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The physicochemical and microbiological status of a restored bog in Québec: Identification of relevant criteria to monitor success

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Abstract

The Bois-des-Bel *Sphagnum* peatland (Rivière du Loup, QC) was restored in 1999 after 20 years of abandonment. Restoration work included not only the blockage of drainage ditches, but also the reintroduction of plant material including *Sphagnum* remains. Following restoration, the physicochemical and microbial characteristics (biomass, activity and composition) of the peat were analysed. The goal was to investigate the functional status of the restored ecosystem. The high N:P (>20) and N:K (>15) ratios indicated possible K and P deficiencies in the restored and the cutover sites, which is mainly associated with the intense leaching and the high degree of decomposition of the peat in these sites. The concentrations of NH_4^+, P and K in the top layer of the restored site were closer to those of the natural site, which indicated a possible effect of restoration on the physicochemistry of the restored site. Microbial biomass values derived from the FE technique followed a gradient natural > restored > cutover through the profile, which was not the case with the SIR technique. Values from SIR varied overall between 0.19 and 4.88 mg C g^{-1} and were significantly higher in the natural site. The natural peatland site had significantly ($P<0.05$) greater cumulative C–CO_2 production (surface aerobic: 4.5–8.7 μg C–CO_2 g^{-1} h^{-1}). The poor organic matter quality was the main explanation for the low respiration rates of the surface layer in the restored and the cutover site. All CO_2 respiration data were plotted against time and the resulting curves were successfully fitted to a global kinetic model. Methane production was detected at low but measurable rates in the natural and the restored samples, but not in the cutover peat. Overall, the results confirmed the existence of a lag between the positive response of vegetation to restoration and that of the microbial compartment. This study also pointed out that some physicochemical dysfunctions remained even after three growing seasons following restoration in the subsurface horizons studied.

Keywords: Ombrotrophic peatland; Ecological restoration; Monitoring; Microbial biomass; Physicochemistry; CO_2 kinetics; Fungal and bacterial activity; Methane fluxes

1. Introduction

Ecosystem-scale restoration of degraded peatlands appears to be an ideal solution for post-harvested sites, as it aims to return the exploited ecosystem to a functional (Erhenfeld, 2001) and self-sustainable state (Wheeler and Shaw, 1995; Rochefort, 2001). In order to evaluate the functional status of these restored ecosystems, it is imperative to establish reliable criteria that will help to assess the success or failure of a given restoration project, and to set realistic and appropriate goals beforehand (Erhenfeld, 2001). Moreover, monitoring of all ecologically relevant properties should be continued long after restoration (Gorham and Rochefort, 2003) to follow the evolution of the system over time, mostly when the restoration approach is novel. Such a trajectory analysis should include surveys of vegetation type and composition, hydrology, biogeochemical cycles, water and peat chemistry as well as microbiology analysis (Chapin et al., 1992).

For the particular case of post-vacuumed *Sphagnum* peatlands (milled peatlands) in Canada, a plant reintroduction method has been developed to favour the revegetation of the residual substrate with bog-specific species (Rochefort et al., 2003). It was used for the first time in a whole ecosystem experiment in 1999, on the Bois-des-Bel peatland. Following restoration, a multidisciplinary research team has started to monitor different aspects of the restored ecosystem, and our

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work focuses on microbial and physicochemical properties and on their potential to be used as indicators of success.

Few studies have been made on the microbial compartment of harvested or restored peatlands, despite its major implication in the organic matter accumulation function of these ecosystems. Croft et al. (2001) found that harvesting activities had a significant negative effect on the size of peat microbial biomass, and detected an increased ammonification activity in exploited sites. Waddington et al. (2001) concluded that cutover peatlands had lower potentials for CO_2 production compared to natural sites because of the low substrate quality remaining after exploitation, less suitable for microbes. However, as abandoned sites are generally lacking photosynthetic activity due to reduced plant cover, they become carbon sources rather than sinks. The recovery of the storage function is one of the goals of restoration. In a rewetted peatland in France, Francez et al. (2000) demonstrated that after 7 years, restoration had influenced the microbial compartment but had not reinitiated the carbon accumulation function. One aim of our project is to evaluate the effects of restoration on the microbial and physicochemical properties associated with this accumulation function. To achieve this goal, the properties of the restored site were compared to those of a non-restored (cutover) site and to those of a natural site, considered in this case as a reference ecosystem (Erhenfeld, 2001).

We believe that in the restored site, the return of the vegetation, the input of fresh organic matter, and the elevation of the water table have modified the conditions in the peat. This should have consequences on the nutrient balance, on the carbon transformations (Francez et al., 2000), on the physicochemical properties of peat (De Mars and Wessin, 1999; Laiho et al., 2004) as well as on the size of the microbial compartment, measured with microbial biomass values. The relationship between microbial biomass values and different nutrient ratios (N:K, C:P, N:P, C:N) was examined to evaluate the availability of resources in the peat from the three sites. Changes of physico-chemistry (Baum et al., 2003), increasing plant cover and also variation in the water table depth (Brake et al., 1999) are thought not only to influence the size of microbial populations, but also their activity. Thus, the potential CO_2 and CH_4 production of different microbial populations was also investigated. As the microbes responsible for methane production and consumption are highly dependant on hydrological conditions, we expected that the effect of restoration on moisture and water availability (Price et al., 2003) might be reflected in CH_4 fluxes. We assumed that restoration improves the quality of organic matter in the top layers through the process of re-vegetation, and that the structure of the microbial communities might consequently be altered in terms of fungal- or bacterial activities. This was shown in forest ecosystems by Blagodatskaya and Anderson (1998) but it has not yet been demonstrated in peatlands.

Specifically, the objectives of our project could be summed up as: (1) to verify if restoration and subsequent vegetation recovery changed the physicochemical conditions in the peat, when compared with an unrestored area and a natural site; (2) to test the hypothesis that restoration, through vegetation recovery and possible physicochemical changes, modified the size of the microbial compartment as well as the microbial activities related to the organic matter accumulation function of the peatland; (3) to question the relevance and accuracy of the investigated microbial and physicochemical variables in monitoring these changes.

2. Material and methods

2.1. Site description

Peat samples were collected from 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 between 1973 and 1980, and thereafter 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. Even 20 years later, no *Sphagnum* species had colonized the site and a large proportion of the site was covered with bare peat (Rochefort, unpublished data). For this reason, in 1999, an ecosystem-scale restoration experiment was set up to re-establish a *Sphagnum*-dominated peat accumulating system in this cutover site. An 8 ha section of the site was restored and a 2 ha area remained unrestored and now serves for comparisons. A buffer area where no sample was collected separates the restored and the cutover sections.

Restoration work included the preparation of the site, the collection and spreading of plant fragments from a nearby donor site, the protection of the plant fragments with straw mulch (3000 kg ha^{-1}), the blockage of drainage ditches done in November 1999 (Rochefort et al., 2003). Phosphorus fertilization was applied by the end of June 2000. Phosphate rock of McInnes (0-13-0) was spread at the rate of 15 g m^{-2}. The Bois-des-Bel field station is surrounded by a natural peatland, which was sampled for the study, and served as a reference ecosystem. The natural peatland vegetation is characterized by *Picea mariana* and *Larix laricina* as dominant tree species, a *Sphagnum* carpet (*S. fuscum*, *S. magellanicum*, *S. rubellum*, and *S. capillifolium*) and a dense cover of ericaceous shrubs on the ground (*Kalmia angustifolia*, *Ledum groenlandicum*, *Chamaedaphne calyculata* and *Vaccinium angustifolium*; Lachance and Lavoie, 2004). *Sphagnum spp.*, *Polytrichum strictum* and *Eriophorum vaginatum* var. *spissum* are among the species that colonized the restored site successfully. Mosses nomenclature follows Anderson et al. (1990); vascular plants identification follows Marie-Victorin (1995). In the cutover site, no vegetation was present on the sampling sites. In 2003, for the whole non-restored site, less than 10% of the site was covered by mosses and trees (gray birch, larch and black spruce) with ericaceous shrubs covered less than 20% of the site. In contrast, the restored site had a good moss cover of 70% (composed of 35% sphagna). Similarly, at the end of 2002, in the sampled areas of the cutover site, total plant biomass was estimated at 13 ± 6 g m^{-2} whereas in the restored site, it was estimated at 422 ± 29 g m^{-2}, with *Sphagnum* biomass being 45 ± 6 g m^{-2} (Rochefort, unpublished data).

2.2. Sampling method

In June 2003, peat was sampled in the natural, the restored and the cutover sites. Three sampling stations separated by approximately 30 m were established in each site during a preliminary study carried out in October 2002. At each station, two sampling points within a 5 m diameter were randomly chosen. At every sampling point, a trench was excavated to a depth of approximately 60 cm. Peat samples were collected on one side of the trench with a sterilised knife at three different depths, using the water table level as a baseline. Depth A represented a constantly aerated layer of peat situated over the water table and under the litter, approximately between 10 and 20 cm below the surface. Depth B represented a peat layer at the water table height between 30 and 40 cm in the restored and the natural section and between 45 and 55 cm in the cutover area, and depth C a peat layer deeper in the profile, under constant waterlogged conditions. The samples consisted in a volume of approximately 2 dm^3 of peat. They were placed in sterilised plastic bags and stored at 4 °C in the dark until they were analysed. Roots were removed from all samples prior to the analyses.

2.3. Physicochemical analyses

All physicochemical analyses were conducted on the peat samples from the three horizons. Peat samples were saturated with water to measure the pH and electrical conductivity (EC). The pH was measured directly in the 1:1 peat:water slurry after it stabilized for 1–4 h, using a pH meter from Denver Instrument (model 225, pH and ISE meter). The slurry was filtered and the filtrate was used to measure EC with a conductivity meter YSI (model 32). Values of EC were corrected for pH according to Sjörs (1950).

Pyrophosphate index was measured to appraise the degree of humification of the different peat samples, following a modified Kaila (1956) procedure. Bulk density was also determined for all samples, based on the difference between the fresh mass and the oven-dry mass of a known volume of peat. After loss on ignition, the peat ash was used to determine total P, K, Ca, Fe, Mg and organic matter using ICP spectroscopy (Optima 4300 DV from Perkin-Elmer). The concentration (mg l^{-1}) of P, K, Ca, Mg, Fe and Mn directly available for plants and microbes was provided by a saturated media extraction (SME) (Bates, 1993). The extracts in water were analysed using a spectrophotometer UV–vis (Ultraspec 200 from Pharmacia Biotech). Total N content was determined following the Kjeldhal method (Bremner and Mulvaney, 1982), assuming that nitrates are too low to contribute to the N pool.

2.4. Microbial biomass and activity

Microbial analyses were only performed on the two upper depths, since results from the preliminary study revealed no difference between the deepest waterlogged layer (depth C) and the layer at the water table (depth B). Microbial C and N contents were estimated for depths A and B on all samples with the fumigation–extraction method, using a peat-modified procedure (Williams and Sparling, 1984; Williams and Silcock, 1997). Total soluble carbon content was determined using a TOC Schimadzu analyser. Soluble nitrates were measured after reduction on a copper–cadmium column with a Jasco analytical chain equipped with a sampler 851-AS and a spectrophotometer V530, and ammonium was measured using the blue indophenol reaction after 30 min incubation at 37 °C. The sum of ammonium and nitrate corresponded to the total mineral soluble nitrogen fraction. The values obtained from non-fumigated samples are referred to as soluble organic carbon (SOC) and soluble organic nitrogen (SON). Microbial biomass C and N result from the difference between fumigated and non-fumigated samples, corrected with the K_{EC} and K_{EN} values of 0.45 (Sparling et al., 1990) and 0.54 (Brookes et al., 1985), respectively.

Three samples from both depths A and B were randomly selected among each of the cutover, the restored and the natural samples to evaluate microbial biomass with the SIR method (Anderson and Domsch, 1978) with the modification suggested by Sparling (1995). The incubation of 20 g of fresh peat with a glucose solution (4 mg/g of fresh peat) lasted 3 h. The concentration of glucose added to the peat corresponds to an average value obtained from other experiment using different types of peat (Francez, unpublished data). Empty jars and jars containing peat without glucose amendment served as control. Gas aliquots were collected and analysed for CO_2 production after 30 min and 3 h. The gas aliquot were analysed with a portable chromatograph (Micro GC CP2002P, Chrompack). In all cases, results are expressed as mg or μg C–CO_2 g^{-1} dry peat. A different experiment generated data that were used to calculate biomass SIR under anaerobic conditions. In this experiment, the jars containing the peat added with glucose amendment were incubated under N_2 atmosphere during 8 h instead of 3 h. Gas aliquots were sampled at 4 and 8 h and were analysed with the same chromatograph.

Finally, the arginine ammonification test (Alef and Kleiner, 1986) was used to evaluate the overall enzymatic activity of all peat samples. The analyses were processed with a spectrophotometer (Uvicon XS, Biotek instruments).

2.5. Selective inhibition and carbon mineralization kinetics

A 21-day selective-inhibition incubation experiment was performed to evaluate the potential activity and CO_2 production of the different microbial communities. Three replicates were randomly selected among all the samples from each site (natural, restored, cutover), for the two upper layers (depth A and B). Ten grams of fresh peat were incubated in plasma bottles with cycloheximide for fungi selective inhibition, streptomycin for bacterial inhibition (both 0.5 mg g^{-1} fresh peat) or none of the two. For depth A, the experiment was only performed in aerobic conditions since this peat layer is constantly above the water table. For depth B subsamples, the peat was incubated in either aerobic

or anaerobic conditions. Thus, for each depth/aeration combination, there were three antibiotic treatments and three sites. These 3×9 different conditions were replicated three times, totalling 81 bottles. Empty bottles served as controls. In all cases, gas aliquots were taken with syringes at times 0, 1, 2, 3, 6, 10 and 21 days, and they were analysed for CO_2 and CH_4 production with the same portable chromatograph GC CP2002P used for biomass SIR measurements. Bottles were kept in the dark at room temperature (20 °C) during the 3 weeks of the incubation experiment. After measurements on day 10, aerobic bottles were opened and then closed again and anaerobic bottles were flushed with N_2 (Magnusson, 1993).

Fungi-to-bacteria ratios were calculated as the ratio between respiration inhibition caused by the bactericide divided by respiration inhibition caused by the fungicide.

To quantify kinetics of C mineralized by the microorganisms, we fitted two models to data on cumulative release over time. The data from aerobically incubated peat samples were fitted to a model based on the one proposed by Andréan and Paustian (1987) and also used by Updegraf et al. (1995):

$$C\text{–}CO_2 = Cm(1 - e^{-kt}) + \alpha t \quad (1)$$

where C–CO_2 is the cumulative C released to time t (days), Cm is the potentially mineralizable C corresponding in this case to the stock of soluble organic carbon (μg g^{-1} dry peat). The instantaneous release rate of this nutrient pool is represented by k (d^{-1}) whereas the more recalcitrant pool mineralization rate would be associated to α (mg C g^{-1} dry peat d^{-1}).

We also fitted the data from anaerobic incubations to a single exponential model that did not include the recalcitrant pool of C, following Updegraf et al. (1995):

$$C - CO_2 = Cm(1 - e^{-kt}) \quad (2)$$

2.6. Statistical analysis

The standard errors of the means were calculated and were used to estimate variability of each parameter in the three sites (cutover, restored and natural) and at the three depths. Rank Spearman correlations were used to determine the relation between relevant properties. Due to non-homogeneity of variances following transformations, non-parametric Kruskal Wallis analyses were performed to detect significant differences between sites or depths. Post hoc pairwise comparisons were used to identify the differences when H value of the Kruskal–Wallis analyses was significant under $\alpha = 0.05$. Statistical analyses were performed with the Minitab+ software package (ECOBIO CNRS WNN1220.00117).

For each depth/aeration combination in the incubation experiment, the main effects of *site* and *antibiotic treatment* and the interaction between *site*×*antibiotic treatment* were tested on cumulative C mineralization using two-way analyses of variance (ANOVAs). All data were log-transformed prior to analyses to correct non-normality. Fisher's LSD multiple comparisons test were performed to discriminate the significant differences detected.

The models for carbon kinetics were fitted using non-linear least square fitting capability of the software Origin 7.1. The Levenberg–Marquardt algorithm and the simplex procedure were both used and generally resulted in the same parameter estimates. Iterative minimization of the residual sum of squares (RSS) was used to obtain convergence, with a tolerance set at 0.001 at the maximum. The R^2 values correspond to 1−(residual SS/total SS). Finally, we plotted predicted and measured data to visually assess goodness of fit.

3. Results

3.1. Physicochemistry and quality of the peat

The main physicochemical properties measured for the different peat samples are found in Table 1. The average peat pH from natural site was 3.7, being slightly more acidic than the restored or cutover sites (around pH 4.5). Electrical conductivity in the peat from the natural site (60–70 μS) was only one-third of that in the peat from the restored site (170–200 μS). Pyrophosphate index was 5–6 times greater in the cutover and the restored sites than in the natural site. Bulk densities of the natural site upper layers were significantly the lowest (60–100 g dm^{-3}). Moreover, in the natural site, densities increased with depth, whereas in restored and cutover site, the values did not vary with depth, and were in all cases equivalent to values obtained for the deepest horizon of the natural site. Peat with higher bulk density was also less acidic, and more minerotrophic (significant positive correlations with pH, N_{tot}, Ca_{tot}, Mg_{tot} and pyrophosphate index).

SOC concentrations varied between 0.5 and 2.5 mg g^{-1}, and were greater in the natural site than in the restored and the cutover sites. They did not vary with depth within sites. SON concentrations were equivalent between the different sites and between the different depths and ranged between 99 and 130 μg g^{-1}. SON was not correlated with P ($r = 0.269$, $P > 0.05$) or K ($r = 0.291$, $P > 0.05$) content. Ammonium concentrations were low in all cases (less than 3.0 mg kg^{-1}) but were significantly higher in the restored and cutover sites. The concentrations did not differ between depths. Nitrate concentrations were in all cases under the limit of detection of the analyser.

In natural peat, P_{tot} and K_{tot} represent a more important proportion of the peat, whereas Mg_{tot} and Ca_{tot} were found in smaller quantities than in the restored and the cutover peat. Except for K_{tot}, which decreased with depth in the natural site, there was no difference between the horizons for these four elements. In its soluble form, P was detected only in the natural site and in the upper layer of the restored site. On the contrary, Mg_{sol} was more concentrated in the two deeper layers of the cutover and restored sites than in the natural site. Soluble K and Ca concentrations were equal between all depths and all sites (Fig. 1).

Table 1
Physicochemical properties (mean ± SE of the mean) of the peat samples taken in June 2003 at the Bois-des-Bel field station in the natural, the restored and the cutover sites ($n=6$)

Depth	A			B			C		
Site	Natural	Restored	Cutover	Natural	Restored	Cutover	Natural	Restored	Cutover
pH	3.80 ± 0.06^{a}	4.46 ± 0.23^{b}	4.14 ± 0.15^{ab}	3.73 ± 0.06^{a}	4.33 ± 0.08^{b}	4.33 ± 0.26^{b}	3.69 ± 0.07^{a}	4.56 ± 0.22^{b}	4.68 ± 0.25^{b}
EC (μS)	70 ± 14^{a}	170 ± 19^{b}	85 ± 13^{a}	63 ± 6^{a}	174 ± 28^{c}	100 ± 29^{b}	66 ± 7^{a}	210 ± 24^{b}	94 ± 26^{a}
Bulk density (mg l^{-1})	66 ± 6^{a}	123 ± 7^{b}	110 ± 9^{b}	61 ± 3^{a}	113 ± 9^{b}	130 ± 11^{b}	106 ± 4^{b}	112 ± 6^{b}	128 ± 6^{b}
Pyrophosphate	0.8 ± 0.2^{a}	4.4 ± 0.8^{b}	4.4 ± 1.2^{b}	0.7 ± 0.2^{a}	4.5 ± 0.5^{b}	7.8 ± 2.9^{b}	ND	ND	ND
C/N_{mic}	17 ± 1^{a}	25 ± 2^{b}	20 ± 3^{ab}	25 ± 3^{b}	13 ± 1^{a}	21 ± 4^{ab}	ND	ND	ND
SOC (mg g^{-1})	1.9 ± 0.2^{b}	0.7 ± 0.03^{a}	0.6 ± 0.09^{a}	1.8 ± 0.1^{b}	0.6 ± 0.04^{a}	0.5 ± 0.1^{a}	ND	ND	ND
SON (μg g^{-1})	122 ± 11	121 ± 5	112 ± 13	130 ± 7	119 ± 9	99 ± 15	ND	ND	ND
N_{tot} (mg g^{-1})	4.8 ± 0.8^{a}	6.5 ± 0.4^{b}	5.7 ± 0.9^{ab}	4.4 ± 0.3^{a}	6.8 ± 0.9^{b}	6.7 ± 0.3^{b}	8.3 ± 0.5^{b}	6.4 ± 0.4^{a}	6.6 ± 0.3^{a}
P_{tot} (μg g^{-1})	470 ± 50^{b}	200 ± 10^{a}	170 ± 50^{a}	440 ± 80^{b}	170 ± 10^{a}	140 ± 10^{a}	450 ± 30^{b}	160 ± 10^{a}	140 ± 20^{a}
K_{tot} (μg g^{-1})	770 ± 90^{b}	240 ± 20^{a}	240 ± 70^{a}	610 ± 80^{b}	260 ± 30^{a}	220 ± 40^{a}	540 ± 70^{b}	330 ± 30^{a}	280 ± 20^{a}
Ca_{tot} (mg g^{-1})	1.3 ± 0.2^{a}	2.8 ± 0.2^{b}	2.9 ± 0.6^{b}	1.3 ± 0.2^{a}	3.5 ± 0.3^{b}	3.1 ± 0.8^{b}	1.3 ± 0.1^{a}	4.4 ± 0.4^{b}	4.3 ± 0.8^{b}
Mg_{tot} (mg g^{-1})	1.0 ± 0.1^{a}	2.1 ± 0.2^{b}	2.5 ± 0.5^{b}	1.3 ± 0.2^{a}	2.9 ± 0.4^{b}	2.5 ± 0.4^{b}	1.1 ± 0.1^{a}	3.4 ± 0.4^{b}	3.4 ± 0.8^{b}
NH_4^+ (mg kg^{-1})	2.2 ± 0.4^{b}	2.7 ± 0.4^{b}	1.3 ± 0.4^{a}	2.9 ± 0.4^{b}	2.3 ± 0.4^{ab}	1.0 ± 0.6^{a}	2.7 ± 0.2^{b}	2.7 ± 0.4^{b}	1.1 ± 0.3^{a}
$NO3^-$ (mg kg^{-1})	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P_{sol} (μg kg^{-1})	140 ± 5^{c}	20 ± 2^{b}	$<0.001^{a}$	200 ± 60^{b}	$<0.001^{a}$	$<0.001^{a}$	100 ± 30^{b}	$<0.001^{a}$	$<0.001^{a}$
K_{sol} (mg kg^{-1})	1.3 ± 0.5	1.1 ± 0.2	0.5 ± 0.2	1.3 ± 0.3	1.4 ± 0.5	0.6 ± 0.3	1.4 ± 0.5	1.3 ± 0.2	0.9 ± 0.4
Ca_{sol} (mg kg^{-1})	3.8 ± 0.7	5.3 ± 0.5	4.4 ± 0.5	3.2 ± 0.9	4.8 ± 0.6	5.1 ± 0.5	3.4 ± 0.5	5.4 ± 0.6	4.4 ± 0.8
Mg_{sol} (mg kg^{-1})	1.4 ± 0.2^{a}	3.3 ± 0.4^{c}	2.2 ± 0.2^{b}	1.1 ± 0.3^{a}	2.9 ± 0.5^{b}	3.4 ± 0.8^{b}	1.2 ± 0.2^{a}	4.1 ± 0.7^{b}	2.9 ± 0.7^{b}

Depth A is the oxic layer above the water table level and beneath the litter horizon. Depth B is the layer at the water table level. Depth C is the deep anoxic waterlogged layer. Results followed by different letters within a depth are statistically different according to the non-parametric multiple comparison test ($\alpha=0.05$). EC, electrical conductivity; pyro, pyrophosphate index; SOC, soluble organic carbon; SON, soluble organic nitrogen, ND, not determined.

3.2. Microbial biomass and nutrient partitioning

Results from microbial biomass and activity measurements are presented in Table 2. According to the fumigation extraction method, the biomass followed a gradient of natural > restored > cutover and was 2–3 times greater in the natural section. With the SIR method, biomass C appeared to be up to 25 times greater in the natural site than in the restored and cutover sites, which had similar values. Depth did not statistically differentiate microbial C

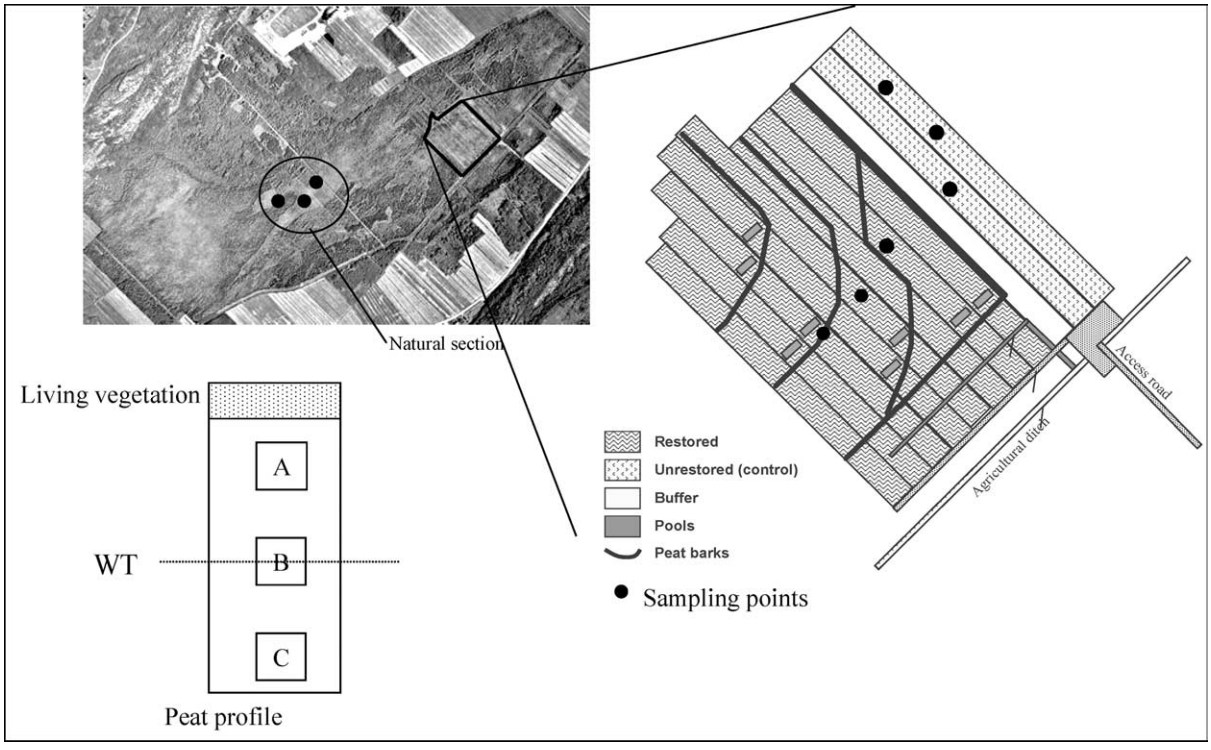

Fig. 1. Aerial photograph of the Bois-des-Bel peatland, schematic enlargement of the restored and the cutover (unrestored) section, and schematic illustration of sampling methodology applied using the water table depth (WT) as a baseline to determine depth A (the oxic layer), depth B (the layer at the water table level) and depth C (the deep anoxic waterlogged layer).

Table 2
Means and standard errors of the different microbial properties of the peat samples taken in June 2003 at the Bois-des-Bel field station

	Depth	A			B		
	Section	Natural	Restored	Cutover	Natural	Restored	Cutover
Biomass	C–FE (mg g^{-1})	5.78 ± 0.26^{a}	2.31 ± 0.22^{b}	1.37 ± 0.17^{c}	4.22 ± 0.26^{a}	2.03 ± 0.26^{b}	0.96 ± 0.22^{c}
	SIR aerobic (mg g^{-1})	4.88 ± 0.15^{a}	0.21 ± 0.12^{b}	0.30 ± 0.17^{b}	3.17 ± 0.15^{a}	0.19 ± 0.16^{b}	0.19 ± 0.16^{b}
	SIR anaerobic (mg g^{-1})	2.61 ± 0.10^{a}	0.23 ± 0.08^{b}	0.28 ± 0.09^{b}	1.68 ± 0.1^{a}	0.17 ± 0.08^{b}	0.32 ± 0.19^{b}
	N–FE (μg g^{-1})	242 ± 14^{a}	160 ± 16^{b}	76 ± 14^{c}	344 ± 16^{a}	50 ± 18^{b}	59 ± 15^{b}
Activity-C							
Aerobic	Basal resp. rate (μg $C–CO_2$ g^{-1} h^{-1})	6.5 ± 1.2^{a}	2.1 ± 0.4^{b}	1.7 ± 0.2^{b}	5.2 ± 1.4^{a}	1.2 ± 0.2^{b}	1.5 ± 0.1^{b}
Anaerobic	Basal resp. rate (μg $C–CO_2$ g^{-1} h^{-1})	ND	ND	ND	1.2 ± 0.2^{a}	0.30 ± 0.02^{b}	0.38 ± 0.03^{b}
	Methane production (ng $C–CH_4 g^{-1}$ d^{-1})	ND	ND	ND	87 ± 15	72 ± 9	0
Ratios							
Aerobic	CO_2/C–FE (mg CO_2 h^{-1} g^{-1} $biomass^{-1}$)	1.1 ± 0.3	1.0 ± 0.2	1.5 ± 0.3	1.4 ± 0.8	0.7 ± 0.2	2.4 ± 0.5
	CO_2/SOC (mg CO_2 h^{-1} g^{-1} SOC^{-1})	2.9 ± 0.9	2.9 ± 0.3	4.7 ± 0.5	3.0 ± 0.3	2.2 ± 0.8	3.6 ± 0.6
Anaerobic	CO_2/C–FE (mg $C–CO_2$ h^{-1} g^{-1} $biomass^{-1}$)	ND	ND	ND	0.27 ± 0.08	0.20 ± 0.08	0.27 ± 0.12
	CO_2/SOC (mg $C–CO_2$ h^{-1} g^{-1} SOC^{-1})	ND	ND	ND	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Activity-N	Arginine ammonification (μg NH_4 g^{-1})	17.1 ± 2.0	21.5 ± 1.1	22.5 ± 2.3	16.8 ± 1.9	18.9 ± 1.7	22.0 ± 2.0
Ratios	Arg/C–FE (μg NH4 g^{-1} $biomass^{-1}$)	3 ± 1	10 ± 2	17 ± 2	4 ± 1	9 ± 2	30 ± 7
	Arg/N–FE (μg NH4 g^{-1} $biomass^{-1}$)	72 ± 18	131 ± 12	339 ± 59	51 ± 9	225 ± 29	423 ± 80

Within a line, results with a different letter are statistically different according to the non-parametric multiple cutover test, using $\alpha = 0.05$. No letter following a result implies that all depth and all sited were equivalent. FE, fumigation–extraction; SIR, substrate induced respiration; CO_2/C–FE, production rate/biomass; CO_2/C–SOC, production rate/soluble organic carbon; Arg/C–FE, ammonium produced/microbial biomass C; Arg/N–FE, ammonium produced/microbial biomass N. Depth A: oxic layer above the water table level and beneath the litter horizen. Depth B: anoxic layer at the water table level.

pools. However, values tended to decrease with increasing depth and this trend was observed in all sites. Microbial N was similarly found in larger quantities in the natural site. In the restored site, the upper layer contained two times more microbial N than the cutover peat. In the deeper layer (depth B) of restored peat, the pool size of microbial N decreased and reached values similar to those of cutover peat. Microbial biomass values from fumigation–extraction were correlated with values obtained with the SIR method ($r = 0.652$, $P < 0.05$). Biomass N (FE) values were correlated positively with biomass C values (FE $r = 0.830$, $P < 0.001$; SIR $r = 0.613$, $P < 0.001$). Microbial biomasses were greater at low pH, low degree of decomposition, and in oligotrophic conditions. Indeed microbial biomass values were correlated negatively with pH, bulk density, pyrophosphate, Ca_{tot}, Mg_{tot}, N_{tot} and C/P_{tot}, and positively with P_{tot}, P_{sol}, K_{tot} (Table 3). Significant correlations have also been observed between the microbial biomass carbon and different ratios of nutrients (Fig. 2). Microbial biomass values decreased when the ratio between C:P, N:P or N:K increased. In the natural site, the ratios were the lowest (C:P, 521–2469; N:P, 5–19; N:K, 4–19). In the restored and cutover site, they were clearly higher, and not significantly different from one another (C:P, 1154–5444; N:P, 20–62 N:K, 15–35).

Arginine ammonification was equivalent in all sites (Table 2). However, the ratio between arginine ammonification and biomass C or N presented significantly lower values in the natural site. The proportion of nitrogen mineralized per unit of biomass was more important with increases in minerotrophy and decreases in oligotrophy. The ratio $N–NH_4$/C–FE was most strongly correlated with P_{sol} ($r = -0.652$, $P < 0.001$) and P_{tot} ($r = -0.701$, $P < 0.001$). The same relations were observed between $N–NH_4$/N–FE and P_{sol} ($r = -0.684$, $P < 0.001$) and P_{tot} ($r = -0.735$, $P < 0.001$).

3.3. Microbial activity

In all cases, the samples from the natural site showed significantly higher cumulative $C–CO_2$ production than the samples from the restored or the cutover sites (Fig. 3). The production of $C–CO_2$ was correlated with biomass C–FE in the upper layer, depth A ($r = 0.767$, $P < 0.05$) but not in the deeper layer, depth B ($r = 0.151$, $P > 0.05$). All samples incubated in anaerobic conditions produced less CO_2 than aerated samples. Within a depth, the aerobic–anaerobic CO_2 production ratios were not influenced by the antibiotic treatments or by the sampling site. They varied from 1.2:1 to 7.7:1 with an overall

Table 3
Rank Spearman correlations between physicochemical properties and microbial biomass

Physicochemical parameter	Biomass		
	NFE (μg g^{-1})	CFE (mg g^{-1})	SIR (mg g^{-1})
EC (μS)	−0.406*	−0.397*	-0.387^{NS}
Pyrophosphate	−0.426*	−0.463**	−0.563*
Bulk density	−0.544**	−0.342*	-0.416^{NS}
pH	−0.462**	−0.483**	-0.418^{NS}
N_{tot} (mg g^{-1})	−0.405*	−0.486**	−0.492*
P_{tot} (μg g^{-1})	0.713***	0.669***	0.684**
K_{tot} (mg g^{-1})	0.733***	0.758***	0.786***
Ca_{tot} (mg g^{-1})	−0.627***	−0.651***	−0.644**
Mg_{tot} (mg g^{-1})	−0.592***	−0.621***	−0.595**
NH_4 (μg g^{-1})	0.404*	0.224^{NS}	0.084^{NS}
P_{sol} (ng g^{-1})	0.452**	0.409*	0.333^{NS}
K_{sol} (μg g^{-1})	0.296^{NS}	0.163^{NS}	0.010^{NS}
Ca_{sol} (μg g^{-1})	-0.242^{NS}	-0.248^{NS}	-0.324^{NS}
Mg_{sol} (μg g^{-1})	−0.431**	−0.431**	−0.543**
n	36	36	18

NFE, microbial biomass nitrogen, fumigation extraction; CFE, microbial biomass carbon, fumigation extraction; SIR, microbial biomass carbon, substrate induced respiration; EC, electrical conductivity; NS, not significant correlation. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

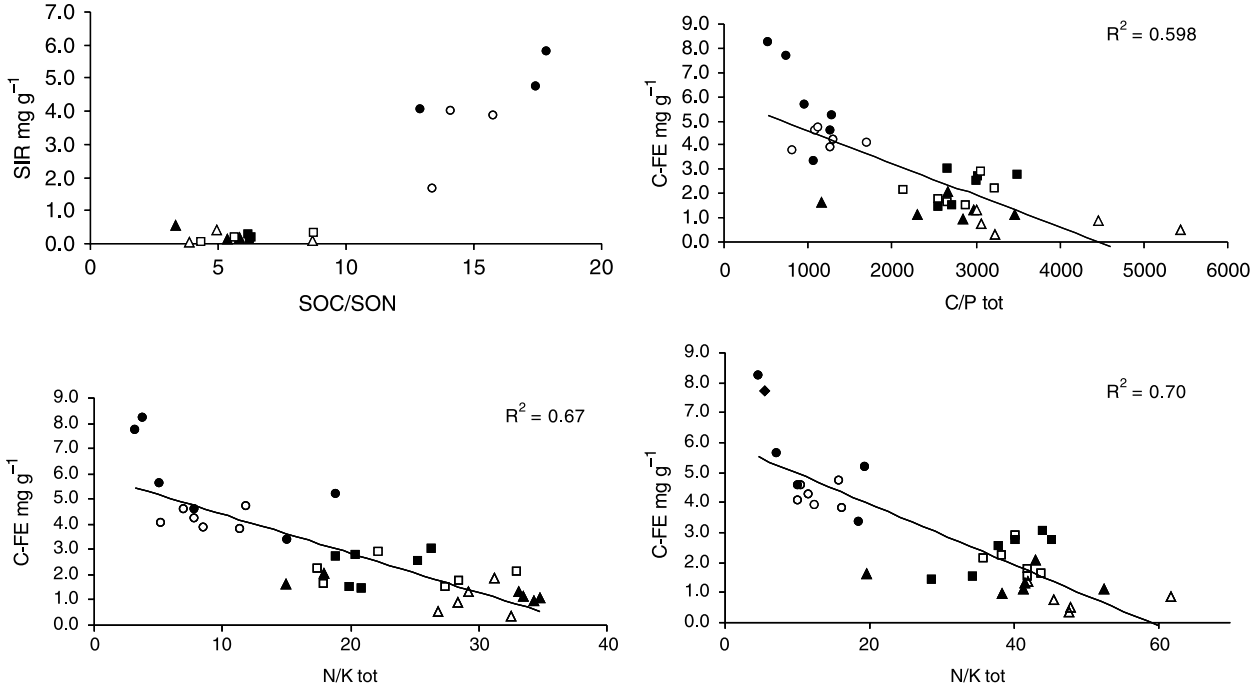

Fig. 2. Relationship between carbon microbial biomass and (a) SOC/SON; (b) C/P total; (c) N/P total; (d) N/K total. R^2 values are presented on the right corner of the graph when the linear relation between the two properties was significant ($P=0.05$). Legend: ● natural depth A; ○ natural depth B; ■ restaured depth A; □ restaured depth B; ▲ cutover depth A; △ cutover depth B. CFE, carbon fumigation–extraction; SIR, substrate induced respiration; N, nitrogen; P, phosphorus; K, potassium.

mean of 4.1:1. Instantaneous mineralization rates estimated for anaerobic data decreased by a factor of 10 in comparison with the parameters estimated in aerobic conditions. Aerobic and anaerobic respiration rates were highly correlated ($r=0.918$, $P<0.001$).

Methane production was very low and it was only detected after 10 days of incubation. In aerobic conditions, the methane production was detected in the restored samples and no production was detected in the cutover site or in the natural site. In anaerobic conditions, the production was limited to the natural and the restored sites, and the rates were more important than in aerobic conditions (Table 2).

Basal respiration rates (μg C–CO_2 g^{-1} dry peat h^{-1}) were always higher in the natural peat and were not correlated with microbial biomass C–FE. The metabolic coefficient (C–CO_2/C–C–FE) evaluated with the fumigation–extraction method was statistically equivalent for all sites. However, the ratios calculated for the cutover peat showed the highest values in depth B. The mineralization index (C–CO_2/SOC) was also evaluated. Highest values of mineralization were observed in the cutover site and lower indexes in the natural one, but the difference was not statistically significant.

The double compartments model described the mineralization data accurately ($R^2>0.87$) when the soluble organic carbon (SOC) mean value was used as a fixed parameter representing the labile carbon pool (Cm, see Eq. (1) in Tables 4a and 4b). The mineralization rates of the labile pool (k) ranged between 0.045 ± 0.009 and 0.115 ± 0.015 kg d^{-1}. The mineralization of the recalcitrant pool (a) were generally greater in the superior layer, and varied between 2.7 ± 2.9 and 37.5 ± 15.1 mg C kg^{-1} d^{-1}. In anaerobic conditions, however, the model did not satisfactorily converge to stable parameter values and the single exponential model better described the mineralization data (Tables 4a and 4b).

3.4. Microbial composition

Samples from the upper layer (depth A) of the restored site had greater cumulative CO_2 production than cutover sites only when incubated with streptomycin or without antibiotics. In all other cases, cutover and restored cumulative CO_2 production was not significantly different. Within a site and for a given incubation condition, there was no significant difference in CO_2 production between samples incubated with cycloheximide, with streptomycin or without antibiotics (Table 3). Similarly, in all cases, the interaction *site*×*depth* tested in the ANOVAs was not significant.

Fungi-to-bacteria (f:b) ratios were >1, indicating a fungi dominated microbial community in all cases except for depth B of the cutover samples in aerobic conditions where the mean value was 0.88 ± 0.11. Values fell within a small interval (0.80 ± 0.07–2.42 ± 0.70) and were not statistically different between all sites and at all depths.

4. Discussion

4.1. Effect of restoration on physicochemistry and quality of the peat

Even though the peat in restored and cutover sites was less acidic than in the natural site, the mean values were still within

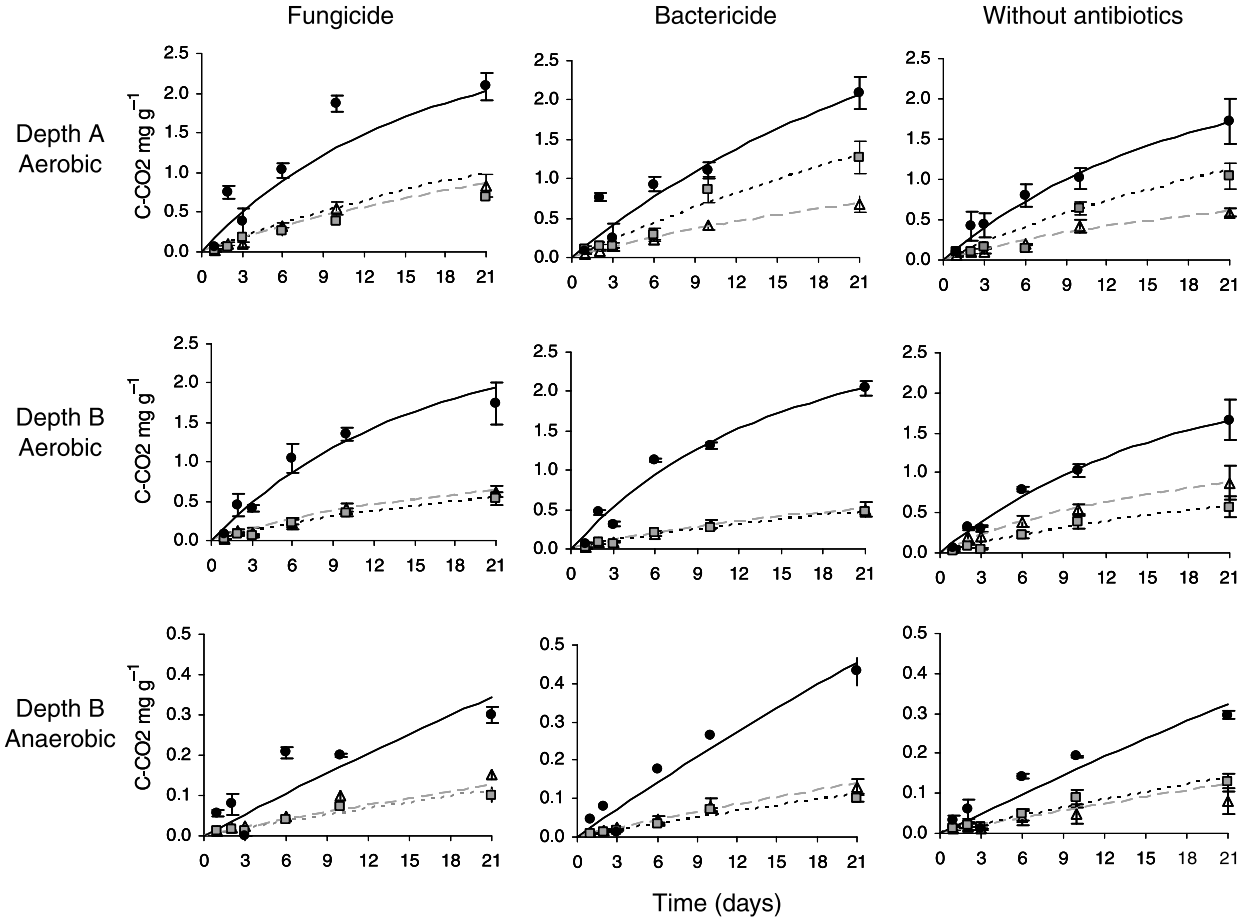

Fig. 3. Cumulative C–CO_2 production vs. incubation time (days) at room temperature for samples from depth A in aerobic conditions, depth B in aerobic conditions and depth B in anaerobic conditions, incubated with bactericide, fungicide or without antibiotic. Lines represent fits for second-order kinetics (aerobic incubation) or single order kinetics (anaerobic). Legend: ○ natural; ■ *Restored*; △ cutover.

Table 4a
Kinetic parameters for C-mineralization models based on Eq. (1) (bi-compartment) for aerobic incubations

Depth	Incubation	Site	Cm (mg C kg^{-1})	$k\times10^{-3}$ (d^{-1})	a (mg C kg^{-1} d^{-1})	Log 2/km (d^{-1})	R^2
A	Peat with cyclo-	Cutover	473±135	78±31	22.5±4.8	3.84	0.97
	heximide	Restored	787±33	53±19	22.2±6.6	5.63	0.98
		Natural	1911±183	87±35	20.2±19.4	3.45	0.89
	Peat with strep-	Cutover	473±135	65±12	15.8±2.0	4.63	0.99
	tomycine	Restored	787±33	51±42	37.5±15.1	5.94	0.95
		Natural	1911±183	59±42	33.8±32.1	5.07	0.87
	Peat without	Cutover	473±135	78±23	10.4±3.5	3.88	0.97
	antibiotics	Restored	787±33	65±40	24.9±10.6	4.62	0.94
		Natural	1911±183	70±15	11.4±10.0	4.30	0.97
B	Peat with cyclo-	Cutover	576±262	85±13	7.17±2.2	3.56	0.99
	heximide	Restored	682±59	55±13	3.8±3.8	5.46	0.98
		Natural	1944±113	85±22	16.3±12.7	3.56	0.94
	Peat with strep-	Cutover	576±262	57±9	4.8±2.2	3.56	0.99
	tomycine	Restored	682±59	45±9	2.7±2.9	6.71	0.99
		Natural	1944±113	94±21	17.9±11.6	3.21	0.96
	Peat without	Cutover	576±262	115±15	16.2±1.9	2.61	0.99
	antibiotics	Restored	684±59	51±17	6.4±5.3	5.90	0.97
		Natural	1944±113	70±9	8.1±6.7	4.34	0.99

The values for Cm were calculated as the mean SOC content of the samples incubated ($n=3$). Values ± asymptotic SE for k (instantaneous mineralization rate of the labile pool of C) and α (instantaneous mineralization of the recalcitrant pool of C) provided the best fit to 21 days cumulative C–CO_2 production data. Log 2/km values represent the labile C pool half-life. R^2 values correspond to 1 − (residual SS/total SS). Depth A: oxic layer above the water table level and beneath the litter horizon. Depth B: anoxic layer at the water table level.

Table 4b
Kinetic parameters for C-mineralization models based on Eq. (2) (single exponential) for anaerobic incubations

Depth	Incubation	Site	Cm (mg C kg^{-1})	km$\times 10^{-3}$ (d^{-1})	Log 2/km (d^{-1})	R^2
B	Peat without antibiotics	Cutover	576±262	11±1	27.37	0.93
		Restored	682±59	11±1	27.37	0.95
		Natural	1944±113	8.6±0.9	35.00	0.89
	Peat with cycloheximide	Cutover	576±262	12±8	25.09	0.87
		Restored	682±59	8±1	37.63	0.93
		Natural	1944±113	9±2	32.37	0.70
	Peat with streptomycine	Cutover	576±262	13±1	23.16	0.98
		Restored	682±59	8±1	37.63	0.95
		Natural	1944±113	13±1	23.16	0.93

the range expected for a *Sphagnum* peatland. Wind-Mulder et al. (1996) also noted such an increase in pH following exploitation. The highest values, recorded in the restored site, might be a consequence of the presence of soluble calcium in higher concentrations, possibly because of the phosphorus fertilization applied after restoration.

Nitrates were low in all sites, as observed by Croft et al. (2001). Ammonium was found in low concentrations in all sites, with lowest average values in the cutover site. This is in contrast with Croft et al. (2001) and Wind-Mulder et al. (1996) who had observed an increase in ammonium levels after exploitation. It could mean that NH_4 formation is limited in this cutover site due to dry conditions (Wind-Mulder et al., 1996), or simply that it is used rapidly by organisms.

As shown by the pyrophosphate indexes, the peat sampled in the restored and in the cutover sites was more humified than the natural site peat. It is known that fresh *Sphagnum* composing surface peat contain the largest concentration of inorganic and easily metabolised organic material (Clymo, 1965), whereas highly decomposed peat, on the contrary, is mainly constituted of sphagnan and other humic acids that do not have labile carbons and are a poor energy source (Fisk et al., 2003; Glatzel et al., 2003). Considering that the surface of the original mire was harvested during exploitation, and that restoration consequently started on what used to be the deep catotelm layer, it is not surprising that SOC is so scarce both in the cutover and in the restored sites. It also explains the higher bulk density of samples from the restored and the cutover sites. The concentrations of total nitrogen reported by Croft et al. (2001) for drained mires correspond to the values obtained here.

Even if restoration brings new plant material into the ecosystem, the average 5 cm layer of fresh organic matter does not seem to be sufficient to contribute to the SOC pool of the deeper horizons of the restored site, which contradicts the first hypothesis. It seems that the new growing plant material is rapidly consumed and does not reach subsequent layers. Glatzel et al. (2004) observed high respiration rates in the surface layer of a restored peatland, which enforces the idea that microbes growing just beneath the living vegetation degrade all the easily accessible carbon.

4.2. Effect of restoration on microbial biomass and nutrient cycles

Values of microbial biomass C obtained by the fumigation extraction method for the natural samples were comparable to those reported in many other studies. Francez et al. (2000) reported values of 1.7–4.2 mg C_{mic} g^{-1} for a 8 years restored *Sphagnum* peatland, which is similar to what was obtained for the Bois-des-Bel field station after only 3 years. Values measured for microbial biomass N were also within the ranges proposed by Francez et al. (2000) and Baum et al. (2003): between 276 and 352 mg N_{mic} kg^{-1} and between 37 and 517 mg N_{mic} kg^{-1}, respectively. Croft (1996) determined that harvesting activities reduced significantly the microbial biomass C in the peat, and our results support the same conclusion. In the restored site, the more stable hydrological conditions and the increase in available P might have improved the fixation of C and N in the microbial biomass, compared to the cutover area. This is supported by the positive correlation between P and microbial biomass values.

The SIR method gave results similar to those obtained by Brake et al. (1999) and Williams and Sparling (1984). In all cases, the biomass measurements derived from the SIR method were lower than the values obtained following the fumigation extraction method. This has been observed and commented on the literature before (Anderson and Domsch, 1978; Williams and Silcock, 1997). According to this technique, the restored and the cutover had equivalent values of biomass, both being lower than the natural values, which contrasts with results from FE. The relation between SOC/SON ratios and biomass (SIR) emphasizes on the importance of the labile C pool to accumulation of C in the biomass. It shows that in a situation where N is the limiting element (higher SOC:SON), microbes will tend to store C. Lower SOC/SON ratios are associated with lower microbial biomass values, and demonstrates that if carbon is not easily accessible, microbes will hardly be able to incorporate it to their biomass and to grow.

The concentrations of P and K found in the natural peat were similar to values reported by Hayati and Proctor (1991) for a blanket bog, while the values obtained for the restored and the cutover peat samples were clearly lower, and suggested

deficiencies. According to Van Duren et al. (1997), intensively drained sites are prone to K limitations, and drainage has been shown to decrease the P availability in the presence of elevated concentrations of Ca or Fe in the upper aerated layer of peat (Richardson and Marshall, 1986). In our case, total Ca and Mg concentrations were higher in the restored and the cutover peat samples but stayed within the ranges reported by Hayati and Proctor (1991) and Laiho et al. (2004) for ombrotrophic peatlands. According to the negative correlation between C–FE, N–FE, SIR and minerals, it appears that the fixation of C and N in the microbial biomass is not favoured by the increase in minerotrophy and pH, which indicates that the population in the study sites are mostly acidophilic and oligotrophic.

Aerts et al. (1992), Bedford et al. (1999) and Baum et al. (2003) suggested that vegetation N:P ratio >20 would indicate P limitation in degraded peat soils, bog surface soil and *Sphagnum* bogs, respectively. In our case, the relationship between microbial biomass (C–FE) and N:P ratio (Fig. 2) presented an interesting pattern: the natural samples had N:P ratios between 4 and 19, while the restored and the cutover samples had N:P ratios varying from 20 to 62. It supports the idea of a P deficiency in the restored and cutover sites. N:K and C:P ratios indicate a difference in the nutritive status of the natural site in comparison with the two others. Their relationship with microbial biomass also supports the idea that greater amount of P and K will increase the fixation of carbon in the biomass. The high values of N:K ratios in the restored and cutover sites clearly indicate a K limitation. As for SOC, restoration does not seem to have modified the concentration of total N, P, or K when compared with the cutover site. Thomas and Pearce (2004) showed that Mg^{2+} is more strongly bound to sphagnan and humic acids than calcium or monovalent cations. Therefore, in the presence of larger concentrations of Mg^{2+} like in the restored and cutover sites, displacement of other cations could make them more vulnerable to depletion.

In natural peatlands, nutrients follow a vertical distribution and their concentration diminishes with depth (Damman, 1978). The exploitation and removal of peat, the absence of continuous plant cover and the consequent reduced biological activity may have limited nutrient replenishment in the cutover and in the restored sites, and could therefore explain their low concentrations (Wind-Mulder et al., 1996). Following the same idea, in the restored site, the increase in P availability in the surface layer could be associated with the growing *Sphagnum* and *Polytrichum* cover, which augments biological activity (Damman, 1978) and substrate stability (Waddington et al., 2003). Interestingly the values of the ratios N–NH_4/biomass (both C and N) followed a gradient natural $<$ restored $<$ cutover, indicating that less nitrogen was potentially mineralized per unit of biomass in the natural site. The relation between these ratios, P and microbial C/N highlights a second key role of P in the regulation of microbial activity: more than just increasing N fixation in the microbial biomass, it also augments N turnover efficiency.

4.3. Microbial activity

4.3.1. C–CO_2 and C–CH_4 production

In all depths and for all incubation conditions, natural peat showed higher CO_2 production rates than cutover and restored sites. It follows Waddington et al. (2001), who observed a significantly greater production of CO_2 in a natural peatland compared to cutover sites in Québec. Similarly, results from the natural site in aerobic incubations were within the range of values proposed by Magnusson (1993) for a forested and an open peatland, whereas production rates in the restored and the cutover site were lower. It also corresponds to a study realized by Glatzel et al. (2004), where abandoned and recently restored subsurface peat samples showed lower CO_2 production rates than peat from pristine sites. CH_4 production has been observed repeatedly at low but measurable rates in many different types of peatlands (e.g. Magnusson, 1993; Moore and Dalva, 1997; Francez et al., 2000; Glatzel et al., 2004).

In the natural site, the presence of roots from *Picea mariana* or small shrubs on the site could have extended the zone of potential methane oxidation and forced the methanogenic population to establish deeper in the peat profile (Watson et al., 1997). Sundh et al. (1995) and Moore and Dalva (1997) observed that in peat profiles, maximum consumption rates of CH_4 by methanotrophs also occurred in samples collected just above or beneath the water table. As methane fluxes are the resulting effect of production and consumption, the methanotrophic activity in the layers sampled could explain the low methane fluxes detected. Francez et al. (2000) observed methane production only in the deepest layers (<75 cm) of a restored peat profile and suggested that the old water level limit could explain this phenomenon. The peat sampling might not have been deep enough to show this functional shift at Bois-des-Bel.

In the restored and the cutover sites, the compaction of peat, detected through the increase in bulk density, might have limited the access to gases like O_2, CO_2 or H_2, essential for respiratory processes. The deficiency of nutrients, especially P and K could also have limited microbial activities (Amador and Jones, 1993; Brake et al., 1999; Francez et al., 2000). However, due to the important concentrations in humic acids and other recalcitrant compounds in the cutover and the restored sites, the microbes might have suffered from the poor substrate quality (De Mars and Wessin, 1999; Fisk et al., 2003), even more than from the low nutrient availability (Bridgham and Richardson, 1992; Waddington et al., 2001). This would explain the very low cumulative C–CO_2 production data and basal respiration rates found in the restored and the cutover sites.

Nevertheless, greater mineralization indexes (CO_2/SOC), suggesting a more recalcitrant fraction hardly accessible to microbes, were observed in the cutover site. Similarly, lower turnover rates (CO_2/C–FE), associated with a more efficient utilization of resources (Brake et al., 1999), were obtained in the restored and the natural sites, which could reflect an effect of restoration on microbial activity. Likewise, even if the restored and the cutover sites presented similar physicochemical properties, only the former emitted CH_4. In the cutover

site, the production of CH_4 is most likely inhibited by the large variations in redox conditions, and by the generally drier conditions (Price et al., 2003), hindering large populations of methanogenes to develop. Finally, the relative short length of the incubation period could have limited methane production in peat from all sites: Updegraf et al. (1995) mentioned that in comparable conditions and for similar peat samples, methanogenesis rates were negligible during the first 30 weeks of their 80-week incubation experiment.

In all cases, anaerobic incubations resulted in respiration rates comparable to those of Magnusson (1993). Aerobic-to-anaerobic C–CO_2 ratios corresponded to values found in the literature (Updegraf et al., 1995; Moore and Dalva, 1997; Glatzel et al., 2004). The high correlation between aerobic and anaerobic C–CO_2 production rates demonstrates the importance of peat quality and nutrient availability for microorganims. Because of the low methane production rates, the CH_4–CO_2 ratios were very low (<0.05). Restored site seems to have a strong potential for CH_4 production, considering that CH_4 was detected even though nutrients and available carbon concentrations were not optimal. This was also concluded by Glatzel et al. (2004), but their study pointed to a strong correlation between CO_2 and CH_4 production rates, which was not the case in our work.

4.4. Composition of the biomass

More information can be drawn from these experiments with the calculations of the fungi-to-bacteria ratios, and the attentive examination of cumulative C–CO_2 productions data in the selective inhibition experiment. However, we should remain careful when interpreting these data, since neither of the antibiotics used kills all the fungi or bacteria. In addition, the organisms killed by the antibiotics could be used as energy sources by other microbes, which would overestimate respiration processes (Thormann, M. personal communication). The f:b ratios indicated either a fungi-dominated activity (f:b>1) or an equal contribution of bacteria and fungi to CO_2 production (f:b$=1$), which is in accordance with the literature (Bailey et al., 2002). In the samples from the upper layer (depth A) of the restored peat, the cumulative production of C–CO_2 was greater than in the cutover peat, when incubated with a bactericide or without antibiotics. This could mean that in this horizon, the fungal activity is more important at the restored site. Studies have shown that the fungi associated with *Sphagnum* are capable of decomposing a large variety of substrates such as lignin and cellulose (e.g. Williams and Crawford, 1993; Thornmann et al., 2002). As restoration has allowed *Sphagnum* to colonize the site, it is possible that the associated fungi developed preferentially and used the large amount of recalcitrant compounds to produce CO_2.

The good fit of the bi-compartmented model to aerobic C mineralization data supports the idea that microbes have access to two different pools of carbon in the soil (Updegraf et al., 1995): one small labile fraction, and one larger and more recalcitrant fraction that contains more humified and insoluble components. In this case, it was assumed that the small fraction (Cm) corresponded to the soluble organic carbon measured in the samples to be incubated. The size of the labile pool of organic matter, larger in the natural and smaller in the two other sites, exerted a strong influence on the carbon mineralization dynamics (Fig. 3). According to these results, as long as the stock of easily accessible carbon is limited, it seems unlikely that microbial populations will colonize the peat. One could note that the α (Eq. (1)) values estimated by the model are highest for the samples taken in the upper layer of the restored and natural sites and incubated with streptomycin. This suggests that these sites experience a more intense mineralization of the stable C pool when the bacteria were inhibited. In other words, when the fungi are favoured by selective inhibition, they attack the stable C pool rapidly in the restored and the natural sites. This supports the idea that *Sphagnum* and fungi are closely associated together in the natural and restored sites. In absence of *Sphagnum*, like in the cutover site, the fungal respiration is the lowest.

In contrast, the two-compartment model did not converge to stable parameter values with anaerobic data, whereas the single exponential model did. The values of k (Eq. (2)) estimated by this model were up to 10 times lower than those of the aerobic mineralization data. Updegraf et al. (1995) had also observed a diminution of the mineralization between aerobic and anaerobic peat samples of different origins. However, in their case, the two-compartment model was more accurate than the single exponential model. The very short duration of our incubations could explain this difference, as their study lasted 80 weeks, while ours lasted only 3 weeks. It therefore represents solely the initial stage of mineralization, during which the soluble labile pool is largely sufficient to maintain the small anaerobic microbial population.

4.5. Conclusion—usefulness of microbial and physicochemical parameters in restoration projects monitoring

Overall, it seems that after three growing seasons post-restoration, the cutover and the restored sites still have great similarities in their physicochemical characteristics. However, microbial biomass, N:P, N:K and C:P and NH_4:biomass ratios of the restored peat showed a tendency to evolve towards values closer to those of the reference site as well as to those found in the literature for natural mires. Nonetheless, they were not clearly different from the cutover values. They could be potentially interesting indicators to monitor during the years following restoration to detect nutrient deficiencies in a restored site, and to compare it to reference or cutover sites. Concerning microbial activity, the utilization of ratios such as C–CO_2/biomass, C–CO_2/SOC or C–CH_4/C–CO_2 could be more easily comparable from one site to another than production rates alone although in this particular case, cumulative production rates, especially methane, were more interesting to discuss. Methane production, as expected, seems to be closely associated with hydrological properties, and a parallel follow-up of these properties would therefore appear to be relevant. This study demonstrated for the first time that

restoration of a cutover peatland resulted in an increase of fungal respiration in subsurface layers, but not in an increase of bacterial C–CO_2 production. Nonetheless, more experiments on microbial community composition are still needed to enhance our understanding of colonization processes occurring in restored and cutover sites.

In a previous study, Waddington et al. (2003) demonstrated that the restored site of Bois-des-Bel was a carbon source to the atmosphere in the years following restoration and suggested that the active decomposition responsible for CO_2 emissions occurred in the top layers of the peatland, where new vegetation and straw mulch were present. Our results showed clearly that the subsurface layers were not enriched in nutrients or in easily metabolised carbon compounds, and did not exhibit large respiration rates. Thus, following restoration, it seems that an active microbiota colonizes these surface horizons and utilizes the new organic material so quickly that nutrients do not reach the subsequent layer, where organic matter is largely more humified. The relationship between nutrients and biomass, particularly P, highlighted its crucial role in carbon and nitrogen cycling. On the other hand, the modelling of C–CO_2 production data confirmed that the SOC pool was limiting in the restored and the cutover sites. The dichotomy between the surface and the subsurface layers explains the limited CO_2 production rates measured in our work and the absence of carbon sequestration confirmed with other studies. Furthermore, the potential of the restored site to emit methane illustrates the complexity of microbial response to restoration: in this case, water table level and stability seemed more important than peat composition.

In conclusion, changes in nutritional status and microbial compartment were detected three growth seasons after restoration. However, there seems to be persistence of dysfunctions in some physicochemical and microbiological characteristics of the subsurface layers. This study follows Francez et al. (2000) who concluded that there was a lag between the positive response of the vegetation to rewetting, and that of the microbes. A survey of microbial diversity based either on carbon utilisation or on functional diversity (Chapin et al., 1992; Bardgett et al., 1996; Grime, 1997) would relevantly complete this study and might shed light over the recovery processes occurring in the microbial compartment.

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