

Integrating organic matter properties, bacteria and testate amoebae (Protista) indicators for assessing peatland regeneration success.

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ABSTRACT

In a range of regeneration stages from a cutover *Sphagnum*-dominated peatbog in the Jura Mountains (Switzerland), contrasting biochemical signatures were recorded by peat organic matter (OM) along the regenerating profiles allowing clear differentiation between the newly regenerated peat and the old peat to be identified. Where peat macrofossils were absent sugar biomarkers allowed to infer peat botanical origin and OM alteration. Over the succession, the OM composition of the new peat differed: peat from the recent stage was more dominated by *Sphagnum*-derived tissues and characterised by lesser carbohydrate preservation and higher bacterial biomass than the advanced regeneration stage. 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) biomass and average size of species declined but were higher in the unexploited site (ii) species richness and diversity increased but density declined.

Although the secondary succession in the cutover bog leads to an ecosystem similar to that of the reference site in terms of surface vegetation, OM and testate amoebae continue 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: a higher testate amoeba diversity is associated with better carbohydrate preservation and a more heterogeneous botanical composition of the peat. The combination of several indicators therefore provides a more complete assessment of ecological conditions which could be valuable for the management of cutover peatlands.

INTRODUCTION

The natural restoration of peatlands has recently become a subject of growing interest, first from a global change perspective, because of the potential of regenerated peatlands to act as carbon sinks, and second, from a more regional perspective, because of the interest represented by such environments as secondary habitats may be important for rare and endangered species (Chapman *et al.* 2003). Much effort is then now directed to the re-establishment of peat-forming vegetation, especially *Sphagnum* mosses (Gorham & Rochefort 2003; Grosvernier *et al.*, 1995; Lavoie *et al.* 2003). However, the outcome of these restoration efforts with respect to key functions such as carbon sequestration remains difficult to predict and, in many cases, local conditions may not allow net accumulation of carbon to occur (Francez *et al.*, 2000; Gorham & Rochefort 2003; Lavoie *et al.* 2003; McNeil & Waddington 2003). Therefore a wider range of physico-chemical, microbial and biological indicators such as vegetation, gas fluxes, microbial communities (including bacteria, fungi, protists and micro-metazoa) and organic matter (OM) must be considered in order to identify the most appropriate indicator(s) for the processes controlling long-term carbon sequestration during peatland regeneration. This was the main goal of the EU-funded project RECIPE (Reconciling commercial exploitation of peat with biodiversity in peatland ecosystems) (Chapman *et al.* 2003). To assess their potential value as indicators of restoration, we studied the OM, testate amoebae and heterotrophic bacteria in peat profiles (up to 70cm depth) from a cutover *Sphagnum*-dominated peatbog in the Jura Mountains (Switzerland). We selected a range of regeneration stages and compared them to reference profiles taken from an undisturbed area

of the peatland. The uppermost soil (0 to 3 cm) corresponding to living *Sphagnum* was analysed for testate amoebae, whereas other analyses were conducted on the whole peat profiles.

MATERIAL AND METHODS

Study site and sampling

La Chaux d'Abel, a cutover peatland in the Jura Mountains, Switzerland (47.09°N, 6.56°E; altitude 1,020m a.s.l.), was sampled in November 2001. 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 various times on different parts of the site. Two regeneration stages: a recent stage (sites 1-2; 29 years) and an advanced one (sites 3-4; 51-58 years) 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. A reference site representing an unexploited area in the same peat bog (hereafter coded « unexploited ») was also selected. Three replicates of 60-70cm peat cores (10-13 samples per profile) were extracted in each community for biochemical, micromorphological and bacterial analyses whereas the uppermost 3cm of living plants were analysed for testate amoebae.

Laboratory and data analyses

Testate amoebae

Testate amoebae were extracted from the samples by sieving on 20µm and 300µm mesh and shells were identified and counted under microscope at 200X and 400X magnifications. Biovolumes of each living (active and encysted) species were estimated by assuming geometrical shapes and converted to carbon using the conversion factor: $1\mu\text{m}^3=0.11 \times 10^{-6} \mu\text{gC}$ (Weisse *et al.* 1990). We compared the sampling sites for a set of five general variables derived from the testate amoebae 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).

Bacterial density

Bacteria were stained with DAPI (4,6 diamino 2 phenylindol), filtered on 0.2µm black membrane filters and examined by epifluorescence microscopy at 1000X magnification for all peat levels. Bacteria numbers and sizes were estimated on a minimum of 10 random fields for each sample. Bacterial biovolumes were estimated by assuming geometrical shapes and converted to carbon using the following conversion factor: $1\mu\text{m}^3=5.6 \times 10^{-7} \mu\text{gC}$ (Bratbak 1985).

Organic matter analyses

Total carbon and nitrogen were determined by combustion at 1100°C with a CNS LECO 2000 apparatus on dried and crushed samples. Identification and quantification of peat constituents were carried out under transmitted light microscope. Bulk peat samples were mounted as smear slides and examined at 20 and 50X magnification. The surfaces covered by the main organic micro-remains were estimated with a grid reticule in the eyepiece of the microscope. A total of 3000 to 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 *et al.*, 2006.

RESULTS AND DISCUSSION

Testate amoebae and bacteria indicators

Clear differences in species richness, diversity, density, biomass and average species size of testate amoebae were observed along the regeneration chronosequence: species richness and diversity increased but density declined from the recent to the advanced regeneration stage and the unexploited site (Laggoun-Défarge *et al.*, in press). The early stages were dominated by *Hyalosphenia papilio*, a species indicative for wet conditions (Mitchell *et al.* 1999). In the intermediate stage, *Archerella flavum* an indicator of wet, acidic conditions reached its highest abundance and two indicators of drier and more acidic conditions, *Nebela tincta*, and *Assulina muscorum* increased in abundance. Advanced stages were dominated by *Nebela tincta*, *Assulina muscorum* and another dry and acidic indicator *Corythion dubium* (Mitchell *et al.* 1999). In the 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. Therefore, 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 colonisers 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.

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 *et al.* (1998) observed lower chemo-heterotrophic 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).

	Site 1			Site 2			Site 3			Site 4			Unexploited		All sites				
	Mean	SE	*	Mean	SE		Mean	SE		Mean	SE		Mean	SE	Min	Max			
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.w. ⁻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.w. ⁻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

à 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.

Table 1. Summary data for testate amoebae extracted from *Sphagnum* samples taken in the five plant communities in La Chaux d'Abel peatland, Switzerland (Laggoun-Défarage *et al.*, in press)

Organic matter and biochemical indicators

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 to 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 (Fig. 1a). 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 mineralisation. The causes of these two dry phases are uncertain but drainage from peat cutting is most likely to be responsible for changes recorded at least 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 palaeo-

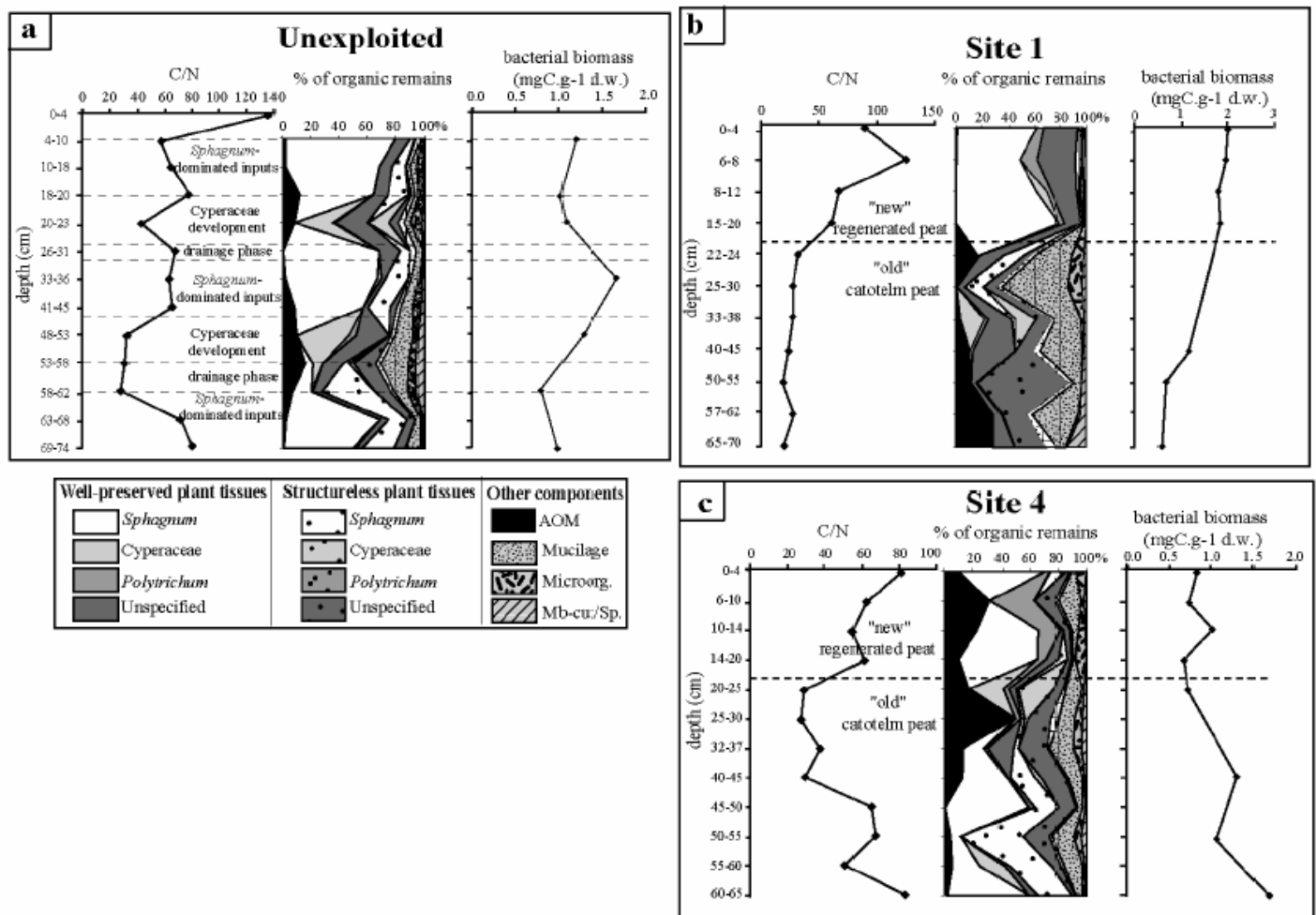


Figure 1. Depth profiles of atomic C/N ratio, relative percentages of organic microremains, and bacterial biomass in the recent regeneration stage ('b' site 1), the advanced regeneration stage ('c' site 4), and the unexploited site ('a' of La Chaux d'Abel peatland. In sites 1 and 2 the dotted line delineates 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, oxidised debris) (modified from Laggoun-Défarge et al., in press)

vegetation which has been well recorded by the composition of the existing peat. In the regenerating sites (1 & 4), vertical patterns of OM composition revealed a limit between the upper “new” peat and the lower “old” catotelm peat (Fig. 1b & c). The latter, especially in site 1, was characterised by a pronounced OM degradation as attested by relative low C/N ratios and sugar contents (Laggoun-Défarge *et al.*, in press) 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 *et al.*, 2006). Moreover, the amount of total sugars recorded in this peat layer, which are 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 at the upper levels of the “old” catotelm peat (Fig. 1c). This overall lack of *Eriophorum* record in the “new” peat can be attributed to its higher decomposability compared to *Sphagnum* mosses (Coulson & Butterfield 1978).

Over the secondary succession, a close examination of organic composition of the “new” peat revealed changes from the recent to the advanced stages. In site 1 (the recent stage) the peat was dominated by *Sphagnum*-remains, while in site 4 (the advanced stage), it had a more heterogeneous botanical composition with better carbohydrate preservation (ca. 337mg.g⁻¹ versus ca. 243mg.g⁻¹ at site 1) (Laggoun-Défarge *et al.*, in press). 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) (Samaritani *et al.*, unpublished data). This explanation was supported by: (i) 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 (Fig. 1); and (ii) a shift in testate amoebae from a fen community towards a more acidic, drier bog community (Laggoun-Défarge *et al.*, in press). 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 in site 1, as a consequence of drainage phases during peat extraction.

CONCLUSIONS

While bulk chemical OM characterisation revealed by C/N profiles, 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 the advanced stages of regeneration. Differences observed in the composition of the newly accumulated peat may be due not only to plant sources but also to trophic and abiotic conditions inducing differences in biodegradation processes between the two stages. This interpretation was supported by testate amoebae communities and bacterial biomass. In 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 than the observation of the present vegetation which is commonly used to assess the state of the ecosystem.

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