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Using "bryophytes and their associated testate amoeba" microsystems as indicators of atmospheric pollution

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ABSTRACT

Testate amoebae (TA) associated with terrestrial mosses are increasingly used in ecological and ecotoxicological studies. The TA community is sensitive to changes in its environment (climate change, metal or gas pollution). In this study, a "bryophyte-TA" microsystem was investigated as an indicator of dry particulate deposition and NO_2 atmospheric concentration over an 8-month period in rural, urban and industrial sites in north-eastern France.

The urban site was characterised by significant contamination with NO_2, and the industrial site by significant contamination with Fe, Pb, Cr and Al. Different ecological descriptors of the TA community can be used as indicators of atmospheric pollution. Simple descriptors (total biomass or total abundance) are useful to indicate atmospheric particulate and gaseous pollution, while a complex descriptor (species richness) is useful to differentiate the source of pollution (urban or industrial) in winter and summer. Moreover, Redundancy Analysis showed that some species are negatively correlated with NO_2 and particulate pollution. Principal Responses Curves revealed contrasting dynamics of the TA species biomass according to the type of pollution (urban or industrial).

Our results bring further support to the use of bryophytes and associated TA as a biomonitoring tool for atmospheric pollution. Further studies are required to develop this tool and standardise it for potential general use.

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1. Introduction

The impact of atmospheric pollution on organisms depends on the type and concentration of pollutants, the exposure period and the characteristics of the organism itself. Each year, many chemical compounds (organic or inorganic) are continuously released into the atmosphere from natural and anthropogenic sources (Thorpe and Harrison, 2008; Blake et al., 2009; Byrd et al., 2010). The potential risk for human health from airborne contaminants is determined by physico-chemical measurements. For instance, the European directive 2008/50/CE fixes the upper limit value for atmospheric concentrations of NO_2 for health protection at 40 $\mu g\,m^{-3}$ as an annual average and the upper limit value for protection of vegetation at 30 $\mu g\,m^{-3}$, as an annual average. The World Health Organization (WHO) recommendations have been defined for PM_{10} (Particulate Matter with a diameter $\leq$10 μm) and fix the upper limit value for health protection at 50 $\mu g\,m^{-3}$ as a daily average (decree no. 1999/30/CE).

Understanding the impact of atmospheric pollutants on ecological systems such as microbial communities is challenging. Particulate pollution is complex because particle composition is heterogeneous (Alves et al., 2001; Gaudry et al., 2008; Slezakova et al., 2008; Han et al., 2009) and varies with primary sources and with the atmospheric environment (Alves et al., 2001). Moreover *in situ* particulate pollution is associated with other pollution, such as gaseous pollution. The measurement of these atmospheric pollutants using physico-chemical techniques alone does not suffice to determine the impact on organisms and the integration of these contaminants on the functioning of ecological systems. Several components of atmospheric pollutants can have an impact on organisms, and the effect of a mixture of pollutants is not equal to the sum of the effects of each of them (Martin-Gonzáles et al., 2006; Khan et al., 2007; Gallego et al., 2007; Nwuche and Ugoji, 2008). The biomonitoring approach, which is based on the sensitivity of organisms, is one solution to estimate the effect of complex air pollution on biological communities (Markert, 2007). Bioindicators, which are defined as an organism or a community that contains

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information on the quality of the environment (Markert, 2007), can integrate pollution over a long period of time. Among microbial organisms, testate amoebae have been shown to be useful for bioindication of anthropogenic pollution in peatland (Gilbert et al., 1999), lakes (Roe et al., 2010) and soils (Asada and Warner, 2009).

Testate amoebae (TA) are unicellular protists characterised by a decay-resistant test (Meisterfeld, 2000a,b). They live in a variety of habitats (aquatic environment, soil, and mosses) where they are directly exposed to pollutants. They are abundant and diverse in mosses (Smith et al., 2008). For example, Vincke et al. (2004) identified 83 taxa in the mosses on Île de la Possession, and Golemansky and Todorov (2000) identified 91 taxa in moss, soil and aquatic samples from Thailand. The unique shape of the rigid test, which remains identifiable even after the death of the organism, allows for species-level identification. TA have been used as a tool for the evaluation of past environmental changes (Mitchell et al., 2008).

Nevertheless, only few studies have been published on the use of TA community as potential bioindicators of pollutants. By the use of different ecological indices (biomass, species-specific response, abundance, and diversity indexes), these studies showed the potential bioindicator properties of the TA community for aquatic (Kauppila et al., 2006; Zhou et al., 2006) and atmospheric pollution (Nguyen-Viet et al., 2004, 2007a, 2008). The authors (Nguyen-Viet et al., 2004, 2007a) showed that species richness and TA abundance negatively correlated with atmospheric concentrations of NO_2 in terrestrial bryophytes (*Tortula muralis*) in France and with atmospheric lead (Pb) accumulated in *Barbula indica* in Vietnam. They showed a species-specific response to each pollutant: *Paraquadrula irregularis* was affected by NO_2, whereas *Euglypha ciliate form glabra* and *E. ciliata* were affected by Pb. Two studies have shown an impact of particulate pollution on the biomasses of microbial communities living in *Pseudoscleropodium purum*. *In situ*, Meyer et al. (2010a) showed that urban and industrial pollution had a negative impact on total TA biomass. Under controlled conditions, these authors showed a negative impact of a common level of urban particulate on the total biomass of an active TA community (Meyer et al., 2010b).

In this context, the aim of this study was to estimate the impact of dry deposition of atmospheric particles and atmospheric NO_2 concentration on the TA community living in *P. purum* and to develop an indicator of atmospheric pollution. The TA community data obtained in our previous *in situ* study (Meyer et al., 2010a) has been reworked and expanded to better determine its role as a potential indicator. The effect of different atmospheric pollutions that were characterised by NO_2 atmospheric concentrations and the concentrations of metal trace elements (MTEs) accumulated in bryophytes were estimated using different ecological indexes for the TA community (biomass, abundance, diversity, and species richness).

The main research questions of this study are:

(1) Is the TA community in *P. purum* affected by atmospheric pollution? If so, can we assume that the various types of pollution (rural, urban and industrial) have the same impact?
(2) Is there a response of the ecological index (e.g., structure of the community and diversity) for the TA community to the atmospheric pollution that could be used as a biological indicator of atmospheric pollution?

2. Methods

The study sites, the moss sampling and analysis and NO_2 sampling and analysis were described in detail in Meyer et al. (2010a) and Fabure et al. (2010).

2.1. Study sites

Three sites were selected in north-eastern France according to their pollution sources. The rural site (R) is located in Montagney (geographical location: N 47°17′11″; E 5°39′40″; at an altitude of 192 m a.s.l.). The urban site (U) is located in Saclay (geographical location: N 48°43′41″; E 2°09′29″; at an altitude of 150 m a.s.l.). The industrial site (I) is located in Dunkirk (geographical location: N 51°2′16″; E 2°22′35″; at an altitude of 6 m a.s.l.) on the North Channel coast.

Meteorological data were collected at each site. Data from Meteo-France stations (temperature and humidity) located at the urban and industrial sites were used. For the rural site, these data were obtained from our own weather station (Data logger: Delta-T DL2e; PC software: LS2e; equipments: relative humidity and air temperature sensors).

2.2. Moss sampling and transplanting

In July 2005, samples of *P.purum* were taken from the Fontainebleau forest, an unpolluted site (geographical location: N 48°24′36″, E 2°37′50″). *P. purum* were placed in small containers (15 cm × 15 cm, 4 cm deep), without being washed beforehand, and were then acclimatised for three months at the rural site. Humidity was maintained in the moss containers using a system of capillarity wicks that absorbed Volvic mineral water (Aboal et al., 2008).

These containers were exposed at each site from October 2005 to June 2006 in three roofed shelters. Each shelter contained five small containers of *P. purum*. In each shelter, a small container was taken out every two months: October (samples T0), December, February, April and June. Each time, all the green parts of the stems were removed and mixed together. Then, random samplings of these moss stems were performed. Fifteen stems were put into 20 mL of glutaraldehyde (2% final concentration) for microbial community analysis. Approximately 50 stems were used for heavy metal analysis.

2.3. Metal trace element analysis

Metal trace elements (MTEs) in bryophytes were analysed by instrumental neutron activation (INAA) and by inductively coupled plasma mass spectrometry (ICP-MS). Concentrations of Al, Cr, Fe, Zn and Br were determined by INAA, while concentrations of Cu and Pb were determined by ICP-MS.

2.4. NO_2 sampling and analysis

Passive samplers were used (Palmes et al., 1976; ADEME, 2002). The passive samplers were removed every two weeks, and absorbed NO_2 was measured by spectrophotometry. Mean concentration of NO_2 ($\mu g\,m^{-3}$) in sampled air was calculated.

2.5. Testate amoeba community extraction and analysis

2.5.1. Extraction

The TA community was extracted from the mosses using the method described by Nguyen-Viet et al. (2007b) and Meyer et al. (2010a,b): each sample was first shaken in a vortex and then filtered through a 180 μm mesh filter. Fifteen mL of glutaraldehyde (2% 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.

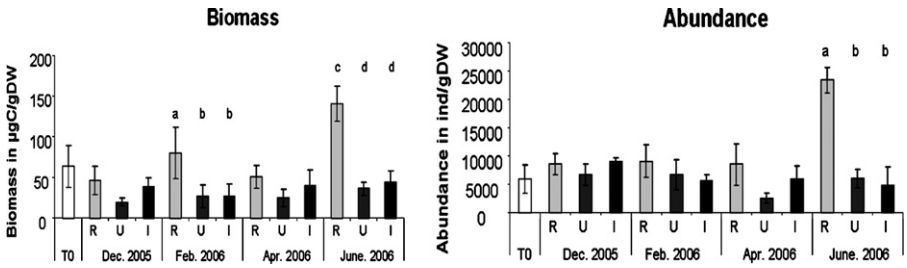

Fig. 1. Comparison of the biomass (μgC gDW^{-1}) and the total abundance (ind gDW^{-1}) of the TA communities (a, b, c, d, e, f: $P < 0.05$: Kruskal–Wallis test) among sites and sampling time (mean ± sd).

2.5.2. Analysis

Ten mL or 15 mL of the final composite sample were analysed at 400× with an inverted microscope in accordance with the Uthermöhl method (Uthermöhl, 1958). The active and encysted tests were counted separately. Between 115 and 384 tests were counted for each sample.

2.5.3. Estimation of biovolume and biomass

The biovolume of each species was considered equivalent to geometrical shapes and then converted to carbon using the following conversion factor: testate amoebae, 1 $\mu m^3 = 1.1 \times 10^{-7}$ μgC (Weisse et al., 1990). These data were expressed as μgC per gram of *P. purum* dry weight (μgC gDW^{-1}).

2.6. Numerical analysis

To analyse the two components of TA community diversity, Hill's numbers and Hill's ratios (Hill, 1973; Alatalo, 1981) were used. The first component, species richness, N_0, is simply the total number of observed species. The second component, species evenness, $E_{2,0} = N_2/N_0$, was computed as the Hill's ratio between inverse Simpson's diversity, N_2, and species richness, N_0, (Gillet et al., 1999). This ratio represents the proportion of dominant species in the community, irrespective of species richness. As in all our statistical analyses, we used biomass instead of abundance compute, N_2. Biomass allows for more weight to be given to species with a big size but a low density. From an ecological point of view, relative biomass is therefore a better representation of the respective contribution of each species in the community than relative abundance.Due to the low number of samples (three per site and per sampling occasion), nonparametric statistical tests were performed. The biomass, abundance, taxa richness and evenness of TA community were compared using Kruskal–Wallis tests. Kruskal–Wallis tests were also used to compare the three sites by their NO_2 and MTE concentrations.

The dynamics of the testate amoeba community in response to environmental conditions was analysed by Redundancy Analysis (RDA) and by Principal Response Curves (PRC). RDA (Ter Braak and Smilauer, 1998) was performed after forward selection of the environmental variables, using the method described in Blanchet et al. (2008) and implemented in the packfor R package (Dray et al., 2007). All biomass data were $\ln(x+1)$ transformed to stabilise variance and reduce the influence of dominant taxa on the ordination.

A PRC analysis was performed using the prc function of the vegan R package (Oksanen et al., 2010). PRC was used to determine the multivariate response of the TA community at the polluted urban and industrial sites over time, as compared to the rural site (Van den Brink and Ter Braak, 1999; Moser et al., 2007). This method makes it possible to summarise effects on all the species of the community and to display them in a single diagram. PRC extracts information from this part of the variance only, which is explained by the treatment (here: site) and time, implemented as covariables. The PRC standardises the control (here: rural site) to be zero-valued for all times, that is, a horizontal line in the PRC diagram. The PRC scores for each of the treatments through time represent compositional deviations from the control.

3. Results

3.1. Characterisation of study sites

Meteorological and atmospheric NO_2 concentrations data were presented in detail by Meyer et al. (2010a).

3.1.1. Meteorological data

The rural site was characterised by lower temperatures than the other two sites, including reaching negative temperatures in the winter.

3.1.2. Atmospheric NO_2 concentrations

During the whole period of exposure, NO_2 concentration was significantly higher at the urban and industrial sites than at the rural site ($P < 0.001$), and from February to June, NO_2 concentration was significantly higher at the urban site than at the industrial site ($P < 0.001$).

3.1.3. Trace element concentrations in P. purum

The analysis by INAA and ICP-MS showed a difference of MTE concentrations in *P. purum*. At the beginning of the study, no differences were observed among the three sites. The data of MTE concentrations in bryophytes are shown in Table 1. After four months of exposure, Fe concentrations were significantly higher at the industrial site than at the urban or rural site, and were significantly higher at the urban site than at the rural site. After six months of exposure, the concentrations of Fe, Pb and Cr were significantly higher at the industrial site than at the urban or rural site, and were significantly higher at the urban site than at the rural site. Finally, after eight months of exposure, the concentrations of Fe, Al and Cr were significantly higher at the industrial site than at the urban or rural site and were significantly higher at the urban site than at the rural site.

3.2. Dynamics of ecological indexes of testate amoeba community

Statistically significant seasonal effects were found for the total biomass and abundance at the rural site (higher in June).

3.2.1. Biomass

At the beginning of the study (samples T0), the biomass of TA was 64 μgC gDW^{-1}. During this study, the biomass evolved differently depending on the site. After four and eight months of exposure, that is, in February and June, biomass was significantly higher at the rural site than at the urban or industrial site ($P = 0.03$ and $P = 0.0002$, respectively) (Fig. 1). In detail, the biomass

Table 1
Variation of trace element concentrations in bryophytes during the study (mean ± sd) (a, b, c: $P < 0.05$, Kruskal–Wallis test).

Element concentrations ($\mu g\,gDW^{-1}$)	October			December			February			April			June		
	Rural	Urban	Industrial	Rural	Urban	Industrial	Rural	Urban	Industrial	Rural	Urban	Industrial	Rural	Urban	Industrial
Fe	176 ± 63	220 ± 76	262 ± 25	367 ± 85	348 ± 35	6772 ± 4705	268 ± 18^{a}	392 ± 60^{b}	9442 ± 3513^{c}	385 ± 7^{a}	572 ± 117^{a}	10947 ± 1439^{c}	378 ± 71^{a}	719 ± 162^{b}	12939 ± 6953^{c}
Cu	8 ± 0.8	8 ± 0.8	9 ± 0.6	8 ± 1	10 ± 2	12 ± 4	7 ± 2	10 ± 2	13 ± 1	8 ± 1	17 ± 1	18 ± 2	8 ± 1	18 ± 4	18 ± 3
Zn	46 ± 26	58 ± 18	64 ± 9	66 ± 11	60 ± 2	102 ± 29	64 ± 4	61 ± 9	122 ± 25	73 ± 14	70 ± 6	131 ± 9	82 ± 15	94 ± 11	148 ± 35
Pb	4 ± 0.4	4 ± 0.5	4 ± 0.6	5 ± 1	5 ± 0.7	10 ± 2	4 ± 0.8^{a}	5 ± 1^{ab}	12 ± 2^{b}	6 ± 0.6^{a}	8 ± 2^{b}	18 ± 2^{c}	5 ± 3	7 ± 1	20 ± 6
Al	459 ± 30	428 ± 31	424 ± 34	586 ± 136	512 ± 22	1023 ± 123	526 ± 27^{a}	631 ± 100^{ab}	1291 ± 371^{b}	637 ± 76^{a}	754 ± 58^{ab}	1712 ± 275^{b}	497 ± 114^{a}	882 ± 190^{b}	1732 ± 353^{c}
Sr	9 ± 0.7	10 ± 0.4	10 ± 0.2	10 ± 1^{a}	11 ± 0.7^{ab}	16 ± 5^{b}	13 ± 0.8^{ab}	14 ± 1^{ab}	20 ± 2^{b}	12 ± 2^{a}	15 ± 2^{ab}	25 ± 2^{b}	14 ± 2	14 ± 3	25 ± 5
Cr	0.8 ± 0.4	1 ± 0.5	1 ± 0.1	1 ± 0.4	1 ± 0.1	7 ± 2	2 ± 0.2^{a}	2 ± 0.3^{a}	10 ± 2^{b}	2 ± 0.1^{a}	3 ± 1^{a}	14 ± 1^{c}	2 ± 0.2^{a}	4 ± 1^{b}	12 ± 4^{c}

of *Trigonopyxis arcula* was significantly higher at the rural site than at the polluted sites in February. In June, the biomasses of seven species were significantly higher at the rural site than at the polluted sites (*Cyclopyxis* sp., *E. ciliata vs glabra, Lesquereusia modesta, Nebela flabellulum, Nebela tincta*, *Trinema* sp. and *T. arcula*).

3.2.2. Abundance

At the beginning of the study (samples T0), the total abundance of the TA species was 5945 ind gDW^{-1}, and the community was heavily dominated by one species: *N. tincta* (22% of the TA community) (Table 2). After eight months of exposure, in June, the total abundance of the TA community was significantly higher at the rural site than at the urban or industrial site ($P = 0.0001$) (Fig. 1). Specifically, the rural community was heavily dominated by three species representing 45% of the TA community: *L. modesta*, *N. tincta* and *Assulina muscorum*; the urban community was heavily dominated by two species representing 55% of the TA community: *Archerella flavum* and *L. modesta*, and the industrial community was heavily dominated by two species representing 53% of the TA community: *L. modesta* and *N. tincta*. In contrast, it would seem that *Hyalosphenia elegans* and *Bullinularia indica* were rarely present at the rural and urban sites, whereas *Difflugia* sp. was rarely present at the industrial site (Table 2).

Moreover, after six months of exposure *B. indica* and *Centropyxis* sp. had disappeared from the industrial site. After eight months, *A. muscorum* and *H. elegans* had also disappeared from the industrial site, and *Difflugia* sp. and *Nebela militaris* had disappeared from the urban site.

3.2.3. Species richness and evenness

At the beginning of the study, species richness was 18 ± 3. At each time of sampling, taxa richness was significantly higher at the rural site than at the urban or industrial site ($P = 0.01$, $P = 0.002$, $P = 0.003$ and $P = 0.007$, respectively). However, at each time of sampling, there was no difference in evenness among sites (Fig. 2).

More specifically, at the urban site, species richness was significantly higher in October and December than in April and June ($P = 0.02$), while evenness was significantly higher in February and June than in October ($P = 0.02$) (Fig. 2). Furthermore, species richness was negatively correlated with time ($r^2 = 0.74$; $P < 0.0001$), while evenness was positively correlated with time. ($r^2 = 0.43$; $P = 0.008$). At the industrial site, species richness was significantly higher in October than in February and June ($P = 0.02$) and was negatively correlated with time ($r^2 = 0.57$; $P = 0.001$).

3.3. Relationships between physico-chemical environmental variables, trace elements accumulated in P. purum and microbial communities

Redundancy Analysis shows that the best model included [NO_2], [Cu], relative humidity (RH), temperature and time as explanatory variables. Put together, they explained 40.5% of the variance in the TA community data ($r^2 = 0.405$, $P = 0.001$). Fig. 3 illustrates the correlations between environmental variables and the TA community over the first two canonical axes. Significant axes 1 and 2 explain respectively 22.16% and 9.69% of the variation of the TA community ($P = 0.001$ for axis 1 and $P = 0.009$ for axis 2, Monte-Carlo permutation test, 1000 permutations). Fig. 3 also clearly indicates that axis 1 is explained mainly by [NO_2] and that axis 2 is mainly explained by time and relative humidity. RDA shows that *N. tincta*, *T. arcula* and *Arcella vulgaris* were strongly negatively correlated with NO_2; *Cyclopyxis* sp. and *Nebela collaris* were strongly positively correlated with relative humidity; *Nebela carinata* was positively correlated with time and *Euglypha strigosa* was negatively correlated with Cu.

Because RDA showed the significant impact of time, Principal Response Curve analysis allowed for the display of the dynamics

Table 2
Testate amoebae abundance (ind gDW^{-1}) at the beginning and the end of exposition (mean ± sd).

Species	October	June		
		Rural	Urban	Industrial
Archerella flavum	54 ± 93		1416 ± 2255	
Arcella vulgaris	421 ± 268	401 ± 179	77 ± 133	131 ± 227
Assulina muscorum	263 ± 294	2237 ± 1551	327 ± 225	
Assulina seminulum	165 ± 167	951 ± 369	498 ± 131	335 ± 217
Bullinularia indica	374 ± 230	71 ± 123	30 ± 53	
Centropyxis sp.	45 ± 77			
Corythion dubium	472 ± 202	944 ± 1034	122 ± 211	440 ± 592
Cyclopyxis sp.	56 ± 96	1566 ± 881		
Difflugia sp.	186 ± 99	742 ± 245		22 ± 38
Euglypha ciliata vs glabra	18 ± 31	1014 ± 413	209 ± 290	
Euglypha compressa	296 ± 253	435 ± 387	339 ± 510	226 ± 210
Euglypha strigosa	357 ± 533	1769 ± 1699	106 ± 117	119 ± 206
Heleopera sp.	22 ± 29			284 ± 491
Hyalosphenia elegans	60 ± 103	60 ± 104	26 ± 44	
Lesquereusia modesta	276 ± 127	3875 ± 3367	1385 ± 384	1181 ± 692
Nebela carinata	159 ± 158	842 ± 365	383 ± 254	359 ± 360
Nebela collaris	239 ± 252	38 ± 66		
Nebela flabellulum	256 ± 386	412 ± 386		
Nebela militaris	26 ± 46	40 ± 69		59 ± 103
Nebela tincta	1313 ± 452	4069 ± 1547	672 ± 337	966 ± 677
Trinema sp.	369 ± 86	2704 ± 2206	167 ± 74	59 ± 103
Trigonopyxis arcula	281 ± 178	774 ± 445		

of the TA community. This analysis considers variations of controls as baseline. In this study, the rural site is the control. Each species is attributed a score according to its contribution to deviations of the community due to the different site conditions. A positive score within the diagram indicates a reduced biomass of the species in the polluted sites compared to the control. The PRC diagram of the TA community living in bryophytes shows a deviation from the control for the urban and industrial sites (Fig. 4). The stars show a significant difference between the total TA biomass at the polluted sites and at the rural site (control).

PRC revealed that 33.4% of the total variance of the TA community data is explained by time and 29.1% by site. The first canonical

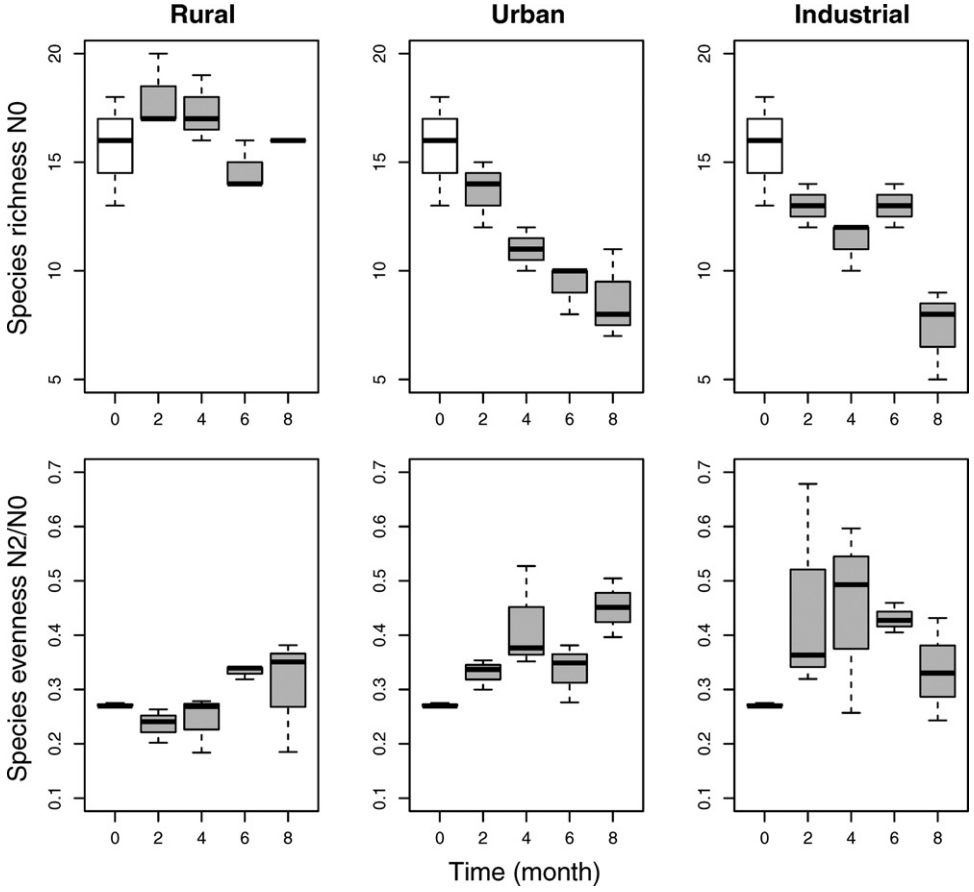

Fig. 2. Comparison of testate amoeba species richness and evenness among sites and over time.

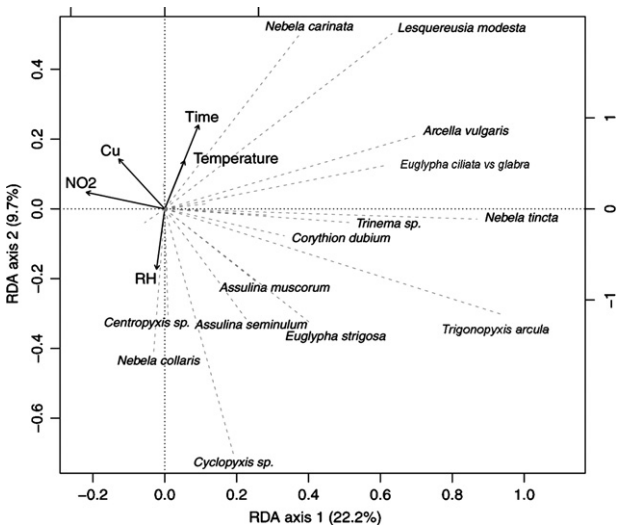

Fig. 3. Redundancy analysis (axes 1 and 2) of the TA community data, after forward selection of the explanatory variables (physico-chemical environmental variables including trace element concentrations in bryophytes). Only species far from the origin are labelled.

axis of the PRC captured the only statistically significant part of the variance explained by the treatment ($P = 0.0014$, Monte Carlo permutation test, 10,000 permutations). The highest responses were obtained for *T. arcula* (1.10), *N. tincta* (0.70) and *A. vulgaris* (0.68).

4. Discussion

4.1. Study sites, metal trace elements accumulation and TA diversity in the microsystem

The characterisation of study sites and MTEs accumulation were discussed in our previous study (Meyer et al., 2010a). Briefly, the three sites were characterised by different climatic conditions and different atmospheric pollution. The urban site showed significant contamination with NO_2, in agreement with its localisation near a highway, while the most serious pollution by MTEs was found at the industrial site, in agreement with the presence of metallurgical plants in the area (Gaudry et al., 2008).

P. purum is used to biomonitor atmospheric pollution (Zechmeister et al., 2005; Amblard-Gross et al., 2002; Fernández and Carballeira, 2001). In France, this bryophyte species is used by

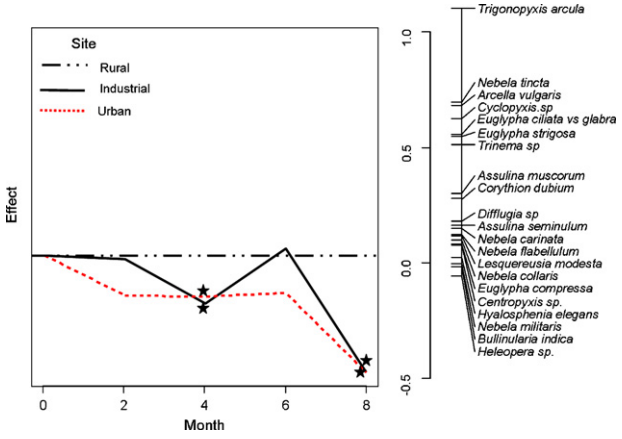

Fig. 4. Principal response curves with species weights for the TA community data, indicating the effect of sites. The star (⋆) shows a significant difference of TA biomass between the control (rural site) and the polluted sites ($P < 0.05$, Kruskal–Wallis test).

the program BRAMM/PIC to monitor MTE atmospheric fallout in a rural zone (Galsomies et al., 1999). Furthermore, the morphology of *P. purum* is adapted to the development of microorganisms: broader leaves and more numerous ramifications (Augier, 1966; Shaw and Goffinet, 2000) can keep humidity higher for greater development of a TA community (18 ± 3 species at the rural site). TA species richness is dependent on moss species identity, geographical location, sampling strategy (length of stem removed, number of samples and number of sampled mosses) and moss moisture (Bonnet, 1973). In the terrestrial mosses, Nguyen-Viet et al. (2004, 2007a) found nine species in *T. muralis* (Besançon, France) and 23 species in *B. indica* (Hanoi, Vietnam), while Mieczan (2009) identified 45 TA taxa in the semi-aquatic mosses (*Sphagnum*). For these reasons, it is difficult to compare diversity of the TA community among different ecotoxicological studies. What is more, in this new field there is no standardised method for bioindication using a TA community?

4.2. Effects of atmospheric pollution on testate amoebae community and using ecological indexes as indicators

Different parameters can be used as indicators of atmospheric pollutants: appearance/extinction of species or decrease in specific biomass/abundance. This study showed a specific impact of atmospheric NO_2 and those particulate pollutions on the TA species living in *P. purum*. *N. tincta*, *T. arcula* and *A. vulgaris* are sensitive to NO_2 while *E. strigosa* is sensitive to Cu (Fig. 3). Moreover, *A. vulgaris* was rarely present and *Difflugia sp.* disappeared at the urban site, while *B. indica* and *Centropyxis* sp. disappeared after six months of exposure and *A. muscorum* after eight months at the industrial site. At the urban site, the species responses are in accordance with the results found in other environments. In waste water and sediments, *Arcella* sp. and *Difflugia* sp. are known to be sensitive to Cu pollution (Nicolau et al., 2005; Kauppila et al., 2006). However, the species responses at the industrial site are in contradiction to the results of Patterson et al. (1996) in water and Asada and Warner (2009) in soil. In these studies, *Centropyxis* sp. was abundant in environments polluted by As, Hg and Cu.

The species-specific response of the TA community can be used as a bioindicator of atmospheric pollutants and, in the "*P. purum*-TA" microsystem, can be used to differentiate the pollution sources (urban or industrial). The diversity and species composition of TA communities varies according to the biotope, so it is difficult to compare different species-specific responses of the TA community in different studies. That is why it is necessary to use global descriptors that are not based on the species composition as indicators of atmospheric pollution and its integration into ecosystems (integrative stress indicators) (Fränzle, 2006).

Our study also showed that atmospheric pollutions have a negative impact on TA community biomass, abundance and species richness (Figs. 1 and 2). Whatever the biotope (other mosses, soil, and sediments), the global responses of the TA community exposed to medium/high-level pollution, such as decrease of total biomass, abundance and species richness, are the same as those observed in our study (Nguyen-Viet et al., 2004, 2007a; Kauppila et al., 2006). The low-level pollution also has similar negative impacts on TA communities, such as a decrease in biomass or abundance of active TA or in the biomasses of different species (Meyer et al., 2010b; Payne et al., 2010). Other global ecological indexes could be used as indicators of atmospheric pollution. TA species found in this study are predators or mixotrophic (predator with algae symbiosis) in the microbial food web, and each species has a different diet (Gilbert et al., 2000). In soil, trophic groups of nematodes have been used for bioindication of pollution (Pen-Mouratov et al., 2008; Heininger et al., 2007). In the same way, trophic groups of TA living in bryophytes could be used in the bioindication of atmospheric pollution. It would seem that the generalist species like *N. tincta* are

more sensitive to pollution, probably because of direct and indirect effect of pollutants. In our previous study (Meyer et al., 2010a), we showed that atmospheric pollution had an impact on the different groups of microbial communities. However, the diet of the species encountered in this study has not been studied sufficiently in the scientific literature. When this type of data is available, an index could be suggested, taking into account the direct and indirect effects of atmospheric pollutants and their integration into ecosystems.

Furthermore, this study showed that climatic conditions, especially temperature and relative humidity, have an impact on the TA community (Fig. 3). These results are in accordance with the results of other studies in other environments (Mitchell et al., 1999; Warner et al., 2007; Beyens et al., 2009), where the diversity and abundance of TA are dependent on environmental conditions such as humidity, pH, soil moisture or temperature.

Nevertheless, temperature and relative humidity were dependent on time. Therefore, it is necessary to consider the dynamics of the community to choose a good indicator of pollution. Time series of total biomass, total abundance and species richness can be used as integrative stress indicators of atmospheric pollution. Moreover, PRC and RDA appear to be good tools to summarise the response of a TA community over time when it is affected by different atmospheric pollutions and could be used as a tool for statistical bioindication. Furthermore, PRC analysis showed that urban pollution did not have the same effect as industrial pollution on TA community biomass (Fig. 4).

5. Conclusion

It is important to have valid indicators for the possible effects of pollution, especially for areas that are polluted with a cocktail of different atmospheric compounds, including particles and gases. The “bryophyte-TA” microsystem combines the advantages of active bioindication methods using bryophytes only with the use of sensitive microorganisms that give earlier responses (Meyer et al., 2010b). Moreover, this microsystem permits the development of a compact system whose size is small and which is easy to manipulate. This microsystem can also be used in an environment where the native bryophytes cannot grow. Furthermore, this study showed that simple parameters such as total biomass or total abundance of TA living in “bryophyte-testate amoebae” microsystems are good indicators of atmospheric pollution. More specifically, species-specific responses of a TA community living in “*P. purum*-TA” microsystems are not only good indicators of atmospheric pollution but also of the sources of pollution (urban or industrial). Nevertheless, other studies are needed to develop a standardised tool for the biomonitoring of air quality using “bryophyte-TA” microsystems. Further studies are needed to improve knowledge of TA ecology and to try to find a trophic diversity index as an indicator of atmospheric pollution.

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