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



<sup>a</sup> Department of Geobotany, University of Trier, Behringstr. 21, D-54296 Trier, Germany <sup>b</sup> Chair of Landscape Ecology, University of Vechta, PO Box 1553, D-49356 Vechta, 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.

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## 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](#page--1-0)). 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 (NH3), have increased mainly due to animal farming [\(Erisman et al., 2008;](#page--1-0) Krupa,  $2003$ ). NH<sub>3</sub> is highly reactive, and preferentially deposited as dry deposition close to the emitted source, whilst its reaction product ammonium (NH $_4^{\scriptscriptstyle\pm}$ ) is principally washed out by precipitation in terms of wet deposition. These two compounds, collectively referred to as  $NH<sub>x</sub>$ , are major contributors to total N deposition ([Asman et al., 1998; Krupa, 2003\)](#page--1-0) and can have an effect on vegetation in high doses [\(Bobbink et al., 2010; Sheppard et al., 2011\)](#page--1-0). 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  $NH<sub>x</sub>$ ([Bobbink et al., 2010; Sheppard et al., 2011; Skinner et al., 2006\)](#page--1-0). Furthermore, [Cape et al. \(2009\)](#page--1-0) defined a lower NH<sub>3</sub> Critical Level

\* Corresponding author.

(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 [\(Hauck, 2010; Turetsky,](#page--1-0) [2003\)](#page--1-0). 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](#page--1-0)). Lichens ([Bruteig, 1993; Frati et al., 2007; Gaio-Oliveira et al., 2001; Gombert](#page--1-0) [et al., 2003; Raymond et al., 2010; Remke et al., 2009; Søchting,](#page--1-0) [1995\)](#page--1-0) and mosses [\(Harmens et al., 2011; Leith et al., 2005; Pesch](#page--1-0) [et al., 2007; Pitcairn et al., 2006](#page--1-0)) have frequently been used in local, national and European wide studies. In addition to this, tree bark offers another resource for the assessment of atmospheric N depositions ([Mitchell et al., 2005; Poikolainen et al., 1998\)](#page--1-0).

To identify underlying atmospheric N sources,  $\delta^{15}$ N signatures of atmospheric N compounds are used [\(Freyer, 1978; Heaton et al.,](#page--1-0) [1997\)](#page--1-0). 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\)](#page--1-0). It is generally accepted that the determined  $\delta^{15}N$  signatures in lichen [\(Boltersdorf and Werner,](#page--1-0) [2013; Fogel et al., 2008; Lee et al., 2009; Russow et al., 2004;](#page--1-0) [Tozer et al., 2005\)](#page--1-0) and in moss tissue ([Bragazza et al., 2005; Liu](#page--1-0) [et al., 2008; Solga et al., 2005; Zechmeister et al., 2008\)](#page--1-0) are able to reflect predominating N isotope sources in the environment. In the context of bark monitoring, the determination of the abun-dance of <sup>15</sup>N has also been applied successfully ([Schulz et al., 2001\)](#page--1-0).

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





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E-mail addresses: [stefanie.boltersdorf@gmx.de](mailto:stefanie.boltersdorf@gmx.de) (S.H. Boltersdorf), [rpesch@iuw.](mailto:rpesch@iuw.uni-vechta.de) [uni-vechta.de](mailto:rpesch@iuw.uni-vechta.de) (R. Pesch), [werner@uni-trier.de](mailto:werner@uni-trier.de) (W. Werner).

picture of the real extent of the prevailing N deposition status over large areas ([Sutton et al., 1998](#page--1-0)). 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 e UBA; [Ihle et al., 2001\)](#page--1-0) (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<sub>3</sub>-N), ammonium – ni-trogen (NH<sub>4</sub>-N) and mean annual precipitation ([UBA, 2004\)](#page--1-0). Deposition data from the years 2006-2007 were considered in order to have reliable data for the majority of the sites. The averaged NH<sub>4</sub> $-$ N deposition thereby ranges from 1.50 kg ha<sup>-1</sup> yr<sup>-1</sup> (Regnitzlosau) to 6.38 kg ha $^{-1}$  yr $^{-1}$  (Schmuecke). The respective NO3–N deposition<br>varies between 0.70 kg ha $^{-1}$  yr $^{-1}$  (Regnitzlosau) and 3.12 kg ha $^{-1}$  yr $^{-1}$  at the Schmuecke site ([Fig. 2](#page--1-0)).

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](#page--1-0)).

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  $1 \times 1$  km<sup>2</sup> 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\)](#page--1-0). The mean (2006-2007), grid cell based, modelled NH<sub>4</sub>-N deposition ranges from 6.50 kg ha<sup>-1</sup> yr<sup>-1</sup> (Zingst) to 35 kg ha<sup>-1</sup> yr<sup>-1</sup> at Kleve site. The NO<sub>3</sub>-N deposition varies between 6.00 kg ha<sup>-1</sup> yr<sup>-1</sup> (Melpitz, Sylt and Zingst) and 16.50 kg ha<sup>-1</sup> yr<sup>-1</sup> (Solling) ([Fig. 2](#page--1-0)). 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<sup>-1</sup> yr<sup>-1</sup> (Zingst) to 38 kg ha<sup>-1</sup> yr<sup>-1</sup> (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.](#page--1-0)

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](#page--1-0)) 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](#page--1-0)). 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 [\(Table 1\)](#page--1-0) 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](#page--1-0)). 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).

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