British Ecological Society
Journal of Applied Ecology

Microbial carbon and nitrogen in abandoned peatlands after peat extraction: patterns of response to regeneration age and plant community at a European scale

Journal:	*Journal of Applied Ecology*
Manuscript ID:	JAPPL-2007-00100
Manuscript Type:	Standard Paper
Date Submitted by the Author:	08-Feb-2007
Complete List of Authors:	Francez, Andre-Jean; University of Rennes 1, ECOBIO JOSSELIN, Nathalie; University of Rennes 1, ECOBIO CHAPMAN, Stephen; The Macaulay Institute, Soil Quality & Protection ARTZ, Rebekka; The Macauley Institute, Soil Quality & Protection LAGGOUN-DEFARGE, Fatima; University of Orléans, Geosciences BUTTLER, Alexandre; EPFL, ECOS GATTINGER, Andreas; Technical University of Münich, Institute of Soil Ecology GILBERT, Daniel; University of Franche-Comté, Biologie Environnementale SIEGENTHALER, Andy; EPFL, ECOS YLI-PETAYS, Mika; University of Helsinki, Forest Ecology VASANDER, Harri; University of Helsinki, Forest Ecology
Key-words:	microbial succession, ecosystem dynamics, ecological succession, basal respiration, microbial metabolic quotient, carbon mineralization rate, aerobiosis vs anaerobiosis, RECIPE

Microbial carbon and nitrogen in abandoned peatlands after peat extraction: patterns of response to regeneration age and plant community at a European scale

ANDRÉ-JEAN FRANCEZ,* NATHALIE JOSSELIN,* STEPHEN J. CHAPMAN,† REBEKKA R.E. ARTZ,† FATIMA LAGGOUN-DÉFARGE,‡ ALEXANDRE BUTTLER,§ ANDREAS GATTINGER,# DANIEL GILBERT,¥ ANDY SIEGENTHALER,§ MIKA YLI-PETÄYS• AND HARRI VASANDER•

*UMR-CNRS Ecobio, University of Rennes 1, Campus de Beaulieu, F-35042 Rennes, France; †The Macaulay Institute, Craigiebuckler AB15 8QH Aberdeen, UK; ‡UMR-CNRS Géosciences, University of Orléans, BP 6759, F-45067 Orléans cedex 2, France; §Swiss Federal Institute of Technology (E.P.F.L.), ENAC-ISTE-ECOS, GR B2-417, CH-1015 Lausanne, Switzerland; #Technical University of Münich, Institute of Soil Ecology, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany; ¥University of Franche-Comté, UMR-INRA Biologie environnementale, Place Leclerc, F-25030 Besançon, France; •Department of Forest Ecology, Latokartanonkaari 7, FIN-00014, University of Helsinki, Finland

Corresponding author: André-Jean Francez, UMR-CNRS Ecobio, University of Rennes 1, fax : +33223231626; e-mail: **andre-jean.francez@univ-rennes1.fr**

Running title: *Microbial carbon and nitrogen in cut-over peatlands*

Word count, including references, tables and figure legends: 7800

Summary

1. Microbial variables are increasingly used to study effects of disturbance on ecosystem functioning or success of recovery processes.

2. The aim of this work was to assess microbial biomass carbon (C) and nitrogen (N) and microbial activity as indicators of cut-over peatland regeneration at a European scale. We hypothethised i) microbial C-N pools and aerobic and anaerobic basal respiration would increase with regeneration age; ii) plant community structure would act as a driving force on microbial variables.

3. Fifteen regeneration stages of different ages and plant recolonization over a chronosequence of 50 years, were investigated. Microbial biomass C and N, basal aerobic and anaerobic respiration, metabolic microbial quotient (MMQ) and soluble organic carbon mineralization rate (C-MR) were measured in surface and deep peat.

4. Microbial biomass dynamics *vs* regeneration age in the surface peat fitted to a logistic function, while the microbial C:N ratio was best described by a gamma function. Other variables were fitted to exponential functions, except aerobic basal respiration which linearly increased over the chronosequence.

5. Plant species richness positively correlated with microbial biomass C, anaerobic basal respiration and C-MR in the surface peat. Botanical composition impacted microbial variables in relation to frequencies of moss and/or ericaceous shrubs. The moss:vascular plant cover ratio positively correlated with microbial biomass C and C-MR under anaerobiosis while MMQ significantly decreased with increasing ericaceous:vascular plant cover ratio, both under aerobiosis and anaerobiosis.

6. *Synthesis and applications.* Results clearly showed i) patterns of response to age of abandonment after peat extraction and ii) a plant-community mediated control on peat microbial pools, suggesting changes in ecosystem dynamics over the succession. The described dynamics of microbial variables over the succession were fitted to relatively simple models that could serve as references in the study of peatland regeneration. We concluded that microbial biomass and basal respiration are among the most efficient variables which could be used as a set of ecological indicators of regeneration dynamics. This should be carried out together with an analysis of the plant community structure, which is easily described by indices integrating the frequencies of key-stone taxa.

Key-words: microbial succession, ecosystem dynamics, ecological indicator, basal respiration, microbial metabolic quotient, carbon mineralization rate, aerobiosis *vs* anaerobiosis, RECIPE

Introduction

Microbial biomass is an important pool in the soil and the interest of estimating it in peat is related to its function in recycling elements, especially in oligotrophic *Sphagnum* mires in which the decomposition processes are slowed down by acidity, hydromorphy (leading to anaerobiosis) and poor-nutrient inputs (Clymo 1983). In these ecosystems peat accumulates at an average rate of 0.10-0.15 yr^{-1} which means that 85-90 % of the annual organic matter produced by plants decayed (Clymo 1984; Francez & Vasander 1995) and the study of the fate of elements such as N in peat showed mineralization takes place in acrotelm despite the important retention of nutrients by *Sphagnum* carpet at the mire surface (Li & Vitt 1997; Francez & Loiseau 1999).

Microbial biomass carbon (C) pools in *Sphagnum* fens and bogs, when estimated with the fumigation-extraction (FE) method range from 0.5 to 14 mg g dry peat $(DP)^{-1}$, depending on peatland nutrient status, season, depth, or degree of disturbance (Williams & Silcock 1997; Baum, Leinweber & Schlichting 2003; Potila & Sarjala 2004; Andersen, Francez & Rochefort 2006). Lower values are generally registered with the substrate-induced respiration (SIR) method (Brake, Hoper & Joergensen 1999, Andersen, Francez & Rochefort 2006). Microbial biomass nitrogen (N) in peat would range from about 200 to 500 µg N g DP^{-1} (Williams & Silcock 1997; Francez, Josselin & Gogo 2000; Baum, Leinweber & Schlichting 2003).

Microbial activity and nutrient cycling in mire ecosystems are controlled by temperature (Williams & Crawford 1983), oxic/anoxic conditions (Scanlon & Moore 2000), water table (Blodeau & Moore, 2003) and nutrient status (Updegraff *et al.* 1995; Chapin *et al.* 2003; Potila & Sarjala 2004), as main factors

acting drastically and with synergy when anthropogenic interventions impact mire dynamics by drainage, peat cutting or fertilization.

Peat extraction strongly disturbs the peat microbial pool. Croft, Rochefort and Beauchamp (2001) demonstrated a negative effect of peat harvesting by vacuum technique on microbial biomass C while ammonification significantly increased. However, abandoned peatlands after extraction function as a C source to the atmosphere because of the lack of vegetation and ongoing CO_2 emissions released by the microbial activity in peat, though the low nutrient quality of peat decreases the potential for C mineralization (Waddington, Rotenberg & Warren 2001).

Abandoned mined peatlands offer poor conditions for regeneration due to bare peat surfaces with unfavourable hydrology, wind erosion and high temperature fluctuations (Campbell, Lavoie & Rochefort 2002; Chapman *et al.* 2003). Nevertheless, there are many examples throughout Europe and North America demonstrating that spontaneous recolonization by bog plants is possible (Lavoie *et al.* 2003), when satisfactory microclimatic conditions (Grosvernier, Matthey & Buttler 1995) and/or substrate quality (Salonen 1994) occur. While vegetal colonization of bare peat is well documented and monitored (see for instance, Robert, Rochefort & Garneau 1999; Tuittila *et al.* 2000), little is known about the microbial communities developing concomitantly. Data on microbial successions in peatlands are very scarce (Thormann, Currah & Bayley 2003) while patterns of litter decomposition and gas emissions are rather well-documented (Aerts, Verhoeven & Whigham 1999; Blodeau 2002; Vasander & Kettunen 2006). Dickinson & Dooley (1967) emphasized the very limited microbial colonization of cutover peat, even after 10 years since abandonment while Maire (1983)

114 demonstrated short-term changes in microbial colonization of sterilized eutrophic
115 peat and interpreted the dynamics as a succession of r and K strategist species.
116 The role of living vegetation on soil processes has received more and more
117 attention in mires and wetlands and the importance of interactions between plants
118 and microbes have now been recognized in peat soils. Root activities regulate
119 microbial metabolic pathway such as Fe(III) reduction (Neubauer *et al.* 2005) or
120 microbial activity by transfers of C-molecules such as acetate into the rhizosphere,
121 controlling partly methanogenesis and methanotrophy (Watson *et al.* 1997; Ström
122 *et al.* 2003). The influence of botanical composition on microbial variables had
123 also been recognized. For instance, Chapman, Campbell & Puri (2003) noted a
124 decreasing C biomass with tree extension while Fisk, Ruether & Yavitt (2003)
125 registered a higher microbial activity in shrub-*Sphagnum* dominated communities
126 compared with sedge-dominated sites.
127 Microbial variables have also been recognized as practical indicators of
128 peatland ecosystem functioning change, being more sensitive than physico-
129 chemical properties (Chapman, Campbell & Puri 2003) and integrative of
130 restoration processes, even if a shift between vegetal and microbial recovery
131 processes persists some years following revegetation management (Andersen,
132 Francez & Rochefort 2006).
133 However, little information is available on the recovery of biomass and
134 activity during microbial succession of abandoned surface peat. The aims of this
135 work were:
136 i) to evaluate the microbial biomass C and N and activity of the microbial
137 communities of abandoned peatlands with different ages after peat cutting in
138 Europe and to reveal patterns of response to regeneration age. This would allow

us to characterize ecosystem dynamics and transformations over secondary succession, from bare peat (low microbial biomass and activity) to later stages of recolonization by vegetation (higher biomass and activity) over a 50 year chronosequence;

ii) to demonstrate the plant-community mediated control on peat regeneration and associated microbial communities by assessing relations between microbial and vegetation variables (species richness, dominance index and plant cover ratios).

iii) to examine the suitability of microbial variables as potential tools in the assessment of peatland regeneration trends.

Material and methods

SITE DESCRIPTION

Five regenerating peatlands with different stages of plant recolonization were selected across Europe (Table 1). They were all derived from past peat extraction of initial raised-bogs and belong to the peatland grid system of the European programme RECIPE (Reconciling Commercial Exploitation of Peat with Biodiversity in Peatland Ecosystems).

EXPERIMENTAL DEVICE

We studied a series of 15 regeneration stages (= sites) that ranged in age from 3 to 50 years following peat extraction (Table 2). The different sites were selected by taking into account the age of abandonment and plant composition in relation to peat-forming key-stone species i.e. *Sphagnum, Eriophorum angustifolium* and *E. vaginatum* which were abundant in every peatland, except in Baupte (FB). Sites

were more or less covered by vegetation and, in addition to the bare peat situation, we distinguished 8 plant communities (Table 2).

A common protocol of peat sampling was performed using the same kind of peat corer (Buttler, Grosvernier & Matthey 1998). A sample dispatching protocol to partners was established by separating aliquots of collected peat slices in order to carry out the different analysis. The work programme consisted of a set of microbial and chemical analyses, performed on the same peat cores extracted from the range of sites, at four depths: 0-5 cm and 5-10 cm (referred as surface peat) , 22.5-27.5 cm and 42.5-47.5 cm (called deep peat).

In each case, 3 plots per site were used as replicates and cores extracted from each site between October and December 2003.

MICROBIAL BIOMASS AND ACTIVITY

C and N pools in the biomass

Microbial biomass C and N were estimated on all peat samples with the fumigation-extraction method using a peat-modified protocol (Williams & Silcock 1997; Andersen, Francez & Rochefort 2006).

Total soluble organic carbon and soluble organic nitrogen were extracted using 0.5M K_2SO_4. C content in the extract was determined using a 1010 TOC Analyser (OI Analytical). N was oxidized with potassium persulphate (Williams *et al.* 1995) and measured colorimetrically as NO_2^- after reduction of NO_3^- on a copper-cadmium column with a Bran+Luebbe analytical chain.

Basal respiration in aerobiosis and anaerobiosis

187 Basal respiration corresponding to CO_2 production in the dark at 20°C was
188 measured during a 7 day experiment, carried out both under aerobic and anaerobic
189 (under N_2 atmosphere) conditions. Twenty-five grams of fresh peat were
190 incubated over 7 days in hermetically-sealed jars. Gas aliquots were collected
191 after 1, 2, 3, 4 and 7 day incubation time, and analysed for CO_2 using a portable
192 gas chromatograph (Chrompack micro GC-CP 2002) fitted with a TCD detector
193 and a Poraplot Q column. The hourly rate of respiration was calculated as the
194 mean of linear CO_2-C production between 2 and 4 days.
195
196 *Microbial ratios*
197 We calculated the microbial metabolic quotient (MMQ) or specific respiration
198 (Insam & Haselwandter 1989; He *et al* 2003) as the ratio of basal respiration (µg
199 C g DP^{-1} h^{-1}) divided by the microbial biomass C (µg C g DP^{-1}) (Anderson 1994).
200 The soluble C-mineralization rate (C-MR) is equal to the daily ratio (d^{-1}) of
201 basal respiration divided by the stock of soluble organic C in the peat.
202
203 *Units*
204 In order to compare our data to the literature and as the results expressed in
205 concentration (µg g DP^{-1}) were highly correlated to those expressed as stocks in
206 peat (g L^{-1}), we only present the results expressed in concentration units (µg g
207 DP^{-1}).
208
209 PLANT COMMUNITY CHARACTERIZATION
210 *Botanical composition*

211 Plant species richness and vegetation composition were recorded at the beginning
212 of the experiment in July 2003 from 1×1 m quadrats in which plant cover (%) was
213 estimated.
214
215 *Vegetation indices*
216 Plant dominance was calculated using the Berger-Parker index (d) as follow:
217 $d = Nmax:N$ eqn 1
218 where Nmax is the percentage cover of the most abundant species and N the sum
219 of covers (%) of all species (Magurran 1988).
220 In order to characterize the plant communities in the different states of the
221 chronosequence, two other ratios were calculated, a moss (m) and a shrub (e)
222 index :
223 $m = \Sigma M:N$ eqn 2
224 where ΣM is the percentage cover of the moss species and N as in eqn 1.
225 $e = \Sigma E:N$ eqn 3
226 where ΣE is the percentage cover of Ericacea and N as in eqn 1.
227 These calculations were justified by the importance of these 2 groups of plants
228 in peatland dynamics: *Sphagnum* species are found all over the mire succession,
229 from open water to fen and bog, and *Polytrichum* species recolonize many
230 disturbed surface peatlands (after peat extraction or fire) while the Ericacea
231 *(Vaccinium, Calluna, Erica)* colonize *Sphagnum* lawns and hummocks (natural
232 dynamics leading to raised-bogs) and, sometimes, bare peat surfaces.
233 In addition, we estimated the percentage of bare peat surface which was also
234 used to separate sites. Then, we firstly distinguished plant communities with bare
235 peat and secondly considered plants of second and third rank in abundance (%

236 cover) in communities without bare peat. The codes of vegetation integrate these
237 2 points, for example SpBp means *Sphagnum* (Sp) dominant in a site with still
238 significant un-colonized bare peat (Bp) while SpEvVa means a first rank
239 dominance for *Sphagnum*, a 2nd one for *Eriophorum vaginatum* and a 3rd one for
240 *Vaccinium* (Table 3).
241
242 STATISTICAL AND REGRESSION ANALYSIS
243 Due to non-homogeneity of variances following transformations, non-parametric
244 Kruskal-Wallis analyses were performed to detect significant differences between
245 depths, regeneration ages and plant communities. Post-hoc pairwise comparisons
246 were used to identify differences when H value of the Kruskal-Wallis test was
247 significant at α=0.05. The Mann-Whitney (Wilcoxon) W-test was used to
248 evaluate significant differences between aerobic and anaerobic conditions.
249 Spearman rank correlation coefficients were calculated to estimate the strength of
250 association between peat properties, vegetation indices, and microbial variables.
251 Statistical analyses were performed with STATGRAPHICS PLUS 2.1 software
252 (Manugistics Inc. 1995). Significance of the analyses was accepted at α=0.05.
253 Depending on the microbial variable responses, time change data were fitted
254 to linear (aerobic basal respiration) or non linear regressions. Thus, we used the
255 following equations:
256 - exponential regressions expressed as:
257 $y=a\ e^{-bAge}$ eqn 4
258 or
259 $y=a\ (1-e^{-bAge)} + c$ eqn 5
260 - gamma function:

$y = aAge^{b}\, e^{cAge^{d}} + f$ eqn 6

or logistic function :

$y = a/(1+(a-b/b)\, exp^{-cAge}$ eqn 7.

where y is the microbial variable, x is the time since abandonment (Age), a–f are fitted constants.

Non-linear regression analysis and fitting procedures were carried out with SYSTAT 10 (SPSS Inc 2000).

Results

MICROBIAL BIOMASS C AND N

Microbial biomass showed significant differences by all the considered factors i.e. depth, regeneration age and vegetation (Table 3).

Microbial biomass decreased with depth. In the surface peat layers (0-10 cm), microbial C and N biomass increased significantly from early to late-successional stages of regeneration and dynamics over time were best described by a logistic function. We registered a first phase (0-10 years) with no change, a second one of strong increasing (10-42 years) and a third one of stabilization. In the deep peat, despite time changes and higher microbial biomass at the intermediate stages, no satisfactory fit was obtained.

Microbial biomass in the surface peat layers also showed patterns of C and N pools with vegetation (Tables 3 & 4, Fig. 1). Lowest values were registered under plant communities characterized by incomplete cover of peat surface, whatever the dominant plant, and highest ones were observed as bare peat decreased and vegetation developed. Microbial C pool showed a significant positive correlation

285 with plant specific richness and increased significantly with increasing the moss
286 index m (Table 4).
287 In the upper part of the peat, changes of microbial C:N ratio over time were
288 significant and fitted to a gamma function (Fig. 1). The same trend was observed
289 in deeper peat, values ranging from 7.1 ± 6.8 to 25.6 ± 6.8 .
290 Microbial biomass C and N negatively correlated with organic C:N ratio and S
291 in the peat but we did not register any correlation between organic N and the
292 microbial N pool (Table 4).
293
294 BASAL RESPIRATION
295 Basal respiration showed significant differences by all the considered factors,
296 except aerobic respiration by depth (Table 3). Basal respiration was significantly
297 higher under aerobiosis compared to anaerobiosis, pooling sites and depths
298 ($W=7685$, $P<0.001$, $n=164$).
299 Basal respiration showed significant changes over time after abandonment,
300 considering the mean peat profile but the best data fittings were described in the
301 surface peat layers (0-10 cm) by exponential (aerobiosis) or linear regression
302 models (anaerobiosis) (Fig. 2).
303 In the surface peat, most of plant communities with *Sphagnum* did not show
304 any significant difference between aerobiosis and anaerobiosis. In sites with bare
305 peat or incomplete plant cover, aerobic respiration was significantly higher than
306 anaerobic respiration (Table 5 and Fig. 2). Only basal respiration under
307 anaerobiosis correlated with plant species richness and bare peat surface (Table
308 5). No relation with other vegetation indices was detected. In deep peat (data not

shown), microbial aerobic respiration was significantly higher than anaerobic respiration, whatever the site and plant cover, except SpCaDe.

Basal respiration was negatively correlated with peat organic sulphur content but did not show any link to peat C:N ratio or N content (Table 4).

Aerobic:anaerobic respiration ratio did not show difference between depths (Table 3). It was significantly higher 10 years after abandonment of extraction (data not shown). It also fluctuated significantly with plant community (Table 3). Highest mean values on the peat profile were registered under EvBp (3.14 ± 0.16) and CaBp (2.99 ± 0.19) while the lowest one was measured in one of the oldest sites with *Sphagnum spp* associated to *Calluna vulgaris* and Poacea (SpCaDe, 0.96 ± 0.19).

MICROBIAL METABOLIC QUOTIENT (MMQ)

MMQ was significantly higher under aerobic conditions ($W= 10968$, $P<0.001$, $n=148$) and we registered significant effects of depth, regeneration age and plant community, both under aerobic and anaerobic conditions (Table 3). MMQ responses to regeneration age in surface peat layers were fitted to exponential regression models (Fig. 2). MMQ decreased with age both under aerobic and anaerobic conditions.

MMQ in surface peat varied significantly among plant communities and we observed significant decreasing quotients with increasing proportion of ericaceous shrubs in the plant community (Table 5 and Fig. 2). MMQ showed higher values as plants did not cover all the peat surface.

MMQ positively correlated with peat C:N ratio and organic S content but decreased with increasing organic N in the peat (Table 4).

334

335 SOLUBLE ORGANIC CARBON-MINERALIZATION RATE (C-MR)

336 C-MR varied significantly with all the considered factors (Table 3) and was
337 significantly higher in aerobic conditions, pooling sites and depths (W=9351,
338 P<0.001, n=160).

339 C-MR changes over time and surface peat results were best described by
340 exponential regressions while in deep peat, C-MR were low and did not show
341 clear trends over time (Fig. 2). Higher C-MR rates were observed in late-
342 successional stages (> 20 years old) with values ranging from 0.329±0.084 to
343 0.507±0.097 d^{-1}.

344 C-MR was higher in aerobic conditions in most of the plant communities
345 (Table 5 and Fig. 2). Increasing plant species richness and mosses positively
346 influenced C-MR under anaerobic conditions (Table 4). No clear trend in relation
347 to vegetation was observed in aerobic conditions, except the fact that C-MR was
348 higher under plant communities mixing *Sphagnum* and *Eriophorum vaginatum*
349 with (SpEvVa, 0.577±0.098 d^{-1}) or without (SpEv, 0.507±0.089 d^{-1}) *Vacinium*
350 *spp.* (Fig. 2). Under anaerobiosis, the mineralization rate increased with plant
351 species richness (Table 4).

352 C-MR under anaerobiosis also significantly decreased with increasing peat
353 C:N ratio.

354

355 **Discussion**

356 MICROBIAL DYNAMICS OVER THE REGENERATION
357 CHRONOSEQUENCE

358 The first aim of our work was to assess responses of microbial variables in
359 abandoned peatlands after peat cutting over a 50 years' chronosequence at a
360 European scale. The analysis of microbial dynamics in the surface peat (0-10 cm)
361 revealed 3 major stages. We distinguished a first phase of ca. ten years, described
362 by a strong increase of basal respiration but at a constant stock of biomass and a
363 MMQ decrease as a consequence. During this early stage of succession, C-MR
364 increased too and the microbial C:N ratio reached its higher values (ca.11). The
365 bare peat situation can be considered as an initial stage of ecological succession
366 after a strong physical disturbance (extraction). One of the main characteristics of
367 the C-N dynamics in regenerating cutover peatlands is the strong change from
368 bare peat, more or less dried and compacted, to a complete revegetation. During
369 succession, matter and nutrient dynamics relate to many processes (Gorham,
370 Vitousek & Reiners 1979). In bare peat areas, microbial biomass represents the
371 most important nutrient living pool and microbial activity is the main factor acting
372 to alter and transform the chemical and physical properties in the peat profile but
373 microbial processes depend on peat chemistry. The correlations between peat
374 properties and microbial variables (Table 4) emphasized the role of substrate
375 quality. The low biomass during early stages of succession could be related to i)
376 the disturbance (exploitation before abandonment) which could limit the
377 development of microbes partly as a consequence of hyphal network disruption
378 during the extraction years (Wardle 1995, van der Wal *et al.* 2006); ii) the poor
379 quality of substrate, bare peat being very humified; iii) conditions of dryness,
380 despite the fact all sites were initially re-wetted. During this phase, the microbial
381 C:N ratio increased, implying changes in the microbial community composition
382 in relation to stress.

In a second phase, microbial biomass increased while aerobic basal respiration and C-MR always increased but at a lower rate, demonstrating a better C-use efficiency and a higher proportion of C incorporated into the biomass (Insam & Haselwandter (1989; Ohtonen *et al.* 1999). The increase of most of microbial variables could indicate that no limiting factors acted as controls of the development of microbial pools and activity. In this stage, significant plant colonization of the sites probably favoured the increasing microbial pool, providing available C-sources (Crow & Wieder 2005).

In a third phase, from 30-40 years after abandonment, the rates of increasing biomass slowed down while aerobic basal respiration and C-MR tended to reach a maximum level, illustrating new processes and changes in the functioning of these regenerating peatlands. MMQ and microbial C:N ratio decreased through the second and third stages. These trends could be linked with progressive growth of roots through peat and revealed the increasing influence of plants in the functioning of regenerating peatlands, especially on the microbial compartment and their activity as new competitors for nutrients (Kaye & Hart 1997). Zak *et al.* (1990) observed a similar trend in an old-field succession. The stabilization in the model could correspond to the maximum carrying capacity, depending on growth control factors (N and P content, organic matter, etc.) and secondly, the existence of threshold values of some key soil properties which need to be exceeded before development of microbes could occur (Van der Wal *et al.* 2006).

Anaerobic basal respiration in the surface peat showed a linear increase over the chronosequence, suggesting that the full potential of anaerobic activity was not reached. In the deeper peat, it was more difficult to bring out patterns of change over time. Re-wetting disturbed peat is the most important factor during

the regeneration of peatland but re-humectation of peat at the local scale would be more difficult and time consuming, in relation to the strong disturbance generated by extraction. With time, anaerobic conditions would become widespread and permanent and corresponding potential activity would increase while aerobic zones stabilize.

MICROBIAL VARIABLES *VS* PLANT COMMUNITY STRUCTURE

Our second objective focused on possible relationships between microbial variables and plant community composition and structure, measured by estimation of species richness and calculation of simple indices, including dominance and frequency of key-stone taxa such as *Sphagnum* and ericaceous shrubs. While the dominance index did not give any information, most of microbial variables correlated with bare peat surface (negatively) and plant species richness (positively) demonstrating the positive effect of plant development and diversity on microbial biomass in regenerating peatlands, as already demonstrated, especially in experimental grassland (Tilman *et al.* 1997; Hooper & Vitousek 1977; Loranger-Merciris *et al.* 2005). Correlations with moss and/or ericaceous indices are of some interest, in relation to peatlands dynamics. The development of shrubs (and trees) had already been recognized as a determinant factor of peat microbial biomass and MMQ changes (Chapman, Williams & Hawkins 2001; Fisk, Ruether & Yavitt 2003).

It was possible to separate the influence of 2 main groups of plant communities, on the one hand, sites with incomplete cover of vegetation and on the other hand, more diversified plant communities without bare peat, on both microbial C and N (Table 4). Despite no direct estimates of plant productivity, our

results probably illustrate the positive influence of increasing plant productivity on microbial compartments (Zak *et al.* 1990 & 2003, Wardle *et al.* 2004). This positive influence is most likely due to a combination of litter quality and quantity inputs and root activities as already demonstrated (Aerts, Verhoeven & Whigham 1999; Ström *et al.* 2003; Crow & Wieder 2005). In the first set of plant communities, the root activity and the input of litter are less important, both in quality and quantity. It was not possible to register any effect of plant composition on microbial biomass, i.e. to separate *Carex, Eriophorum* or *Sphagnum* when these plants were the dominant species on sites with incomplete colonization by plants. Nevertheless, different MMQ suggested differences in this set of plants. For instance, MMQ under Sphagnum was very low, while MMQ under *Carex* was the highest, suggesting different potentiality of energy sources for microbes. In the second set more consequent and diversified litter inputs would support higher microbial C and N biomass. Nevertheless, the EaPoCa site did not show significant difference of microbial biomass (C and N) with sites of the first set. This could be due to the contribution of *Polytrichum* species by which inhibition of microbes by allelopathic processes is recognized (Rozé 1987).

MICROBIAL VARIABLES AS INDICATORS OF CHANGE IN REGENERATING PEATLANDS

The third objective of this work was to assess the ability of microbial variables to be suitable indicators of peatland regeneration at a European scale. Microbial variables are increasingly used as ecological indicators of change after disturbance or stress (He *et al.* 2003) and soil quality (Winding, Hund-Rinke & Rutgers 2005). There are several definitions of ecological indicators that have been applied in

458 many ways (Niemi & McDonald 2004). He *et al.* (2003) and Winding, Hund-
459 Rinke & Rutgers (2005) reviewed the relationships between a set of
460 microbiological variables and their role in the soil and concluded of the interest in
461 studying several variables together. Here, we would especially focus on the
462 existence (or not) of thresholds or trends which could be used in relation to
463 disturbance and resilience as time of recovery ("engineering resilience",
464 Gunderson 2000). This aspect implies the existence of sufficient knowledge in
465 order to assess the results in comparison with reference systems. The variables
466 used need to keep the same accuracy over time in order to minimize the deviation
467 in assessment of responses to disturbance and resilience.

468 MMQ was one of the first variables used to study microbial succession and
469 changes over time (Insam & Haselwandter 1989; Anderson 1994) but its
470 disadvantages have been discussed (Wardle and Ghani 1995; Ohtonen *et al* 1999).
471 These authors suggested the MMQ would not be efficient in characterizing
472 microbial succession after disturbance because this quotient integrates both stress
473 and disturbance effects. In our chronosequence, MMQ fitted well to an
474 exponential regression, but high fluctuations were observed in the earlier-stages,
475 probably for both stress and disturbance effects.

476 In peatlands, a few authors have already used microbial variables as ecological
477 indicators in the context of land-use changes or to monitor the success of
478 restoration and wetland creation (Brake, Hoper & Joergensen 1999; Chapman,
479 Campbell & Puri 2003;Croft, Rochefort & Beauchamp 2001; Andersen, Francez
480 & Rochefort 2006).

481 Peat microbial pools (mainly C-N estimated with the fumigation-extraction
482 method), show significant higher values in pristine mires compared to peatlands

disturbed by mining (Croft, Rochefort & Beauchamp 2001) or young restored peatlands (Andersen, Francez & Rochefort 2006). Land-use changes impacted microbial C that ranged from a few hundred µg to about 14 mg g DP^{-1}, depending on the water regime (undrained *vs* drained, Baum, Leinweber & Schlichting 2003), nutrient content (pristine *vs* fertilized, Karsisto 1992, Williams & Silcock 1997) or vegetation (Fisk, Ruether & Yavitt 2003). Our models illustrated that microbial biomass levelled off around 5000 (C) and 1000 (N) µg gDP^{-1}. This level would correspond to a significant new equilibrium, following resistance and resilience processes, as theoretically described by Herrick (2000). As we did not know what were microbial C stocks before peat cutting in the different peatlands, we can only consider values of pristine peatlands published in literature as reference systems. Thus, microbial C biomass in natural raised-bogs would range from 3 to 6 mg C gDP-1 in the upper part (0-30 cm) of the peat profile (Karsisto 1992, Croft, Rochefort & Beauchamp 2001; Andersen, Francez & Rochefort 2006). More investigation is needed in order to propose detailed models, especially around the crucial question of thresholds (Groffman *et al.* 2006). Whatever it will be, our study illustrated that microbial biomass is of strong interest to model responses of peatland functioning after peat-cutting. It also showed that others variables such as basal respiration measured in both aerobic and anaerobic conditions constituted helpful complementary tools, demonstrating the validity of considering a set of variables as ecological indicators in the assessment of responses to changes.

Acknowledgements

507 This study was part of the RECIPE program funded by the EC (Contract n°
508 EVK2-CT-2002-00154) and by the Swiss Federal Office for Education and
509 Science. We are grateful to Mr Denis Le Gouix (Degussa Company Ltd, France),
510 Vapo Ltd. (Finland), Mr. George Watson (Scotland) and Espace Naturel Comtois
511 (France) for authorizing the different peatland access. The authors also thank
512 Marie-Paule Briand and Guillaume Morillon for their help with laboratory
513 analyses. Myriam Bormans and Cédric Wolf contributed to modelling.
514

514 **References**

515 Aerts, R., Verhoeven, J.T.A. & Whigham, D.F. (1999) Plant-mediated controls on
516 nutrient cycling in temperate fens and bogs. *Ecology,* **80**, 2170-2181.
517 Andersen, R., Francez, A.-J. & Rochefort, L. (2006) The physicochemical and
518 microbiological status of a restored bog in Québec: identification of relevant
519 criteria to monitor success. *Soil Biology & Biochemistry,* **38**, 1375-1387.
520 Anderson T.H. (1994) Physiological analysis of microbial communities in soil:
521 applications and limitations. *Beyond the biomass. Compositional and functional*
522 *analysis of microbial communities* (eds K. Ritz, Dighton J. & Giller K.E.), pp.
523 67-76. British Society of Soil Science, John Wiley and Sons, Chichester, New-
524 York, 2nd edition.
525 Baum, C., Leinweber, P. & Schlichting, A. (2003) Effects of chemical conditions
526 in re-wetted peats on temporal variation in microbial biomass and acid
527 phosphatase activity within the growing season. *Applied Soil Ecology,* **22**, 167-
528 174.
529 Blodeau, C. (2002) Carbon cycling in peatlands – A review of processes and
530 control. *Environmental Review,* **10**, 111-134.
531 Brake, M., Hoper, H. & Joergensen, R.Z.G. (1999) Land use-induced changes in
532 activity and biomass of microorganisms in raised bogs at different depths. *Soil*
533 *Biology & Biochemistry,* **31**, 1489-1497.
534 Buttler, A., Grosvernier, P. & Matthey, Y. (1998) A new peat sampler for
535 extracting undisturbed surface peat cores for growth pot experiments. *New*
536 *Phytologist,* **140**, 355-360.

537 Campbell, D.R., Lavoie, C. and Rochefort, L. (2002) Wind erosion and surface
538 stability in abandoned milled peatlands. *Canadian Journal of Soil Science,* **82**,
539 85-95.
540 Chapin, C.T., Bridgham, S.D., Pastor, J.& Updegraff, K. (2003) Nitrogen,
541 phosphorus, and carbon mineralization in response to nutrient and lime
542 additions in peatlands. *Soil Science,* **168**, 409-420.
543 Chapman, P.J., Williams, B.L. & Hawkins, A. (2001) Influence of temperature
544 and vegetation cover on soluble inorganic and organic nitrogen in a spodosol.
545 *Soil Biology & Biochemistry,* **33**, 1113-1121.
546 Chapman, S.J., Campbell, C.D. & Puri, G. (2003) Native woodland expansion:
547 soil chemical and microbiological indicators of change. *Soil Biology &*
548 *Biochemistry,* **35**, 753-764.
549 Chapman, S.J., Buttler, A., Francez, A.J., Laggoun-Défarge, F., Vasander, H.,
550 Schloter, M., Combe, J., Grosvernier, P., Harms, H., Epron, D., Gilbert, D. &
551 Mitchell, E. (2003) Exploitation of northern peatlands and biodiversity
552 maintenance: a conflict between economy and ecology. Frontiers in Ecology
553 and the Environment, **1**(10), 525-532.
554 Clymo, R.S. (1983). Peat. *Ecosystems of the world 4A ; Mires : swamp, bog, fen*
555 *and moor.* (ed. A.J.P. Gore), pp.159-217. Elsevier, New-York.
556 Clymo, R.S. (1984). The limits to peat bog growth. *Philosophical Transactions of*
557 *the Royal Society of London,* **B303**, 605-654.
558 Comont, L., Laggoun-Défarge, F. & Disnar, J.R. (2006) Evolution of organic
559 matter indicators in response to major environmental changes : the case of a
560 formerly cutover peatbog (Le Russey, Jura Mountains, France*). Organic*
561 *Geochemistry,* **37**, 1736-1751.

562 Croft, M., Rochefort, L. & Beauchamp, C.J. (2001) Vacuum-extraction of
563 peatlands disturbs bacterial population and microbial biomass carbon. *Applied*
564 *Soil Ecology,* **18**, 1-12.
565 Crow, S.E.& Wieder, R.K. (2005) Sources of CO_2 emission from a northern
566 peatland: root respiration, exudation, and decomposition. *Ecology,* **86**, 1825-
567 1834.
568 Daniels, R.E. & Eddy, A. (1985) *Handbook of European Sphagna.* Institute of
569 Terrestrial Ecology, Natural Environment Research Council. Cambrian News,
570 Aberystwyth, UK.
571 Dickinson, C.H. & Dooley, M.J. (1967) The microbiology of cut-away peat. I.
572 Descriptive ecology. *Plant and Soil,* **27**, 172-186.
573 Fisk, M.C., Ruether, K.F. & Yavitt, J.B (2003) Microbial activity and functional
574 composition among northern peatland ecosystems. *Soil Biology & Biochemistry,*
575 **35**, 591-602.
576 Francez, A.-J. & Loiseau, P. (1999) Le devenir de l'azote minéral dans une
577 tourbière à *Sphagnum fallax* et *Carex rostrata* du Massif central français.
578 *Canadian Journal of Botany,* **77**: 1136-1143.
579 Francez, A.J., Gogo, S. & Josselin, N. 2000. Distribution of potential CO_2 and
580 CH_4 production, denitrification and microbial biomass C and N in the profile of
581 a restored peatland in Brittany (France). *European Journal of Soil Biology,* **36**,
582 161-168.
583 Francez A.-J. & Vasander H. (1995) Peat accumulation and peat decomposition
584 after disturbance in French and Finnish mires. *Acta Oecologica,* **16**, 599-608.
585 Groffman, P., Baron, J.S., Blett, T., Gold, A.J., Goodman, I., Gunderson, L.H.,
586 Levinson, B.M., Palmer, M.A., Paerl, H.W., Peterson, G.D., LeRoy Poff, N.,

587 Rejeski, D.W., Reynolds, J.F., Turner, M.G., Weathers, K.C. & Wiens, J. (2006)
588 Ecological thresholds: the key to successful environmental management or an
589 important concept with no practical application? *Ecosystems,* **9**, 1-13.
590 Gorham, E., Vitousek, P.M. & Reiners, W.A. (1979) The regulation of chemical
591 budgets over the course of terrestrial ecosystem succession. *Annual Review of*
592 *Ecology and Systematics,* **10**, 53-84.
593 Grosvernier, P., Matthey, Y. & Buttler, A. (1995) Microclimate and physical
594 properties of peat : new clues to the understanding of bog restoration processes.
595 *Restoration of temperate wetlands* (eds B.D. Wheeler, S.C. Shaw, W.J. Fojt
596 W.J. & R.A. Robertson), pp. 435-450. John Wiley & Sons, Chichester, U.K.
597 Gunderson, L.H. (2000) Ecological resilience-theory to practice. *Annual Review*
598 *of Ecology and Systematics,* **31**, 421-439.
599 He, Z.E., Yang, X.E., Baligar, V.C. & Calvert, D.V. (2003) Microbiological and
600 biochemical indexing systems for assessing quality of acid soils. *Advances in*
601 *Agronomy,* **78**, 89-137.
602 Herrick, J.E (2000) Soil quality: an indicator of sustainable land management.
603 *Applied Soil Ecology,* **15**, 75-83.
604 Hooper, D.U. & Vitousek, P. M. (1997) The effects of plant composition and
605 diversity on ecosystem processes. *Science,* **277**, 1302-1305.
606 Insam H. & Haselwandter, K. (1989) Metabolic quotient of the soil microflora in
607 relation to plant succession. *Oecologia,* 79, 174-178
608 Karsisto, M. (1992) Microbiological and organic characterization of peat. *Suo*
609 *Mires and Peat,* **43**, 217-220.
610 Kaye, J.P. & Hart, S.C. (1997) Competition for nitrogen between plants and soil
611 organisms. *Trends in Ecology and Evolution,* **12**, 139-143.

612 Lavoie, C., Grosvernier, P., Girard, M. & Marcoux, K. (2003) Spontaneous
613 revegetation of mined peatlands: an useful restoration tool? *Wetlands Ecology*
614 *and Management,* **11**, 97-107.
615 Li, Y. & Vitt, H. (1997) Patterns of retention and utilization of aerially deposited
616 nitrogen in boreal peatlands. *Ecoscience,* **4**, 106-116.
617 Loranger-Merciris, G., Barthes, L., Gastine, A. & Leadley, P. (2006) Rapid
618 effects of plant species diversity and identity on soil microbial in experimental
619 grassland ecosystem. *Soil Biology & Biochemistry,* **38**, 2336-2343.
620 Magurran, A.E. (1988) *Ecological diversity and its measurement.* Croom Helm,
621 London.
622 Maire, N. (1983) Etude du repeuplement d'une tourbe stérilisée, par quatre
623 méthodes biologiques globales (ATP, dégagement de CO2, phosphatase et
624 uréase). *Recherche agronomique en Suisse,* **22**, 221-246.
625 Manugistics Inc (1995) *Stagraphics Plus for Windows, version 2.* Sigma Plus
626 distribution, Paris.
627 Neubauer, S.C., Givler, K., Valentine, S.K. & Megonical, P. (2005) Seasonal
628 patterns and plant-mediated controls of subsurface wetland biogeochemistry.
629 *Ecology,* **86**, 3334-3344.
630 Niemi, G.J. & McDonald, M.E. (2004) Application of ecological indicators.
631 *Annual Review of Ecology, Evolution and Systematics,* **35**, 89-111.
632 Ohtonen, R., Fritze H., Pennanen, T., Jumpponen, A. & Trappe, J. 1999.
633 Ecosystem properties and microbial community changes in primary succession
634 on a glacier forefront. *Oecologia,* **119**, 239-246.

635 Potila, H. & Sarjala, T. (2004) Seasonal fluctuation in microbial biomass and
636 activity along a natural nitrogen gradient in a drained peatland. *Soil Biology &*
637 *Biochemistry,* **36**, 1047-1055.
638 Robert, E.C., Rochefort, L. & Garneau, M. (1999) Natural revegetation of two
639 block-cut mined peatlands in eastern Canada. *Canadian Journal of Botany,* **77**,
640 447-459.
641 Rozé, F. (1987) Effets comparés des différentes litières sur la minéralisation et la
642 nitrification nettes de l'azote dans un sol de lande haute. *Revue d'Écologie et de*
643 *Biologie du Sol,* **24**, 273-282.
644 Salonen, V. (1994) Revegetation of harvested peat surfaces in relation to substrate
645 quality. *Journal of Vegetation Science,* **5**, 403-408.
646 Scanlon, D. and Moore, T. (2000) Carbon dioxide production from peatland soil
647 profiles : the influence of temperature, oxic/anoxic conditions and substrate. *Soil*
648 *Science,* **165**, 153-160.
649 Ström, L., Ekberg, A., Mastepanov, M. & Christensen, T.R. (2003) The effect of
650 vascular plants on carbon turnover and methane emissions from a tundra
651 wetland. *Global Change Biology,* **9**, 1185-1192.
652 SPSS Inc. (2000) *Systat 10.* SPSS Science Marketing, Chicago, USA.
653 Thormann, M.N., Currah, R.S. & Bayley, S.E. (2003) Succession of microfungal
654 assemblages in decomposing peatland plants. *Plant and Soil,* **250**, 323-333.
655 Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Sieman, E. (1997) The
656 influence of functional diversity and composition on ecosystem processes.
657 *Science,* **277**, 1300-1302.

658 Tuittila, E.-S., Rita, H., Vasander, H. & Laine, J. (2000) Vegetation patterns
659 around *Eriophorum vaginatum* L. tussocks in a cut-away peatland in southern
660 Finland. *Canadian Journal of Botany,* **78**, 47-58.
661 Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M. &
662 Webb, D.A. 51964-1980). *Flora europaea.* Volumes 1-5. Cambridge University
663 Press, Cambridge London.
664 Updegraff, K., Pastor, J., Bridgham, S. & Johnston, C. (1995) Environmental and
665 substrate controls over carbon and nitrogen mineralization in northern wetlands.
666 *Ecological Applications,* **5**, 151-163.
667 Van der Wal, A., van Veen, J.A., Smant, W., Boschker, H.T.S., Bloem, J., Kardol,
668 P., van der Putten & de Boer, W. (2006) Fungal biomass development in a
669 chronosequence of land abandonment. *Soil Biology & Biochemistry,* **38**, 51-60.
670 Vasander, H. & Kettunen, A. (2006) Carbon in Boreal Peatlands. *Boreal Peatland*
671 *Ecosystems* (eds R.K. Wieder & D.H. Vitt), Ecological Studies, 188, pp. 165-
672 194. Springer-Verlag, Berlin, Heidelberg.
673 Waddington, J., Rotenberg, P.A. & Warren, F.J. (2001) Peat CO_2 production in a
674 natural and cutover peatland: Implications for restoration. *Biogeochemistry,* **54**,
675 115-130.
676 Wardle, D.A. (1995) Impacts of disturbance on detritus food webs in agro-
677 ecosystems of contrasting tillage and weed management practices. *Advances in*
678 *Ecological Research,* **26**, 105-185.
679 Wardle, D.A. & Ghani, A. (1995) A critique of the microbial metabolic quotient
680 (qCO_2) as a bioindicator of disturbance and ecosystem development. *Soil*
681 *Biology & Biochemistry,* **27**, 1601-1610.

682 Wardle, D.H., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H.,
683 Wall, D.H. (2004) Ecological linkages between aboveground and belowground
684 biota. *Science,* **304**, 1629-1633.
685 Watson, A., Stephen, K.D., Nedwell, D.B. & Arah, J.R.M. (1997) Oxidation of
686 methane in peat: kinetics of CH_4 and O_2 removal and the role of plant roots. *Soil*
687 *Biology & Biochemistry,* **29**, 1257-1267.
688 Williams B. L., Shand, C.A., Hill M., O'Hara C., Smith, S. & Young, M.E. (1995)
689 A procedure for the, simultaneous oxidation of total soluble nitrogen and
690 phosphorus in extracts of fresh and fumigated soils and litters. *Communications*
691 *in Soil Science and Plant Analysis,* **26**, 91-106.
692 Williams, B.L. & Silcock, D.J. (1997) Nutrient and microbial changes in the peat
693 profile beneath *Sphagnum magellanicum* in response to addition of ammonium
694 nitrate. *Journal of Applied Ecology,* **34**, 961-970.
695 Williams, R.T. & Crawford, R.L. (1983) Effects of various physiochemical
696 factors on microbial activity in peatlands: aerobic biodegradative processes.
697 *Canadian Journal of Microbiology,* **29**, 1430-1437.
698 Winding, A., Hund-Rinke, K. & Rutgers, M. (2005) The use of microorganisms in
699 ecological soil classification and assessment concepts. *Ecotoxicology and*
700 *Environmental Safety,* **62**, 230-248.
701 Zak, D.R., Grigal, D.F., Gleeson, S. & Tilman D. (1990) Carbon and nitrogen
702 cycling during old-field succession: constraints on plant and microbial biomass.
703 *Biogeochemistry,* **11**, 111-129.
704 Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D. & Tilman, D. (2003) Plant
705 diversity, microbial communities, and ecosystem function: are there any links?
706 *Soil Science Society American Journal,* **61**, 2042-2050.
707

707 **Figure Legends**

708

709 **Fig. 1.** Responses of microbial biomass carbon (C) and nitrogen (N) and C:N ratio
710 *vs* a) time over the chrono-sequence (P<0.01, n=7; ■=surface, ▲=deep peat) and
711 b) plant communities (dotted histograms=surface, grey=depth) (detailed
712 abbreviations, see Table 3). Histograms with different letters are statistically
713 different (P<0.01).

714

715 **Fig. 2.** Responses of basal respiration, MMQ and C-MR to: a) time after
716 abandonment (square=surface, triangle=deep peat; black=aerobiosis,
717 open=anaerobiosis); b) plant community (only surface results shown; histograms
718 with different letters are statistically different at P<0.05).

719

Table 1. Peatland location and general characteristics.

Peatlands (Country code)	Coordinates	Altitude m	10-year average air T °C	Precipitation mm yr^{-1}	Water table depth (annual range) cm[#]	Bulk density (g DP L^{-1})*	C:N*†	N (%)*†	S (%)*†
Aitoneva (FIN)	62°12N-23°18E	156	4.2	694	-9.4±14.5(-32/36)	141±30	44±2	1.3±0.4	0.227±0.045
Middlemuir Moss (UK)	57°36N-2°9W	110	8.0	1109	14±14 (-51/-6)	147±87	38±5	1.5±0.2	0.395±0.037
Baupte (FB)	49°17N-1°21E	4	11.4	890	58±7 (-95/-15)	121±32	22±2	2.4±0.2	0.500±0.053
Le Russey (FR)	47°10N-6°47E	867	7.7	1417	11±7 (-26/0)	119±40	29±4	1.9±0.4	0.028±0.047
La Chaux d'Abel (CH)	47°10N-6°57E	1040	6.4	1463	16±7 (-41/-4)	101±53	27±6	2.1±0.6	0.074±0.045

[#] Negative value indicates site with periodic flooding

* mean ± SD; DP = dry peat

† Total organic element in peat, performed by combustion at 1100°C with a CNS-2000 LECO apparatus (Comont, Laggoun-Défarge & Disnar 2006).

Table 2. Description of studied sites and used codes in modelling of microbial variables responses to regeneration age and plant community structure (index mean ± SD).

Peatland (Code)	Sites	Age*	Dominant plant species	Vegetation code	Bare peat cover (%)	Richness S	Indices d	m	e
Aitoneva	A	10	*E. vaginatum*†, *Utricularia*	EvBp	21	8.0±2.0	0.42	0.25	0.00
(FIN)	B	10	*E. vaginatum*	EvBp	81	4.7±1.5	0.95	0.00	0.00
	C	10	*Carex rostrata, Sphagnum*	CaBp	47	6.3±0.6	0.42	0.42	0.00
	D	10	*Sphagnum* spp	SpBp	10	6.7±1.5	0.61	2.30	0.00
	E	10	Bare peat	Bp	100	0	-	-	-
Middlemuir	A	3	Bare peat	Bp	95	1	-	-	-
Moss	B	7	*Sphagnum spp*	SpBp	12	8.3±0.6	0.29	0.413	0.22
(UK)	C	7	*E. angustifolium,, Sphagnum spp*	EaBp	14	7.7±2.3	0.57	0.313	0.08
	D	55	*Sphagnum* spp, *C. vulgaris*#, *D. flexuosa*ᚴ	SpCaDe	0	11.3±2.1	0.33	0.487	0.37
Baupte	A	7	Bare peat	Bp	100	0	-	-	-
(FB)	B	7	*E. angustifolium*	EaBp	22	6.7±1.1	0.574	0.314	0.00
Le Russey	B	22	*E. angustifolium, Polytrichum ssp, C. vulgaris*	EaPoCa	0	7.5±2.1	0.478	0.600	0.20
(FR)	C	34	*Sphagnum* spp, *E. vaginatum, V. oxycoccos*‡	SpEvVa	0	9.3±0.5	0.577	2.025	0.48
Chaux d'Abel	B	42	*Sphagnum* spp, *E. vaginatum*	SpEv	0	6.5±1.3	0.605	1.642	0.00
(CH)	C	55	*Sphagnum* spp, *E. vaginatum, V.* spp	SpEvVa	0	5.3±2.2	0.641	2.946	0.19

* mean age estimation from local surveys and dendrochronology

† *E. = Eriophorum ; # C. = Calluna,* ᚴ*D. = Deschampsia,* ‡ *V. = Vaccinium* (nomenclature after Daniels & Eddy 1985; Tutin *et al.* 1985).

Table 3. H-statistics and P-values of Kruskal-Wallis test for microbiological variables by depth, age of regeneration and vegetation.

Effect:	Depth	Age	Plant
Variables:			
Microbial biomass			
Carbon (µg C g DP^{-1})	24.6***	68.2***	71.3***
Nitrogen (µg N g DP^{-1})	12.5**	41.3***	45.5***
Microbial C:N	6.0 NSD	6.4 NSD	10.0 NSD
Basal respiration (µg CO_2–C g DP^{-1} h^{-1})			
Aerobiosis	7.8 NSD	81.7***	57.2***
Anaerobiosis	15.7**	34.5***	31.5***
Aerobic:anaerobic basal respiration	5.8 NSD	109.0***	72.1***
MMQ (d^{-1}) †			
Aerobic MMQ	21.2***	84.4***	75.9***
Anaerobic MMQ	17.0***	73.8***	77.1***
C-MR (d^{-1}) ‡			
Aerobic C-MR	13.5**	60.1***	43.9***
Anaerobic C-MR	34.7***	26.9***	16.4*

† Microbial Metabolic Quotient, ‡ Soluble organic C Mineralization Rate
NSD, no significant difference; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Matrix of Spearman rank correlation coefficients between microbial variables *vs* peat properties (surface + deep peat; 34 ≤ n ≤ 57) and vegetation indices over the succession (n=7, peat surface only).

	C:N	N (%)	S (%)	Bare peat cover (%)	Dominance (d)	Richness (S)	Moss index (m)	Ericacaeous index (e)
C-FE microbial biomass	-0.357*	0.253 NSD	-0.605***	-0.847*	0.318 NSD	0.728*	0.700*	0.610 NSD
N-FE microbial biomass	-0.338*	0.257 NSD	-0.446**	-0.712*	0.316 NSD	0.586 NSD	0. 333 NSD	0.475 NSD
Aerobic respiration	-0.027 NSD	-0.056 NSD	-0.411***	-0.441 NSD	0.467 NSD	-0.167 NSD	0.483 NSD	-0.051 NSD
Anaerobic respiration	-0.119 NSD	0.016 NSD	-0.374**	-0.746*	-0.183 NSD	0.728*	0.833*	0.458 NSD
MMQ aerobiosis †	0.380**	-0.300**	0.423**	0.797*	-0.001 NSD	-0.879*	-0.667 (*)	-0.797*
MMQ anaerobiosis	0.365**	-0.279*	0.496***	0.848*	-0.133 NSD	-0.946**	-0.583 (*)	-0.763*
C-MR aerobiosis ‡	-0.049 NSD	0.019 NSD	0.197 NSD	-0.288 NSD	0.600 (*)	-0.042 NSD	0.367 NSD	-0.051 NSD
C-MR anaerobiosis	-0.312*	0.261 NSD	-0.159 NSD	-0.932**	-0.367 NSD	0.778*	0.767*	0.661 NSD

† Microbial Metabolic Quotient, ‡ Soluble organic C Mineralization Rate
NSD, no significant difference; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 5. W-statistics and P-values of Mann-Whitney Wilcoxon test for aerobiosis *vs* anaerobiosis microbial variables in surface layers by vegetation. "Bare peat" results are added as a comparison, see Table 2 about vegetation codes.

Vegetation	Basal respiration	MMQ	C-MR
SpBp	NSD, P=0.609	NSD, P=0.936	NSD, P=0.999
Carex	A>aN, 0**	A>aN, 0**	A>aN, 0**
EaBp	A>aN, 22**	A>aN, 41*	A>aN, 36*
EvBp	A>aN, 0**	A>aN, 0**	A>aN, 0**
SpEv	NSD, P=0.701	NSD, P=0.753	NSD, P=0.936
EaPoCa	NSD, P=0.470	NSD, P=0.194	NSD, P=0.312
SpEvVa	A>aN, 2*	A>aN, 3*	NSD, P=0.144
SpCaDe	A<aN, 30*	NSD, P=0.531	NSD, P=0.531
Bare peat *(Bp)*	A>aN, 80**	A>aN, 74 **	A>aN, 88*

† See Table 2 for codes

NSD, no significant difference; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

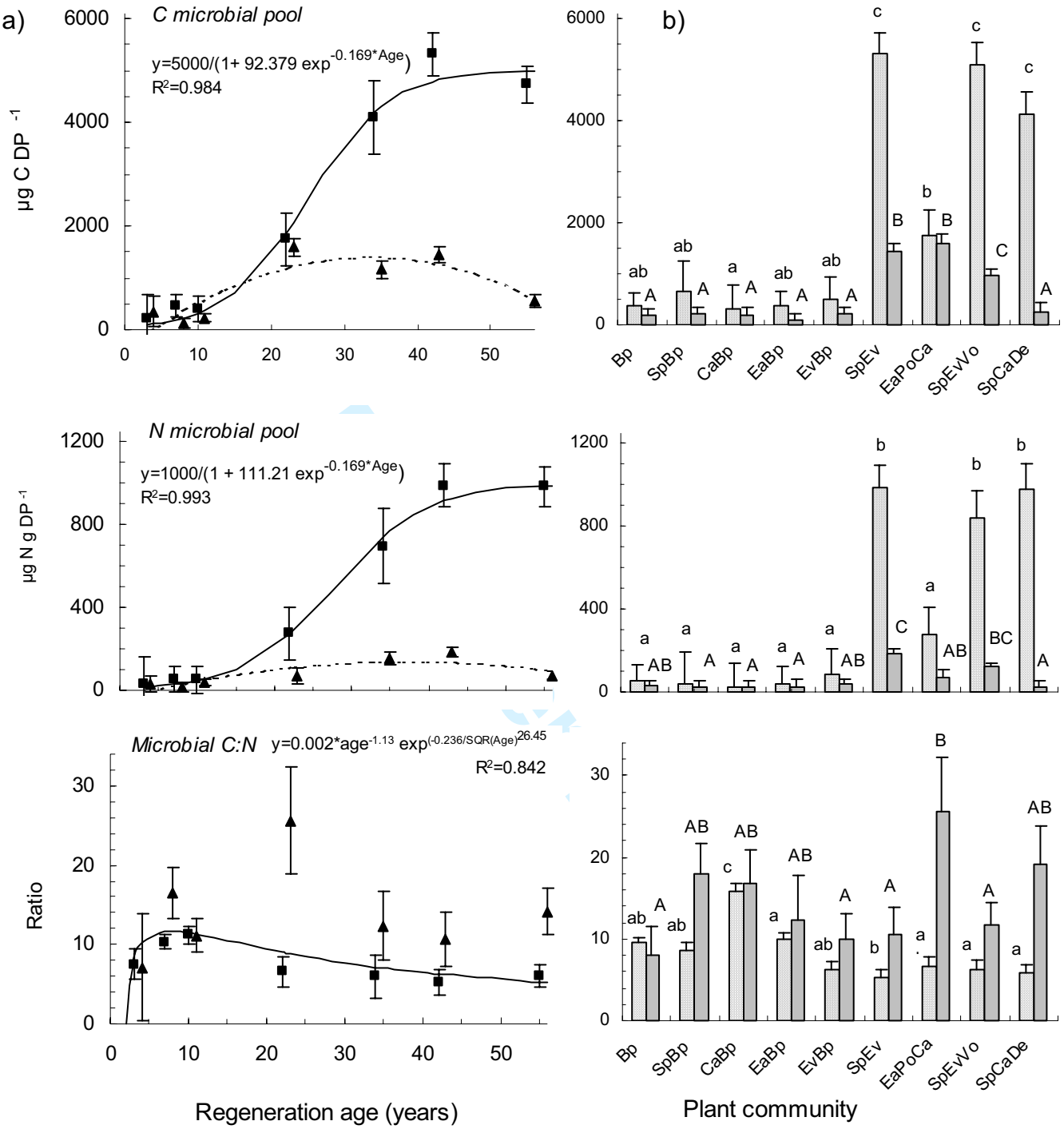

Fig. 1. Responses of microbial biomass carbon (C) and nitrogen (N) and C:N ratio *vs* a) time over the chrono-sequence (P<0.01, n=7; ■=surface, ▲=deep peat) and b) plant communities (dotted histograms=surface, grey=depth) (detailed abbreviations, see Table 3). Histograms with different letters are statistically different (P<0.01).

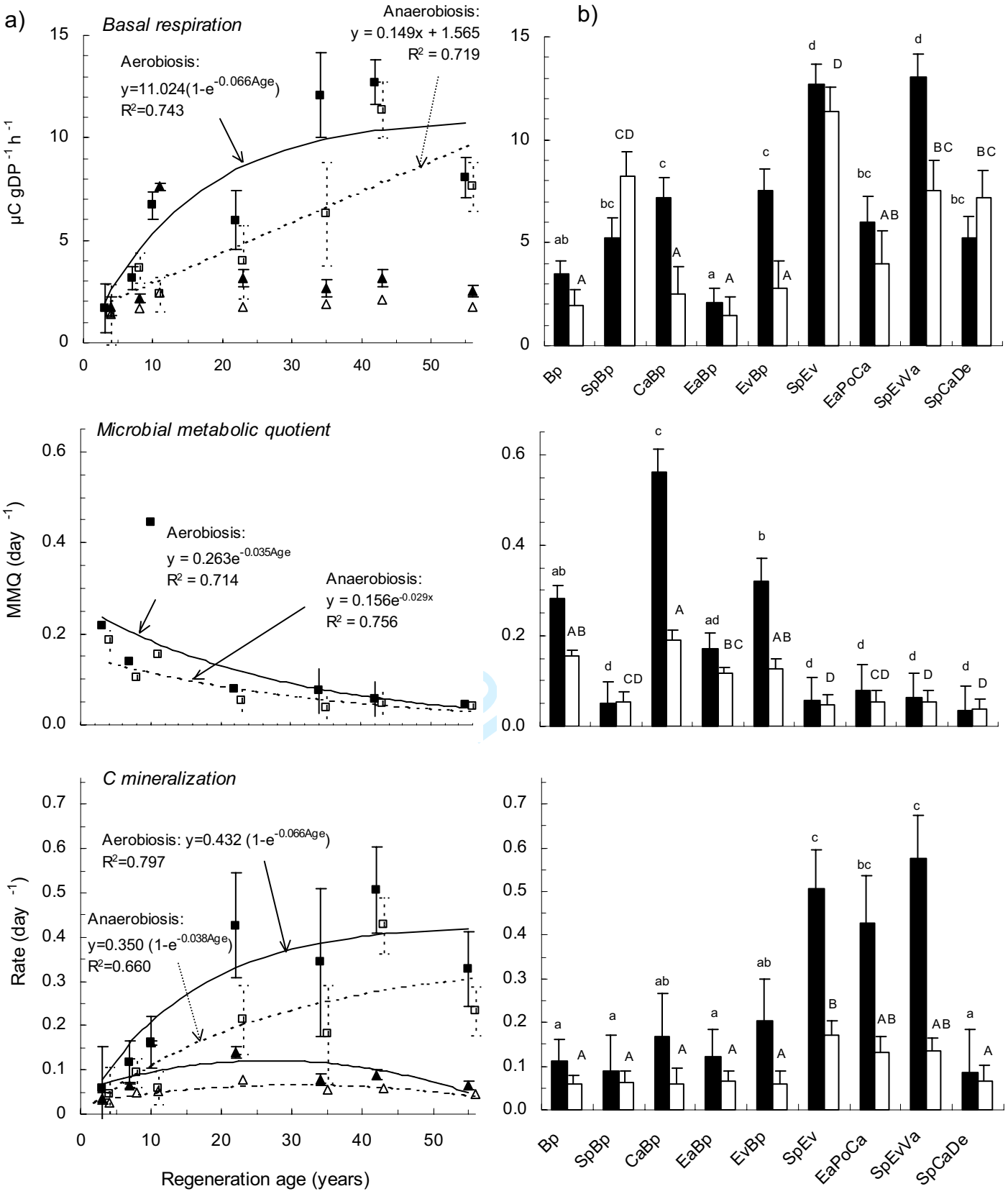

Fig. 2. Responses of basal respiration, MMQ and C-MR to: a) time after abandonment (square=surface, triangle=deep peat; black=aerobiosis, open=anaerobiosis); b) plant community (only surface results shown; histograms with different letters are statistically different at $P<0.05$).