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Evaluation of lichen species resistance to atmospheric metal pollution by coupling diversity and bioaccumulation approaches: A new bioindication scale for French forested areas

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ABSTRACT

In order to evaluate the metal resistance or sensitivity of lichen species and improve the bioindication scales, we studied lichens collected in eight plottings in French and Swiss remote forest areas. A total of 92 corticolous species was sampled, grouped in 54 lichen genera and an alga. Various ecological variables were calculated to characterize the environmental quality – including lichen diversity, lichen abundance, and Shannon index –, as well as lichen communities. Average ecological features were estimated for each study site and each of the following variables – light, temperature, continentality, humidity, substrate pH, and eutrophication – and they corresponded to lichen communities. Based on lichen frequencies, we calculated the index of atmospheric purity (IAP) and lichen diversity value (LDV). These two bioindication indices were closely related to lichen diversity and lichen abundance, respectively, due to their calculation formula. It appeared that LDV, which measures lichen abundance, was a better indicator of metal pollution than IAP. Coupling lichen diversity and metal bioaccumulation in a canonical correspondence analysis, we evaluated the resistance/sensitivity to atmospheric metal pollution for the 43 most frequent lichen species. After validation by eliminating possible influences of acid and nitrogen pollutions, we proposed a new scale to distinguish sensitive species (such as *Physconia distorta*, *Pertusaria coccodes*, and *Ramalina farinacea*) from resistant species (such as *Lecanactis subabietina*, *Pertusaria leioplaca*, and *Pertusaria albescens*) to metal pollution, adapted to such forested environment.

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1. Introduction

Atmospheric deposition of chemicals impacts natural ecosystems over a long-term, and biological species are more or less susceptible to these pollutants (Schulze et al., 1989; Tyler, 1989). Lichens are considered sensitive organisms because of their biological features. The absence of protective cuticle or root system results in a high sensitivity to anthropogenic disturbances, such as atmospheric pollutants (Bajpai et al., 2010; Conti and Cecchetti, 2001; Shukla et al., 2014; Szczepaniak and Biziuk, 2003). The loss of lichen diversity constitutes one of the main markers of atmospheric pollution on the biosphere, as revealed since the first observations in the late 19th century in Paris (Nylander, 1866). Because assessment of atmospheric pollution is complex and expensive, biomonitoring is a helpful support technique. Several biomonitoring approaches are used to evaluate the level of atmospheric pollution, in relation to lichen diversity (i.e., bioindication; Geiser and Neitlich, 2007; Pinho et al., 2004) or accumulation of pollutants (i.e., bioaccumulation; Conti et al., 2011; Hissler et al., 2008). Lichens are relatively good candidates frequently used to monitor atmospheric deposition in various environmental contexts: e.g., forested (Gauslaa, 1995; Giordani et al., 2012), rural (Bosch-Roig et al., 2013; Vonarb et al., 1990), and urban (Gombert et al., 2004; Loppi et al., 2004) areas.

Atmospheric acid deposition in Europe several decades ago, linked to man-made SO_2 and NOx emissions, was responsible for several disturbances on forest diversity (Schulze et al., 1989). More specifically, many authors reported that some lichen species have disappeared because of their susceptibility to acid pollutants (Piervittori et al., 1997; Sigal and Johnston, 1986). In this

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context, a first biomonitoring scale was developed in England and Wales by Hawksworth and Rose (1970), associating common lichen species for different atmospheric SO_2 concentrations. More recently, in Germany, Wirth (1991) developed a toxitolerance index for more than 750 lichen species also based on the acid pollution criteria. With the generalized decrease of SO_2 concentration in the atmosphere since the 1980's (Berge et al., 1999), a change in biomonitoring scale was needed. Several scales were developed following the relative importance of nitrogen compounds in the atmosphere (i.e., NOx and NH_4; Lallemant et al., 1996; van Haluwyn and Lerond, 1993). Nevertheless, these various scales do not take into account other pollutants such as metals (e.g., lead, zinc, cadmium) or organic pollutants (e.g., polycyclic aromatic hydrocarbons [PAH] and polychlorinated biphenyl [PCB]), and little is known about the sensitivity or resistance to such pollutants for lichen species commonly found in northern countries. Consequently, the development of new scales integrating these changes in sulfur and nitrogen compounds as background levels and the occurrence of emerging pollutants is therefore required.

In the meantime, several indices of atmospheric air quality were established based on lichen richness and abundance, such as the lichen diversity value (LDV; Asta et al., 2002) and the index of atmospheric purity (IAP; LeBlanc and Sloover, 1970). These indices attempt to evaluate a general degree of atmospheric pollution. The limit of such indices, however, is that they do not point to the exact pollutants caused by disturbance. A qualitative ecological characterization of lichen occurrence should also be employed as an additional tool to complete the quantitative evaluation, as being more frequently done.

In this study, we sampled lichen species in open forest sites from various remote regions of France and neighboring country to characterize the current degree of recent atmospheric pollution based on several approaches of lichen biomonitoring. Assuming a response to a gradient of metal bioaccumulation on lichen richness and abundance, our main objective was to evaluate the resistance/sensitivity of lichen species to atmospheric metal pollution by coupling both lichen diversity and bioaccumulation of metals in a multivariate analysis, and to propose a new resistance/sensitivity scale adapted to present-day environmental conditions to further assess the critical loads using lichens.

2. Materials and methods

2.1. Study area

Eight unmanaged open-forested sites were monitored, of which seven sites from various regions of France, and one site located in Switzerland (Fig. 1). The French sites (SP 11, EPC 63, EPC 74, HET 54a, EPC 08, PM 72, and CHS 35) belong to the French monitoring network of forest ecosystems RENECOFOR (Réseau National de suivi des Écosystèmes Forestiers), which is part of the International Cooperative Programme Forest network (ICP-Forest). The sites included both coniferous forests (*Abies alba* Mill. in SP 11, *Picea abies* (L.) H. Karst in EPC 63, EPC 74, and EPC 08, and *Pinus pinaster* Aiton in PM 72) and hardwood forests (*Quercus petraea* (Matt.) Liebl. in CHS 35 and *Fagus sylvatica* L. in BEX and HET 54a). Despite the dominant trees, a mixed of species were found with generally both coniferous and hardwood trees in each study site.

The sites considered various environmental conditions (Table 1). The elevation was from 80 m a.s.l for CHS 35–1210 m a.s.l for EPC 74. The Northwestern sites (PM 72 and CHS 35) were influenced by an oceanic climate with low annual precipitation (<840 mm), while the Northeastern (HET 54a and EPC 08) and central (EPC 63) ones were under semi-continental climate. The climate was more of mixed influences for the mountainous sites (SP 11, EPC 74, and BEX). Several types of bedrock were concerned, from sedimentary (limestone or sandstone) to magmatic (basalt) substratum.

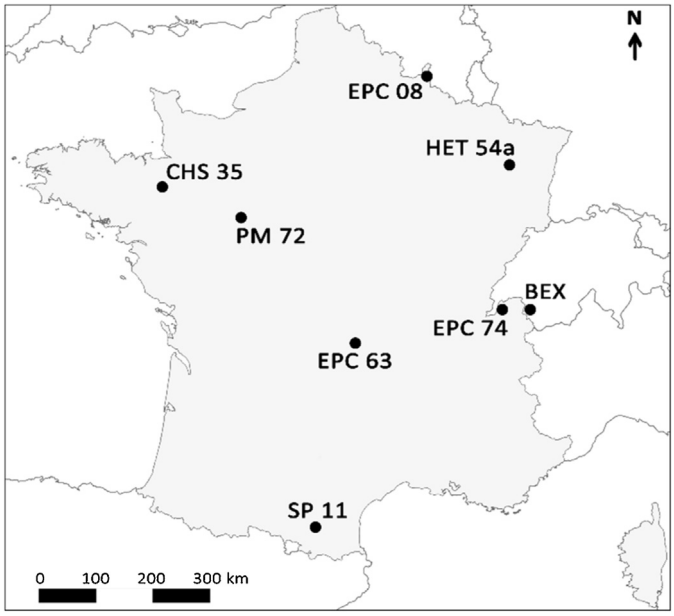

Fig. 1. Location of the study sites sampled for lichen diversity: seven sites are located in various regions of France and one in nearby Switzerland.

Metal atmospheric pollution has already been studied for these sites through surface horizons of soils (Gandois et al., 2010a; Hernandez et al., 2003), bulk atmospheric deposition (Gandois et al., 2010b), and lichen bioaccumulation (Agnan et al., 2015). The metal concentrations registered in lichens collected on the trees considered for bioindication are given in Table 2. Differences between sites were observed with a higher anthropogenic influence in the North-Eastern part of the country, particularly for Pb and Cd in EPC 08, while a greater dust deposition was observed in the Southern regions (e.g., in SP 11). The availability of lichen bioaccumulation data (i.e., metal concentrations in lichens though accumulation from the environment) was a central part of this study to determine both lichen resistance and lichen sensitivity in coupling lichen diversity to the degree of metal concentrations.

2.2. Sampling procedure

Because microclimate and bark properties are known to influence lichen diversity (Ellis, 2012; Giordani, 2006), each study site encompassed a representative area of about 250000 m^2 in open field at the edge of a forest to both maximize the number of sampling species and preserve the forest influence (Poličnik et al., 2008). Twelve trees avoiding young and disturbed specimens for lichen sampling (i.e., circumference > 40 cm, inclination < 10 °, trunk without mosses and damages) of various species were sampled (Bargagli and Nimis, 2002; Giordani et al., 2011), including both deciduous and coniferous trees (Table 1) to improve the representativeness of local lichen diversity (Daillant et al., 2007; Deruelle and Garcia Schaeffer, 1983). We followed the standardized European protocol (EN 16413, 2014), leaving the random sampling to maximize the number of lichen species by increasing the tree diversity (Moreau et al., 2002). Since we aimed to evaluate the metal resistance and sensitivity of lichens by combining bioaccumulation and diversity approaches, we thus followed the same procedure as for bioaccumulation study (Agnan et al., 2015). The four cardinal points of the tree trunks were sampled using a ladder grid of five vertical squares of 10 cm × 10 cm to cover an area of 500 cm^2 per tree side and a total area of 24000 cm^2 (i.e., 240 squares) for

Table 1
Summary of geographical and environmental characteristics for each study sites.

site	coordinates	elevation (m)	annual precipitation (mm)	lithology	tree species sampled
SP 11	2°05′40″E/ 42°52′15″N	990	1200	limestome/marble	*Abies alba* Mill., *Corylus avellana* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Malus pumila* Mill.
EPC 63	2°58′05″E/ 45°45′00″N	950	1100	basalt	*Crataegus monogyna* Jacq., *Fraxinus excelsior* L., *Picea abies* (L.) Karst., *Pinus* sp.
EPC 74	6°21′00″E/ 46°13′30″N	1210	1300	sandstone/schist	*Abies alba* Mill., *Acer* sp., *Fagus sylvatica* L., *Picea abies* (L.) Karst., *Prunus avium* L., *Salix* sp., *Sorbus aucuparia* L.
BEX	6°58′30″E/ 46°13′00″N	945	1000	limestone/schist	*Acer* sp., *Betula pendula* Roth, *Fagus sylvatica* L., *Fraxinus excelsior* L., *Salix* sp.
HET 54a	6°43′10″E/ 48°30′50″N	320	900	limestone	*Fagus sylvatica* L., *Fraxinus excelsior* L., *Quercus* sp.
EPC 08	4°47′50″E/ 49°57′00″N	475	1300	clay loam	*Betula pendula* Roth, *Corylus avellana* L., *Fagus sylvatica* L., *Picea abies* (L.) Karst., *Prunus avium* L., *Quercus* sp., *Rhus hirta* (L.) Sudw., *Salix caprea* L., *Syringa vulgaris* L.
PM 72	0°20′00″E/ 47°44′25″N	155	800	schist	*Castanea sativa* Mill., *Pinus pinaster* Ait., *Quercus petraea* (Mattus.) Liebl., *Quercus rubra* L.
CHS 35	1°32′50″W/48°10′10″N	80	840	clay	*Fagus sylvatica* L., *Pinus pinaster* Ait., *Quercus petraea* (Mattus.) Liebl.

Table 2
Summary of metal bioaccumulation (mean ± standard deviation, in $\mu g\,g^{-1}$) in three foliose lichen species (i.e., *X. parietina*, *P. sulcata*, and *H. physodes*) from the investigated forest areas (from Agnan et al., 2015).

element	SP 11		EPC 63		EPC 74		BEX		HET 54a		EPC 08		PM 72		CHS 35	
Al	2364.1	±1054.3	988.3	±299.4	1126.7	±412.0	1157.9	±277.8	192.4	±603.1	1072.7	±591.1	426.1	±46.0	397.7	±62.2
As	0.7	±0.2	0.7	±0.4	0.3	±0.1	0.3	±0.0	0.3	±0.1	0.6	±0.2	0.2	±0.0	0.2	±0.0
Cd	0.1	±0.0	0.1	±0.0	0.7	±0.7	0.1	±0.1	0.2	±0.2	0.6	±0.1	0.4	±0.1	0.1	±0.1
Co	0.4	±0.2	0.3	±0.1	0.4	±0.1	0.3	±0.1	0.3	±0.1	0.4	±0.1	0.1	±0.0	0.2	±0.0
Cr	3.7	±1.3	2.2	±1.0	1.8	±0.6	2.8	±1.0	1.8	±0.7	2.5	±0.9	0.8	±0.1	0.7	±0.1
Cs	0.3	±0.1	0.3	±0.2	0.2	±0.1	0.2	±0.1	0.2	±0.1	0.2	±0.1	0.1	±0.0	0.1	±0.0
Cu	4.7	±0.9	6.9	±2.1	10.4	±3.2	7.1	±2.4	7.9	±3.1	7.3	±1.0	7.2	±1.0	5.1	±1.5
Fe	1347.1	±595.6	759.1	±262.2	618.5	±208.4	687.0	±158.2	617.6	±334.8	631.0	±328.5	278.6	±35.7	240.9	±36.4
Mn	29.3	±14.6	25.1	±4.0	142.0	±126.8	102.7	±74.7	69.5	±73.2	45.7	±11.3	45.3	±4.4	346.8	±117.7
Ni	1.7	±0.5	1.3	±0.5	2.1	±0.7	2.1	±0.7	1.7	±0.6	2.0	±0.3	0.6	±0.1	1.4	±0.3
Pb	2.3	±1.5	2.5	±1.1	7.3	±3.5	5.1	±2.7	16.1	±13.4	5.2	±1.1	1.5	±0.3	6.2	±10.4
Sb	0.1	±0.1	0.1	±0.0	0.1	±0.0	0.2	±0.0	0.2	±0.1	0.3	±0.1	0.2	±0.0	0.1	±0.0
Sn	0.4	±0.2	0.3	±0.2	0.5	±0.1	0.6	±0.2	0.4	±0.2	0.7	±0.1	0.3	±0.0	0.2	±0.0
Sr	8.5	±3.4	44.4	±29.8	16.9	±9.3	16.8	±5.6	10.4	±6.3	10.7	±2.0	4.4	±0.4	30.7	±22.5
Ti	187.9	±82.9	123.2	±51.2	62.6	±20.5	80.3	±19.8	85.6	±43.8	73.3	±42.5	32.3	±2.6	33.6	±5.4
V	4.1	±2.0	2.4	±0.5	2.4	±0.7	2.2	±0.5	2.6	±1.3	2.4	±0.4	1.0	±0.1	1.3	±0.2
Zn	22.1	±9.6	30.0	±18.1	69.0	±43.4	35.1	±14.4	47.9	±20.8	108.4	±11.8	72.8	±16.2	30.0	±4.2

each study site (Asta et al., 2002; Fig. 2). The ladder was placed at minimum 1 m above the ground level to avoid soil influence (Bargagli and Nimis, 2002). We determined the presence of lichen species in each 100 cm^2 noticed in a sampling sheet: 0 if absent, 1 if present. This allowed obtaining the frequency of each species by site, averaging all values: from 0 (totally absent in the study site) to 1 (present in every 10 cm × 10 cm squares). The average values are given in Table 3. We used a 10- or 30-fold hand lens to identify all the species. Lichen specimens were collected using a knife, and preserved in a plastic bag until complete identification.

2.3. Species identification

Lichen species identification was performed in laboratory using a stereomicroscope (from 20- to 60-fold) and microscope (100-fold). Determination guides (Clauzade and Roux, 1985; Dobson, 2011; Smith et al., 2009; van Haluwyn and Lerond, 1993), and chemicals – potassium hydroxide 10% (K), sodium hypochlorite (C), and paraphenylenediamine (P) – were used to distinguish the different genera and/or species. Only genera were identified for immature specimens. Conversely, we identified the sub-species when possible. The nomenclature used was based on Roux (2012).

2.4. Index calculations and statistical treatment

For each site, we determined the number of species found and the abundance of each species calculated by adding each frequency, determined using the field ladder grid (see above). We also calculated the Shannon's diversity index H' based on the following formula:

$$H' = -\sum_{i=1}^{i=R} (p_i \times \log_2 p_i)$$

where p_i is the proportion of characters of the species i, and R is the species richness.

Two bioindication indices were calculated: the lichen diversity value (LDV; Asta et al., 2002), which represents the sum of frequencies, and the index of atmospheric purity (IAP; LeBlanc and Sloover, 1970) as follows:

$$IAP = \frac{1}{10}\sum_{i=1}^{i=n} (Q_i \times f_i)$$

where n is the number of species, Q_i is the ecological index of each species i (corresponding to the total number of companion species present at all studied sites), and f_i is the frequency of species i.

Table 3
Site and average (avg.) frequencies for each lichen species.

species	code	SP 11	EPC 63	EPC 74	BEX	HET 54a	EPC 08	PM 72	CHS 35	avg.
Acrocordia gemmata (Ach.) A. Massal.	Age			0.092		0.071		0.067	0.021	**0.031**
Alyxoria varia (Pers.) Ertz et Tehler	Ava								0.025	**0.003**
Amandinea punctata (Hoffm.) Coppins et Scheid.	Apu	0.200	0.008	0.242		0.058		0.021		**0.066**
Anisomeridium biforme (Borrer) R. C. Harris	Abi						0.054			**0.007**
Arthonia atra (Pers.) A. Schneid.	Aat								0.063	**0.008**
Arthonia radiata (Pers.) Ach.	Ara	0.171		0.021		0.054			0.021	**0.033**
Aspicilia coronata (A. Massal.) Anzi	Aco		0.013							**0.002**
Buellia disciformis (Fr.) Mudd	Bdi	0.104		0.046						**0.019**
Calicium salicinum Pers.	Csa		0.029						0.046	**0.009**
Caloplaca cerina (Ehrh. ex Hedw.) Th. Fr.	Cce				0.004					**0.001**
Caloplaca ferruginea (Hudson) Th. Fr.	Cfe	0.008			0.029					**0.005**
Candelaria concolor (Dicks.) Stein	Cco			0.154						**0.019**
Candelariella reflexa (Nyl.) Lettau	Cre							0.025		**0.003**
Candelariella vitellina (Hoffm.) Müll. Arg.	Cvi	0.046								**0.006**
Chaenotheca ferruginea (Turner ex Sm.) Mig.	Chf							0.075		**0.009**
Chrysothrix candelaris (L.) J. R. Laundon	Cca	0.117	0.242	0.042	0.133	0.029	0.063	0.067		**0.086**
Cladonia fimbriata (L.) Fr.	Cfi					0.050	0.317	0.171	0.046	**0.073**
Dendrographa decolorans (Turner et Borrer ex Sm.) Ertz et Tehler	Dde	0.117				0.025			0.046	**0.023**
Enterographa crassa (DC.) Fée	Ecr								0.196	**0.024**
Evernia prunastri (L.) Ach.	Epr	0.025	0.238	0.104	0.108	0.058	0.042			**0.072**
Fuscidea cyathoides subsp. *corticola* (Fr.) Cl. Roux comb. nov.	Fcy		0.033							**0.004**
Graphis elegans (Borrer ex Sm.) Ach.	Gel								0.175	**0.022**
Graphis scripta (L.) Ach.	Gsc	0.042				0.242			0.042	**0.041**
Haematomma ochroleucum (Neck.) J. R. Laundon	Hoc								0.042	**0.005**
Hypocenomyce scalaris (Ach.) M. Choisy	Hsc							0.013		**0.002**
Hypogymnia physodes (L.) Nyl.	Hph	0.067	0.358	0.313			0.017	0.075		**0.104**
Hypotrachyna laevigata (Sm.) Hale	Hla							0.008		**0.001**
Lecanactis subabietina Coppins et P. James	Lsu	0.008						0.038	0.050	**0.012**
Lecanora albella (Pers.) Ach.	Lab					0.067				**0.008**
Lecanora allophana Nyl.	Lal	0.054						0.033		**0.011**
Lecanora argentata (Ach.) Malme	Lar	0.113		0.254		0.017		0.033		**0.052**
Lecanora barkmaniana Aptroot et Herk	Lba			0.063		0.038				**0.013**
Lecanora carpinea (L.) Vain.	Lca	0.088	0.004		0.008					**0.013**
Lecanora chlarotera Nyl.	Lch	0.150	0.008	0.246	0.788	0.054	0.071	0.004		**0.165**
Lecanora compallens van Herk et Aptroot	Lcm					0.133				**0.017**
Lecanora conizaeoides Nyl. ex Cromb.	Lcn	0.025	0.004		0.021					**0.006**

Table 3 (*Continued*)

species	code	SP 11	EPC 63	EPC 74	BEX	HET 54a	EPC 08	PM 72	CHS 35	avg.
Lecanora dispersa (Pers.) Sommerf.	Ldi			0.025						**0.003**
Lecanora expallens Ach.	Lex	0.008		0.033						**0.005**
Lecanora hagenii (Ach.) Ach.	Lha			0.042						**0.005**
Lecanora horiza (Ach.) Linds.	Lho			0.021						**0.003**
Lecanora intumescens (Rebent.) Rabenh.	Lit	0.075								**0.009**
Lecanora leptyrodes (Nyl.) Degel.	Lle				0.008					**0.001**
Lecanora subcarpinea Szatala	Lsc		0.025							**0.003**
Lecanora subrugosa Nyl.	Lsr		0.025							**0.003**
Lecidea sp.	Lec				0.021					**0.003**
Lecidella elaeochroma (Ach.) M. Choisy	Lel	0.013			0.692	0.038	0.113			**0.107**
Lepraria incana (L.) Ach.	Lic	0.450	0.238	0.483	0.142	0.679	0.458	0.625	0.671	**0.468**
Leptogium teretiusculum (Wallr.) Arnold	Lte	0.163								**0.020**
Melanelixia glabratula (Lamy) Sandler et Arup	Mgl	0.121	0.179	0.042	0.442	0.121	0.142			**0.174**
Melanohalea exasperata (DeNot.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. et Lumbsch	Mea				0.008					**0.001**
Melanohalea exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. et Lumbsch	Meu					0.138		0.008		**0.018**
Melanohalea laciniatula (Flagey ex H. Olivier) O.Blanco, A. Crespo, Divakar, Essl., D. Hawksw. et Lumbsch	Mla			0.004						**0.001**
Micarea prasina Fr.	Mpr					0.021				**0.003**
Naetrocymbe punctiformis (Pers.) R. C. Harris	Npu							0.013		**0.013**
Ochrolechia androgyna (Hoffm.) Arnold	Oan	0.013				0.021				**0.004**
Ochrolechia pallescens (L.) A. Massal.	Opa	0.025								**0.003**
Ochrolechia pallescens subsp. *parella* (L.)	Opp	0.004				0.029				**0.004**
Ochrolechia turneri (Sm.) Hasselr.	Och				0.033	0.029				**0.008**
Ochrolechia sp.	Otu								0.029	**0.004**
Opegrapha rufescens Pers.	Oru					0.038				**0.005**
Parmelia sulcata Taylor	Psl	0.075	0.442	0.213	0.579	0.488	0.454	0.050	0.008	**0.289**
Parmelina carporrhizans (Taylor) Poelt et Vězda	Pca				0.096			0.113		**0.026**
Parmeliopsis ambigua (Wulfen) Nyl.	Pab	0.004								**0.001**
Pertusaria albescens (Huds.) M. Choisy et Werner	Pal	0.033				0.175		0.021	0.083	**0.039**
Pertusaria amara (Ach.) Nyl.	Paa	0.125				0.046		0.058		**0.029**
Pertusaria coccodes (Ach.) Nyl.	Pco	0.008	0.488					0.071		**0.071**
Pertusaria flavida (DC.) J. R. Laundon	Pfl								0.013	**0.002**
Pertusaria hemisphaerica (Flörke) Erichsen	Phe							0.013		**0.002**
Pertusaria leioplaca DC.	Pli	0.013							0.025	**0.005**
Pertusaria pertusa (Weigel) Tuck.	Ppe					0.088				**0.011**
Phaeographis smithii (Leight.) B. de Lesd.	Psm					0.154				**0.019**
Phlyctis argena (Spreng.) Flot.	Par	0.004		0.117		0.221		0.046		**0.048**
Physcia adscendens (Fr.) H. Olivier	Pad	0.025	0.417	0.192		0.154				**0.098**

Table 3 (*Continued*)

species	code	SP 11	EPC 63	EPC 74	BEX	HET 54a	EPC 08	PM 72	CHS 35	avg.
Physcia clementei (Turner) Lynge	Pcl							0.267		**0.033**
Physcia leptalea (Ach.) DC.	Plp		0.004							**0.001**
Physcia tenella (Scop.) DC.	Pte		0.013				0.229			**0.030**
Physconia distorta (With.) J. R. Laundon	Phy		0.025		0.008					**0.004**
Physconia enteroxantha (Nyl.) Poelt	Pdi			0.042						**0.005**
Physconia sp.	Pen			0.013						**0.002**
Pleurosticta acetabulum (Neck.) Elix et Lumbsch	Pac		0.025	0.196						**0.028**
Pseudevernia furfuracea (L.) Zopf	Pfu		0.063	0.304						**0.046**
Punctelia subrudecta (Nyl.) Krog	Psb					0.021				**0.003**
Pyrenula laevigata (Pers.) Arnold	Pla								0.083	**0.010**
Ramalina farinacea (L.) Ach.	Rfr	0.175	0.329		0.017	0.033				**0.069**
Ramalina fastigiata (Pers.) Ach.	Rfs			0.004						**0.001**
Schismatomma cretaceum (Hue) J. R. Laundon	Scr			0.117					0.075	**0.024**
Tephromela atra (Huds.) Hafellner	Tcr							0.046		**0.006**
Thelotrema lepadinum (Ach.) Ach.	Tat								0.021	**0.003**
Usnea sp.	Usn			0.004	0.046					**0.006**
Xanthoria parietina (L.) Th. Fr.	Xpa		0.013	0.025	0.017	0.075	0.025			**0.019**
Zwackhia viridis (Pers. ex Ach.) Poetsch et Schied.	Zvi								0.071	**0.009**
Pleurococcus viridis Ag.	Pvi	0.188	0.092	0.146		0.304	0.188	0.063	0.017	**0.124**
all		**2.854**	**3.313**	**3.596**	**3.200**	**3.767**	**2.171**	**2.067**	**1.867**	

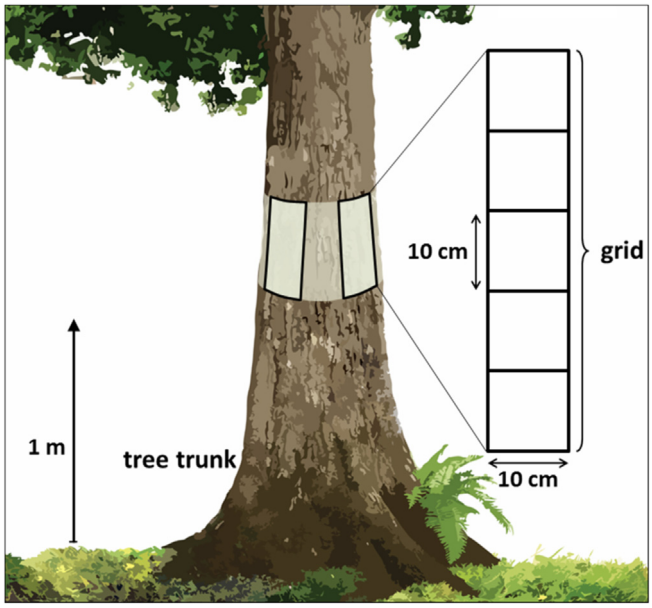

Fig. 2. Sampling procedure using a 10 cm × 50 cm grid on the tree trunk in the four cardinal directions.

A Student *t*-test was applied on lichen diversity between each tree genus ($\alpha = 0.05$). The lichen frequencies did not follow a normal distribution (Shapiro-Wilk test); then, data were log-transformed for the multivariate analyses. Principal component analysis (PCA) was performed on ecological and environmental data (Dobson, 2011; Nimis and Martellos, 2008; Smith et al., 2009; Wirth, 2010) based on lichen species frequency. Canonical correspondence analysis (CCA) was used to evaluate the resistance or sensitivity of the 43 most abundant lichen species to metal atmospheric pollution based on species frequency. Statistical analyses were carried out using RStudio 0.98 (RStudio Inc., Boston, Massachusetts, USA) and ade4 package (Dray and Dufour, 2007).

3. Results

3.1. Ecological indices

3.1.1. Lichen and tree diversities

The identified lichen species and their respective frequency for each study site are reported in Table 3. A total of 54 lichen genera, distributed in 92 corticolous species, and an alga (*Pleurococcus viridis* Ag.) were sampled (Fig. 3a). The most abundant species were *Lepraria incana* (L.) Ach. (observed in 8 sites with a total frequency of 3.75), *Parmelia sulcata* Taylor (8 sites, frequency of 2.31), *Lecanora chlarotera* Nyl. (7 sites, frequency of 1.32), and *Melanelixia glabratula* (Lamy) Sandler & Arup (6 sites, frequency of 1.05). Some species were found in only one site with a very low frequency (<0.005): e.g., *Ramalina fastigiata* (Pers.) Ach., *Physcia leptalea* (Ach.) DC, and *Parmeliopsis ambigua* (Wulfen) Nyl. Overall, the lichen species were distributed into 64 crustose, 20 foliose, 6 fruticose, and one squamulose morphologies (Fig. 3b). The foliose/crustose thallus ratios were from 0.05 to 1 and decreased as follows: CHS 35 < SP 11 < HET 54a < PM 72 < BEX < EPC 74 < EPC 63 < EPC 08.

Biological richness and abundance (sum of frequencies) showed a high heterogeneity among the study sites: from 13 to 35 species encountered by individual site and the abundances ranged between 1.87 and 3.77 (Table 4). SP 11, PM 72, and CHS 35 showed a

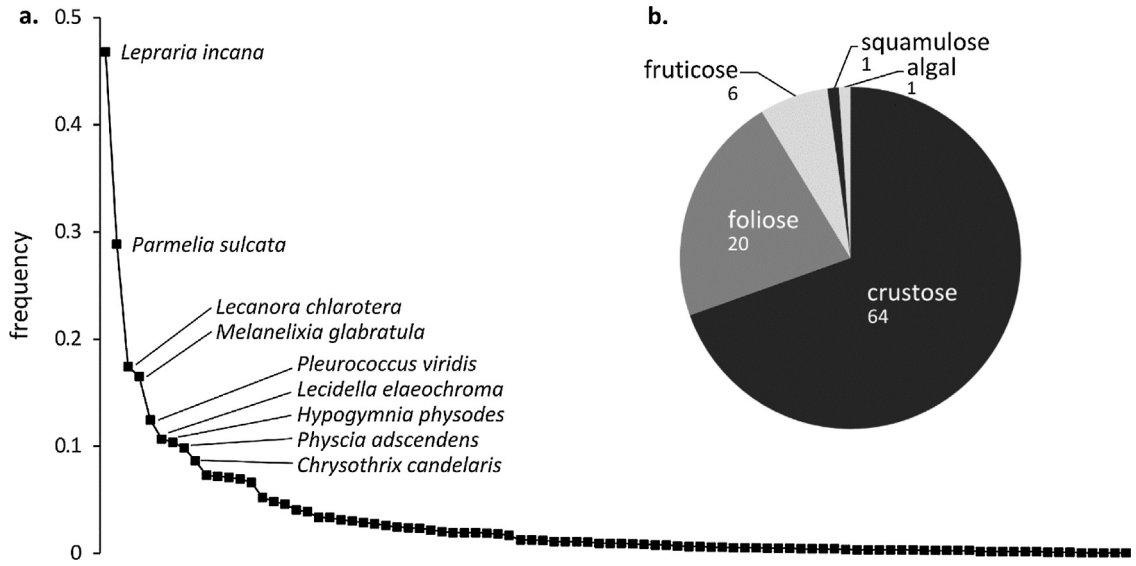

Fig. 3. Lichen diversity found in the eight study sites: average abundance of each lichen species (a) and relative proportion of each type of morphology (b).

Table 4
Summary of main ecological, bioindication indices, and values of the six environmental variable from Wirth, 2010 of each plotting area.

study site	ecological indices			bioindication indices		Wirth, 2010's environmental indices (%)					
	lichen richness	lichen abundance	Shannon index	IAP	LDV	light	temperature	continentality	humidity	pH	eutrophication
SP 11	35	2.85	4.43	241	57	55.8	52.3	44.8	26.3	32.6	43.2
HET 54a	33	3.77	4.30	263	75	62.7	51.1	42.9	21.8	37.6	47.5
EPC 74	30	3.60	4.27	227	72	76.4	50.1	45.8	18.5	36.5	46.0
PM 72	26	2.02	3.71	159	40	69.0	50.2	38.5	26.2	25.8	38.6
EPC 63	25	3.31	3.66	157	66	75.1	50.0	41.9	26.6	37.1	51.8
CHS 35	23	1.87	3.52	137	37	45.2	51.3	39.1	37.2	25.9	23.0
BEX	20	3.20	3.02	117	64	64.5	49.9	50.0	14.1	37.0	57.1
EPC 08	13	2.17	3.16	94	43	77.3	50.0	50.0	15.6	32.4	56.5

relatively low lichens abundance for a same range of richness (richness/abundance ratio from 12.3 to 12.9) compared to the other sites (ratio from 6.0 to 8.8). The Shannon index, ranged between 3.02 and 4.43. It followed the lichen diversity values with the exception of BEX site, which may be due to a higher abundance (Table 4).

The main lichen communities observed in the study sites were commonly found in France (Coste, 2001; van van Haluwyn and Lerond, 1993; van Haluwyn et al., 2009): *Leprarion incanae* Almborn 1948 (except in BEX and PM 72), including sciaphilous species (*Lepraria incana*), and *Lecanorion carpinae* (Ochsn.) Barkm, 1958 (except in EPC 63 and CHS 35), including heliophilous, nitrophilous and toxitolerant species (such as *Lecanora carpinea*, *Lecanora chlarotera*, and *Lecidella elaeochroma*). *Parmelion acetabuli* Barkman 1958 was found in four sites (BEX, HET 54a, EPC 08, and PM 72), including *Parmelia sulcata*, *Melanelixia glabratula*, as well as *Melanohalea exasperatula* and *Physcia adscendens*, mainly heliophilous and slightly neutrophilous and toxitolerant species. Other nitrophobous and poleophobous communities were found locally: *Graphidion scriptae* Oschner 1928 (with *Arthonia*, *Graphis*, *Enterographa* and *Opegrapha*; in HET 54a and CHS 35), *Cladonion coniocraeae* Duvigneaud ex James, Hawksworth & Rose, 1977 (with *Cladonia fimbriata*; in EPC 08 and PM 72), and *Calicion viridis* Černh. & Hadač 1944 (with *Chrysothrix candelaris*; in BEX).

The sampling procedure, including both hardwood and conifer trees as far as possible (Table 1), attempted to reduce the tree bark influence by limiting to only sample the main representative tree species in each site (i.e., fir in SP11, spruce in EPC 63, beech for HET 54a, oak in CHS 35, etc.), and thus, the lichen communities adapted to these tree species. We collected lichen samples on a total of 21 different tree species, from 3 to 9 by site. Considering dominant tree species ($n \geq 5$, Fig. 4), lichen richness observed on hardwood trees was usually greater compared to richness on conifers, except for *Abies*: $p < 0.05$ (Student test). *Fraxinus* was the tree species with the greater lichen richness (9.6 species on average). Also, the lichen communities found on deciduous trees (*Lecanorion carpinae* and *Parmelion acetabuli* associated with other foliose and fruticose species) differed from those on conifers (generally *Leprarion incanae*).

3.1.2. Bioindication indices

The highest IAP (>200) were found in HET 54a, SP 11, and EPC 74, while EPC 08 and BEX showed the lowest values (<120), following a similar trend as lichen richness (Table 4). The different sampling and/or calculation methods may limit the data comparison (Scerbo et al., 1999). Lichen diversity values were also highest (>70) in HET 54a and EPC 74, but the lowest values (≤40) were for the two Western stations (CHS 35 and PM 72), following the lichen abundance trend.

3.1.3. Ecological features

For each lichen species, we studied ecological features through six environmental parameters described by Wirth (2010): light, temperature, continentality, humidity, pH, and eutrophication. When ecological data were absent (i.e., for 25 species), we used data from Nimis and Martellos (2008) database, as well as other references (Clauzade and Roux, 1985; Dobson, 2011;

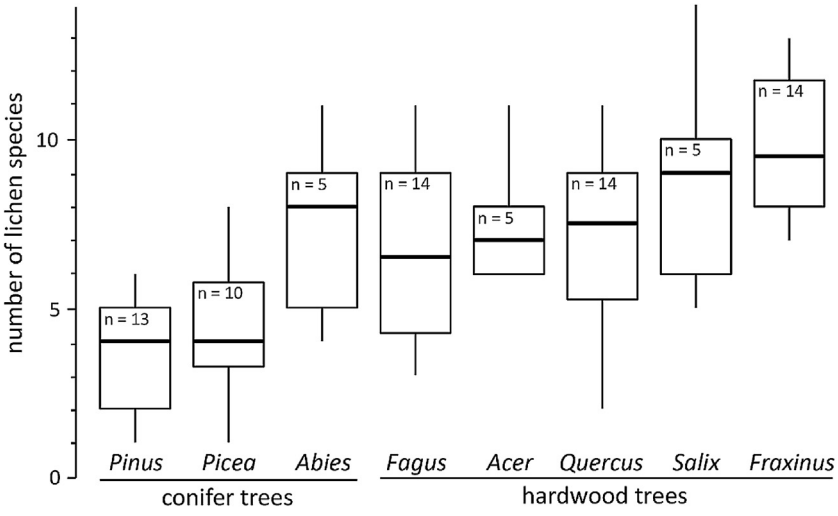

Fig. 4. Lichen diversity by tree-support species (n indicates the number of individuals for each tree genus).

Smith et al., 2009; van Haluwyn and Lerond, 1993). An average ecological value of each parameter was calculated for each station based on individual value and frequency of each lichen species. To better homogenize the indices between these different references and to reduce the wide ranges (generally nine levels are reported by Wirth, 2010), we introduced a new scale of three levels (e.g., xerophytic/mesophytic/hygrophytic species, acid/neutral/basic substrate pH, etc.). The results were expressed using the frequency of each lichen species (Table 4).

The most important gradient were found for eutrophication (from low, i.e., CHS 35 and PM 72, to moderate eutrophic species, i.e., BEX and EPC 08) and light (with high proportions of heliophilous species, i.e., EPC 08, EPC 74, and EPC 63, and species with moderate light affinity, i.e., CHS 35 and SP 11). In contrast, mesophytic species were dominant indicating a low difference in temperature among sites. On overall, lichen species were, on average, mostly acidophilic, xerophilic and moderately oceanic in all the stations.

3.2. Coupling ecological and biogeochemical approaches

To determine the resistance or sensitivity of each lichen species to metal atmospheric pollution, we performed multivariate statistical analyses including the three diversity variables previously studied (lichen richness, lichen abundance, and Shannon index), the six ecological parameters mentioned above, the two bioindication indices (IAP and LDV), and metal bioaccumulation data measured in foliose lichen species (i.e., *Xanthoria parietina*, *Parmelia sulcata*, or *Hypogymnia physodes*) estimated using the sum of enrichment factors (EF) for 17 metals (Al, As, Cd, Co, Cr, Cs, Cu, Fe, Mn, Ni, Pb, Sb, Sn, Sr, Ti, V, and Zn; see Agnan et al. (2015)). A PCA was then performed and the first two components (81% of the data variance) were represented (Fig. 5).

The first component (45% of the data variance) was influenced by lichen abundance and LDV with negative scores. It was associated to lichen species living on basic bark and eutrophic, continental, and bright environments, as illustrated by EPC 74, BEX, HET 54a, and EPC 63 sites. The positive scores were characterized by hydrophilic species and metal EF data from bioaccumulation in lichen, influencing the two Western sites (CHS 35 and PM 72). The second component (36% of the data variance) grouped the two diversity indices (lichen richness and Shannon index), as well as IAP and lichen species living in warmer environments. The temperature could not explain this component due to the lack of ecological contrast in the study sites. This component distinguished SP 11 and HET 54a with positive scores, and EPC 08, and to a lesser extent BEX, with negative scores.

A CCA was performed on metal bioaccumulation data and lichen species frequencies found for each study site (Fig. 6a,b). This method was already used for lichen sensitivity to nitrogen by Glavich and Geiser (2008). Only lichen species presented in at least two different study sites were included in the CCA. We added in the analysis the sum of EF of the 17 metals previously cited (Agnan et al., 2014) and the two bioindication indices (IAP and LDV). The IAP was explained by the first axis (26% of the data variance), while the second axis (21% of the data variance) evidenced an opposite pattern between LDV and EF (Fig. 6a). Each lichen species was represented by a three letter code on Fig. 6b (see Table 5 for the species correspondence). Since IAP was a diversity index (Fig. 5), it was proved difficult to classify the lichen species following the first axis. Using the EF position in the first plot as factor of metal pollution (Fig. 6a), however, we determined the degree of metal influence for each lichen species depending on the position of the species in the second plot (Fig. 6b). To scale this influence, we applied a geometric rotation using EF as the new y axis (y'). The rotated coordinates allowed differentiation of sensitive vs resistant species based on the EF values (i.e., projection on the EF gradient, $y = 2.28\,x$; Fig. 6b). The lowest and negative y' indicated a resistant species to metal atmospheric pollution and the highest and positive y' a sensitive species (Table 5). Given the range of y' values of 3 (between −1.5 to +1.5), we determined three groups of identical ranges as follows: $y' < -0.5$ for resistant species, $-0.5 < y' < 0.5$ for intermediate species, $y' > 0.5$ for sensitive species. The list of resistant species included various crustose lichens, while only two crustose species were present in the sensitive list (*Pertusaria coccodes* and *Caloplaca ferruginea*). Two foliose (*Melanohalea exasperatula* and *Physcia tenella*) and one fruticose (*Cladonia fimbriata*) species, however, were found as resistant species. The number of sites where each lichen species was present was given as confidence information of y'.

4. Discussion

4.1. Lichen diversity and communities

The diversity of corticose lichen species observed in the eight forest study sites was generally lower on coniferous trees compared to hardwood trees (Fig. 4), confirming literature observations (Selva, 1994). Lichen communities were likewise different between

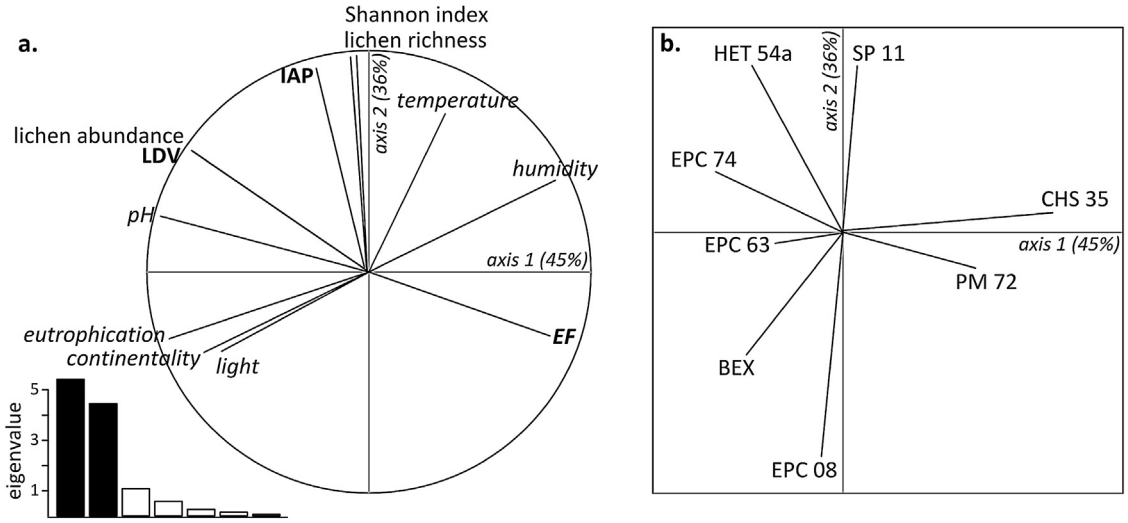

Fig. 5. Principal component analysis (PCA) including ecological characteristics (normal), ecological indices (italic), bioindication indices (bold), and the sum of enrichment factors of 17 metals (EF, bold and italic).

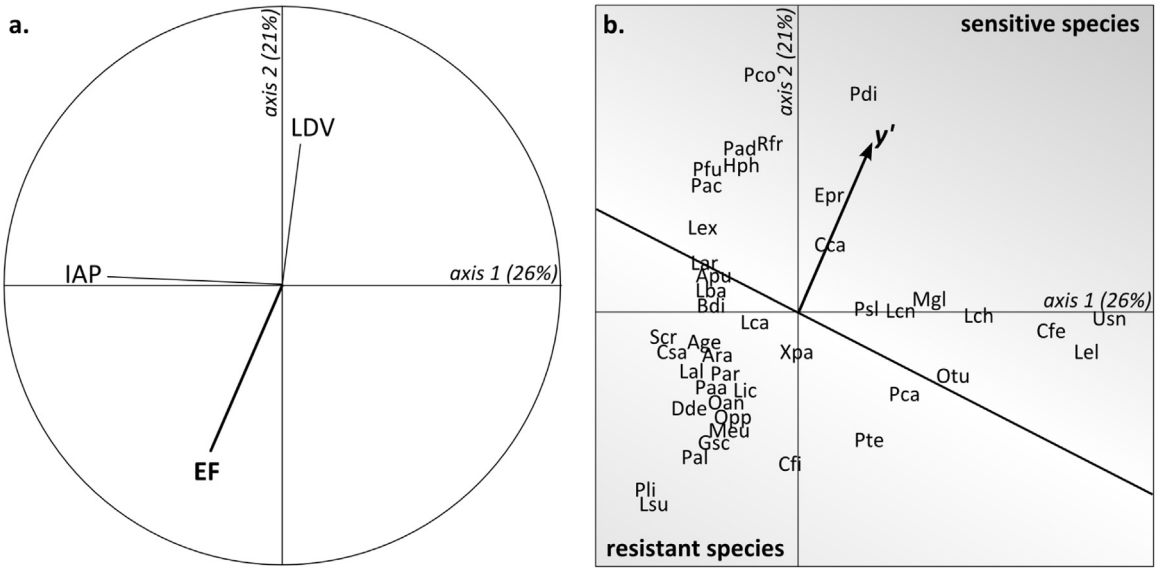

Fig. 6. Canonical correspondence analysis based on frequency of the 43 main lichen species (presented in more than two different sites, with a three letter code, see Table 4 for the species correspondence), bioindication (IAP and LDV) and bioaccumulation (sum of enrichment factors [EF] of 17 metals) indices for each study site.

these two types of trees with mostly sciaphilous communities on conifers and heliophilous species on hardwood trees (e.g., *Leprarion incanae* vs *Lecanorion carpinae* in EPC 74, respectively). Our sampling method in open areas bordering forests allowed therefore maximizing lichen diversity and communities: both sciaphilous and heliophilous lichens were found as dominant species (*Lepraria incana* and *Parmelia sulcata*, respectively; Fig. 3).

Overall, the lichen diversity observed in the study sites was high. The number of lichen species was in the same range as those observed in other European forests: e.g., in Italy (Giordani, 2007), Slovenia (Poličnik et al., 2008), or Portugal (Pinho et al., 2004); the Shannon index, however, showed higher values compared to other European and North American forested sites (Mulligan, 2009; Peterson and McCune, 2001). But, this range was higher than in boreal environments (Kuusinen, 1996), probably in relation to specific climate conditions in cold regions. Indeed, the diversity data from the literature are not always comparable since the sampling methods used can sometimes lead to discrepancies between the observations (e.g., Kuusinen and Siitonen, 1998; Selva, 1994).

Based on the indices of Nimis and Martellos (2008), 12% of the overall taxa were pioneer species, with the maximum proportion for BEX and SP 11 (25 and 20%, respectively) and the minimum for CHS 35 (4%). In BEX, two common lichen species (*Lecanora chlarotera* and *Lecidella elaeochroma*) were responsible for 98% of the pioneer frequency, but these species can also be found in non-pioneer environments (Pirintsos et al., 1995). The pioneer frequency was not directly positively correlated with lichen richness (Table 4), as suggested by Selva (1994). This can be explained either by our sampling protocol in open field limiting forest, or by inadequate Nimis and Martellos (2008) pioneer index applied in our study sites.

Differences were, conversely, observed among study sites regarding ecological characteristics. For example, SP 11 and EPC 08 showed both nitrophilous and poleotolerant communities, while nitrophobous species were found in CHS 35 and HET 54a. This agreed with observations in atmospheric deposition sometimes different from modeled estimates, particularly under-estimated in the Pyrenees (SP 11) and over-estimated in the Armorican Mas-

Table 5
List of resistant, intermediate, and sensitive lichen species relative to atmospheric metal pollution based on a bioaccumulation–lichen diversity coupling method. *y'* value indicates the new scale of lichen resistance/sensitivity to metals. The number of sites where each lichen species was present gives a confidence information of *y'*.

	lichen species	number of sites	code	*y'*
resistant species	*Lecanactis subabietina*	3	Lsu	−1.442
	Pertusaria leioplaca	2	Pli	−1.402
	Pertusaria albescens	4	Pal	−1.093
	Graphis scripta	3	Gsc	−0.919
	Cladonia fimbriata	4	Cfi	−0.893
	Melanohalea exasperatula	2	Meu	−0.854
	Dendrographa decolorans	3	Dde	−0.799
	Ochrolechia pallescens subsp. *parella*	2	Opp	−0.781
	Ochrolechia androgyna	2	Oan	−0.656
	Pertusaria amara	3	Paa	−0.620
	Lepraria incana	8	Lic	−0.615
	Lecanora allophana	2	Lal	−0.608
	Physcia tenella	2	Pte	−0.555
	Calicium salicinum	2	Csa	−0.551
	Acrocordia gemmata	4	Age	−0.537
	Schismatomma cretaceum	2	Scr	−0.525
	Arthonia radiata	4	Ara	−0.513
intermediate species	*Lecidella elaeochroma*	4	Lel	−0.494
	Chrysothrix candelaris	7	Cca	−0.482
	Lecanora chlarotera	7	Lch	−0.428
	Melanelixia glabratula	6	Mgl	−0.389
	Lecanora conizaeoides	3	Lcn	−0.284
	Lecanora expallens	2	Lex	−0.234
	Parmelia sulcata	8	Psl	−0.187
	Lecanora argentata	4	Lar	−0.021
	Ochrolechia turneri	2	Otu	−0.018
	Amandinea punctata	5	Apu	0.066
	Lecanora barkmaniana	2	Lba	0.080
	Buellia disciformis	2	Bdi	0.124
	Lecanora carpinea	3	Lca	0.170
	Parmelina carporrhizans	2	Pca	0.204
	Xanthoria parietina	5	Xpa	0.244
	Phlyctis argena	4	Par	0.493
sensitive species	*Pleurosticta acetabulum*	2	Pac	0.519
	Caloplaca ferruginea	2	Cfe	0.524
	Pseudevernia furfuracea	2	Pfu	0.590
	Hypogymnia physodes	5	Hph	0.706
	Evernia prunastri	6	Epr	0.732
	Usnea sp.	2	Usn	0.739
	Physcia adscendens	4	Pad	0.771
	Ramalina farinacea	4	Rfr	0.824
	Pertusaria coccodes	3	Pco	1.256
	Physconia distorta	2	Pdi	1.405

sif (CHS 35; Boutin et al., 2015; Pascaud et al., 2016). Even though no obvious correlation was observed with lichen richness, lichen abundance, or foliose/crustose thallus ratio, lichen communities agreed with the ecological features described by Wirth (2010): e.g., CHS 35 had a low percentage of eutrophic species, unlike EPC 08. These ecological observations were therefore a complementary description to assess environmental quality that cannot be illustrated by lichen richness or abundance only.

Results of bioindication indices showed that IAP were largely higher than data from French urban areas (Gombert et al., 2004), and LDV were generally in the upper range compared to other forest sites in Europe (Giordani, 2007; Pinho et al., 2004; Poličnik et al., 2008). These indices were closely related to lichen richness and lichen abundance, respectively (Table 4), which was supported by the PCA results (Fig. 5). This is most likely due to their calculation method: only frequencies were used in LDV whereas Qi (i.e., the number of companion species, largely influenced by lichen diversity) is considered in IAP. Thereby, the difference of results between IAP and LDV, already observed by Poličnik et al. (2008), can be attributed to the difference between lichen richness and lichen abundance strongly highlighted with the Northwestern sites (PM 72 and CHS 35) and SP 11, showing a high number of lichen species weakly abundant. Each index was mainly influenced by one principal component (Fig. 5): axis 1 for LDV (45% of the data variance) and axis 2 for IAP (36% of the data variance). Based on lichen ecological features (Nimis and Martellos, 2008), the signs of environmental alteration (e.g., acid or poor nutrient environment) were mainly influenced by the positive scores of the first component, i.e., opposed to the LDV. It is likely that IAP, and thus lichen diversity, were mostly driven by climate variable (temperature) despite a low gradient of temperature among lichen species. The sites PM 72 and CHS 35, both positively influenced by the first axis, may either reflect an environmental alteration (i.e., more acid conditions), or be driven by the continentality–humidity axis due to their location with Atlantic influence.

4.2. Resistance and sensitivity of lichen species to metal atmospheric pollution

As observed in the PCA (Fig. 5), the LDV was opposed to the sum of metal enrichment factors in the axis 1 vs axis 2 plot. This implies that, in addition to the response toward the general alteration of environment, this index better responds to metal pollution as well. Indeed, the three lowest LDV were observed in CHS 35, PM 72, and EPC 08 (positive scores of the first axis of the PCA and negative scores of the second axis), that correspond to the highest EF and metal deposition (as observed in EPC 08; Gandois et al., 2010b).

The northeastern France is impacted by various activities (local industries, metallurgy, and mining), while both energy and metallurgy may explain such contamination in the northwestern France (already observed in upper horizons; Hernandez et al., 2003). This may be the dominant influence for CHS 35 and PM 72 in the PCA toward other environmental variables. Thus, it can be supposed that metal pollution affects more lichen abundance (illustrated by LDV) than lichen richness (IAP). This is in agreement with results from Jeran et al. (2002), who had previously observed that IAP was not a good index for metal pollution.

Based on the CCA, we evaluated the resistance or sensitivity of each lichen species to metal pollution (Fig. 6 and Table 5). Very few literature observations, however, allowed supporting our results: *Cladonia fimbriata* (present in 4 sites, $y' = -0.893$) is a well-known species able to grow on cadmium, lead, and zinc enriched substrates (Cuny et al., 2004; Tyler, 1989), whereas conversely, *Hypogymnia physodes* (present in 5 sites, $y' = 0.706$), is known as a metal sensitive species, particularly for copper (Hauck and Zöller, 2003). To validate our results, we verified any correlations with other pollutants: in both resistant and sensitive groups. There were both acidophilic (e.g., *Graphis scripta*, *Pertusaria albescens*, *Pertusaria coccodes*) and nitrophilic (*Dendrographa decolorans*, *Physcia adscendens*, *Physconia distorta*; Gombert et al., 2004) species, as well as both tolerant (*Melanohalea exasperatula*, *Physcia adscendens*) and sensitive (*Ochrolechia pallescens*, *Lecanora allophana*, *Physconia distorta*; Wirth, 1991) to SO_2/NO_2 pollution species. This implies that we cannot attribute the y' values to sulfur and nitrogen pollution influence, these elements being well known as major atmospheric pollutants. In this way, our method allowed correct evaluation of the influence of metal without other major disturbance. However, organic pollutants also accumulated by lichens (Bajpai et al., 2010; Harmens et al., 2013), were not investigated here. By applying the frequencies of studied species to these indices, and comparing to the enrichment factors from Agnan et al. (2015), we observed that the four more polluted sites (i.e., HET 54a, EPC 08, CHS 35, and PM 72) as evidenced by bioindication, obtained negative scores (i.e., dominated by resistant lichen species), while several less contaminated sites (e.g., EPC 63 and EPC 74) obtained positive values (i.e., dominated by sensitive lichen species).

These preliminary data need to be completed and compared with additional data from other European forest sites. Thus, it will be possible to determine the maximum exposure of metal pollution without significant harmful effects (also called critical load) as already done for nitrogen (Geiser et al., 2010).

5. Conclusions

This study aimed to evaluate the resistance or sensitivity of lichen species to atmospheric metal pollution. We performed eight lichen plottings in French and Swiss forested sites, and used different biomonitoring approaches (lichen richness, lichen abundances, lichen community description, ecological features, bioindication indices, as well as metal bioaccumulation) for a complete environmental description. Each method provided its own contribution to this investigation; similar results were demonstrated by lichen communities and ecological features. Ninety-two corticolous species were sampled, including 70% of crustose lichens. The abundance was higher on hardwood trees compared to conifers. The lichen diversity value (LDV) showed a better response to both ecological disturbances (largely influenced by light and nutrient conditions, such as eutrophication and pH) and metal pollution compared to the index of atmospheric purity (IAP).

Using a multivariate approach coupling frequencies of each lichen species and metal bioaccumulation data, we performed an innovative scale of resistance/sensitivity to metals for the 43 more frequent lichen species, distinguishing sensitive, intermediate, and resistant species to metal pollution. To validate these results, we compared to the few data available in the literature, and checked any correlation with sensitivity to acid and nitrogen pollution. This approach constitutes a first insight into the investigation of resistance and sensitivity of lichen species to metals in open forested sites far from local pollution sources, which should be enhanced by results with data from other European forests in future researches.

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