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Structure of Microbial Communities in *Sphagnum* Peatlands and Effect of Atmospheric Carbon Dioxide Enrichment

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ABSTRACT

Little is known about the structure of microbial communities in *Sphagnum* peatlands, and the potential effects of the increasing atmospheric CO_2 concentration on these communities are not known. We analyzed the structure of microbial communities in five *Sphagnum*-dominated peatlands across Europe and their response to CO_2 enrichment using miniFACE systems. After three growing seasons, *Sphagnum* samples were analyzed for heterotrophic bacteria, cyanobacteria, microalgae, heterotrophic flagellates, ciliates, testate amoebae, fungi, nematodes, and rotifers. Heterotrophic organisms dominated the microbial communities and together represented 78% to 97% of the total microbial biomass. Testate amoebae dominated the protozoan biomass. A canonical correspondence analysis revealed a significant correlation between the microbial community data and four environmental variables (Na^+, DOC, water table depth, and DIN), reflecting continentality, hydrology, and nitrogen deposition gradients. Carbon dioxide enrichment modified the structure of microbial communities, but total microbial biomass was unaffected. The biomass of heterotrophic bacteria increased by 48%, and the biomass of testate amoebae decreased by 13%. These results contrast with the absence of overall effect on methane production or on the vegetation, but are in line with an increased below-ground vascular plant biomass at the same sites. We interpret the increase in bacterial biomass as a response to a CO_2-induced enhancement of *Sphagnum* exudation. The causes for the decrease of testate amoebae are unclear but could indicate a top-down rather than a bottom-up control on their density.

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Introduction

Because of their fast turnover rates, cosmopolitan distribution, and ubiquity, soil microorganisms may have a potential as biomonitors of the effects of global change or other perturbations on ecosystems [18, 35]. However, the value of microorganism community structure as early indicators of environmental changes is hardly explored. Among the exceptions is the use of testate amoebae in ecological and paleoecological studies of peatlands [8, 40]. The present relative scarcity of baseline data makes it difficult to assess the magnitude of the response of soil microbes to global change [48], and this is especially true in nonagricultural ecosystems, such as peatlands.

Sphagnum peatlands are widely distributed in the temperate and boreal zones of the Northern Hemisphere where variations in climatic and geomorphologic conditions give rise to different peatland types [11]. In addition to this, human activities have affected peatlands both directly (drainage and peat harvesting) and indirectly (e.g., N deposition) [24]. Natural *Sphagnum* peatlands are nutrient-poor ecosystems, and are usually N-limited, except in areas with high rates of N deposition resulting from human activities [1]. We hypothesized (hypothesis 1) that microbial communities differed among peatland sites across Europe and that these differences could be related to moisture conditions and water chemistry.

Elevated atmospheric CO_2 was shown to stimulate the photosynthesis rate of C-3 plants, including *Sphagnum* [52]. However, this "CO_2 fertilization" was also shown to cause nutrients to become even more limited owing to increased nutrient uptake by plants to balance their C supply [33, 34]. Elevated CO_2 is therefore unlikely to increase plant production in nutrient-poor ecosystems, but may nevertheless affect soil organisms indirectly through changes in soil or litter chemistry [5, 16] or increased availability of labile C through exudation [13]. This increased input of C in the soil may in turn stimulate mineralization [4] or N fixation [17]. An increased input of labile C due to elevated CO_2 is therefore likely to have a significant impact on heterotrophic microorganisms. Different microbial functional groups living in *Sphagnum* are likely to react differently to CO_2 enrichment. Under low N concentrations, photosynthetic microorganisms compete well for N, and, owing to a greater surface-to-volume ratio, smaller species have a competitive advantage over larger species [27]. Different CO_2 effects may therefore be expected within photosynthetic microorganisms: cyanobacteria are able to fix atmospheric N and would have a competitive advantage over other photosynthetic microorganisms under low-N conditions [19]. We therefore hypothesized that CO_2 enrichment would have two main indirect effects: (hypothesis 2) an increase in the biomass of heterotrophic microorganisms through increases in the supply of labile C, and (hypothesis 3) a decrease in the biomass of autotrophic microorganisms other than cyanobacteria through a reduction of available N.

To assess the relationships between microbial communities and environmental variables and the sensitivity of these communities to high atmospheric CO_2, we analyzed microbial groups in *Sphagnum* samples taken after 3 years of CO_2 enrichment in five peatlands in Europe. The results we present here were obtained from the first *in situ* CO_2 enrichment in *Sphagnum* peatlands [28]. To our knowledge, this is one of the few studies on microbial communities in *Sphagnum*, including heterotrophic bacteria, cyanobacteria, microalgae, heterotrophic flagellates, ciliates, testate amoebae, fungi, and two metazoan groups of microbial size, nematodes and rotifers, and the first study of CO_2 effect on these communities.

Methods

Study Sites

Five sites were chosen to be representative of local *Sphagnum* lawn communities in ombrotrophic or near-ombrotrophic peatlands in the Swiss Jura Mountains (La Chaux-des-Breuleux), eastern Finland (Salmisuo Mire), the Netherlands (Dwingeloo), south Sweden (Kopparås Mire), and northwest England (Roudsea). The Swiss site (CH) is situated on the bottom of a shallow valley, on impermeable marl deposits. The mire was drained and peat was mined until the end of World War II. Between the drainage ditches a secondary bog vegetation has reestablished [26] and a mosaic of lawn, hummocks, and depressions is now well developed with *Eriophorum vaginatum*, *Carex nigra*, *Vaccinium oxycoccos*, *Sphagnum fallax*, and *Polytrichum strictum*. The Finnish site (FI) is mainly open with scattered small *Pinus sylvestris* trees. Most parts are slightly minerotrophic, but small ombrotrophic spots exist. The vegetation is dominated by *Eriophorum vaginatum* and *Carex pauciflora* and, to a lesser extent, by dwarf Ericaceae shrubs. On lawn communities, the dominant peat mosses are *Sphagnum balticum* and *Sphagnum papillosum* with the subdominants *Sphagnum magellanicum* and *Sphagnum rubellum*. The Dutch site (NL) is a small (7500 m^2) peat area that developed in a depression over loamy sand and boulder clay. Up to 1955 the site was used for peat cutting. The vegetation consists of a mosaic of pools, carpets, and hummocks. Common species are *Erica tetralix*, *Vaccinium oxycoccos*, and *Sphagnum magel-*

lanicum. Monoliths 1.1 m in diameter were extracted from the sites and kept in large containers outside the University of Wageningen. The Swedish site (SE) is slightly minerotrophic, but smaller spots are ombrotrophic. The lawn vegetation at the studied part of the mire is dominated by *Eriophorum angustifolium*, *Calluna vulgaris*, *Andromeda polifolia*, *Narthecium ossifragum*, *Scirpus caespitosus*, *Sphagnum magellanicum*, *Sphagnum papillosum*, and *Sphagnum rubellum*. The British site (UK) has been drained, but the drainage ditches were blocked and secondary vegetation is well established. Lawn communities with *Eriophorum vaginatum*, *Scirpus caespitosus*, *Erica tetralix*, and *Sphagnum papillosum* dominate the site. Authorities for plant species follow Corley et al. [15] for mosses and Tutin et al. [50] for vascular plants.

Experimental Setup and Variables Measured

The experiments started in spring 1996 and ended in autumn 1998. At each site 10 mini-FACE rings [38] with a diameter of 1 m (in NL 1.1 m) were randomly laid out on the bog surface. In five rings the atmospheric CO_2 concentration was kept at ambient levels (about 360 ppm while in the other rings the CO_2 concentration was maintained at 560 ppm for 24 h day^{-1}. A CO_2 concentration of 560 ppm represents a doubling of preindustrial atmosphere CO_2 concentrations and corresponds to the projected concentration for year 2120 approximately at the current annual increase of atmospheric CO_2 of 3.3 petagrams (Pg) yr^{-1} [45] (1 Pg = 10^{15} g). The elevated CO_2 rings were located at a distance of at least 6 m from ambient air rings to prevent CO_2 pollution. Blowers next to each FACE ring supplied ambient air or CO_2-enriched air to circular tubes resting on the bog surface on which 72 small venting pipes were mounted. The venting pipes had small holes at 6 and 12 cm height above the moss surface. Air was sampled in the middle of elevated CO_2 rings at 7.5 cm above the moss surface (mid-canopy level) and analyzed for CO_2 with an infrared gas analyzer. Based on the measured CO_2 concentration and wind speed, the CO_2 supply was adjusted automatically via a PC and mass flow controllers to maintain the target concentration of 560 ppm. During winter months the FACE system was turned off because of minimal biological activity and snow cover at the FI, SE, and CH sites. Calibration and test experiments were conducted to optimize and evaluate the performance of the FACE equipment. Further details on the miniFACE system and its performances are given in Miglietta et al. [38]. To avoid edge effects, no samples were collected in the outer 15 cm of each plot. The choice of working *in situ* and with miniFACE systems [38] allowed us to keep the disturbance and artificiality of the experiment at a minimum level. This is especially important in the case of microorganisms, which may be affected by changes in physicochemical conditions inherent in laboratory conditions. Further description of the five sites and the relationships among the vegetation, testate amoebae communities, and water chemistry was presented previously [39]. Water table depths were monitored weekly in piezometers inserted into each plot. In all sites the samples were taken. Mean water table depths (WTD), snow cover duration, and levels of N deposition are given in Table 1.

Water Chemistry

Water samples were collected from all plots at the five sites using Millipore soil moisture samplers (Rhizon, Eijkelkamp B.V., The Netherlands). The samplers were inserted in the moss carpet as close as possible to the water table and connected to pre-evacuated glass bottles. The water samples were analyzed at Wageningen Agricultural University for DOC (dissolved organic C), pH, total N and P, and major cations and anions following standard protocols [12] (Table 2). DOC was calculated as total C (measured by NDIR following oxidation) minus inorganic C (measured by NDIR following mineralization by H_2PO_4). Nitrate was measured with an HPLC by means of separation on an ion-exchange column and detection with refractive index [12]. Ammonium and sulfate were measured colorimetrically. Total N, chloride, and aluminum were measured using an elemental analyzer. Sodium and potassium were measured by flame emission spectroscopy. Calcium, magnesium, and iron were measured on an atomic absorption spectroscopy.

Microbial Community Analyses

At the end of the experiment (end of summer 1998) 10–20 *Sphagnum* mosses were collected in each plot in *Sphagnum* lawn communities. The top 5 cm of the mosses were fixed in glutaraldehyde solutions (2% final concentration) in the field. Six samples (three of five elevated [CO_2] plots and three of five ambient [CO_2] plots) from each site were analyzed for microorganisms. Microbial communities were analyzed using a similar approach to the one used previously for *Sphagnum* mosses by Gilbert et al. [20, 21].

Samples were homogenized for 1 min with a vortex before sampling for the different microbial groups. Although we believe that this procedure removes most of the microorganisms living in the pore space between the leaves, it is unlikely to be sufficient to remove the microorganisms living in the large, dead *Sphagnum* cells (hyalocysts). The smaller microorganisms, such as bacteria, cyanobacteria, fungi, the smaller protists, and even some micro-metazoans, are able to enter the pores (typically 5–10 μm in diameter). Thus our counts most likely underestimate to some extent the actual numbers.

For each sample, three different slide preparations were analyzed: (1) Heterotrophic bacteria were stained with DAPI (4,6-diamino-2-phenylindole), filtered on 0.2-μm black membrane filters, and examined by epifluorescence microscopy. The image was recorded using a digital camera. Bacteria numbers and sizes were estimated using the LEICA QWIN image analysis program. A minimum of 10 random fields were counted for each sample. (2) Heterotrophic flagellates and the smaller (<20 μm) cyanobacteria and algae were stained with primulin solution (Direct Yellow 59), filtered on 0.8-μm membrane filters, and examined by epifluorescence microscopy. Autotrophic microorganisms were sepa-

Table 1. Location, climatic data, nitrogen deposition, and water table depth at the five BERI sites

Site	location	Mean daily temperature (°C)		Mean annual precipitation (mm y^{-1})	Number of days with snow cover	N deposition (g N m^{-2} y^{-1})	Water table depth[a] (mm)
		Coldest month	Warmest month				
CH	La Chaux-des-Breuleux, Swiss Jura (47°13′ N, 07°03′ E)	−5	15	1390	80–120	1.8	324
FI	Salmisuo, Ilomantsi, eastern Finland (62°47′ N, 30°56′ E)	−12	16	600	183	0.4	106
NL	Peat from Dwingeloo (52°49′ N, 6°25′ E) transplanted to Wageningen (51°99′ N, 05°70′ E)	2	17	805	<10	3.9	92
SW	Kopparåsmyre, southern Sweden (57°08′ N)	−2	16	800	90–100	0.8	102
UK	Roudsea bog, North West England (54° 14′N, 03°	1	13	1800	20	2	106

[a] Average over 3 years 1996–98 weekly measurements during the growing season.

rated from similar-sized heterotrophic microorganisms (e.g., flagellates) by their autofluorescence. A variable number of random fields were analyzed. The total number of fields counted was different for each group and depended on its abundance in order to ensure a reliable estimate. (3) Densities of testate amoebae, ciliates, nematodes, rotifers, fungi, and the larger (>20 μm) cyanobacteria and algae were estimated in plankton chambers at 400× magnification. A 1-mL subsample was allowed to settle during 24 h in a plankton chamber. The slide was then scanned either completely for the larger groups (testate amoebae, nematodes, rotifers, the larger algae), or, as for the second slide, a variable number of random fields was analyzed for the smaller, more numerous microorganisms (algae, fungi, cyanobacteria). The first author carried out all analyses to ensure comparability among samples.

Although protozoa, cyanobacteria, and microalgae all contain mixotrophic species that are able to use both CO_2 and organic C as a source of C [3], we use the categories heterotrophic (for heterotrophic bacteria, fungi, micrometazoans, and protozoa) and autotrophic (for cyanobacteria and microalgae). Biovolumes of each community were estimated by assuming geometrical shapes and converted to carbon using the following conversion factors: heterotrophic bacteria, 1 $\mu m^3 = 5.6 \times 10^{-7}$ μg C [10] cyanobacteria and algae, 1 $\mu m^3 = 1.2 \times 10^{-7}$ μg C; flagellates, 1 $\mu m^3 = 2.2 \times 10^{-7}$ μg C [9]; ciliates and testate amoebae, 1 $\mu m^3 = 1.1 \times 10^{-7}$ μg C [53]; fungi, 1 $\mu m^3 = 2.5 \times 10^{-7}$ μg C; nematodes and rotifers, 1 $\mu m^3 = 1.25 \times 10^{-7}$ μg C [20]. Biovolumes of fungi were estimated by measuring the length and diameter of hyphae and by counting and measuring spores. These data were expressed as μg C per gram of *Sphagnum* dry mass.

Numerical Analyses

The relationships between microbial communities and environmental variables (water chemistry and water table depth) across the five sites were analyzed using a partial canonical correspondence analysis (CCA) in which the CO_2 treatment was treated as a co-variable (to focus only on patterns unrelated to the CO_2 treatment), and water chemistry variables and the mean water table depth were used as environmental variables. A forward selection procedure was used to determine to which explanatory variables the community data were most strongly correlated. Monte-Carlo permutation tests were used to determine the significance of the variables. Nonsignificant variables were included as passive variables as in a correspondence analysis. To analyze the effect of elevated CO_2 on microbial communities while accounting for intersite and within-site variability, we performed a MANOVA, with site (= country; nominal variable), treatment (elevated CO_2; ambient CO_2), and treatment * site as factors using the program JMP 3.2.6. Carbon biomass data were transformed using $\ln(x+1)$, square root, or 4th-order root to homogenize variances. The total microbial biomass was analyzed using an ANOVA with the same factors as in the MANOVA. The significance level was set at $P < 0.05$.

Table 2. Fall water chemistry from the samping sites (mg L^{-1} exept for pH)[a]

	CH		FI		NL		SE		UK	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
pH	5.56	0.22	4.29	0.13	4.13	0.08	4.45	0.03	4.09	0.03
DOC	44.73	2.56	26.53	0.83	71.79	5.86	20.64	2.23	44.24	1.44
N_{tot}	0.62	0.06	0.14	0.02	1.13	0.37	0.32	0.02	0.72	0.07
DIN[b]	0.11	0.03	0.01	0.01	0.08	0.05	0.02	0.01	0.13	0.04
P	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
K	0.06	0.01	0.00	0.00	0.08	0.03	0.06	0.01	0.07	0.02
Ca	1.97	0.25	0.26	0.09	0.48	0.15	0.65	0.15	0.80	0.28
Mg	0.07	0.01	0.06	0.00	0.24	0.03	0.25	0.05	0.25	0.01
Na	0.13	0.03	0.05	0.00	0.73	0.04	0.44	0.09	0.84	0.03
Al	0.28	0.03	0.22	0.10	0.25	0.08	0.07	0.02	0.22	0.07
Fe	0.70	0.09	0.52	0.03	2.73	1.08	0.38	0.15	0.83	0.07
NH_4	0.12	0.03	0.02	0.01	0.11	0.06	0.02	0.01	0.16	0.05
NO_3	0.07	0.04	0.00	0.00	0.01	0.00	0.02	0.02	0.02	0.01
SO_4	0.60	0.15	0.00	0.00	0.13	0.13	1.57	0.60	1.15	0.22
Cl	0.45	0.11	0.42	0.04	11.08	1.29	4.90	1.65	8.70	0.25

[a] $N = 6$ per site.
[b] DIN = NO_3^- - N + NH_4^+ - N.

Results

Water Chemistry

The Swiss site had higher pH and P and had a lower mean water table than the other four sites (Tables 1 and 2). The Dutch site had the highest DOC and total N, as well as most measured chemical species. The oceanic–continental gradient was illustrated by the concentrations of Na^+ and Cl^-: high in NL and UK, intermediate in SE, and low in FI and CH. The N pollution gradient was best reflected by total N and DIN (dissolved inorganic N: NH_4^+-N + NO_3^--N). Total N decreased in the order NL>UK>CH>SE>FI. The concentrations of DIN followed a similar pattern, with higher values in UK, CH, and NL and low values in SE and FI.

Microbial Communities

Heterotrophic bacteria were the dominant microbial group in all sites (Fig. 1). Fungi, microalgae, or testate amoebae were the second dominant group, depending on the site. Microalgae represented a significant proportion of the total microbial biomass only in the Swedish and British sites. On average over the five sites, the microalgae biomass was dominated by Desmidiaceae, such as *Penium* sp., Euglenophyceae, such as *Euglena* sp. and *Trachelomonas* sp., and Bacillariophyceae (diatoms), such as *Eunotia* sp. and *Pinnularia* sp. Other algal groups represented a smaller proportion of the microalgae biomass (details not illustrated). A total of 58 testate amoebae species were recorded in the samples. Most frequent species overall were *Euglypha strigosa*, *Euglypha laevis*, *Assulina muscorum*, *Hyalosphenia elegans*, *Euglypha compressa*, *Nebela tincta* var. *major*, *Hyalosphenia minuta*, *Assulina seminulum*, *Nebela militaris*, *Hyalosphenia papilio*, *Heleopera sylvatica*, *Nebela griseola*, and *Nebela tincta*. The five study sites had characteristic testate amoebae species assemblages and plant communities, which were detailed in a previous study [39]. Cyanobacteria represented a marginal proportion of the total microbial biomass in all sites and were dominated by Chroococcales, such as *Chroococcus* sp., *Merismopedia* sp., and *Microcystis* sp., and Nostocales species, such as the nitrogen-fixing *Anabaena* sp. and *Nostoc* sp. Rotifera were dominated by bdelloids, among which was the test forming species *Habrotrocha angusticollis* Murray. Other types, such as *Colurella* sp., were also recorded. Nematodes ranged from under 100 μm to over 1 mm in length, but most individuals counted (58%) were between 200 and 400 μm in length.

Intersite Differences, and Correlation with Water Chemistry and Water Table Depth

The total microbial biomass (Fig. 2) varied among sites (significant site effect in the ANOVA model; $P < 0.0001$). The structure of microbial communities also varied among sites (significant site effect in the whole MANOVA model and for every microbial group; Fig. 2, Table 3). These differences were most clearly illustrated by the relative contribution of different microbial groups to the total biomass (Fig. 1). The Swiss site stood out as having a greater relative proportion of fungi than the other sites.

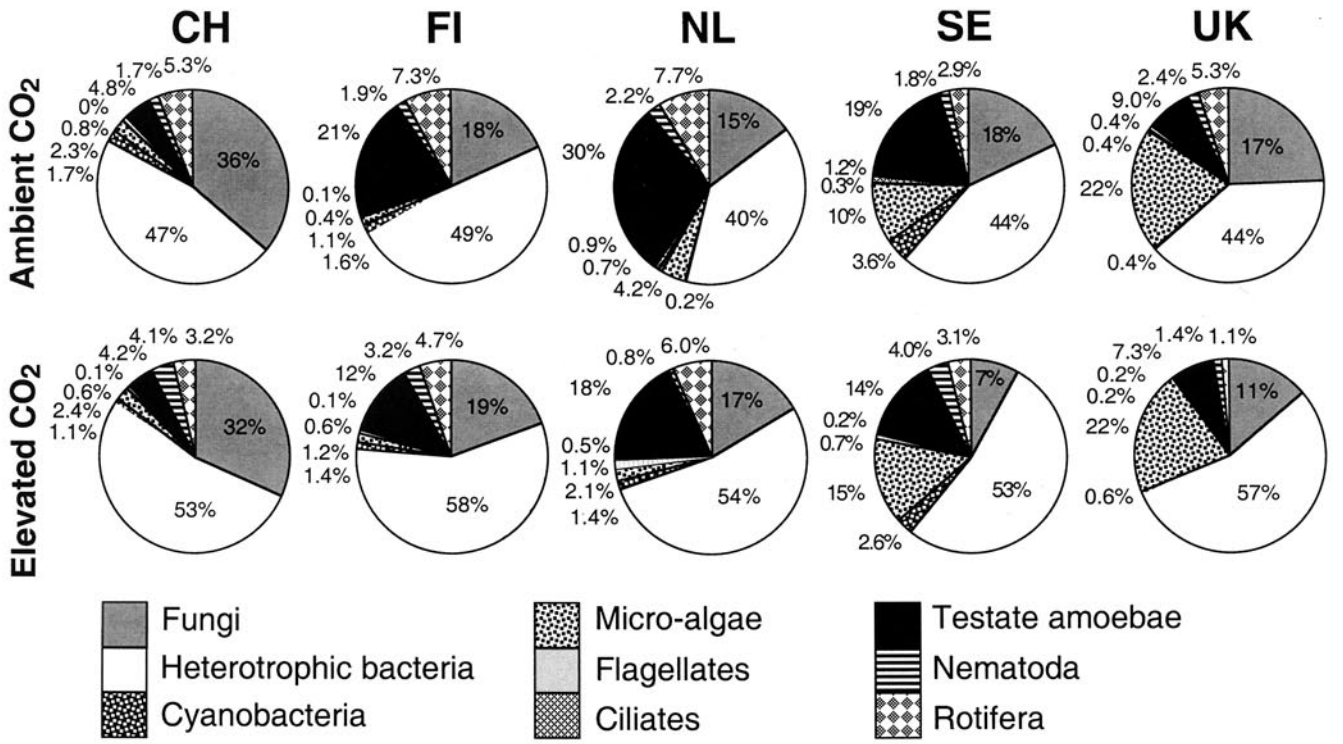

Fig. 1. Relative proportion of different microbial groups (% of total microbial carbon biomass) in *Sphagnum* samples taken after three growing seasons, in ambient (360 ppm) and elevated(560 ppm) CO_2 plots in five peatlands across Europe.

The Swedish and British sites had a greater proportion of algae than the other three sites. Four variables were significant in the partial CCA: Na^+, DOC, WTD (water table depth), and DIN. Together, these variables explained 46.8% of the variation in the microorganism data. The first three axes were significant ($P < 0.05$, Monte-Carlo test, 9999 permutations) and are illustrated in Fig. 3. Axis 1 reflects mainly an oceanic–continental gradient (Na^+), separating the oceanic sites UK and NL (to a lesser extent SE) from the continental sites CH and FI. Axis 1 also represents a moisture gradient (WTD), although axis 3 clarifies the relationship between microbial groups and WTD better. Axis 2 is positively correlated with DOC, which was highest in NL, and is negatively correlated with DIN (NH_4^+-N + NO_3^--N). Axis 3 is positively correlated with WTD, DIN, and DOC. Axis 3 clarifies which microbial groups are correlated with WTD and which ones are correlated with Na^+. These two factors were not separated in the diagram of axes 1 and 2 (see the relative position of fungi and cyanobacteria). Among photosynthetic groups, cyanobacteria were negatively correlated with DIN and DOC, while algae were correlated with Na^+ and negatively with WTD (i.e., wetter conditions). Among heterotrophic groups, bacteria were only weakly positively correlated with DIN, DOC, and WTD. Fungi were more clearly correlated with WTD and also weakly with DIN and DOC. Ciliates were correlated to DOC and Na^+ and negatively to WTD; flagellates were weakly correlated to DOC; and testate amoebae were weakly negatively correlated to WTD. No clear correlation emerged for nematoda and rotifera.

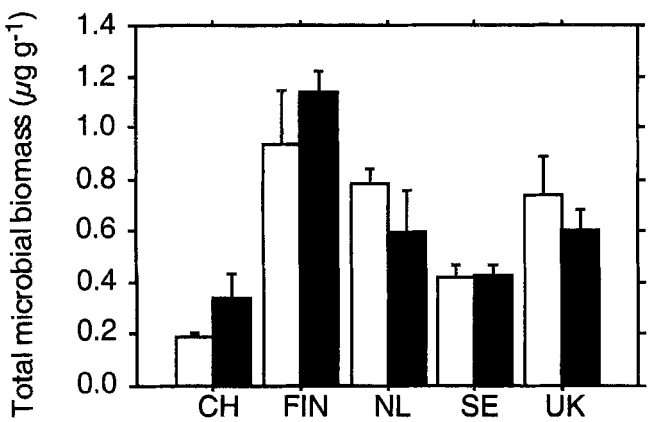

Fig. 2. Total carbon biomass (μg C g^{-1}, mean ± 1 S.E.) of all microbial groups in *Sphagnum* samples taken after three growing seasons, in elevated (560 ppm, black bars) and ambient (360 ppm, white bars) CO_2 plots in five peatlands across Europe. See text for ANOVA summary.

Table 3. Effect of elevated CO_2 on microbial communities in five European sphagnum peatlands[a]

	Whole model		Site		Treatment		Treatment * Site	
	F	*P*	F	*P*	F	*P*	F	*P*
Overall test	2.82	<0.001	9.55	<0.001	4.23	0.012	1.43	0.108
Bacteria	5.31	0.001	10.37	<0.001	4.58	0.045	0.43	0.783
Cyanobacteria	9.22	<0.001	17.28	<0.001	3.23	0.088	2.66	0.063
Microalgae	12.08	<0.001	25.73	<0.001	0.06	0.810	1.42	0.262
Flagellates	4.95	0.001	8.50	<0.001	2.08	0.165	2.13	0.115
Ciliates	2.49	0.043	4.71	0.008	0.52	0.481	0.76	0.562
Testate amoebae	19.29	<0.001	40.25	<0.001	4.47	0.047	2.04	0.127
Nematoda	4.80	0.002	5.13	0.005	2.54	0.127	5.04	0.006
Rotifera	2.55	0.039	4.68	0.008	0.67	0.424	0.89	0.489
Fungi	4.20	0.004	7.18	0.001	0.84	0.369	2.07	0.123

[a] *P* values: tests with Pillai's Trace; F and *P* values from the MANOVA.

Effect of CO_2 Enrichment on Microbial Communities

The total microbial biomass was not affected by the treatment (CO_2 and CO_2 * site effects nonsignificant), but the structure of microbial communities was modified (overall treatment effect in the MANOVA $P = 0.012$; Table 3). Bacterial biomass increased significantly on average by 48% (Fig. 4). The contribution of bacterial biomass to total biomass also increased from between 40 and 49% in the control plots to between 53 and 58% in the elevated CO_2 plots (Fig. 1). Testate amoebae biomass decreased significantly on average by 13%. Cyanobacteria increased in four sites, but this overall trend (+117%) was not significant ($P = 0.088$). With the exception of bacteria, which increased across all sites, all groups reacted differently in at least one site (Fig. 4). Nevertheless, the overall treatment * site effect was not significant.

Discussion

Patterns of Microbial Communities across the Five Sites

The Swiss site, which was much drier than the other four sites, had the lowest total microbial biomass, the lowest proportion of photosynthetic organisms, and the highest relative proportion of fungi of the five sites. The higher biomass of cyanobacteria in the two sites with the lower N concentrations (SE and FI) is in agreement with the competitiveness of these microorganisms for N under low N conditions [19]. These results support our first hypothesis that microbial communities differed among peatland sites across Europe and that these differences could be related to moisture conditions and water chemistry.

Peatlands are often described as being intermediate between terrestrial and aquatic ecosystems. However, many different types of peatlands exist depending on the hydrology, nutrient availability and pH [11], and each type is characterized by a mosaic of hummocks or tussocks, lawns, and depressions (hollows or pools). These different microsites are likely to be colonized by different microbial communities, but little is known on these patterns to this date. To our knowledge, the few existing studies on microbial communities in *Sphagnum* peatlands showed that pigmented organisms represent a small proportion of the total microbial biomass [20, 21]. We used the mean values of the control plots (Fig. 1) to compare our results with those of other studies. Heterotrophic organisms (heterotrophic bacteria, fungi, protozoa, and micrometazoa) dominated the microbial communities and together represented between 79% and 97% (average 91% over the five sites) of the total microbial biomass. By comparison to the studied sites, in a more nutrient-rich (annual mean DIN 0.6 mg L^{-1}) *Sphagnum fallax–Carex rostrata* fen (French Massif Central), heterotrophic microorganisms represented 65% of the total microbial biomass excluding fungi [20]. In another part of the same peatland, heterotrophic microorganisms represented 51% of the total microbial biomass including fungi [21]. Taking the average between these two values, we tentatively estimate the contribution of heterotrophic microorganism to the total microbial biomass to be about 58% in oligotrophic *Sphagnum* fens. Thus the ecological gradient for fen to bog is reflected in the following approximate relative proportions of autotrophic vs heterotrophic microorganisms: wet, oligotrophic fen (42%/58%) [20, 21], bog (9%/91%). This ratio illustrates the increasing importance of microbial heterotrophic assimilation toward the more terrestrial ecosystems.

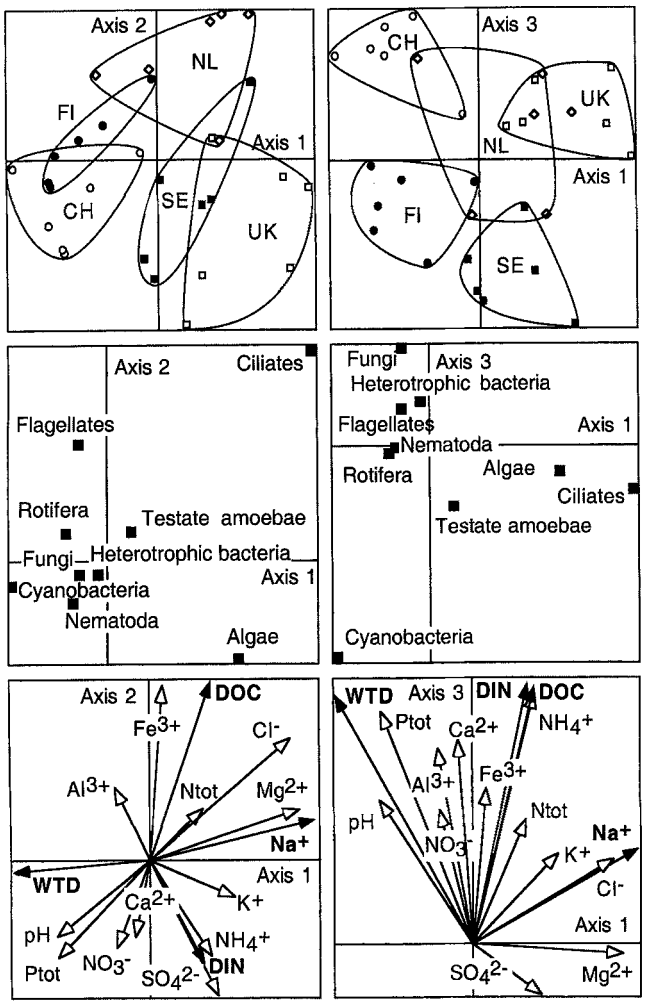

Fig. 3. Scatter diagrams of the partial canonical correspondence analysis on microbial community data based on biomass in the five sites. Dummy binary variables were used to remove the effect of the CO_2 treatment. Significant variables are represented with closed arrowheads and bold labels. Nonsignificant variables are projected passively in the ordination space and are represented with open arrowheads and plain labels. The overall analysis and the first three axes are significant (Monte-Carlo permutation test, 999 permutations, $P < 0.003$). Axes 1–3 explain respectively 22.2%, 15.4%, and 7.8% of the variation in the microbial community data. The left figures illustrate the position of samples, microbial groups, and environmental variables in axes 1 and 2. The right figures show axes 1 and 3. WTD, average water table depth.

We hypothesize that the primary limiting factor for autotrophic microbial assimilation in *Sphagnum* is the thickness of the water film on the *Sphagnum* mosses. Where these mosses are submerged, such as in bog pools, a larger volume of water may be colonized by algae and cyanobacteria. Where only a thin capillary water film is present, such as in the capitulum of *Sphagnum* mosses growing on the top of hummocks, autotrophic microorganisms will represent only a marginal proportion of the total microbial biomass. Other factors, often resulting from the physical, chemical, and physiological characteristics of *Sphagnum* itself, contribute to making *Sphagnum* an extremely unfavorable environment to many organisms. These conditions include low pH, nutrient concentrations, temperature, and, deeper down the peat profile, anoxic conditions [51]. But we believe that water availability is the primary limiting factor. Manipulative experiments could be used to test this hypothesis.

Effect of Elevated CO_2 on Microbial Communities

Soil organisms may be affected indirectly by CO_2 enrichment through changes in the quality or quantity of above- and below-ground litter [5, 16] and of exudation [13]. Increased exudation and investment in roots may allow plants to increase nutrient availability, through a stimulation of decomposition, in order to balance their increased C supply caused by higher photosynthetic rates under elevated CO_2 [7, 47, 54]. Therefore, although elevated atmospheric CO_2 may increase the input of organic matter into the soil, it may also accelerate C losses by stimulating the decomposition of soil organic matter [2]. Such effects are not necessarily reflected by clear changes in standing biomass or growth of plants [6]. Furthermore, the effect of CO_2 enrichment on exudation is not easy to study because microorganisms rapidly use labile C compounds. Finally, total microbial biomass may not be affected, while the structure of microbial communities may change [36]. A more detailed analysis of the different microbial functional groups is therefore useful as an indirect indicator of changes in the functioning of ecosystems [14]. For example, a positive effect of elevated CO_2 on bacteria but no effect on total microbial biomass was observed in a tropical model ecosystem [32]. The effect of CO_2 may also be detected only within a microbial group as changes in metabolic activity or community composition, while the total biomass may remain constant [25, 37]. This study did not include a assessment of possible changes in the community structure of individual microbial groups. For most groups, and especially for fungi and bacteria, this would have required a molecular approach that was beyond the scope of this study.

The increased protozoan biomass but lack of change in microbial biomass observed by Lussenhop et al. suggested

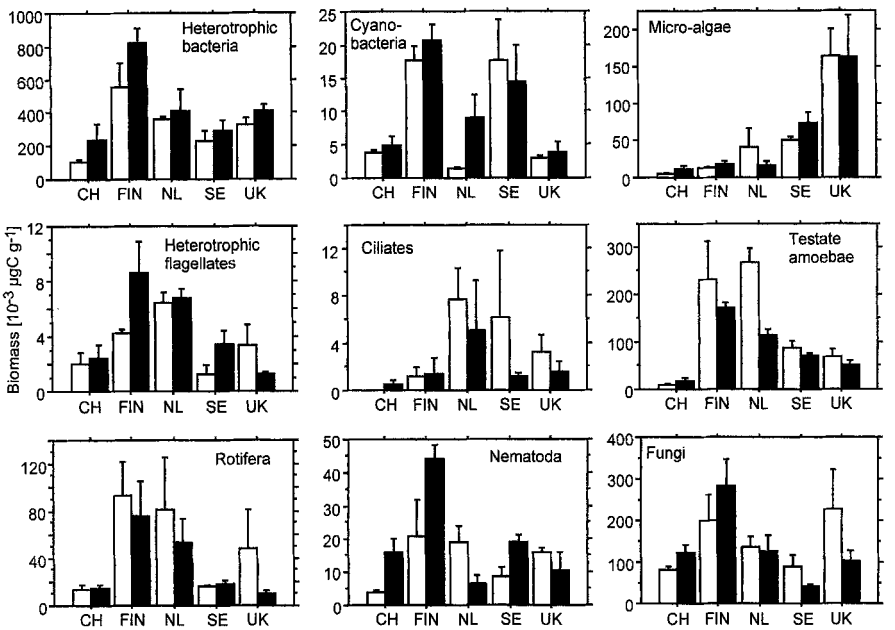

Fig. 4. Carbon biomass (10^{-3} µg C g^{-1}, mean ± 1 S.E.) of microbial groups in *Sphagnum* samples taken after three growing seasons, in elevated (560 ppm, black bars) and ambient (360 ppm, white bars) CO_2 plots in five peatlands across Europe. For statistical analyses, see Table 3.

an increased turnover of bacteria [36]. Our results are similar to that and other studies in that elevated CO_2 modified the structure of microbial communities in the five studied sites, but not the total microbial biomass. Contrary to Lussenhop et al. [36], however, we see an increase in bacteria but a decrease in testate amoebae. Increased microbial (not strictly bacterial) biomass has been found to be associated with enhanced N uptake by plants and available C for microorganisms, but reduced microbial respiration per unit biomass, indicating an alteration of the plant–microbe interaction in favor of the plant N uptake. These effects suggest a reduction of soil organic carbon mineralization [31]. Following this reasoning our results may indicate a decrease in decomposition. In support for this interpretation, elevated CO_2 reduced the decomposition of *Polytrichum strictum*, one of the dominant bryophytes of the Swiss site [43].

By contrast to microbial communities, no significant overall CO_2 effect was observed on the biomass [29] and growth [6] of *Sphagnum* or on the vascular plant biomass [29] across the same five sites. However, the root + rhizome biomass was found to be consistently and significantly greater in the elevated CO_2 treatments than in the controls [6]. This increased root + rhizome biomass may represent compensatory growth for greater nutrient uptake to match an enhancement in the photosynthetic rate [46]. Elevated CO_2 increased methane production in three of the sites, but this effect was not statistically significant [44].

The apparent contradiction between CO_2 effects on plants or methane production and on soil microorganisms may illustrate the sensitivity of microorganisms to ecosystem perturbation. Our results suggest an enhanced exudation of labile C to which bacteria responded. Although measurements of exudates or labile C in *Sphagnum* bogs under elevated CO_2 are lacking, laboratory experiments provide some indirect clues. In a growth chamber experiment, CO_2 enrichment was shown to increase the soluble sugar content in *Sphagnum* [52]. Furthermore, CO_2 enrichment caused an increase in C assimilation in *Sphagnum*, as determined by gas exchange measurements, but only about 30% of this enhanced C uptake was reflected by increased biomass, the rest being likely lost as exudates or respiration (Bjartmar Sveinbjörnsson, pers. comm.). This suggested that an important proportion of the additional C fixed though photosynthesis under elevated CO_2 was exuded or lost by the plant.

Under low N concentrations, the smaller photosynthetic microorganism species have a competitive advantage over larger species [27]. In accordance with this, following experimental N addition in a *Sphagnum* peatland the biomass of larger photosynthetic microorganisms increased and that of smaller photosynthetic microorganisms decreased [20]. However, contrary to our second hypothesis on the effects of elevated CO_2 on microorganisms, microalgae did not react to elevated CO_2 and cyanobacteria increased slightly, although this effect was not (or only marginally) significant ($P = 0.088$). However, the available data suggests that the N availability was not

significantly modified by elevated CO_2. Elevated CO_2 had no significant effect on the NO_3^-, NH_4^+, P, and K^+ concentrations of surface layer water throughout the second growing season of the experiment in the five sites [30]. In addition, the N content of *Sphagnum* and vascular plants was not significantly reduced under elevated CO_2 across the five sites [6, 29]. Therefore the absence of a significant effect of CO_2 on photosynthetic microorganisms does not contradict our third hypothesis that elevated CO_2 would decrease in the biomass of autotrophic microorganisms other than cyanobacteria through a reduction of available N.

Elevated CO_2 reduced the biomass of testate amoebae, the dominant protozoan group. Testate amoebae feed on a wide range of microorganisms, but bacteria are probably not an important part of their diet, except for the smaller (length range: 20–50 μm) species such as *Corythion dubium*, *Trinema* sp., or small *Euglypha* species [22]. Together, these species represented less than 5% of the total C biomass of testate amoebae, whereas the larger species (length range: 70–200 μm) such as *Hyalosphenia elegans*, *Hyalosphenia papilio*, *Bullinularia indica*, *Nebela tincta* var. *major*, and *Heleopera sphagni* together accounted for over 60% of the testate amoebae C biomass. Furthermore, their response to elevated CO_2 accounted for 80% of the CO_2 effect on the total testate amoebae C biomass. Thus if these dominant species were not feeding directly on bacteria, this would explain why the total testate amoebae C biomass did not increase. But this does not explain their decrease. Interestingly, a negative effect on higher trophic levels of soil microbial communities was also found in experiments simulating environmental warming [41, 42] and elevated CO_2 [49] in other ecosystems. To this date, satisfactory explanations for these changes are lacking. By contrast, in mineral soils planted with poplar, one season of CO_2 enrichment caused an increase in soil protozoa (mostly amoebae and flagellates, no indication of size, but assumed to be bacterivorous) but no change in bacterial biomass. This suggested an increased growth and turnover of bacteria [36]. Anderson and Griffin [4] observed a 56% increase in protozoan abundance in the rhizosphere of wheat plants grown in the laboratory under elevated CO_2, suggesting an increase in microbial activity caused by higher rates of root exudation. However, this effect was due to the smaller groups, flagellates (of which 90% were under 10 μm) and gymnamoebae (of which 50% were under 20 μm). By contrast, the abundance of ciliates (which were on average larger than the flagellates or gymnamoebae) tended to decrease, although this effect was not significant. Our longer-term results show that either the biomass of direct bacterial predators did not increase significantly (heterotrophic flagellates), or they tended to decrease (rotifers) (Fig. 4). A positive effect on these groups might have been expected in response to the increased bacterial biomass if their abundance was controlled by resource availability (bottom-up control). The absence of response could indicate that this was not the case. The lack of significant effect on heterotrophic flagellates may be due to a dilution effect of the CO_2-induced enhancement of bacterial biomass. Alternatively the abundance of flagellates, rotifers, and other groups such as testate amoebae may rather be controlled by predation (top-down control). Microcosm studies would be needed to assess the relative importance of top-down vs bottom-up controls over microbial groups in *Sphagnum*.

Changes in microbial community structure may provide evidence for changes in soil function such as nutrient or C turnover [25, 36, 49]. If the changes we see indeed indicate increased labile carbon input into the soil, this may lead to increased decomposition and nutrient cycling, although N limitation of microbial decomposition may lead to the opposite effect. Given the importance of peatlands in the global C cycle [23], changes in decomposition rates would affect (1) aboveground productivity, (2) plant community structure, and ultimately (3) peatland–atmosphere carbon exchange. But these changes would become clear on a longer time scale than the duration of most experiments simulating global change. Potential altered rates of C sequestration by *Sphagnum* peatlands could have far-reaching consequences on the C cycle and global warming [6]. Rates of C sequestration by *Sphagnum* peatlands may increase, due to an increased competitiveness of *Sphagnum* over vascular plants, feeding back negatively to atmospheric [CO_2] and hence to warming. Alternatively C sequestration could decrease if *Sphagnum* was out competed by vascular plants, possibly due to increased decomposition providing nutrient for vascular plants, feeding back positively to [CO_2] and to warming. We clearly need more work to understand how these important ecosystems will respond and feed back to global change. Finally, if we understood better how microbial communities relate to plant communities, or key ecosystem processes such as nutrient cycling and C sequestration rates, they could be used to monitor environmental changes or patterns across broad geographical and ecological scales.

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