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Cut-over peatland regeneration assessment using organic matter and microbial indicators (bacteria and testate amoebae)

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Summary

1. Cut-over peatlands cover large surfaces of high potential value for enhancing biodiversity and carbon sequestration if successfully restored. Unfortunately, evaluation of restoration success is not straightforward. We assessed the bioindicator value of organic matter (OM), testate amoebae (protozoa) and bacteria in peat from two regeneration stages and a reference site of a cut-over bog.
2. Contrasting biochemical signatures of peat OM were observed along the regenerating profiles, allowing clear differentiation between the newly regenerated peat and the old peat. Where peat macrofossils were absent sugar biomarkers were used to infer peat botanical origin and OM alteration.
3. Over the succession, the OM composition of the new peat differed. Peat from the more recent stage was dominated by *Sphagnum*-derived tissues and characterized by lower carbohydrate preservation and higher bacterial biomass than the advanced regeneration stage.
4. Surface testate amoeba communities also changed from the recent to the advanced stages of regeneration, indicating a shift from wet and moderately acidic conditions to drier and more acidic conditions. Over this regeneration sequence (i) the biomass and average size of species declined but were higher at the unexploited site and (ii) species richness and diversity increased but density declined.
5. *Synthesis and applications.* Although secondary succession in the cut-over bog led to an ecosystem similar to that of the reference site in terms of surface vegetation, OM and testate amoebae continued to reflect disturbances associated with peat harvesting. Nevertheless, the described dynamics of both microbial and biochemical variables over the succession showed similarities between the advanced stage and the reference site: a higher testate amoeba diversity was associated with better carbohydrate preservation and a more heterogeneous botanical composition of the peat. The inferred water table depth and pH based on testate amoebae indicators proved to be an alternative approach for assessing restoration processes, in contrast to labour-intensive repeated measurements in the field. The botanical and biochemical composition of peat OM provided additional information on past anthropogenic perturbations of the bog and could be used for restoration monitoring. The combination of several indicators therefore provides a more complete assessment of ecological conditions that could be valuable for the management of cut-over peatlands.

Key-words: biomarkers, botanical composition of peat, micro-organisms, RECIPE, restoration ecology, secondary succession, *Sphagnum*

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Introduction

Regenerating peatlands may act as carbon sinks and can be important for rare and endangered species (Chapman *et al.* 2003). Therefore much effort is directed to encouraging the re-establishment of peat-forming vegetation, in particular *Sphagnum* mosses (Grosvernier, Matthey & Buttler 1995; Gorham & Rochefort 2003; Lavoie *et al.* 2003). However, the outcome of these restoration efforts with respect to carbon balance remains difficult to predict and, in many cases, local conditions may not allow net accumulation of carbon to occur (Francez, Gogo & Josselin 2000; McNeil & Waddington 2003).

Most of the work on carbon dynamics in peatlands has been approached by measurement of processes occurring either on the surface or in the peat, in most cases by estimating surface fluxes of CO_2 and CH_4 (Harriss *et al.* 1985; Knowles & Moore 1989; Moore & Knowles 1989; McNeil & Waddington 2003). Little is known about the quality of the organic matter (OM) and the characteristics of the microbial communities responsible for its biochemical transformation, in natural peatlands in general and cut-over sites in particular. Studies suggest that these aspects have important implications for the functioning of the ecosystem. Indeed, the physiochemical and botanical properties of the underlying peat have been shown to affect strongly (i) the growth of *Sphagnum* mosses (Grosvernier, Matthey & Buttler 1997; Buttler, Grosvernier & Matthey 1998) and (ii) gas fluxes to the atmosphere (Buttler, Dinel & Lévesque 1994; Charman, Aravena & Warner 1994) while microbial communities have been shown to respond to ecological gradients as well as ecosystem perturbations, such as nitrogen input and elevated atmospheric CO_2 concentrations (Gilbert *et al.* 1998a,b; Mitchell *et al.* 2003).

Clearly, in order to refine management strategies for cut-over peatlands, more information is needed on (i) the patterns of changes in the community structure of different taxonomic groups and in the biochemical characteristics of the peat OM, and (ii) the processes controlling long-term carbon sequestration during peatland regeneration. These are two of the main goals of the European Union (EU)-funded project RECIPE (reconciling commercial exploitation of peat with biodiversity in peatland ecosystems; Chapman *et al.* 2003). Our focus is on testate amoebae (Protista), bacteria and biochemical characteristics of peat OM. These indicators are not generally considered together in peatland restoration studies but they may react faster than other indicators to changes occurring during peatland regeneration and are likely to provide valuable information on processes occurring in the soil (Warner & Chmielewski 1992; Chapman *et al.* 2003). In addition, the shells (test) of testate amoebae are preserved in peat, thus allowing comparison of modern assemblages with pre-disturbance assemblages and showing changes in communities through time (Buttler *et al.* 1996; Davis & Wilkinson 2004).

We studied the abiotic and biotic aspects of peat in a suite of secondary plant communities situated on a cut-over peatland in the Swiss Jura Mountains: (i) the depth-related changes of biochemical and micromorphological characteristics of peat OM; (ii) the bacteria carbon biomass at different depths; and (iii) the abundance, diversity and community structure of testate amoebae living in the *Sphagnum* mosses at the surface. Our aims were to assess how these different variables were correlated and identify specific indicators of changes in the structure or functioning of the ecosystem. Because of clear changes that can be observed directly from the structure of the vegetation along a regeneration sequence, we hypothesized that both the accumulated OM and the related microbial indicators would also differ, but we could not predict how the different data sets might compare and to what extent they would reflect ecosystem recovery. Our results illustrate how a single type of indicator may not suffice to assess the state of an ecosystem.

Materials and methods

STUDY SITE AND SAMPLING

La Chaux d'Abel, a cut-over peatland in the Jura Mountains, Switzerland (47·09° N,6·56° E; altitude 1020 m a.s.l.), was sampled in November 2001. The mean annual precipitation and temperature are 1463 mm and 6·4 °C, respectively. The site was abandoned after active peat cutting ceased in 1963, with only a small area remaining intact. Subsequently, spontaneous regeneration took place and, at present, moss and vascular plant communities have developed for variable lengths of time on different parts of the site (Matthey 1996). Dendrochronological investigations on the largest trees showed that at sites 1 and 2 (Table 1), which are contiguous, the time for regeneration to occur since peat cutting stopped has been about 29 years, assuming a lag time of 7 years for tree establishment (A. Siegenthaler, unpublished data). At sites 3 and 4, regeneration time has ranged between 51 and 58 years. Therefore two regeneration stages (sites 1–2 and sites 3–4) were selected by taking into account the age of abandonment and plant composition in relation to peat-forming key-species, i.e. *Sphagnum* and *Eriophorum* species (Table 1). A reference site representing an unexploited area in the same peat bog was also selected.

Three replicate peat cores, 13 cm in diameter, were extracted in each community, totalling 15 cores. The 60–70-cm long cores were cut into 2-cm thick slices. One part of each subsample was used for chemical analyses after air-drying, pulverizing into a powder and storage at 4 °C, and the other part was used for micromorphology and bacteria analyses after fixation in a 2% glutaraldehyde solution and storage at 4 °C. The uppermost 3 cm of living plants were analysed for testate amoebae, whereas OM and bacteria analyses were conducted on the whole profile (10–13 samples per profile).

LABORATORY AND DATA ANALYSES

Testate amoebae

Testate amoebae were extracted from the samples by sieving through 20 µm and 300 µm meshes without boiling (Hendon & Charman 1997). Both living and dead shells were identified and counted under a microscope at 200× and 400× magnifications. Biovolumes of each living (active and encysted) species were estimated by assuming geometrical shapes and were converted to carbon using the conversion factor 1 $\mu m^3 = 1{\cdot}1\ 10^{-7}$ µg C (Weisse *et al.* 1990). Nomenclature for testate amoebae followed Meisterfeld (2000a,b).

Table 1. Characteristics of the sampling sites in La Chaux d'Abel peatland, Swiss Jura Mountains

Site	General description	Vegetation (dominant plants)	Age (years)*
Site 1	Regenerating zone in a part of the mire where no intact portion remains. Fen vegetation	*Polytrichum strictum*, *Polytrichum commune*, *Sphagnum fallax*, *Carex nigra*, etc.	Early stage (*c.* 29)
Site 2	Same zone as stage 1, but different dominant plants species	*Eriophorum vaginatum*, *Sphagnum fallax*, etc.	Early stage (*c.* 29)
Site 3	Regenerating zone at the base of a peat extraction wall, open mixed forest of birch and pine	*Eriophorum vaginatum*, *Sphagnum fallax*, *Betula pubescens*, *Pinus rotundata*, etc.	Advanced stage (51–58)
Site 4	Same zone, but drier and with different dominant plant species	*Polytrichum strictum*, *Polytrichum commune*, *Sphagnum fallax*, *Betula* sp., *Pinus rotundata*, etc.	Advanced stage (51–58)
Unexploited	Intact raised bog, but under the influence of lateral drainage from the peat cutting wall. Tall pine forest with dense shrub cover	*Pinus rotundata*, *Picea abies*, *Vaccinium uliginosum*. Moss layer dominated by non-*Sphagnum* mosses, with discontinuous *Sphagnum* patches	

*Mean ages of regeneration estimated from local surveys and dendrochronology.

We compared the sampling sites for a set of five general variables derived from the testate amoeba data: total density (living + dead), percentage of living species, carbon biomass, species richness and the Shannon-Wiener diversity index (H', using the base 2 logarithm). The average values were compared using an ANOVA followed by comparison of all pairs (Turkey–Kramer HSD). To assess how communities changed during the regeneration sequence, we inferred the water table depth (WTD) and pH using a transfer function based on a data set from an earlier study in the same region (Mitchell *et al.* 1999, 2001). The calculations were performed using the software WA-CALIB (Line, ter Braak & Birks 1994). The resulting values were used to draw a plot of inferred depth to water table (DWT) vs. pH for the samples.

Bacterial density

Bacteria were stained with DAPI (4,6 diamino 2 phenylindol), filtered on 0·2-µm black membrane filters and examined by epifluorescence microscopy at 1000× magnification for all peat levels. The images were recorded using a digital camera. Bacteria numbers and sizes were estimated in a minimum of 10 random fields for each sample. Bacterial biovolumes were estimated by assuming geometrical shapes and converted to carbon using the conversion factor 1 $\mu m^3 = 5{\cdot}6 \times 10^{-7}$ µg C (Bratbak 1985).

Organic matter: carbon, nitrogen, micromorphological and sugar analyses

Total carbon (C) and nitrogen (N) were determined by combustion at 1100 °C with a LECO CNS 2000 (Lakehead University Instrumentation Laboratory) apparatus on dried and pulverized samples. Because of the absence of inorganic carbon (carbonates), the determined total carbon represents the total organic carbon and was used for C/N calculations.

Light microscopy observations enabled organic components (plant organ-derived tissues, amorphous material and micro-organisms) to be identified and quantified. Bulk peat samples were mounted as smear slides and examined at 20× and 50× magnification. The surfaces covered by the main organic microremains were estimated with a grid reticule in the eyepiece of the microscope. A total of 3000–5000 items per sample were counted to calculate relative frequencies with an estimated error of about 10%.

A detailed procedure for sugar analysis is given in Comont, Laggoun-Défarge & Disnar (2006). Briefly, two aliquots (*c.* 100 mg) of a given sample are hydrolysed (4 h at 100 °C) in 1·2 M H_2SO_4 solution, one after previous soaking with 24 NH_2SO_4 (12 h at room temperature) and the other without previous soaking. After hydrolysis and adequate sample treatment, individual sugars were silylated and quantified by gas chromatography using an internal standard, the individual compound response coefficients being determined independently with a mixture of eight common monosaccharides. The two hydrolyses release the total and hemicellulosic sugars, respectively. The cellulosic sugars are determined by difference. Replicate analyses gave an analytical precision of 10–15%. Sugar analyses were first conducted on characteristic peat-forming plants sampled from Le Russey peatland, in the French Jura, about 15 km away from the study site (Comont, Laggoun-Défarge & Disnar 2006). The following plant source signatures were identified: xylose and arabinose for *Eriophorum vaginatum* L. and *Eriophorum angustifolium* Honck., mannose for *Polytrichum strictum* Menz. Ex. Brid. and rhamnose and galactose for *Sphagnum fallax* (H. Klinggr.) H. Klinggr. Together with microscopic investigations of peat samples, which enabled in some cases a 'direct' identification of the botanical composition of the peat, these specific signatures were also used to reconstruct past vegetation changes in the underlying peat.

Results

TESTATE AMOEBAE

A total of 22 testate amoeba taxa were observed in the samples (Table 2). Significant differences in species richness were found among the five sites. The highest species richness was found at site 3 and the unexploited site, while the two recent succession sites (sites 1 and 2) had low species richness (Table 3). The pattern of diversity (H) was similar to that of species richness (Table 3).

The percentage of living and encysted testate amoebae was higher at sites 1 and 2 (63–64%) than in the other three sites (49–52%) but this difference was not significant. The highest overall densities were observed at sites 1 and 2, and carbon biomass was significantly higher at these sites than at the other sites and lower at site 4 than at the unexploited site (Table 3). The differences between the density and carbon biomass results were due to change in community structure: smaller species such as *Assulina muscorum* and *Nebela tincta* increased at the expense of the larger species *Hyalosphenia papilio*. The significantly higher biomass recorded at the unexploited site

Table 2. Overall density and relative frequency of testate amoebae taxa identified in the *Sphagnum* samples from La Chaux d'Abel peatland

		Overall density (individuals/g dry wt)				Overall relative frequency (%)			
Taxon	*n*	Mean	SE	Minimum	Maximum	Mean	SE	Minimum	Maximum
*Archerella flavum**	4	1438	691	0	7704	2·35	1·19	0·00	26·16
Arcella catinus	7	357	141	0	1531	1·00	0·46	0·00	10·00
Assulina muscorum	15	3146	954	274	15406	18·71	4·59	0·26	73·48
Assulina seminulum	8	459	231	0	2914	1·62	1·02	0·00	23·03
Bullinularia indica	5	136	79	0	1166	0·71	0·42	0·00	9·21
Centropyxis aculeata	3	119	109	0	1636	0·25	0·10	0·00	1·89
Centropyxis aerophila var. *sphagnicola*	1	9	9	0	136	0·01	0·01	0·00	0·16
Corythion dubium	11	978	518	0	8044	4·40	1·10	0·00	24·31
Euglypha ciliata-type	11	746	206	0	2693	4·83	1·29	0·00	19·79
Euglypha compressa	5	150	78	0	919	0·51	0·21	0·00	3·81
Euglypha laevis†	3	45	25	0	273	0·06	0·02	0·00	0·37
Heleopera sylvatica	7	311	138	0	1996	1·23	0·46	0·00	9·52
Heleopera rosea	10	318	138	0	2045	0·63	0·14	0·00	2·36
Hyalosphenia elegans	3	219	129	0	1572	0·27	0·12	0·00	1·87
Hyalosphenia papilio	13	30556	8542	0	91693	33·82	7·20	0·00	91·43
Hyalosphenia subflava	1	7	7	0	98	0·02	0·01	0·00	0·33
Nebela militaris	12	1087	651	0	10089	2·10	0·55	0·00	11·65
Nebela penardiana	1	41	41	0	615	0·04	0·03	0·00	0·65
Nebela tincta	14	4888	1139	0	12815	20·85	4·58	0·00	86·13
Nebela tincta var. *major*	14	3067	959	0	10756	4·69	1·04	0·00	19·52
Phryganella acropodia	9	446	218	0	3136	1·32	0·43	0·00	8·94
Trigonopyxis arcula	5	64	29	0	333	0·59	0·20	0·00	3·13

*Synonym *Amphitrema flavum*.
†Includes *Euglypha rotunda.*

was because of the presence of large species (e.g. *Bullinularia indica*) and the dominance of medium-sized taxa (e.g. *Nebela tincta*).

Clear differences in communities were found along the regeneration sequence (Fig. 1). The recent stages were dominated by *Hyalosphenia papilio*, a species indicative of wet conditions (Mitchell *et al*. 1999). At site 3, *Archerella flavum*, an indicator of wet, acidic conditions (Mitchell *et al*. 1999), reached its highest abundance, and two indicators of drier and more acidic conditions, *Nebela tincta* and *Assulina muscorum*, increased in abundance. This site was also the most heterogeneous, based on the position of individual samples in the inferred water table depth × pH biplot. Site 4 was dominated by *Nebela tincta*, *Assulina muscorum* and another dry and acidic indicator, *Corythion dubium* (Mitchell *et al*. 1999). In the inferred water table depth × pH biplot, the samples were aligned from the recent to advanced succession stages, suggesting a continuous trend towards drier and more acidic conditions (Fig. 1). Illustrations of the key testate amoeba indicator species are given in Fig. 4.

BACTERIA

Bacterial density and biomass averaged $4{\cdot}84 \times 10^{10}$ cells and 1·24 mg C g dry weight $^{-1}$ (d wt) of peat, respectively, and were not significantly different among the sites. Bacterial biomass tended to decline from the recent to the advanced regeneration stages but this trend was not significant (Fig. 2). Biomass decreased with depth in the top 40 cm in the recent regeneration stages (respectively 1·91, 1·44 and 1·91 in the upper 25 cm and 0·81, 1·13 and 0·61 mg C g dry wt^{-1} below 25 cm depth at sites 1–3) while it was more stable in the top 40 cm, with a rise at lower levels in the advanced regeneration stage (site 4) and the unexploited site. As a consequence of these vertical patterns, bacterial biomass was significantly higher in the upper peat (top 25 cm) of sites 1–3 than in the upper peat of site 4 (ANOVA with Fisher's protected least-square differences, respectively, $P = 0{\cdot}0009$, $P = 0{\cdot}029$ and $P = 0{\cdot}002$) and in the upper peat of sites 1 and 3 compared with the upper peat of the unexploited site (respectively $P = 0{\cdot}018$ and $P = 0{\cdot}025$). In contrast, in the lower sections of the cores the differences were no longer correlated with the regeneration sequence. However, bacterial biomass was positively correlated with the C/N ratio and the relative percentage of *Sphagnum* remains in the peat (Fig. 3).

PEAT ORGANIC MATTER

Carbon and nitrogen contents

Overall, total organic carbon contents were high (40–51%). At the unexploited site, C/N ratios varied little with depth (60–80) except at 20–25 cm and 48–62 cm, where they significantly decreased (30–40), revealing higher OM degradation and/or changes in the OM sources (Fig. 3). In contrast, in formerly exploited zones (sites 1 and 4), a contrasting pattern occurred between two sections, the upper section (0 to –25 cm depth), corresponding with the 'new' regenerated peat, and

Table 3. Summary data for testate amoebae extracted from *Sphagnum* samples taken in the five plant communities in La Chaux d'Abel peatland, Switzerland

	Site 1			Site 2			Site 3			Site 4			Unexploited			All sites			
	Mean	SE*†		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	Minimum	Maximum
Species richness	8·3	1·3	a	7·3	3·5	a	14·7	1·4	b	10	1·2	ac	14	0·87	bc	10·8	0·9	4	17
H′ diversity index	1·1	0·3	a	0·9	0·3	a	2·7	0·3	b	2·1	0·2	b	2·6	0·35	b	1·9	0·2	0·5	3·2
% living and encysted	63	2·9	a	64	5·8	a	49	9·8	a	50	2·3	a	52	1·15	a	55·6	2·7	32	76
Density (10^3 ind. g d wt^{-1})	83	7·3	a	84	12	a	53	17	a	7·9	4·5	b	16·3	2·42	b	48·6	9·4	3·3	104
C biomass (mg g d wt^{-1})	0·83	0·12	a	1·0	0·2	a	0·3	0·04	bc	0·08	0·05	b	0·45	0·02	c	0·54	0·10	0·02	1·37
Average biovolume per individual amoeba (10^4 µm^{-3})	14·4	0·5	ac	13·5	0·2	ac	9·7	1·4	ab	5·9	1·1	b	18·1	3·10	c	12·3	1·3	3·77	23·7

*Standard errors; $n = 3$ in all cases.
†Lowercase letters indicate significant differences among sites in the ANOVA and Turkey–Kramer HSD comparison among pairs of sites.

the 'old' catotelm peat (Fig. 3). The latter horizon was characterized by rather low C/N ratios (20–30, particularly at site 1), unlike the regenerated peat where the ratios were much higher (60–100).

MICROMORPHOLOGICAL CHARACTERISTICS

Characteristic tissues deriving from vascular plants, particularly from *Cyperaceae* (Fig. 4g) and mosses (Fig. 4h–j), were quantified in the peat samples. Most of the observed *Sphagnum*- and *Polytrichum*-derived tissues consisted of characteristic leaf cell walls (Fig. 4h–j). Well-preserved and degraded tissues were counted as two distinct classes (Fig. 4g–o). Amorphous OM flakes (Fig. 4l-o) present in various proportions consisted of a complete amorphization of the cell walls. Another 'amorphous' component, namely the mucilage (Fig. 4k,n,o), was also present as slimy and translucent substances with no internal structure. This component is more likely to be derived, at least partly, from *in situ* microbial syntheses of bacteria, fungi and/or plant roots (Decho 1990; Défarge *et al*. 1996; Laggoun-Défarge *et al*. 1999). Many tangled masses of melanized fungal hyphae (Fig. 4o), often associated with decayed plant rootlets, were also present in the so-called 'old' peat. Micro-organisms (Fig. 4a–f,j) were represented by algae, testate amoebae, diatoms and the rotifer *Habrotrocha angusticollis*.

The depth evolution of the relative abundance of these organic microremains showed contrasting signatures between the profiles (Fig. 3). At the unexploited zone, peat was mostly composed of morphologically well-preserved *Sphagnum* tissues, except at the two levels (depth of 20–25 cm and 48–62 cm) where the C/N ratios significantly decreased. These two levels were characterized by (i) a higher degradation, as revealed by the abundance of structureless plant tissues, amorphous OM and mucilage, and (ii) the occurrence of well-preserved Cyperaceae tissues. The OM composition in these two levels may indicate events such as natural and/or anthropogenic drainage phases, which would have allowed the establishment of specific vascular plants such as sedges. The peat OM composition of the profiles from formerly exploited sites (sites 1 and 4; Fig. 3) confirmed the C/N results. Two sections were distinguished: a deeper 'old' peat characterized by strong OM degradation, as shown by high amounts of amorphous OM, structureless plant tissues and mucilage, and an upper 'new' regenerated peat composed mainly of well-preserved plant tissues. According to the regeneration stage, the new peat showed different OM compositions: site 1 was composed exclusively of *Sphagnum* mosses, while its composition was more heterogeneous at the more advanced regeneration stage (site 4) (Fig. 3a).

SUGAR CONTENTS AND DISTRIBUTIONS

The results of sugar analyses from characteristic peat-forming plants as reported previously (Comont, Laggoun-Défarge & Disnar 2006) are summarized in Table 4. The quantitative and qualitative evolution of peat carbohydrates

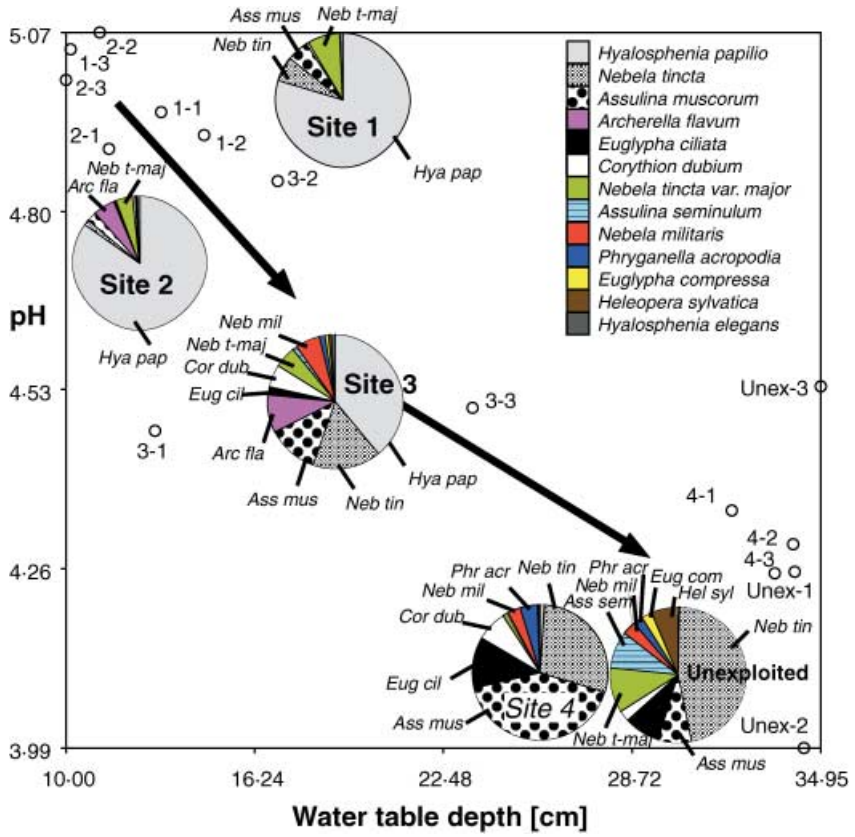

Fig. 1. Changes in testate amoeba community structure and inferred water table depth and pH in the regenerating vegetation and non-harvested bog. The water table and pH were inferred using a transfer-function from the Jura Mountains (Mitchell *et al.* 1999, 2001). Sample codes are as follows: 1–1, site 1 replicate 1; ... 4–3, site 4 replicate 3; Unex, unexploited.

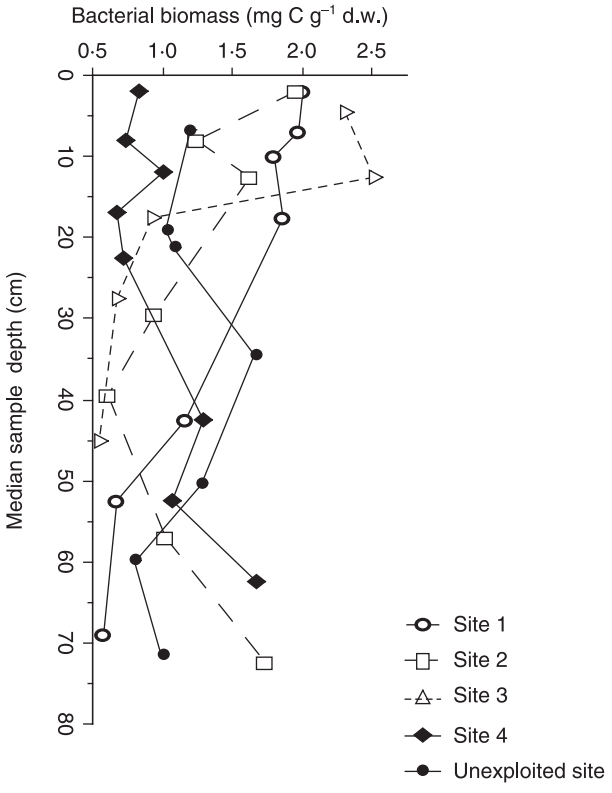

Fig. 2. Depth evolution of bacteria biomass in the recent and advanced regenerating stages (sites 1,2 and 3,4, respectively) and the unexploited site.

in the peat profiles of two regeneration stages (sites 1 and 4) and the unexploited site are presented in Fig. 5.

At site 1 (and to a lesser extent at site 4), the depth-related changes in total sugar yields allowed two peat sections to be distinguished along the profiles. The uppermost peat (0–20/25 cm depth) contained high sugar contents (250–400 mg g^{-1}) that were in the same range as those found in living plants: 282, 326, 400 and 357 mg g^{-1} for *Eriophorum angustifolium, Eriophorum vaginatum, Polytrichum strictum* and *Sphagnum fallax*, respectively (Comont, Laggoun-Défarge & Disnar 2006). In contrast, in the underlying peat sugar yields strongly decreased down to *c.* 70 mg g^{-1} at site 1 and *c.* 160 mg g^{-1} at site 4; at the bottom of the latter profile, they increased up to 300 mg g^{-1} (Fig. 5). At the unexploited site, the amounts of sugars progressively decreased with increasing depth from 327 to 200 mg g^{-1}, except at 20–25 cm and 48–62 cm, where they decreased slightly to 200 and 147 mg g^{-1}, respectively.

In the whole profile, the total hemicellulose content was higher than that of total cellulose sugars (Fig. 5). Moreover, a greater discrepancy between their amounts occurred only in the upper sections of sites 1 and 4 (*c.* 200–300 mg g^{-1} for hemicelluloses vs. *c.* 50–100 mg g^{-1} for cellulose, respectively). However, both in the deeper peat and throughout the unexploited site profile they showed similar patterns (Fig. 5).

The relative percentages of individual hemicellulose sugars (wt%) also differed amongst the three profiles. At sites 1 and 4, mannose, and to a lesser extent galactose and rhamnose, were present in a relatively high proportion in the surface peat

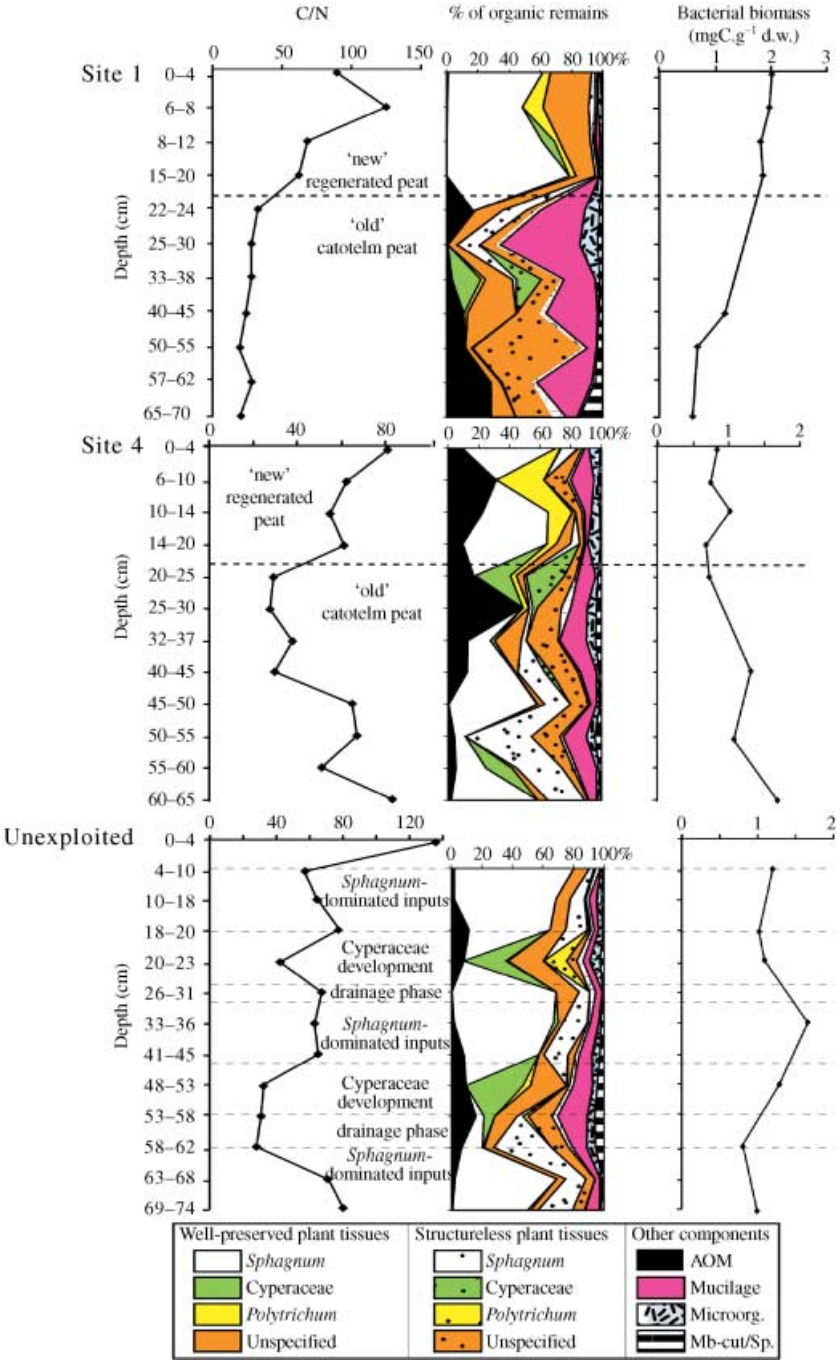

Fig. 3. Depth profiles of atomic C/N ratio, relative percentages of organic microremains and bacterial biomass in the recent regeneration stage (site 1), the advanced regeneration stage (site 4) and the unexploited site of La Chaux d'Abel peatland. The dotted line delineates the threshold between the uppermost 'new' regenerating peat and the 'old' catotelm peat. AOM, amorphous organic matter; Mb, Cut, Sp, membranes, cuticles, spores; GD, OD, gelified debris, oxidized debris.

(< 20 cm; mannose up to 40% at site 4). In contrast, xylose and arabinose concentrations, which were relatively low in this peat section, significantly increased below between 20 and 45 cm depth (Fig. 5). At the unexploited site, relative hemicellulose sugar contents showed almost constant values along the profile, except at (i) 20 cm depth, where rhamnose and mannose slightly decreased and increased, respectively, and (ii) *c*. 32 cm depth, where xylose significantly increased.

Discussion

To our knowledge this is the first study to compare variation in microbial, biochemical and micromorphological indicators in a cut-over peatland where the surface is in different stages of regeneration. Both biological and biochemical indicators were consistent in showing clear differences between the profiles from the unexploited part of the peatland and those from its regenerating parts.

TESTATE AMOEBAE AND BACTERIA INDICATORS

Testate amoebae can be identified to species level and can be related to regional ecological calibration data sets developed for palaeoecological studies (Charman & Warner 1992; Charman 1997, 2001; Booth 2001, 2002; Lamentowicz & Mitchell 2005; Payne *et al*. 2006). This represents a clear

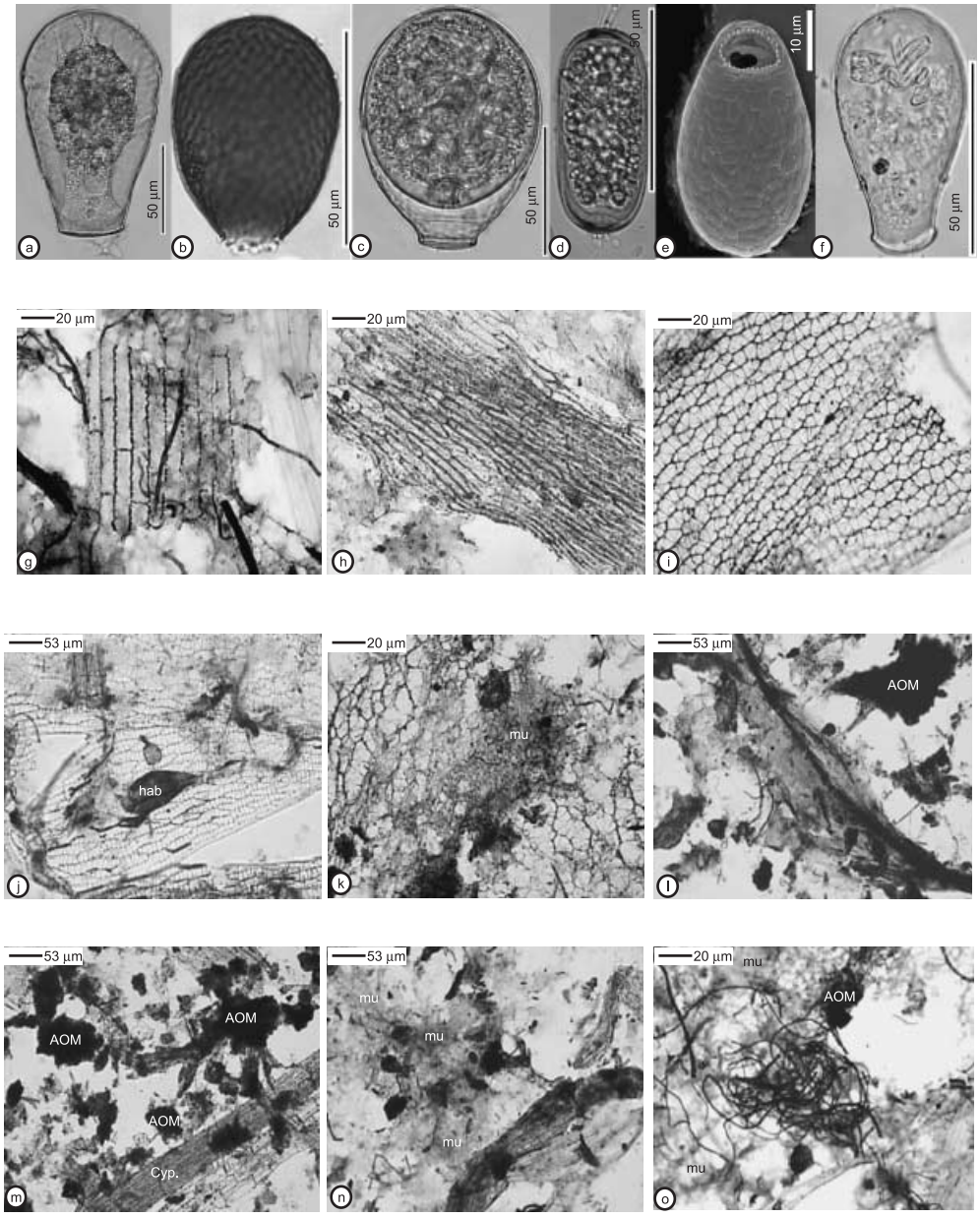

Fig. 4. Characteristic taxa of testate amoebae and main organic components identified in *Sphagnum* mosses and bulk peat samples, respectively. (a) *Hyalosphenia papilio*; (b) *Assulina muscorum*; (c) *Nebela tincta*; (d) *Archerella flavum*; (e) *Corythion dubium*; (f) *Nebela militaris* [scale bars = 50 µm except for *C. dubium* (10 µm)]; (g) Cyperaceae-derived leaf tissues; (h) *Polytrichum*-derived leaf tissues; (i) *Sphagnum*-derived leaf tissues; (j) shell of the bdelloid rotifer *Habrotrocha angusticollis* (hab) associated with *Sphagnum*-derived leaf tissues; (k) degraded *Sphagnum*-derived leaf tissues and mucilage (mu); (l) structureless unspecified plant tissue and an amorphous OM (AOM) flake; (m) AOM flakes and Cyperaceae-derived sheath tissue; (n) mu and structureless unspecified plant tissue; (o) fungal hyphae, mu and an AOM flake.

advantage of using testate amoebae as management tools for peatland resources (Mitchell, Charman & Warner 2008). The quantitative inference of water table depth and pH based on testate amoebae indicators represents an alternative to labour-intensive repeated measurements of these variables in the field (Charman *et al*. 2004).

Nevertheless, to date testate amoebae have only rarely been included in studies of cut-over peatland regeneration (Buttler *et al*. 1996; Davis & Wilkinson 2004). In a study of naturally regenerating cut-over bogs in the Jura Mountains, Buttler *et al*. (1996) observed that testate amoebae responded rapidly to changes occurring at the surface. This study also provided data on recent stages of succession not included in this study. The authors observed a convergence of vegetation and testate amoeba community structure in the advanced regeneration stage, regardless of the initial conditions.

In this study, we observed clear differences in species richness, diversity, density, biomass and average species size. Species richness and diversity increased but density declined from the recent to the advanced regeneration stages and the

Table 4. Yields of total sugars, cellulosics and hemicellulosics (mg g^{-1}) and concentrations of hemicellulosic sugars (wt%) for characteristic peat-forming plants collected in Le Russey, a peatland near La Chaux d'Abel in the Jura (modified from Comont, Laggoun-Défarge & Disnar 2006)

	S. fallax	*P. strictum*	*E. vaginatum*	*E. angustifolium*
Total sugars (mg g^{-1})	357	401	326	282
Total cellulosics (mg g^{-1})	142	112	224	218
Total hemicellulosics (mg g^{-1})	215	289	102	64·5
Hemicellulosic glucose (wt%)	27·3	30·6	13·5	15·2
Xylose (wt%)	16·2	4·4	52·4	47·9
Arabinose (wt%)	4·1	2·6	16·1	11·9
Mannose (wt%)	11·1	41·5	4·5	6·7
Rhamnose (wt%)	12·7	5·1	3·2	5·0
Ribose (wt%)	0·4	1·0	0·6	0·0
Fucose (wt%)	1·4	0·9	0·7	1·4
Galactose (wt%)	27·6	13·8	8·9	11·9

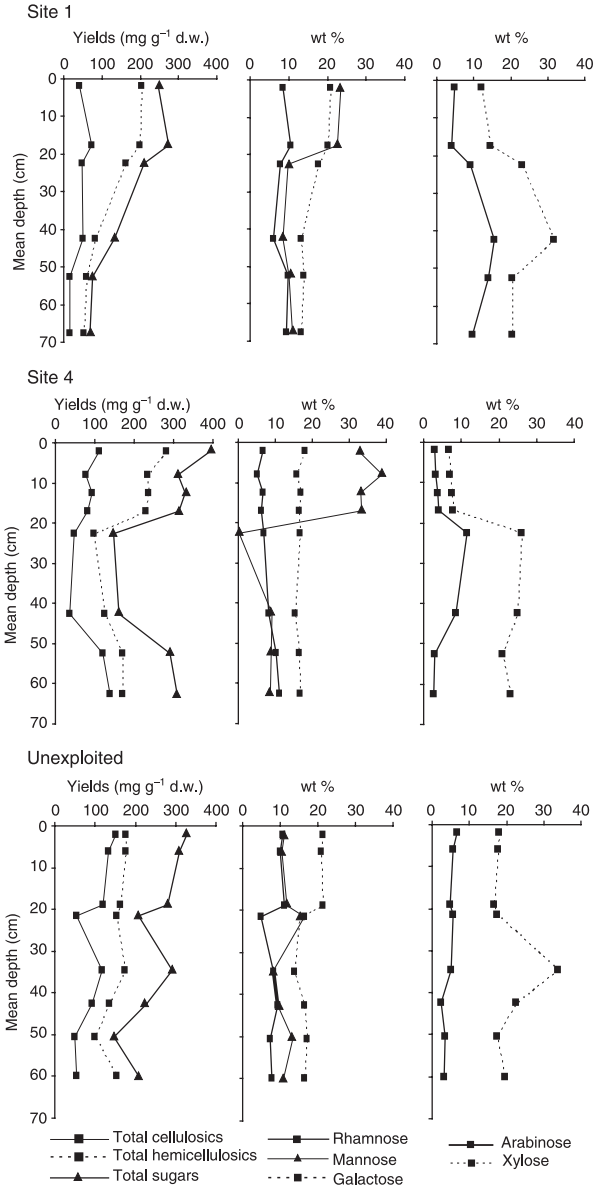

Fig. 5. Depth evolution of total sugar, cellulosics and hemicellulosics yields (mg g^{-1}) and concentrations (wt%) of moss and Cyperaceae markers in the recent and advanced regenerating stages (sites 1 and 4) in comparison with the unexploited site.

unexploited site. This result is in agreement with patterns of community assembly of various groups of organisms during primary and secondary succession (Odum 1971). However, recent studies of testate amoeba diversity and community structure in chronosequences, and relationships between plant and testate amoeba diversity, have shown contrasting responses, suggesting that testate amoebae might not respond in the same way as larger organisms (Ledeganck, Nijs & Beyens 2003; Wanner & Xylander 2005). In the cut-over peatland secondary succession sequence, we found shifts in community composition, rather than simply an addition of new taxa as observed by Wanner & Xylander (2005) in sand dunes. However, peatlands are quite different from sand dunes and mineral soils in their evolution. It can be assumed that the changes in ecological conditions (e.g. moisture and pH) associated with the development of a new, actively growing peat layer acts as a strong ecological filter that causes early colonizers to disappear from the community.

Biomass and the average size of species declined over the regeneration sequence but were higher in the unexploited site of the peatland. These changes also agree well with the changes in ecological conditions over the regeneration sequence. Testate amoebae are aquatic organisms and respond to soil moisture content in a size-specific way: larger species are more stimulated by wet conditions than smaller ones (Lousier 1974). However, larger species are also less numerous and heavier than smaller ones and therefore they are less likely to be transported over long distances. In a broader context, it has been suggested that testate amoebae larger than 100–150 μm may not be cosmopolitan (Wilkinson 2001). The processes responsible for such biogeographical patterns probably affects the recolonization of secondary habitats at a finer scale. Therefore we would not expect large species to colonize favourable habitats very quickly. This hypothesis is supported by the available data on primary succession. In a series of primary colonization experiments in mineral soils, Wanner and co-workers have observed that all successional stages are dominated by small Euglyphid testate amoebae, while larger taxa characteristic of forest humus only occur in late succession stages (Wanner & Dunger 2001; Wanner & Xylander 2005). Such differences in the size and quality of the testate population may serve as predictors of the rate and directions of

change as the regenerating peat community becomes more established. Beyond the quantitative inference of key ecological variables, the structure of the testate amoeba community might provide information on the degree of 'naturalness' of a site.

Bacterial biomass is a relatively crude measure of microbial activity in ecosystems including secondary succession in cut-over peatlands. Nevertheless clear changes were observed. Beyond biomass, changes in bacterial community structure and associated processes can be expected. A community approach for bacteria was beyond this study. However, low densities of bacteria were recorded during the apparently drier phases (see below) and in the more advanced regeneration stages (albeit not significant). Similarly, Gilbert (1998) observed lower chemoheterotrophic assimilation (mainly bacterial) during the dry period of mid-summer in a *Sphagnum*-dominated peatland. This apparent negative effect of dry conditions on bacteria density and production parallels the pattern of testate amoeba density where low numbers were found in the more advanced, drier secondary sites and in the unexploited site. Testate amoebae feed on a broad range of micro-organisms, thus the lower density of testate amoebae in the drier sites matches the density patterns of at least some of their prey (the bacteria) and microbial secondary production (Yeates & Foissner 1995; Gilbert *et al*. 2000). These results could also suggest that the larger species of testate amoebae that are characteristic of the unexploited site may be less directly dependant on the abundance of bacteria and instead feed more (or perhaps exclusively) on fungi. Such an assumption is commonly made, although there is still little reliable data on the feeding habits of most testate amoeba species.

ORGANIC MATTER AND BIOCHEMICAL INDICATORS

The high preservation of organic material in peat that results from low pH and anoxia make the peat archives particularly useful for palaeoenvironmental reconstructions. Nevertheless, to date the biochemical composition of peat OM has rarely been used as an indicator for past environmental conditions, particularly in ombrotrophic peatlands (Morita & Montgomery 1980; Nott *et al*. 2000; Pancost *et al*. 2002; Nichols *et al*. 2006), and none of these studies concern formerly cut-over sites. Recently, a study of a regenerating cut-over bog in the Jura Mountains allowed Comont, Laggoun-Défarge & Disnar (2006) to obtain insights into changes in the OM sources and dynamics of inherited biopolymers along the regeneration sequence.

Field observations did not suggest that the 'old' peat beneath the regenerated peat was different between the recent and advanced regeneration stages. However, from detailed microscopic observations the proportion of *Sphagnum* (either well-preserved or not) was higher in the catotelm peat of the advanced stage than of the recent stage. In the recent stage, in contrast, the peat contained a higher proportion of unidentifiable remains. However, the biochemical signature revealed that, in both stage, the new peat was of comparable origin. We interpret this as an indication of a similar original composition but differential preservation because of the conditions of the sites at the onset of regeneration

At the unexploited site, the irregular but overall progressive decrease of total sugars with increasing depth depicts typical diagenetic evolution. Nevertheless, the high and nearly constant C/N ratio values (i.e. 60–80) recorded along the peat profile, and the abundance of well-preserved tissues mainly derived from *Sphagnum* mosses, are typical of rather well-preserved inherited plant material. In contrast, the two sections taken between 20–25 and 48–62 cm depth that display much lower C/N ratios, lower total sugar yields and OM dominated by decomposed plant tissues, suggest an increasing degradation of OM. At the top of these two sections well-preserved Cyperaceae-derived tissues replaced the *Sphagnum*-derived tissues. These features suggest a change in vegetation and environmental conditions that might have been provoked by drier phases in the history of the bog. Such a dry event would have shifted the competition between *Sphagnum* and *Eriophorum* in favour of the latter and increased peat mineralization. The causes of these two dry phases are uncertain but drainage from peat cutting is most likely to be responsible for changes recorded in the upper peat. Taken together, these results illustrate well the fact that, although this part of the bog has not been exploited for peat, drainage related to peat harvesting affected the vegetation and therefore the botanical composition of the peat. These changes were well recorded in the existing peat.

In the regenerating sites (sites 1 and 4), vertical patterns of OM composition revealed a limit between the upper 'new' peat and the lower 'old' catotelm peat. The latter, especially at site 1, was characterized by a pronounced OM degradation, as attested by relative low C/N ratios and sugar contents and a predominance of amorphous OM and mucilage. In contrast, the new regenerated peat was dominated by moss-derived tissues. This was confirmed by distributions of individual hemicellulose sugars displaying high proportions of mannose and, to a lesser extent, galactose compounds typical for mosses (Comont, Laggoun-Défarge & Disnar 2006). The new peat also exhibited much higher yields of total hemicellulosics in comparison with total cellulosic sugars. Such a discrepancy might reflect a higher contribution of moss to the peat, these plants being richer in hemicellulosic sugars than sedges (Comont, Laggoun-Défarge & Disnar 2006). However, a relative enrichment of the hemicellulosic carbohydrate pool as a result of cellulose destruction cannot be excluded. Nevertheless, the amount of total sugars recorded in this peat layer, which were in the same range as in living plants, are indicative of a good OM preservation. Surprisingly, although the vegetation cover is currently dominated by mosses and sedges, no evidence of any Cyperaceae-derived material, and/or related sugar biomarkers, was identified by analyses. In fact, sugar markers of Cyperaceae, i.e. xylose and arabinose (Bourdon *et al*. 2000), were not present in the new peat but in the upper levels of the old catotelm peat (Fig. 5). This overall lack of *Eriophorum* record in the new peat can be attributed to its higher decomposability compared with *Sphagnum* mosses (Coulson & Butterfield 1978; Clymo & Hayward 1982; Chague-Goff & Fyfe 1996).

Over the secondary succession, a close examination of organic composition of the new peat revealed changes from the recent to the advanced stages. At site 1 (the recent stage) the peat was dominated by *Sphagnum* remains, while at site 4 (the advanced stage) it had a more heterogeneous botanical composition, with better carbohydrate preservation (*c.* 337 mg g^{-1} vs. *c.* 243 mg g^{-1} at site 1). In addition to the original botanical composition, such contrasting composition might also be related to abiotic factors, i.e. trophic conditions inducing differences in biodegradation processes between the two sites. The surface vegetation suggests that the environmental conditions of the recent regenerating stage (site 1) are probably more minerotrophic and consequently more favourable to microbial activity than the more advanced regenerating stage (site 4) (E. Samaritani, A. Siegenthaler, Y. M. li-Petäys, A. Buttler, P.-A. Christin and E. A. D. Mitchell, unpublished data). This explanation was supported by (i) the bacterial biomass, which was about twice as high in the new peat of the recent regenerating stage as in the advanced stage and in the unexploited site, and (ii) a shift in testate amoebae from a fen community towards a more acidic, drier bog community. In the same way, when considering the whole profiles (new and old peat), it appeared that the highest bacterial biomass was recorded in the *Sphagnum*-dominated peat layers and the lowest in the highly decomposed and deeper peat layers. This was interpreted, at least at site 1, as a consequence of drainage phases during peat extraction.

CONCLUSIONS AND IMPLICATIONS FOR MANAGEMENT

Our aims were to assess how testate amoebae, bacteria and peat OM were correlated and to identify specific indicators of changes in the structure or functioning of the ecosystem. While bulk chemical OM characterization allowed the newly regenerated peat to be differentiated from old peat, OM indicators (carbohydrates and botanical composition of the peat) combined with heterotrophic bacteria biomass and testate amoebae diversity revealed contrasting signatures between the recent and advanced stages of regeneration. At the natural unexploited site, specific OM indicators provided information on past changes in vegetation and related environmental conditions, well recorded in the accumulated peat.

This study illustrates how biochemical markers and testate amoebae could provide additional information on the functioning of the ecosystem as well as the observation of the present vegetation, which is commonly used to assess the state of the ecosystem. Testate amoebae appear to be particularly useful because (i) they provide information on the soil biota, (ii) they are preserved in the peat deposits, thus allowing palaeoenvironmental reconstruction, and (iii) their analysis does not require expensive equipment or consumables. Indicators from OM also appear useful because they allow the botanical and biochemical composition of even quite decomposed peat to be determined. Unlike accepted ideas regarding the rapid consumption of carbohydrates in modern environments, their good preservation in peats provides additional information on past anthropogenic perturbations in bogs and their consequence in terms of OM recycling and storage.

Understanding ecosystem dynamics in secondary ecosystems is challenging because we rarely have accurate information on the nature of the ecosystem prior to disturbance and a detailed account of human impact. It is valuable to compare independent lines of evidence to determine such characteristics of the site. A multidisciplinary assessment approach may therefore prove useful for the management of abandoned cut-over peatlands.

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