MICROBIAL ECOLOGY

Microb Ecol (1998) 35:83–93

The Microbial Loop at the Surface of a Peatland: Structure, Function, and Impact of Nutrient Input

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Received: 12 August 1996; Accepted: 1 April 1997

ABSTRACT

The aim of this study was to assess the structure and function of the microbial loop in a peatland of the French Massif central, and the impact of fertilization on the different microbial communities.

In terms of biomass, testate Amoeba (48% of the total microbial biomass), heterotrophic bacteria (15%), cyanobacteria (14%) and Bacillariophyceae (13%) were the dominant groups of microorganisms. Other microalgae (7%), ciliates (2%) and heterotrophic flagellates (1%) accounted for only a low proportion of total microbial biomass. The relative importance of heterotrophic microorganisms was higher than in marine or lacustrine environments. In addition, ciliates and heterotrophic flagellates only constituted a small proportion of the total protozoan biomass, which was heavily dominated by testate amoeba. Thus, the structure of the protist community in the subaquatic peatland was completely different from that reported for lakes or marine environments.

In other aspects, the supply of nutrients (PKCa and NPKCa) resulted in increases of the relative biomasses of heterotrophic bacteria, Bacillariophyceae, and ciliates and by a decrease in the relative proportion of testate amoeba and of other microalgae.

Introduction

It is essential to understand the flow of matter and energy within aquatic ecosystems in order to study how these environments function. These flows transit, in part, through the following microbial loop (dissolved organic matter → bacteria → heterotrophic protists (ciliates, flagellates, amoeba) → macrozoorganisms) [7].

The importance of this food chain, which is superimposed on the classical pathway based on photosynthetic assimilation by autotrophs, was first demonstrated in pelagic marine ecosystems [22, 31, 42]. More recently, the microbial loop has been studied in lacustrine environments [2, 3, 33, 41] and in soil [12]. However, to our knowledge, no work concerning the microbial loop has been conducted in subaquatic habitats such as peatlands, although the various groups of microorganisms composing this trophic pathway have been observed there [13, 17, 18, 23, 44].

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As peatland ecosystems are environments deficient in various inorganic nutrients and have very high organic matter loadings, it is likely that the microbial loop plays a key role in their functioning. Furthermore, from the works of Simek and Straskrabova [37], it would appear that Protozoa, the central players in the microbial loop, are less sensitive to the acidity of the medium than Metazoa. They could, thus, have a dominant role in the trophic pathways in peatlands. In addition, according to Munawar and Weisse [27], the relative importance of the microbial loop and the way it functions could be early indicators of human disturbances to aquatic biological communities. For these reasons, a study of the structure and function of the main groups of microorganisms forming the microbial loop in a peatland was undertaken.

Materials and Methods

The experiments were carried out in the Pradeaux peatland (Puy-de-Dôme, France, 3°55 E, 45°32 N), a drained peatland with an area of 10 ha, that was 9,000 years old [20] and situated at an altitude of 1,300 m. During 1994, total precipitation exceeded 1,780 mm, the mean temperature was −0.6°C in January and 17.3°C in July. The work was carried out on a *Carex rostrata–Sphagnum fallax* fen.

The three study plots were rectangular in shape, 9 m long, and 1.50 m wide. Two of them had been fertilized biannually since 1986. The "PKCa" plot received 100 kg of P_2O_5 ha^{-1} $year^{-1}$ in the form of slag (containing 2% MgO), 180 kg of K_2O ha^{-1} $year^{-1}$ in the form of chloride, and 2,000 kg of CaO ha^{-1} $year^{-1}$. The NPKCa plot, in addition, received 180 kg N ha^{-1} $year^{-1}$ in the form of 33% NH_4 NO_3. The nutrients were spread two times per year: in June and July [20].

Each plot was divided into three subplots 3 m × 1.5 m. An integrated sample was collected each month by combining five samples, taken at random, within each of the subplots, in October and November 1993, and from March to November 1994. These subplots did not constitute a true replication. However, three measurements for each parameter verified the accuracy of the methods and the observed values.

Sampling

Because of the dominance of water in this biotope, we used the below-mentioned hydrobiological methods on water samples extracted from the *Sphagnum* carpet and waterlogged soil. The water was sampled using a specially modified syringe with a press system attached to the needle base. This system is composed of a circular box (5 cm in diameter) on which the syringe is fixed, and provides for efficient water extraction from the *Sphagnum* carpet. Indeed, preliminary experiments allowed us to verify that this extraction procedure did not destroy fragile microorganisms such as flagellates and ciliates.

Abiotic Variables

Temperature, Oxygen, pH, and Conductivity. These variables were measured in situ with a YSI-GRANT/3800 multiparametric probe with the following precision: T, ± 0.4°C; O_2, ± 0.03 mg $liter^{-1}$, pH, ± 0.04 unit; conductivity, ± 0.004 mS cm^{-1}.

Water Chemistry. Total nitrogen (TN), ammonia-nitrogen (NH_4^+), nitrate nitrogen NO_3^-, nitrite nitrogen (NO_2^-), total phosphorus (TP), orthophosphates (PO_4^{3-}), silica, and inorganic carbon (HCO_3) were analyzed on filtered (Whatman GF/C) water, using standard methods [5].

Community Counts and Estimation of Biomass

Samples were fixed with a 2% (final concentration) solution of glutaraldehyde and stored at 4°C in the dark.

Heterotrophic Bacteria. Subsamples (0.25 ml) were homogenized for 1 min with a vortex in 3 ml distilled and sterilized water. Heterotrophic bacteria were stained with DAPI (4,6 diamino-2-phenylindol), according to Porter and Feig [30] and filtered using 0.2-µm-pore-size filters. Preparations were made within 24 h after sampling and stored at −25°C, in the dark. Slides were examined at 1250 × magnification by epifluorescence microscopy, in a dark room, using an Olympus HBS microscope equipped with an epifluorescence illuminator HB2-RFL, a mercury lamp HBO-100W, and a neofluor objective lens 100/1.25. Epifluorescence microscopy on vortexed samples allowed us to quantify both unattached (i.e., suspended in the peatland water) and attached bacteria (i.e., fixed on suspended matter). In this way, only bacteria directly fixed on the living part of *Sphagnum* were not taken into account.

Cyanobacteria, Heterotrophic Flagellates, and Microalgae. Subsamples (0.5 ml) were stained with primulin solution (0.1 M buffer Trizma, pH 4) [11], and microorganisms were collected on 0.8-µm-pore-size black Nucleopore filters. The slides were observed using the method described above.

Ciliates and Testate Amoeba. The biomass of ciliates and testate amoeba and their community composition were determined using Utermöhl's [43] method. Samples were stored at 4°C, in the dark. Preliminary experiments to determine potential losses during storage confirmed that losses were < 8% during 3 months' storage [38]. Therefore, counts were made within 2 months. Storage volumes of 3 ml were allowed to settle for at least 24 h in plankton chambers. Cell densities were estimated with an inverted Leitz microscope at 400 ×, by scanning the whole chamber area to account for any heterogeneous distribution of the cells.

Biovolumes and Estimation of Biomass. Biovolumes of each community were estimated by assuming geometric shapes and converting to carbon using the following conversion factors: heterotrophic bacteria, 1 $mm^3 = 0.56 \times 10^{-6}$ mgC [10]; cyanobacteria and algae, 1 $mm^3 = 0.12 \times 10^{-6}$ mgC; flagellates, 1 $mm^3 = 0.22 \times 10^{-6}$ mgC [9]; ciliates and testate amoeba, 1 $mm^3 = 0.11 \times 10^{-6}$ mgC [45]. The reported biomasses of heterotrophic bacteria, cyanobacteria, microalgae, and heterotrophic flagellates were the mean of three

replicates. The ciliates and testate amoeba counts were conducted on a single assay by combining three samples from each plot.

Bacteria were separated into three groups: cocci, bacilli, and vibrios. Approximately 20 cells were examined to estimate the average length of the different morphotypes.

Chlorophylls

Chlorophylls were determined spectrophotometrically from samples (40–100 ml) collected on Whatman GF/C glass fiber filters. Pigments were extracted with 90% acetone and concentrations were calculated from Scor-Unesco [36] equations. The measurements were conducted in triplicate.

Auto- and Heterotrophic Activities

Photosynthetic and heterotrophic activities were measured from the assimilation of $NaH^{14}CO_3$ and ^{3}H-amino acids using the double-labeling technique. Tritium labeling was chosen, first, because the specific activity of ^{3}H is up to tenfold greater than that of ^{14}C-labeled compounds [6], and, second, because possible refixation by photosynthesis of inorganic products derived from bacterial heterotrophy [25] or from algal respiration [32] is avoided or minimized. Activities were estimated using a protocol derived from Steemann-Nielsen's method [39, 40]. For each plot, three 125-ml glass-stoppered bottles (two light and one dark) were injected with $NaH^{14}CO_3$ (10 μCi/100 ml, specific activity: 56 mCi/mmol) and ^{3}H-amino-acids (10 μCi/100 ml, specific activity: 38 Ci/mmol). The bottles were then incubated for 3 h at solar midday. At the end of incubation, aliquots (5 ml) were taken from the bottles and filtered, in parallel, through Millipore membrane filters of 0.45-μm poresize, at a vacuum pressure of less than 100 mm Hg. The radioactivity retained on the filters was counted using a liquid scintillation counter. According to the works of McKinley [24] and McKinley and Wetzel [25], the values for photoheterotrophic assimilation were calculated from the difference between the activity measured in the light bottle and that measured in the dark bottle. The calculation was based on: (a) activity in the light bottle = potential photoheterotrophic activity + potential chemoheterotrophic activity + background, and (b) activity in the dark bottle = potential chemoheterotrophic activity + background.

Results

The Physicochemical Environment

The temperature at the surface of the peatland fluctuated between 0.1 and 29.4°C during the course of the study (Table 1). The pH values were characteristic of an acid, oligotrophic, low-lying peatland. The pH was slightly higher, on average, in the fertilized plots (between 5.6 and 5.8) than in the control plot (4.7) (Table 1). The mean conductivity, about 25 μS cm^{-1}, was very low (Table 1). The mean value of the dissolved oxygen concentration for all the samples was higher in the control plot (8.2 mg liter^{-1}) than in the PKCa (7.4 mg liter^{-1}) and NPKCa plots (6.8 mg liter^{-1}) (Table 1).

The total nitrogen concentrations varied between 0.52 and 2.59 mg liter^{-1}. They were highest in summer. The mean NO_3^- and NH_4^+ concentrations were of the same order of magnitude (about 0.20 mg liter^{-1}). The mean concentrations of NH_4^+ were, however, higher in the fertilized plots. The reverse trend was observed for the mean concentration of NO_3^-. The NO_2^- concentrations were low (Table 1).

The concentrations of orthophosphates and total phosphorus were, on the whole, higher in summer. They were much higher in the two fertilized plots than in the control plot (Table 1).

Structure and Function of the Microbial Loop Communities

The mean bacterial abundance was 7.29×10^6 cells ml^{-1} in the control plot, 8.57×10^6 and 11.0×10^6 cells ml^{-1} in the PKCa and NPKCa plots, respectively. Among the different morphotypes observed, cocci dominated in all three plots and accounted for approximately 89% of the total.

The bacterial biomass fluctuated between 0.21 and 1.90 μgC ml^{-1}. It was higher, on average, in the NPKCa plot (0.73 ± 0.59 μgC ml^{-1}) than in the PKCa plot (0.54 ± 0.31 μgC ml^{-1}) and in the control plot (0.50 ± 0.32 μgC ml^{-1}). The highest bacterial biomasses were recorded at the beginning of summer (Fig. 1).

The population density of cyanobacteria fluctuated between 0.16×10^4 and 22.4×10^4 cells ml^{-1}. It was higher on average, in the PKCa plot (5.55×10^4 cells ml^{-1}) than in the control plot (4.50×10^4 cells ml^{-1}) and in the NPKCa plot (3.93×10^4 cells ml^{-1}). On average, the biomasses were much higher in the PKCa (mean = 0.58 ± 0.92 μgC ml^{-1}) and control plots (mean = 0.48 ± 0.49 μgC ml^{-1}), than in the NPKCa plot (mean = 0.28 ± 0.32 μgC ml^{-1}) (Fig. 1).

Cyanobacteria appeared at the end of June and were present until August. During this period, filamentous forms belonging to the genera *Anabaena* and *Gloetrichia* dominated. Many akinetes were observed from July onwards, with a peak abundance at the end of September.

The abundance of the Bacillariophyceae varied from 0.16×10^4 to 4.39×10^4 cells ml^{-1}. It was higher on average, in the NPKCa plot (1.90×10^4 cells ml^{-1}) than in the control plot (1.57×10^4 cells ml^{-1}) and in the PKCa plot (0.77×10^4 cells ml^{-1}). The biomasses were also higher in the NPKCa plot (mean = 1.08 ± 0.71 μgC ml^{-1}) than in the PKCa (mean = 0.70 ± 0.54 μgC ml^{-1}) and control plots (mean = 0.44 ± 0.24 μgC ml^{-1}) (Fig. 2).

Table 1. Spatial and temporal variations in physicochemical variables in the control, PKCa, and NPKCa plots

Control	Temp (°C)	O_2 (mg l^{-1})	Cond (µS cm^{-2})	pH	$N\text{-}NH_4^+$ (mg l^{-1})	$N\text{-}NO_2^-$ (mg l^{-1})	$N\text{-}NO_3^-$ (mg l^{-1})	Total N (mg l^{-1})	PO_4^{3-} (mg l^{-1})	Total P (mg l^{-1})
12/10/93	10.2	5.9	24	4.4	0.38	0.008	0.23	1.08	0.10	0.10
15/11/93	0.1	8.1	22	4.1	0.19	0.008	0.22	1.01	0.08	0.20
21/03/93	ND[a]	ND	ND	ND	0.08	0.010	0.17	0.59	0.03	0.06
27/04/94	8.5	9.4	10	5.8	0.06	0.010	0.17	0.62	0.03	0.07
27/05/94	9.2	6.4	22	4.1	0.05	0.012	0.17	0.69	0.04	0.18
29/06/94	16.7	3.3	30	5.1	0.13	0.014	0.18	1.12	0.09	0.14
27/07/94	29.4	4.7	ND	4.6	0.47	0.016	0.34	2.59	0.05	0.32
30/08/94	15.8	11.7	40	4.5	0.18	0.015	0.17	1.68	0.04	0.30
27/09/94	11.6	4.8	27	5.0	0.15	0.014	0.17	1.30	0.05	0.30
24/10/94	7.3	13.7	26	4.5	0.02	0.008	0.22	0.71	0.04	0.16
29/11/94	2.9	13.7	30	5.1	0.04	0.013	0.56	1.29	0.06	0.14
PKCa										
12/10/93	9.8	6.8	36	5.4	0.47	0.093	0.22	1.09	0.11	0.36
15/11/93	1.4	7.4	18	4.7	0.30	0.016	0.21	0.91	0.12	0.18
21/03/93	ND	ND	ND	6.0	0.10	0.008	0.17	0.60	0.09	0.11
27/04/94	7.8	7.4	14	6.5	0.05	0.010	0.16	0.52	0.09	0.12
27/05/94	11.3	4.9	20	4.3	0.07	0.013	0.18	0.95	0.08	0.26
29/06/94	16.0	3.0	24	5.6	0.20	0.015	0.17	1.26	0.54	0.73
27/07/94	19.6	4.2	ND	7.0	0.64	0.024	0.35	2.56	0.35	0.72
30/08/94	14.9	10.4	32	6.7	0.14	0.01	0.17	1.56	0.40	0.54
27/09/94	11.8	2.9	31	6.1	0.16	0.010	0.16	1.08	0.26	0.40
24/10/94	7.6	12.9	22	4.2	0.02	0.005	0.19	0.79	0.17	0.21
29/11/94	3.0	13.6	16	5.0	0.01	0.004	0.18	0.62	0.12	0.17
NPKCa										
12/10/93	9.7	3.4	36	5.5	0.87	0.009	0.21	1.32	0.40	0.60
15/11/93	0.4	6.1	34	5.3	0.26	0.007	0.24	0.80	0.16	0.26
21/03/93	ND	ND	ND	5.8	0.12	0.010	0.17	0.66	0.10	0.14
27/04/94	7.8	6.8	14	6.3	0.05	0.010	0.17	0.53	0.09	0.11
27/05/94	10.2	3.6	18	4.4	0.06	0.013	0.19	0.85	0.13	0.32
29/06/94	16.8	2.9	18	6.0	0.83	0.019	0.28	2.04	0.47	0.66
27/07/94	17.6	4.7	ND	7.2	0.16	0.017	0.17	1.44	0.60	0.92
30/08/94	15.3	10.5	24	7.0	0.15	0.013	0.17	1.39	0.39	0.54
27/09/94	11.3	3.2	35	6.6	0.14	0.009	0.15	0.95	0.35	0.40
24/10/94	7.3	13.2	24	4.3	0.01	0.005	0.20	0.59	0.09	0.17
29/11/94	2.9	13.7	20	5.1	0.01	0.004	0.18	0.62	0.12	0.17

[a] ND, Not determined

Although some dominant species such as *Eunotia pectinalis* and *Pinnularia viridis* occurred in all plots others only developed in one treatment or the other: *Frustulia rhomboïdes, Navicula* sp. and *Pinnularia subcapitata* in the control plot, *Nitzchia* sp. and *Achnanthes* sp. in the fertilized plots.

Bacillariophyceae were detected from June onward and reached a peak in density at the end of July. Species of a size < 60 µm (mainly *Pinnularia subcapitata, Navicula* sp., and *Frustulia rhomboïdes*), accounted for most of the biomass of Bacillariophyceae up until the end of August, slowly declined in the control plot. Larger species (mainly *Pinnularia viridis* and *Eunotia pectinalis*) were present until the end of the study and were then the dominant size class.

The abundance of the other microalgae was relatively high and varied from 0.37 to 12.6 × 10^4 cells ml^{-1}, for all plots combined. This community accounted for a small fraction of the biomass, which was much higher, on average, in the control plot (0.24 ± 0.27 µg C ml^{-1}) than in the PKCa plot (0.06 ± 0.10 µgC ml^{-1}) and in the NPKCa plot (0.04 ± 0.04 µgC ml^{-1}) (Fig. 2). The species encountered were mainly euglenophyta, desmids (e.g., *Closterium* sp.) and other small chlorophyta. In the fertilized plots, species belonged to the genus *Cryptomonas.*

The biomass of pigmented organisms was much higher in all plots from June to September than at other periods of the year.

The chlorophyll *a* and *c* concentrations showed similar

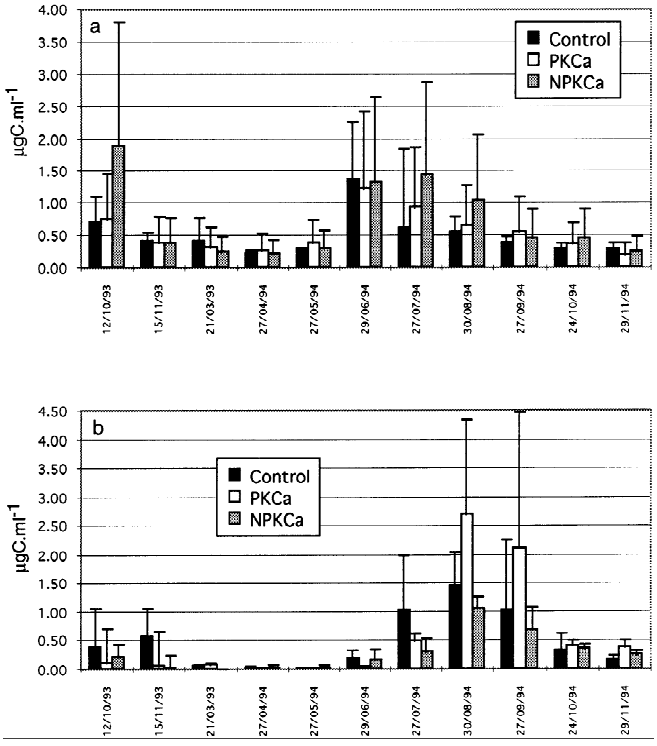

Fig. 1. Spatiotemporal variations in bacterial (**a**) and in cyanobacteria biomass (**b**) (µgC ml^{-1}) in the control, PKCa, and NPKCa plots.

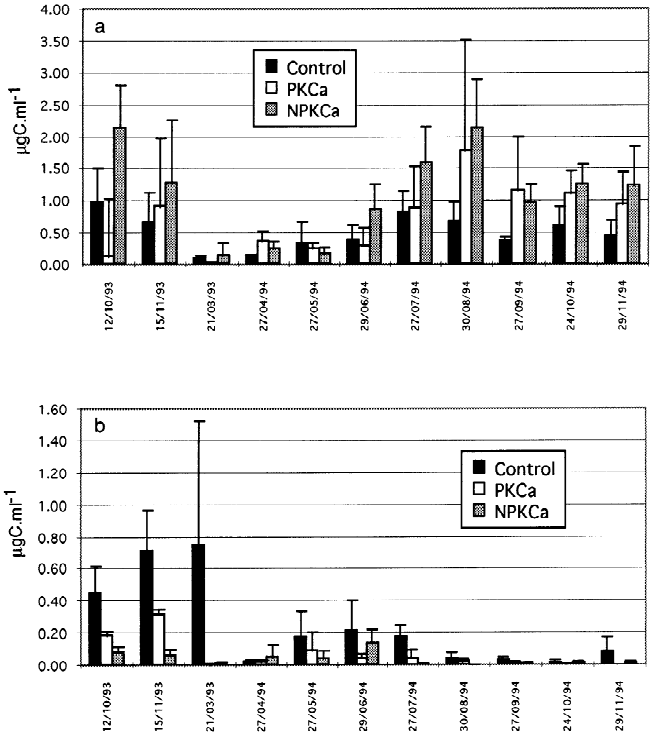

Fig. 2. Spatiotemporal variations in the biomass of Bacillariophyceae (**a**) and of other microalgae (**b**) (µgC ml^{-1}) in the control, PKCa, and NPKCa plots.

seasonal patterns of change. The chlorophyll *a* concentrations were higher, on average, in the NPKCa plot (95.7 ± 59.7 µgliter^{-1}) than in the control plot (79.6 ± 41.9 µgliter^{-1}) and in the PKCa plot (55.8 ± 33.8 µgliter^{-1}) (Fig. 3). Active chlorophyll *a* accounted for 32–34% of total chlorophyll *a,* depending on plot. This percentage was higher in summer (about 40%), than in winter (about 20%). The mean chlorophyll *b* concentrations varied from 5.7 µg liter^{-1} in the PKCa plot to 13.5 µgliter^{-1} in the NPKCa plot (Fig. 3) and the mean chlorophyll *c* concentrations ranged from 36.4 µg liter^{-1} in the PKCa plot to 57.8 µgliter $^{-1}$ in the NPKCa plot (Fig. 3).

It was also found that the chlorophyll *c* concentrations varied over the season in a pattern similar to Bacillariophyceae abundance (Figs. 2 and 3).

The density of heterotrophic flagellates was much higher, on average, in the PKCa plot (7.12 × 10^3 cells ml^{-1}) and in the NPKCa plot (7.44 × 10^3 cells ml^{-1}) than in the control plot (2.85 × 10^3 cell ml^{-1}). Their biomass was low and, on average, only accounting for 0.02–0.03 µgC ml^{-1} depending on the plot (Fig. 4). The highest biomass concentration, were recorded in summer.

The average abundance of ciliates was also much higher in the NPKCa plot (6.45 × 10^2 cells ml^{-1}) and in the PKCa plot (3.63 × 10^2 cells ml^{-1}) than in the control plot (2.55 × 10^2 cells ml^{-1}). The same was true for the biomass values (0.24 ± 0.26, 0.19 ± 0.19, and 0.08 ± 0.07 µgC ml^{-1} in the NPKCa, PKCa and control plots, respectively) (Fig. 4). The biomass concentrations were much higher until September, during which period when large species belonging to the group Hypotricha (*Paramecium* sp.) (>100 µm) appeared.

The density of the testate amoeba varied from 0.11 × 10^2 to 11.4 × 10^2 cells ml^{-1}. On average there was little variation between the plots (1.39 × 10^2 cells ml^{-1} for the control plot and 2.44 × 10^2 and 2.60 × 10^2 cells ml^{-1} for the PKCa and NPKCa plots, respectively).

In contrast, the mean biomass concentration was much higher in the control plot (1.63 ± 0.80 µgC ml^{-1}) than in the PKCa (1.11 ± 0.70 µgC ml^{-1}) and NPKCa plots (0.56 ± 0.32 µgC ml^{-1}) (Fig. 4). This was because the dominant species in the control plot (*Quadrullela* sp. [length: 100 µm], *Cyphoderia* sp. [length: 180 µm], *Nebela* sp. [length: 120 µm]) were larger than the main species in the fertilized plots (*Trinema* sp. [length: 20 µm], *Euglypha* sp. [length: 35 µm], *Difflugia* sp. [length: 80 µm]).

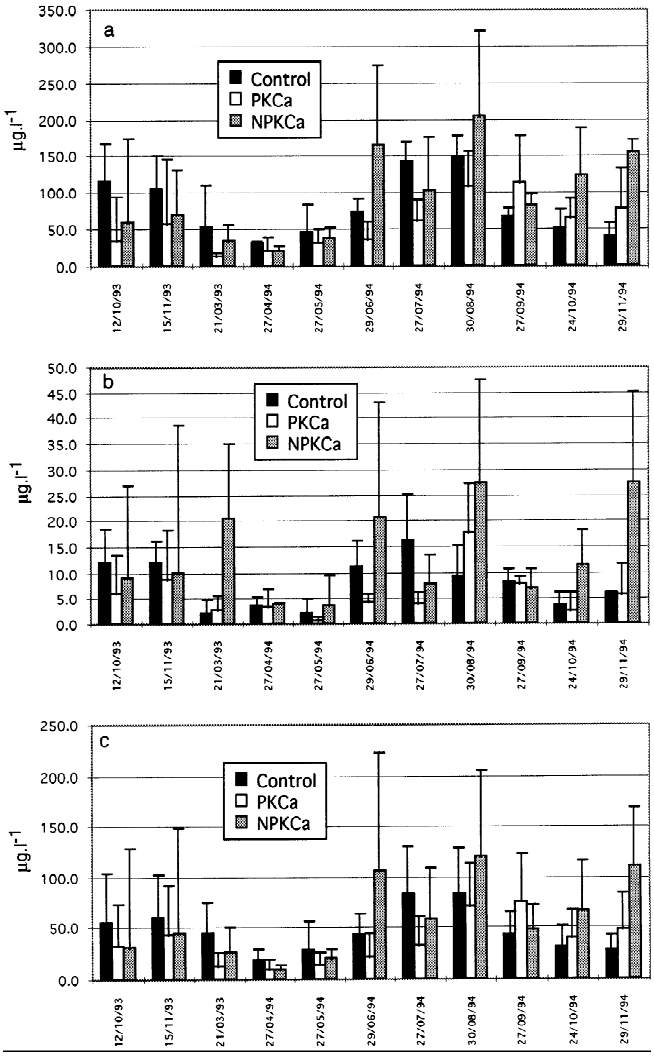

Fig. 3. Spatiotemporal variations in chlorophyll *a* (**a**), chlorophyll *b* (**b**) and chlorophyll *c* (**c**) concentrations (µg liter^{-1}) in the control, PKCa, and NPKCa plots.

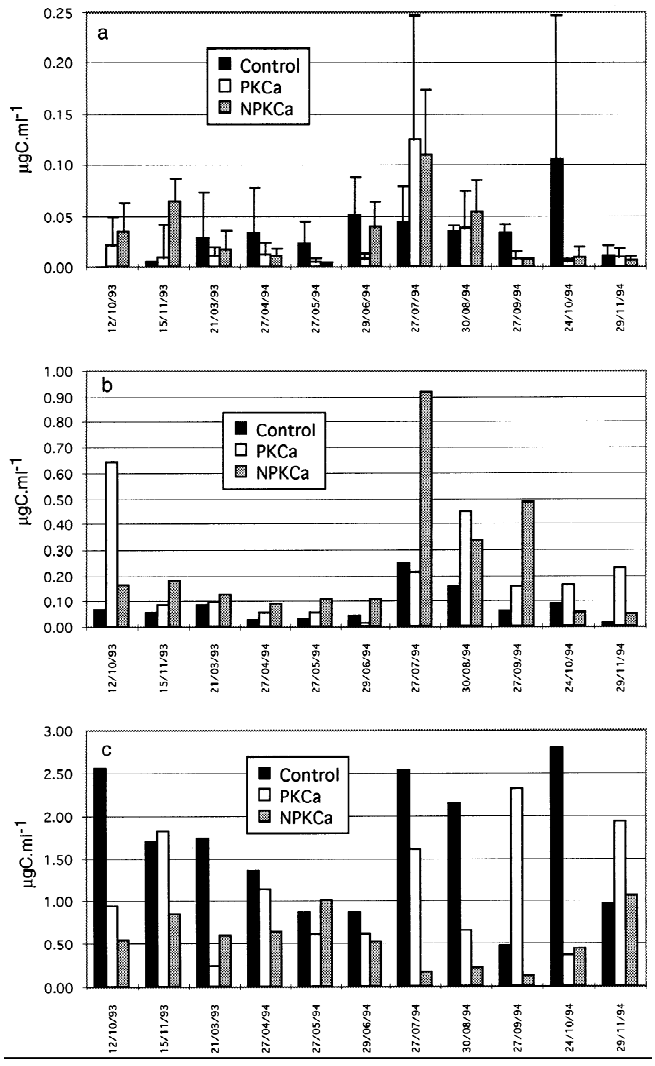

Fig. 4. Spatiotemporal variations in the biomass of heterotrophic flagellates (**a**), ciliates (**b**) and testate amoeba (**c**) (µgC ml^{-1}) in the control, PKCa, and NPKCa plots.

Photosynthetic and Heterotrophic Assimilation

The values for photosynthetic assimilation varied from 5 to 261 mgC m^{-3} h^{-1}. They were much higher in summer. The average values of photosynthetic assimilation were 67.8 ± 44.5, 77.8 ± 60.9, and 86.7 ± 82.5 mgC m^{-3} h^{-1} in the PKCa, control, and NPKCa plots, respectively (Fig. 5).

The values of potential photoheterotrophic assimilation varied from 0 to 0.91 µgC m^{-3} h^{-1}. On average, the values were lower in the control plot (0.36 ± 0.39 µgC m^{-3} h^{-1}) than in the PKCa and NPKCa plots (0.44 ± 0.23 µgC m^{-3} h^{-1} and 0.46 µgC m^{-3} h^{-1} ± 0.58 µgC m^{-3} h^{-1}, respectively) (Fig. 6).

The values of potential chemoheterotrophic assimilation varied from 0.68 to 4.24 µgC m^{-3} h^{-1}. On average, the values were higher in the control plot (2.17 ± 1.02 µgC m^{-3} h^{-1}), than in the NPKCa plot (1.54 ± 0.56 µgC m^{-3} h^{-1}) and in the PKCa plot (1.47 ± 0.41 µgC m^{-3} h^{-1}) (Fig. 6).

Photoheterotrophic assimilation was higher in summer in the control and NPKCa plots. It varied little seasonally in the PKCa plot. Chemoheterotrophic assimilation fluctuated relatively little with time, with the exception of the control plot (where it was higher during spring and autumn).

Discussion

Structure and Function of the Microbial Loop in Peatland Subaquatic Environments

In terms of biomass, four groups of microorganisms were dominant. These were the testate amoeba which, on average,

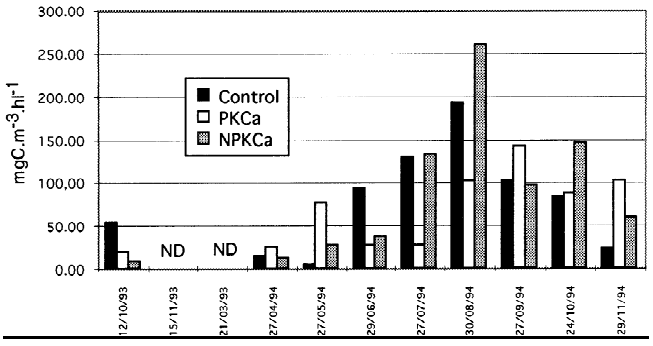

Fig. 5. Spatiotemporal variations in photosynthetic assimilation (mgC m^{-3} h^{-1}) in the control, PKCa, and NPKCa plots (ND, not determined).

accounted for 48% of the total biomass heterotrophic bacteria (15%), cyanobacteria (14%), and Bacillariophyceae (13%). Other microalgae (7%), ciliates (2%), and heterotrophic flagellates (1%) only accounted for a low proportion of total microbial biomass (Fig. 7). It should be noted, however, that not all groups of microorganisms were considered in our study. Some of the groups not examined in our study

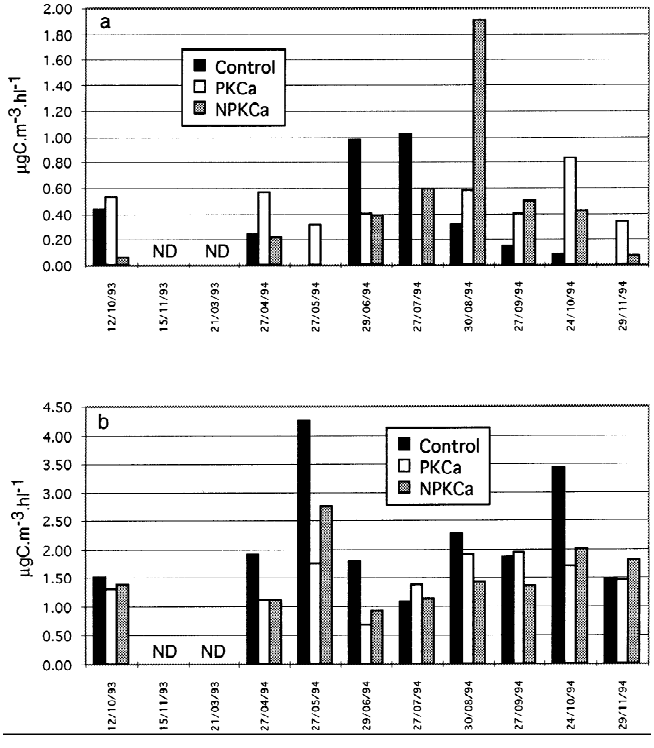

Fig. 6. Spatiotemporal variations in potential photoheterotrophic assimilation (**a**) and potential chemoheterotrophic assimilation (**b**) (µgC m^{-3} h^{-1}) in the control, PKCa, and NPKCa plots (ND, not determined).

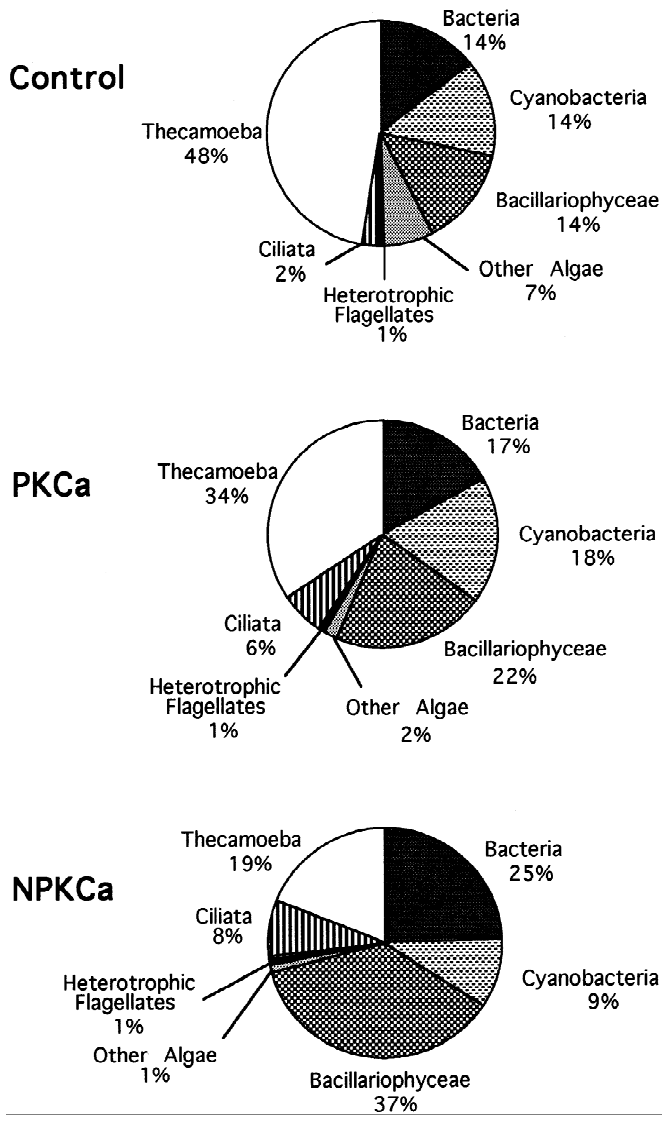

Fig. 7. Relative proportions of the biomasses of different microbial groups in the control, PKCa, and NPKCa plots.

such as the fungi, can attain high biomass concentrations in peatlands [16, 28, 48, 49].

Heterotrophic bacteria developed in very large numbers in June, as a result of warm temperatures and nutrient availability. Their biomass was correlated with the concentration of ammonium ($r = 0.61$, $p < 0.05$) and orthophosphate ($r = 0.86$, $p < 0.001$) in the environment. From July onward, the bacterial biomass was probably limited by the development of bacterivorous microorganisms (heterotrophic flagellates, ciliates, testate amoeba, rotifera, etc.).

The apparent density of the *Sphagnum* in the superficial layer of the peatland (0–5 cm) was estimated from 29 samples to be 13.5 ± 4.4 $gliter^{-1}$ in dry weight. We can,

therefore, calculate that the average bacterial biomass estimated from epifluorescence counts to be 0.5 μgC liter^{-1}, was equivalent to 37 μgC g^{-1} of dried peat; and the mean bacterial abundance (11.0×10^6 cells ml^{-1}) was about 8×10^8 cells g^{-1} of dried peat. These values are higher than those reported by Martin et al. [26], who gave an estimated bacterial abundance of 26×10^6 bacteria g^{-1} of dry peat. This is due to the considerable underestimation of bacterial density by the standard method of culturing used by these authors. Alternatively, the values of bacterial biomass that we report are consistent with the values of biomass for the entire microorganism assemblage (300 at 2,310 μgC g^{-1} of dry peat) obtained by Williams and Sparling [46], using an indirect method of fumigation-extraction.

Bacillariophyceae and cyanobacteria accounted for most of the biomass of pigmented microorganisms. The abundance values were 1,000-fold higher than those obtained by Duthie [17] on the surface of upland peatbog. The development of autotrophic microorganisms is closely related to the concentrations of inorganic nutrients present in the environment. For example, the biomasses of Bacillariophyceae and cyanobacteria were correlated with the concentrations of total N ($r = 0.78$, $p < 0.01$, and $r = 0.64$, $p < 0.05$, respectively), of total P ($r = 0.88$, $p < 0.001$, and $r = 0.82$, $p < 0.01$, respectively) and with conductivity ($r = 0.75$, $p < 0.05$, and $r = 0.87$, $p < 0.01$, respectively). Furthermore, the concentrations of chlorophylls *a* and *c* were closely correlated with the biomass concentrations of cyanobacteria and of Bacillariophyceae ($0.70 < r < 0.96$, $p < 0.05$).

Testate amoeba, apparently a very specific group characteristic of subaquatic peatlands, accounted for nearly one-half of the microbial biomass. They were more or less constantly present throughout the year. This is also true for many soils [34, 35]. The diversity of trophic behavior in these organisms and their capacity to encyst to resist changes in temperature and humidity certainly explains the constancy of their presence. Testate amoeba have been described as able to grow on diverse food sources, including: woody debris, bacteria, microalgae, ciliates, other testate amoeba, or even rotifers or nematodes [15]. According to Couteaux and Devaux [14], testate amoeba may also consume fungi.

The densities of testate Amoeba recorded were slightly lower than those reported by Warner [44], who estimated about 10,000 cells liter^{-1} in the top 5–10 cm of an upland *Sphagnum magellanicum* bog. The biomass of testate amoeba was correlated with the biomasses of cyanobacteria ($r = 0.53$, $p < 0.1$), Bacillariophyceae ($r = 0.64$, $p < 0.05$), heterotrophic flagellates ($r = 0.74$, $p < 0.01$), and ciliates ($r = 0.76$, $p < 0.01$). These microorganisms are probably the preferred prey of testate amoeba.

The abundance of the ciliates was close to that reported by Groliere [23]. This author counted up to 1,100 ciliates ml^{-1} in habitats situated within *Sphagnum* carpet in the peatlands of the Massif central (France). The maximum densities that were recorded in July and August occurred at a time when ciliates larger than 20 μm were developing. Our results show that the biomass of ciliates was closely correlated with biomass concentrations of cyanobacteria ($r = 0.92$, $p < 0.001$) and Bacillariophyceae ($r = 0.81$, $p < 0.01$). The correlation was even stronger between the biomass of ciliates larger than 20 μm and that of Bacillariophyceae of a size less than 60 μm ($r = 0.89$, $p < 0.001$). If these statistical relations are assumed to reflect trophic interactions, the consumption of Bacillariophyceae of a size less than 60 μm, and, particularly, of the smallest of them, by testate amoeba and large-sized ciliates would favor the dominance of the larger Bacillariophyceae (*Pinnularia viridis* and *Eunotia pectinalis*). This dominance was observed until mid-autumn.

There are no data relating to the abundance of heterotrophic flagellates to their ecologic role in peatland aquatic environments. The biomass of heterotrophic flagellates of a size < 20 μm was weakly correlated with that of bacteria ($r = 0.50$, $p < 0.1$). It is probable that, as has been observed in aquatic environments [8], heterotrophic flagellate protozoa have a diet that consists mainly of bacteria. This predator-prey relationship is difficult to demonstrate in peatlands because of the relatively low biomass of flagellates; and, because the populations of flagellates are simultaneously subjected to a high predation pressure by testate amoeba and, to a lesser extent, by ciliates and micrometazoans.

Inorganic carbon fixation by photosynthetic assimilation greatly dominated, compared to fixation of organic carbon by the photo- and chemoheterotrohic pathways. The heterotrophic activities were underestimated by the experimental protocol that was used, as pointed out by Amblard et al. [1].

The values of photosynthetic assimilation were of the same order of magnitude as those recorded by Pârvu [29] who estimated the primary production in a *Sphagnum magellanicum* peatland to be 40 mgC m^{-3} h^{-1} using Vinberg's method, which measures the quantity of oxygen released by photosynthesis.

Photosynthetic assimilation was correlated with the biomass of cyanobacteria ($r = 0.92$, $p < 0.001$) and Bacillari-

ophyceae ($r = 0.77$, $p < 0.05$), and with the chlorophyll *a* concentration ($r = 0.94$, $p < 0.001$).

We have compared the structure and the functioning of the microbial loop community at the surface of the peatland and in an acid humic lake in the Massif Central of France [4]. The structure of the peatland protozoa communities was different from that recorded in lake environments. For example, the biomass of heterotrophic flagellates was of the same order of magnitude in the peatland as in the humic lake, whereas the biomass of ciliates was, on the average, tenfold greater in the peatland. Moreover, testate amoeba, which were practically absent from the lacustrian plankton, accounted for nearly one-half of the biomass of microorganisms in the peatland (Fig. 7). It should be noted that the subaquatic peatland environment is typified by its unusually pigmented to heterotrophic microorganism-biomass ratio. In this environment, heterotrophic microorganisms accounted for 65% of the total biomass, and pigmented microorganisms for only 35%. In the mesotrophic lake environment, however, the relative proportions of these two communities were reversed (29% and 71%, respectively) [4]. To our knowledge, there is no information available regarding potential heterotrophic assimilation on the surface of peatlands. On average, potential photoheterotrophic assimilation per unit volume was twice as high in the humic lake than at the surface of the peatland. In contrast, potential assimilation by chemoheterotrophy was tenfold higher in the peatland [4].

Impact of Nutrient Input

The comparison of the mean values obtained in the different treatments shows that fertilization of the plots led to an increase in pH and a reduction in the dissolved oxygen concentration (Table 1). The supply of phosphorus resulted in a increase in the concentrations of orthophosphates and total phosphorus in the PKCa and NPKCa plots. On the other hand, the addition of nitrogen did not result in higher total N total, NH_4^+, NO_3^-, or NO_2^- values in the NPKCa plot (Table 1). Similar observations have already been made in the same peatland by Francez (Francez AJ, Ph.D Thesis, Université Pierre et Marie Curie, Paris, 1991; [19]) in the dead part of the *Sphagnum* layer (10–20 cm depth). Nitrogen, which is a limiting nutrient in unaltered peatlands and is quickly consumed by the plants and microorganisms, is discharged into the atmosphere by denitrification, or is leached to greater depths [47].

In terms of the microorganisms, the greater supply of nutrients was reflected by an overall increase in microbial biomass, if testate amoeba are not considered (increase of 15% in the PKCa plot and 31% in the NPKCa plot). When testate amoeba were included, the total microbial biomass decreased by 7% and 15% in the PKCa and NPKCa plots, respectively. The relative proportion of pigmented microorganisms increased in the fertilized plots, accounting for 42 and 47% of the total biomass in the PKCa and NPKCa plots, respectively, compared to 35% in the control plot. More precisely, the supply of nutrients resulted in increases in the relative biomasses of bacteria (14%, 17%, and 25% in the control, PKCa, and NPKCa plots), Bacillariophyceae (14%, 22%, and 37%, respectively) and, to a lesser extent, of ciliates (2%, 6%, and 8%, respectively); and by a decrease in the relative proportion of testate amoeba (48%, 34%, and 19%, respectively) and of other microalgae (7%, 2%, and 1%, respectively) (Fig. 7). The relative biomass of cyanobacteria was also higher in the PKCa plot (18%), where there was no addition of nitrogen, than in the NPKCa plot (9%) (Fig. 7). The supply of nitrogen, no doubt, negated any competitive advantage that nitrogen-fixing cyanobacteria may have possessed.

Regarding the contribution of the different size classes, it should be observed that the relative biomass of picoorganisms (0.2–2 μm) was higher in the NPKCa plot (24.3%) than in the PKCa (16.9%) and control plots (14.5%). The relative biomass of nanoorganisms (2–20 μm) was higher in the PKCa plot (21.4%) than in the control (16.5%) and NPKCa plots (13.7%), and that of the microorganisms (20–200 μm) was higher in the control plot (69.0%) than in the NPKCa (62.0%) and PKCa plots (61.7%). Overall, the relative biomass of picoorganisms was higher at the end of spring and in early summer; that of nanoorganisms was higher in summer to early autumn. It was during the rest of the year (i.e., the cold season) that the relative biomass of microorganisms was highest.

The statistical relations observed for the data collected from the control plot, which partly reflected the trophic dependencies between the various groups of microorganisms, were not really changed in the fertilized plots. In contrast to the bacterial biomass, the biomass of bacterivorous organisms was, however, higher in the control plot than in the fertilized plots. The biomass of protozoa larger than 60 μm was, as with the bacterial biomass, higher in the NPKCa plot (M = 0.347 μgC ml^{-1}) and the PKCa plot (M = 0.305 μgC ml^{-1}) than in the control plot (M = 0.241 μgC ml^{-1}).

The decrease in the relative proportion of testate amoeba in the fertilized plots no doubt accentuated the role of other protozoa in predation on bacteria. For example, in the NPKCa plot, the biomass of heterotrophic flagellates was very closely correlated with the bacterial biomass ($r = 0.91$, $p < 0.001$), whereas it was probable that predation by the testate amoeba limited the development of heterotrophic flagellates in the control plot, as has been observed in lake environments in periods when the density of cladocera is high [21].

The functioning of the communities was only slightly changed. Only the potential chemoheterotrophic assimilation was significantly lower in the PKCa and NPKCa plots than in the control plot.

Our study is, to the best of our knowledge, the first that has been conducted on the microbial loop in peatlands. In this ecosystem, where a large proportion of primary production is by *Sphagnum,* the microbial loop seems to play an important role in the flow of matter and energy. The trophic chain based on photosynthetic assimilation by algae is relatively poorly developed, whereas, in terms of biomass, the relative importance of heterotrophic microorganisms is higher than in marine or lacustrine environments.

However, ciliates and heterotrophic flagellates compose only a small proportion of the total protozoan biomass. It is heavily dominated by testate amoeba, microorganisms having a varied diet. These latter are, therefore, of importance in the structure and functioning of the microbial loop in this environment. This aspect is completely different from observations conducted in marine or lacustrine environments. The input of nutrients resulted in an overall modification of the environment, which was reflected, at first, by a change in the community structure.

Acknowledgments

This study has been supported by the European Community (Environmental program, 3rd Framework, Contract no. EV5V-CT92-0099, CEREMCA Association).

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