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ENVIRONMENTAL MICROBIOLOGY

Relationship of Atmospheric Pollution Characterized by Gas (NO_2) and Particles (PM10) to Microbial Communities Living in Bryophytes at Three Differently Polluted Sites (Rural, Urban, and Industrial)

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Abstract Atmospheric pollution has become a major problem for modern societies owing to its fatal effects on both human health and ecosystems. We studied the relationships of nitrogen dioxide atmospheric pollution and metal trace elements contained in atmospheric particles which were accumulated in bryophytes to microbial communities of bryophytes at three differently polluted sites in France (rural, urban, and industrial) over an 8-month period. The analysis of bryophytes showed an accumulation of Cr and Fe at the rural site; Cr, Fe, Zn, Cu, Al, and Pb at the urban site; and Fe, Cr, Pb, Al, Sr, Cu, and Zn at the industrial site. During this study, the structure of the microbial communities which is characterized by biomasses of microbial groups evolved differently according to the site. Microalgae, bacteria, rotifers, and testate amoebae biomasses were significantly higher in the rural site. Cyanobacteria biomass was significantly higher at the industrial site. Fungal and ciliate biomasses were significantly higher at the urban and industrial sites for the winter period and higher at the rural site for the spring period. The redundancy analysis showed that the physico-chemical variables ([NO_2], relative humidity, temperature, and site) and the trace elements which were accumulated in bryophytes ([Cu], [Sr], [Pb]) explained 69.3% of the variance in the microbial community data. Moreover, our results suggest that microbial communities are potential biomonitors of atmospheric pollution. Further research is needed to understand the causal relationship underlined by the observed patterns.

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Introduction

In the last few years, atmospheric pollution has become a serious problem of society because of its drastic effects on both human health and ecosystems [7, 17, 30, 43]. Atmospheric environment is a mixture of gases and particles of mineral or organic origin. The types of atmospheric pollutants and the pollution levels depend on many factors, such as emission sources, physical conditions, and meteorological parameters. The concentrations of the principal atmospheric gas pollutants have been increasing due to the increase of anthropogenic sources like traffic and industry [4, 30]. For instance, nitrogen dioxide (NO_2) is

considered as the indicator of road traffic pollution [4]. More recently, particulate air pollution has become one of the most important issues in air quality. Particles can be generated by natural (volcanism, forest fire...) and anthropogenic (industry, traffic...) sources [2, 29, 50, 52]. In the case of anthropogenic pollution, the particles can contain many elements such as heavy metals like Cu, Zn, Ni, and Pb [19, 38, 50, 53]. The measurement of particles has been included in the panel of air quality monitoring and norms but only according to size fraction [27]. The World Health Organization (WHO) recommendations have been defined for PM10 (Particulate Matter with a diameter ≤10 μm). WHO fixes for PM10 the upper limit value for protection of health at 50 μg m^{-3} on a daily average (decree n°1999/30/CE). However, the measurement of these particle concentrations by physical techniques alone does not show the impact on organisms and ecosystems. Indeed, the relative magnitudes of the PM10 deposition modes (dry and wet) vary with ecosystem type, altitude, primary source location, and chemical burden of the atmosphere [27].

The effect of atmospheric pollutants could thus be evaluated by using specific organisms. The "biomonitoring approach," which is based on the sensitivity of organisms, is one solution to estimate the effect of air pollution. It is proven a complementary method to the usual chemical analysis. It integrates the pollution level over a long period of time and therefore provides data about an average pollution level for a given place. Several groups have been used, such as vascular plants [12], lichens [13], and mosses [37] which accumulate heavy metals thanks to their anatomical and physiological characteristics [11, 37, 42]. However, studies carried out at the scale of simple organisms or at the scale of populations do not take into account the complex effects of pollution, i.e., the direct and the indirect effects resulting from modifications of interactions between the species. A possible way to understand the effect of complex pollution on a complex functioning ecosystem could be to study simple systems but which nonetheless include different species belonging to the different trophic groups (primary producers, predators, and decomposers). Under these conditions, the microbial communities living in terrestrial bryophytes could represent a good compromise. Many species of mosses are ubiquitous and cosmopolitan and can be found in many natural and anthropogenic ecosystems. They shelter a large number of microbial species, including auto- and heterotrophic bacteria, algae, protozoa, fungi, and small metazoans like rotifers or nematodes, with a very high growth rate living in a small area.

Some studies have been done on the effect of atmospheric pollution on microbial communities in mosses. Gilbert et al. [25] showed that the enrichment in nitrogen affected a structure of microbial communities living in *Sphagnum fallax*. This nitrogen enrichment involved an increase in the relative importance of cyanobacteria and microalgae and a decrease in the relative importance of bacteria and testacean. Furthermore, nitrogen enrichment modifies the functioning of peatland. The change in the structure of microbial communities leads to increased activity of decomposition [9] and thus to greater release of CO_2. During two studies which were carried out respectively in France and in Vietnam, Nguyen-Viet et al. [33] showed that species richness and testate amoebae abundance in terrestrial mosses were negatively correlated with atmospheric concentrations of NO_2 and with atmospheric lead (Pb) accumulated in bryophytes (*Barbula indica*) [34]. Moreover, they showed that the species reacted differently according to the type of pollutant. This same team has shown in experimental conditions that the bryophyte Pb concentration was negatively correlated with the biomasses of bacteria, microalgae, ciliates, and testate amoebae living in *Sphagnum fallax* [35]. To our knowledge, the effect of particle deposition on microbial communities in mosses has not been studied. However, the nature and the size of particles accumulated in mosses could have a specific impact on their microbial communities. Indeed, particles in mosses could release pollutants or could be ingested by filtering or phagotrophic microbial predators.

In this study, we evaluated the relationships between atmospheric pollution (PM10 particles and NO_2 concentrations) and microbial communities in *Pseudoscleropodium purum* (Hewd.) at three differently polluted sites (rural, urban, and industrial). To allow comparison of microbial communities at three sites, we used transplanted bryophytes coming from the same unpolluted place. We hypothesized that (1) atmospheric pollution would be different at the three sites, and thus accumulated elements in mosses would vary according to the site, (2) the structure of microbial communities would vary among sites in relation to the type of pollution (rural, urban, industrial), and (3) the effect of atmospheric pollutants would be different for each functional microbial group according to its ecology and position in a microbial food web.

Methods

Study Sites

The study was conducted at three differently polluted sites in northeast of France (Fig. 1). The rural site (R) is localized in Montagney (geographical localization: Lambert II: X: 851727, Y: 2259120; at an altitude of 192 m), 30 km to the west of Besançon (110,000 inhabitants). The landscape around this site consists of meadows, and the main human activity is cattle farming. The urban site (U) is localized in Saclay (geographical localization: Lambert II:

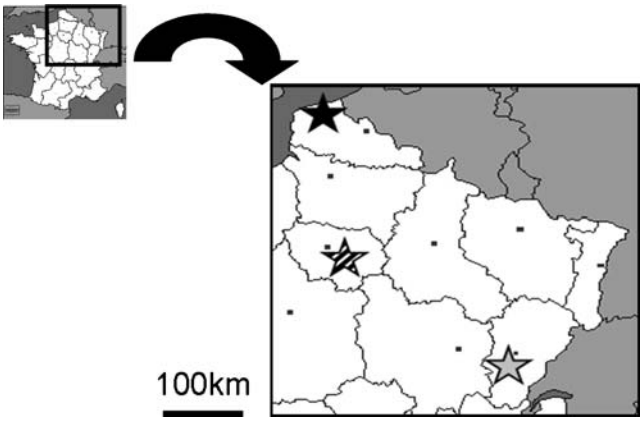

Figure 1 Location of study sites (rural, urban, and industrial) in France (www.hist-geo.com)

X: 587564, Y: 2414660; at an altitude of 150 m), a small city (28,000 inhabitants) situated 20 km to the south of Paris, 100 m distance from the N118 highway where the traffic density is close to 65,000 vehicles per day and 7 km from a waste incinerator. The industrial site (I) is localized in Dunkirk (geographical localization: Lambert II: X: 598633, Y: 2672262; at an altitude of 6 m) at the North Channel coast where the main activities are the steel industry (6.7 Mtons/year), the aluminum industry (0.3 Mtons/year), and petroleum refinery. The chemical industry and the food industry are also prevalent.

Moss Sampling and Transplanting

Transplants of *P. purum* (Hewd.) were taken in the "Fontainebleau forest" in July 2005, a zone which is distant from fixed and mobile pollution sources. The technique used in this study was slightly different from the "moss-bag" technique used by Couto et al. [15]. Pieces of moss carpets were placed in small containers (15×15 cm, 4 cm in depth), but were not washed before, and were then acclimatized for 3 months at the rural site. Humidity was maintained in the moss containers using a system of capillarity wicks which absorbed Volvic mineral water [1].

These containers were exposed at each site from October 2005 to June 2006 in three roofed shelters, allowing air circulation but preventing contamination by atmospheric particles carried by rain. Each shelter contained five small containers of *P. purum* (Hewd.). In each shelter, a small container of *P. purum* (Hewd.) was taken out every 2 months (October, December, February, April, and June). Each time, all the green parts of the stems (about 4 cm) were removed and mixed together. Random samplings of moss stems were carried out. The first was about 15 stems, put into 20 ml of glutaraldehyde (2% final concentration) for microbial community analysis. The second was approximately 50 stems, used for heavy metal analysis.

Meteorological Data, NO_2 Sampling and Analysis, Metal Trace Element Analysis

Meteorological Data Data from Meteo France stations (temperature, humidity, rain, wind speed, solar radiation) located in urban and industrial sites were used. For the rural site, these data were obtained from our own weather station.

NO_2 Passive samplers are calibrated tubes, in which gases move only by molecular diffusion [14, 39]. A triethanolamine solution fixed the NO_2. Mean concentration of NO_2 ($\mu g\ m^{-3}$) in sampled air was calculated on the basis of the amount of collected pollutant, exposure time and gas collection rate in the tube. Passive samplers were set up vertically and at 2 m from the ground [8, 14]. The passive samplers were removed every 2 weeks, and absorbed NO_2 was measured by spectrophotometry.

Metal Trace Elements Metal trace elements (MTE) in mosses were analyzed by instrumental neutron activation (INAA) and by inductively coupled plasma mass spectrometry (ICP-MS) by the Commisariat à l'Energie Atomique (CEA).

Instrumental Neutron Activation Fifty milligrams of dry powder for each sample was mixed to an equivalent weighed amount of ultra pure cellulose. A pellet of the mixture was prepared by means of a press. Each pellet was wrapped in a plastic bag under a thermal neutron flux of 1.2×10^{13}n $cm^{-2}s^{-1}$ in the CE/Saclay Orphee reactor. Four radioactivity countings, at four different decay times were performed. Each sample was irradiated simultaneously with a precise amount (2 to 5 μg) of gold (IRMM reference material Au 0.1%/Al). Pellets were wrapped in high purity aluminum foil in a thermal flux of about 2×10^{13}n $cm^{-2}s^{-1}$ in the CE/Saclay Orphee reactor. Three radioactivity countings were performed. Standardization was done by means of the k_0 technique [40] with a flux monitor using about 10 mg of pure Fe.

Concentrations of Al, Cr, Fe, Zn, and Br were determined by this method.

Inductively coupled plasma mass spectrometry ICP-MS measurements were performed using a quadrupole ICP-MS spectrometer X7 series. Concentrations of Cu and Pb were determined by ICP-MS after acid digestion.

This method requires the use of internal standards. The quality control of the analytical technique was ascertained by applying the same analytical methods to the following certified reference materials: lichen 336, soil 7, and algae *Fucus* 140, all provided by the International Atomic Energy Agency.

Microbial Communities' Extraction and Analysis

Extraction

All microbial organisms were extracted from the mosses using the method of Nguyen-Viet et al. [35]: each sample was first shaken in a vortex and then filtered through a 180 µm mesh filter. Fifteen milliliters of glutaraldehyde (2% of final concentration) were added to the sample. Afterwards, the sample was shaken and filtered again. The process was repeated six times, and all filtrate fractions were combined to obtain a final composite sample of 110 ml.

Analysis

For heterotrophic bacteria, 0.5 ml of the final solution added to 0.1 ml of DAPI (4,6 diamino 2 phenylindol, 0.2% of final concentration) was exposed to darkness for 15 min and then filtered through 0.2 µm black membrane filters [41]. The black membrane filters were examined by epifluorescence microscopy at ×1,000 magnification. Bacteria numbers and sizes were estimated by an image analysis program (LUCIA 4.0). Between 366 and 1,100 bacteria were counted and measured for each sample.

For the other microorganisms, 10 or 15 ml of the final composite sample were analyzed at ×400 with an inverted microscope in accordance with the Uthermöhl method [46]. For fungi, hyphae and spores were counted and measured. For testate amoebae, living and encysted tests were counted separately.

Estimation of Biovolume and Biomass

The biovolume of each groups was first estimated by assimilation with geometrical shapes and then converted to carbon using the following conversion factors: heterotrophic bacteria, $1\,\mu m^3 = 5.6 \times 10^{-7}\,\mu gC$ [10]; cyanobacteria and algae, $1\,\mu m^3 = 1.2 \times 10^{-7}\,\mu gC$; ciliates and testate amoebae, $1\,\mu m^3 = 1.1 \times 10^{-7}\,\mu gC$ [49]; fungi, $1\,\mu m^3 = 2.5 \times 10^{-7}\,\mu gC$; nematodes and rotifers, $1\,\mu m^3 = 1.25 \times 10^{-7}\,\mu gC$ [26]. These data were expressed as microgram of Carbon (µgC) per gram of *P. purum* dry weigh (µgC gDW^{-1}).

Numerical Analysis

The element concentrations in mosses were transformed into enrichment factors (EF) by dividing the trace element (TE) contents determined for each moss at the end of an exposure period (June 2006) by the corresponding initial contents (October 2005) [6]: $[\text{final TE}]_{\text{June}}/[\text{initial TE}]_{\text{October}}$. The initial contents of chemical elements in bryophytes (three samples per site) were analyzed in October 2005 after acclimatization of mosses at the rural site and before exposure at the polluted sites.

Kruskal–Wallis tests were used at each time of sampling to compare the three sites according to biomasses of different microbial groups and the concentrations of NO_2.

To analyze the relationship between microbial biomass and environmental data, Linear Model (LM) and Linear Model with Random Effect were compared to determine the importance of the random effects (the variable "replicate" was considered as a random variable) as described by Venables and Ripley's study [47]. As the random effects were negligibly small, LM was performed between microbial community data (biomasses of microbial groups) and environmental data (temperature, relative humidity, NO_2, and trace element concentrations in bryophytes).

Finally, to assess the relationships between the composition of microbial communities and environmental variables, a redundancy analysis (RDA) was carried out using the program CANOCO 4. Detail of this method can be found in Ter Braak and Smilauer's study [45]. Briefly, the importance of the environmental variables was determined by stepwise forward selection. At each step, the "extra fit" was determined for each variable. The variable with the largest extra fit, if significant (Monte Carlo permutation test, 999 permutations), was then included, and the process was repeated until no variables remained that could significantly improve the fit. All biomass data were $\ln(x+1)$ transformed to stabilize variance and reduce the influence of dominant taxa on the ordination. In this study, the variable "site" is a nominal variable. To analyze a nominal response variable with CANOCO, each nominal variable must be represented by a series of dummy variables, each representing a category. For the analyses by CANOCO, the categories of different nominal variables must be assigned different numbers. One can number then consecutively from 1 to m with m being the total number of categories.

Results

Meteorological Data, Atmospheric Pollution, and Trace Element Concentrations in *P. purum* (Hewd.)

For autumn and winter period (October to February), the average temperature was higher at the industrial site (9.0°C from October to December, 4.1°C from December to February) than at the urban site (respectively, 6.5°C and 2.3°C) or at the rural site (respectively, 5.6°C and −0.3°C). Moreover, during this same period, the minimal temperatures were lower at the rural site (respectively, −3.2°C and −8.8°C) than at the urban site (respectively, −0.4°C and −3.8°C) or at the industrial site (respectively, 2.7°C

and −0.9°C). From February to April, the average temperatures were similar at the three sites, but the minimal temperature was lower at the rural site (−2.80°C) than at the urban site (−0.50°C) or at the industrial site (0.1°C). From April to June, the average temperatures were similar, and the minimal temperatures were never negative.

During this study, the average and the standard deviation values of relative humidity were similar for all sites (86±7% at the rural site, 80±6% at the urban site, and 81±2% at the industrial site).

Atmospheric NO_2 concentration levels varied from 7 to 13 µg m^{-3} at the rural site, from 56 and 68 µg m^{-3} at the urban site, and from 37 and 48 µg m^{-3} at the industrial site. During the whole period of exposure, the NO_2 concentration was significantly higher at the urban and industrial sites than at the rural site ($p<0.01$).

The analysis by INAA and ICP-MS showed an accumulation of particle chemical elements in *P. purum* (Hewd.). At the beginning of the study, the initial concentrations (average±standard deviation) of Al, Cu, Zn, Cr, Fe, Pb, and Sr in bryophytes were 437.21±37.57, 8.57±0.92, 56.00±21.81, 0.98±0.40, 219.44±72.40, 3.73±0.55, and 10.02±0.73 µg g^{-1}, respectively. No differences were observed between the three sites.

Metal concentrations in bryophytes were presented in Fig. 2. The enrichment factors were calculated for Fe, Cr, Pb, Al, Sr, Cu, and Zn at each site. The bryophytes exposed at the industrial site accumulated Fe, Cr, Pb, Al, Sr, Cu, and Zn (EFs were 49, 11.4, 5.3, 4, 2.5, 2.03, and 2.04). Those exposed at the urban site accumulated Cr (EF=3.48), Fe (EF=3.27), Zn (EF=2.20), Cu (EF=2.20), Al (EF=2.06), and Pb (EF=2.03). The bryophytes exposed at the rural site accumulated Cr (EF=2.24) and Fe (EF=2.15).

Evolution of Microbial Community Biomasses

At the beginning of the study (samples T0), the microbial community was composed of 64% decomposers, among which were 53% bacteria and 11% fungi; 3% primary producers, among which were 2.7% microalgae; and 33% predators, among which were 23% testate amoebae and 9% rotifers (Table 1).

The total microbial biomass changed in relation to the evolution of temperatures, increasing significantly from December to June at the rural site ($p=0.01$). After 8 months of exposure, there were no significant differences in the total biomass among the three sites (Fig. 3).

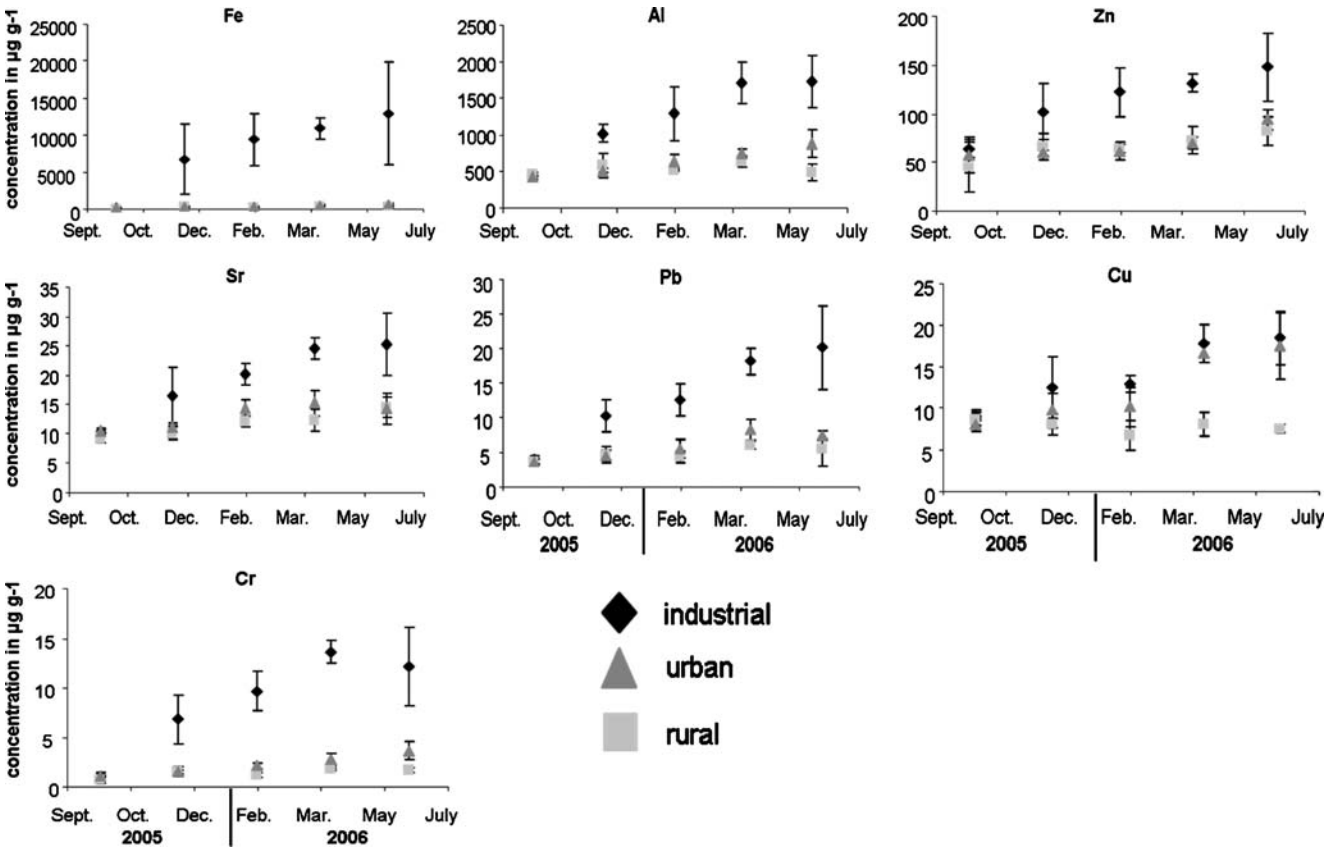

Figure 2 Evolution of chemical element concentrations in bryophytes (average ± SD) in micrograms per gram during the study for each site

Table 1 Relative importance of microbial groups (average and standard deviation) at the beginning and the end of exposition

Groups	October 2005		June 2006					
			Rural		Urban		Industrial	
	Average	SD	Average	SD	Average	SD	Average	SD
Bacteria	52.90	11.91	38.53	6.72	41.41	1.76	38.06	7.65
Fungi	10.67	2.66	26.08	2.68	26.45	0.49	27.32	7.28
Cyanobacteria	0.38	0.10	7.15*	1.66	9.23*	2.55	17.88*	6.73
Microalgae	2.72	0.67	8.18	0.58	11.95	3.18	5.41	2.82
Testate amoebae	23.43	3.73	16.53	5.61	8.72	3.20	9.62	4.17
Ciliates	0.94	0.58	0.23*	0.06	0.05*	0.03	0.06*	0.04
Rotifers	8.62	6.59	3.16	1.61	2.13	1.28	1.59	0.23
Nematodes	0.33	0.18	0.13	0.04	0.07	0.03	0.06	0.05

*$p<0.05$, Kruskal–Wallis test

Primary Producers During the study, the total biomass of primary producers increased at the three sites ($p<0.05$). However, the biomass of cyanobacteria tended to increase at the polluted sites at the end of the study ($p=0.02$ in April compared to the rural site; Fig. 3), and the relative importance of cyanobacteria was significantly higher at the industrial site (18%) than at the rural site (7%) in June ($p=0.05$; Table 1). Conversely, the biomass of microalgae tended to decrease at the industrial site in June compared to the urban and rural sites ($p=0.06$; Fig. 3).

Decomposers At the rural site, the total biomass of decomposers increased significantly during the spring period (in April and June; $p=0.01$). Bacterial biomass was not significantly different at the end of the study at the three sites. Fungi biomass tended to decrease in April ($p=0.17$) and in June ($p=0.05$) at the industrial site compared to the rural site (Fig. 3).

Predators The total biomass of the predators did not increase significantly at the studied sites during the spring period. However, the biomass of ciliates decreased significantly at the urban and industrial sites ($p=0.04$ in April and June; Fig. 3). The relative importance of ciliates also decreased to a significant degree at the urban and industrial sites ($p=0.05$; Table 1). In the same way, the biomass of testate amoebae was significantly higher at the rural site than at the urban and industrial sites ($p=0.0008$) in June (Fig. 3). Conversely, no significant differences were observed for nematodes and rotifers at the end of the study.

Relationships Between Physico-chemical Environmental Variables, Trace Elements Accumulated in *P. purum* (Hewd.), and Microbial Communities

Redundancy Analysis [NO_2], [Cu], [Sr], [Pb], relative humidity (RH), temperature, time, and site were significant and, taken together, explained 69.3% of the variance in the microbial community data. Pollutants explained 23.0% of this percentage.

Figure 4 illustrates the correlations between environmental variables and microbial communities over the first two canonical axes. Axes 1 and 2 explain, respectively, 41.0% and 12.1% of the variation of microbial communities ($p=0.001$ for each ax, Monte Carlo permutation test, 999 permutations). Figure 4 also clearly indicates that axis 1 is mainly explained by temperature and that axis 2 is mainly explained by NO_2. Therefore, the position of the species along these axes reflects their correlation with temperature and [NO_2]. The RDA shows that testate amoebae and nematodes are strongly negatively correlated with NO_2; fungi and microalgae are strongly positively correlated with temperature and time.

The Linear Model shows that the variance of biomass of cyanobacteria was significantly explained by the regression with relative humidity, [Al], [Cr], [Fe], [Cu], [Zn], [Sr], and [Pb]. The variance of biomasses of microalgae, bacteria, and fungi were significantly explained by the regression with relative humidity and temperature. The variance of testacean biomass was significantly explained by the regression with [NO_2] and [Cu]. The variance of biomass of rotifers was significantly explained by the regression with [Cu] and [Pb]. The variance of biomass of nematodes was significantly explained by the regression with [NO_2].

Discussion

Characterization of the Study Sites and Metal Trace Element Accumulation in Bryophytes

Although the three sites were all located in the northern part of France, they were characterized by different climatic conditions, especially during the wintry periods when

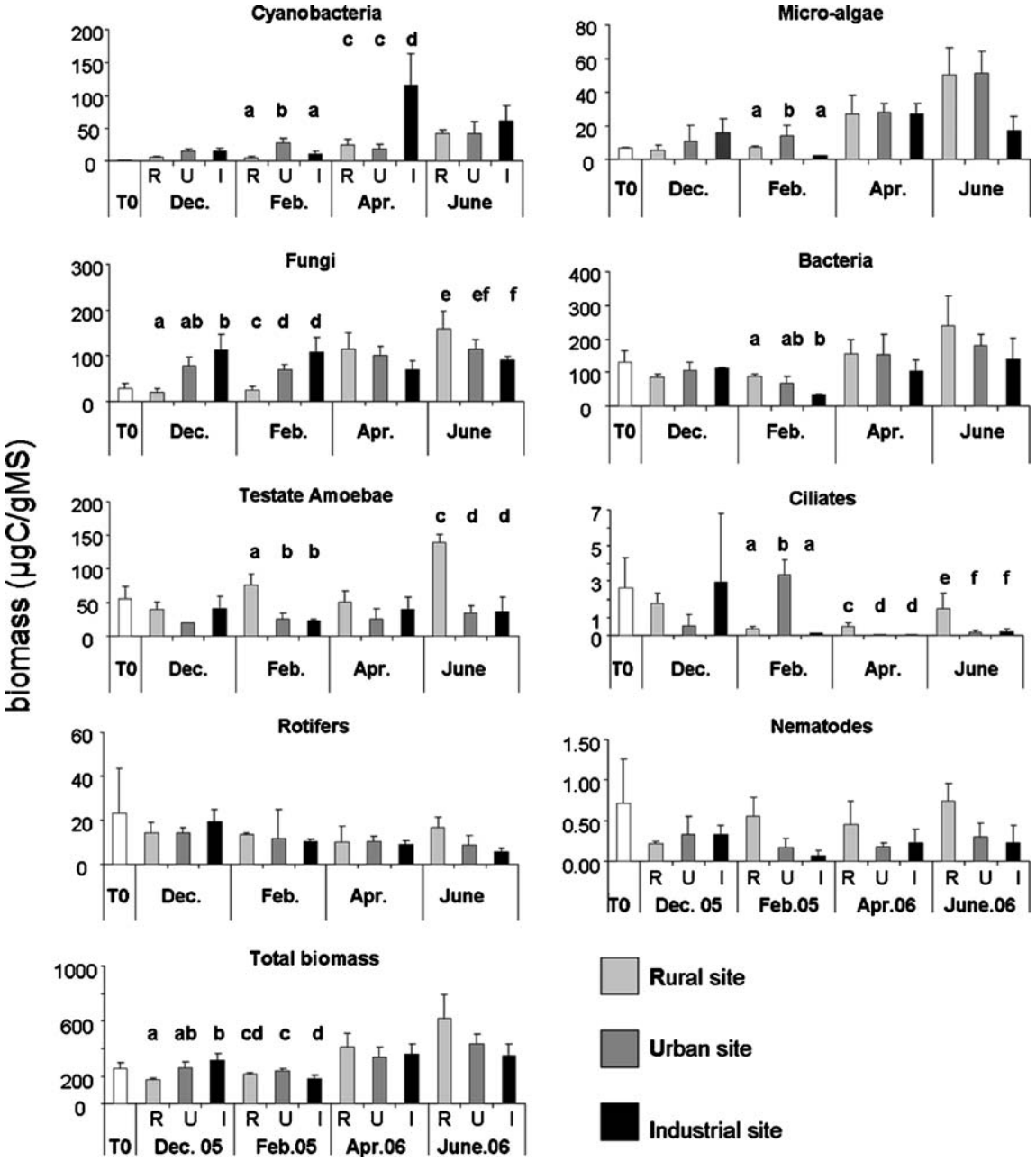

Figure 3 Evolution of biomasses (average ± SD) of microbial communities (µgC/gDW) (*a*, *b*, *c*, *d*, *e*, *f* $p<0.05$, Kruskal–Wallis test)

temperatures were perceptibly lower at the rural site. The three sites were also characterized by different atmospheric pollutions. On one hand, NO_2 concentration was significantly higher at the urban and industrial sites due to traffic activity. However, all the NO_2 concentration values measured were under the official EC norm (decree n°1999/30/CE) for protection of health (mean emission for 1 h: 200 µg/m^3). On the other hand, the depositions of atmospheric particulate chemical elements were quite different in relation to industrial and traffic activity. In accordance with our working hypothesis, MTE accumulated in *P. purum* (Hewd.) differently according to the emission source. The bryophytes exposed at the rural site accumulated Cr and Fe; those exposed at the urban site accumulated Cr, Fe, Zn, Cu, Al, and Pb; and those exposed at the industrial site accumulated Fe, Cr, Pb, Al, Sr, Cu, and Zn (Fig. 2). Bryophytes are well known for their ability to accumulate MTE. So, Galsomies et al. [23] measured the Cr, Cu, Fe, and Zn concentrations accumulated in *P. purum* (Hewd.) in France (Ile-de-France area). Their results showed lower Cu and Zn contents than those measured in the bryophytes exposed at the urban site in this study, whereas Cr and Fe contents were higher. The dissimilarity between the results of these studies can be explained by an

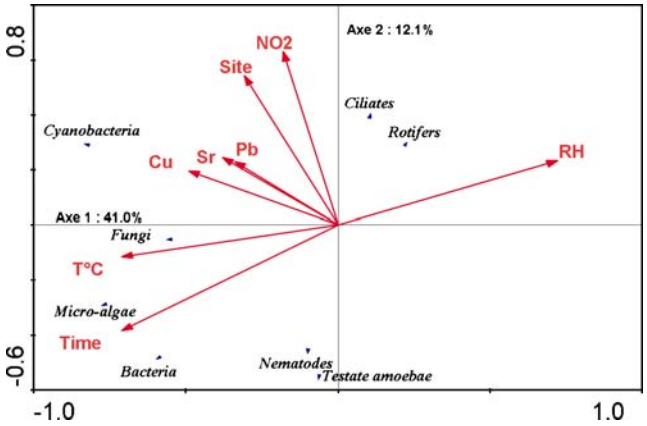

Figure 4 Redundancy analysis biplots (*axes 1* and *2*) of microbial community data, physico-chemical environmental variables, and trace element concentrations in bryophytes

increase in road traffic between 1999 and 2006. Furthermore, Fernandez et al. [20] showed that *P. purum* which were exposed during 30 days in the industrial area in Galicia accumulated MTE. However, in this study, the value of EF for Zn was three times bigger than in our study, while the EF of Cr was 11 times smaller. The MTE concentrations were also measured on other bryophyte species like *Brachythecium populeum* (Hedw.) in India [44] or *Sphagnum girgensohnii* in Romania [16]. However, it is difficult to compare MTE concentrations in mosses between different studies because of the difference in bryophyte species used, pollutant emissions, time/space scale of the studies, and environmental conditions (i.e., meteorological), which can influence metal bioaccumulation in mosses.

The results of this study could complete the others because transplanted bryophytes were used, avoiding genetic variability of mosses. Furthermore, roofed shelters were used to remove rain washing effect, and accumulation of MTE was observed over a long time span of four consecutive periods of 2 months. Under these conditions, the results show regular accumulations of MTE in the upper part of the bryophytes in relation to industrial emissions or traffic activity at the studied sites. These results are in accordance with those obtained by Gaudry et al. [24], who showed that the atmospheric concentration in PM10 particles sampled by *Partisol* pump at the same three sites was considerably higher at the industrial and at the urban sites. These results confirm the interest of using of bryophytes as a method to evaluate metal trace element accumulation at polluted sites.

Relationships Between Environmental Variables, Atmospheric Pollution, and Microbial Biomasses

In this study, we analyzed the biomass evolution of the entire microbial communities in terrestrial mosses in three very different situations. Two major elements could explain the variation we observed: the climatic variables and the atmospheric pollution.

Growth of the bryophytes is strongly related to air temperature and to the presence of water [3, 22, 28]. These factors could probably also limit the development of microbial communities. In this study, temperature played a strongly limiting role during the autumn and winter periods (October to February). Thus, the reduction in the total microbial biomass which occurred at all the studied sites is more marked at the rural site, which was frequently exposed to negative temperatures in winter. This negative effect of cold temperatures on microbial communities is well known, especially for heterotrophic bacteria [51]. In the same way, significant relations between the biomasses of fungi and microalgae and temperature were also observed in the autumn and winter periods at the three sites. Consequently, the biomass of the microbial predators remained low during these periods. During the spring period, the total microbial biomass increased in relation to the cooler temperature.

However, in spring, when temperatures and humidity were similar, the increase in biomass of the different groups was different at the different sites. These variations could be explained by gas and TE concentrations in mosses. Our results show that the NO_2 atmospheric concentrations were negatively correlated with the biomasses of testate amoebae and nematodes, while they were positively correlated with the biomasses of fungi and cyanobacteria for the winter period. For the testate amoebae, these results confirm the observations of Nguyen-Viet et al. [33], which were obtained under similar conditions. For fungi and cyanobacteria, these results are similar to other studies in relation with the impacts of pollutants of atmospheric origin [35]. NO_2 is considered to be an indicator of urban atmospheric pollution due to traffic and could have a direct effect on microbial communities. Indeed, for the time being, no datum concerning the direct effect of this gas on microorganisms is available.

In addition, *P. purum* (Hewd.) accumulated MTE during the period of exposure, in relation to PM10 particle deposition. These MTE constitute the atmospheric pollution at the industrial and urban sites only in part, where many organic pollutants are probably also emitted. Be that as it may, in accordance with our working hypothesis, the structure of microbial communities seems to have been significantly influenced by particulate TE deposition (Fig. 4). However, we do not know if these atmospheric particles have a direct impact on the microbial communities by contact or an indirect effect through the microbial food web.

In the primary producers group, cyanobacterial biomass tends to increase, while eukaryotic autotrophic microorganism biomass seems to be not significantly affected by pollutant

deposition. The cyanobacterial biomass was positively correlated with the bryophyte concentrations of Pb, Cu, and Cr, and the relative importance of cyanobacteria—especially *Nostoc* genus—was higher at the industrial site than at the rural site. It is possible that these microorganisms are resistant because of their capacity to accumulate the metal adsorbed in the available nutritive elements [18]. El-Sheekh et al. [18] showed for example that *Nostoc muscorum* and *Anabaena subcylindrica* can eliminate part of the Pb, Cu, Co, and Mn contained in sewage wastewater. In soils, Viti et al. [48] showed that some genera of cyanobacteria—in particular *Nostoc* genus—increased in soil contaminated with Cr.

In the decomposers group, our results show that the bacterial biomass was negatively correlated with the Zn, Br, and Al atmospheric concentrations. In their study, Baldi et al. [5] have showed that there are positive correlations between atmospheric pollutants (boron, arsenic, and mercury) and the abundance of the resistant bacteria of the phyllosphere. However, it is impossible to know whether resistant bacteria developed in bryophytes due or not to our global method of counting bacteria. In the present study, the biomass of fungi increased during the winter period at the polluted sites but decreased during the spring period at these same sites. Frey et al. [21] showed that during a long-term experiment (4 years), the biomass of microbial communities—especially the fungi biomass—decreased, while metal TE concentrations increased. For these authors, the MTE increased the soil pH and decreased the solubility of these elements for the microbial communities. In our study, it would appear that fungi are more sensitive to temperature variations than particulate MTE accumulated in bryophytes.

Following our hypothesis, the microbial predator would be more affected by the pollution resulting from two different parameters: a direct toxic effect and an indirect effect resulting from the modification of the interactions—in particular predation—between the different microbial groups. Indeed, our results show that the biomass of the unicellular predators (ciliates and testate amoeba) was the most significantly affected in the microbial communities. Conversely, the biomass of micrometazoa was no different at the three sites at the end of the study. These results are similar as those obtained in a previous study [34] and confirm that the microbial loop could be an early warning indicator of anthropogenic stress [25, 32]. Concerning the testate amoebae communities, our results are in accordance with several studies in different ecosystems. Nguyen-Viet et al. [34] showed a decline of testate amoebae species richness, abundance, and diversity index with increasing Pb concentration in mosses. In addition, a reduction of abundance of *Difflugia* sp., in activated sludge, was observed in response to copper pollution [36]. The present study confirms this interest to consider the moss testate amoebae communities as good bioindicators of atmospheric pollution. Indeed, in addition to being sensitive to pollutants, observing and determining them is easier than for ciliates. More, their tests could be preserved in some habitats—like peat from *Sphagnum* peatlands—and could be used for paleoecological studies to reconstruct historical evolution of atmospheric pollutant deposition.

In general, the studies have focused on one type of pollutant [25, 31], one group of microorganisms [34] or have been done under controlled conditions [21, 35]. Our study showed correlations between atmospheric pollutants, metal trace elements accumulated in bryophytes, biomasses, and the structure of microbial communities. NO_2 atmospheric concentrations and Cu, Sr, and Pb accumulated in bryophytes affected the structure of microbial communities. Our results suggest that microbial communities and, in particular, testate amoebae, may be useful as biomonitors of atmospheric pollution from metal trace element particulates and NO_2. Other atmospheric pollutants (PAH, VOC,...) could also strongly influence the structure of the microbial communities in bryophytes. Further work should be undertaken, such as experiments under controlled conditions, which would allow these results to be completed. Additional studies could also be done to determine if predators absorb the atmospheric particles, many of them being the same size as microbial preys. More globally, studies are needed to understand the possible direct and indirect effects of atmospheric particle deposition on microbial communities.

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