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Comparative use of lichens, mosses and tree bark to evaluate nitrogen deposition in Germany

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ABSTRACT

To compare three biomonitoring techniques for assessing nitrogen (N) pollution in Germany, 326 lichen, 153 moss and 187 bark samples were collected from 16 sites of the national N deposition monitoring network. The analysed ranges of N content of all investigated biomonitors (0.32%–4.69%) and the detected $\delta^{15}N$ values (−15.2‰–1.5‰), made it possible to reveal species specific spatial patterns of N concentrations in biota to indicate atmospheric N deposition in Germany. The comparison with measured and modelled N deposition data shows that particularly lichens are able to reflect the local N deposition originating from agriculture.

1. Introduction

Increasing intensification of agricultural land through the application of nitrogen (N) fertilizer, caused by the growing demand for agricultural products, has had major impacts on ecosystems worldwide (Galloway et al., 2008; Godfray et al., 2010). Future predicted consumption growth of the human population is expected to further exacerbate this problem, making the monitoring and control of N emissions a high priority for environmental science. Particularly nitrogenous gases such as ammonia (NH_3), have increased mainly due to animal farming (Erisman et al., 2008; Krupa, 2003). NH_3 is highly reactive, and preferentially deposited as dry deposition close to the emitted source, whilst its reaction product ammonium (NH_4^+) is principally washed out by precipitation in terms of wet deposition. These two compounds, collectively referred to as NH_x, are major contributors to total N deposition (Asman et al., 1998; Krupa, 2003) and can have an effect on vegetation in high doses (Bobbink et al., 2010; Sheppard et al., 2011). In comparison to vascular plants, lower plants such as lichens and mosses depend on atmospheric inputs as their primary source of nutrients, and can be highly sensitive to direct impacts of NH_x (Bobbink et al., 2010; Sheppard et al., 2011; Skinner et al., 2006). Furthermore, Cape et al. (2009) defined a lower NH_3 Critical Level (CLE) for this sensitive vegetation type. Mosses and lichens are therefore suitable to indicate the N input at ecosystem level due to their specific physiology and ecology (Hauck, 2010; Turetsky, 2003). Such organisms that can be used for the quantitative determination of contaminants in the environment are referred to as accumulative biomonitors (Conti and Cecchetti, 2001). Lichens (Bruteig, 1993; Frati et al., 2007; Gaio-Oliveira et al., 2001; Gombert et al., 2003; Raymond et al., 2010; Remke et al., 2009; Søchting, 1995) and mosses (Harmens et al., 2011; Leith et al., 2005; Pesch et al., 2007; Pitcairn et al., 2006) have frequently been used in local, national and European wide studies. In addition to this, tree bark offers another resource for the assessment of atmospheric N depositions (Mitchell et al., 2005; Poikolainen et al., 1998).

To identify underlying atmospheric N sources, $\delta^{15}N$ signatures of atmospheric N compounds are used (Freyer, 1978; Heaton et al., 1997). The abundance of ^{15}N is a valuable and widely used indicator of sources and pathways of N in organisms and ecosystems (Högberg, 1997; Robinson, 2001). It is generally accepted that the determined $\delta^{15}N$ signatures in lichen (Boltersdorf and Werner, 2013; Fogel et al., 2008; Lee et al., 2009; Russow et al., 2004; Tozer et al., 2005) and in moss tissue (Bragazza et al., 2005; Liu et al., 2008; Solga et al., 2005; Zechmeister et al., 2008) are able to reflect predominating N isotope sources in the environment. In the context of bark monitoring, the determination of the abundance of ^{15}N has also been applied successfully (Schulz et al., 2001).

Due to their high costs, current deposition measurement stations are not widespread and therefore provide only a partial

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picture of the real extent of the prevailing N deposition status over large areas (Sutton et al., 1998). However, biomonitors may serve as possible alternatives to get a spatially representative picture of the deposition conditions. This study therefore compares the ability of the three biomonitors – lichens, mosses and tree bark – to reflect the atmospheric deposition of N compounds in terrestrial ecosystems. Furthermore, we compare the spatial patterns of $\delta^{15}N$ with potential sources of N deposition. These two research topics may be subdivided into the following objectives:

i. Assessing the level of N deposition in Germany by tissue N content of lichens, mosses and tree bark.
ii. Identifying the key contrasting sites with respect to N depositions using these biological indicators.
iii. Indicating the main sources of N pollution and their different spatial patterns in Germany using $\delta^{15}N$ measurements.
iv. Testing whether data obtained from these bioindicators (N% and $\delta^{15}N$) correlate with measured and modelled data from N deposition assessment programmes.

2. Material and methods

2.1. Site description and N deposition data

Data was collected from 16 deposition measurement sites of the Air Monitoring Network of the Federal Environment Agency of Germany (Umweltbundesamt – UBA; Ihle et al., 2001) (Fig. 1). The study sites are situated in different topographical areas, including coastal and plain areas in the north, low mountain range landscapes in the central area of Germany and highly mountainous areas in the south. Besides the topographical differences (1 m–1205 m above sea level), the wet-only deposition measurement network reflects different land use related influences of N pollution. In addition to areas relatively clean air, which are typically forestry dominated areas (e.g. the Black Forest or Bavarian Forest), the research also includes sites that are highly affected by agricultural and long-range transboundary emissions, especially in the north-western and eastern areas of Germany. The wet-only deposition data (not collected with continuously open collectors and data only from precipitation events) include nitrate – nitrogen (NO_3–N), ammonium – nitrogen (NH_4–N) and mean annual precipitation (UBA, 2004). Deposition data from the years 2006–2007 were considered in order to have reliable data for the majority of the sites. The averaged NH_4–N deposition thereby ranges from 1.50 kg ha^{-1} yr^{-1} (Regnitzlosau) to 6.38 kg ha^{-1} yr^{-1} (Schmuecke). The respective NO_3–N deposition varies between 0.70 kg ha^{-1} yr^{-1} (Regnitzlosau) and 3.12 kg ha^{-1} yr^{-1} at the Schmuecke site (Fig. 2).

The German deposition network contributes to the European Monitoring and Evaluation Programme (EMEP), which operates under the Long-Range Transboundary Air Pollution (LRTAP) convention in Europe. The objective of the programme is to model and predict the deposition of acidifying and eutrophying pollutants on a European scale (Simpson et al., 2006).

In addition to measured data, modelled N deposition data from the project Modelling of Air Pollutants and EcoSystem Impact (MAPESI), providing deposition information for different ecosystem types at national, regional and local scale, were included in the analysis. Here, total N deposition is modelled for a 1 × 1 km^2 grid by consolidating information of bulk deposition (taking into account permanently open collectors), dry and occult deposition of oxidised and reduced N compounds. Besides, nine Corine Landcover 2000 land use classes were taken into account by the chemistry transport model Long Term Ozone Simulation and European Ozone Simulation (LOTOS–EUROS), in order to model dry deposition (UBA, 2011). The mean (2006–2007), grid cell based, modelled NH_4–N deposition ranges from 6.50 kg ha^{-1} yr^{-1} (Zingst) to 35 kg ha^{-1} yr^{-1} at Kleve site. The NO_3–N deposition varies between 6.00 kg ha^{-1} yr^{-1} (Melpitz, Sylt and Zingst) and 16.50 kg ha^{-1} yr^{-1} (Solling) (Fig. 2). In the present study, total N deposition data from the year 2007 were included relating to semi-natural vegetation as receptor surface. The corresponding modelled total N deposition ranges from 13 kg ha^{-1} yr^{-1} (Zingst) to 38 kg ha^{-1} yr^{-1} (Kleve).

2.2. Lichen, moss, bark sampling and N analysis

Lichen, moss and bark samples were collected at 16 deposition sites across Germany in September and October 2008 (Fig. 1). At each deposition measurement site we attempted to sample ten replications of each epiphytic lichen species listed in Table 1.

In total, 326 lichen samples were collected. The pooled lichen samples (3–5 thalli per one sample tree) were collected with a knife from free-standing trees that met the requirements for bioindication with lichens (VDI, 2005) within a 2 km radius around the deposition measurement field station. Lichens were sampled on trunks and twigs over 1.50 m above ground level. Along with the lichens, bark samples were taken using a drawknife. The sampling was carried out by removing 2–3 mm shavings of bark on trunks or on branches over 1.50 m above ground level. The sample size ranged from 6 to 13 sample trees per site (considered tree species were listed in Table 1). All investigated tree species were analysed separately, but they were averaged finally per respective deposition measurement station.

Simultaneously to the collection of lichens and tree bark, the moss species (Table 1) were collected mostly in mixed forests in the same radius around the deposition monitoring sites. A detailed description of the sampling procedure and preparations for chemical analyses is given in the European moss survey protocol 2005/2006 (ICP Vegetation, 2005). Accordingly, samples were taken at least in 3 m distance from the nearest tree, in small open areas. Five replications per moss species were collected on-site. We avoided collecting lichen and bark samples in the stem runoff, and in areas which were colonised by algae or covered by other epiphytes than lichens. Certainly not all investigated lichen and moss species were present on all deposition measurement stations along Germany. So the sampling pattern unfortunately considers not everywhere the same species.

After removal, all samples were put into paper bags, labelled and stored firstly in a refrigerator and finally in a freezer at −20 °C. For the moss analyses, the green and green–brown shoots from the last 3 years growth were included. All samples were dried with a freeze-dryer (Martin Christ GmbH, type 101541, Osterode, Germany), pulverised for homogenisation using an agate-type ball mill (Fritsch GmbH,

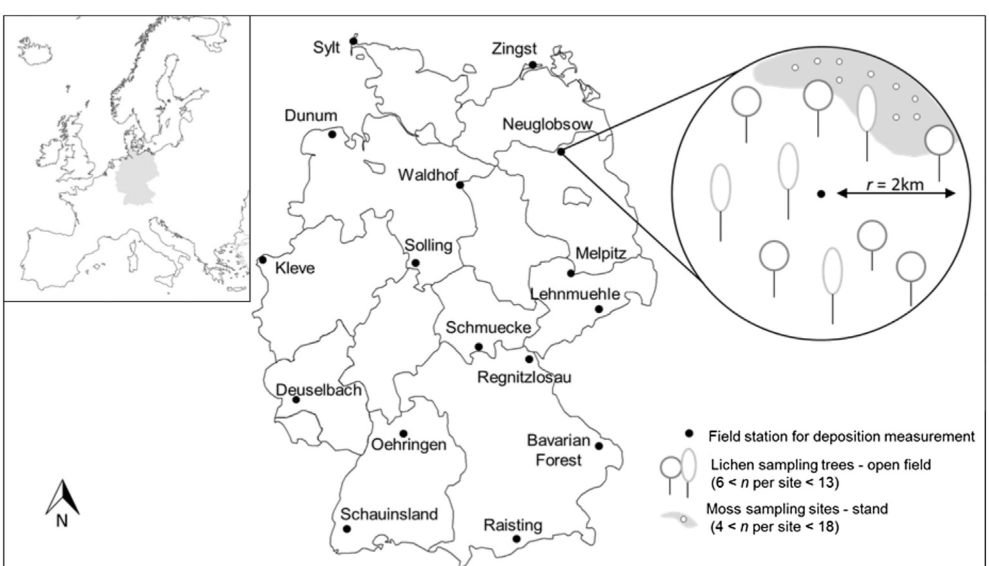

Fig. 1. The field sites for deposition measurement ($n = 16$, UBA) in Germany and the design of lichen and moss sampling (data Environmental Systems Research Institute-ESRI, USA).

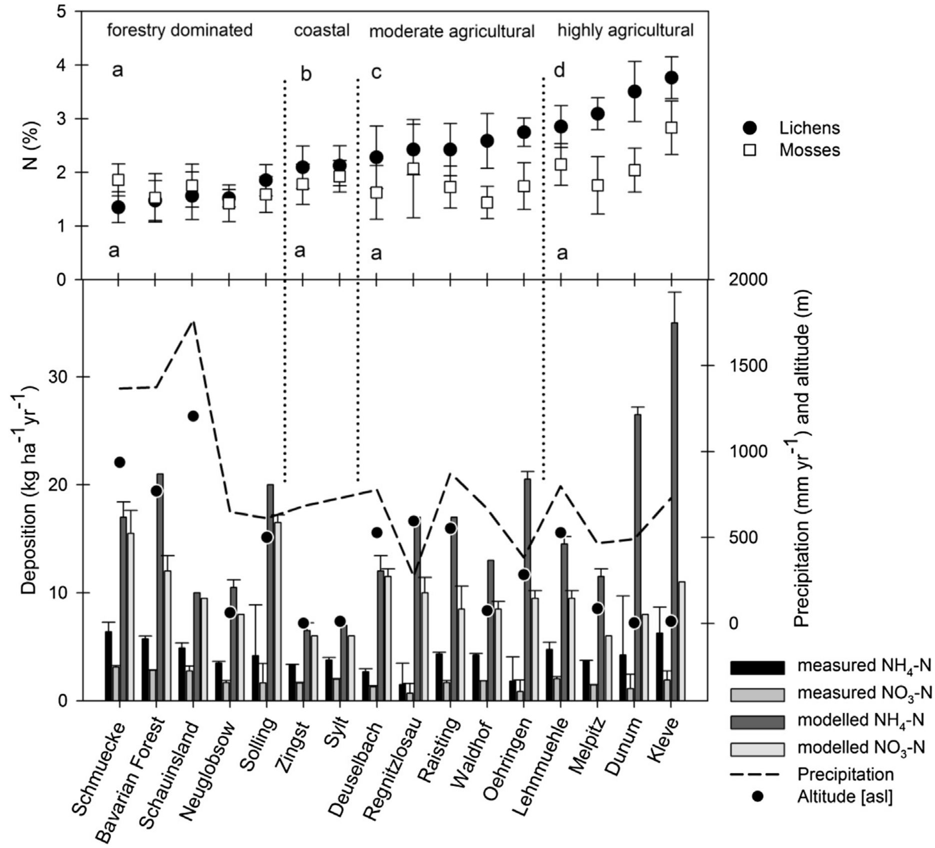

Fig. 2. Average annual measured NH_4–N and NO_3–N wet-only deposition from 2006 to 2007 and average annual total NH_4–N and NO_3–N deposition from 2006 to 2007 as modelled by MAPESI (Mean ± *SD*). Mean annual precipitation of the years 2006 and 2007 (Mean ± *SD*) and altitude of field site for deposition measurement (bottom). Average N content of lichen and moss samples (Mean ± *SD*) are presented and different letters refer to significant (ANOVA, $P < 0.001$) differences among the monitoring organisms and the four different site groups (forestry dominated, coastal, moderate agricultural and highly agricultural areas) (top).

Pulverisette 9, Idar-Oberstein, Germany) and finally preserved in a desiccator. Subsequently, subsamples of 3–4 mg of each sample were put into tin capsules and total N concentration and ^{15}N natural abundance was analysed using an elemental analyser coupled with an isotope-ratio mass spectrometer (Thermo Scientific, Flash EA 1112 Series + IRMS Delta VI Advantage-Isotope ratio MS, Waltham, USA). The N concentration is expressed as percentage (%) N from dry weight (analytical precision ($n = 2$) is ±0.1%). The determination of $^{15}N/^{14}N$ is denominated as $\delta^{15}N$. The $\delta^{15}N$ values are reported in per mil (‰) relative to air as the international standard for N and the analytical precision ($n = 2$) is ±0.1‰. IAEA (International Atomic Energy Agency) certified and internal laboratory reference material was used for quality

Table 1
Summary of all investigated biomonitor species and their percentage share in the investigation (%). *Physcia adscendens* and *Physcia tenella* were merged and named *Physcia* spp. in the present study. The selection of tree species was based on the VDI Guideline 3957/13 (VDI, 2005) and was grouped by acidity. Additional tree species were marked with asterisk (*).

Lichen species		Moss species		Tree species		
				±Acid		
Parmelia sulcata	41%	*Hypnum cupressiforme*	49%	*Alnus glutinosa*	2%	42%
Physcia adscendens	28%	*Pleurozium schreberi*	33%	*Betula pendula*	11%	
Physcia tenella		*Pseudoscleropodium purum*	18%	*Pinus sylvestris**	1%	
Xanthoria parietina	31%			*Prunus avium*	4%	
				Prunus domestica	5%	
				Quercus robur	7%	
				Quercus petraea	8%	
				Salix spp.*	4%	
				±Acid/subneutral		
				Tilia cordata	2%	4%
				Tilia platyphyllos	2%	
				Subneutral		
				Acer platanoides	4%	54%
				Acer pseudoplatanus	21%	
				*Carpinus betulus**	1%	
				*Corylus avellana**	1%	
				Fraxinus excelsior	7%	
				Malus domestica	11%	
				Populus × canadensis agg.	7%	
				*Sorbus torminalis**	2%	

assurance in the analyses: IAEA-N_1 ($\delta^{15}N$ = 0.4‰), acetanilide (N% = 10.36%, $\delta^{15}N$ = 1.8‰) and barley (N% = 1.85%, $\delta^{15}N$ = 5.2‰).

2.3. Statistical analyses

Normal distribution of data was tested using the Kolmogorov–Smirnov test. Two-way variance analysis (ANOVA) followed by Scheffé's post-hoc test was used to examine initial differences in N chemistry between the biomonitors. This analysis was carried out in SPSS (IBM SPSS Statistics, Armonk, USA). Pearson's correlations were also performed to establish relationships between N deposition and indicator chemistry and logarithmic regression was analysed with indicator chemistry data carrying out by Sigmaplot (Systat Software Inc., Chicago, USA).

3. Results

3.1. Species- and spatial-specific accumulative responses to N

Within the taxonomic group, interspecific differences with respect to N% were only found at Kleve site within the lichens. *P. sulcata* and *X. parietina* show significant less tissue N content than *Physcia* spp. ($P < 0.05$) (Fig. 3). The N content in lichen tissue ranged between 0.93% (*P. sulcata*, Bavarian Forest) and 4.69% (*Physcia* spp., Dunum), with an average of 2.48% ± 0.79 (n = 326). N content (averaged over all sites) in *P. sulcata* ranged from 0.93% to 3.94% (n = 134), in *X. parietina* tissue N content between 1.45% and 4.38% was detected (n = 100) and *Physcia* spp. showed a range from 1.32% to 4.69% (n = 92).

A comparison of the mean values (averaged over all sites) revealed significant differences ($P < 0.001$) in N tissues between the *P. sulcata* (lowest N concentrations) and the both more N enriched species *X. parietina* and *Physcia* spp.

Considering all lichen N contents per investigated site and the different characteristic N affected sites categories (highly and moderate agricultural areas, coastal and forestry dominated areas), lichens showed clearly significant differences ($P < 0.001$) (Fig. 2).

Tissue N content of all moss samples varied from 0.81% (*H. cupressiforme*, Bavarian Forest) to 3.64% (*H. cupressiforme*, Regnitzlosau). The average is 1.77% ± 0.52 (n = 153). Studying the species separately, *H. cupressiforme* N content ranged between 0.81% and 3.64% (n = 74), *P. schreberi* between 0.98% and 2.53% (n = 51) and *P. purum* between 1.08% and 3.13% (n = 28). On average (averaged over all sites), *P. schreberi* showed significantly the lowest N concentrations ($P < 0.05$) whereas highest tissue N content was found in *H. cupressiforme*. No significant differences were found

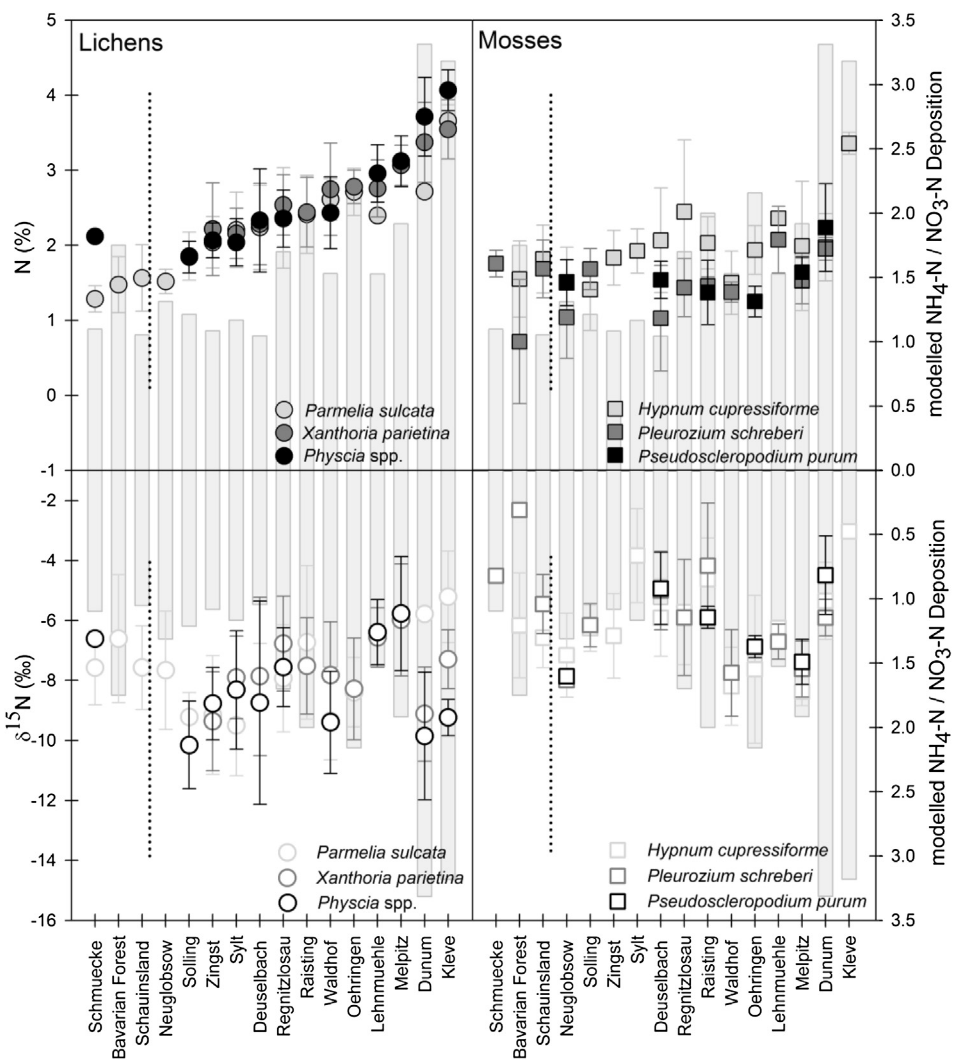

Fig. 3. Average N concentrations and $\delta^{15}N$ values of the lichen species (*P. sulcata*, *X. parietina* and *Physcia* spp.) and moss species (*H. cupressiforme*, *P. schreberi* and *P. purum*) (Mean ± *SD*) at various sites in Germany. Grey bars show the ratio of MAPESI modelled reduced and oxidised N at the respective study site. Dotted line demarcates areas with precipitation >1000 mm yr^{-1} (Schmuecke, Bavarian Forest and Schauinsland).

according to the various N affected site categories ($P < 0.001$) (Fig. 2).

In the bark samples, an average of 0.91% ± 0.25 ($n = 187$) was measured, which is lower than for lichens and mosses. The full range amounted from 0.32% (*Pinus sylvestris*, Neuglobsow) to 1.56% (*Salix* sp., Melpitz) (Fig. 4). A comparison of the mean values of the bark N content revealed a significant difference ($P < 0.001$) between both moderate and highly agriculture affected areas on the one hand, and the coastal areas on the other. The forestry dominated areas ranked between these two groups.

3.2. Species- and spatial-specific natural abundance of ^{15}N

Within the taxonomic group, interspecific differences with respect to δ^{15}N were only found at Kleve site within the lichens. *Physcia* spp. showed highly negative δ^{15}N values, followed by *X. parietina* and finally *P. sulcata* which showed less negative δ^{15}N values ($P < 0.05$) (Fig. 3). The most negative δ^{15}N signatures were detected in lichen samples. These δ^{15}N signatures ranged between −15.2‰ (*Physcia* spp., Deuselbach) to −1.5‰ (*P. sulcata*, Raisting) ($n = 326$). The measured δ^{15}N values of the individual lichen species ranged from −12.1‰ to −1.5‰ for *P. sulcata* ($n = 134$) and from −14.2‰ to −2.6‰ for *X. parietina* ($n = 100$). The δ^{15}N signatures of *Physcia* spp. ($n = 92$) varied from −15.2‰ to −3.2‰. There were no significant differences between the lichen species δ^{15}N by comparison of their mean values (averaged over all sites). With the help of the isotopes, we were able to find three groups regarding N deposition on-site. The coastal areas had significantly lower N loads ($P < 0.05$) (less negative δ^{15}N values) than forestry dominated and moderate agriculture affected areas. The latter two groups were also significantly lower than the highly agriculture affected sites (highly negative δ^{15}N values).

The moss δ^{15}N values varied from −10.5‰ (*H. cupressiforme*, Oehringen) to −0.9‰ (*H. cupressiforme*, Kleve) ($n = 153$). Considering the δ^{15}N signatures of the moss species separately, the largest range was found in *H. cupressiforme* (−10.5‰ to −0.9‰; $n = 74$), followed by *P. schreberi* with a range from −8.8‰ to −1.9‰ ($n = 51$) and finally *P. purum* which varied from −8.3‰ to −3.6‰ ($n = 28$) (Fig. 3). The δ^{15}N signatures between the moss species did not differ

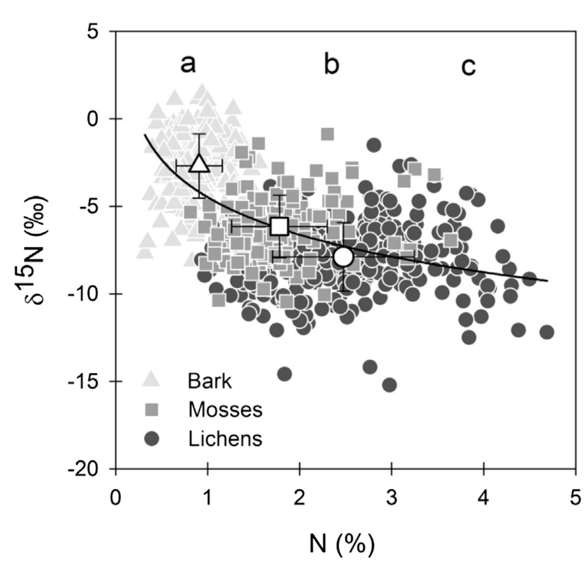

Fig. 4. Total N and δ^{15}N values of all lichen ($n = 326$), moss ($n = 153$) and bark samples ($n = 187$) and the means ± *SD* (white symbols). Different letters indicate significant statistical difference at level $P < 0.001$. Logarithmic regression ($r = 0.56$, $P < 0.0001$) for all data is shown.

from each other. Additionally no differences were detected regarding the N affected site categories and the respective δ^{15}N signatures in mosses.

From all the biomonitors tested, the bark samples showed the weakest negative δ^{15}N signatures. The δ^{15}N values ranged from −8.1‰ (*Betula pendula*, Neuglobsow) to 1.5‰ (*Acer pseudoplatanus*, Lehnmuehle), with an average of −2.7‰ ± 1.9 ($n = 187$) (Fig. 4). No differences were found concerning the various site categories.

3.3. Relationship between tissue N, δ^{15}N patterns and N deposition data

In the assessments below, only sites with precipitation <1000 mm yr^{-1} were taken into account, due to a study which detected limited diagnostic N specific values in mosses relating to wet deposition dominated sites (Pitcairn et al., 2006). The mean N content in lichens per study site showed a highly significant relationship with the reduced and oxidised N compounds in measured wet-only deposition ($r = 0.77$, $P < 0.01$) and with the MAPESI modelled total N deposition ($r = 0.70$, $P < 0.001$). The best positively correlated relationship was with MAPESI modelled ratio of NH_4–N and NO_3–N ($r = 0.86$, $P < 0.001$). Regarding the N content in mosses, a strong correlation was found between N content and modelled MAPESI total N data ($r = 0.71$, $P < 0.01$) and modelled MAPESI NH_4–N/NO_3–N deposition data ($r = 0.71$, $P < 0.05$) (Fig. 5; Fig. 6). A non-significant trend was found with measured on-site wet-only N deposition data ($r = 0.52$, $P = 0.067$) (Fig. 5). Bark samples only showed a positive trend between N content and both reduced N compounds and total N deposition, modelled by MAPESI (Figs. 5 and 6).

No significant correlation was found between δ^{15}N signatures and atmospheric enrichment of agriculture-related reduced N deposition. There was only a non-significant positive tendency between ^{15}N enriched moss data and measured and modelled N deposition data (Figs. 5 and 6). Bark samples show a non-significant negative trend (Fig. 5).

4. Discussion

4.1. Patterns of N concentrations in different biomonitors in comparison to N deposition

Considering the N concentration in tissue of each monitor group separately, this study found elevated levels in comparison to the results of other biomonitoring studies. The mean N of 2.48% ± 0.79 determined in lichen tissue is rather high, and comparable with studies which were conducted in highly urbanised and predominantly agricultural areas (Boltersdorf and Werner, 2013; Franzen-Reuter, 2004) in Germany. N contents measured in *Physcia* spp. and *X. parietina* are comparable to N contents measured close to industrial livestock farming units (Frati et al., 2007; Gaio-Oliveira et al., 2001; Ruoss, 1999), highly traffic impacted regions (Gombert et al., 2003) or even to data from fertiliser experimental studies (Gaio-Oliveira et al., 2005). Regarding the bryophytes, similar concentration ranges were found in the German moss survey 2005 (Pesch et al., 2007), although the present study showed higher average values. Compared to the European moss survey by the UNECE ICP Vegetation Programme 2005/6 in Finland, Sweden and Norway, or to the moss survey 2010/11 in Estonia and Finland (Harmens et al., 2011, 2013), higher N concentrations were found in the present study, though compared to survey results of Poland and France, the present tissue N contents in mosses were rather low (Harmens et al., 2013). Taking into account the results of the Whim Moss field experiment by Skinner et al. (2006), similarly high N concentrations were observed for *H. cupressiforme*, where this

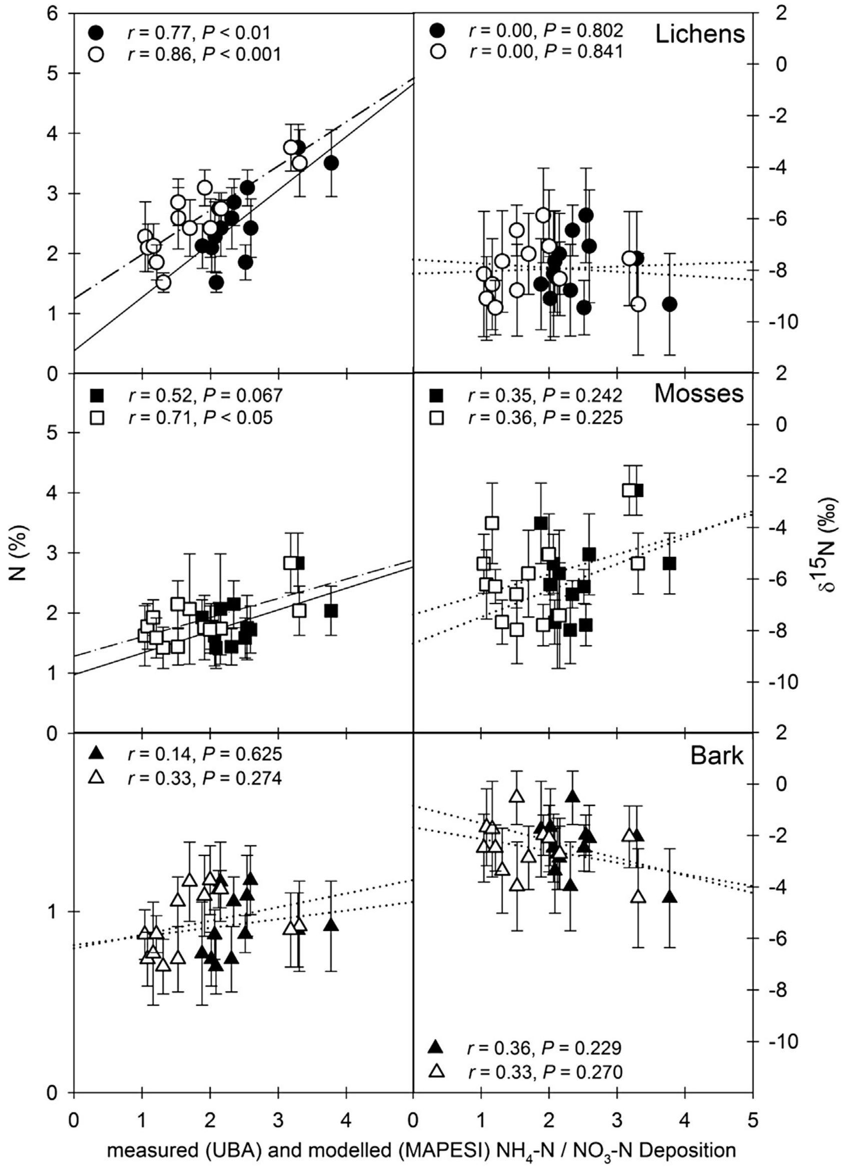

Fig. 5. Total nitrogen concentration and $\delta^{15}N$ values of lichens (circles), mosses (squares) and bark samples (triangles) (Mean ± *SD*) of the study sites ($n = 13$) plotted against measured wet-only deposition ratio of NH_4–N/NO_3–N (UBA) (black symbols and black solid line) and MAPESI modelled total deposition quotient of NH_4–N/NO_3–N (white symbols and dash dot line).

experiment has its highest induced NH_3 concentration. Finally, the N content of bark indicates an N enriched environment at the chosen study sites in Germany in comparison to other studies. The N concentrations in the tree bark in this study are around four times higher than those measured in Scots pine in East and South Germany sampled from 1988 to 1997. These were at the time sampled from anthropogenic N impacted areas (Schulz et al., 2001). A comparison with a Finnish survey of pine bark samples in 1985 (Poikolainen et al., 1998) also shows that the bark N concentrations in Germany seem to be strongly influenced by anthropogenic N loads nowadays. The cited comparisons of tree bark refer all to conifers. The literature regarding deciduous trees with respect to N concentration is rare and this shows the urgent need for research in this context. Thus, we opine that the appraisal of tree bark samples is less substantiated as for lichens and mosses.

Comparing the N concentration in the three studied biomonitors, it can be concluded that they differ significantly from each other ($P < 0.001$) (Fig. 4). In addition to empirical reasons and the low number of chosen sites, this might be due to different N uptake mechanisms. The three biomonitors can be divided into those which can actively absorb N and those which passively take up N (Table 2).

Bark, as a biologically inert surface is primarily exposed to stem deposition, dry deposition and throughfall (Schulz et al., 1999). N compounds from the direct surroundings cannot be accumulated effectively. Due to their physiology and ecology, bryophytes obtain nutrients from precipitation and dry deposition, and only have a low absorption rate from their substrate (Ayres et al., 2006; Harmens et al., 2011; Pitcairn et al., 2006). Based on the lack of specialised structures for water and gas exchange, most moss species and lichens are poikilohydric organisms that absorb water and nutrients by wet and dry deposition. Both mosses and lichens allow free exchange of solutions and gases across their cell surface (Bargagli and Mikhailova, 2002; Hauck, 2010; Turetsky, 2003), therefore have a greater ability to take up N compared to bark.

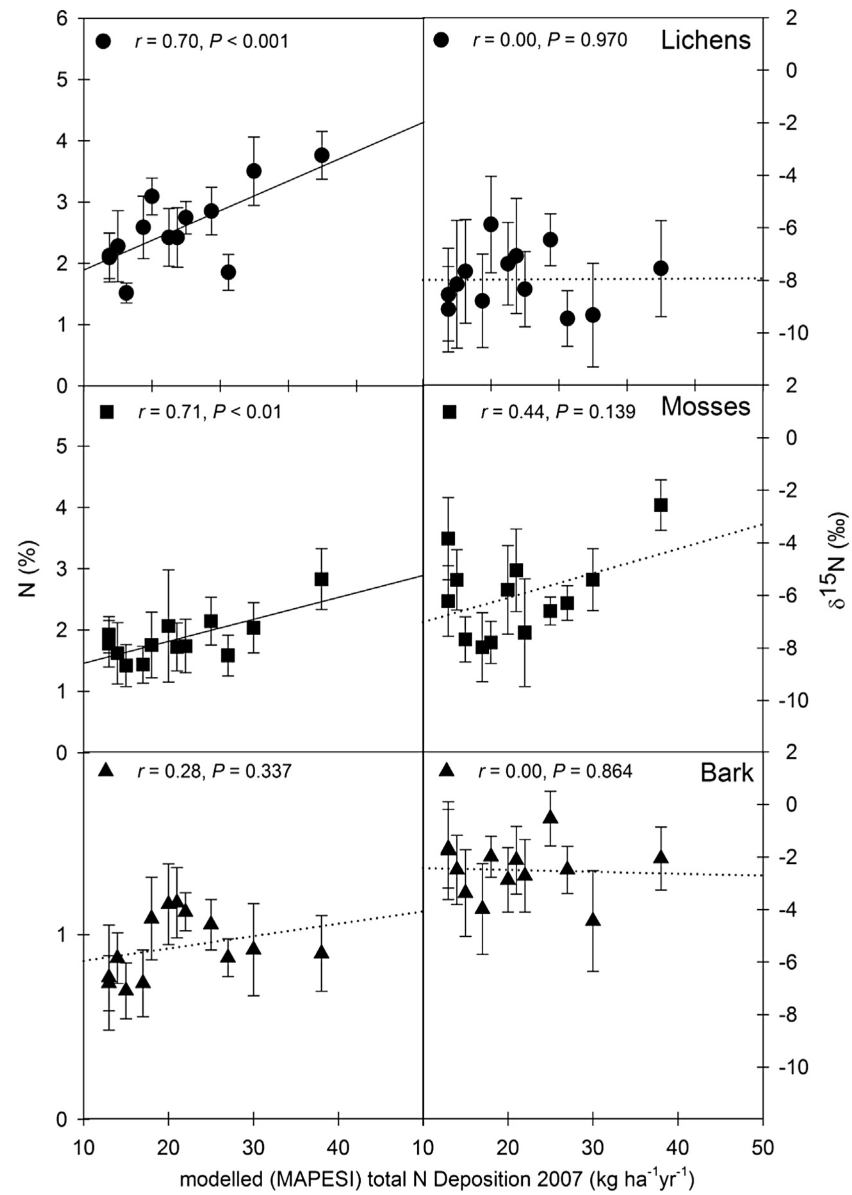

Fig. 6. Total nitrogen concentration and $\delta^{15}N$ values of lichens (circles), mosses (squares) and bark samples (triangles) (Mean ± *SD*) of the study sites ($n = 13$) plotted against modelled MAPESI total N deposition data (relating to semi-natural vegetation as receptor surface) from 2007.

The present study reveals that Germany has a high N load, and that there are site-specific differences regarding the N concentration in the three biomonitors. In this study, especially the average N content in lichens exhibits significant site differences between highly (>2.9%) and moderate agricultural areas, as well as coastal and finally forestry dominated areas (<1.9%) (Fig. 2). The identified areas with highest N contents (Kleve, Dunum, Melpitz and Lehnmuehle) are located in intensely used agricultural areas which are characterized by high densities of livestock units (Statistisches Bundesamt, 2010; Statistische Ämter des Bundes und der Länder, 2011).

Mosses show no significant differences regarding the various categories of agricultural land use and intensity. These findings are in contrast to results obtained by Schröder et al. (2010), where significant Spearman correlation coefficients of above 0.5 could be detected between the N concentration in mosses and the density of agricultural activity. In a following study, the European moss data was therefore successfully applied to map spatial patterns of total N deposition throughout Europe (Schröder et al., 2011). Compared to Harmens et al. (2011), who reported highest N concentrations (≥1.6%) in predominantly agricultural countries like Belgium, France and Germany, almost all sites in the present study showed average N content in mosses above 1.6%. Only the N content at the Waldhof, Neuglobsow and Bavarian Forest site were lower. In a recent study, Kluge et al. (2013) found that canopy drip effects strongly and significantly influence the concentration of N in mosses. At 30 sites in a region of North-western Germany, significant differences were found between mosses sampled in forest stands and in open fields and clearings, ranging from an average of 2.2% within forest stands to 1.1% on open fields. Open fields were thereby defined as areas at least 10 m from the nearest tree crown. Sites 3 m from the nearest tree crown projection, as described in ICP Vegetation (2005) (as applied in this study) still reflected the canopy drip effects in the measured N concentration in the mosses. The results by Kluge et al. (2013) underline that canopy drip effects should be accounted for when investigating spatiotemporal trends of N concentration in mosses.

The regional patterns of N content in bark significantly differentiated potentially less N affected sites (forestry dominated and

Table 2
Qualitative assessment of the characteristics of the three biomonitors – epiphytic lichens (green algal), mosses (on tree stump and ground) and tree bark – relating to their N accumulation in an agriculture affected area.

	Lichen species (On single trees in open fields)	Moss species (Small open areas in stands)	Tree bark (On single trees in open fields)
Surface	Hydrophilic fungal layer of polysaccharides (chitin) and hydrophobic structures which allow gas exchange[a,b]	Hydrophilic layer of polysaccharides (cellulose)	Hydrophilic layer of polysaccharides (cutin: cellulose, pectin, wax)
	No stomata, no cuticle[b,c]	No gametophyte stomata, no cuticle[d]	
	Rhizines[a]	Rhizoids[e]	
N sources	Atmospheric sources[f]	Atmospheric sources + soil pool[g]	Atmospheric sources + soil pool[h]
N deposition type	Wet + dry deposition[f]	Wet + dry deposition[i]	Wet + dry deposition[h]
N uptake	NH_4^+, NH_3>NO_3^-[c,j,k,l]	NH_3>NH_4^+>NO_3^-[m,n,o]	NH_4^+, NH_3>NO_3^-[h]
NH_3, NH_4^+, NO_3^-	Active	Active	Passive

[a] Büdel and Scheidegger (2008).
[b] Purvis (2000).
[c] Tozer et al. (2005).
[d] Turetsky (2003).
[e] Goffinet et al. (2008).
[f] Bargagli and Mikhailova (2002).
[g] Harmens et al. (2011).
[h] Schulz et al. (1999).
[i] Bates (2008).
[j] Dahlman et al. (2004).
[k] Frati et al. (2007).
[l] Boltersdorf and Werner (2013).
[m] Nordin et al. (2006).
[n] Pitcairn et al. (2006).
[o] Skinner et al. (2006).

coastal areas) from highly to moderately agriculturally affected areas. This indicates that bark samples are able to reflect the surrounding N concentration in the atmosphere when they are strongly exposed to agriculture-based dust and corresponding stem and crown deposition of N, which is in line with other studies conducted in Germany (Boltersdorf and Werner, 2013; Schulz et al., 1999, 2001).

The overall picture of measured N contents in the different biomonitors suggests differences in the regional patterns of N deposition without including the measured and modelled deposition data. Using modelled and measured N deposition data and taking into account the low number of compared values ($n = 13$), it was shown that lichens and mosses show significant relationships between increased content in plant tissue and N deposition. Highest correlations in both cases were found with the modelled NH_4–N/NO_3–N values (Fig. 5). Furthermore, the tissue N concentration in lichens and mosses were significantly correlated with modelled total N deposition (Fig. 6). Also other studies document strong relationships between N in lichens and N deposition. Lichens react with high tissue N enrichment particularly in regions with a high proportion of wet deposition (Bruteig, 1993; Franzen-Reuter, 2004; Boltersdorf and Werner, 2013; Remke et al., 2009). Studies from Sweden and the Antarctic have shown that lichens are able to capture NH_4^+ and NO_3^- simultaneously under natural N deposition conditions (Crittenden, 1998; Johansson et al., 2010). Studies have also revealed that NH_4^+ uptake prevailed in lichens that were exposed to high N doses (Dahlman et al., 2004; Ellis et al., 2005; Palmqvist and Dahlman, 2006).

Mosses generally are documented to be efficient in assimilating atmospherically deposited N and are considered well-suited indicators for increased N deposition in agricultural areas. Studies have shown that especially the existence of reduced N compounds in dry deposition stimulate an increase in tissue N accumulation (Bragazza et al., 2005; Raymond et al., 2010; Skinner et al., 2006). This might explain the less clear correlations with the wet-only deposition data (Fig. 5).

4.2. Patterns of $\delta^{15}N$ in biomonitors and the source of N input

With the help of isotope research, it is possible to identify potential atmospheric N sources, to which the biomonitor organisms are exposed (Robinson, 2001). This is well described in the scientific literature with regard to lichens (Boltersdorf and Werner, 2013; Fogel et al., 2008; Lee et al., 2009; Russow et al., 2004; Tozer et al., 2005), mosses (Bragazza et al., 2005; Liu et al., 2008; Solga et al., 2005; Zechmeister et al., 2008) and tree bark (Schulz et al., 2001). As previously mentioned, mosses and lichens are very efficient in taking up atmospheric N, mainly as NH_x (Hauck, 2010; Li and Vitt, 1997; Turetsky, 2003) and therefore they can provide a fairly accurate picture of the surrounding airborne N environment and the prevailing N sources by help of $\delta^{15}N$ signatures (Boltersdorf and Werner, 2013; Lee et al., 2009; Skinner et al., 2006; Zechmeister et al., 2008). In the present study, almost all investigated biomonitors show depletion in ^{15}N, varying from −15.2‰ to 1.5‰. The few positive $\delta^{15}N$ values are all among the bark samples. N pollution dominated by N oxides (NO_y) mostly lead to positive $\delta^{15}N$ values (enrichment in ^{15}N), while pollution based on agriculture which is associated with NH_4^+, yield negative isotopic values (depletion in ^{15}N) (Freyer, 1978; Heaton et al., 1997).

The measured $\delta^{15}N$ values for all three biomonitors therefore describe areas that are exposed to agricultural N sources in Germany (Boltersdorf and Werner, 2013; Solga et al., 2005; Schulz et al., 2001). It is documented that measured $\delta^{15}N$ in plant material next to agricultural NH_3 sources are enriched in ^{15}N and measured $\delta^{15}N$ values downwind from a source exhibit less ^{15}N (Erskine et al., 1998; Lee et al., 2009; Skinner et al., 2004, 2006). A similar pattern in the isotope values was investigated by comparing urban N emissions (mostly N oxides), leading to positive $\delta^{15}N$

values in mosses to agriculture emissions resulting in negative isotopic values in the plant tissue (Gerdol et al., 2002; Pearson et al., 2000). Pearson et al. (2000) identified differences between the $\delta^{15}N$ values in mosses collected in urban areas (average of +3.7‰) and rural areas (average of −7.8‰).

Other studies show that epiphytic plants, which are not in contact with the soil and which are further up the canopy or stem have more depleted isotopic signals compared to plants in herb and moss layer (Hietz et al., 2002; Wania et al., 2002). Moreover, the surrounding environment can be added as a decisive factor for specific isotope patterns. Mosses can exhibit higher $\delta^{15}N$ values especially at open and dryer sites, and furthermore particularly under thick canopies (Liu et al., 2007). Since the investigated moss samples were collected preferably in small open areas in forest stands, the influence of canopy drip could not be completely excluded so this might also explain the higher enrichment of ^{15}N in mosses compared to the lichens. Furthermore, lichens growing on stems and branches have a higher incoming flow of the N compounds locally present, and are able to represent deposition conditions more accurately than mosses grown within the stand, where they are often covered by other vegetation that intercepts N.

The large isotopic ranges determined, of up to 14‰ within lichens and nearly 10‰ within the mosses and bark samples, suggest that these biomonitors are highly sensitive to different types of atmospheric N pollution. The wide ranges in N stable isotope values indicate the spatially very variable sensitivity of N related monitor organisms (Lee et al., 2009; Skinner et al., 2006; Stewart et al., 2002).

When comparing $\delta^{15}N$ values of the sites, lichens are clearly able to distinguish agriculturally affected areas from background areas ($P < 0.05$) (Schmuecke, Schauinsland and Bavarian Forest). Especially the sites which are located in the western part of Germany (Deuselbach, Solling, Waldhof, Dunum and Kleve) are characterized by strong negative $\delta^{15}N$ values in the nitrophytic lichens *X. parietina* and *Physcia* spp. (Fig. 3). This is probably due to either high densities of local industrial farming activities, and/or the influence of agriculture from neighbouring countries (The Netherlands, France and Belgium) through long-range transport of N pollution caused by prevailing westerlies.

Nevertheless, no relationships were found between the investigated tissue $\delta^{15}N$ and measured and modelled N deposition data. Only a slight trend was found with regard to moss and bark samples. The moss samples show heterogeneous patterns relating to the various N characterized areas and they differ from lichen and bark relating to $\delta^{15}N$ values (Fig. 5). Other studies document better correlations between moss N chemistry and dry deposited NH_x compounds (Bragazza et al., 2005; Pitcairn et al., 2006). For example, Schröder et al. (2010) used decision tree models to show that tissue N contents in mosses were best explained by NH_4^+ and NO_2 concentrations in air, sampled moss species and total dry N deposition at the European scale.

5. Conclusions

This is the first nationwide survey of Germany comparing lichens, mosses and tree bark samples as biomonitors for N deposition. The detected N concentration ranges of all investigated biomonitors reflect the high anthropogenic dimension of N pollution in Germany. The tissue N contents of all biomonitors identify the N deposition originating from intensive agriculture on terrestrial ecosystems and indicate the degree of N deposition in Germany. Sites documented to have a high agricultural influence could be clearly distinguished from less agriculture-affected regions with the help of lichens. The study also shows that dealing with different biomonitors is a difficult task due to their variety of N responses. The specific receptor surfaces of the indicators and therefore their different strategies of N uptake are responsible for the particular tissue N concentration of each organism group.

The isotopic signatures could be potentially used as a complementary instrument to assist in interpretations of the prevailing N source on-site, especially in combination with data of the N content in plants or N deposition data. This study has shown that the $\delta^{15}N$ values depended on its N origin and the specific N transformations in each organism system, so that a direct comparison between atmosphere and ecosystems is not possible. Nevertheless, the $\delta^{15}N$ values in the biological monitors were able to detect that it is the high agriculture-related reduced N deposition which is responsible for the increased N load at the investigated deposition sites in Germany.

All considered biomonitors represent a low cost alternative to the expensive and sporadically installed stations for deposition measurements in Germany. Particularly considering the small number of available N deposition stations that monitor the German area of ca. 35,7100 km^2 (Statistische Ämter des Bundes und der Länder, 2013), the additional use of biomonitors should be considered.

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