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# Integrated Monitoring of the Effects of Airborne Nitrogen and Sulfur in the Austrian Limestone Alps

## Is Species Diversity a Reliable Indicator?

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Many forested areas in industrial countries are exposed to excess deposition of airborne nitrogen (N) and sulfur (S). Biodiversity decline is one of many observed effects. Unlike N deposition, S loads have been decreasing

continuously over the last 25 years. In this paper, we evaluated the use of species diversity in 4 taxonomic groups (vascular plants, lichens, bryophytes, birds) as indicators of N and S deposition. Long-term monitoring data from 1992 to 2005 were taken from an intensively monitored site in Austria. Both temporal changes and determinants of diversity were explored. On average, diversity declined from the beginning of

the 1990s until the year 2005, but there was a considerable variation among the organisms and the diversity indicators. Few changes in diversity were statistically significant. Strongest changes occurred at the level of single species, as an increase or decrease of their abundance. Factors other than N and S deposition—particularly historical forest management and natural disturbances—were significant, complicating interpretation of the observed diversity changes. We conclude that species diversity alone is not a reliable indicator of N and S impacts on forests, particularly if few indicator species groups are used and the observation period is short.

**Keywords:** Air pollution; eutrophication; acidification; forest biodiversity; long-term monitoring; Austria.

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## Introduction

Nitrogen (N) and sulfur (S) emissions increased dramatically during the second half of the 20th century, causing excess deposition of N and S in natural and seminatural ecosystems, particularly in industrialized countries (Bouwman et al 2002; Galloway et al 2004). Owing to the canopy characteristics of forests, deposition of airborne pollutants is significantly elevated in these areas. Although direct pollution sources are less abundant in mountain areas, mountain forests are exposed to high precipitation and thus higher deposition loads (Lovett 1984; Erisman and de Vries 2000).

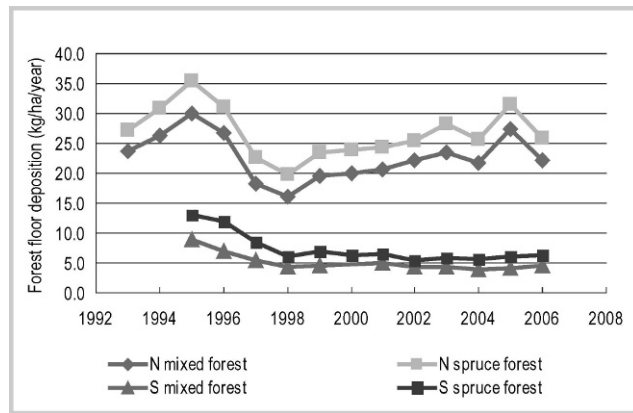
Chronic N deposition may cause soil acidification, disrupt the soil nutrient balance, increase susceptibility to parasite attack as well as emissions of nitrogenous greenhouse gases from the soil, and elevate nitrate loss to groundwater (Fenn et al 1998; Erisman and de Vries 2000; Aber et al 2001; Bobbink et al 2003). Significant changes of lichen, bryophyte, and vascular plant species composition in response to chronic N deposition have been reported for many ecosystems (Bobbink et al 2003). Since N emissions are predicted to increase globally during the decades to come, N excess will be among the major threats to biodiversity in future (Bouwman et al 2002; Galloway et al 2004; MEA 2005). Elevated S

deposition, causing soil acidification, the depletion of base cations from the soil, and the leaching of aluminum and heavy metals into groundwater, has been recognized as a major environmental problem since the 1970s. As for N, detrimental effects on living organisms have been found in freshwater and terrestrial ecosystems (van Dobben et al 1999; Legge and Krupa 2002), including biodiversity loss (Zvereva et al 2008).

Unlike N emissions, S emissions have been successfully abated in Europe through internationally ratified protocols (United Nations Economic Commission of Europe/Convention on Long-Range Transboundary Air Pollution [UN-ECE/CLRTAP]) and related measures. Loads of S have been decreasing continuously during the last 25 years (EMEP 2006; Rogora et al 2006; Figure 1).

After the World Summit on Sustainable Development and the Convention on Biological Diversity (CBD), which aims at significantly reducing the rate of biodiversity loss by the year 2010 (CBD 2003), the effects of air pollutants on biodiversity have become a central issue. Although the term biodiversity takes into account “variation within species, between species, and of ecosystems” (Article 2 of the Convention on Biological Diversity), species diversity is most often used as the key indicator. A change in the diversity of species involves complex population processes such as migration, extinction, and colonization.

**FIGURE 1** Forest floor deposition (throughfall plus stemflow) of nitrogen (sum of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N) and sulfur ( $\text{SO}_4^{2-}$ -S) for 2 forests (see intensive plots below) in the study area. Mixed forest: mixed beech–spruce–maple–ash forest. Spruce forest is predominantly Norway spruce with some beech.



In most organisms, these processes are not well studied, often involve response lags, and determinants vary with different spatial and temporal scales (Rosenzweig 1995; Crawley and Harral 2001; Ibáñez et al 2006).

In this paper, we evaluate the use of diversity as an indicator of the effects of N and S deposition in an intensively monitored site in the Northern Limestone Alps in Austria. Long-term measurements of deposition, climate, and forest management have been combined with the monitoring of several taxonomic groups and biodiversity. Our first question is therefore whether the diversity of different taxonomic groups exhibits the same temporal changes. As diversity indices, we used species numbers (SN) and Shannon Index of Diversity (SH) (Margurran 1988). The second purpose of this paper was to quantify the major determinants of diversity in the study area by including impacts through airborne N and S deposition (Figure 2).

## Material and methods

### Area description

The size of the site is 90 ha, and it is situated in the Northern Limestone Alps National Park (47°50'30"N, 14°26'30"E) (Figure 3). The altitude ranges from 550 m to 956 m above sea level (masl). The main type of rock is Norian dolomite, partly overlaid by limestone. The catchment area is divided into a steep (30–70°) slope from 550–850 masl and an almost flat plateau (850–956 masl). The long-term average annual temperature is 7.2°C. The coldest monthly temperature at 900 masl is –1°C (January), and the highest monthly temperature is 15.5°C (August). Annual rainfall ranges from 1500 to 1800 mm. Monthly precipitation ranges from 75 mm (February) to 182 mm (July). Snowfall occurs between October and May, and the average duration of snow cover is about 4

months. The slope is mainly covered by mixed mountain forest with beech (*Fagus sylvatica*) as the dominant species, Norway spruce (*Picea abies*), maple (*Acer pseudoplatanus*), and ash (*Fraxinus excelsior*), whereas *Picea abies* predominates on the plateau following plantation after a clear-cut around the year 1910. Long-term trends of N and S deposition are given in Figure 1.

### Monitoring of bioindicators

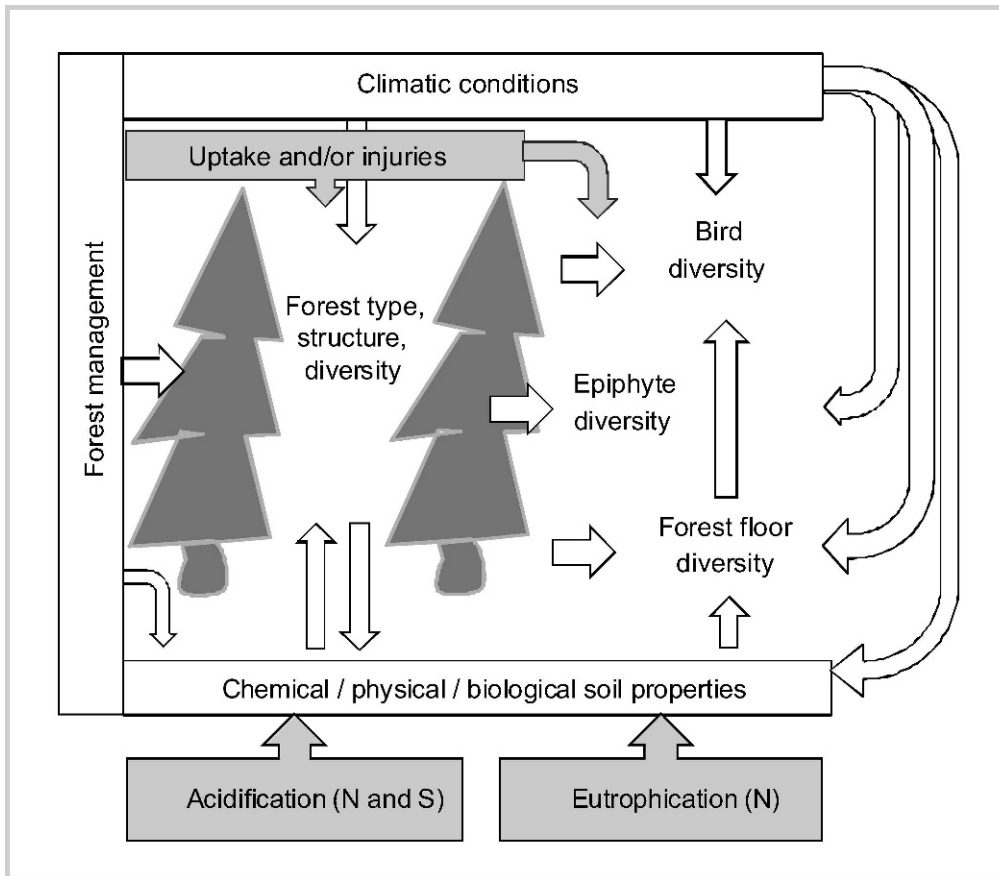
Forest floor vegetation, epiphytic lichens and bryophytes on tree trunks, forest floor bryophytes, and birds were monitored using permanent plots distributed over the entire area (Figure 3; Table 1). Methodological details concerning plot selection can be found in Dirnböck et al (2007), Zechmeister et al (2007), and Hülber et al (2008).

### Statistical analyses

Temporal change in diversity was assessed using those plots that were recorded in each observation year. The number of species per permanent plot (SN) and the Shannon diversity index per plot (SH) were used (Margurran 1988; Oksanen 2005). The total number of species in the study area (PSN) was calculated as the cumulative sum of species occurring on all of these plots. Changes of SN and SH between surveys were tested using a one-sided paired Wilcoxon rank sum test for SN and one-sided paired *t*-test for SH after testing for normality. For each taxon, only the difference between first and last observation year was tested.

The diversity indices were further analyzed with respect to the major environmental and anthropogenic determinants: forest floor vegetation (mean and standard deviation of the soil pH value and C/N ratios and the radiation, and tree-layer diversity as the “stand diversity index” according to Jaehne and Dohrenbusch [1997]); epiphytic lichens (radiation and tree diversity, tree species identity, stem diameter, and direct air pollution effect indicated by injuries to lichen individuals); and birds (tree-layer diversity, gross forest type, tree density, SH). Data on these predictor variables were not available for all plots (bryophytes were not analyzed due to a lack of data). For more details, see Dirnböck et al (2007). Generalized linear models (GLM) with SN and SH as responses and the previously described predictor variables were fitted. We used the log-link function with Poisson error distribution for SN and the identity link with Gaussian error for SH. Second-order polynomials were used for all continuous predictor variables. Explained deviance of all partial effects was calculated by dropping terms from the full model. The significance of single predictors was tested with a Chi-square test for SN and F-test for SH (McCullagh and Nelder 1989). The GLM function of R 2.6.2 was used for fitting the models.

**FIGURE 2** Conceptual model of the major factors controlling the diversity of epiphytes, forest floor vegetation, and birds in temperate forest ecosystems. Effects and their direction are illustrated with arrows; effects through airborne N and S are shown in the gray boxes. Diversity is controlled by a number of direct and indirect factors. The habitat exerts a direct influence through climate, soil, and substrate condition. Apart from average habitat conditions, heterogeneity is an important factor. Indirect effects occur via trophic interactions and the tree layer. Tree-layer diversity in turn is controlled by soil and climate, but also to a great extent by forest management and natural disturbances. Major effects of airborne N and S likely occur through direct uptake of deposited substances—causing various injuries—and indirect uptake through soil acidification and eutrophication.



## Results

### Diversity changes

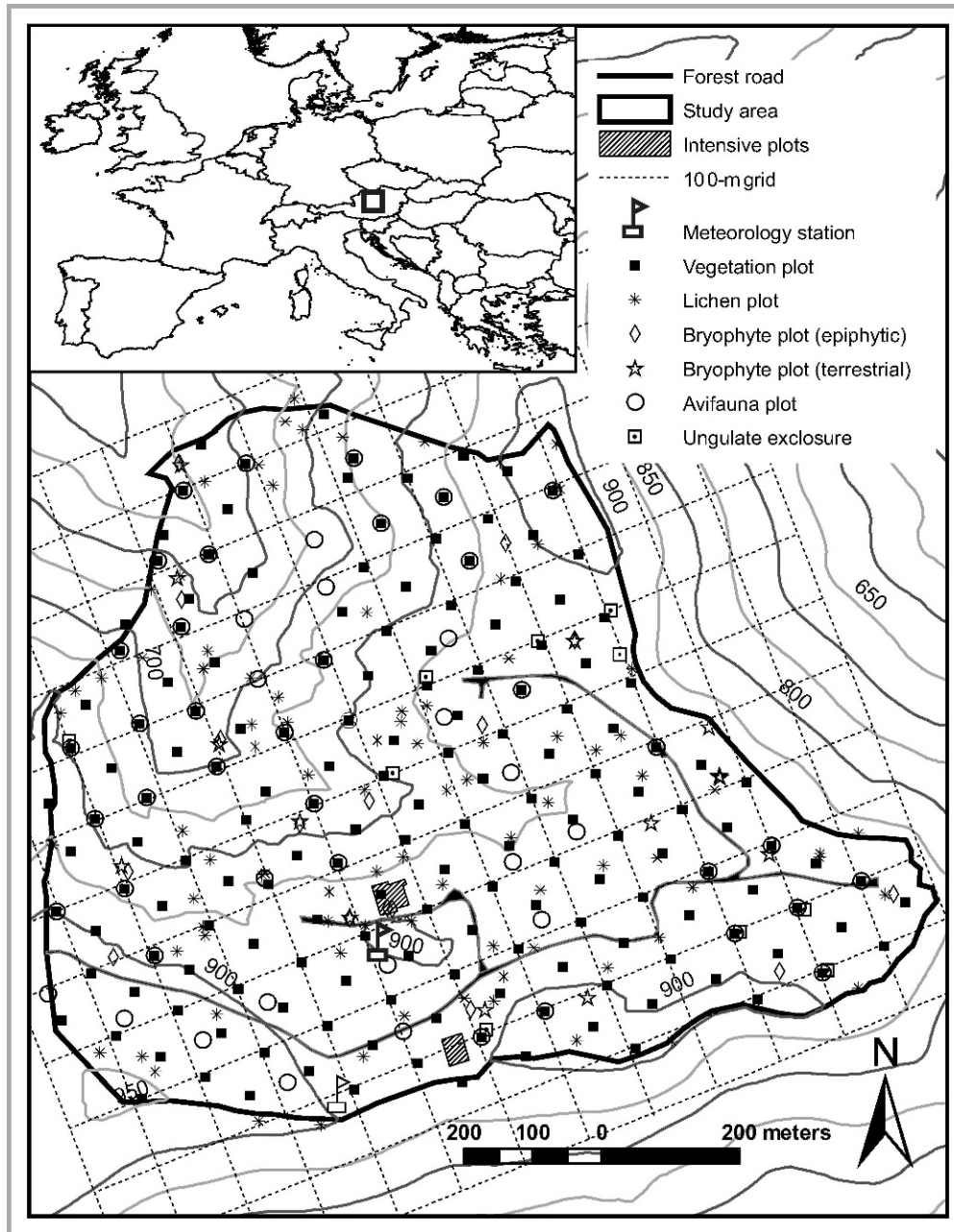
Averaged over all organisms, diversity declined from the beginning of the 1990s until 2005. For all but the epiphytic lichens, PSN decreased. There are fewer species per plot (SN) today than at the beginning of the observations. SH showed predominantly decreasing values (Table 2). Epiphytic lichens were an exception since their species number increased in the entire study area (PSN) and at the plot scale (SN). Although SH decreased, statistical significance was not achieved. In general, only a few diversity trends are statistically significant.

### Factors controlling biodiversity

The predictor variables explained between 22% and 45% of the variation of SN and SH in epiphytic lichens, forest floor vegetation, and birds (Table 3). The SH of forest floor plots showed the weakest relationship and the SH of epiphytic lichens showed the strongest relationship with the predictors. Only a few single predictors were significantly related to SN and SH due to the low number of replicates and the high number of predictors used.

Forest type and forest floor diversity explained most of the diversity of birds. Mixed conifer–deciduous forests hold more species with higher abundance diversity than coniferous forests. Forest floor diversity was positively correlated with SN and SH of bird plots (Table 3).

**FIGURE 3** The Austrian UN-ECE Integrated Monitoring site of Zöbelboden, showing the location of the main meteorological measurements, the 2 intensive plots for deposition measurements, and the distribution of all permanent plots for the monitoring of bioindicators. (Map by Thomas Dirnböck)



Epiphytic lichen diversity was most strongly and directly influenced by air pollution. The higher the air pollution impact, the fewer the species and the more homogeneous the plots. The SN value of lichen plots, unlike the SH value, depended very much on the species identity of the host tree (more than on air pollution; higher SN and SH on deciduous trees) and the tree-layer

diversity in the surroundings of the plot. Climatic factors codetermined diversity, which can be seen from the reasonable proportion of the deviance explained by solar radiation (Table 3).

SN and SH values of forest floor vegetation were rather differently related to the predictor variables. Whereas tree-layer diversity was the most important



**TABLE 1** Bioindicator groups and years of recording (dark gray cells) in the study area.

Year of survey	1991–1999									2000–2006						
Bioindicator group	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6
Forest floor vegetation																
Terrestrial and epiphytic bryophytes																
Epiphytic lichens																
Birds																

predictor for SN, SH was almost not influenced. SH was mostly affected by soil diversity, so that SH was higher in plots with a higher variability of soil properties. The second most important predictor of SH was the pH value of the soil—more acidic soils resulted in fewer species. Nitrogen availability, as indicated by the C/N ratio of the soils, had no effect on SN and a weak effect on SH (Table 3).

## Discussion

On average, diversity declined from the beginning of the 1990s until the year 2005, but there was considerable variation among the organisms and the diversity indicator. All bioindicator groups except epiphytic lichens decreased regarding PSN and SN (Table 2). The decreasing trend is less consistent at the plot scale because SH—though mostly decreasing—increases for forest floor vegetation and is stable for terrestrial bryophytes. Compared with the variability of diversity between permanent plots, the temporal changes are rather weak.

The diversity of birds, epiphytic lichens, and forest floor vegetation is to a reasonable extent related to habitat conditions and their heterogeneity. The unexplained variation is probably due to random processes, measurement errors, and variables not included in the models (eg microclimate). Indirect effects (Figure 2) become increasingly important, progressing from epiphytic lichens to forest floor vegetation to birds. Tree-layer diversity is among the main factors controlling the diversity of forest organisms (see also Neumann and Starlinger 2001). The tree layer can change drastically through forest management and natural or human disturbances. In the study area, wood production and hunting had a strong effect on the structure and composition of the forests. In addition, repeated wind throw and bark beetle calamities occurred. As in many forests, both human use and natural disturbance are the dominant determinants of diversity (Lindenmayer and Franklin 2002). Potentially, N and S deposition is able to totally deteriorate the tree layer, and thus exert a strong indirect effect on diversity, but loads have to be long-lasting and high (Wright and Rasmussen 1998; Magill et al 2004).

The temporal development of the diversity of bryophytes remains idiosyncratic. Whereas epiphytic bryophyte diversity decreased, terrestrial bryophytes exhibited a considerable peak in the year 1999 and strongly decreased thereafter. Zechmeister et al (2007) showed that species composition varied considerably with time and that these changes were not attributable to the observed deposition trends. Effects of airborne N and S are limited to only some species such as *Hypnum cupressiforme*, which is a well-established indicator species (Kuhn et al 1987). Bryophyte diversity and the composition of the communities proved to be rather insensitive to airborne N and S deposition in the study area. Zechmeister et al (2007) discussed this insensitivity with regard to the seasonal timing of the photosynthetic activity of bryophytes and the deposition. During periods of growth, the monthly background deposition was found to be rather low, ranging between 1–2 kg N ha<sup>-1</sup> and 0.5–1 kg S ha<sup>-1</sup> (data not shown). In contrast, fog deposition with disproportionally high concentrations of pollutants occurs in autumn, when bryophytes are rather inactive. This is a typical deposition regime in many temperate mountain forests (Lovett 1984), and thus comparable results may be found more often.

The increase of lichen diversity would, at first glance, be in line with many studies carried out in industrialized areas, where recovery of lichen diversity followed the decrease of SO<sub>2</sub> concentrations after emission reduction (van Dobben and Ter Braak 1998). In contrast to the deposition measurements, which clearly show a rising pH value, lichen communities and single species are continuously affected by acid deposition (eg extinction of *Bryoria* sp, *Usnea* sp). Also, eutrophication effects through excess N pollution are clearly indicated (eg a decrease of *Hypogymnia physodes*) (data not shown). Time lags of local extinctions and fast colonization of nonsensitive species seem to be responsible for the observed discrepancy. Lichen diversity would thus be misleading as a single indicator for airborne S and N effects.

Strong effects through N and S deposition on forest floor vegetation are improbable since soils are mostly well buffered against acid deposition, and N limitation is rather weak (Diekmann and Dupré 1997). Nevertheless,

**TABLE 2** Time trends of the species number in the entire study area (PSN, sum of the species of all plots), species number per plot (SN, mean and standard deviation), and Shannon diversity per plots (SH, mean and standard deviation) for forest floor vegetation, epiphytic bryophytes, terrestrial bryophytes, epiphytic lichens, and birds.

Bioindicator	Diversity index	1992/ 1993	1997/ 1999	2004/ 2005	Increase (+)	P-value <sup>e)</sup>
					Decrease (-)	
Forest floor vegetation ( <i>n</i> = 124)	PSN <sup>a), b)</sup>	195	–	181	–	–
	SN	31 ± 10.0	–	30.4 ± 8.0	–	0.100
	SH	2.30 ± 9.93	–	2.39 ± 8.07	+	<b>0.014</b>
Epiphytic bryophytes ( <i>n</i> = 11)	PSN <sup>a)</sup>	16	17	15	–	–
	SN	4.4 ± 2.0	4.1 ± 2.5	3.8 ± 2.4	–	0.373
	SH	1.09 ± 0.57	0.98 ± 0.62	0.87 ± 0.57	–	0.302
Terrestrial bryophytes ( <i>n</i> = 9)	PSN <sup>a)</sup>	53	66	45	–	–
	SN	2.9 ± 2.0	4.1 ± 3.1	2.6 ± 2.4	–	0.373
	SH	0.43 ± 0.55	0.52 ± 0.65	0.43 ± 0.51	±	–
Epiphytic lichens ( <i>n</i> = 80)	PSN <sup>a), c)</sup>	(40)	65	69	+	–
	SN <sup>d)</sup>	(4.4 ± 2)	6.0 ± 3.1	6.7 ± 3.4	+	<b>0.014</b>
	SH <sup>d)</sup>	(0.58 ± 0.43)	0.88 ± 0.51	0.86 ± 0.54	–	0.484
Birds ( <i>n</i> = 55)	PSN <sup>a)</sup>	–	50	48	–	–
	SN	–	14.4 ± 4.9	13.1 ± 3.4	–	<b>0.015</b>
	SH	–	2.48 ± 4.91	2.23 ± 3.4	–	< <b>0.001</b>

<sup>a)</sup> Only plots that were recorded in all observation years were used.

<sup>b)</sup> Only plots with tree cover > 10% and only herb layers with a height < 60 cm were taken into account.

<sup>c)</sup> Represents pooled species numbers derived from different methods.

<sup>d)</sup> In the years 1992/1993 only one method was applied, so these values were not used for further analyses.

<sup>e)</sup> Significances of the changes between the first and the last observation year were tested using 1-sided paired Wilcoxon rank sum test for SN and 1-sided paired t-test for SH.

Hülber et al (2008) found a weak trend in single species abundances, such as an increase of basiphilous species. In addition, the species composition exhibited homogenization in response to soil eutrophication. Homogenization of N availability in soils may be a trigger for diversity loss in forest ecosystems (Gilliam 2006). The temporal change in the diversity of forest floor vegetation is probably too weak to infer a clear effect of N and S deposition. The decrease of species in the entire study area would fit into the picture of homogenization, but then the plots should also become more homogeneous. However, this is not the case (SH increases).

The diversity of birds decreased significantly from the year 1997 to 2005. This is most probably due to changes in the structure and diversity of the tree layer, the main determinant for variation in bird diversity (eg Verschuyt et al 2008). Dead wood increased, while the stand diversity index in the area decreased significantly between 1992

and 2005 (Dirnböck et al 2007). Changes in bird diversity are rather strongly influenced by forest floor and tree-layer diversity, both of which are sensitive to acidification and eutrophication. Indirect effects of N and S via the tree layer and the forest floor may be relevant in the long-term.

## Conclusion

Forests are complex ecosystems where numerous direct and indirect effects simultaneously influence the diversity of organisms. Effects of airborne N and S on the diversity of forest organisms are thus difficult to detect and prone to misinterpretation. In order to distinguish among effects using long-term monitoring, certain conditions have to prevail: the impact must be strong enough to cause a measurable effect within a reasonable time. In reality, these three conditions seldom occur

**TABLE 3** Relationships among species number (SN), Shannon diversity (SH), and the predictor variables. The strength of the predictor is represented as the percentage change of the deviance when dropping the respective variable from the full generalized linear model. Significance levels (Chi-square test for SN and F-test for SH) are given as n.s. > 0.1, \* < 0.1, \*\* < 0.05, \*\*\* < 0.001; *P*-values < 0.1 are shown in bold; SD, standard deviation.

	Birds ( <i>n</i> = 32)		Epiphytic lichens ( <i>n</i> = 42)		Forest floor ( <i>n</i> = 59)	
	SN	SH	SN	SH	SN	SH
Null deviance	148.7	14.0	44.2	0.1	35.9	3.4
Deviance explained (%)	<b>35.0</b>	<b>38.4</b>	<b>43.1</b>	<b>45.3</b>	<b>26.9</b>	<b>22.4</b>
Air-quality class	–	–	<b>17.4*</b>	<b>41.8**</b>	–	–
Deadwood floor	10.5 <sup>n.s.</sup>	7.1 <sup>n.s.</sup>	–	–	–	–
Deadwood standing	0.1 <sup>n.s.</sup>	1.2 <sup>n.s.</sup>	–	–	–	–
Forest floor vegetation diversity	24.2 <sup>n.s.</sup>	21.2 <sup>n.s.</sup>	–	–	–	–
Forest type (deciduous/coniferous)	<b>36.4**</b>	<b>34.2**</b>	–	–	–	–
Mean C/N ratio	–	–	–	–	0.0 <sup>n.s.</sup>	1.3 <sup>n.s.</sup>
Mean pH	–	–	–	–	<b>14.0*</b>	23.3 <sup>n.s.</sup>
Mean solar radiation	–	–	18.9 <sup>n.s.</sup>	13.7 <sup>n.s.</sup>	<b>15.7*</b>	2.1 <sup>n.s.</sup>
SD solar radiation	–	–	–	–	9.2 <sup>n.s.</sup>	10.2 <sup>n.s.</sup>
SD soil depth	–	–	–	–	<b>34.9**</b>	24.1 <sup>n.s.</sup>
Tree-layer diversity	4.8 <sup>n.s.</sup>	6.3 <sup>n.s.</sup>	15.4 <sup>n.s.</sup>	1.4 <sup>n.s.</sup>	<b>42.7***</b>	1.2 <sup>n.s.</sup>
Tree stem count	14.0 <sup>n.s.</sup>	9.7 <sup>n.s.</sup>	–	–	–	–
Tree stem diameter	–	–	1.0 <sup>n.s.</sup>	5.3 <sup>n.s.</sup>	–	–
Tree type (conifer/deciduous)	–	–	<b>29.9**</b>	10.0 <sup>n.s.</sup>	–	–

simultaneously. In our case study, we assumed effects from N and S deposition, since deposition loads were high or had changed reasonably. However, changes were restricted to single species, as seen in increase or decrease of their abundance. Rarely did diversity change significantly. Confounding factors, particularly forest management and natural disturbances, complicated the

interpretations. Species diversity alone is not a reliable indicator of the effects of airborne N and S in forests, particularly if few indicator species groups are used and the observation period is short. This is why integrated and long-term monitoring approaches that capture different response groups and timescales are attractive.

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