (*Picea mariana* (Mill.)) and in some cases, *Larix laricina* (Du Roi). For post-vacuum extracted peatlands, vegetative cover is sparse and consists of species like *Eriophorum spissum*, small *Betula papyrifera* Marsh., and occasionally by *Vaccinium angustifolium* Ait. The peatland section in process of restoration sampled in 1995 (Rocheffort et al., 1997) was dominated by mosses like *Polytrichum strictum* and by *Sphagnum* sp. mainly *S. fuscum*. A more detailed description of the vegetation for some of the peatlands sampled has been reported by Wind-Mulder et al. (1996; Rivière-Ouelle and Sainte-Monique), Gauthier and Grandtner (1975; Rivière-Ouelle and Rivière-du-Loup), Lavoie and Rocheffort (1996; Caouna) and Desrochers et al. (1998; all post-mined peatlands).

From 1993 to 1998, investigations were carried out in one peatland, at Rivière Ouelle, that harbored natural, restored and post-vacuum extracted treatments as described by Rocheffort et al. (1997). The restored site was sampled the year of plant reintroduction, and three (1995) and 6 years (1998) latter. On average, for the three sites sampled, plant cover was only 10%, where *Polytrichum strictum* was the dominant moss (70% of the total cover), *Spagnum* mosses contributed for about 15% of the total cover and vascular plants, mostly *Chamaedaphne calyculata* with few plants of *Ledum groenlandicum*, *Larix laricina*, *Betula* sp. *Vaccinium oxycoccos* and *V. angustifolium*, and *Drosera rotundifolium*, for the remaining 15%.

2.2. Peat sampling and characteristics

Three peat cores per treatment were extracted with a rectangular sampler to avoid compaction of the sample (Sheppard et al., 1993). In natural peatlands, peat cores were taken along transect lines of three sampling points distant by 10 m. In post-vacuum extracted peatlands, three sub-samples were taken in a transect line in the middle of the former peat file. The sampler was

disinfected with ethanol 70% between each sampling to avoid crossed contamination between the different peat layers and decrease risks of contamination. Cores of peat were wrapped in aluminum paper and placed in plastic bags. They were stored on ice and carried to the laboratory. Peat cores were maintained at 4°C and analysed as soon as possible (in days for microbial and N analyses and weeks for the other physico-chemical analyses). All results are expressed on a dry weight (dw) basis.

Each peatland was characterized for the depth, decomposition and bulk density of its oxic zone (Table 1). The oxic zone was determined using steel rods that were placed into the peat at each site, 1 month before sampling. The rod oxidation zone was used to estimate the oxic zone and depth in the peatland (Trettin et al., 1996). The degree of decomposition was determined according to the von Post method (Parent and Caron, 1993). Briefly, a peatland treatment is first observed to describe the plant residues, ranging from unaltered (H1) to non-recognizable (H10). Then, a fresh peat sample is pressed in the hand and, the extruded matter, ranging from clear water to all peat material extruded, and the appearance of the residues, ranging from non-pasty to no residue, are determined. The H-value can be used to classify peatlands into fibric (H1–H4), mesic (H5–H6) and humic (H7–H10).

In 1993, the depth of peat core analysed was of 15 cm for post-vacuum extracted peatlands excluding the first 2 cm and of 15 cm under the euphotic layer (surface to a depth of 2–5 cm below living *Sphagnum*) for natural peatlands. In 1994 and 1995, analyses were averaged over the oxic zone of each peat core. The peat sample analysed was a composite one taken from the centre of core at regular intervals (every 3 cm for shallow peat column and every 5 cm for deep peat column). In 1998 at Rivière Ouelle, two samples were taken per treatment; one as in 1993 and another as in 1995.

2.3. Physicochemical analyses

The pH and electrical conductivity were determined in a suspension of peat in double distilled water (peat:water, 1:5, v/v) (Karam, 1993). Moisture content was calculated as percent fresh weight after drying 10 g of peat at 105°C until constant weight. Total carbon and nitrogen contents were measured with LECO CNS-1000 analyser (Leco Corporation, Michigan,

USA) on samples of dried and ground peat (0.12 mm). The ammonium and nitrate contents were extracted with 2 M KCl and measured with an auto-analyser (Technicon Industrial Systems, Tarrytown, NY, USA).

2.4. Microbial counts

Microbial counts were done using the plate-dilution frequency method of Harris and Sommers (1968), based on the most probable number of microorganisms. Briefly, 10 g of fresh peat were aseptically sampled from the centre of each peat core. Serial dilutions of 10^{-1} to 10^{-6} were carried out in phosphate saline solution (Page et al., 1982) and 10 μ l of each dilution were distributed on solid media. To compare results with other studies, standard growth media were used. The Tryptic Soy medium (10% Tryptic Soy Agar, Difco, Detroit, MI) was employed for total bacterial counts and the Rose Bengal medium for fungi (Page et al., 1982), cellulose and hemicellulose media (Page et al., 1982) were used to count microorganisms implicated in the carbon cycle. Petri plates were incubated to 27°C (± 2) in darkness. Total bacteria and fungi were counted on day 7 and hemicellulolytic and cellulolytic microorganisms on day 28. Counts were expressed in exponential number of colony forming units by peat dry weight (cfu g^{-1} (dw)).

2.5. Microbial biomass carbon

Microbial biomass C was determined according to the fumigation and extraction method of Vance et al. (1987). This method is adapted for acidic soils as used by Sparling et al. (1990) and discussed by Voroney et al. (1993). Briefly, 20 g of fresh peat were fumigated with ethanol-free CHCl_3 for 24 h to kill and lyse microbial cells. Carbon content from fumigated and non-fumigated samples was extracted with 75 ml of 0.25 M K_2SO_4 (Angers et al., 1995) and measured with a carbon analyser (total organic carbon, TOC-5050 Shimadzu, Kyoto, Japan). A proportionally constant of 0.45 was used to calculate the microbial biomass carbon (Wu et al., 1990). The fumigated extracts were analysed by GC-MS to detect residual chloroform and it did not represent >5% of the C present in these extracts.

2.6. Isolation and identification of microorganisms

Bacteria, actinomycetes and fungi were isolated and identified in natural, restored and post-vacuum extracted treatments of one peatland. Bacteria were isolated by successive passages on Tryptic Soy medium (10% Tryptic Soy Agar, Difco, Detroit, MI), actinomycetes on the selective medium Actinomycetes Isolation Agar (Difco, Detroit, MI) and fungi were isolated on Potato Dextrose Agar medium (Difco, Detroit, MI). Bacteria and actinomycetes were identified by the fatty acid analysis of their cell membrane using the microbial identification system and the similarity index of the computer libraries (Bacterial Strain Identification and Mutant Analysis Service, Department of Plant Pathology, Auburn University, Auburn, AL). To separate the actinomycetes from the other bacteria, the classification of Balows et al. (1992) was used. Fungi were identified to the genus by their microscopic characteristics (Barron, 1968).

2.7. Nitrogen mineralization

Nitrogen mineralization was determined following an in vitro (in laboratory) and in situ (in field) incubation. For the in vitro incubation, a preliminary study was performed in 1993, where peat samples were adjusted to 60% water holding capacity (Salonen and Setälä, 1992). However, this process involved in our case, a drying–re-wetting cycle, which can alter N mineralization (data not shown). For this reason, no attempt was made, in 1994, to correct peat moisture and samples were incubated at field moist condition (Williams, 1992). For this in vitro incubation, 80 g of peat were placed in 500 ml glass jars and incubated at room temperature using three samples per site (Salonen and Setälä, 1992). Incubations were maintained for 42 days and then, 10 g of peat were collected for analyses (see below). For the in situ incubation, approximately 80 g of fresh peat were placed in a polyethylene bag (Ziploc, sandwich bags), which allows an aerobic incubation (Humphrey and Pluth, 1996). Once closed, the bags, three per site, were laid down and buried. The bags were buried at 1 cm depth for the post-vacuum extracted peatlands and below the green living *Sphagnum* in natural peatlands (about 15–20 cm depth) to put the bags in similar peat structure. The ammonium and nitrate contents were

extracted and measured on days 0 and 42 as described above. The net nitrogen mineralized was calculated by subtracting the ammonium or nitrate contents at day 0 from day 42.

2.8. Statistical analyses

The different peatland sites constituted replicates using three to six sub-samples per site (Table 1). The paired comparisons of natural and post-vacuum extracted peatlands were made with analysis of variance, ANOVA (F -test with 1 d.f.), using the GLM procedure of SAS (Statistical Analysis System Institute, 1990). As the analyses were performed on different peat depth over years, no attempt was made to combine years in 1993 and 1994. Homogeneity of variance was verified by a Bartlett's test (Snedecor and Cochran, 1980) and logarithm transformations were used to obtain homogenous variances for the microbial populations. For nitrogen mineralization data, common transformations did not lead homogeneity of variances, so the ANOVA was done following a rank transformation (Conover and Iman, 1981). Levels of significance were set at $P < 0.10$ and less (Steel and Torrie, 1980) due to the expected great variability of soil microbial populations (Atlas and Bartha, 1987).

3. Results

3.1. Physicochemical parameters

In 1994, similar pH and electrical conductivity values were obtained for natural and post-vacuum

extracted treatments, averaging 4 and 40 $\mu\text{S cm}^{-1}$, respectively (Table 2). Peat moisture was 7–8% higher in natural peatlands than post-vacuum extracted ones for 1993 and 1994, respectively (Table 2). Total C and N were higher in post-vacuum extracted peatlands than in natural ones. Mean contents of total organic C for the 2 years was 4% higher in post-vacuum extracted peatlands than in natural peatlands.

Peat ammonium content was higher in the post-vacuum extracted peatlands than in the natural peatlands in 1993 and 1994 (Table 2). However, nitrate content of peat was similar in natural and post-vacuum extracted peatlands. Furthermore, on average, in post-vacuum extracted peatlands, nitrate content was lower than ammonium content by 57 $\mu\text{g g}^{-1}$ (dw) (Table 2). In natural peatlands, peat nitrate and ammonium contents were quite similar.

3.2. Microbial populations

The populations of all microorganisms were lower in post-vacuum extracted peatlands than in natural peatlands (Table 3). Bacterial populations of post-vacuum extracted peatlands were lower than those of the natural peatlands in 1993 and 1994, respectively. The hemicellulolytic and cellulolytic populations were also lower in post-vacuum extracted than in natural peatlands. In 1994, fungi were lower in post-vacuum extracted peatlands than in natural peatlands. Despite the absence of significant difference in 1993, the population of fungi was lower in post-vacuum extracted peatlands compared to natural peatlands.

Table 2
Physicochemical properties of the natural and post-vacuum extracted peatlands in 1993 and in 1994 (mean \pm standard error of the mean)

Parameter	1993		1994	
	Natural	Post-vacuum extracted	Natural	Post-vacuum extracted
pH	N.D. ^a	N.D.	4.0 \pm 0.2	4.2 \pm 0.4
Specific conductivity ($\mu\text{S cm}^{-1}$)	N.D.	N.D.	34 \pm 14	46 \pm 10
Peat moisture (%) ^b	91 \pm 4 b	83 \pm 4 a	91 \pm 3 b	83 \pm 5 a
Total C (%) ^b	48 \pm 1 b	52 \pm 2 a	48 \pm 1 b	52 \pm 2 a
Total N (%) ^b	0.94 \pm 0.15 b	1.11 \pm 0.40 a	0.86 \pm 0.11 b	1.22 \pm 0.35 a
Initial $\text{NH}_4^+\text{-N}$ ($\mu\text{g g}^{-1}$ (dw)) ^b	23 \pm 19 b	115 \pm 98 a	6 \pm 2 b	52 \pm 53 a
Initial $\text{NO}_3^-\text{-N}$ ($\mu\text{g g}^{-1}$ (dw))	20 \pm 6	37 \pm 29	13 \pm 7	17 \pm 15

^a N.D.: no determination.

^b Values within a sampling year with a different letter are significantly different at $P < 0.05$, except for total N in 1994, where $P < 0.10$.

Table 3

Microbiological parameters measured in natural and post-vacuum extracted peatlands in 1993 and in 1994 (mean \pm standard error of the mean, and level of significance)

Parameter	1993		P-value	1994		P-value
	Natural	Post-vacuum extracted		Natural	Post-vacuum extracted	
Bacteria (\log_{10} cfu g^{-1} (dw)) ^a	6.6 \pm 0.2 b	5.9 \pm 0.6 a	<0.05	6.8 \pm 0.6 b	5.8 \pm 0.9 a	<0.10
Hemicellulolytic microorganisms (\log_{10} cfu g^{-1} (dw)) ^a	6.6 \pm 0.2 b	5.2 \pm 0.5 a	<0.05	6.8 \pm 0.2 b	6.1 \pm 0.5 a	<0.10
Cellulolytic microorganisms (\log_{10} cfu g^{-1} (dw)) ^a	6.4 \pm 0.2 b	5.8 \pm 0.7 a	<0.10	7.0 \pm 0.2 b	6.2 \pm 0.5 a	<0.01
Fungi (\log_{10} cfu g^{-1} (dw)) ^a	4.3 \pm 1.5	4.0 \pm 0.9	<0.01	5.5 \pm 0.3 b	4.7 \pm 0.5 a	<0.01
Microbial biomass C ($\mu g g^{-1}$ (dw)) ^a	9064 \pm 2129 b	2750 \pm 566 a	<0.001	5219 \pm 1265 b	881 \pm 655 a	<0.001

^a Values within a sampling with a different letter are significantly different.

In 1995, within a single site, where the three different types of peatlands were compared, bacterial population was lower in the post-vacuum extracted peatland than in the natural one. The bacterial population of the peatland under restoration was intermediate to the natural and post-vacuum extracted treatments (Fig. 1). The population of fungi was similar in the three peatland sections. The actinomycete population was the smallest in quantity of all microbial populations and counted the lowest population in the natural peatland treatment.

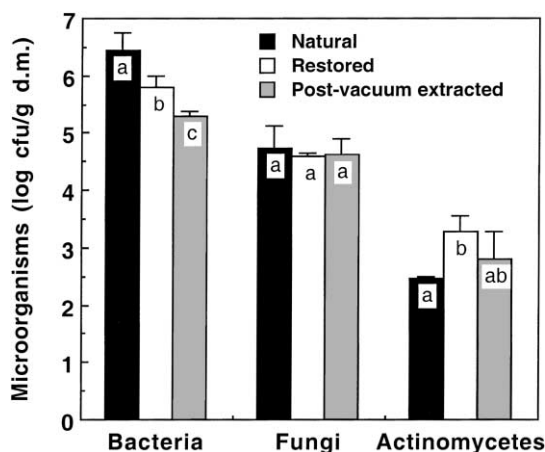


Fig. 1. Microorganisms in the natural, restored and post-vacuum extracted treatments of the peatland located in Rivière-Ouelle in July 1995 (mean populations of bacteria, fungi or actinomycetes followed by the same letter are not significantly different at $P < 0.05$ according to LSD test).

3.3. Microbial biomass carbon

Microbial biomass C of the post-vacuum extracted peatlands was lower than in natural peatlands for 1993 and 1994 (Table 3). Also, in post-vacuum extracted peatlands in 1994, significant and positive correlations were obtained between bacteria, hemicellulolytic and cellulolytic microorganisms and MB-C (data not shown).

The MB-C was compared in 1993 and 1998 for the 0–15 cm depth, and in 1995 and 1998 for the 0–30 cm depth (both oxic zones) and a quotient was calculated to determine the percentage of microbial carbon in the total carbon from one peatland harboring a natural, restored and post-vacuum extracted section. For all comparisons, the post-vacuum extracted and restored treatments were similar whereas the natural treatment showed the highest MB-C and quotient (Table 4).

3.4. Isolation and identification of microorganisms

For bacteria, a total of five to nine different species were isolated and three species were specific to natural peatland treatments; *Staphylococcus epidermidis* and *Paenibacillus pabuli* (Table 5). For actinomycetes, a total of six to seven different species per type of peatland treatments were isolated and only *Micrococcus luteus* was specific to the natural peatlands (Table 6). The total number of fungal species isolated in each type of peatland ranged from seven species in the natural treatment, five species in the restored treatment

Table 4

Total carbon, microbial biomass carbon and their quotient at two sampling depths in natural, restored and in post-vacuum extracted treatments of Rivière Ouelle peatland in 1993, 1995 or 1998 (mean \pm standard error of the mean)

Peatland	Year	0–15 cm			Year	0–30 cm		
		Total carbon (%)	Microbial biomass carbon ($\mu\text{g g}^{-1}$ dry peat)	Quotient		Total carbon (%)	Microbial biomass carbon ($\mu\text{g g}^{-1}$ dry peat)	Quotient
	1993				1995			
Natural		48.6 c ^a	7569 b	1.38 b		48.7 c	10894 a	2.23 a
Restored		54.8 a	1377 c	0.28 c		53.2 ab	6590 b	1.24 b
Post-vacuum extracted		54.5 a	2361 c	0.43 c		51.5 b	4406 bc	0.85 bc
	1998				1998			
Natural		45.8 d	13873 a	3.10 a		45.9 d	11841 a	2.59 a
Restored		55.0 a	4077 c	0.74 c		55.0 a	3548 bc	0.64 bc
Post-vacuum extracted		51.9 b	2477 c	0.48 c		52.5 b	2934 c	0.56 c

^a Values within a sampling depth with a different letter are significantly different at $P < 0.05$ according to LDS test.

and six species in the post-vacuum extracted treatment (Table 7). The identification of fungi was limited to the genus. Only one *Penicillium*, with yellow and white mycellium forming asci and producing an abundant red exudate (no. 4), was isolated specifically in the natural peatlands.

3.5. Nitrogen mineralization

In 1994, the mineralized ammonium contents was $6 (\pm 9) \mu\text{g g}^{-1}$ in natural peatlands compared to $43 (\pm 29) \mu\text{g g}^{-1}$ (dw) in post-vacuum extracted peat-

lands following an incubation in situ ($F = 8.3$, $P < 0.05$, d.f. = 1). However, in vitro, the ammonification was similar for natural and post-vacuum extracted peatlands, with a mean content of $121 (\pm 43)$ and $74 (\pm 45) \mu\text{g g}^{-1}$, respectively. When the ammonification in vitro and in situ were compared, a 2–20-fold greater rate was measured in vitro. The mineralization of ammonium to nitrate was similar for natural ($10 \pm 38 \mu\text{g g}^{-1}$) and for post-vacuum extracted ($25 \pm 38 \mu\text{g g}^{-1}$) peatlands following in situ incubation, and for natural ($-5 \pm -9 \mu\text{g g}^{-1}$) and post-vacuum extracted ($24 \pm 41 \mu\text{g g}^{-1}$) peatlands

Table 5

Bacteria identified in natural, restored and in post-vacuum extracted treatments of Rivière Ouelle peatland in 1995

Bacteria	Peatlands		
	Natural	Restored	Post-vacuum extracted
<i>Bacillus chitinosporus</i>		×	
<i>Bacillus pumilus</i>		×	×
<i>Bacillus thuringiensis</i>	×	×	×
<i>Brevibacterium linens</i> or <i>B. epidermis</i>	×	×	
<i>Brevibacterium acetylicum</i>		×	
<i>Janthinobacterium lividum</i>		×	
<i>Paenibacillus pabuli</i>	×		
<i>Pantoea ananas</i>			×
<i>Pseudomonas fluorescens</i>	×	×	×
<i>Pseudomonas putida</i>	×	×	×
<i>Staphylococcus epidermidis</i>	×		
<i>Staphylococcus warneri</i>		×	
Total	6	9	5

Table 6
Actinomycetes identified in natural, restored and in post-vacuum extracted treatments of Rivière Ouelle peatland in 1995

Actinomycetes	Peatland		
	Natural	Restored	Post-vacuum extracted
<i>Actinomadura yumaensis</i>		×	
<i>Cellulomonas turbata</i>			×
<i>Micrococcus luteus</i>	×		
<i>Nocardia asteroides</i>	×	×	×
<i>Nocardia otitidiscavarium</i>		×	×
<i>Rhodococcus erythropolis</i>			×
<i>Rhodococcus luteus</i> fascians	×	×	×
<i>Streptomyces halstedii</i> ssp. <i>Scabies</i>	×	×	
<i>Streptomyces lavendulae</i>	×		×
<i>Streptomyces violaceusniger</i> ssp. <i>hygroscopicus</i>		×	
<i>Streptomyces violaceusniger</i> ssp. <i>violaceusniger</i>	×		×
Total	6	6	7

Table 7
Fungi identified in natural, restored and in post-vacuum extracted treatments of Rivière-Ouelle peatland in 1995

Fungi	Peatland		
	Natural	Restored	Post-vacuum extracted
<i>Aspergillus</i> sp.	×	×	×
<i>Penicillium</i> sp. no. 1	×	×	×
<i>Penicillium</i> sp. no. 2	×		×
<i>Penicillium</i> sp. no. 3		×	
<i>Penicillium</i> sp. no. 4	×		
<i>Pythium</i> sp.	×	×	
<i>Trichoderma</i> sp. no. 1	×		×
<i>Trichoderma</i> sp. no. 2			×
<i>Verticillium</i> sp.	×	×	×
Total	7	5	6

following in vitro incubation. These negative values for natural peatlands represent a loss of nitrate following the incubation in vitro.

4. Discussion

4.1. Microbial modifications in peatlands

Results of the present study demonstrate that peat mining decreased bacterial population as well as populations of hemicellulolytic and cellulolytic microorganisms of peatlands as determined by the dilution plate counts. Thus, they contribute to the proposition that microbial populations are an effective

bio-indicator of ecological disturbances of soils (Smith and Paul, 1990; Granatstein and Bezdicek, 1992; Maltby, 1992; Babich et al., 1996). Also, since several factors may influence the microflora such as climatic conditions or botanical composition, we suggest that a natural (undisturbed, i.e. control) peatland neighboring the post-vacuum extracted one should be used as a reference to compare the microbial populations.

The aerobic bacterial population of all peatland sites and types confounded were similar to those observed by Waksman and Stevens (1929), Waksman and Purvis (1932), Collins et al. (1978) and Martin et al. (1982) in natural ombrotrophic peatlands. Bacterial counts between \log_{10} 5.5 to 7.7 g^{-1} (dw) are common in

peatland surface of bogs (Given and Dickinson, 1975). Fungal populations were also in the range measured by others in natural ombrotrophic peatlands varying between \log_{10} 4.0 to 6.1 g^{-1} (dw) (Holding et al., 1965; Collins et al., 1978). Fungal populations were low in all cases and possibly related to very acid pH (≤ 4) and ammonium concentration of peat. The concentration of ammonium could be deleterious to fungi (Alexander, 1977). Quantities of hemicellulolytic and cellulolytic microorganisms of all peatland sites and types confounded were higher than those measured by Collins et al. (1978) who observed a population of \log_{10} 3.3 cfu g^{-1} . This difference could be related to the degree of peat decomposition and selective media.

Microbial biomass C confirms the results obtained from the dilution plate counts where a lower concentration of MB-C was obtained in post-vacuum extracted peatlands relatively to natural ones. Average values for quantity of MB-C content for natural peatlands is usually between 1000 and $15\,000 \mu\text{g g}^{-1}$ (dw) (Brake et al., 1999; Willams and Silcock, 1997; Groffman et al., 1996; Hart et al., 1986; Clarholm and Rosswall, 1979). Values obtained in this study were within these limits. Microbial biomass and activity of microorganisms is dependent of several factors including available carbon that is the fraction of soil organic carbon that heterotrophic microorganisms can easily use as carbon and energy sources (Davidson et al., 1987). The presence of *Sphagnum* and its litter under decomposition, in natural peatlands, brings C available to microorganisms.

The reduction of microbial populations following peat mining does not seem irreversible. However, only the actinomycete population and MB-C increased in the treatment under restoration of the peatland. These increases can be linked to the peat water content and the nature of available carbon, more suitable for actinomycetes. Evaluation of the degree of decomposition of peat with von Post scale demonstrated that the surface of post-vacuum extracted peatlands is more decomposed than the natural peatlands. In peatlands, total organic C increases during the decomposition process of peat (Bohlin et al., 1989) and the restoration efforts seems to increase, in this study, this decomposition. This increase in total organic C is linked to the accumulation of compounds resistant to degradation, like lignin and bitumen (Nordén et al., 1992). In addition to nutritional competition, the acti-

nomycetes are well-known to produce antibiotics that prevent bacterial growth.

An absolute value of MB-C to characterize the microbiological state of a post-vacuum extracted peatland and measure the disturbances brought by man would be difficult to establish since this parameter varies with climate and regions (Insam et al., 1989); also, more information is required on the origin of the extracted C (Willams and Silcock, 1997). Differences in soluble organic matter have been noted among soils in the fumigation and extraction extract (Parkinson and Coleman, 1991). In peatlands, we can expect differences in soluble C composition, but such information is currently lacking in the literature. Nevertheless, we suggest the use of MB-C or the quotient MB-C to total C to discriminate natural peatland from post-vacuum extracted or peatlands under restoration, using a paired natural peatland.

The identification of microorganisms has not allowed to described some species that could be used as bio-indicators for natural, restored and post-vacuum mined peatlands. Similarly, Dooley and Dickinson (1970) obtained no increase in microflora species in a complex of ombrotrophic peatlands exploited and abandoned for 10 years. Most microorganisms isolated in this study are ubiquitous in peatland (Ivarson, 1977; Collins et al., 1978; Martin et al., 1982; Tahvonen, 1982). Also, they are related to the carbon cycle such as pectinic substances, cellulose, hemicellulose or lignin decomposition. In addition, some isolates from the post-vacuum extracted peatlands are involved in the turnover of organic matter, such as *Actinomadura* and *Nocardia* (Balows et al., 1992). In addition to the source of carbon available, the low genus richness of this study can be linked to the extreme acidic conditions (about pH 4) brought by the acidic *Sphagnum* exudates.

The bacteria found exclusively in the natural peatland were *Bacillus pabuli*, *Paenibacillus pabuli* and *Staphylococcus epidermidis*. However, these bacteria are widespread in nature including water, soil and plant habitats (Balows et al., 1992). Their wide distribution discredits them as potential bio-indicators. Also, among the isolated actinomycetes, only *Micrococcus luteus* was exclusive to the natural peatland. However, *M. luteus* has been isolated from several habitats such as skin, soil, plants and water (Balows et al., 1992), and peatlands (Martin et al., 1982; Collins et al., 1978).

Again, this microorganism was not considered as potential bio-indicator. Among the fungi isolated, one *Penicillium* sp. forming asci and a red exudate, was isolated exclusively in the natural peatland. However, since *Penicillium* sp. are common in peatland (Ivarson, 1977; Collins et al., 1978; Tahvonen, 1982) and their identification to the species level requires special training, this potential bio-indicator was not considered worthwhile. All microorganisms isolated in this study were able to grow on rich culture media, but the presence of more fastidious and non-culturable microorganisms should be investigated. In future studies, the new technologies of 16S-DNA and 18S-DNA profiles should be investigated to overcome the absence of growth of certain microorganisms on culture media.

4.2. Nitrogen cycling

In post-vacuum extracted peatlands, mineralization of organic nitrogen appears to be stopped after the ammonification process. In fact, the ammonium content of peat was 5–9-fold higher in post-vacuum extracted peatland than in the natural one. In addition, in the in situ incubation study, our results indicated a higher mineralization potential of peat nitrogen to ammonium in the post-vacuum extracted peatland than in the natural one. In contrast, in our in vitro incubation study, the ammonification potentials of peat nitrogen from natural and post-vacuum extracted peatlands were similar. Therefore, the enrichment in ammonium content can be the result of a greater mineralization caused by differences in temperature or peat moisture. The reduced peat moisture combined with the large temperature fluctuation (post-vacuum extracted peatland; mean: 18°C; minimum: 9.7°C; maximum: 27.6°C and natural peatlands mean: 17°C; minimum: 15.7°C; maximum: 20.0°C; J. Price, personal communication) in the zones of incubation of post-vacuum extracted peatland can increase the rate of ammonification, as suggested by Updegraff et al. (1995) and Wheeler and Shaw (1995). Other factors in post-mining peatlands enhancing oxidation and drying–re-wetting cycles of the peat, created by greater fluctuations of the watertable (Price, 1996), could enhance the ammonification potential in situ. Finally, the low population of microorganisms and plants to assimilate this ion can be partly related to this increase in concentration in post-vacuum ex-

tracted peatlands relatively to natural ones (Money, 1995; Wind-Mulder et al., 1996).

There were no differences in nitrate content in the two types of peatland at the beginning of the incubation studies, i.e. at sampling time. Increases in nitrification activity in peatlands are only measured following an increase of the pH by liming (Ivarson, 1977; Küster, 1975). However, the nitrate content of peat was 2–120-fold lower than the ammonium content. This can be explained by a lower population of nitrifiers than ammonifiers in ombrotrophic peatlands (Collins et al., 1978). A loss of nitrate was observed following the in vitro incubation as denitrification occurred. Other studies of nitrogen mineralization have also demonstrated denitrification activity following aerobic incubations in vitro (Groffman et al., 1996; Hart et al., 1986) or in situ (Martikainen, 1996; Tietema et al., 1990).

In conclusion, the present study has shown that mining of peatlands decreased the populations of total bacteria, hemicellulolytic and cellulolytic microorganisms and MB-C. Also, when *Sphagnum* is reintroduced, the actinomycete population and MB-C increased, whereas the bacterial population decreased, relatively to the natural peatland. These results suggest a link with the accumulation of resistant C and support their possible use as ecological indicators to monitor site disturbance using paired natural peatland. Compared to natural peatlands, mining modified nitrogen cycling, where the mineralization of peat nitrogen to ammonium, and peat ammonium concentration increased. Other physiological groups of microorganisms involved in the mineralization of N, such as the ammonifiers, should be targeted to determine if they could be used as potential bio-indicators. To avoid culturing of peat microorganisms, the use of ammonifier-PCR probes can be an interesting avenue to study.

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