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A critical appraisal of accumulative biomonitors  
to assess and to map sources and rates  
of atmospheric nitrogen deposition  
on different regional scales  
in Germany



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‘The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.’

William Lawrence Bragg [1890-1971]

# Summary

Mankind has dramatically influenced the nitrogen (N) fluxes between soil, vegetation, water and atmosphere – the global N cycle. Increasing intensification of agricultural land use, caused by the growing demand for agricultural products, has had major impacts on ecosystems worldwide. The predicted increase in human consumption is expected to exacerbate these problems, underlining the need for monitoring and control of N emissions. Particularly nitrogenous gases such as ammonia (NH<sub>3</sub>) have increased mainly due to industrial livestock farming. NH<sub>3</sub> is highly reactive, and mainly deposited as ‘dry deposition’ close to the source of emission, whilst its reaction product ammonium (NH<sub>4</sub><sup>+</sup>) is principally washed out by precipitation in ‘wet deposition’. These two compounds, collectively referred to as NH<sub>x</sub>, are the major contributors to total N deposition.

Countries with high N deposition rates require a variety of deposition measurements and effective N monitoring networks to assess N loads. Due to high costs, current ‘conventional’-deposition measurement stations are not widespread and therefore provide only a patchy picture of the real extent of the prevailing N deposition status over large areas. Coupled with many uncertainties in the whole chain of processes – emissions, dispersion, chemistry and the different methods of data handling – the assessment of deposition on-site is still a major challenge. Although global and regional scale models of N deposition exist, they cannot reflect small-scale variations in deposition rates.

One tool that allows quantification of the exposure and the effects of atmospheric N impacts on an ecosystem is the use of bioindicators. Due to their specific physiology and ecology, especially lichens and mosses are suitable to reflect the atmospheric N input at ecosystem level.

The present doctoral project began by investigating the general ability of epiphytic lichens to qualify and quantify N deposition by analysing both lichens and total N and δ<sup>15</sup>N along a gradient of different N emission sources and severity. The results showed that this was a viable monitoring method, and a grid-based monitoring system with nitrophytic lichens was set up in the western part of Germany. Finally, a critical appraisal of three different monitoring techniques (lichens, mosses and tree bark) was carried out to compare them with national relevant N deposition assessment programmes. In total 1057 lichen samples, 348 tree bark samples, 153 moss samples and 24 deposition water samples, were analysed in this dissertation at different investigation scales in Germany.

The study identified species-specific ability and tolerance of various epiphytic lichens to accumulate N. Samples of tree bark were also collected and N accumulation ability was detected in connection with the increased intensity of agriculture, and according to the presence of reduced N compounds (NH<sub>x</sub>) in the atmosphere. Nitrophytic lichens (*Xanthoria parietina*, *Physcia* spp.) have the strongest correlations with high

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agriculture-related N deposition. In addition, the main N sources were revealed with the help of  $\delta^{15}\text{N}$  values along a gradient of altitude and areas affected by different types of land use ( $\text{NH}_3$  density classes, livestock units and various deposition types). Furthermore, in the first nationwide survey of Germany to compare lichens, mosses and tree bark samples as biomonitors for N deposition, it was revealed that lichens are clearly the most meaningful monitor organisms in highly N affected regions. Additionally, the study 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 tissue N concentration of each organism group. It was also shown that the  $\delta^{15}\text{N}$  values depend on their N origin and the specific N transformations in each organism system, so that a direct comparison between atmosphere and ecosystems is not possible.

In conclusion, biomonitors, and especially epiphytic lichens may serve as possible alternatives to get a spatially representative picture of the N deposition conditions. Furthermore, bioindication with lichens is a cost-efficient alternative to physico-chemical measurements to comprehensively assess different prevailing N doses and sources of N pools on a regional scale. They can at least support on-site deposition instruments by qualification and quantification of N deposition. The ease of applicability of a 'can be used anyway' monitoring method with lichens is advantageous in heterogeneous areas with diverse local wind conditions and topographic variations.

# Zusammenfassung

Die Menschheit beeinflusst aktiv und nachdrücklich die Stickstoff (N) -flüsse zwischen Boden, Vegetation, Wasser und Atmosphäre - den globalen N-Kreislauf. Die zunehmende Intensivierung der landwirtschaftlichen Flächen, verursacht aufgrund der wachsenden Nachfrage nach Agrarprodukten, hat große negative Auswirkungen auf die Ökosysteme weltweit. Aufgrund der zukünftig vorhergesagten Konsumsteigerung der Bevölkerung wird erwartet, dass diese Probleme verschärft werden, so dass eine Überwachung und Kontrolle der N-Emissionen unabdingbar ist und von hoher Priorität. Besonders stickstoffhaltige Gase wie Ammoniak ( $\text{NH}_3$ ) liegen aufgrund expandierter Massentierhaltung deutlich erhöht vor.  $\text{NH}_3$  ist hoch reaktiv und wird vorzugsweise über ‚trockene Deposition‘ in der Nähe der Emissionsquelle abgelagert während sein Reaktionsprodukt Ammonium ( $\text{NH}_4^+$ ) hauptsächlich durch Niederschlag in Form von ‚nasser Deposition‘ fern von der eigentlichen Emissionsquelle abgelagert wird. Diese beiden N-Verbindungen, die gemeinsam als  $\text{NH}_x$  bezeichnet werden, tragen hauptsächlich zu erhöhten Gesamt-N-Depositionen bei. Länder mit hohen N-Depositionsraten erfordern eine Vielzahl von Depositionsmessungen und effektive N-Depositionsmessnetze, um vorliegende N-Belastungen beurteilen zu können. Angesichts der hohen Kosten, sind die derzeitigen ‚konventionellen‘ Depositionsmessstationen nicht weit verbreitet und spiegeln daher nur ein unvollständiges Bild des realen Ausmaßes der vorherrschenden N-Depositionen über große Flächen wider. Gekoppelt mit vielen Unsicherheiten in der gesamten Prozesskette - Emission, Dispersion, chemische Analyse und Datenbearbeitung -, ist die Beurteilung von Depositionen vor Ort derzeit noch eine große Herausforderung. Obgleich auch globale und regionale Modelle für N-depositionen existieren, erfassen diese jedoch keine kleinräumigen Schwankungen von Depositionsraten.

Eine Methode, die eine Quantifizierung der Aussetzung und zugleich der Auswirkung des atmosphärischen N-Einflusses in Ökosystemen zulässt, ist der Einsatz von Bioindikatoren. Aufgrund ihrer besonderen Physiologie und Ökologie eignen sich Flechten und Moose besonders gut um den atmosphärischen Stickstoffeintrag auf Ökosystemebene anzuzeigen.

Im vorliegenden Promotionsprojekt wurde zunächst untersucht, ob es generell möglich ist mit epiphytisch wachsenden Flechten und in ihnen ermittelten N-Gesamtgehalten und  $\delta^{15}\text{N}$  Signaturen im Gewebe, N-Depositionen entlang eines Gradienten von verschiedenen N-Emissionsquellen zu quantifizieren und zu qualifizieren. Mit Hilfe der gewonnenen Ergebnisse konnte eine Monitoringmethode entwickelt werden, die in einem geeigneten flächenbasierten Raster und mit Hilfe von nitrophytischen Flechten in Deutschland umgesetzt wurde. Schließlich wurden drei verschiedene Monitoringsysteme (Flechten, Moose und Borke), unter Berücksichtigung von national relevanten N-Deposition-Assessment-Daten, angewendet und ihre Beurteilungskraft kritisch hinterfragt. Insgesamt wurden in der vorliegenden Dissertation 1057 Flechtenproben, 348 Borkenproben, 153 Moosproben und 24 Depositionswasserproben in unterschiedlich räumlich angeordneten Untersuchungsansätzen in Deutschland, berücksichtigt.

Innerhalb der Studie konnte an unterschiedlichen epiphytischen Flechten art-spezifische Akkumulationsfähigkeit und Toleranz gegenüber N festgestellt werden. Auch mit Hilfe von N-Gehalten in Borkenproben konnte ein Zusammenhang zwischen intensiver landwirtschaftlichen Nutzung und den daraus resultierende N-Verbindungen in der Atmosphäre dargestellt werden. Die nitrophilen Flechtenarten (*Xanthoria parietina*, *Physcia* spp.) spiegeln, die durch intensive

Landwirtschaft verursachten erhöhten N-Depositionen, am besten wider. Darüber hinaus konnten die vorherrschenden N-Quellen mit Hilfe der  $\delta^{15}\text{N}$ -Werte entlang eines Höhengradienten und durch verschiedene Landnutzungen gekennzeichnete Gebiete ( $\text{NH}_3$ -Emissionsdichten, Großvieheinheiten und verschiedene Depositionstypen) ermittelt werden. Ein Vergleich mit modellbasierten N-Depositionskarten, konnte belegen, dass Flechten in der Lage sind ein ähnliches räumliches N-Depositionsmuster auf regionaler Ebene abzubilden.

Zudem konnte der erste bundesweite Vergleich von drei unterschiedlichen Monitoringverfahren (Flechten-, Moos- u. Borkenmonitoring) in Deutschland zeigen, dass Flechten sowohl in stark als auch in gering belasteten Gebieten die aussagekräftigsten Monitororganismen bezüglich einer Beurteilung von N Deposition sind. Darüber hinaus zeigt die Studie, dass der Umgang mit verschiedenen Bioindikatoren aufgrund der unterschiedlichen N-Aufnahmestrategien keine leichte Aufgabe ist. Die spezifischen Rezeptor-Oberflächen der Bioindikatoren sind für den Gesamt-N-Gehalt der jeweiligen Organismusgruppe verantwortlich. Es hat sich auch gezeigt, dass die  $\delta^{15}\text{N}$ -Werte zum einen stark von der N Quelle und zum anderen von der spezifischen N-Umsetzung in jedem Organismussystem abhängig ist, so dass ein direkter Vergleich zwischen der Atmosphäre und Ökosystemen nicht möglich ist.

Die vorliegende Arbeit zeigt, dass vor allem epiphytische Flechten eine Alternative zu herkömmlichen Monitoring-Stationen darstellen, um N-Depositionsbedingungen weiträumlich abzubilden. Zudem stellt ein Monitoringverfahren mit Flechten eine kostengünstige Alternative dar bezüglich Beurteilung und Einschätzung verschieden vorliegender N-Dosen und N-Quellen im regionalen Maßstab, im Vergleich zu ‚konventionellen‘-Depositionsmessanlagen. Sie können Depositionsmessanlagen vor Ort zusätzlich unterstützen, durch Qualifizierung und Quantifizierung der jeweiligen N-Deposition. Die einfache Anwendbarkeit bzw. Implementierung einer ‚can be used anyway‘- Monitoringmethode mit Flechten ist gerade in sehr heterogenen Landschaften mit diversen lokalen Windverhältnisse und topographischen Variationen erfolgversprechend.

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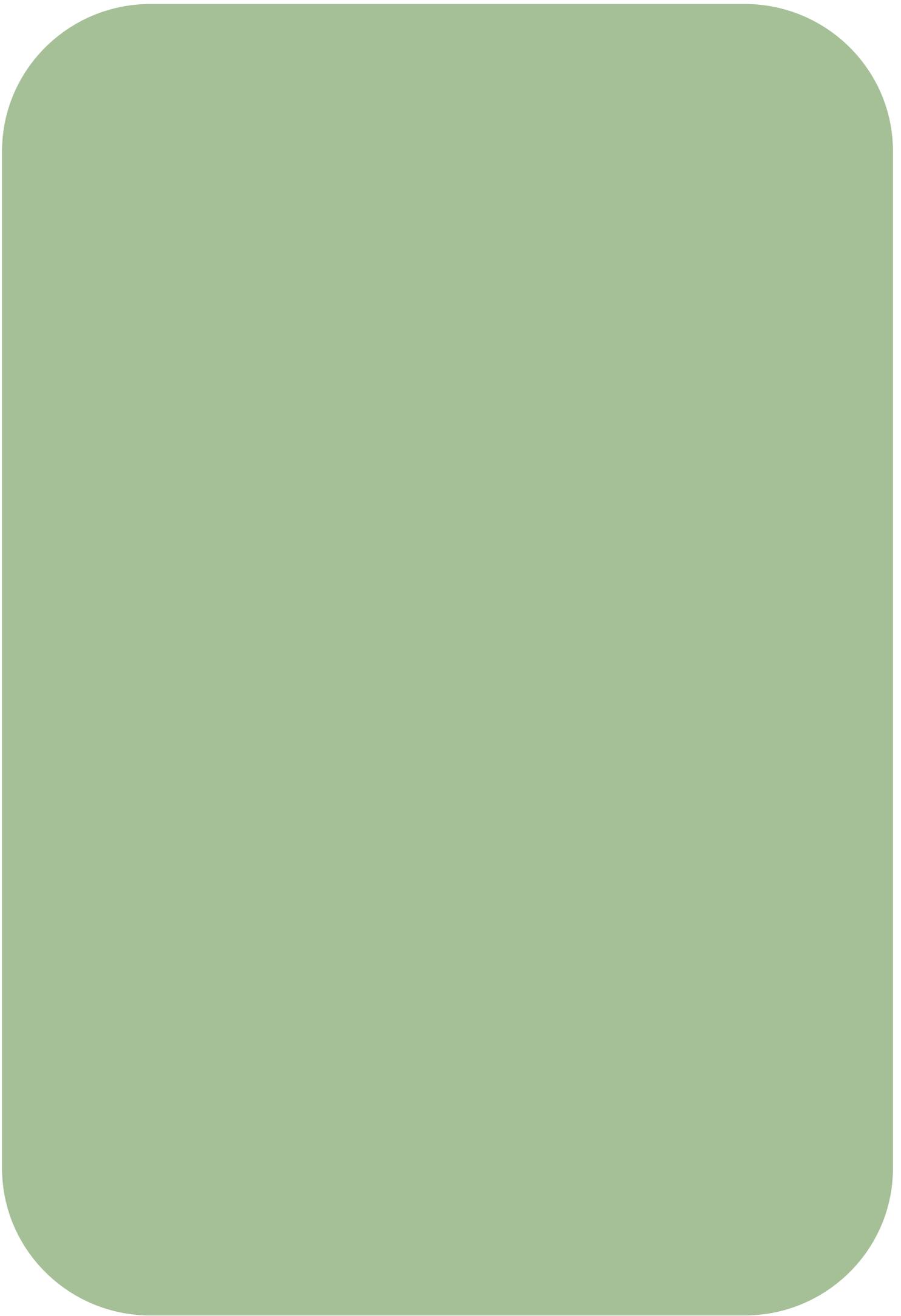
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# Chapter **1**

General introduction

S H Boltersdorf

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## 1. Why Nitrogen? The nitrogen problem from a global and national perspective

### 1.1 Fundamental facts - 'All we are is a lot of talking nitrogen' Arthur Miller [1915-2005]

Nitrogen (N) is one of the essential building blocks of life on earth. All living things need N as a reactive element to make proteins and for the genetic material deoxyribonucleic acid (DNA). N is involved in many key metabolic processes of growth and energy transfer. Also, the green pigment chlorophyll, which is needed for the essential and self-sustaining photosynthesis, contains N (Evans, 1989). N can be found in air, water and soil; in plants, animals and humans. The air we breathe consists mostly of N. The total amount of N on our planet remains constant. Only the chemical forms, in which the element occurs, are different and essential for the distribution of N between the different environmental media (water, air and soil) and the effects on all living creatures and the environment.

Around 98% of the world's N is located in the lithosphere. The remaining content is integrated in a global dynamic cycle including the atmosphere, oceans, lakes, streams and all living creatures (Nieder & Benbi, 2008). The atmosphere contains the largest N pool ( $3.87 \times 10^{21}$  Tg (Schlesinger & Bernhardt, 2013)), mainly in molecular form ( $N_2$ ) which comprises 78% of all atmospheric gases (Nieder & Benbi, 2008).  $N_2$  is not very reactive and cannot be used directly by most organisms. The so-called 'reactive N ( $N_r$ )' behaves quite differently and is able to form bonds with a variety of organic and inorganic compounds and is able to convert from one specific N form into another (UBA, 2011a).  $N_r$  is defined as all other N forms in the environment apart from  $N_2$  and includes primarily the oxidized forms: nitrogen monoxide (NO), nitrogen dioxide ( $NO_2$ ), nitrate ( $NO_3^-$ ), the reduced forms: ammonium ( $NH_4^+$ ) and ammonia ( $NH_3$ ) and finally organic N forms (e.g. proteins and enzymes); all with different oxidation states and properties (Butterbach-Bahl et al., 2011a; Erisman et al., 2011).

N occurred in two stable isotopes, the lighter  $^{14}N$  and the heavier  $^{15}N$ . The proportion of the two stable isotopes compared to a standard (expressed as  $\delta^{15}N$ ) varies according to the source of fixed N (Dawson et al., 2002; Dawson & Siegwolf, 2007; Robinson, 2001). Positive  $\delta^{15}N$  values indicate  $^{15}N$  enrichment while a negative value indicates  $^{15}N$  depletion (Robinson, 2001).

Very few processes in nature are able to convert atmospheric  $N_2$  into reactive N forms. For example,  $N_2$  can be oxidized to N oxides ( $NO_x$ ) by lightning strikes, or specific bacteria (rhizobia) are able to bind  $N_2$  (biological N fixation) (Socolow, 1999; Galloway & Cowling, 2002) making it available for plants. Additionally, volcanoes, biomass burning (Sutton et al., 2008) and the weathering of rocks (Hollaway & Dahlgren, 2002), are limited natural  $N_r$  sources. Even though bacteria and volcanoes are able to provide reduced  $N_r$  forms, it is mainly the oxidized N forms that are emitted by abiotic natural sources (Galloway et al., 2003; Schlesinger, 2009).

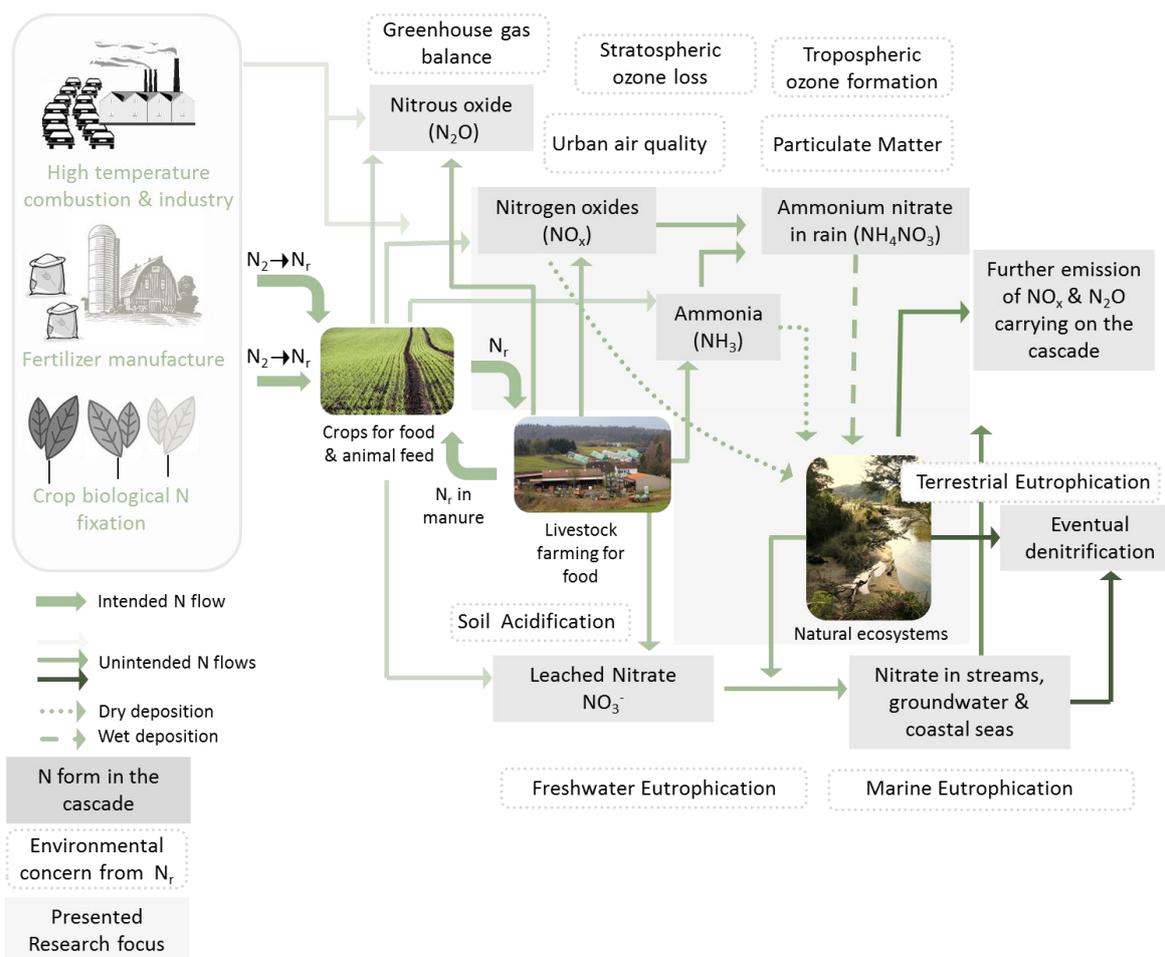
Man has greatly increased the rate of conversion of elemental N into reactive compounds. This conversion is primarily connected – under high energy expenditure – to the production of fertilizers. However, the burning of fossil and renewable fuels is also responsible for the emission of  $N_r$ , in this case mainly into the atmosphere. In these combustion processes not only the stored N in the fuel reaches the atmosphere, but also airborne elemental N is converted, mainly into N oxides. Biological  $N_2$  fixation was an important input pathway of  $N_r$  creation in terrestrial ecosystems before anthropogenic activities severely altered these systems (Cleveland et al., 1999). The cultivation of leguminous plants which fix  $N_2$  through their root symbiosis with the bacterium *Rhizobium*, was the only source of  $N_2$  fixation for humans in preindustrial times. In this period, the total biological  $N_2$  fixation due to agriculture was estimated to be around 15 Tg  $N\ yr^{-1}$  (Smil, 1999). One century later, the estimated agricultural  $N_2$  input is now around 40 Tg  $N\ yr^{-1}$  (Galloway et al., 1995).

In the 19<sup>th</sup> century, natural biomass production and agricultural income were limited by the availability of N in the soil. This means of all the elements which were necessary for plant growth, measured by demand, N was least available and limited the net primary production in many ecosystems (De Vries et al., 2006; Vitousek & Howarth, 1991).

Overall, the worldwide release of  $N_r$  has multiplied tenfold since the mid-19<sup>th</sup> century (Galloway & Cowling, 2002; Galloway et al., 2003). The massive  $N_r$  release began with the onset of industrialization about 150 years ago, through the increasing use of fossil fuels in industry and transport.

Especially the production of ammonia ( $NH_3$ ) in large amounts for synthetic N fertilizer led to an enhanced release of  $N_r$  compounds through the development of the Haber-Bosch process in 1910 (Erismann et al., 2008). The introduction of the Haber-Bosch process and its rapid spread was an important step in the development of the industrial agricultural production that increasingly characterizes the land management in Europe, North America and other parts of the world, especially since the late 1960s. The Haber-Bosch process annually converts about 80 million tons of atmospheric  $N_2$  to  $N_r$  (Rockström et al., 2009).

Local, regional and global N cycling has changed dramatically through the intensification of agriculture. Human land use and land management have altered the biosphere-hydrosphere-atmosphere exchange. Physical and chemical processes in soils, waters and the atmosphere modify the N cycle, which in turn have effects on all living things, including humans. An overview of examples of losses, transformation and effects of  $N_r$  fertilizers in the environment, is given in the ‘N cascade’ of Galloway et al. (2003) (Fig. 1.1).



**Figure 1.1** Illustration of the N cascade showing movements of human-produced  $N_r$  through various environmental media – atmosphere, terrestrial and aquatic ecosystem – and main atmospheric pathways of  $N_r$  of the Earth (modified after Sutton et al., 2011; Source: Galloway et al., 2003; Hertel et al., 2006).

Once emitted different N compounds enter the atmosphere, where they are affected by transmission, before returning back to the ecosystems via various deposition pathways. The present research pays special attention to the entry pathway of N (Fig. 1.1).

The  $N_r$  input via atmospheric deposition occurs through ‘wet’ or ‘dry’ deposition (Asman et al., 1998; Hertel et al., 2006; Krupa, 2003; Loubet et al., 2009).  $N_r$  compounds mainly enter ecosystems indirectly through precipitation or in the form of cloud droplets (wet deposition). The two wet deposition pathways that remove gases and aerosols from the

atmosphere are termed below-cloud and in-cloud scavenging. In-cloud processes are mainly characterized by incorporation of aerosols into cloud droplets within clouds – ('rainout'). Below-cloud processes are known as 'washout', where aerosols are removed through interception by e.g. rain events (Hertel et al., 2006; 2011; Krupa, 2003; Reay et al., 2008). N in aerosols mainly occurs as  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Hertel et al., 2006; 2011; Van der Erden et al., 1998) with a size range of 0.1 – 1.0  $\mu\text{m}$  (diameter) (Hertel et al., 2011). The wet removal pathway of gases depends on solubility and reactivity. Highly soluble  $\text{NH}_3$  is removed effectively from the air by clouds and rain (Hertel et al., 2011). In reactions with acid gases and aerosols,  $\text{NH}_3$  can be transformed into wet deposited  $\text{NH}_4^+$  bound in aerosols. In general these compounds have a long lifetime in the atmosphere and therefore play an important role in long-range transport of pollutants (<1000 km) (Hertel et al., 2006; 2011; Sutton et al. 1998; Van den Erden et al., 2001).

In contrast to the wet deposition process, dry deposition is a direct deposition of gases and aerosols on different receptor surfaces (e.g. terrestrial or marine surfaces) (Hertel et al., 2011). This continuous process is determined by air concentrations, turbulent transport processes in the boundary layer, chemical and physical traits of the depositing species and the capability of the receptor surface to capture or absorb the gases and particles (Hertel et al., 2011). The drifting range of gaseous compounds is usually less than 100 km, although components dissolved in clouds can be drifted up to 1000 km or more. (Van den Erden et al., 2001).

Ammonia mainly originates from agricultural hot-spots (intensive area or point source) and may be recaptured and dry deposited locally by the surrounding vegetation (Asman et al., 1998; Hertel et al., 2011; Krupa, 2003; Loubet et al., 2009; Skinner et al., 2006). Regions with lower levels of agriculture can be affected by additional N loads through substantial long range transport via  $\text{NH}_4^+$  bound in aerosols. Thus, dry deposition of  $\text{NH}_3$  is most important close to the source of emission, whilst wet deposition of the  $\text{NH}_3$  reaction product  $\text{NH}_4^+$  is most important some distance downwind from the source of emission (Asman et al., 1998). The existence of hot spot sources and effective deposition processes leads to  $\text{NH}_x$  (gas phase ( $\text{NH}_3$ ) and aerosol phase ammonia ( $\text{NH}_4^+$ )) sources and sinks that are spatially very heterogeneous at a scale of one square kilometre (Sutton et al., 1998; Dragosits et al., 2002). Gaseous  $\text{N}_r$  compounds which are generally dry deposited, are  $\text{NH}_3$  of agricultural origin and  $\text{HNO}_3$  and  $\text{NO}_2$ , originating from traffic or industry (Hertel et al., 2006; 2011).

The N compounds  $\text{NH}_3$ ,  $\text{NO}_x$  and  $\text{N}_2\text{O}$ , as well as smaller contributions from organic compounds (e.g. amines), are the  $\text{N}_r$  key parts from the atmosphere (Hertel et al., 2011). In the following section, the focus is particularly on agriculture-related  $\text{NH}_x$  deposition and to a lesser extent on  $\text{NO}_y$  ( $\text{NO}_x$  and reaction products), which is the major source of

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atmospheric N pollution in industrial and congested areas.  $\text{NO}_y$  compounds are mostly generated under high temperatures in combustion processes mainly through the oxidation of free atmospheric N (Hertel et al., 2011).

Given that nitrogen occurs in different stable isotopes, a higher concentration of  $\text{NO}_y$  compounds in the atmosphere leads to more positive  $\delta^{15}\text{N}$  signatures, whilst more  $\text{NH}_x$  compounds leads to more negative  $\delta^{15}\text{N}$  signatures. Thus, the analysis of  $\delta^{15}\text{N}$  values in different media can potentially provide on-site information about the source and of course of the prevailing N deposition type (Freyer, 1978; Heaton et al., 1997, Kendall et al., 2007).

## 1.2 Global facts

The intensification of agricultural production and industrial combustion has led to an increased accumulation of  $\text{N}_r$  compounds in all environmental media, and has therefore been influencing the Earth's climate and ecosystem for the past 150 years (Erisman et al., 2007; 2011; Rockström et al., 2009). Anthropogenic activities nowadays convert more  $\text{N}_2$  from the atmosphere into  $\text{N}_r$  than all terrestrial processes on Earth combined (Rockström et al., 2009). Four processes are primarily responsible for this conversion: industrial fixation of atmospheric  $\text{N}_2$  to  $\text{NH}_3$  ( $\sim 80 \text{ Mt N yr}^{-1}$ ), agricultural fixation of atmospheric  $\text{N}_2$  based on cultivation of leguminous crops ( $\sim 40 \text{ Mt N yr}^{-1}$ ), fossil-fuel combustion ( $\sim 20 \text{ Mt N yr}^{-1}$ ) and biomass burning ( $\sim 10 \text{ Mt N yr}^{-1}$ ) (Rockström et al., 2009).

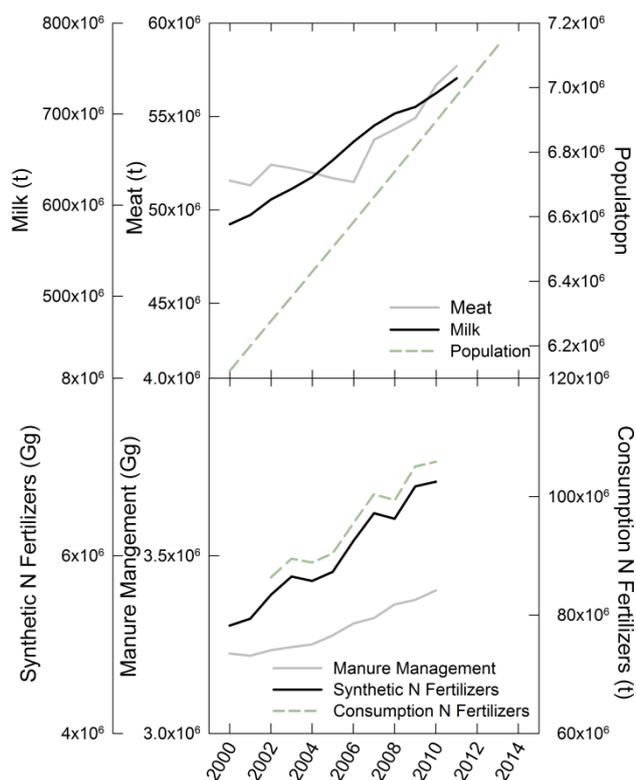
The rapid growth in the global population is made possible by the intensification and spread of agriculture and the availability of fertilizers (Erisman et al., 2008). The 'Food and Agriculture Organization of the United Nations' (FAO) currently estimates that to meet the demand for food caused by the projected population growth, global agricultural production will have to increase by 60% from its 2005–2007 levels (FAO, 2013).

The availability of fossil fuel, the transport of food, feed, goods and products, and the displacement of nutrients from areas without need to areas of intensive agriculture throughout the world, has made globalization possible (Galloway et al., 2008; Godfray et al., 2010) (Fig. 1.2). About 30 to 50% of agricultural income is now attributed to the use of mineral fertilizers (Stewart et al., 2005) and nearly half of the world population is nourished today by the help of artificially produced N fertilizers (Erisman et al., 2008).

The agricultural livestock sector is globally highly dynamic and rapid (Thornton, 2010). The increase of livestock amounts is driven by the fast increasing demand for livestock products. This demand is driven by rapid population growth, urbanization and growing incomes in developing countries (Delgado, 2005).

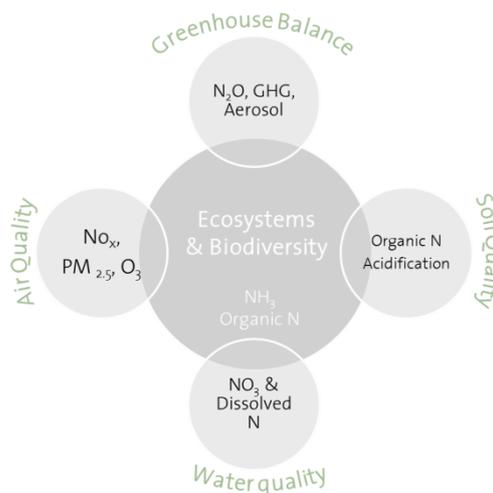
In comparison, a stagnation of livestock production is observed in developed countries, while many production systems increase their efficiency and environmental sustainability (Thornton, 2010). In agriculture, N for crops is supplied mainly by synthetic fertilizers and manure. However, only a fraction of the applied N is actually absorbed by plants and then removed by harvest. A large proportion of the used N, the so-called N excess, escapes unused into the atmosphere, is washed out by water or remains in the soil. Presently, very high N excesses are produced by the intense supply of industrial fertilizers and the concentration of livestock in some areas, which leads to far-reaching environmental problems. Currently, the  $N_r$  excess disturbs and destabilises ecosystem functions and ecosystem services to people, threatens biodiversity in water and on land, accelerates climate change and is harmful to human health. There are environmental problems in which humanity has significantly exceeded the capacity of the earth today (Rockström et al., 2009) (Fig. 1.1, Fig. 1.3).

The most important direct effects of inefficient use of  $N_r$  on ecosystems are ozone damage, acidification (decreases in pH values and accordingly, a decline of many important functions and reactions in the respective medium), eutrophication (nutrient oversaturation, thus overproduction of organic matter and simultaneously increased oxygen consumption), N saturation, biodiversity impacts and damage by plaques and diseases (Cowling et al., 1998; Fields, 2004; Galloway et al., 2003; Rockström et al., 2009). The negative influences on water, soils and air have, in turn, a negative impact on



**Figure 1.2** Global development of population, meat (t), milk production (t) and emissions (CO<sub>2</sub> eq. in Gg) of synthetic fertilizers and manure management. Furthermore the world consumption of N fertilizers is presented (Data source: FAO, 2013).

agricultural production and human well-being (FAO, 2013). The five key societal threats of excess  $N_r$  presented in Figure 1.3 illustrate the multi-pollutant and the multi-phase complexity of the  $N_r$ - related N cycle and the overlapping consequences.



**Figure 1.3** Overview of the key societal threats of excess  $N_r$  (modified after Sutton et al., 2011).

*Water quality*, which affects ecosystems and human health, is detrimentally affected by the intensification of agricultural activities, waste water discharges and fossil fuel combustion (Lavelle et al., 2005). Rivers, lakes, aquifers, coastal and marine waters are impaired through the increased  $N_r$  input. One of the main problems is the phenomenon of eutrophication (Grizzetti et al., 2011, Smith, 2003). Typical effects caused by excessive inputs of N into aquatic ecosystems are the increased biomass production of phytoplankton and macrophytic vegetation, consumer species, and benthic and epiphytic algae. Furthermore, shifts to bloom-forming algal species that might be toxic or inedible, reduction in species diversity, changes in species composition and loss of coral reef communities are documented outcomes (Smith, 2003; Smith & Schindler, 2009). The disease infant methaemoglobinaemia is one example of a direct effect on human health due to increased  $NO_3^-$  contamination in drinking water (blue baby syndrome) (Ward et al., 2005).

Even though the N supply causes primarily a positive effect on *soil quality*, i.e. by enhancing soil fertility for crop growth, high doses of N in the form of animal manure, chemical fertilizer or high atmospheric N deposition can also cause negative effects (acidification, loss of soil diversity, changes in soil organic content) (Matzner & Murach, 1995; Raubuch & Beese, 2005; Velthof et al., 2011).

*Air quality* is mainly affected by primary emissions of N compounds ( $NO_y$ ,  $NH_x$ ), but also by secondary pollutants like ground level ozone ( $O_3$ ) and secondary particulate matter (PM). Problems caused by air pollution include negative effects on human health, vegetation, buildings and visibility (Moldanová et al., 2011). Ozone, which is formed photochemically in the presence of  $NO_2$  and volatile organic compounds (VOC), is one of the most important global air pollutants in terms of impacts to health, croplands, biodiversity and ecosystem services (Mills et al., 2013; Moldanová et al., 2011). Through

the oxidation of  $\text{NO}_2$  to nitric acid ( $\text{HNO}_3$ ), particulate nitrate can be formed and additionally react with  $\text{NH}_3$  to form ammonium nitrate or can be absorbed on PM. These urban aerosol particles can absorb terrestrial radiation or scatter solar radiation and are therefore responsible for low visibility in cities and scenic areas (photochemical smog, regional hazes). A depletion of stratospheric ozone and a cooling of the regional climate are also caused by aerosols (Cowling et al., 1998; Moldanová et al., 2011, Sutton et al., 2011). Furthermore, material deterioration can occur through a result of weathering and corrosion caused by the acidifying N pollutants (acid rain) (Moldanová et al., 2011). Apart from these effects, the anthropogenic N has adverse effects on human health (Moldanová et al., 2011; Townsend et al., 2003; WHO, 2013), whether in developed or developing countries, indoors or outdoors (WHO, 2013). Symptoms include bronchitis, reduced lung function, lung cancer, asthma, breathing problems, cardiovascular and respiratory diseases (Fields, 2004; Moldanová et al., 2011; WHO, 2013). Apart from the predominant respiratory diseases caused by the inhalation of N containing substances, the ecological reactions to excess N can increase allergenic pollen production and potentially affect the dynamics of several vector-borne diseases, including West Nile virus, malaria, and cholera (Townsend et al., 2003).

$\text{N}_r$  compounds alter the Earth's *greenhouse balance* or rather the global radiative balance, directly and indirectly (Butterbach-Bahl et al., 2011b; Schulze et al., 2009). The direct effects are generally related to the formation of the so-called greenhouse gas (GHG)  $\text{N}_2\text{O}$ , which is emitted by microbial processes and is roughly 296 times as powerful as carbon dioxide ( $\text{CO}_2$ ) on a 100-yr timescale and per unit of weight (Butterbach-Bahl et al., 2011b; IPCC, 2007). The GHG  $\text{N}_2\text{O}$  contributes to global warming, in the same way as the tropospheric GHG  $\text{O}_3$ , which causes a reduction of the biospheric  $\text{CO}_2$  sink and thereby an additional warming effect (Butterbach-Bahl et al., 2011b). A parallel cooling-effect also occurs, resulting from emissions of  $\text{NO}_x$  and  $\text{NH}_3$ . The atmospheric deposition of these  $\text{N}_r$  compounds increases the biospheric  $\text{CO}_2$  sink, and in aerosol form they are responsible for reflecting light before it reaches the Earth's surface (Butterbach-Bahl et al., 2011b).

In addition to climate change and habitat conversion, enhanced  $\text{N}_r$  deposition is a major contributor to the reduction of *global biodiversity* (Dise et al., 2011; Galloway et al., 2003; Rockström et al., 2009; Sala et al., 2000, Vitousek et al., 1997). Increased  $\text{N}_r$  concentrations in *ecosystems* cause direct damage to vegetation, eutrophication of ecosystems, alteration of nutrient ratios in soil and vegetation, increasing soil acidity and exacerbating the impact of other stressors like pathogens, frost and drought (Bobbink et al., 1998; Dise et al., 2011; Galloway et al., 2003). Ecosystems vary widely in their

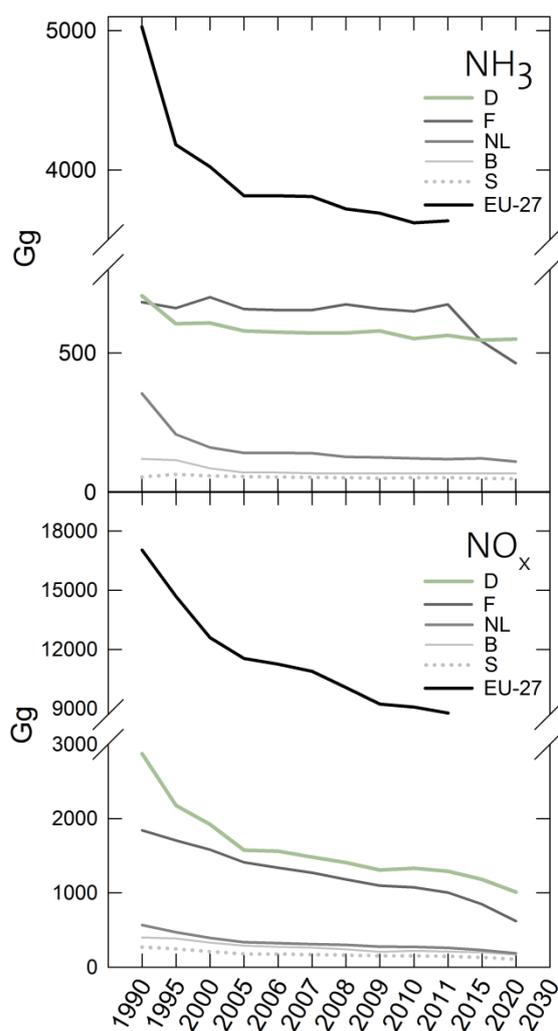
sensitivity to atmospheric N deposition due to different abiotic and biotic characteristics (Bobbink et al., 2010), but generally, the biodiversity of terrestrial ecosystems decreases if N deposition increases (Bobbink, 2004; De Schrijver et al., 2011; Duprè et al., 2010; Klimek et al., 2007; Phoenix et al., 2012). The inputs of reduced N compounds have stronger negative effects on biodiversity compared to oxidized N forms (Paulissen et al., 2004, Pitcairn et al., 2003; Sheppard et al., 2011). The most vulnerable ecosystems are those naturally adapted to low nutrient levels or poorly buffered against acidification. Grassland, heathland, peatland, forests, arctic and montane ecosystems are among these highly endangered ecosystems (Dise et al., 2011). Typical effects on plant diversity in these ecosystems are a reduction in species richness and changes in species composition, often coupled with invasion of nitrophilic species (Bassin et al., 2007; Britton & Fisher, 2007; Duprè et al. 2010; Edmondson et al., 2010; Mitchell et al., 2002; 2005; Nordin et al., 2006; Stevens et al., 2010). Lower plant species like mosses, lichens and liverworts, which obtain N mainly from rainfall, also react highly sensitively to enhanced N deposition (Davies et al., 2007; Johansson et al., 2012; Pitcairn et al., 2006) and are vulnerable to direct effects of excess N deposition (Britton & Fisher, 2010). Even low to medium N deposition influences the species richness of lichens and mosses (Remke et al., 2009). In remote and pristine areas, distant N loads can also threaten the species based on factors like orographic enhancement of deposition at high altitudes, through N pollutants transported in fog or high levels of N deposited over a short period during snowmelt (Dise et al., 2011; Taylor et al., 1999). The devastating major changes in vegetation diversity are also linked to changes in above-ground faunal communities (Dise et al., 2011) e.g. declines of butterflies, beetles, moths and bird populations in areas of high N deposition (Beusink et al., 2003; Nijssen et al., 2001; Ockinger et al., 2006; Weiss, 1999).

### 1.3 National facts

In Germany, approximately 3200 Gg N per year enter the N cycle in form of  $N_r$  compounds, whereby the majority is bound in mineral fertilizers (~1808 Gg N). Additional N inputs are attained from other countries or regions mainly by transport of feedstuffs (~370 Gg N) or via atmospheric deposition (~260 Gg N) (UBA, 2011a). Biological N fixation by agriculture and natural ecosystems amounts to 300 Gg N per year. Around 520 Gg N per year are released by combustion processes in industry,

energy-supply, transport and households. It is expected that approximately 2000 Gg N leaves the N cycle again (UBA, 2011a). This includes the transboundary atmospheric transport to neighbouring countries (~700 Gg N), the N release via rivers to the oceans (450 Gg N) and the conversion of  $N_r$  into molecular N ( $N_2$ ) (850 Gg N) (UBA, 2011a). The release of gaseous N compounds ( $NH_3$ ,  $NO_x$  and  $N_2O$ ) from combustion processes and agriculture amounts to 1200 Gg N per year (about 63% of all German N emissions) and represents the most significant flow of the N cycle in Germany (UBA, 2011a).

Among the EU-27 countries, Germany emitted most  $NO_x$  emissions in 2011 (1293 Gg), followed by the United Kingdom, Spain and France (EEA, 2013). Figure 1.4 illustrates a decreasing trend in  $NO_x$  emissions in total and relative to total EU-27 emissions. The  $NO_x$  emissions in Germany decreased from 1990 to 2011 by about 55% (EEA, 2013; UBA, 2013a). Fuel combustion accounted for over 93% of  $NO_x$  emissions in 1990, and even after a 60% reduction between 1990 and 2011, it is still the main driver of  $NO_x$  in Germany (UBA, 2013a). Half of the 1990 emissions from fuel combustion originate from the road transportation: this has similarly reduced by 64% between 1990 and 2011 (UBA, 2013a). The emissions reductions of traffic are due to stricter regulations and emission standards resulting in technical improvements and improved fuels. The additional decline of using solid and liquid fuels significantly affects the trend (EEA, 2013; UBA, 2013a). Concerning  $NH_3$  emissions, France, Germany, Spain and Italy are the EU member states which have contributed most (more than 10%) in the year 2011. France (18.5% of EU-27 emissions) was the largest emitter, followed by Germany (15.5%) (EEA, 2013). Agriculture is the most important source of  $NH_3$  in the



**Figure 1.4** Contributions of  $NH_3$  and  $NO_x$  emissions in Gg from Germany (D), France (F), the Netherlands (NL), Belgium (B) and Sweden (S) to EU-27. The total  $NH_3$  and  $NO_x$  emission trend of total EU-27 is also shown. Data for the years 2015 and 2020 are based on projections. (Data source: European Environment Agency, Copenhagen, Denmark).

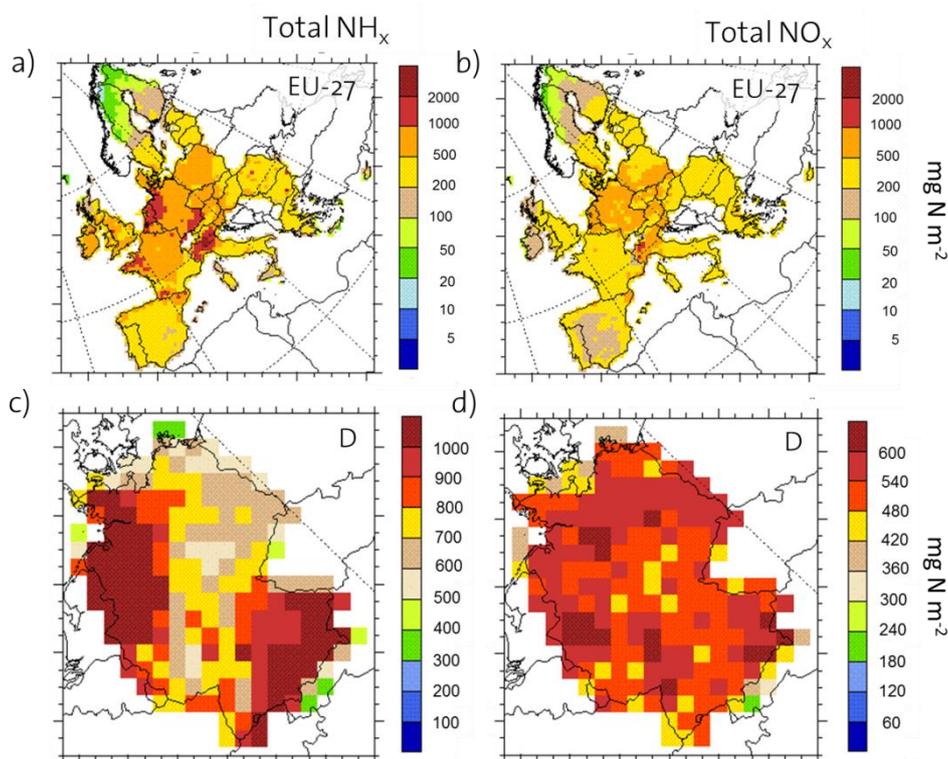
EU-27, causing 93% of emissions (EEA, 2013). In Germany the overall NH<sub>3</sub> emission trend follows agricultural emissions which are mainly derived from manure management (with 85% of total NH<sub>3</sub> emissions in 1990 and a 26.6% reduction from 1990-2011) and agricultural soils (UBA, 2013a). Figure 1.4 shows a decline in NH<sub>3</sub> emissions between 1990 and 1995 due to reduced livestock populations following the German reunification (1990), while there has been only a slightly decreasing trend since then (EEA, 2013; UBA, 2013a).

Regulations for the reduction of N emissions in Germany are linked to several protocols e.g. the ‘Gothenburg Protocol’ of the United Nations Economic Commission for Europe (UNECE) also known as the ‘Multi-effect Protocol’, which is part of the ‘Convention on Long-Range Transboundary Air Pollution’ (CLRTAP). It is a multi-pollutant protocol aiming to reduce acidification, eutrophication and ground-level ozone by setting emission ceilings (UNECE, 2013). NO<sub>x</sub> and NH<sub>3</sub> emissions should be minimised by 2010. The ‘Gothenburg Protocol’ and the directive on ‘National Emission Ceilings’ (NEC directive 2001/81/EC) (adopted in the German law through the 33<sup>rd</sup> Ordinance – 33. BImSchV / 39. BImSchV) (UBA, 2013a,b) have stipulated a NH<sub>3</sub> emission ceiling of 550 Gg for NH<sub>3</sub> and 1081 Gg (1051 Gg concerning NEC directive 2001/81/EC) for NO<sub>x</sub> in Germany in 2010 (UBA, 2013b, c). In 2010 and 2011, the NH<sub>3</sub> and NO<sub>x</sub> emission ceilings were exceeded in Germany (EEA, 2013). Further efforts to reduce emissions are therefore necessary. International agreements on air pollution by the United Nations Economic Commission for Europe (UNECE), such as the Geneva CLRTAP of 1979 and their subordinate protocols as well as the legally binding setting of emission limits for the member states of the European Union (NEC directive of the EU) became the basis for political and economic measures to reduce atmospheric pollution in Germany (UBA, 2010). A scientifically based and policy driven programme under the CLRTAP for international co-operation to solve transboundary air pollution problems, is the ‘European Monitoring and Evaluation Programme’ (EMEP) (EMEP, 2013). The EMEP programme focuses on assessing the transboundary transport of acidification and eutrophication, the formation of ground level ozone, persistent organic pollutants (POPs), heavy metals and particulate matter.

The Federal Environment Agency (UBA) gives top priority to the measuring and monitoring of these large-scale air pollution problems in Germany. The UBA-air monitoring network is especially interested in cross-border air pollution. Therefore, the Federal Environment Office operates its monitoring stations (so-called rural background stations) far away from urban areas and major local emitters such as power stations, heavy industry or major roads (UBA, 2013d).

Nonetheless, the area of Germany should be covered representatively regarding characteristic landscapes and altitudes. The EMEP prescribes one station per 50 000 km<sup>2</sup>, whilst background measurement obligations under EU legislation stipulate one station per 100 000 km<sup>2</sup>. While the north of Germany meets the requirements of the EMEP station density quite well, gaps are found in the west and southeast. There are also no monitoring stations in the altitude range between 200 and 800 m above sea level, the typical low mountain range that occupies at least 40% of Germany (UBA, 2013d). In former times the UBA air monitoring network counted nine personnel occupied stations, 23 container sites and the measured power central office or pilot station in Offenbach. Today, only seven manned stations and the monitoring network centre in Langen are still operated (UBA, 2013d).

In Germany, reduced atmospheric deposition (NH<sub>x</sub>) and oxidized (NO<sub>x</sub>) N compounds still remain at a very high level. Gauger et al. (2008) estimated an average of total N input of around 26 kg ha<sup>-1</sup>yr<sup>-1</sup> in Germany, but that the input into forest ecosystems is on average about 40 kg N ha<sup>-1</sup>yr<sup>-1</sup> (ranging from 15 to >100 kg N ha<sup>-1</sup>yr<sup>-1</sup>) (UBA, 2010). Especially the amount of diffuse nutrient sources is highest in locations prone to leaching, with high concentrations of livestock.



**Figure 1.5** EMEP model results for **a)** total reduced N deposition in the EU-27, **b)** total oxidized N deposition in the EU-27, **c)** total reduced N deposition in Germany (D) and **d)** total oxidized N deposition in Germany (D) for the year 2011. Modelled with EMEP/MSC-W model version rv4.4 and grid resolution 50 × 50 km<sup>2</sup> (Data source: Norwegian Meteorological Institute, Oslo, Norway).

This is the case in the entire north-western Germany (sandy soils) and in some areas of the foothills of the Alps (high runoff) (UBA, 2011b). For an overview of how the different N loads are distributed in the EU-27 and in Germany, modelled EMEP results are shown for both regions in Figure 1.5. Compared to most other EU countries, very high deposition rates of reduced N compounds as well as of oxidised N compounds are generally found in Germany. It becomes apparent that large gradients of N deposition occur across EU-27 and that areas in north-west and central Europe receive the highest loads of N deposition (Fig. 1.5). It is noticeable that the deposition of  $\text{NH}_x$  is highest in Central Europe, as it contains the intensive agrarian countries Germany, the Netherlands, Belgium, France and Italy. Concerning Germany, the north-western areas and the southern areas in the foothills of the Alps are characterized by high deposition rates of reduced N compounds from agriculture. The spatial distribution of  $\text{NO}_x$  compounds in Germany is quite homogenous due to urban sprawl and widespread land use. The Rhine-Ruhr metropolitan region and the metropolitan areas around the cities Berlin, Hamburg, Munich and Frankfurt (Main) have the highest  $\text{NO}_x$  deposition rates.

In the late 19<sup>th</sup> century, the average of agricultural N fertilization in Germany amounts to less than  $20 \text{ kg ha}^{-1}\text{yr}^{-1}$  (BMELF, 1956). By the end of the 20<sup>th</sup> century, this was on average  $220 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Bach et al., 1997), reaching a maximum of  $300 \text{ kg ha}^{-1}\text{yr}^{-1}$  in areas with specialised cultivation conditions or intensive livestock farming (SRU, 1985; UBA, 2009). Agricultural production takes place mostly in open systems and over long cycles. Not all N compounds are equally available for plants as nutrients. Introduced substances, including N, are not exploited completely. Considering this, the German federal government had the objective to reduce the overall net N surpluses in agricultural production to  $80 \text{ kg ha}^{-1}$  per year by 2010. (BMU, 2007; 2010). Relating to the N surplus, a decline from  $132 \text{ kg ha}^{-1}$  to  $105 \text{ kg ha}^{-1}$  (three-year rolling average) from 1991 to 2007 represented a decrease of more than 20% (BMU, 2010). However, in the year 2010, the N surplus was still  $96 \text{ kg ha}^{-1}$ , with significantly higher N surpluses in the intensive livestock farming regions of north-western Germany. The objective to reduce N surplus up to  $80 \text{ kg ha}^{-1}$  per year has not been reached and further reductions are intended by 2015 (BMU, 2010; UBA, 2013f).

The eutrophying and acidifying effects of N emissions and the resulting immissions and depositions exert considerable pressure on biodiversity (BMU, 2010). More than half of the existing vascular plants in Germany naturally grow under low-nutrient conditions. Many populations are endangered because of excessively high N loading rates (BMU, 2007). Many animal species depending on specific plant species are affected indirectly (BMU, 2010). Water pollution with N is caused by entries via groundwater, erosion and

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runoff from predominantly agricultural land and drainage (UBA, 2011b). A survey of groundwater quality according to EU Water Framework Directive in 2009 revealed that over one quarter (26.5%) of the total groundwater resources in Germany is in poor chemical status due to high nitrate levels (UBA, 2013f). Also the protection of marine and limnic ecosystems from N induced eutrophication and the accompanying biological degradation is not yet sufficient. So far, the international targets to reduce the input of  $N_r$  in coastal ecosystems have not been reached in Germany (UBA, 2009). The protection of human health from hazards induced by nitrate in drinking water, in vegetables and fruits, PM and ozone in the ambient air is not yet sufficient. Applicable guiding and limiting values are still exceeded in Germany (UBA, 2009).

As part of an indicator system, the internationally called ‘critical loads’ (CLOs) describe ecosystem-specific load limits for atmospheric inputs of harmful substances and nutrients. CLO reflect quantitative estimations of deposition rates of a substance per unit area and time (Cape et al., 2009, BMU, 2010). A comparison between the amount of N deposition and CLOs for eutrophication shows that the CLOs are still exceeded today on almost the entire surface of sensitive ecosystems in Germany. The excess in some parts of north-west Germany, where intensive livestock farming on sensitive soils is common and the deposition of N is particularly high, are drastic (UBA, 2011b). While airborne N emissions from transport, energy conversion and industry have decreased in terrestrial ecosystems in recent years, the inputs of  $NH_3$  and  $NH_4^+$  from agriculture have stagnated at a high level. Given that Germany still has difficulties achieving various national and international N-related aims for environmental quality and activity treatments, the development and implementation of additional measures to reduce the quantified emissions and the subsequent implementation processes is necessary. The patchy and low availability of deposition measurement stations underlines the need for alternative and cost-efficient monitoring systems in Germany.

## 2 Uncertainties ... and how lichens, mosses and tree bark come into consideration

The previous sections described the diversity of problems caused by human-accelerated environmental N change at a global and national level. The annual costs for  $N_r$ -related damage in the EU-27 is thought to range between 70 and 320 billion Euro (150-750 €/capita) (Brink et al., 2011). Highest costs are caused by health impacts from  $NO_x$  emissions (10-30€ per kg of pollutant  $N_r$  emission), followed by health impacts from

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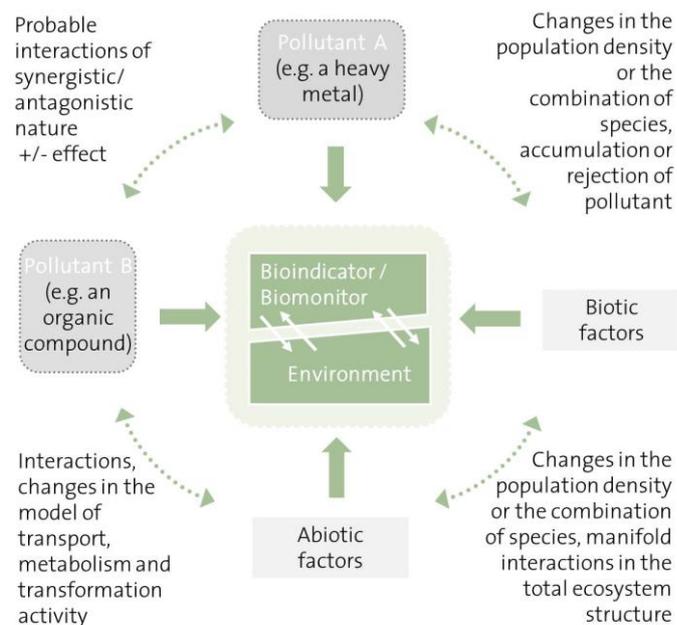
secondary  $\text{NH}_4^+$  particles (2-20€ per kg N), from GHG balance effects (5-15€ per kg N), from ecosystem impacts via N runoff (5-20€ per kg N) and finally from N deposition (2-10€ per kg N) (Brink et al., 2011).

The dramatic problems as well as the resulting costs, demonstrate that the degree of N enrichment has to be reduced and that the undesirable effects and N input have to be monitored correctly, in a realistic way and at best at an ecosystem level.

Observations can only partly address the complex process chain of the atmospheric N<sub>r</sub> deposition, which includes atmospheric deposition, emissions, dispersion, chemical transformation and eventual loss (Hertel et al., 2011; Simpson et al., 2011). This complexity makes a precise estimation of N loads very difficult. Furthermore, observations are severely restricted in spatial extent and type.

Often, the insufficient resolution of monitoring networks causes significant uncertainties in mapping and assessing N deposition. Particularly with regard to the close-range  $\text{NH}_3$  inputs, higher resolution inventories are needed. More difficulties occur if a spatial extrapolation is calculated from fine scaled and often process-based monitoring data to a larger scale (Lindenmayer & Likens, 2010). Strong uncertainties arise when estimating global, regional and local  $\text{NH}_3$  emissions, which increase at smaller scales (Erisman et al., 2007). Global and regional scale models cannot be expected to reproduce small-scale variations in deposition regimes, caused by factors like local inputs close to source (e.g.  $\text{NH}_3$ ) (Sutton et al., 1998) and the topography effect (Hertel et al., 2011), especially based on the fact that N compounds can be highly variable, spatially and temporally (Simpson et al., 2011).

Based on the limitation of the spatial resolution of deposition measurement stations, the prediction of atmospheric transport models is not accurate, particularly in very heterogeneous landscapes. These models underestimate hotspots especially of dry deposition which commonly results in the under prediction of critical loads excesses (Hertel et al., 2011). Estimates of deposition by state-of-the-art models are particularly uncertain (+/- 50%) (Hertel et al., 2011). The low reliability of modelled data can only be supported with the help of comparative measurement data. Unfortunately comprehensive evaluations are not ensured, although a validation between the modelled and observed values could assess the degree of agreement (Simpson et al., 2011). N monitoring should be feasible even in remote locations that are hard to reach and in a wide variety of sensitive ecosystems. Furthermore, an additional indication of predominant N sources on-site would enhance the usefulness of the monitoring procedures. A low cost approach suitable for large areas and for heterogeneous landscapes is urgently required for a robust quantitative and qualitative assessment of N deposition.



**Figure 1.6** Simplified illustration of complex (eco-) system interrelations with regard to pollutants, and consequences for bioindication and biomonitoring (modified after Markert., 1996; Markert et al., 2003).

Possible alternatives to produce a spatially representative picture of N deposition conditions are bioindicators, i.e. organisms which contain information on the quality of the environment. In contrast, biomonitors are organisms which include information on the quantitative aspects of the quality of the environment. Accordingly, biomonitors are always bioindicators, but bioindicators are not always biomonitors (Conti & Cecchetti, 2001; Markert et al., 2003). Organisms that can be used for the quantitative determination of contamination in the environment are defined as

accumulative biomonitors (Conti and Cecchetti, 2001). These accumulative biomonitors have the ability to incorporate contaminants due to equilibrium process of biota compound intake/discharge from and into the surrounding environment (Conti and Cecchetti, 2001). Bioindicators are able to offer a sensitive signal to exposure and ecosystem effects and they reflect many of the clearest demonstrable biological and ecological responses to enhanced N depositions (Sutton et al., 2004a). These responses include accumulation of N, changes in biochemical processes and changes in community species composition (Conti & Cecchetti, 2001; Nimis et al., 2002; Sutton et al., 2004b). An overview of the interrelations concerning pollutants and consequences for bioindicators/biomonitoring in the environment is given in Figure 1.6.

Lower plants such as mosses and lichens are, compared to vascular plants, relatively dependent on atmospheric inputs as their primary source of nutrients. They can react highly sensitively especially to  $\text{NH}_x$  (Bobbink et al., 2010; Sheppard et al., 2011; Skinner et al., 2006). Due to their specific physiology and ecology (Hauck, 2010; Turetsky, 2003), lichens and mosses are predestined to indicate the N inputs at ecosystem level. Because of the specialized symbiosis between algae and fungi, lichens are ubiquitous and are able to colonize extreme habitats. However, they are also very sensitive to environmental changes. Due to their lack of a protective outer cuticle, these perennial organisms are able to absorb both nutrients and pollutants. Furthermore, they can be sampled

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throughout the year (Nimis & Purvis, 2002). Lichens as bioindicators, particularly as accumulative bioindicators of N inputs, have not been used for long.

Especially epiphytic lichen species have mostly been used for monitoring purposes since the 1960s (Conti & Cecchetti, 2001; Nimis & Purvis, 2002; Schöller, 1997). As industrialization and air pollution increased, so did the field of research with lichens. Scientists have mainly studied lichen bioindication in connection with sulphur dioxide (SO<sub>2</sub>) emissions (Carreras & Pignata, 2002; Hawksworth & Rose, 1970; Geiser & Neitlich, 2007; Nash III, 1973; Nash & Gries, 2002; Raymond et al., 2010, Salemaa et al., 2004; Takala et al., 1985) and heavy metals in metropolitan areas (Achotegui-Castells, 2013; Brown & Beckett, 1983; Carreras & Pignata, 2002; Guttová et al., 2011; Salemaa et al., 2004; Salo et al., 2012, Scerbo et al., 2002). With the steep decline in SO<sub>2</sub> pollution in recent years, airborne N compounds have become the most important acidifying and eutrophying air pollutants and thereby have become an increasingly interesting research objective in biomonitoring sciences.

The majority of studies have directed their attention to the effect of increased N influence on changes in lichen species composition (Davies et al., 2007; Fenn et al., 2003; Gadson et al., 2010; Geiser et al., 2010; Johansson et al., 2012; Jovan & McCune, 2005; Jovan et al., 2012; Pinho et al., 2008, 2011; Ruoss, 1999; Sparrius, 2007; Van Herk, 1999; Van Herk et al., 2003; Vilsholm, 2009). One of the closest links to the influence of atmospheric N deposition in ecosystems is the method of using accumulative bioindicators. Using lichens as accumulative bioindicators in conjunction with the use of classical physico-chemical methods for monitoring air concentrations and deposition, provides a more accurate estimation of the current N status on-site (Sutton et al., 2004b). The main advantage of these biomonitors compared to physical sampling is dealing with situations of complex terrain (e.g. forest edges, near sources etc.), where the physical determination of deposition is extremely difficult. By using bioindicators, a site-level risk assessment can be supported with the critical loads/levels approach because these organisms contain information about concentrations of atmospheric N deposition. The derived site-level estimate of N exposure is followed by a comparison with the appropriate critical limit/load (Sutton et al., 2004b). Several studies have been conducted in different study areas, which were characterized by various N sources (agriculture, traffic and industry) revealing a clear relationship between atmospheric N deposition and N content in lichens (Britton & Fisher, 2010; Bruteig, 1993; Franzen-Reuter, 2004; Frati et al., 2007; Gaio-Oliveira et al., 2001; Gombert et al., 2003; Hyvärinen & Crittenden, 1998; Kubin, 1990; McMurray et al., 2013; Raymond et al., 2010; Remke et al., 2009; Root et al., 2013; Ruoss, 1999; Søbchting, 1995; Tozer et al., 2005). Nationwide surveys

have only been performed by Bruteig (1993) and Kubin (1990) in less N affected Norway and Finland. To date, no comprehensive study exists in highly N-influenced countries, such as Germany. The purpose of an excellent monitoring tool should not only address to a quantitative assessment of N deposition, but also to identify the prevailing N source on-site. In this context, the field of isotope research is highly promising.

As mentioned in section 1.1, different N sources can be distinguished on the basis of stable isotope analysis. Extensive studies using this highly powerful technique have been carried out to interpret the interrelations between plants and environment, as well as the relationships between elemental concentrations and isotopic signatures (Cuna et al., 2007; Dawson et al., 2002; Evans, 2001; Michener & Lajtha, 2007; Peterson & Fry, 1987; Strauch et al., 2011). The abundance of  $^{15}\text{N}$  is a valuable and widely used indicator for N sources and N pathways in different organisms and ecosystems (Hietz et al., 2002; Högberg, 1997; Robinson, 2001; Wania et al., 2002). As lichens obtain their nutrients basically from the atmosphere it is obvious that these organisms can help to identify the source of N added to the environment by anthropogenic activities, based on isotope research. At present, only a few reports on lichen research have used the natural abundance of  $^{15}\text{N}$  and integrated it in N monitoring surveys to identify various N sources in field experiments (Fogel et al., 2008; Franzen-Reuter, 2004; Lee et al., 2009; Russow et al., 2004; Skinner et al., 2006; Tozer et al., 2005). Mosses have also been frequently used as accumulative bioindicators with regard to N in local, national and Europe-wide studies (Baddeley et al., 1994; Delgado et al., 2013; Foan et al., 2014; Harmens et al., 2011, 2013; Leith et al., 2005; Pesch et al., 2007; Pitcairn et al., 2001, 2006; Raymond et al., 2010; Schröder et al., 2011). Various studies have shown, that mosses could be used for a quantitative and qualitative assessment of N deposition (Bragazza et al., 2005; Delgado et al., 2013; Foan et al., 2014; Liu et al., 2008; Pearson et al., 2000; Solga et al., 2005; Zechmeister et al., 2008). Besides lichens and mosses, also tree bark was used for biomonitoring of N deposition in several studies (Mitchell et al., 2005; Poikolainen et al., 1998, Schulz et al., 1999, 2001). However, there has so far been little comparative assessment of the tissue  $\text{N}/\delta^{15}\text{N}$  response of lichens to atmospheric N deposition in relation to other bioindicators such as bryophytes and tree bark.

According to cited literature, there are still questions which have not been answered in biomonitoring research so far. Especially the species-specific response of lichens and their differing abilities of N accumulation have not been investigated in-depth. Thereby, special N-tolerant bioindicator organisms for the use in highly affected N regions have not been identified. Performing comparisons with different bioindicators at different N impacted sites at various investigation scales could enable an ease applicability of a ‘can be used anyway’ monitoring method. Improving bioindicator methods in the context of

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measured and modelled deposition data based on physico-chemical air monitoring programmes is an important and necessary component for the validation and assessment of different monitoring techniques.

### 3 Research strategy

The preceding sections have illustrated the disastrous problems with respect to the continuing  $N_r$  enrichment and the necessity to develop alternatives for an area-wide assessment of the human-accelerated N depositions.

The following research approaches were developed based on the mentioned problems and uncertainties, as well as new methodological possibilities. The present thesis emerged as part of the project 'Abschätzung von Stickstoffdepositionen mit Hilfe der epiphytisch wachsenden Flechten *Xanthoria parietina*, *Physcia adscendens*, *Parmelia sulcata* und *Hypogymnia physodes* und ihrer stabilen Isotopenmuster' funded by the German Federal Environmental Foundation (DBU) within the framework of a doctoral scholarship programme, and was conducted at the Department of Geobotany, Faculty of Regional and Environmental Sciences, Trier University. The research objectives have been addressed in form of specific publications and international conference contributions. Three following publications (Chapter 2-4) make up the present cumulative dissertation. The internal coherence of the publications is presented in Figure 1.7.

#### 3.1 Study objectives and chapter outline

The main research intention of the present study was to develop and test a widely applicable and integrated monitoring method to assess N loads and to identify prevailing atmospheric N sources with the help of N content and stable nitrogen isotopes in different epiphytic lichen species.

Specific research objectives were developed, which are discussed in detail with the help of research hypotheses and based on statistics in the publications, respectively. The underlying research objectives investigated on different spatial scales were formulated based on the following issues:

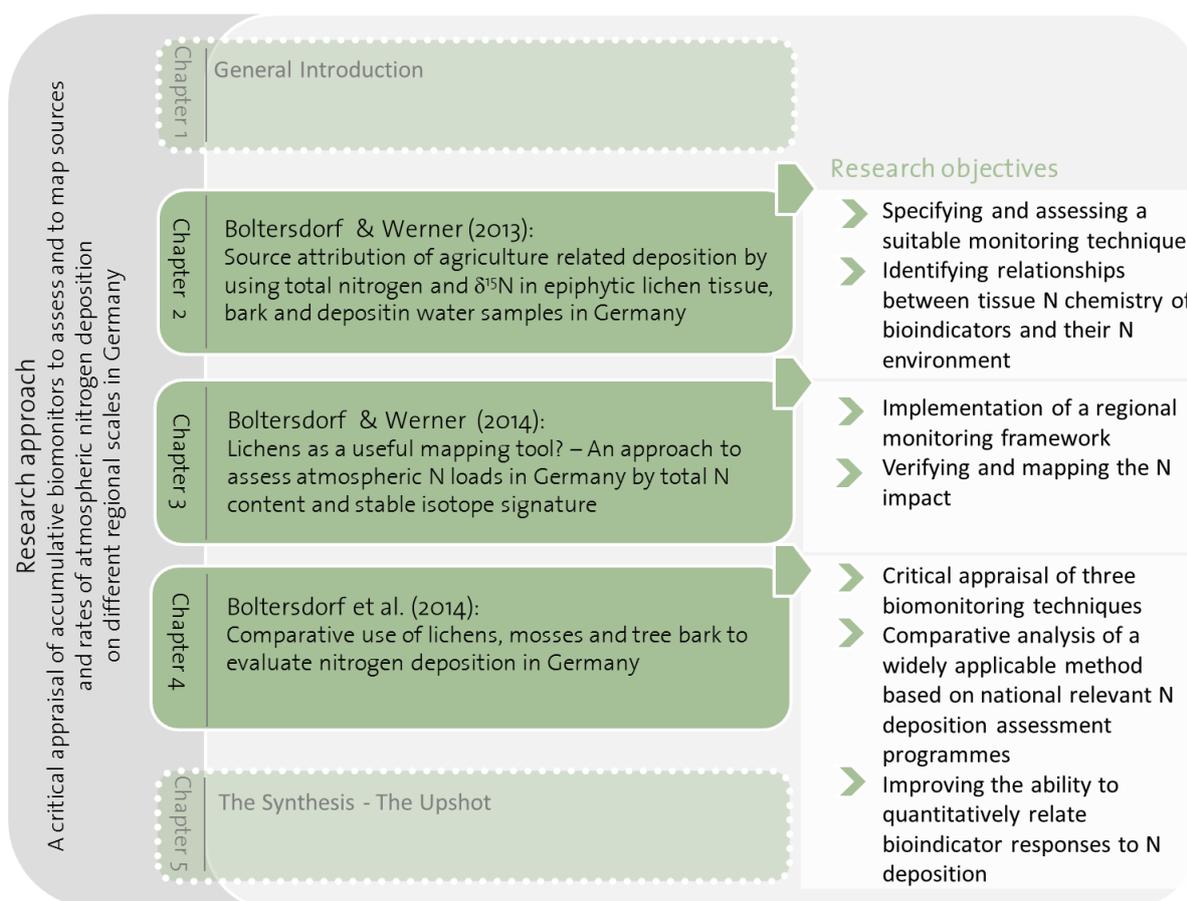
- i. Specifying and assessing a suitable monitoring technique – identifying relationships between the tissue N chemistry (N%,  $\delta^{15}\text{N}$ ) of different monitor organisms (lichens and tree bark) and their N environment (Chapter 2)
- ii. Implementation of a regional monitoring framework – verifying and mapping the N impact (Chapter 3)
- iii. Critical appraisal of three biomonitoring techniques – comparative analysis of a widely applicable method based on measured and modelled data of the national N deposition assessment programme; improving the ability to quantitatively relate bioindicator responses to N deposition (Chapter 4)

Chapter 2 investigates the ability of tissue N content and the  $\delta^{15}\text{N}$  signature of different lichen species to act as quantitative and qualitative indicators for the estimation of N deposition along a gradient of increased agricultural activities.

The first step was to identify species-specific ability and tolerance of various epiphytic lichens to accumulate N. It was tested if lichens respond species-specifically under increased N deposition rates with an increase in the total tissue N concentration. Samples of tree bark were collected and tested simultaneously. With the help of stable isotope analyses of lichens, bark and deposition water samples, this study attempted to identify and assess the main source of N deposition in the chosen study area. In doing so, this study revealed that lichens and tree bark exhibit different concentrations of N and  $\delta^{15}\text{N}$  signatures, despite being exposed to similar N deposition, due to different receptor surfaces.

To prove whether lichens and/or tree bark samples are able to reflect the predominant N components of the atmosphere on-site, data obtained from lichen and bark monitoring were correlated with data obtained from N monitoring networks based on physico-chemical measurements.

Key influencing variables which are responsible for an enrichment of N in lichen and bark tissues were detected previously by investigating relationships between lichen, bark and deposition water samples and their analysed chemical variables (N%,  $\delta^{15}\text{N}$ ,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ). Simultaneously an international integration and comparison with data from literature was conducted. Analysing the N content and especially the  $\delta^{15}\text{N}$  values in lichens is an unusual practice in lichen research, and only little is known about how well lichens can be used to map and to reflect different area-wide N loads and sources based on their tissue N chemistry so far.



**Figure 1.7** Structure and main research issues of the thesis.

The second objective (Chapter 3) addressed the mentioned research gap by developing a regional monitoring grid within which the utility of nitrophytic lichens as monitor organisms was tested. Surveys from the first study (Chapter 2) formed the basis for this regional implementation. This framework, including spatial and comprehensive data, was set up to verify if it is possible to characterize and map different N loads by analysing the N content of lichens on a regional scale. Furthermore, chapter 3 tests whether  $\delta^{15}\text{N}$  values of lichens can be used to differentiate between the main N sources and types of deposition along an altitudinal gradient (from lowland to submountainous areas) and between areas affected by different land use types, which are categorized by various  $\text{NH}_3$  density classes (Rösemann et al., 2011), numbers of livestock units (Kreins, 2013) and various deposition types (Wellbrock et al., 2005). Geostatistical and Partial Least Square Regression (PLS-R) analyses were applied in this survey.

Chapter 4 deals with a comparative analysis of the applicability of three biomonitoring techniques (lichen, moss and bark monitoring) and gives a critical appraisal. Initially, the level of N pollution in Germany was assessed by tissue N content of lichens, mosses and tree bark. Using these biological indicators, this study intended to identify the key

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contrasting sites with respect to N depositions. Different spatial patterns of the prevailing N source were indicated by  $\delta^{15}\text{N}$  analyses.

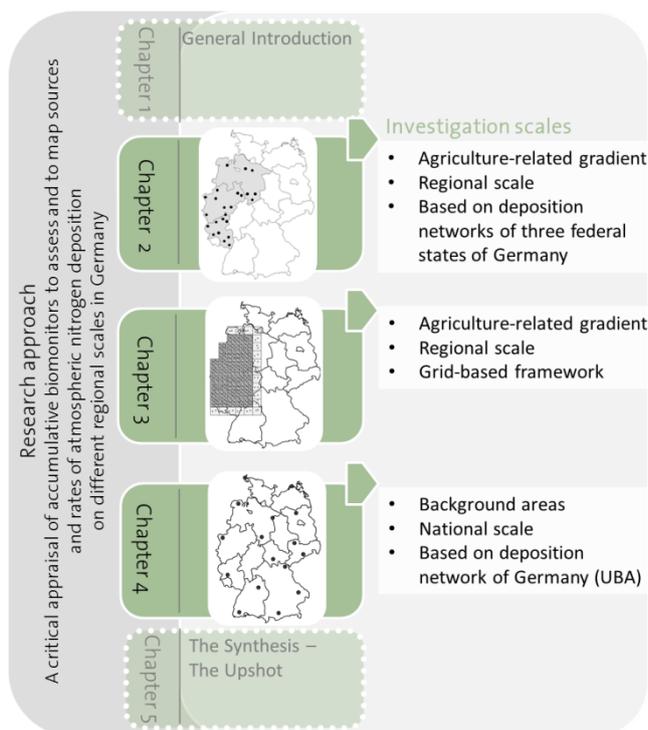
Finally, the explanatory power of the different biomonitoring techniques was compared by consulting measured and modelled data of the national as well as internationally relevant air monitoring network which are based on background stations in Germany.

Using these data from N deposition assessment programmes, correlations were performed with data obtained from the investigated bioindicators (N% and  $\delta^{15}\text{N}$ ) to determine possible dependences.

The main results and conclusions of all studies (Chapters 2 – 4) are summarized in the concluding Chapter 5 (Synthesis – The Upshot).

### 3.2 Methodology– Research design

The studies are based on different spatial reference scales. The first research objective was pursued by adapting the survey to regional deposition monitoring networks of the three federal states Rhineland-Palatinate, North Rhine-Westphalia and Lower Saxony in Germany. The co-operating institutions were: Research Institute for Forest Ecology and Forestry of Rhineland-Palatinate (FAWF-RLP), State Agency for Nature, Environment and Consumer Protection of North Rhine-Westphalia (LANUV-NRW) and Northwest German Forest Research Station (NW-FVA). In total, 24 deposition measurement stations were taken into consideration for the first study. Besides, 383 lichen samples, 161 bark samples and 24 deposition water samples were tested for usability. The selection of these stations was due to an increased influence of N deposition by agriculture from south to north and due to the proximity to neighbouring countries with intense agriculture. Based on the first study (Chapter 2) and with the help of geostatistical analysis, a suitable size of the respective grid cells for the implemented monitoring framework (Chapter 3) was developed. The sampling grid covered  $\sim 1425000 \text{ km}^2$  and was based on a regular  $25 \times 25 \text{ km}$  and on the outer edge a  $50 \times 50 \text{ km}$  grid cell system. Finally, a total of 174 grid cells were recorded in the western part and towards the north of Germany. In total, 348 lichen samples were analysed. The last study (Chapter 4) was adapted to the existing nationwide air monitoring programme of the Federal Environment Agency of Germany (UBA) and considered 16 deposition measurement stations in so-called ‘background areas’ with relatively clean air. In this investigation, 326 lichen samples, 153 moss samples and 187 tree samples were analysed.



**Figure 1.8** Overview of the research design

(L.) Gaertn., *Betula pendula* Roth, *Fraxinus excelsior* L., *Fraxinus ornus* L., *Juglans regia* L., *Malus domestica* Borkh., *Populus × canadensis* agg. Moench, *Prunus avium* L., *Prunus domestica* L., *Pyrus communis* L., *Quercus petraea* (Mattuschka) Liebl., *Quercus robur* L., *Tilia cordata* Mill., *Tilia platyphyllos* Scop. and *Ulmus* sp. L. Additionally, the following species were taken into account in order to achieve the relevant number of lichen samples and to ultimately ensure the statistical significance per site relating to lichen sampling: *Carpinus betulus* L., *Castanea sativa* Mill., *Corylus avellana* L., *Fagus sylvatica* L., *Pinus sylvestris* L., *Robinia pseudoacacia* L., *Salix* spp. L. *Sambucus nigra* L. and *Sorbus torminalis* (L.) Crantz.

Only epiphytic lichens were collected for all investigations (*Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor, *Xanthoria parietina* (L.) Th.Fr., *Physcia adscendens* (Fr.) Oliv. and *Physcia tenella* (Scop.) DC.). All species reflect more or less relatively tolerant and also widespread lichen species in Germany. Two acidophilic species (*Hypogymnia physodes* (L.) Nyl. and *Parmelia sulcata* Taylor) and three nitrophilic species (*Xanthoria parietina* (L.) Th.Fr., *Physcia adscendens* (Fr.) Oliv. and *Physcia tenella* (Scop.)) were used for the investigations. Within the scope of the last study (Chapter 4), the moss species *Hypnum cupressiforme* Hedw., *Pleurozium schreberi* (Brid.) Mitt., and *Pseudoscleropodium purum* (Hedw.) M. Fleisch were added for the comparison between different biomonitor techniques. In total, 1057 lichen samples, 348 tree bark samples,

In Chapters 2 and 4 the same methodology was applied for comparisons of deposition data of deposition collectors. Within a radius of 2 km around each respective deposition station, lichens were collected from carefully selected trees. All selected trees met the requirements for bioindication with lichens (VDI, 2005). The VDI Guideline 3957/13 (VDI, 2005), does not only include well-defined requirements relating to tree characteristics, it stipulates the tree species that may be sampled. These are: *Acer platanoides* L., *Acer pseudoplatanus* L., *Alnus glutinosa*

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153 moss samples and 24 deposition water samples, were analysed for the present doctoral thesis.

The above description is intended an initial overview of the research design and the main research organisms. Detailed descriptions regarding data acquisition and preparation are found in each of the following publications.

This thesis is clearly focused on lichens in relation to N depositions and the associated chemical N parameters. Additional data sets concerning the various investigated tree species (pH, conductivity,  $\delta^{13}\text{C}$  ratios and specific bark characteristics) and also additional  $\delta^{13}\text{C}$  analyses of lichens are presented in part in the final report of the DBU-project and they will be used in future publications.

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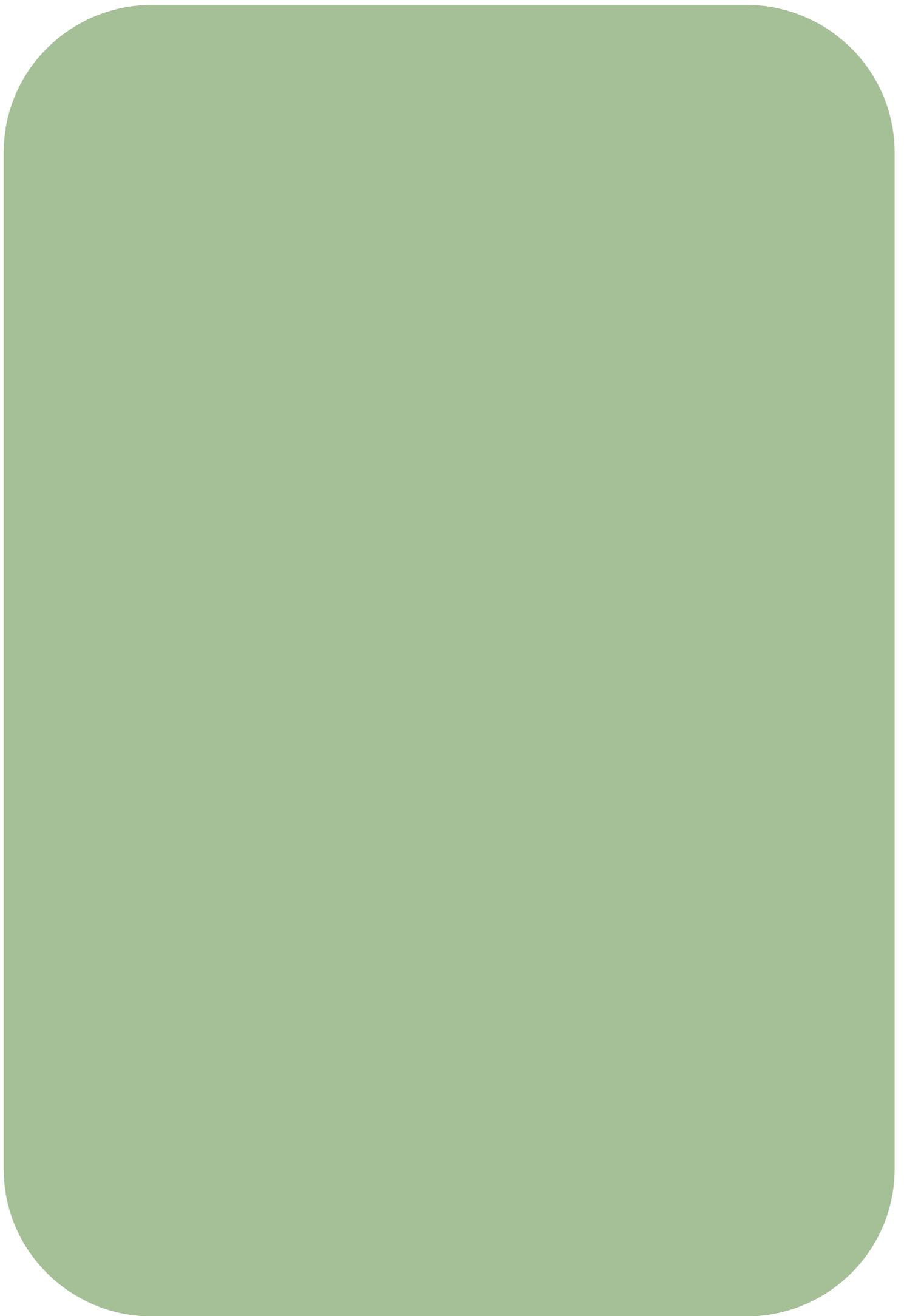
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# Chapter 2

Source attribution of agriculture related deposition by using total nitrogen and  $\delta^{15}\text{N}$  in epiphytic lichen tissue, bark and deposition water samples in Germany

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## Source attribution of agriculture related deposition by using total nitrogen and $\delta^{15}\text{N}$ in epiphytic lichen tissue, bark and deposition water samples in Germany

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**Abstract** Compared with physico-chemical deposition measurement methods, lichens are able to identify the longterm overall effects of high N pollution concentrations in the air. In addition, the natural abundances of the stable isotope of N,  $^{15}\text{N}$ , are being widely used in research on N cycling in ecosystems. They can also be used as instruments for source attribution. In this study, epiphytic lichens were tested to determine whether their respective N content and  $\delta^{15}\text{N}$  ratios can be used to estimate N deposition rates and to locate various sources of N compounds. Epiphytic lichen and bark samples were collected from around various deposition measurement field stations at different sites in the western part of Germany. The N content of epiphytic lichens reflects the species-specific, agriculture-related circumstances of N deposition at various sites in Germany. At the same time,  $\delta^{15}\text{N}$  signatures of the different investigated epiphytic lichen species and bark samples are highly depleted in  $^{15}\text{N}$  under high ammonium deposition. The different surface types of lichens and barks exhibit different concentrations of N and  $\delta^{15}\text{N}$  ratios, despite being exposed to similar N deposition rates. The verification of highly negative  $\delta^{15}\text{N}$  ratios at sites with local and regional emitters shows that source attribution is possible by comparing different  $\delta^{15}\text{N}$  signatures in areas with a wide range of different N deposition types and the corresponding differences in  $\delta^{15}\text{N}$  among various source N pools. Especially nitrophytic lichens can support the on-site instrumentation measuring N deposition by qualification and quantification.

**Keywords** Agriculture Deposition; Bark; Deposition Water; Isotope Ecology; Lichen; Nitrogen-15; Germany

### 1. Introduction

Over the past century, humans have made immense and all-time changes to the global nitrogen (N) cycle, using the Haber–Bosch process to convert atmospheric di-nitrogen ( $\text{N}_2$ ) into many reactive nitrogen ( $\text{N}_r$ ) compounds [1]. The atmospheric deposition of oxidised ( $\text{NO}_y$ ) and reduced N ( $\text{NH}_x$ ) has increased about threefold between pre-

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industrial times and the early 1990s [2], and it is clear that nowadays the rates of N deposition are steadily becoming a bigger problem with the growth in the world population [1,3]. The main risks of N<sub>r</sub> for the world population include the negative impacts on water quality, air quality, greenhouse balance, ecosystems, biodiversity and soil quality [4]. Without doubt, the extensive eutrophication of habitats has a negative impact on protected area systems (e.g. ecological network Natura 2000) and impairs the functioning and structure [5] and, more broadly, the self-regulation of ecosystems. Almost all natural terrestrial ecosystems in the temperate zones are N limited. Significantly, more than 70% of red-listed endangered species in Germany are N deficiency indicator plants [6]. The loss of biodiversity and self-regulation of various ecosystems necessitates an urgent qualitative and quantitative research into N inputs. Since pollution control measures in Europe have been more successful in controlling emissions of oxidised N forms than those of reduced N forms [7], areas with high rates of N deposition, where most of the N originates from NH<sub>x</sub> from agricultural activities, are increasing continuously or remain at a very high rate [8,9].

Agricultural activities are the dominant sources of the reactive pollutant ammonia (NH<sub>3</sub>) and its reaction product ammonium (NH<sub>4</sub><sup>+</sup>). Ammonia is primarily emitted near the ground level in rural environments [8,10]. Because of the afore-mentioned characteristics of NH<sub>3</sub>, researchers have to deal with a long-range pollutant transport (NH<sub>4</sub><sup>+</sup>), on the one hand, and with the major effect of NH<sub>3</sub> on a local scale, on the other hand, with emission and receptor areas often being situated closely together [10]. The spatial variability of NH<sub>3</sub> and the knowledge that often a large part of the deposition measurements is probably outside the reach and influence of substantial NH<sub>3</sub> or NH<sub>4</sub><sup>+</sup> deposition, due to the most inadequate and not widespread number of measurement sites [10], make it more urgent to find a reliable alternative to test the agriculture-related impacts satisfactorily.

Epiphytic lichens have been shown to be outstanding indicator organisms in a wide range of air pollution studies [11–20]. They are being used effectively and successfully in air pollution control in the context of environmental impact studies, in immission impact land registers and in regional/urban planning for the identification and delineation of polluted areas, as well as for the long-term documentation of changes in the environment [16,21–23]. The main application area of lichens as indicators of air pollution is in the assessment of immissions in regional conurbations; they are rarely used in rural regions [24]. Moreover, most of the research studies on lichens in the past few decades have dealt with sulphur and heavy metal concentrations in the atmosphere and only few studies with N [16]. But in the past few years, monitoring of N deposition using lichens has increased, and diverse studies have confirmed that lichens provide a fairly precise

picture of the atmospheric N deposition in the environment. Due to their lack of cuticles, stomata and roots, lichens are not limited in terms of where they absorb nutrients and are therefore very active metabolically and open to receive various atmospheric N-containing compounds from both wet and dry depositions [25,26]. The fact that epiphytic lichens reflect the surrounding N deposition, especially from agriculture-derived N-containing compounds, has been confirmed in many studies [27–37].

In order to not just assess N deposition quantitatively, but also identify the qualitative N source, isotope research can be used. For explaining the interrelations between plants and the environment, the relationships between elemental concentrations and isotopic signatures have been studied extensively [38–41]. The abundance of  $^{15}\text{N}$  is a valuable and widely used indicator of the sources and pathways of N in organisms and ecosystems [42,43]. Currently, there are only a few reports on lichen research that has used the natural abundance of  $^{15}\text{N}$  and integrated it with N monitoring to identify various N sources in field experiments [33,44–48]. Because ‘conventional’ deposition measurement methods are affected by the sources of methodical and analytical errors, the present study researched the effects of atmospheric N deposition on epiphytic lichens. Thereby, it was explored whether it is possible that the tissue N content and the  $\delta^{15}\text{N}$  signature of different lichen species can act as quantitative and qualitative indicators for the estimation of N impact in agriculturally related areas. This study focused on different but narrowly related hypotheses:

- (i) It is assumed that lichens, under currently increased N deposition rates, react by increasing the N concentration in their tissue and that this response is species-specific.
- (ii) Data obtained from lichen monitoring and from the bark samples taken correlate with data obtained from N monitoring networks based on physico-chemical measurements. Furthermore, the N content of lichens and barks reflects the influence of various components of the N atmosphere.
- (iii) Different  $\delta^{15}\text{N}$  values of lichen and bark samples allow researchers to identify the main N source of deposition on a gradient of increased agricultural activities.
- (iv) Different receptor surfaces (lichens and barks) exhibit different concentrations of N and  $\delta^{15}\text{N}$  signatures, despite being exposed to similar N deposition.

These research issues were tested with 383 lichen samples, 161 bark samples and 24 deposition water samples from 24 sampling sites based on the national monitoring networks in western Germany.

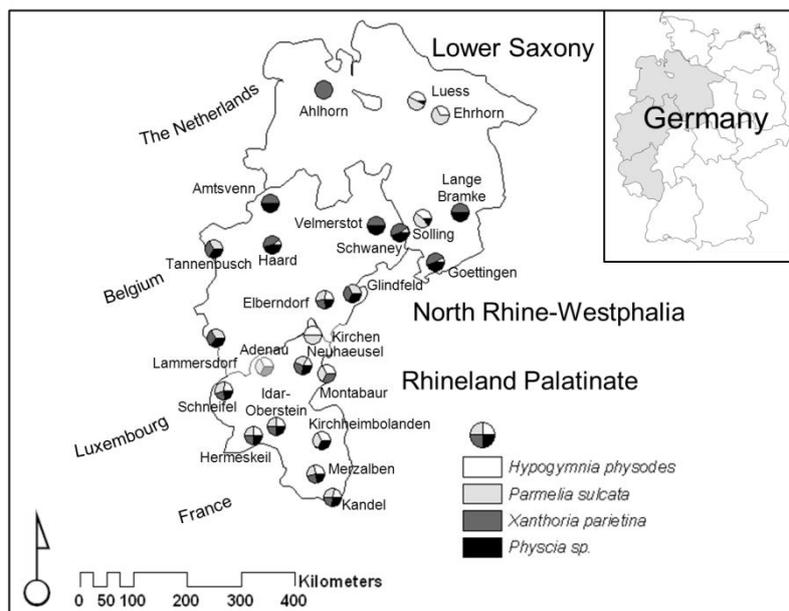
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## 2. Material and methods

### *2.1. Study area*

The investigation in the western part of Germany was adapted to different wet-only deposition measurement networks to monitor the inputs of pollutants reaching the soil, vegetation and surface water via precipitation (wet deposition). These networks were established by different institutes of the federal states Rhineland-Palatinate (RP), North Rhine-Westphalia (NW) and Lower Saxony (NI). The selected 24 reference deposition stations (Fig. 2.1) are located in open fields and in relatively rural areas, where the deposition levels of air pollutants transported over long distances and across national borders can be measured. The sites were chosen by present chemical analyses, different data clusters and a north–south gradient network of stations that detects a wide range of different N deposition type spectra in the area of interest. The main focus was on whether there were any specific kinds of agriculture-affected areas. The investigated sites vary in elevation between 30m a.s.l. at the sites Tannenbusch (Lower Rhine region, NW) and Ahlhorn (Geest, NI) and up to 690 m a.s.l. at the site Schneifel (in the Eifel mountains, RP). The investigation area is mainly characterised by the central uplands and the northern lowlands of western Germany. A temperate seasonal climate is dominant in this area, in which humid westerly winds are prevalent. Precipitation distribution is associated with landform configuration.

The highest average amount of annual precipitation was measured at the Elberndorf (NW) site, with 1629 mm yr<sup>-1</sup>, while the lowest amount of precipitation was 745 mm yr<sup>-1</sup> measured at the Kirchheimbolanden (RP) site. The study area is mainly characterised by heterogeneous rural areas with extensive agricultural areas and forests in the south and by the regional conurbations called the ‘Rhine-Ruhr’ metropolitan region in NW, with a high traffic volume. In the northern part of the study area, data collection sites are mainly located in high-intensity agricultural areas with animal husbandry and arable farming (Westphalian Lowland, Lower Rhine Plain, Duemmer and Ems-Hunte Geest and East Frisian Geest).



**Figure 2.1** Sampling sites and distribution of collected lichens within the study area in north-western part of Germany.

## 2.2. Deposition data

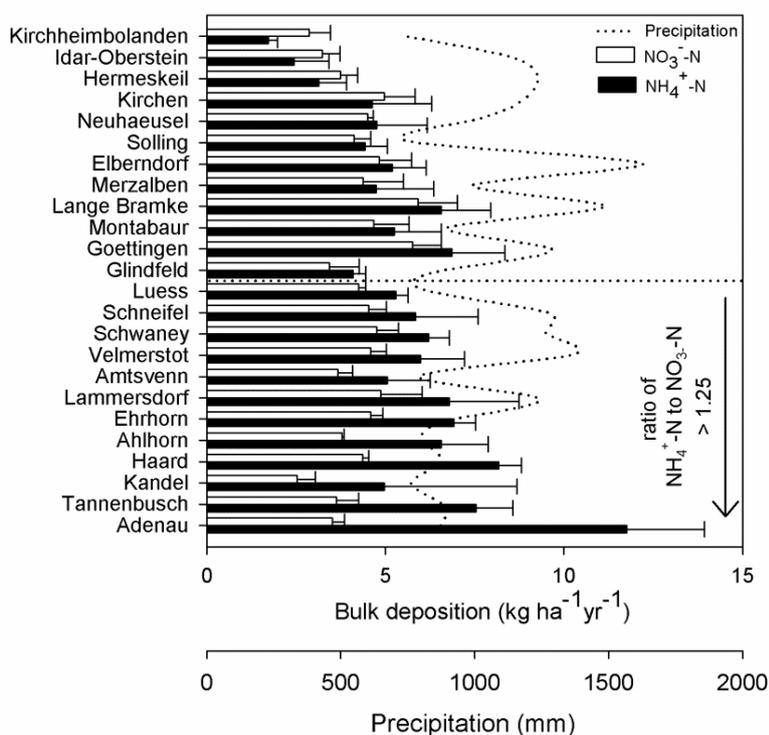
Deposition data were obtained from each responsible institution in the monitoring network, in the respective federal state. The wet-only deposition data, which include nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ) and mean annual precipitation, were available from 24 open field sites in the study area. The installation and methods of measurement employed at each field deposition site are described by the Norwegian Institute for Air Research [49]. They are furthermore adjusted to research instructions laid down in the International Cooperative Programme on assessment and monitoring air pollution effects on forests [50].

Deposition data from the years 2006–2008 were considered. Average ammonium–nitrogen ( $\text{NH}_4^+\text{-N}$ ) deposition ranged from  $1.73 \text{ kg ha}^{-1} \text{ yr}^{-1}$  (Kirchheimbolanden, RP) to  $11.76 \text{ kg ha}^{-1} \text{ yr}^{-1}$  (Adenau, RP). Nitrate–nitrogen ( $\text{NO}_3^-\text{-N}$ ) deposition varied from  $2.53 \text{ kg ha}^{-1} \text{ yr}^{-1}$  at the Kandel site (RP) to  $5.92 \text{ kg ha}^{-1} \text{ yr}^{-1}$  at Lange Bramke (NI) (Fig. 2.2). In addition to this, deposition water samples from March 2010 (RP, NW and NI) were analysed for  $\text{NO}_3^-$  (at wavelengths of 218 nm and 228 nm) and  $\text{NH}_4^+$  using a photometer [51] (Shimadzu, UV-160A, Kyoto, Japan). The  $\delta^{15}\text{N}$  values of nitrate–nitrogen ( $\delta^{15}\text{N}\text{-NO}_3^-\text{-N}$ ) and ammonium–nitrogen ( $\delta^{15}\text{N}\text{-NH}_4^+\text{-N}$ ) were measured using the extraction method of Stark and Hart [52], followed by determination 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 analytical precision ( $n = 4$ ) was  $\pm 0.1\%$ .

### 2.3. Field sampling and chemical analyses

From November 2008 to February 2009, samples of the following epiphytic lichens were collected: *Hypogymnia physodes* (L.) Nyl., *Parmelia sulcata* Taylor, *Xanthoria parietina* (L.) Th. Fr., *Physcia adscendens* (Fr.) Oliv. and *Physcia tenella* (Scop.) DC (Fig. 2.1). The latter two lichen species were put together in the *Physcia* sp. category in this investigation. The decisive main criteria for the selection of these lichen species were their levels of dispersion and the ease of sample collection and preparation. Samples were collected from trees that met the requirements for bioindication with lichens within a 2 km radius around the 24 deposition measurement field stations [53]. Besides the tree species listed in the VDI guideline 3957/13 [53], the following tree species were studied: *Castanea sativa*, *Corylus avellana*, *Fagus sylvatica*, *Robinia pseudoacacia*, *Salix* sp., *Sambucus nigra* and *Sorbus torminalis*. Along with the lichens, bark samples were taken from the respective trees. In order to guarantee a representative sampling, five replications per lichen species were collected at each field site.



**Figure 2.2** Overview of average precipitation,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  deposition at the investigated sites. Data are expressed in means  $\pm$   $SD$  (period 2006–2008). Study sites are ordered from the lowest  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratio to the highest  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratio of bulk deposition. (Data were obtained from the Research Institute for Forest Ecology and Forestry – Rhineland-Palatinate, the State Office for Nature, Environment and Consumer Protection – North Rhine-Westphalia and the Northwest German Forest Research Station – Lower Saxony).

Complete lichen thalli were removed neatly. After removal, the materials were put into paper bags, labelled and stored in a cold environment. After field work, the samples were

stored in a toploader freezer at  $-18^{\circ}\text{C}$ . Before being analysed, the lichen and bark samples were cleaned from obvious contaminants, and then all the samples were freeze-dried (Martin Christ GmbH, type 101541, Osterode, Germany) and pulverised for homogenisation using an agate-typed ball mill (Fritsch GmbH, Pulverisette 9, Idar-Oberstein, Germany). Small sample sizes were additionally pulverised with a vibrating teflon-typed ball mill (Retsch GmbH, type MM2, Haan, Germany). Subsequently, all the samples were preserved in a desiccator, and subsamples of 3–4 mg of each sample were put into tin capsules ( $6 \times 6 \times 12$  mm, IVA Analysentechnik e. K., Duesseldorf, Germany) and their total N concentration was analysed. The  $^{15}\text{N}$  natural abundance was analysed using the isotope-ratio mass spectrometer (Thermo Scientific, Flash EA 1112 Series + IRMS Delta VI Advantage-Isotope ratio MS, Waltham/USA) at the Department of Geobotany, University of Trier. The  $\delta^{15}\text{N}$  values are reported in per mill (‰) relative to air as the international standard for N and the analytical precision ( $n = 2$ ) is  $\pm 0.1\%$ . In addition, the diluted bark samples [54,55] were tested for  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N by continuous flow analysis with integrated proteolysis (Alpkem Corporation, RFA-300, Oregon, USA) after the bark extractions were centrifuged at 4000 rpm for half an hour (Thermo Scientific, Heraeus Multifuge X3R, Waltham, USA).

#### 2.4. Data analyses

Data qualifications of normality and homogeneity of variance were tested with the aid of the Kolmogorov–Smirnov test and the Levene test, respectively. Significant differences between mean values were analysed using a one-way variance analysis (ANOVA) followed by Scheffé's or Tukey's *post hoc* test. Pearson product-moment correlation coefficients were calculated to study the relationship between lichen and bark tissue concentrations and parameters derived from physical and chemical measurements as well as data obtained from other sources. Significance levels were  $< 0.05$  and  $p < 0.001$  for statistics. Critical  $p$  values between  $p > 0.05$  and  $< 0.1$  were counted as marginally significant (+)

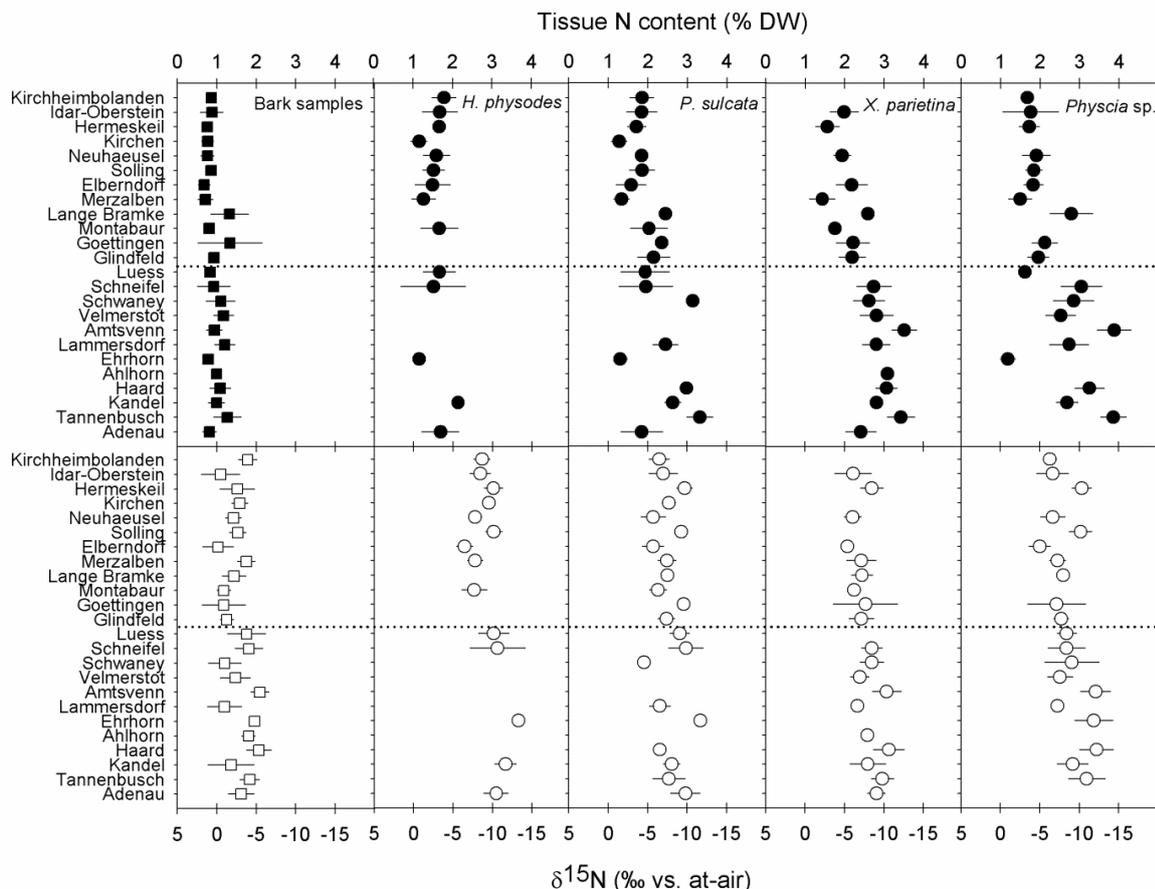
### 3. Results

#### 3.1. N content in the epiphytic lichen and bark samples

*Lichens.* The distribution of lichen species (Fig. 2.1) shows that the proportion of nitrophytic lichen species (*X. parietina* and *Physcia* sp.) increased from south to north. In less N-loaded low mountain ranges, acidophytic lichen species (*H. physodes* and *P.*

*sulcata*) were frequently found and used as samples. Figure 2.1 presents a first indication of the different sensitivities of various lichen species to increased N deposition rates. In what follows, the measured N data are itemised as mean  $\pm$  *SD*. The N content in the lichen *H. physodes* ( $n = 80$ ) ranged between 0.71% and 2.64%, with both extremes of N concentrations being found at the Schneifel (RP) site. The average was  $1.57\% \pm 0.42$  for *H. physodes*. *P. sulcata* ( $n = 110$ ) showed ranges between 1.01% at the Kirchen site in RP and 3.78% at Tannenbusch in NW. On average,  $1.94\% \pm 0.60$  was measured in *P. sulcata*. The results obtained for the nitrophytic lichen *X. parietina* ( $n = 96$ ) varied from 0.93% (Merzalben, RP) to 3.83% (Amtsvenn, NW). The mean N concentration was  $2.48\% \pm 0.65$ . *Physcia* sp. ( $n = 97$ ) showed concentrations of 1.04% (Merzalben, RP) and 4.61% (Amtsvenn, NW). The mean value for *Physcia* sp. was calculated to be  $2.41\% \pm 0.81$  (Fig. 2.3). Multiple comparisons revealed significant differences ( $p < 0.05$ ) in N tissue concentrations between the lichen species. The nitrophytic lichens *X. parietina* and *Physcia* sp. were significantly enriched in N. The epiphytic lichen *P. sulcata* ranked in the middle, while this study was able to detect the lowest concentrations of N in *H. physodes*.

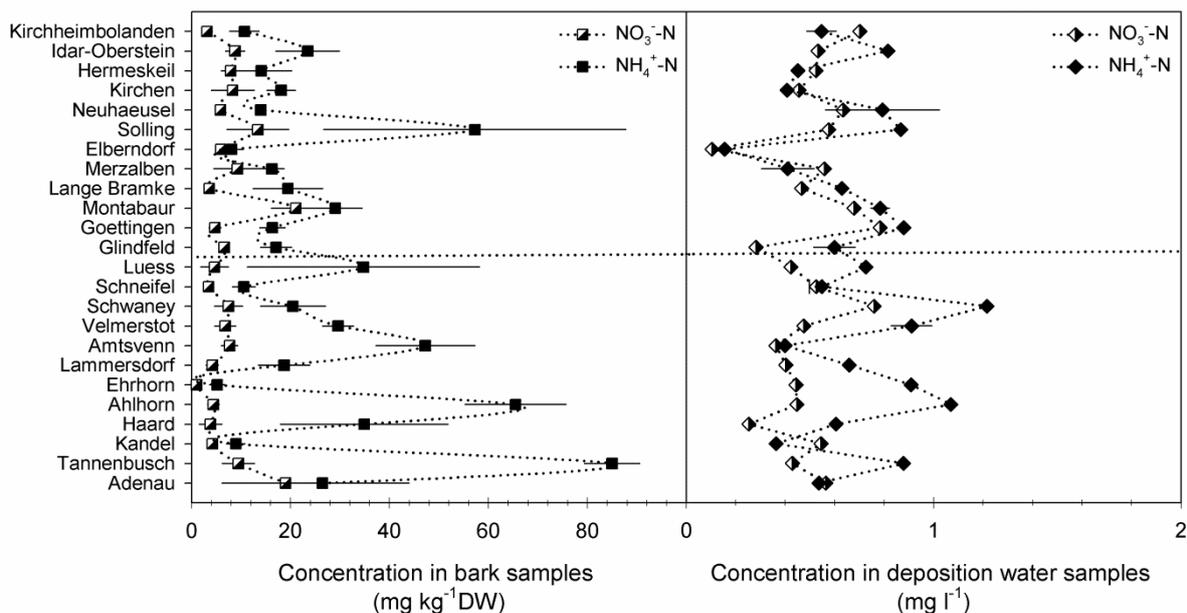
*Bark.* The concentrations of N in the bark samples ( $n = 161$ ) ranged from 0.37% (Idar-Oberstein, RP) to 2.50% (Goettingen, NI) with an average of  $0.92\% \pm 0.30$ . The mean values of the analysed bark samples per site ( $n = 24$ ) varied from 0.68% (Elberndorf, NW) to 1.33% (Goettingen, NI). The N% in the bark samples was, on average, significantly lower in RP and higher in NI. The results for the sites in NW ranked in the middle. A closer look indicated that the concentrations of N in lichens as well as in the bark samples were highest at the sites located in the western part of the study area. Basically, a higher N content could be determined in lichens at the study sites where the ratio of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in the deposition was above 1.25. Especially in NW, the highest N concentrations were measured in the nitrophytic lichens *X. parietina* and *Physcia* sp. The bark samples showed marginally higher concentrations of N in NW and NI. Figure 2.3 shows a ranking of the study sites by the increase in the  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratio.



**Figure 2.3** N content and  $\delta^{15}\text{N}$  ratios of all the investigated lichen species and N content and the respective  $\delta^{15}\text{N}$  ratios of the bark samples at each study site. Data of lichens are expressed in means  $\pm$  *SD* (*H. physodes* 5 < *n* > 12, *P. sulcata* 1 < *n* > 15, *Physcia* sp. 2 < *n* > 6 and *X. parietina* 1 < *n* > 6). Data of the bark samples represent means  $\pm$  *SD* (5 < *n* > 16).

### 3.2. $\delta^{15}\text{N}$ values of lichens, bark samples and deposition waters and their respective measured ammonium and nitrate concentrations

*Lichens.* The  $\delta^{15}\text{N}$  signatures of *H. physodes* ( $n = 80$ ) varied from  $-14.4\text{‰}$  (Ehrhorn, NI) to  $-5.1\text{‰}$  (Elberndorf, NW). The mean  $\delta^{15}\text{N}$  for *H. physodes* was  $-9.5\text{‰} \pm 2.1$ . *P. sulcata* ( $n = 110$ ) showed isotopic signatures between  $-12.5\text{‰}$  (Schneifel, RP) and  $-3.5\text{‰}$  (Elberndorf, NW). On average,  $-8.1\text{‰} \pm 2.1$  was measured in *P. sulcata*. In *X. parietina* ( $n = 96$ ), this study detected  $\delta^{15}\text{N}$  signatures between  $-12.8\text{‰}$  at the Amtsvenn site in NW and  $-2.4\text{‰}$  (Idar-Oberstein) in RP. The mean  $\delta^{15}\text{N}$  value was  $-7.8\text{‰} \pm 2.1$ . The  $\delta^{15}\text{N}$  signatures of *Physcia* sp. ( $n = 97$ ) varied from  $-14.6\text{‰}$  (Amtsvenn, NW) to  $-1.9\text{‰}$  (Goettingen, NI) (average  $-8.5\text{‰} \pm 2.6$ ) (Fig. 2.3). The  $\delta^{15}\text{N}$  ratios measured in *H. physodes* were, in general, significantly ( $p < 0.05$ ) more negative than the  $\delta^{15}\text{N}$  ratios determined in the other investigated lichen species.

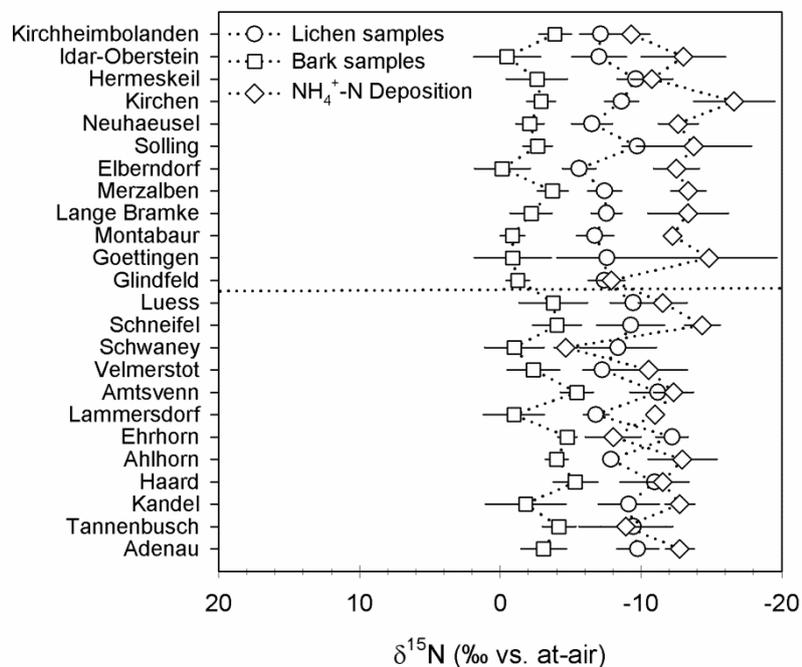


**Figure 2.4** Concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in the deposition water samples from the year 2010 (means  $\pm$  *SD*,  $n = 3$ ) and concentrations of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  of the collected bark samples (2008/2009) at the respective study sites (means  $\pm$  *SE*,  $5 < n > 16$ ).

*Bark.* The  $\delta^{15}\text{N}$  signatures of the sample trees ( $n = 161$ ) ranged from  $-8.0\text{‰}$  at the Kandel site in RP to  $4.0\text{‰}$  at Elberndorf in NW. The averaged  $^{15}\text{N}$  signature of all trees was  $-2.6\text{‰} \pm 2.1$  (Fig. 2.3). The average  $\text{NH}_4^+\text{-N}$  concentration in the barks of the sample trees at their respective sites ( $n = 24$ ) ranged between  $5.19 \text{ mg kg}^{-1}$  (Ehrhorn, NI) and  $84.96 \text{ mg kg}^{-1}$  (Tannenbusch, NW). The mean  $\text{NH}_4^+\text{-N}$  value was  $26.29 \text{ mg kg}^{-1} \pm 19.67$ . Regarding the  $\text{NO}_3^-\text{-N}$  concentration, the smallest concentration of  $1.04 \text{ mg kg}^{-1}$  was measured at the Ehrhorn site (NI), while the largest concentration of  $21.06 \text{ mg kg}^{-1}$  was detected in RP (Montabaur). The mean  $\text{NO}_3^-\text{-N}$  value was  $7.28 \text{ mg kg}^{-1} \pm 4.75$  (Fig. 2.4).

*Deposition waters.* Regarding the analysed deposition water samples of the study sites in RP, NW and NI for the year 2010 ( $n = 24$ ), the  $\delta^{15}\text{N}\text{-NH}_4^+\text{-N}$  varied from  $-16.6\text{‰}$  at Kirchen (RP) to  $-4.7\text{‰}$  at Schwaney (NW), while its average was  $-11.7\text{‰} \pm 2.8$ . The  $\delta^{15}\text{N}\text{-NO}_3^-\text{-N}$  found in the deposition water samples ranged from  $-15.3\text{‰}$  (Idar-Oberstein, RP) to  $-7.3\text{‰}$  (Glindfeld, NW). The mean  $\delta^{15}\text{N}\text{-NO}_3^-\text{-N}$  was  $-11.5\text{‰} \pm 1.9$ . The measured concentrations of  $\text{NH}_4^+\text{-N}$  in the deposition samples ranged from  $0.16$  to  $1.22 \text{ mg l}^{-1}$ . Both extremes were measured in NW (mean =  $0.67 \text{ mg l}^{-1} \pm 0.25$ ). The concentrations of  $\text{NO}_3^-\text{-N}$  were found to be between  $0.10 \text{ mg l}^{-1}$  (Elberndorf, NW) and  $0.78 \text{ mg l}^{-1}$  (Goettingen, NI) (mean =  $0.48 \text{ mg l}^{-1} \pm 0.14$ ) (Fig. 2.4). The results of the one-way ANOVA and the Levene test used for the comparison of different  $\delta^{15}\text{N}$  values of the

lichen, bark and deposition water samples revealed a significant variance ( $p < 0.05$ ) for the three analysed variables. The  $\delta^{15}\text{N}$ - $\text{NH}_4^+$ -N ratios in the deposition water samples were more negative in comparison with the  $\delta^{15}\text{N}$  signatures measured in the lichen samples. The  $\delta^{15}\text{N}$  ratios in the bark samples were less negative (Fig. 2.5).



**Figure 2.5** Tissue  $\delta^{15}\text{N}$  ratios of all the analysed lichens (means ( $5 < n > 32$ )  $\pm$   $SD$ ), tissue  $\delta^{15}\text{N}$  ratios of the sample trees (means ( $5 < n > 16$ )  $\pm$   $SD$ ) and  $\delta^{15}\text{N}$  signatures of  $\text{NH}_4^+$ -N measured in the deposition water samples (means ( $n = 4$ )  $\pm$   $SD$ ) for each study site.

### 3.3. N content and $\delta^{15}\text{N}$ values of the lichen and bark samples in comparison with N related data

*Lichens.* At sites with high N deposition, this study was able to find an increase in N content irrespective of the lichen species. Especially  $N_{\min}$  (sum of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N) ( $r = 0.232$ ,  $p < 0.01$ ),  $\text{NH}_4^+$ -N deposition ( $r = 0.304$ ,  $p < 0.01$ ) and the ratio of  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N ( $r = 0.335$ ,  $p < 0.01$ ) showed a significant linear relationship. There was only a weak correlation between the  $\delta^{15}\text{N}$  values of  $\text{NH}_4^+$  in the deposition waters and the N content of the investigated lichens ( $r = 0.187$ ,  $p < 0.01$ ). However, similarly significant relationships were found between the  $\delta^{15}\text{N}$  signatures of  $\text{NO}_3^-$  in the deposition waters and the N content of lichen tissues ( $r = 0.102$ ,  $p < 0.05$ ) (Tab. 2.1). By considering each lichen species, positive correlations of the N content of *Physcia* sp. ( $r = 0.622$ ,  $p < 0.01$ ) and *X. parietina* ( $r = 0.447$ ,  $0.1 > p < 0.05$ ) with  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N ratio in the deposition water samples were found. The results obtained for both nitrophytic lichen species also indicated a relationship between  $\text{NH}_4^+$ -N deposition and N content in their thalli (Fig. 2.6). When considering N content and  $\text{NO}_3^-$ -N deposition, only *H. physodes* ( $r = -0.786$ ,  $p < 0.01$ ) showed a negative correlation. There was no correlation

between  $\text{NO}_3^-$ -N and N content in the other researched lichen species (Tab. 2.1). The measured N content in lichen species at their respective sites correlated positively with the N content of the other predominant lichen species. The N contents of *H. physodes* and *P. sulcata* were similar and correlated strongly ( $r = 0.929$ ,  $p < 0.01$ ). In addition to this result, positive correlations were detected between the N content (%) of *X. parietina* and that of *Physcia* sp. ( $r = 0.956$ ,  $p < 0.01$ ), followed by *P. sulcata* and *Physcia* sp. ( $r = 0.879$ ,  $p < 0.01$ ) or rather *P. sulcata* and *X. parietina* ( $r = 0.825$ ,  $p < 0.01$ ) (Tab. 2.1). The  $\delta^{15}\text{N}$  values showed a similar trend in each lichen species at the respective sites. Isotopic signatures correlated strongly between both the nitrophytic lichens *X. parietina* and *Physcia* sp. ( $r = 0.965$ ,  $p < 0.01$ ), followed by *H. physodes* and *P. sulcata* ( $r = 0.885$ ,  $p < 0.01$ ). *H. physodes* also showed  $\delta^{15}\text{N}$  signatures similar to those shown by *Physcia* sp. ( $r = 0.897$ ,  $p < 0.01$ ) and *X. parietina* ( $r = 0.873$ ,  $p < 0.01$ ) at the same study sites (Tab. 2.1). Concerning  $\delta^{15}\text{N}$  signatures determined in lichen tissues and present N deposition, a significant ( $p < 0.001$ ) slightly negative trend could be detected for all the lichen samples, by an increased input of  $\text{N}_{\text{min}}$ ,  $\text{NH}_4^+$ -N deposition and quotient of  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N in the deposition. The strongest negative correlation was detected between the  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N ratio in the deposition waters and nitrophytic lichens (Fig. 2.6). With an increase in  $\text{NH}_4^+$ -N/ $\text{NO}_3^-$ -N in the deposition, a simultaneous depletion of  $^{15}\text{N}$  for *X. parietina* ( $r = -0.532$ ,  $p < 0.05$ ) and *Physcia* sp. ( $r = -0.584$ ,  $p < 0.01$ ) was observed. With an increase in  $\text{NH}_4^+$ -N deposition, only a marginal negative trend with the  $\delta^{15}\text{N}$  values of *Physcia* sp. ( $r = -0.405$ ,  $0.1 > p < 0.05$ ) was observed (Tab. 2.1). With an increase of nitrate in the bulk deposition, a weak enrichment of  $\delta^{15}\text{N}$  in all the lichen species ( $r = 0.137$ ,  $p < 0.01$ ) was observed simultaneously. The  $\delta^{15}\text{N}$ - $\text{NO}_3^-$ -N in the deposition correlated in the same positive way ( $r = 0.166$ ,  $p < 0.01$ ) (Tab. 2.1). Moreover, a relationship between the increase in the N content in nitrophytic lichens and simultaneous  $^{15}\text{N}$  depletion in lichen tissues was observed. The N content of *X. parietina* showed a significantly negative trend with the  $\delta^{15}\text{N}$  value of *X. parietina* ( $r = -0.617$ ,  $p < 0.01$ ) and *Physcia* sp. ( $r = -0.592$ ,  $p < 0.05$ ). The N content of *Physcia* sp. also correlated with the isotopic signatures of *X. parietina* ( $r = 0.763$ ,  $p < 0.01$ ) (Tab. 2.1).

**Bark.** By investigating the relationship between the N content (%) in the bark samples and nitrogenous compounds in the deposition, this study detected that  $\text{N}_{\text{min}}$  ( $r = 0.405$ ,  $p < 0.05$ ) and  $\text{NH}_4^+$ -N ( $r = 0.348$ ,  $0.1 > p < 0.05$ ) had a marginal influence on the increase in the N content (%) in the bark samples. Concerning the analysed bark samples, the study additionally revealed that the N content of all the researched lichens correlated significantly with the mean N content measured in the respective sample trees at the respective sites ( $r = 0.469$ ,  $p < 0.01$ ). Linear increases in N concentrations in the lichens

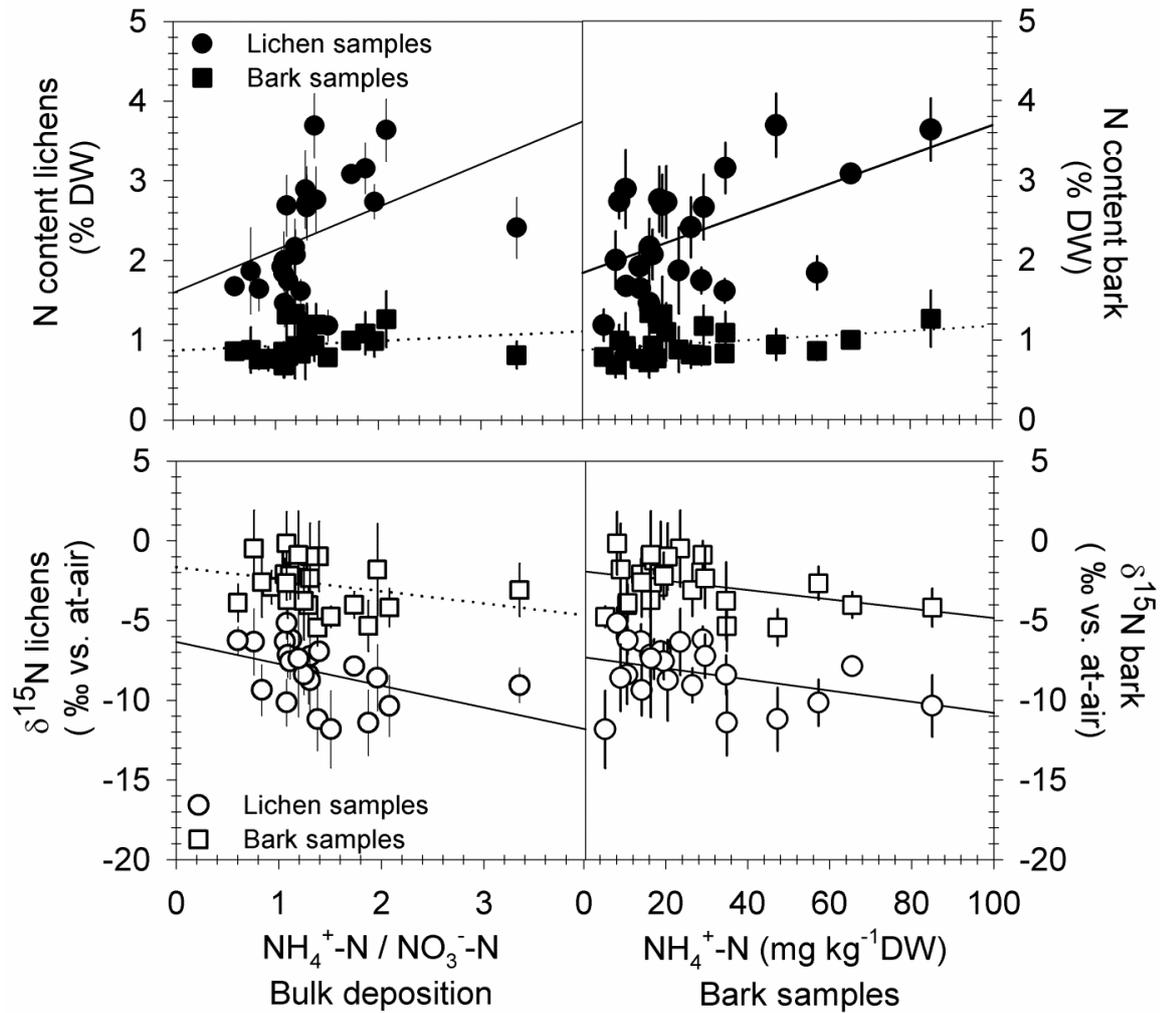
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coincided with increases in N concentration in the sample trees for all species ( $r = 0.508$ ,  $p < 0.01$ ) with a better fit for *P. sulcata* ( $r = 0.790$ ,  $p < 0.01$ ) and *H. physodes* ( $r = 0.632$ ,  $p < 0.05$ ). Less clear relationships were detected in *X. parietina* ( $r = 0.579$ ,  $p < 0.01$ ) and *Phycia* sp. ( $r = 0.616$ ,  $p < 0.01$ ) (Tab. 2.1). Regarding the  $\delta^{15}\text{N}$  values of the bark samples and the associated  $\delta^{15}\text{N}$  values of the lichen species, positive correlations were found. If depletion of  $^{15}\text{N}$  in the lichens existed, the bark samples also showed depletion of  $^{15}\text{N}$  at the study site ( $r = 0.485$ ,  $p < 0.01$ ). The closest relationship was found in *X. parietina* ( $r = 0.795$ ,  $p < 0.01$ ), followed by *Phycia* sp. ( $r = 0.698$ ,  $p < 0.01$ ) and *H. physodes* ( $r = 0.606$ ,  $p < 0.05$ ). *P. sulcata* showed less significant correlations ( $r = 0.441$ ,  $p < 0.05$ ) (Tab. 2.1). Simultaneously, the increase in the analysed  $\text{NH}_4^+\text{-N}$  concentrations in the bark samples correlated significantly with the N content (%) of *X. parietina* ( $r = 0.621$ ,  $p < 0.01$ ) and *Phycia* sp. ( $r = 0.538$ ,  $p < 0.05$ ) (Fig. 2.6) and correlated less significantly with that of *P. sulcata* ( $r = 0.481$ ,  $p < 0.05$ ). The nitrophytic lichens *X. parietina* ( $r = -0.501$ ,  $p < 0.05$ ) and *Phycia* sp. ( $r = -0.482$ ,  $p < 0.05$ ), however, were the only epiphytic lichens to show a negative correlation between the increases in the  $\text{NH}_4^+\text{-N}$  concentration in the sample trees and parallel decreases in the  $\delta^{15}\text{N}$  in lichen tissues (Fig. 2.6).

**Table 2.1** Linear correlation coefficients (Pearson  $r$ ) of N deposition data, N related data and N content of all the researched lichen species and bark samples and the respective  $\delta^{15}\text{N}$  signatures ( $n$  = number of samples).

Deposition	2006–2008	Lichens														All						
		2006–2008		2010		Bark		<i>H. physodes</i>		<i>P. sulcata</i>		<i>X. parietina</i>		<i>Physcia</i> sp.								
		$\text{NH}_4^+ - \text{NO}_3^-$ N	$\text{N}_{\text{min}}$	$\text{NH}_4^+ - \text{N} / \text{NO}_3^- - \text{N}$	$\delta^{16}\text{N} - \text{NH}_4^+ - \text{NO}_3^- - \text{N}$	$\delta^{15}\text{N} - \text{NH}_4^+ - \text{NO}_3^- - \text{N}$	$\text{N} / \text{N} \%$	$\delta^{15}\text{N} - \text{N} \%$		$\delta^{15}\text{N} - \text{N} \%$												
Deposition	2006–2008	$n$	24	24	24	24	24	24	24	14	14	21	21	19	19	20	20	383	383			
	2010	$\delta^{15}\text{N} - \text{NH}_4^+ - \text{N}$	24	1	0.276	0.943**	0.867**	0	0.728	0.241	0.207	0.348+–0.208	–0.110	–0.336	0.333	0.237	0.426	–0.454	0.511*	–0.405+	0.304**	–0.216**
Bark	2006–2008	$\text{NO}_3^- - \text{N}$	24	1	0.579**	–0.222	–0.222	–0.063	0.241	–0.111	0.32	0.218	–0.786*	0.233	–0.03	0.033	–0.135	0.265	0.014	0.153	–0.075	0.137**
	2010	$\text{N}_{\text{min}}$	24	1	0.659**	–0.076	0.042	0.143	0.138	0.405*	–0.101	–0.33	–0.236	0.273	–0.19	0.321	–0.297	0.377	–0.237	0.232**	–0.137**	
Lichens	2006–2008	$\text{NH}_4^+ - \text{N} / \text{NO}_3^- - \text{N}$	24	1	0.052	0.079	0.312	0.295	0.174	–0.273	0.225	–0.413	0.335	–0.264	0.447+	–0.532*	0.622**	–0.584**	0.335**	–0.276**		
	2010	$\delta^{15}\text{N} - \text{NH}_4^+ - \text{N}$	24	1	0.468*	0.391+–0.145	0.141	0	0.147	–0.281	0.414*	0.233	0.161	–0.177	0.033	–0.225	0.187**	–0.073	0.166**			
Bark	2006–2008	$\delta^{15}\text{N} - \text{NO}_3^- - \text{N}$	24	1	–0.173	0.07	0.096	0.215	0.306	0.194	0.208	0.336	–0.038	0.055	0.036	0.208	0.102*	0.166**				
	2010	$\text{NH}_4^+ - \text{N}$	24	1	0.275	0.304	–0.368	0.044	0.038	0.481*	0.001	0.621**	–0.501*	0.538*	–0.482*	0.508**	0.075					
Lichens	2006–2008	$\text{NO}_3^- - \text{N}$	24	1	–0.276	0.215	0.08	0.289	–0.087	0.053	–0.33	0.036	0.051	–0.065	–0.180*	0.003						
	2010	$\delta^{15}\text{N}$	24	1	0.069	0.632*	–0.501	0.790**	0.083	0.579**	–0.285	0.616**	–0.119	0.469**	0.085							
<i>H. physodes</i>	2006–2008	$\delta^{15}\text{N}$	24	1	0.351	0.606*	0.036	0.441*	–0.523*	0.795**	–0.280	0.698**	–0.084	0.485**								
	2010	$\text{N} \%$	14	1	–0.007	0.929**	0.223	0.558	–0.249	0.513	0.172											
<i>P. sulcata</i>	2006–2008	$\text{N} \%$	21	1	–0.13	0.885**	–0.599+	0.873**	–0.111	0.897**												
	2010	$\delta^{15}\text{N}$	21	1	0.358	0.825**	–0.596*	0.879**	–0.342													
<i>X. parietina</i>	2006–2008	$\text{N} \%$	19	1	0.028	0.825**	–0.596*	0.879**	–0.342													
	2010	$\delta^{15}\text{N}$	19	1	0.365	0.825**	–0.596*	0.879**	–0.342													
<i>Physcia</i> sp.	2006–2008	$\text{N} \%$	20	1	–0.617**	0.956**	–0.592**															
	2010	$\delta^{15}\text{N}$	20	1	0.763**	0.965**																
All	2006–2008	$\text{N} \%$	383	1	–0.434+																	
	2010	$\delta^{15}\text{N}$	383	1	0.033																	

+0.1 >  $p$  < 0.05.  
\*  $p$  < 0.05.  
\*\*  $p$  < 0.01.  
\*\*\*  $p$  < 0.001.



**Figure 2.6** Positive correlation between averaged tissue N content of the nitrophytic lichens *X. parietina* and *Physcia* sp. ( $r = 0.439$ ,  $p < 0.05$ ,  $n = 23$ ) and N content of suitable trees at sites ( $r = 0.174$ , n.s.,  $n = 24$ ) with quotient  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  in the bulk deposition. The respective  $\delta^{15}\text{N}$  ratios of lichens ( $r = -0.425$ ,  $p < 0.05$ ,  $n = 23$ ) and  $\delta^{15}\text{N}$  ratios of the bark samples ( $r = 0.273$ , n.s.,  $n = 24$ ) show negative tendencies. The N% of lichens ( $r = 0.538$ ,  $p < 0.01$ ,  $n = 23$ ) correlates positively with the  $\text{NH}_4^+\text{-N}$  concentration of the bark samples. The associated N% of the bark samples ( $r = 0.304$ , n.s.,  $n = 24$ ) does not. Slightly negative correlations are found between the  $\text{NH}_4^+\text{-N}$  concentration of the bark samples and the  $\delta^{15}\text{N}$  ratios of the lichens ( $r = -0.383$ ,  $0.05 < p > 0.1$ ,  $n = 23$ ) and the  $\delta^{15}\text{N}$  ratios of trees ( $r = -0.368$ ,  $0.05 < p > 0.1$ ,  $n = 24$ ). Data are expressed in means  $\pm$  SD (lichens) and  $\pm$  SD (bark samples).

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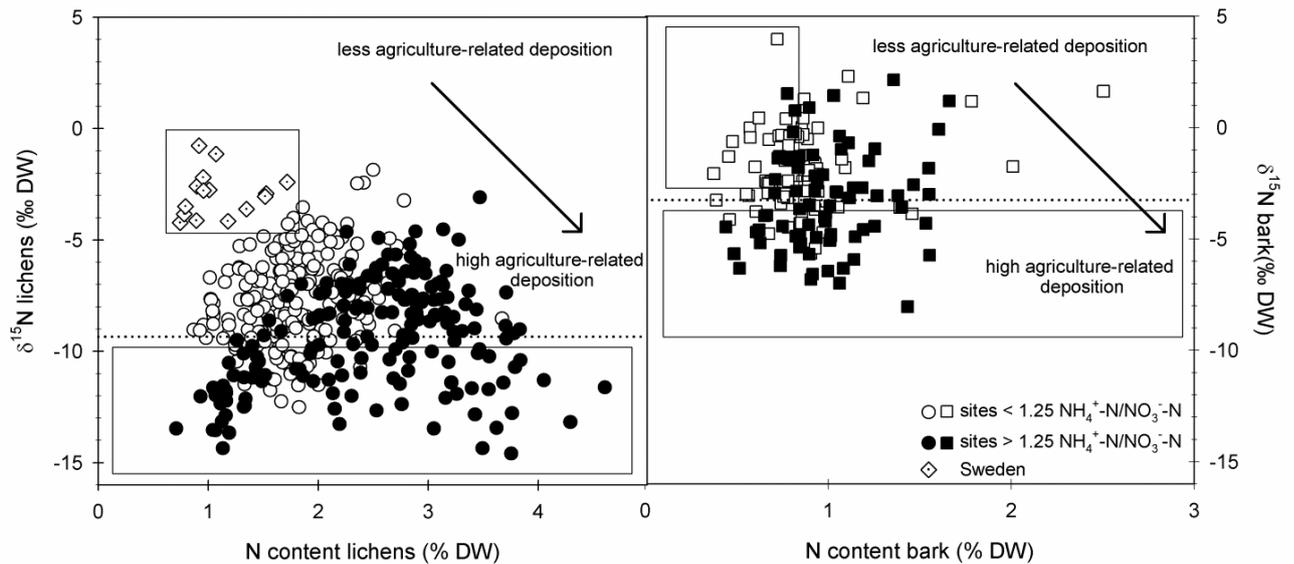
## 4. Discussion

### 4.1. N content as a monitor for atmospheric N input

The various ranges of N concentrations in the different lichen species at the same study sites suggest that the ability to accumulate N is species-specific. Figure 2.3 clearly shows the different abilities of accumulation of non-nitrophilous lichens (*H. physodes* and *P. sulcata*) and nitrophilous lichens (*X. parietina* and *Physcia* sp.) at the same study sites. Lichens can use  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , organic N and gaseous N (relating to cyanolichens) in order to cover the demand for N. Especially  $\text{NH}_4^+$  can be absorbed well and very quickly by lichens [26]. N compounds deposited from exogenous sources on the surface of the lichens are first absorbed into a fungal apoplastic continuum upon the rehydration of the thallus that encloses the photobiont cells. They are then absorbed by lichen symbionts [25,56]. The apoplast possesses multitudinous cation exchange sites so that  $\text{NH}_4^+$  ions can be ligated to the extracellular space of the thallus readily, but there is a chance that the  $\text{NH}_4^+$  ions can be lost to the environment [57]. One of the main reasons for the different N absorption rates of the nitrophytic lichens and less N-tolerant lichens is that the former exhibit a lower extracellular cation exchange capacity in the apoplast and therefore their ability to capture N is limited. As a consequence of this, these nitrophytic lichens are more protected against toxic effects of increased N impacts [34]. Furthermore, nitrophytic lichens can increase their photosynthetic capacity and consequently the  $\text{NH}_4^+$  assimilation even at sites with increased  $\text{NH}_4^+$  deposition rates [35].

Compared with data collected in the same survey year in Sweden (Juånaeset, Oestavall) (Tab. 2.2), we were able to demonstrate in this research that the sites investigated in the western part of Germany can be referred to mainly as N-enriched sites and not as sites with an unpolluted background (Fig. 2.7).

The epiphytic lichen tissues generally show rather high concentrations of N compared with those investigated in other studies focusing on source allocation in rural areas, especially in Scandinavia [27–30]. Concerning the very high N content principally measured in *X. parietina* and *Physcia* sp., we were able to detect N values that were comparable to those obtained in studies conducted in highly urbanised and predominantly agricultural areas or in fertiliser experimental studies (Tab. 2.2). Finally, all the data listed in Table 2.2 show a strong relationship with the subject of N patterns in lichens and the possibility of drawing reliable conclusions about the N depositions in the environment and in experimental field studies.



**Figure 2.7** Comparison of the measured N concentrations and  $\delta^{15}\text{N}$  ratios in lichens ( $n = 383$ ), as well as in the bark samples ( $n = 161$ ), separately for areas  $<1.25$  ( $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ) and areas  $>1.25$  ( $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ). Swedish data (*P. sulcata*, *H. physodes* and *X. parietina*,  $n = 15$ ) are used to classify the N load in the study area.

By tracking the important factors for the increase in N in lichen tissues, we were merely able to find mean linear, but very significant relationships between  $\text{NH}_4^+\text{-N}$  and  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratios in the bulk deposition. When considering the species-specific data, we were able to identify a similar trend for these two main factors in almost all the lichen species, but only in *X. parietina* and *Physcia* sp., a marginal or rather strong significant correlation was observed. Lichens can capture  $\text{NO}_3^-$  and  $\text{NH}_4^+$  at the same time [58], but will absorb  $\text{NH}_4^+$  when both N sources are available [25,59]. When comparing the calculated linear relationship between  $\text{NH}_4^+\text{-N}$  deposition and N content in lichens with that reported in other studies, no strong tendency could be clearly identified. Figure 2.3 shows, however, quite clearly that, in general, higher concentrations are found in lichens where  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratios greater than 1.25 are measured. These were mainly found in NW and NI, at sites located in the western part of the study area. When comparing the N deposition data derived from the deposition monitoring networks in the different federal states, no statistical differences were found between these states. This result contradicts the fact that especially the northern parts of NW and NI are characterised by large sections of intensive agriculture, which causes an enrichment of agriculture-derived  $\text{NH}_3$  and  $\text{NH}_4^+$  compounds and which is in contrast to the sites in RP.

**Table 2.2** Mean N concentrations and  $\delta^{15}\text{N}$  ratios of epiphytic lichen species of various investigations.

Lichen species	N content (%)	$\delta^{15}\text{N}$ (‰)	Study area	References
<i>Physcia caesia</i>	4.2-4.4		Near intensive $\text{NH}_3$ source (Switzerland)	[31]
<i>Xanthoria parietina</i>	2.36-3.34		Field experiment next to industry and livestock (Portugal)	[34]
<i>X. parietina</i>	2.06-3.61	-10.8-(-2.8)	Urbanised and agriculturally influenced region (Germany)	[33]
<i>P. adscendens</i>	1.75-4.21		City Grenoble (France)	[32]
<i>X. parietina</i>	1.66-2.35		Around a pig farm (Italy)	[36]
<i>X. parietina</i>	1.43-3.51	-10.6-(-5.3)	Agriculturally influenced region (Germany)	Own investigation
<i>X. parietina</i>	1.34-3.34		Fertiliser experiment (Portugal)	[78]
<i>P. sulcata</i>	1.28-3.31	-11.6-(-4.5)	Agriculturally influenced region (Germany)	Own investigation
<i>Physcia</i> sp.	1.18-3.87	-12.2-(-5.0)	Agriculturally influenced region (Germany)	Own investigation
<i>X. parietina</i>	1.18-1.72	-4.2-(-2.4)	Forest at Juånaset, Oestavall (Sweden)	Own investigation
<i>H. physodes</i>	1.16-2.14	-13.3-(-6.5)	Agriculturally influenced region (Germany)	Own investigation
<i>P. sulcata</i>	1.09-3.16	-8.9-(-2.8)	Urbanised and agriculturally influenced region (Germany)	[33]
<i>H. physodes</i>	0.90-2.46	-8.5-(-1.5)	Urbanised and agriculturally influenced region (Germany)	[33]
<i>P. sulcata</i>	0.90-1.01	-2.8-(-0.8)	Forest at Juånaset, Oestavall (Sweden)	Own investigation
<i>Ramalina</i> sp.	0.75-1.35	-20.5-(-18.3)	At various distances from an active fumarole (New Zealand)	[45]
<i>H. physodes</i>	0.75-1.07	-4.2-(-1.1)	Forest at Juånaset, Oestavall (Sweden)	Own investigation
<i>Usena</i> sp.	0.61-1.12	-20.5-(-18)	At various distances from an active fumarole (New Zealand)	[45]
Diverse epiphytic lichen species		-21-0.4	Fringe, floc and dwarf zone of mangrove forest (Belize)	[47]

Moreover, it must be mentioned that these two federal states are very much exposed to westerly winds with additional N loads from neighbouring countries with intensive agriculture (Belgium, France and the Netherlands). As has been mentioned above,  $\text{NH}_3$  is mainly emitted by agriculture and returns to ecosystems primarily in the form of dry deposition of  $\text{NH}_3$ , which is highly variable and has a key function close to the emitted source. Moreover,  $\text{NH}_3$  also returns in the form of wet deposition of  $\text{NH}_4^+$ , which plays an important role some distance downwind from the source [8,10]. Based on these facts and the determined N accumulation in lichens, it could be assumed that the underlying deposition data of this study do not reflect the actual realistic  $\text{NH}_4^+$  concentrations in the respective environment and that these data have been substantially underestimated.

Many regions in the agricultural landscape are frequently exposed to N loads that are bigger than those actually measured in on-site bulk deposition measurement stations. In particular, measurements of gaseous deposition are not often carried out and therefore easily omitted. In Central Europe, wet deposition dominates dry deposition, but the latter increases when the area is exposed to a bigger agricultural impact. This circumstance leads to erroneous estimations of  $\text{NH}_3$  deposition or even  $\text{NH}_4^+$  deposition. Ammonia favours the production of  $\text{NH}_4^+$ .  $\text{NH}_3$ , however, originates from a process dependent on temperatures or rather meteorological processes, which can be extremely variable in space and time. It also depends on the production or rather chemical composition of the fertiliser itself and the specific agricultural practices. Both play an important process-influencing role [8,10]. The different factors cause strong regional, spatially limited variations of N deposition conditions, which can only be determined with difficulty and by using the present sporadic bulk samplers [10]. Instead of this process, the analysis of N concentrations in epiphytic lichens can be a powerful and practical alternative compared with conventional deposition measurements.

Especially nitrophytic lichens can be a manageable and meaningful instrument for the determination of N deposition in regions with a wide range of  $\text{NH}_4^+$  inputs. Many studies have shown that epiphytic lichens have a very high potential to explain and indicate the currently prevailing actual conditions in terms of N (Tab. 2.2). Similar studies have dealt with interrelations between N concentrations in barks and different N depositions, for example, the work of Poikolainen *et al.* [30], Schulz *et al.* [60] and Mitchell *et al.* [61]. Poikolainen *et al.* [30] detected N contents that varied between 0.18 and 0.90% in pine (*Pinus sylvestris* L.) samples in Finland in the year 1985. With a simultaneous decrease in agricultural activities in the north of Finland, lower concentrations of N in the bark samples were measured. Furthermore, present data obtained from the western part of Germany show N concentrations that are up to four times higher than those in background areas in Russia and Bulgaria or in N impact areas in eastern and southern Germany [60]. Mitchell *et al.* [61] detected N contents between 0.34 and 0.43% in oak bark, along with an N deposition gradient in Scotland, but also measured concentrations of N similar to those found in this study with a much larger range of anthropogenic N impacts on the atmosphere and consequently on ecosystems. Linear significant correlations between  $N_{\min}$  deposition and N content of the bark samples were determined in the present study and they support the above-mentioned statement. The measured  $\text{NH}_4^+$ -N concentrations in the bark samples and the correlation with the respective  $\text{NH}_4^+$ -N concentration of the deposition data reflect that the highest N concentrations in the bark samples were found where generally the highest N loads are

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expected, originating from agriculture. Besides, with an increase in the  $\text{NH}_4^+\text{-N}$  concentrations of the respective trees, we observed a contemporaneous increase in lichen N content.

While comparing the N content of the bark samples with the N content of lichens, we were able to detect a correlation of similar increased N conditions in the environment, especially at the sites in Goettingen, Lange Bramke, Schwaney, Lammersdorf, Velmerstot, Tannenbusch and Schneifel (Fig. 2.3). The simultaneously high N contents in the lichen and bark samples at the identical study sites show inferences of an exposure of similar N loads strongly influenced by agriculture. The fact that the quantities of N differ between the bark and lichen samples has to do with the different characteristics of N uptake and the accumulation ability of two distinct surface structures (Fig. 2.3). In barks, mainly airborne matter is accumulated in the porous outermost sphere. This is subject to a periodic repulsion, so that there are no possible long-term exposure and associated accumulation with respect to N-containing compounds. The external bark layer is primarily influenced by bark deposition, which is composed of stem deposition, dry deposition and throughfall [62]. These factors influence each other strongly and can affect the process of accumulation through time and varying individual events, so that the actual N load is often removed or influenced negatively. In addition, the bark surface is not subject to metabolic processes, so that the adsorption of N related compounds is smaller on the tree bark than in the lichens. The N adsorption by the bark is an inactive process and it is mainly dry deposition that is responsible for the presence of N. In return, lichens do not have a protective epidermal tissue and are directly tied to the water vapour pressure in their environment. They absorb water and dissolved nutrients from precipitation, dew and fog and also through the air from which ions can freely enter the interior of the lichens. Because of the prevailing low nutrient concentrations coupled with the absence of physiological adaptations to eliminate detrimental compounds, lichens are able to accumulate ions very effectively and favour a strong incorporation [23,26]. This active process of N adsorption or rather N uptake in lichens caused by metabolic processes and the characteristics of lichens to fulfil their N demand with different N sources [26] can be given as reasons for the higher N concentration in the lichens than in the bark samples. Different receptor surfaces (lichens and barks) exhibit different N concentrations, despite being exposed to similar N deposition. The existing results of the bark samples indicate that the N deposition situation especially in the present study area in Germany can be classed as very high. Only the nitrophytic lichens (*X. parietina* and *Physcia* sp.) showed correlations between data obtained from lichen monitoring and data obtained from N monitoring networks based on physicochemical

measurements. In terms of the bark samples taken and a possible correlation with data obtained from N monitoring networks, no significant relationships were found. But the lichen and bark N contents reflect the influence of agriculture-related N components on the atmosphere.

#### 4.2. $^{15}\text{N}$ for indicating agriculture-related N source in the lichen, bark and deposition water samples

Essentially, plants with access to  $\text{N}_2$  (molecular N from air) should be close to 0‰, considering that the  $\delta^{15}\text{N}$  value of atmospheric  $\text{N}_2$  is 0‰ [41]. If other sources (e.g.  $\text{NH}_x$  mainly from agriculture or  $\text{NO}_y$  from traffic, industry and power production [63]) are taken into account for N uptake by plants except atmospheric  $\text{N}_2$ , the  $\delta^{15}\text{N}$  value will be different from 0‰ [41]. Generally, it could be shown that  $\delta^{15}\text{N}$  of  $\text{NO}_y$  from atmospheric pollution is mostly enriched, and  $\delta^{15}\text{N}$  of atmospheric  $\text{NH}_x$ , for example,  $\text{NH}_3$  originating from agriculture, is usually depleted [43,64,65]. Accordingly, epiphytes broadly take up N directly from the atmosphere, so it is generally accepted that the  $\delta^{15}\text{N}$  signatures of these organism groups reflect those of various N depositions from the atmosphere [45,47,66,67]. With this background knowledge, epiphytic lichens can identify, with the help of isotope analyses, the available N source on-site. N monitoring with lichens and the use of stable isotopes for source attribution are not frequently used research approaches. To date, only a few studies have been published [33,44–48]. The most frequently reported N isotope ratios in plants are roughly between  $-10$  and  $+10$ ‰ [38]. Some research that has dealt specifically with the environmental N inputs and pathways in vascular epiphytes or lichens could detect primarily  $\delta^{15}\text{N}$  ratios often lower than  $-10$ ‰, caused by a high depletion of  $^{15}\text{N}$  [44,45,47,66,67]. In contrast, ectomycorrhizal fungi, mycorrhizal fungi and saprophytic fungi are generally enriched in  $^{15}\text{N}$  [68,69]. This study investigated  $\delta^{15}\text{N}$  in lichen samples that were all in the negative range or rather depleted in  $^{15}\text{N}$ . In lichen tissues collected in the same survey year in Sweden (Tab. 2.2),  $^{15}\text{N} / ^{14}\text{N}$  ratios that were less depleted than those in the western part of Germany (Fig. 2.7) were identified. Among all the investigated lichen species, similar isotopic abundances in regions with related N impacts were recognised (Fig. 2.3). For comparison of the measured  $\delta^{15}\text{N}$  values with data obtained in similar N-influenced areas in Europe, we are able to cite studies of bryophytes in Germany and Austria that determined  $\delta^{15}\text{N}$  values from  $-7.9$  to  $-2.9$ ‰ [70] and from  $-10$  to  $-2.5$ ‰ [71] for moss

species at different N-affected areas. Bragazza *et al.* [72] have shown distinct  $\delta^{15}\text{N}$  signatures of *Sphagnum* mosses on different Europe-wide N deposition levels. Moss tissues had  $\delta^{15}\text{N}$  signatures ranging from  $-8.0$  to  $-3.5\text{‰}$  and showed much lower  $\delta^{15}\text{N}$  values in countries with intensive agriculture such as in the Netherlands, Denmark, Switzerland and Italy in contrast to Fennoscandia. The highest correlation between  $\text{NH}_x/\text{NO}_y$  ratios was found in dry deposition, which reflects a high influence of local  $\text{NH}_3$  emissions [72]. With regard to epiphytic lichens, Franzen-Reuter [33] reported a similar range of  $\delta^{15}\text{N}$  signatures (Tab. 2.2) in an area exposed to variable N sources and loads. A decrease in  $^{15}\text{N}$  content with increasing  $\text{NH}_4^+$ -N deposition was observed. Fogel *et al.* [47] were able to detect the existence of isotopically depleted  $\text{NH}_3$  in the atmosphere coinciding with simultaneously highly depleted  $^{15}\text{N}$  signatures in lichens (Tab. 2.2) in a mangrove forest on offshore islands in Belize, Central America. A study conducted by Lee *et al.* [48] on King George Island, Antarctica, which is located far from any possible anthropogenic inputs of N, showed a similar relationship between isotopically depleted  $\text{NH}_3$  sources and a strong increase in the very negative  $\delta^{15}\text{N}$  values in lichens near local seabird colonies. The measured data for the western part of Germany show very similar patterns in comparison with those of other studies dealing with N input and source identification. Significantly, a relationship between  $\delta^{15}\text{N}$  signatures in all the researched lichens and the  $\text{NH}_4^+/\text{NO}_3^-$  ratios in wet deposition could be established within the scope of this work. Specifically, significant trends for nitrophytic *Physcia* sp. could be observed, followed by a very similar, but less significant relationship for *X. parietina*. A similar result was also reported by Solga *et al.* [73]. With the help of  $\delta^{15}\text{N}$  signatures, moss species have been able to reflect an increased shift of  $\text{NH}_x/\text{NO}_y$  in bulk deposition in the last 140 years for the western part of Germany.

To clarify the precise correlation of  $^{15}\text{N}$  signature of lichen tissues with reduced N compounds in the atmosphere, we have to consider that the N supply of epiphytic lichens primarily depends on dry and wet depositions. An explanation for the determined negative  $\delta^{15}\text{N}$  signatures in lichens can therefore only be found in these two atmospheric pathways. Due to the absence of stomata, cuticle and roots, the lack of connection to soil and the inert substrate surface on which epiphytic lichens grow, they reflect an isotopic abundance similar to that in the surrounding atmosphere [45]. Besides, lichens can incorporate  $\text{NO}_3^-$  and  $\text{NH}_4^+$  simultaneously. Research on different lichen groups has investigated that  $\text{NH}_4^+$  uptake is preferentially higher and, relative to other N sources, to a greater extent passive [25]. The higher  $\text{NH}_4^+$  absorption can also be explained by a larger adhesion of this cation to the negatively charged cell walls in the apoplast of lichens [57]. While analysing  $\delta^{15}\text{N}$  signatures in lichens in New Zealand, Tozer *et al.* [45]

were able to detect that  $\text{NH}_4^+$  is readily taken up and that, in addition, absorption of  $\text{NH}_3$  via diffusion may occur in lichens. In connection with these characteristics, lichens are ideal and powerful models for examining and reflecting agricultural impacts. Especially when considering that both  $\text{NH}_3$  and  $\text{NH}_x$   $\delta^{15}\text{N}$  signatures are closely coupled and typically depleted [45], the relationship between isotopically depleted atmospheric N compounds from agriculture and the measured depleted  $\delta^{15}\text{N}$  signatures in lichens is obvious.

Ammonia is one of the main  $\text{N}_r$  compounds emitted by anthropogenic interventions, mainly from agriculture, and is often not routinely measured by air quality monitoring networks. If data are monitored, it is usually not done so on an area-wide basis or in an adequate spatial and temporal resolution [10,74]. Ammonia emissions in Germany for the year 2000 were calculated to be around 575 Gg N  $\text{yr}^{-1}$  [75]. Within the EU (27 countries), Germany had the highest  $\text{NH}_3$  emissions, followed by France. In comparison, Sweden had a total  $\text{NH}_3$  emission of 47 Gg N  $\text{yr}^{-1}$  for the same year [75]. In regions with high  $\text{NH}_3$  emissions, the dry deposition of  $\text{NH}_3$  is very important close to local sources and wet deposition of  $\text{NH}_4^+$  is most important some distance downwind from sources [8,74].

Especially in the context of  $\text{NH}_4^+$  deposition monitoring, due to the afore-mentioned facts, deposition can be estimated easily with a limited number of sites, but for measuring  $\text{NH}_3$  deposition, many measurement sites would be essential, because there are many scattered local sources of  $\text{NH}_3$ . So, the given circumstances make it difficult to draw realistic conclusions about agriculture-related N depositions for an entire area [8]. Furthermore, precipitation sampling often includes a component of dry deposition on standard collectors [76]. In addition, errors may appear in the following logistical and chemical treatments, so all things considered, the determination of realistic N conditions in the environment is a difficult undertaking. Upon consideration of the present obtained N deposition data, it can be noticed that even in the areas that are strongly dominated by agriculture (the northern part of NW and NI), no elevated concentrations of  $\text{NH}_4^+$  are detected compared with less agriculture-affected areas in RP. Although the available N deposition data did not show a highly significant correlation – just a tendency between  $^{15}\text{N}$  in the tissues of lichens and the respective deposition data – it is likely that isotopically depleted N in atmospheric deposition is responsible for the observed negative  $\delta^{15}\text{N}$  values of the exceedingly  $^{15}\text{N}$ -depleted epiphytic lichens, especially at sites that are influenced by local and regional agricultural emitters. With reference to the work of Sah and Brumme [77], who detected  $\delta^{15}\text{N}\text{-NH}_4^+\text{-N}$  ratios from  $-12.5$  to  $+12.6\%$  and  $\delta^{15}\text{N}\text{-NO}_3^-\text{-N}$  ratios from  $-3.7$  to  $+2.5\%$  in bulk precipitation of beech and spruce

forests in Germany, this study can assert that all the analysed rainwater samples were more depleted in  $^{15}\text{N}$ . Freyer [64] observed, on average,  $-12.1\%$  for  $\delta^{15}\text{N-NH}_4^+\text{-N}$  and  $-3.1\%$  for  $\delta^{15}\text{N-NO}_3^-\text{-N}$  in precipitation in NW. He found that N isotope data of rain reflected gaseous N compounds emitted from local N sources and that  $\text{NH}_4^+$  concentrations in rain mainly and simultaneously correlated with excessive ammonia emissions, especially at times of fertiliser application or in areas with large amounts of livestock. Moreover, Heaton *et al.* [65] found that  $\delta^{15}\text{N}$  values for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ions in precipitation tend to be similar to the supposable composition of the precursor gases. Based on this knowledge, for the measured N deposition data of the study sites, we can assume that  $\text{NH}_3$  accounts for lower  $\delta^{15}\text{N}$  signatures in rainfall, particularly in largely agricultural regions. Kinetic resolution and absorption effects, in which the lighter  $^{14}\text{N}$  isotope is chosen, are responsible for isotope fractionation between gaseous  $\text{NH}_3$  and dissolved rain  $\text{NH}_4^+$  and therefore consequently for a depletion [64]. Due to the fact that this study was able to determine depleted  $^{15}\text{N}$  values in all the deposition water samples, it can be asserted that we are additionally dealing with very high atmospheric  $\text{NH}_3$  concentrations in the present investigation area. Samples in NW that result in significantly less negative data are an exception and may be due to the higher incidence of N oxides caused by the presence of an area of high population density and thus higher emissions from traffic (Rhine-Ruhr metropolitan region) and consequently less depletion. In contrast to the work of Russow *et al.* [44] and Tozer *et al.* [45], this study could not identify less depletion of  $^{15}\text{N}$  in the deposition water samples, as it had in the lichen tissues. Tozer *et al.* [45] advanced a two-step fractionation model, which includes first a fractionation factor associated with  $\text{NH}_3$  volatilisation from source and second a fractionation step by diffusive  $\text{NH}_3$  uptake into lichens (sink), which is in the end responsible for a higher depletion in the lichens than in the deposition water samples. In this context, no clues could be offered for this fact. Perhaps this was due to the different sampling periods of lichen material (November 2008 – February 2009) and deposition water samples (March 2010).

Supplemental analyses, particularly in relation to  $\text{NH}_3$ , could have given additional information within the framework of the present study. Besides, it must be stated that the study of Russow *et al.* [44], which was conducted in the north-western Negev Desert, Israel, was carried out under different environmental conditions in comparison with the present study. The bulk deposition mainly showed positive  $\delta^{15}\text{N}$  values, which were due to dust influence of the nearby arable land. Furthermore, we found that the sampled nitrophytic lichens, which are highly depleted in  $^{15}\text{N}$  relative to atmospheric  $\text{N}_2$ , showed a significant correlation with the  $\text{NH}_4^+\text{-N}$  concentrations in the bark samples. Both tissue

types seem to be mostly exposed to reduced N forms. It has also been shown that the lichen and bark samples at the respective sites have similar patterns of  $\delta^{15}\text{N}$  with respect to the parallel appearance of highly negative signatures at the specific sites. Significantly, this study was able to detect a correlation between  $\delta^{15}\text{N}$  signatures in the bark samples and  $\delta^{15}\text{N}$  signatures in the lichen samples.

Particularly, nitrophytic lichens showed a strong depletion in their tissues. We also observed a depletion of  $^{15}\text{N}$  in their surrounding substrate. Although  $\delta^{15}\text{N}$  signatures in both the bark and lichen tissues had a similar pattern at each study site, the corresponding bark samples showed less negative  $\delta^{15}\text{N}$  values than epiphytic lichens growing in the same site. The different receptor surfaces exhibit different  $\delta^{15}\text{N}$  signatures despite being exposed to similar N depositions. Similar observations were also recently made by Fogel *et al.* [47]. Further  $\text{NH}_4^+$ -N concentrations in the bark are related to  $\delta^{15}\text{N}$  signatures in nitrophytic lichens, so that it could be assumed that  $\text{NH}_4^+$  deposition on the bark surface will account for the decrease in the  $^{15}\text{N}$  abundance in *X. parietina* and *Physcia* sp. The fact that it is only a trend that accumulated  $\text{NH}_4^+$  in the bark could be observed must be taken as an additional indicator for why bark samples are mostly depleted in  $^{15}\text{N}$ . Schulz *et al.* [60] could prove that a mixture of isotopically different N compounds (rest-N of the bark and anthropogenic atmospheric  $\text{NH}_4^+$  on the bark surface) is responsible for the specific  $\delta^{15}\text{N}$  values of the bark samples. In conclusion, the resulting  $\delta^{15}\text{N}$  signatures in lichens, bark samples and deposition water samples reflect the relative extent of reduced N forms in the atmosphere or rather the extent of anthropogenic impact on ecosystems. The N uptake of lichens and the consequential and representational  $\delta^{15}\text{N}$  values can be seen as an efficient system to indicate differences in  $\delta^{15}\text{N}$  among potential N sources. Different  $\delta^{15}\text{N}$  values of the lichen and bark samples allow the identification of the main N deposition source across a gradient of increased agricultural activities.

## 5. Conclusion

The ability of lichens to absorb N compounds solely from the atmosphere and the subsequent accumulation of N in their tissues are what researchers used to determine N pollution in the environment. This study showed an increase in agriculture-derived N impacts on ecosystems by using the N content and isotopic abundance of N in different epiphytic lichens, bark samples and wet deposition samples. The species-specificity of N concentration in lichen tissues along a gradient of diverse N depositions or rather

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different agriculture-affected areas could also be detected. The influence of intensive agriculture was readily identifiable, especially with the aid of nitrophytic lichens (*X. parietina* and *Physcia* sp.).

Furthermore, it was determined that the comparison of lichens and the corresponding data relating only to wet N deposition data is a difficult task. The comparability of N deposition data with lichen N content is not guaranteed satisfactorily. Since epiphytic lichens can absorb a large range of different N compounds, and since monitoring networks do not frequently measure a variety of different N compounds on-site, lichens will reflect both dry and wet depositions. Often a large part of the deposition measurements is likely to be outside the reach and influence of substantial  $\text{NH}_3$  or  $\text{NH}_4^+$  deposition. Besides, the monitoring network frequently does not boast a sufficient resolution, especially in very heterogeneous regions. In addition to this, the dispersal of nitrogenous air pollution over long distances plays an important role, which has so far been strongly underestimated. It will also not be picked up by temporarily installed deposition measurement devices.

Given this, bioindication with lichens is a cost-effective alternative to physico-chemical measurements to assess differences in  $\delta^{15}\text{N}$  among different source N pools in a large area. Although bark and water samples can reflect the impact of agriculture, lichens are, due to their species-specific strong absorption and efficient incorporation of N, powerful indicators of the accumulation of N, in real terms, as a consequence of increasing human intervention relating to N. Therefore, we conclude that lichens can support on-site deposition instruments measuring N deposition by qualification and quantification of the respective N deposition. Monitoring with lichens is advantageous in heterogeneous areas with diverse local wind conditions and topographic variations. Because of the errors in deposition measurements and because the non-, or rather poorly, determinable diffuse  $\text{NH}_3$  deposition, it is better to assess the time-integrated N impact on ecosystems with lichens. It would, however, be even more effective to reduce N emissions in general and to protect ecosystems and human health, rather than calculate N loads with rough models. Finally, the focus should be on stringent overall emission reductions. National reductions of N emissions in heavily affected areas are in the end more cost-effective than other measures.

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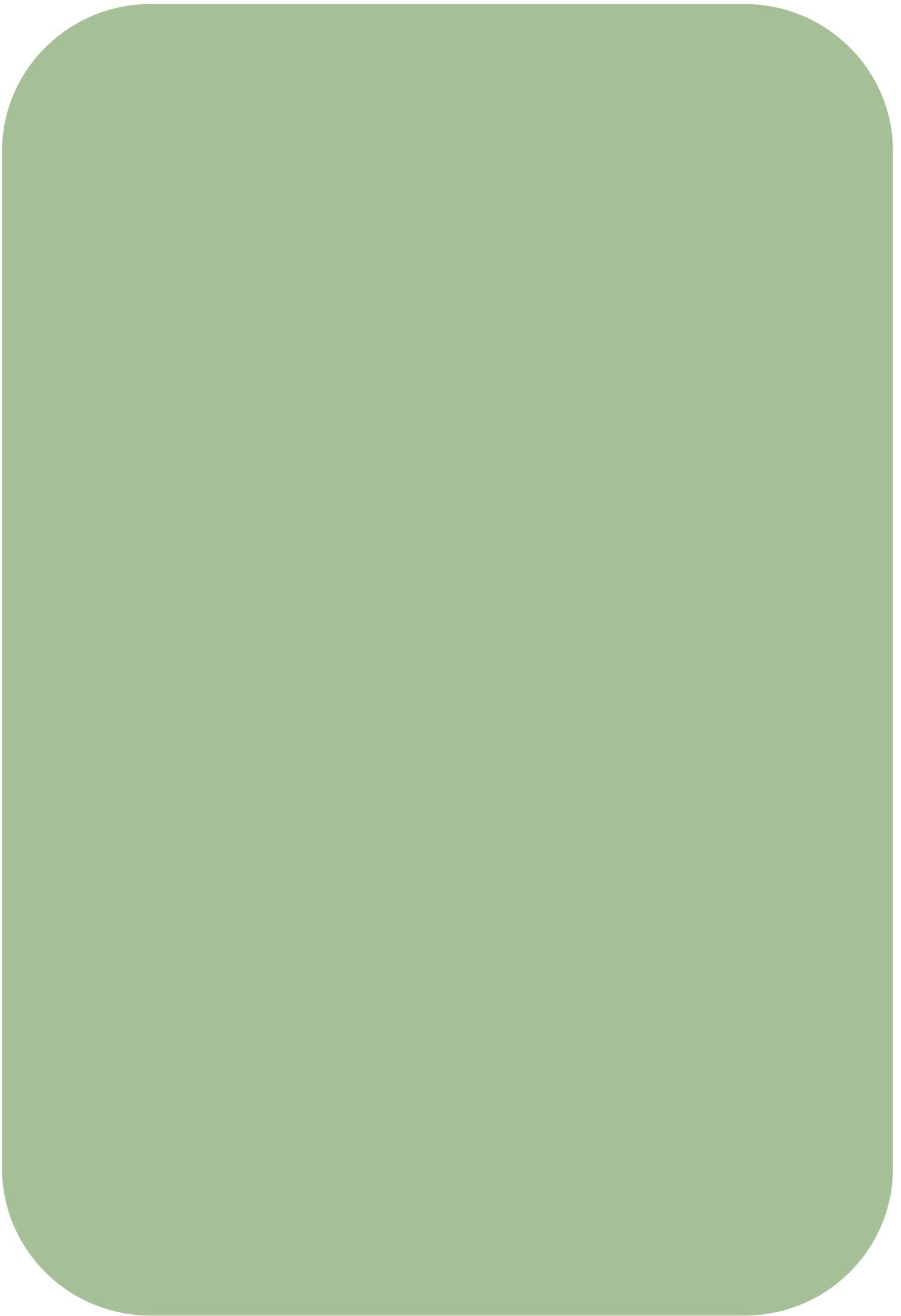
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# Chapter 3

Lichens as a useful mapping tool? – An approach to assess atmospheric N loads in Germany by total N content and stable isotope signature

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## Lichens as a useful mapping tool? – An approach to assess atmospheric N loads in Germany by total N content and stable isotope signature

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**Abstract** To assess whether nitrogen (N) content and  $\delta^{15}\text{N}$  ratios in nitrophytic lichen species (*Xanthoria parietina* (L.) Th. Fr. (1860) and *Physcia* spp. (Schreb.) Michaux (1803)) reflect the quantity and quality of atmospheric N loads, 348 lichen samples from 174 sampling grid cells were investigated in the western part of Germany. The analysed lichen N content ranged between 0.98 and 4.28% and  $\delta^{15}\text{N}$  ratios between -15.2 and -1.3‰. Based on the N concentrations and the  $\delta^{15}\text{N}$  ratios of lichens, different landscape categories and coupled N deposition rates could be inferred for different regions of Germany. By analysing environmental variables like altitude, ammonia emission density, livestock unit and different defined deposition types, a direct relationship was found between lichen chemistry and N compounds produced from agricultural activity. The results support the development of a monitoring method which could be used nationally or even internationally to support current N deposition measurements, by providing reliable information on the quantity and quality of N deposition in high N environments.

**Keywords** Lichens; Nitrogen Deposition; Tissue N Content; Stable isotopes  $^{15}\text{N}$ ; Geostatistical Analysis

### 1. Introduction

The dramatic increase of biologically reactive nitrogen (N) in the atmosphere due to anthropogenic activity has greatly impacted the N status of ecosystems (De Schrijver et al. 2011; Erisman et al. 2008; Krupa 2003). Atmospheric deposition of both oxidized N ( $\text{NO}_x$ ), predominantly originating from combustion processes, and reduced N ( $\text{NH}_y$ ), mainly derived from agriculture (Hertel et al. 2006), tripled between 1860 and the early 1990s, on a global scale (Galloway et al. 2004).

The rapid increase of the N load represents a considerable threat to the environment, such as loss of biodiversity due to eutrophication of both terrestrial and aquatic systems and declines in water quality with serious implications for human health (Galloway et al.

2003; Townsend et al. 2003). Countries with high N deposition rates therefore require a variety of deposition measurements and effective N monitoring network that can assess the N loads.

Typical current N measurement networks only provide a partial and often fairly inaccurate picture of the local N deposition rates in a limited specific area (Sutton et al. 1998). Coupled with many uncertainties in the whole chain of processes – emissions, dispersion, chemistry and the different methods of data handling – assessment of deposition on-site is still a major challenge (Monks et al. 2009). Although global and regional scale models of N deposition exist, they cannot be expected to represent small-scale variations in deposition rates. In addition, they do not take biosphere-atmosphere exchange into account; therefore, these models cannot provide an accurate evaluation of local N impacts. Such mathematical data-based models can therefore only be used for mapping of N deposition in conjunction with local measurements (Simpson 2011). One tool that allows the quantification of the exposure to and effects of atmospheric N impacts in an ecosystem is bioindicators (Pesch et al. 2008). Lichens are one of such biological monitoring organisms, well suited as indicators of air pollution (Conti and Cecchetti 2001). Their sensitivity to various common pollutants means that they show measurable changes in physiology in response to even tiny changes in atmospheric concentrations.

Lichens are often used in monitoring studies investigating the impacts of N on ecosystem scales, measuring changes based on biodiversity and composition of lichen species (Giordani et al. 2012; Johansson et al. 2012; Mayer et al. 2009). Besides, studies often investigate the variations in physiological parameters (Johansson et al. 2011) or the ability to accumulate N-related pollutants. The capability of lichens to quantitatively reflect N deposition rates through N concentrations in their tissue has been frequently discussed (Bruteig 1995; Conti and Cecchetti 2001; Frati et al. 2007; Johansson et al. 2010; Hyvärinen and Crittenden 1998; Ochoa-Hueso and Manrique 2011; Poikolainen et al. 1998; Remke et al. 2009; Skinner et al. 2006; Søbchting 1995). Due to their lack of cuticles, stomata and roots, lichens absorb nutrients over the whole of their surface area, and are therefore very highly metabolically active and can freely absorb various atmospheric N-containing compounds from both wet and dry deposition (Dahlman et al. 2004; Hauck 2010).

To provide not only information about the general quantitative N input in an area but also about the predominant N sources, isotope analysis can be used. Relationships between elemental concentrations and isotopic signatures have been extensively studied to investigate the links between plants and the environment (Dawson et al. 2002; Evans

2001). The abundance of  $^{15}\text{N}$  is a valuable and widely used indicator of the sources and pathways of N in organisms and ecosystems (Robinson 2001). Up to now there have been only few lichen studies that use the natural abundance of  $^{15}\text{N}$  and integrate it with N monitoring to identify various N sources in field experiments (Fogel et al. 2008; Lee et al. 2009; Russow et al. 2004; Skinner et al. 2006; Tozer et al. 2005). In addition, lichen diversity patterns and resulting air quality indices have been only regularly shown on maps in relation to N pollution (Davies et al. 2007; Estrabou et al. 2011; Frati and Brunialti 2006; Gombert et al. 2004; Pinho et al. 2004; Van Dobben and Ter Braak 1998). Therefore the main aim of this study is to present an easy method with which to monitor and classify areas in relation to N loads on ecosystem level. Results from N-tolerant lichens (*Xanthoria parietina*, *Physcia* spp.) monitored in a region affected by various N doses and N sources are presented, providing a more complete spatial pattern of air quality and atmospheric N deposition.

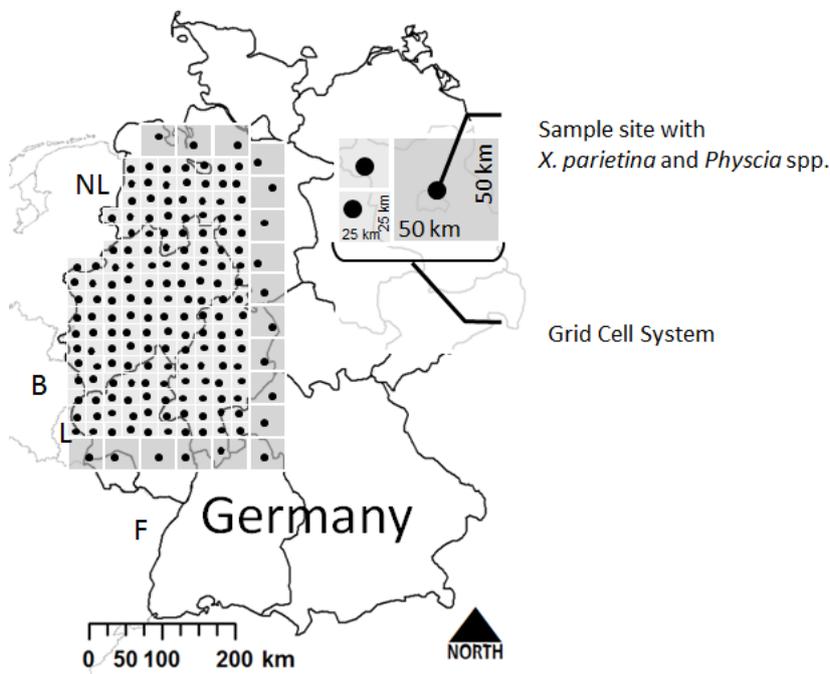
The present study focused on (i) testing the utility of nitrophytic lichens as monitor organism and (ii) verifying if it is possible to characterize and map the N impacts by analysing the N content of lichens on a regional scale. Furthermore, it was investigated (iii) whether  $\delta^{15}\text{N}$  ratios of lichens can be used to differentiate the main N sources and types of deposition along an altitudinal gradient (from lowland to submountainous areas) and through different land use affected areas.

## 2. Material and methods

### 2.1 Sampling grid

The sampling grid covered approximately 142,500 km<sup>2</sup> in the western part of Germany. Systematical sampling was based on a regular 25 × 25 km and on the outer edge a 50 × 50 km grid cell system, in which a total of 174 grid cells were recorded (Fig. 3.1). The sampling grid covered a climatic gradient from maritime climatic conditions in the north, followed by sub-maritime and finally more mountainous climatic conditions in the south. The winds come predominantly from the west. The altitude of the sampling sites ranged from 1 to 660 m asl. The selection of the study area was based on different characteristic land uses, such as urban agglomerations and industrial areas (e.g. the Rhine-Ruhr metropolitan region), very intensive agricultural areas (e.g. the pork belt in the Oldenburg Muensterland) or very heterogeneous structured areas with varying land uses. Different land uses are associated with various characteristic N compounds (e.g. NO<sub>x</sub>

often from traffic and industry emissions and  $\text{NH}_y$  compounds mainly originating from agriculture). These typical N compounds and the regional patterns are crucial for the identification of different deposition types (Wellbrock et al. 2005). The study area is characterized by the ‘moderate’ deposition type 1 in the southern part and especially in the low mountain regions, followed by ‘mainly reduced N’ deposition type 3 in the northern lowlands and finally the ‘mainly ammonia N’ deposition type 4 in the northern coastal area (Wellbrock et al. 2005). The study area is additionally affected by long-range transport of N from neighbouring N sources. The main contributors of reduced N loads in Germany are the Netherlands and France. Oxidized N loads are mainly emitted by France, Great Britain and the Netherlands (EMEP 2012a).



**Figure 3.1** Overview of the location of the sampling grid with 174 sample sites in Germany and the arrangement of the different grid cell sizes.

## 2.2 Lichen survey

Within each grid cell, a pooled sample (three to five thalli per one sample tree) of each lichen species (N-tolerant *X. parietina* (L.) Th. Fr. (1860) and of nitrophytic *Physcia adscendens* (Fr.) Oliv. (1882) and/or *Physcia tenella* (Scop.) DC. (1805)) was sampled in February and March 2010. The latter two lichen species were summarized as *Physcia* spp. for the present analysis. The decisive main criteria for the selection of these lichen species were their wide area of distribution, as well as the ease of sample collection and preparation. Samples were collected from free-standing trees that met the requirements

for bio-indication with lichens (VDI 2005). No sampling trees were selected close to local N emitters. The largest proportions of sampled trees are described by the species *Acer pseudoplatanus* L. (1753) (21%), followed by *Tilia cordata* Mill. (1768) (18%) and *Quercus robur* L. (1753) (15%).

Complete lichen thalli were removed neatly. After removal, the material was packed into paper bags, labelled and stored in a refrigerator before being transferred to a freezer at -18°C. Before analysing, lichen samples were cleaned from obvious contamination and then freeze-dried (CHRIST type 101541, Osterode, Germany). All sample sites were assigned values for ammonia emission density per administrative district (Rösemann et al. 2011), livestock units per administrative district (Kreins 2013) and to regions which were subdivided into the following categories: submountainous areas, heterogeneous structured areas, urban agglomerate and industrial areas, areas along the river Rhine (railway, shipping and automobile traffic line), sparsely settled regions, intensive agricultural and border areas, agriculturally dominated North German Plain region and finally coastal areas.

### 2.3 Chemical analyses of lichen samples (N content, $\delta^{15}\text{N}$ )

Before the chemical analyses, lichen samples were pulverised for homogenisation by an agate-type ball mill (FRITSCH Pulverisette 9, Idar-Oberstein, Germany), and smaller sample volumes were pulverised with a vibrating teflon-type ball mill (RETSCH MM2, Haan, Germany). Subsequently, all samples were dried in a desiccator, and subsamples of 3 to 4 mg of each sample were weighed into tin capsules and analysed for total N concentration and  $^{15}\text{N}$  natural abundance by an elemental analyser coupled with an isotope ratio mass spectrometer (IRMS) (THERMO Scientific, Flash EA 1112 Series + IRMS Delta VI Advantage-Isotope ratio MS, Waltham, MA, USA). The  $\delta^{15}\text{N}$  values are given in per mill (‰) relative to air international standard and to International Atomic Energy Agency (IAEA) reference material. In addition, an internal laboratory reference material was used to guarantee the quality of the measurement. The standard deviation for analytical precision was  $\pm 0.1\%$ . The natural abundance of  $^{15}\text{N}$  was calculated according to the following formula ( $R$  defined as the atomic  $^{15}\text{N}/^{14}\text{N}$  ratio):

$$\delta^{15}\text{N}(\text{‰ vs at - air}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} \right) \times 1000 \quad (1)$$

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#### 2.4 Statistical analyses

Normality of data was tested using the Kolmogorov-Smirnov test. Significant differences between mean values were analysed using Student's *t*-test and the one-way variance analysis (ANOVA) followed by Scheffé's or Tukey's *post-hoc test*. Pearson correlation was calculated to study relationships between parameters derived from chemical measurements (N content,  $\delta^{15}\text{N}$ ) and data obtained from other sources. Moreover, the method of partial least square regression (PLS-R) was applied by Unscrambler (CAMO Software AS., Oslo, Norway). For this operation, data were standardized by Z transformation.

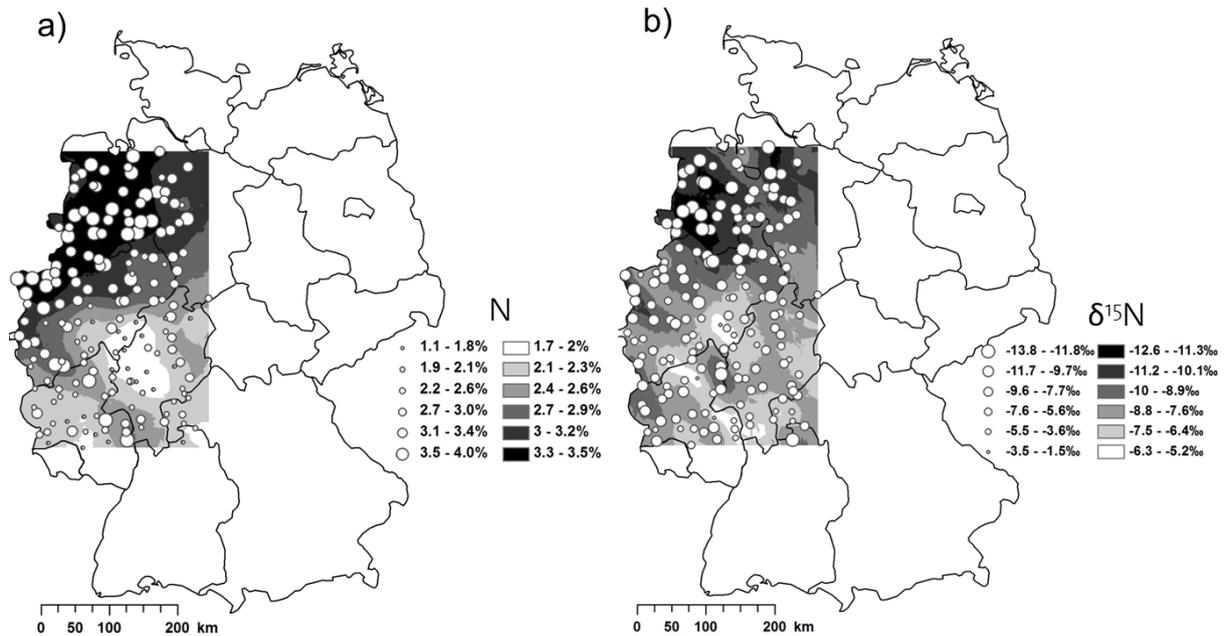
#### 2.5 Geostatistical analyses

This calculation deals with the analysis and prediction of spatial or spatio-temporal phenomena using the property of spatial dependence and the use of random functions (Webster and Oliver 2007). With the help of 'block kriging', it is possible to use the estimates of the average expected value in a given location, such as a block (e.g. 25 × 25 km), around a point (Cressie 2006). In the present study, the geostatistical analyses were performed using a geographic information system (GIS) (ESRI Inc. ArcMap 10, Redlands, CA, USA).

### 3. Results

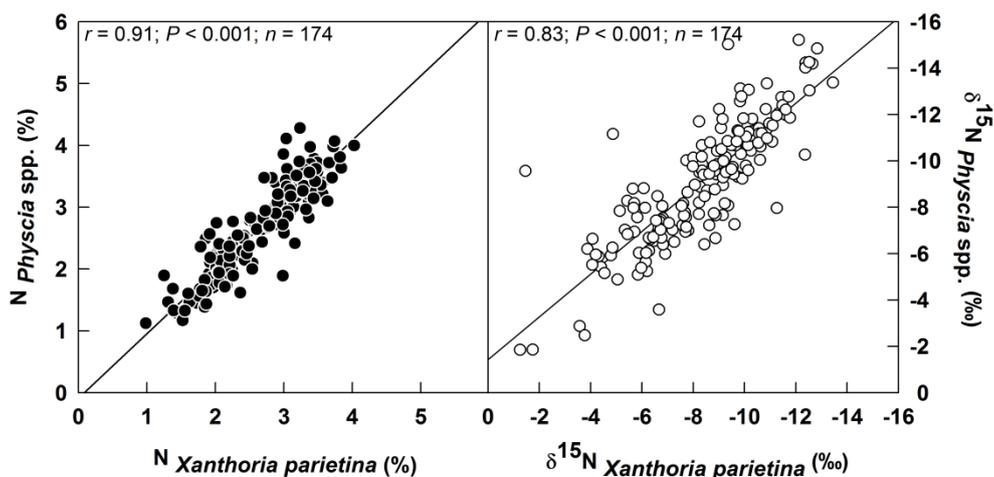
#### 3.1 N content and $\delta^{15}\text{N}$ in lichen thalli

The N content in all lichen samples ( $n = 348$ ) ranged between 0.98 and 4.28%, with an average  $\pm$  *SD* of  $2.68 \pm 0.72\%$ . Site averages ranged from  $1.10 \pm 0.09$  to  $4.01 \pm 0.02\%$  (Fig. 3.2a). The  $\delta^{15}\text{N}$  ratios in lichen tissues ( $n = 348$ ) varied from -15.2 to -1.3‰ (average  $-8.8 \pm 2.5\%$ ). Mean  $\delta^{15}\text{N}$  ratios of the sample sites ranged between  $-13.8 \pm 1.4$  and  $-1.5 \pm 0.4\%$  (Fig. 3.2b).



**Figure 3.2** a Averaged N content and b  $\delta^{15}\text{N}$  of all lichen samples (*X. parietina* and *Physcia* spp.) at 174 sampling sites in north-western Germany and interpolation by ordinary kriging;  $\text{N}\%$  = nugget:sill = 0.39; cross validation  $r = 0.67$ ; root-mean-square standardized = 1.035;  $\delta^{15}\text{N}$  = nugget:sill = 1.27; cross validation  $r = 0.50$ ; root-mean-square standardized = 1.022.

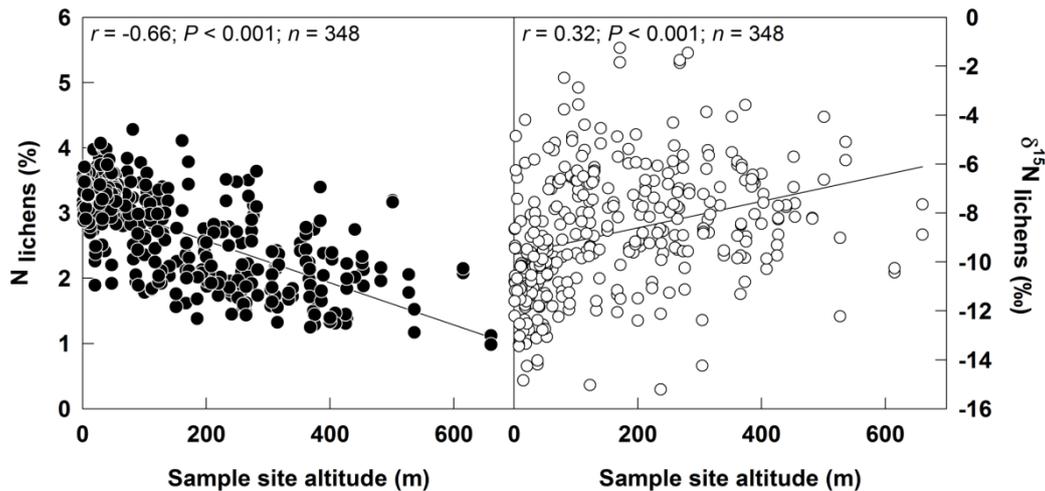
The two lichen species did not differ significantly from each other in terms of N content (*Physcia* spp.  $n = 174$ ,  $2.69 \pm 0.76\%$ ; *X. parietina*  $n = 174$ ,  $2.66 \pm 0.67\%$ ) and  $\delta^{15}\text{N}$  signature (*Physcia* spp.  $n = 174$ ,  $-9.2 \pm 2.6\text{‰}$ ; *X. parietina*  $n = 174$ ,  $-8.4 \pm 2.4\text{‰}$ ) (data not shown). The interspecific relationship of tissue N content and  $\delta^{15}\text{N}$  signature for studied grid cell was highly significant in both cases (Fig. 3.3).



**Figure 3.3** Correlation between the N contents of the lichen pairs per grid cell and correlation between  $\delta^{15}\text{N}$  values of the lichen pairs per grid cell.

### 3.2 Comparison of lichen and site data

A general gradient could be detected from high N concentrations in the northern lowlands to low N concentrations in the southern mountain regions of the study area (Fig. 3.4). The  $\delta^{15}\text{N}$  signatures revealed similar gradient but less pronounced relationships to the sample site altitude with very strong negative  $\delta^{15}\text{N}$  signatures in the northern part of the study grid compared to less negative values in the southern region (Fig. 3.4).



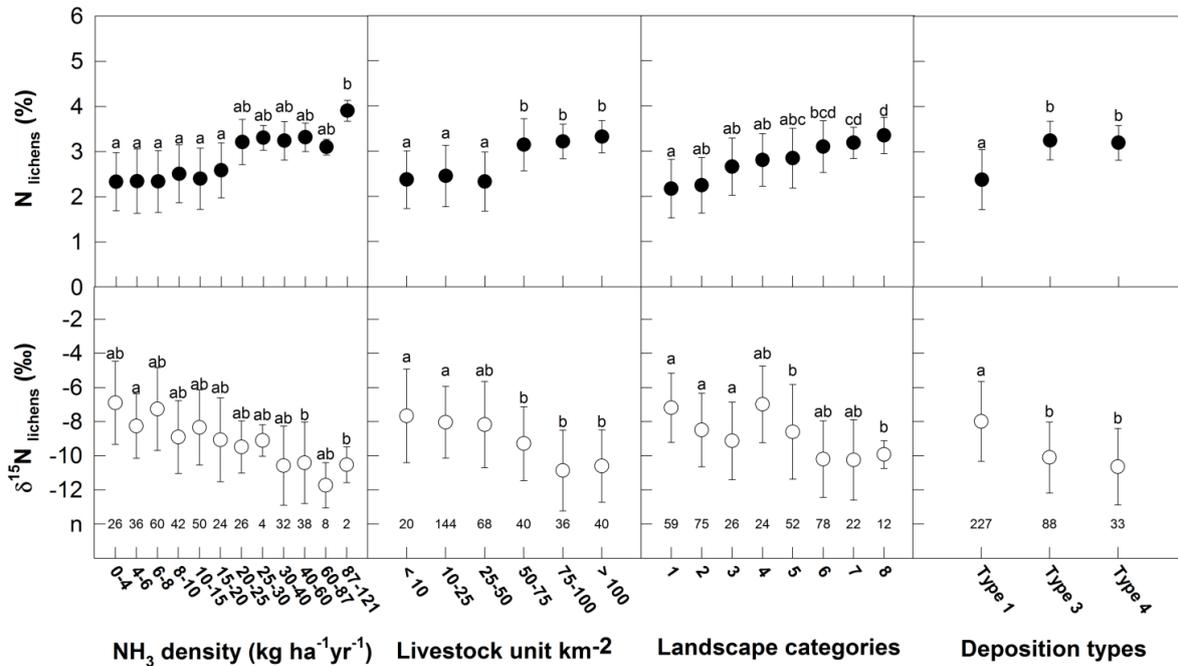
**Figure 3.4** Linear relationships between sample site altitude and tissue N content, and altitude and  $\delta^{15}\text{N}$  ratio in the lichens *X. parietina* and *Physcia* spp. for the western part of Germany.

The total tissue N content related to several  $\text{NH}_3$  emission density classes differed significantly between the lowest classes and the highest class (Fig. 3.5).

Regarding the livestock units per square kilometre, two groups could be separated. Less dense classes ( $< 50$  livestock unit /  $\text{km}^2$ ) differentiate significantly from highly dense classes ( $> 50$  livestock unit /  $\text{km}^2$ ) in terms of N content in lichens.

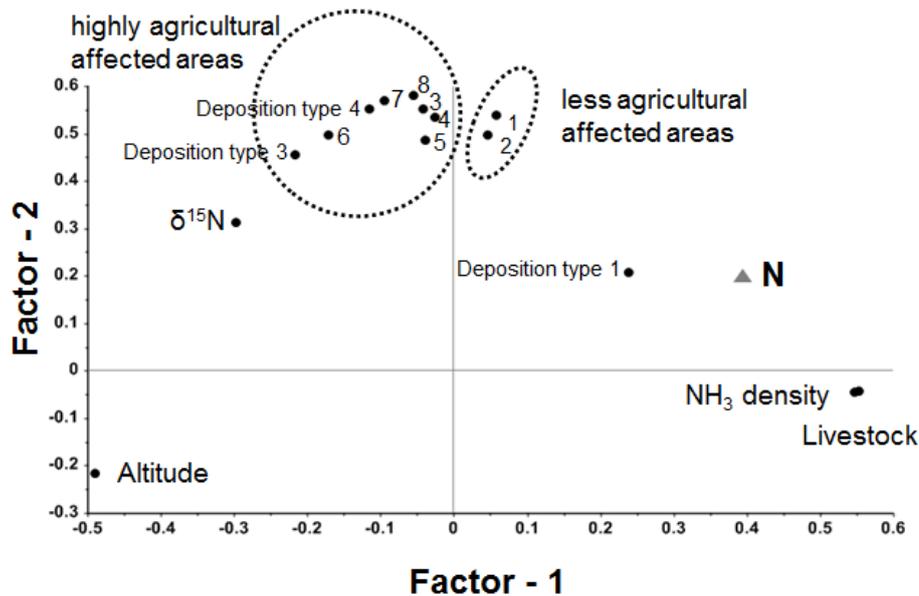
There was an increasing trend of total tissue N content with intensification of agriculture and accountable for significant differences between submountainous areas, heterogeneous and more agricultural affected areas and finally agriculturally dominated North German Plain ( $P < 0.05$ ). Significant differences of  $\delta^{15}\text{N}$  ratios between all considered categories ( $\text{NH}_3$  density, livestock unit per  $\text{km}^2$  and landscape categories) were detected ( $P < 0.05$ ). With an increase of agricultural activity,  $\delta^{15}\text{N}$  signatures in lichens become highly negative (Fig. 3.5).

Total N content as well as  $\delta^{15}\text{N}$  signature were significantly different between deposition type 1 and the other two deposition types (3, 4) ( $P < 0.05$ ) (Fig. 3.5).



**Figure 3.5** Averaged N content and  $\delta^{15}\text{N}$  ratio of both summarized lichen species (*X. parietina*, *Physcia* spp.) plotted against different  $\text{NH}_3$  density-, livestock unit per  $\text{km}^2$ -, landscape- (1 submountainous areas; 2 small-scale heterogeneous areas; 3 agglomerative and industrial areas; 4 areas along the Rhine; 5 sparse settlement; 6 intensive agricultural areas/border regions; 7 coastal areas; 8 agriculturally dominated North German Plain) and deposition type- categories. Data are expressed as means  $\pm$  *SD* of *n* sample range per category. Different letters above the bars indicate a significant statistical difference of  $P < 0.05$ .

PLS-R analyses revealed that tissue N content was mainly influenced by livestock and  $\text{NH}_3$  emission density. Altitude was inversely related to N content (Fig. 3.6). When used the  $\delta^{15}\text{N}$  signature as the explanatory variable, altitude was positively correlated with  $\delta^{15}\text{N}$ . Livestock unit,  $\text{NH}_3$  emission density and N content were inversely related to  $\delta^{15}\text{N}$  (data not shown).



**Figure 3.6** Loading plot based on a PLS-R model fit with the variables altitude, NH<sub>3</sub> density, livestock, δ<sup>15</sup>N values, landscape categories (1 submountainous areas; 2 small-scale heterogeneous areas; 3 agglomerative and industrial areas; 4 areas along the Rhine; 5 sparse settlement; 6 intensive agricultural areas/border regions; 7 coastal areas; 8 agriculturally dominated North German Plain) and deposition types. The explained variable is tissue N content (triangle gray) ( $n = 348$ ). The first two dimensions are presented.

## 4. Discussion

### 4.1 Biomonitoring species

Several studies have shown that increased atmospheric N deposition is responsible for a decrease of lichen abundance and diversity and parallel to that for an increase of more N-tolerant lichen species (Geiser et al. 2010; Larsen et al. 2009; Van Dobben and Ter Braak 1998; Van Herk 1999). Due to the globally increased rates especially of reduced N in the environment (Erisman et al. 2008) and the resulting widespread distribution of such N-tolerant lichen species, they are very suitable as monitor organisms in highly N-affected areas. The widespread presence of the two investigated nitrophile lichen species (*X. parietina* and *Physcia* spp.) testifies to the fact that the present study area is characterized by enriched N loads, and that the species are suitable bioindicators in areas affected by N deposition. Moreover, these nitrophytic lichen species have not yet reached to the final N saturation limit, and the authors argue that these lichens are still able to accumulate additionally N (Boltersdorf and Werner 2013).

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#### 4.2 Quantity - Characterizing different atmospheric N depositions by N content in lichens.

The ability of lichens to accumulate different atmospheric N compounds was species-specific (Johansson et al. 2010, 2012; Dahlman et al. 2004; Gaio-Oliveira et al. 2001, 2005). The analysed lichen species clearly showed similar reactions to N deposition at each study site. Therefore, this study suggested that these nitrophytic lichens had small variations in tissue N content within the sample grid cell and were able to reflect atmospheric N impact and to respond quite similarly to their environment. The interspecies relationship ( $n = 174$ ,  $r = 0.910$ ,  $P < 0.001$ ) showed a clear evidence of this. Furthermore, both N-tolerant lichens represent a study area which was strongly affected by N deposition. This was confirmed by the N content average of  $2.68 \pm 0.72\%$  and the fact that the lowest analysed N concentration was 1%, and the majority of the monitored areas exhibit N concentrations above 2%. Compared to other studies focusing on estimation of N deposition with the help of lichen tissues, this study was comparable to N concentrations found in highly urbanized and predominantly intensive agricultural areas (Fрати et al. 2007; Gaio-Oliveira et al. 2001; Gombert et al. 2003). Studies focusing on source allocation in lichens and at the same time cover a large area along an N deposition gradient were quite rare. Comparable studies originated mainly from Scandinavia and were carried out with the acidophytic lichen species *Hypogymnia physodes* (L.) Nyl. (1896) (Bruteig 1995; Poikolainen et al. 1998; Søchting 1995). These studies showed rather low concentrations of N, in contrast to the present study. Even in areas affected by urban, agricultural and marine emissions in Canada (Georgia Basin, British Columbia), the measured lichen N contents in the analysed lichens were lower than in the present investigation area (Raymond et al. 2010). These facts led to the conclusion that most of the investigated sites in the western part of Germany can be referred to as mainly N-enriched sites.

The fact that lichens reliably reflect the predominant N deposition rates has been frequently discussed for a long time (Bruteig 1995; Conti and Cecchetti 2001; Frати et al. 2007; Gaio-Oliveira et al. 2001; Johansson et al. 2011, 2012; Poikolainen et al. 1998; Remke et al. 2009; Skinner et al. 2006; Søchting 1995).

Studies have shown that lichens are able to assimilate  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and organic N (Dahlman et al. 2004; Johansson et al. 2010). At the ecologically relevant N doses, lichens have similar uptake rates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Johansson et al. 2010). However, if high artificially N doses are given, lichens will prefer  $\text{NH}_4^+$  uptake which is more detrimental to lichen vitality than  $\text{NO}_3^-$  (Dahlman et al. 2004; Sheppard et al. 2011).

Ammonia ( $\text{NH}_3$ ), primarily from the volatilization of livestock waste, has a high impact near agricultural sources due to dry deposition rates. Its reaction product ammonium ( $\text{NH}_4^+$ ), which returns to ecosystems via wet deposition, may constitute a significant fraction of the ambient aerosol concentration in the environment (Skjøth et al. 2011; Sutton et al. 1998).

The distribution of different lichen N contents in the investigation area showed that in regions strongly affected by intensive agriculture, i.e. with large amounts of  $\text{NH}_3$  and  $\text{NH}_4^+$  in the atmosphere, N concentrations were consistently high in lichen samples. This was in line with the findings of Skinner et al. (2006) and Frati et al. (2007). Also in the present study, a clear relationship was found between agriculture and increased tissue N content in lichens, which was proven by correlations between N concentration in the species and  $\text{NH}_3$  emission density categories, livestock units and different deposition types. At the sample sites in regions characterized by high percentage of reduced N ( $\text{NH}_3$ ,  $\text{NH}_4^+$ ) of the total N input (deposition type 3 and type 4 after Wellbrock et al. (2005)), on the one hand N concentrations were higher than other regions and, on the other hand, both deposition types were not different from each other. This revealed a significant influence of the prevailing high reduced nitrogen compounds on lichens (Fig. 3.5). In addition, as altitude increased, there was a decrease in lichen N content, consistent with the concentration of agricultural, urban and industrial N emissions at or near sea level with attenuation towards upland unpolluted sample sites (Fig. 3.4). A similar relationship was also shown by Raymond et al. (2010) concerning the lichen species *Platismatia glauca* (L.) W.L. Culb. & C.F. Culb. (1968) in a complex topographical region characterized by marine and urban emissions in south-western British Columbia, Canada. If we set the individual areas with their different forms of land use and the corresponding N rates in contrast to the analysed N concentrations, it is obvious that lichen N content will be decrease from the northern sample sites with intensive regional and local emitters of reduced N compounds, followed by more heavily industrial and urban influenced areas with predominantly oxidized N compounds and finally to rather less N affected areas, mainly at higher altitudes.

The widespread presence of N-tolerant lichen species in the whole investigation area is an indication for a general strong influence of anthropogenic N deposition. Due to the different species-specific sensitivity to N stress (Davies et al. 2007; Johansson et al. 2012; Sheppard et al. 2011; Sparrius 2007), it is possible to make a quantitative assessment in N-affected regions. Furthermore, the N content of lichens reflects well the N loads from the atmosphere to the landscape surface quantitatively at a spatial resolution of

25 × 25 km. With the help of lichens as deposition sampler per grid cell of 625 km<sup>2</sup>, a region can be monitored approximately and comprehensively along an atmospheric N deposition gradient.

#### *4.3 Quality - assessing the source of N input by $\delta^{15}\text{N}$ ratios in lichens*

In the present study, all lichen samples were depleted in <sup>15</sup>N, varying from -15.2 to -1.3‰. The interspecies relationship ( $n = 174$ ,  $r = 0.832$ ,  $P < 0.001$ ) showed similar  $\delta^{15}\text{N}$  signatures of the two investigated lichen species on-site. In comparison to other studies dealing with lichens and the identification of different N sources, especially reduced N sources (Fogel et al. 2008; Lee et al. 2009; Skinner et al. 2006; Tozer et al. 2005), this study showed very similar patterns. Rainwater analyses revealed that in general different  $\delta^{15}\text{N}$  ratios can occur in the two main atmospheric forms of N deposition, oxidized ( $\text{NO}_x$ ) and reduced ( $\text{NH}_y$ ) compounds.  $\text{NH}_y$ , mainly derived from animal breeding, tends to have particularly in wet deposition lower  $\delta^{15}\text{N}$  ratios (depletion in <sup>15</sup>N).  $\text{NO}_x$ , however, has usually high  $\delta^{15}\text{N}$  ratios (enriched in <sup>15</sup>N) (Heaton et al. 1997; Freyer 1978). Due to the fact that epiphytic lichens broadly take up N directly from the atmosphere (Dahlman et al. 2004; Hauck 2010), it is generally accepted that  $\delta^{15}\text{N}$  signatures of these organisms reflect the on-site existing N deposited compounds from the atmosphere. With the help of isotope analyses, the predominating N sources can be identified in the field (Fogel et al. 2008; Tozer et al. 2005).

Furthermore, studies with heather and lichen species could detect increasing depletion in <sup>15</sup>N in sample tissues with increasing distance from a  $\text{NH}_3$  point source (Skinner et al. 2004, 2006). With this in mind, it is obvious that the analysed lichens were exposed to an N mainly from reduced N compounds.

To characterize qualitatively the relevant N sources for the sampled lichens, it is important to know that atmospheric  $\text{NH}_3$  is subject to transport and dispersion, but also scavenging by dry deposition, which shows a high spatial variability and is most important close to the emitting source (Asman et al. 1998; Hertel et al. 2006). Moreover,  $\text{NH}_3$  can be scavenged by transformation to aerosol bound  $\text{NH}_4^+$  in reactions with acid gases and aerosols. The  $\text{NH}_4^+$  containing aerosols are mainly removed by wet deposition. They generally have a long lifetime and therefore can be transported over long distances (>1,000 km) in the atmosphere, and thus, the influence of  $\text{NH}_4^+$  containing aerosols is more important some distance from the N source (Asman et al. 1998; Hertel et al. 2006). In this regard, it should be considered that in particular the heavily agriculturally affected

region in the north of the study area, where we detected primarily  $\delta^{15}\text{N}$  ratios often lower than  $-10\text{‰}$  in lichen samples, is subject to a variety of influences which support the high local N deposition rate. In the present study, we have to assume that local and regional variations of present N deposition were first and foremost due to emissions of local N sources by intensive arable farming and industrial livestock farming, followed by the enriched frequency and intensity of precipitation in the northern parts in Germany (EMEP 2012b) and through long-range transport mechanisms from highly agricultural affected neighbouring countries (The Netherlands, France and Belgium) (EMEP 2012a). Especially the latter influences the local N input load and simultaneously the  $\delta^{15}\text{N}$  ratios in lichens. It is likely that both lichen species represent locally emitted  $\text{NH}_3$  or rather the reaction product  $\text{NH}_4^+$  and N from distant  $\text{NH}_3$  emitters carried by westerly winds. The existence of significant relationships between the analysed  $\delta^{15}\text{N}$  ratios in lichens and  $\text{NH}_3$  densities, livestock units and the characteristic deposition types, verifies this.

The fact that lichens in the present study were more enriched in  $^{15}\text{N}$  at higher altitudes testifies to a change in the prevailing deposited N compounds and its isotopic composition, due to the occurrence of other land use-related emitters, and because of an increase of orographic effects on precipitation associated with the uplands. Orographic effects favour washouts from low-level clouds by precipitation falling from higher-level clouds by seeder-feeder scavenging (Fowler and Battarbee 2005). Especially oxidized products of emitted N pollutants are generally too small ( $0.1\text{-}1.0\ \mu\text{m}$ ) to deposit at significant rates and rely on precipitation scavenging for their removal from the atmosphere, so that furthermore the direct deposition of cloud droplets (occult deposition) in the uplands are responsible for a contamination with mainly oxidized N compounds in this region (Fowler and Battarbee 2005). In addition, it is known that occult and dry depositions are very similar in terms of emission sources and formation processes (Simeonov et al. 2003). Accordingly, the analysed  $\delta^{15}\text{N}$  ratios in lichens point to a shift from highly wet deposition affected regions in the lowland to a region which is characterized by an occult/dry deposition in the uplands. A similar relationship was found in moss species along an altitudinal transect in a nationwide study in Austria (Zechmeister et al. 2008). Simultaneously, we detected lichens with less negative  $\delta^{15}\text{N}$  ratios along the river Rhine. This, in turn, also suggests that the predominant N oxides from traffic, industrial emissions and also from surrounding metropolitan areas (e.g. the Rhine-Ruhr metropolitan region) are responsible for the enrichment of  $^{15}\text{N}$  and correspondingly less negative  $\delta^{15}\text{N}$  ratios in lichens.

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## 5. Conclusion

On the basis of this research, it can be stated that the ability of lichens to absorb N compounds solely from the atmosphere and the subsequent accumulation of N in their tissues and the resulting  $\delta^{15}\text{N}$  ratio make it possible to identify the quantity and quality of agriculture-derived N deposition along a gradient. Based on a comparison of model-based maps documenting the N impacts in the north western part of Germany (De Vries et al. 2011; Gauger et al. 2001), it can be determined that lichens are able to provide a similar picture of the N deposition patterns on a regional scale. Monitoring the atmospheric N deposition is severely restricted in spatial extent and type, especially in very heterogeneous regions. The nitrophytic lichens (*X. parietina* and *Physcia* spp.) represent, however, in a more cost-effective manner an alternative to physico-chemical measurements to comprehensively assess different N doses and sources of N pools on a wide regional scale.

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# Supplementary material

## Environmental Monitoring and Assessment

Lichens as a useful mapping tool? – An approach to assess  
atmospheric N loads in Germany by total N content and stable  
isotope signature

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**3 total pages**

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## S1. Description of NH<sub>3</sub> density

Ammonia emission density per administrative district (kg ha<sup>-1</sup>yr<sup>-1</sup>) implies the emissions from livestock (stable, storage, application, grazing) and emissions from mineral fertilizer application and additional emissions from cultivation farming of legumes (this does not play an important role in terms of quantity) (Rösemann et al. 2011). Data from the year 2007.

## S2. Livestock Unit

The regionalized Agrarian and Environmental Information System (RAUMIS) was used to extract data on livestock units per km (Gömann et al. 2013). RAUMIS is a mathematical model that maps data on the German agricultural sector at the regional level (administrative district).

The basic data for RAUMIS are based on the agrarian structure census 2007 and livestock census 2007 (all farms > 2 ha). RAUMIS is designed for continuous use for long-term simulation analyses in the agricultural and environmental sectors. It provides a computerized information system that assists the agricultural policy makers in decision-making (Gömann et al. 2013). Data from the year 2007.

## S3. Deposition types

Based on modelled total deposition rates, the input in forest monitoring plots (1800 sites in a systematic grid of 8×8 km<sup>2</sup> of the National Forest Soil Inventory of Germany) was characterised and load areas in Germany were delimited.

The soil inventory plots were grouped into specific deposition types using multivariate statistical methods. The multivariate analysis consisted firstly of a factor analysis, which was applied to deposition data and to deposition components, and secondly the resulting deposition factors were used to group the soil inventory plots into deposition types by applying a cluster analysis.

With the help of multivariate statistics on modeled deposition data, Wellbrock *et al.* (2005) characterized six deposition types for Germany which delimit load areas. The three relevant deposition types for the present study are described as follows:

Deposition type 1 (moderate atmospheric input) was located in the southern part and especially in the low mountain regions.

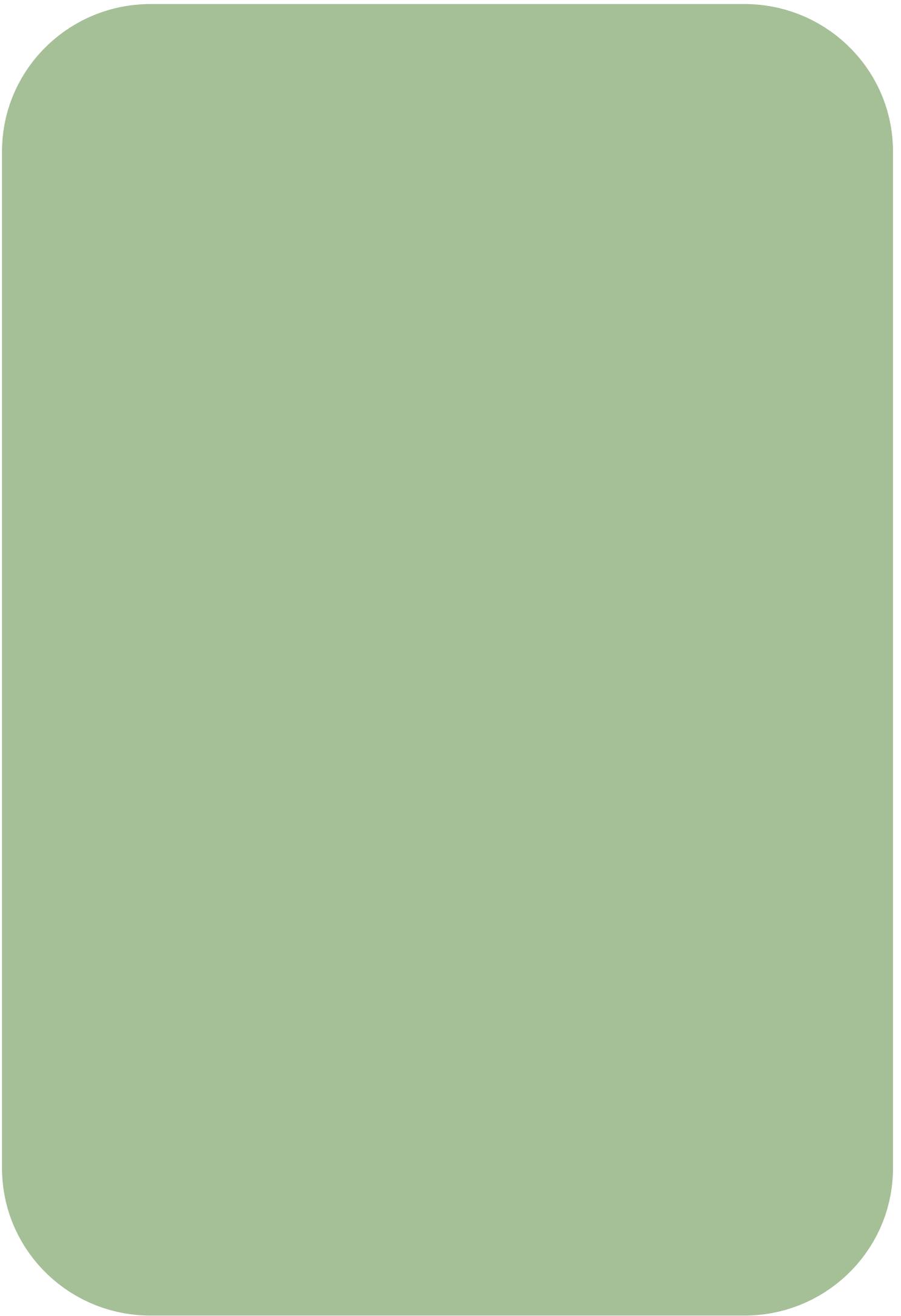
Deposition type 3 (high percentage of reduced N of the total N input together with low calcium and magnesium input) dominated in the northern lowlands.

The deposition type 4 (high sea-borne sodium as well as magnesium input and high percentage of ammonia nitrogen of total nitrogen input) situated in the northern coastal area (Wellbrock et al. 2005).

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# Chapter 4

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

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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  $\delta^{15}\text{N}$  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.

**Keywords** Nitrogen deposition; Stable isotopes  $^{15}\text{N}$ ; Lichens; Mosses; Bark

**Capsule** First nationwide comparison of lichens, mosses and tree bark to assess the N deposition in Germany by analysing N content and  $\delta^{15}\text{N}$  values.

### Highlights

- We investigated N pollution with the help of bioindicators in Germany.
- The N load was monitored with lichens, mosses and bark by tissue N content.
- Main source of N pollution was revealed by tissue  $\delta^{15}\text{N}$  values.
- Particularly the N content and  $\delta^{15}\text{N}$  in lichens reflected agriculture-related N deposition

## 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 ( $\text{NH}_3$ ), have increased mainly due to animal farming (Erisman et al., 2008; Krupa 2003).  $\text{NH}_3$  is highly reactive, and preferentially deposited as dry deposition close to the emitted source, whilst its reaction product ammonium ( $\text{NH}_4^+$ ) is principally washed out by precipitation in terms of wet deposition. These two compounds, collectively referred to as  $\text{NH}_x$ , 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  $\text{NH}_x$  (Bobbink et al., 2010; Sheppard et al., 2011; Skinner et al., 2006). Furthermore, Cape et al. (2009) defined a lower  $\text{NH}_3$  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,  $\delta^{15}\text{N}$  signatures of atmospheric N compounds are used (Freyer, 1978; Heaton et al., 1997). The abundance of  $^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $^{15}\text{N}$  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 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  measurements.
- iv. Testing whether data obtained from these bioindicators (N% and  $\delta^{15}\text{N}$ ) correlate with measured and modelled data from N deposition assessment programmes.

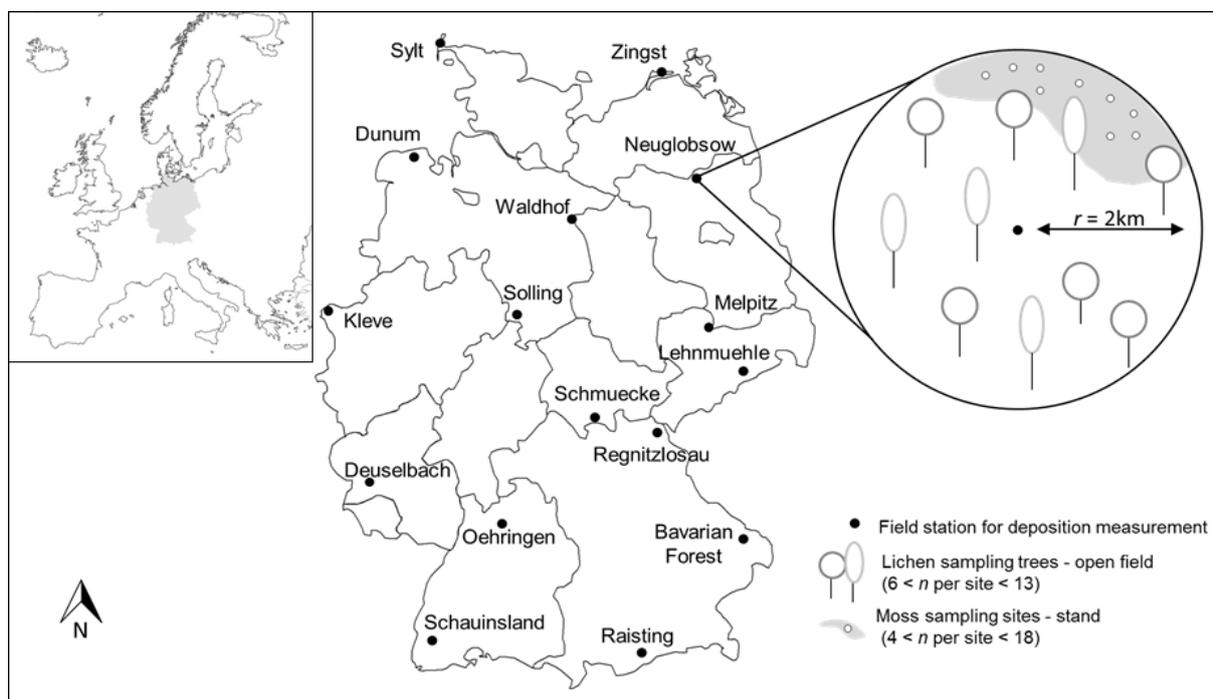
## 2. Material and methods

### *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. 4.1). 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 to 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 ( $\text{NO}_3\text{-N}$ ), ammonium - nitrogen ( $\text{NH}_4\text{-N}$ ) 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  $\text{NH}_4\text{-N}$  deposition thereby ranges from  $1.50 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Regnitzlosau) to  $6.38 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Schmuecke). The respective  $\text{NO}_3\text{-N}$  deposition varies between  $0.70 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Regnitzlosau) and  $3.12 \text{ kg ha}^{-1}\text{yr}^{-1}$  at the Schmuecke site (Fig. 4. 2).

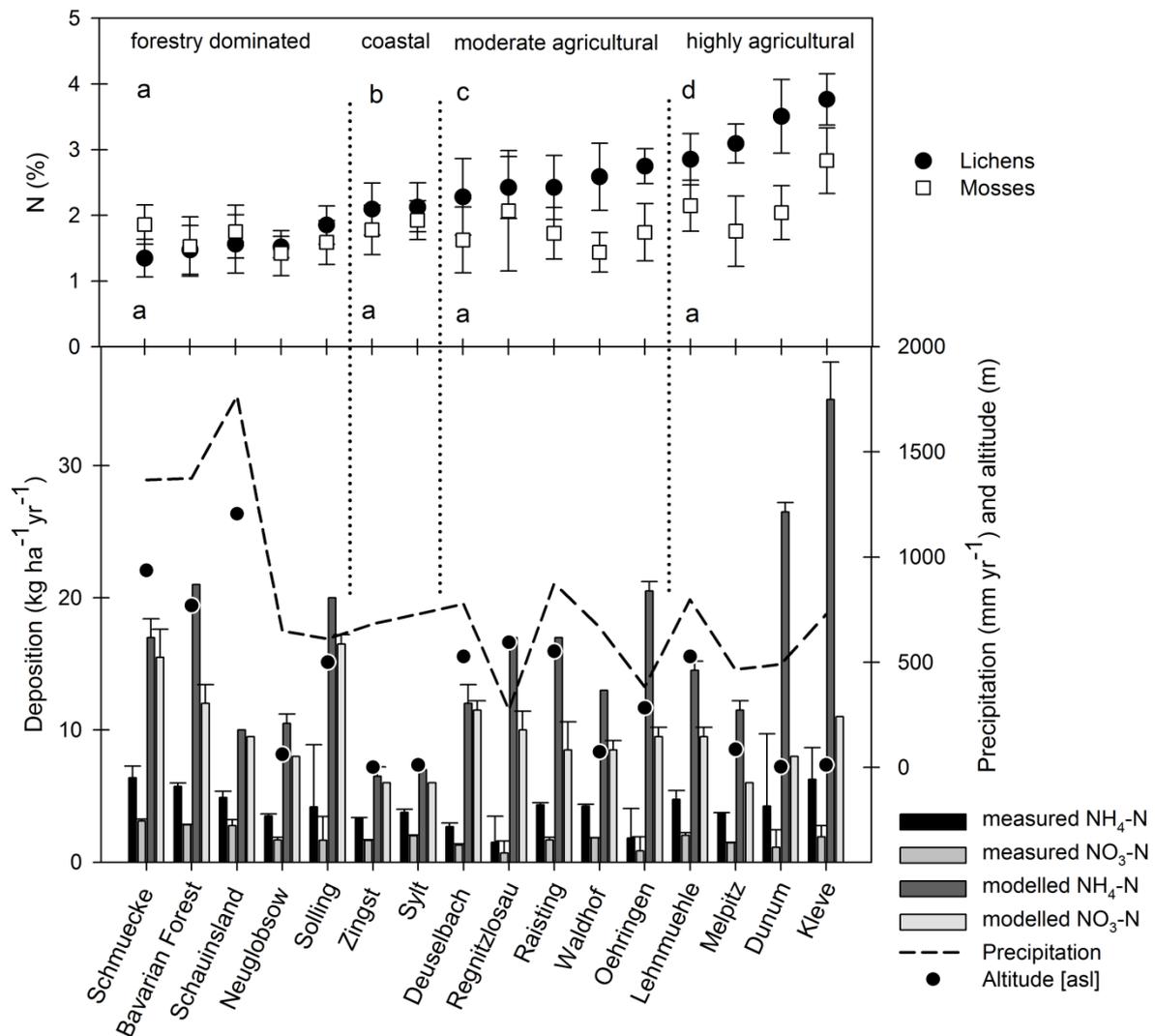
The German deposition network contributes to the European Monitoring and Evaluation Programme (EMEP), which operates under the Long-Range Transboundary Air Pollution (LRTAP) convention in Europe. The objective of the programme is to model and predict the deposition of acidifying and eutrophying pollutants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



**Figure 4.1** 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).

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  $1 \times 1 \text{ km}^2$  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  $\text{NH}_4\text{-N}$  deposition ranges from  $6.50 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Zingst) to  $35 \text{ kg ha}^{-1}\text{yr}^{-1}$  at Kleve site. The  $\text{NO}_3\text{-N}$  deposition varies between  $6.00 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Melpitz, Sylt and Zingst) and  $16.50 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Solling) (Fig. 4.2). 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  $13 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Zingst) to  $38 \text{ kg ha}^{-1}\text{yr}^{-1}$  (Kleve).



**Figure 4.2** Average annual measured  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  wet-only deposition from 2006 – 2007 and average annual total  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  deposition from 2006 – 2007 as modelled by MAPESI (Mean  $\pm$  *SD*). Mean annual precipitation of the years 2006 and 2007 (Mean  $\pm$  *SD*) and altitude of field site for deposition measurement (bottom). Average N content of lichen and moss samples (Mean  $\pm$  *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).

### 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. 4.1). At each deposition measurement site we attempted to sample ten replications of each epiphytic lichen species listed in Table 4.1.

In total, 326 lichen samples were collected. The pooled lichen samples (3 to 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 Tab. 4.1). All investigated tree species were analysed separately, but they were averaged finally per respective deposition measurement station.

**Table 4.1** 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
<i>Parmelia sulcata</i>	41% <i>Hypnum cupressiforme</i>	49% <i>Alnus glutinosa</i>
<i>Physcia adscendens</i>	28% <i>Pleurozium schreberi</i>	33% <i>Betula pendula</i>
<i>Physcia tenella</i>		18% <i>Pinus sylvestris</i> *
<i>Xanthoria parietina</i>	31% <i>Pseudoscleropodium purum</i>	1% <i>Pinus sylvestris</i> *
		4% <i>Prunus avium</i>
		5% <i>Prunus domestica</i>
		7% <i>Quercus robur</i>
		8% <i>Quercus petraea</i>
		4% <i>Salix</i> spp.*
		± acid/subneutral
		2% <i>Tilia cordata</i>
		2% <i>Tilia platyphyllos</i>
		subneutral
		4% <i>Acer platanoides</i>
		21% <i>Acer pseudoplatanus</i>
		1% <i>Carpinus betulus</i> *
		1% <i>Corylus avellana</i> *
		7% <i>Fraxinus excelsior</i>
		11% <i>Malus domestica</i>
		7% <i>Populus × canadensis</i>
		agg.
		2% <i>Sorbus torminalis</i> *

Simultaneously to the collection of lichens and tree bark, the moss species (Tab. 4.1) 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  $-20^{\circ}\text{C}$ . 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, 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  $^{15}\text{N}$  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  $\pm 0.1\%$ ). The determination of  $^{15}\text{N}/^{14}\text{N}$  is denominated as  $\delta^{15}\text{N}$ . The  $\delta^{15}\text{N}$  values are reported in per mil (‰) relative to air as the international standard for N and the analytical precision ( $n = 2$ ) is  $\pm 0.1\%$ . IAEA (International Atomic Energy Agency) certified and internal laboratory reference material was used for quality assurance in the analyses: IAEA-N<sub>1</sub> ( $\delta^{15}\text{N} = 0.4\%$ ), acetanilide (N% = 10.36%,  $\delta^{15}\text{N} = 1.8\%$ ) and barley (N% = 1.85%,  $\delta^{15}\text{N} = 5.2\%$ ).

### *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

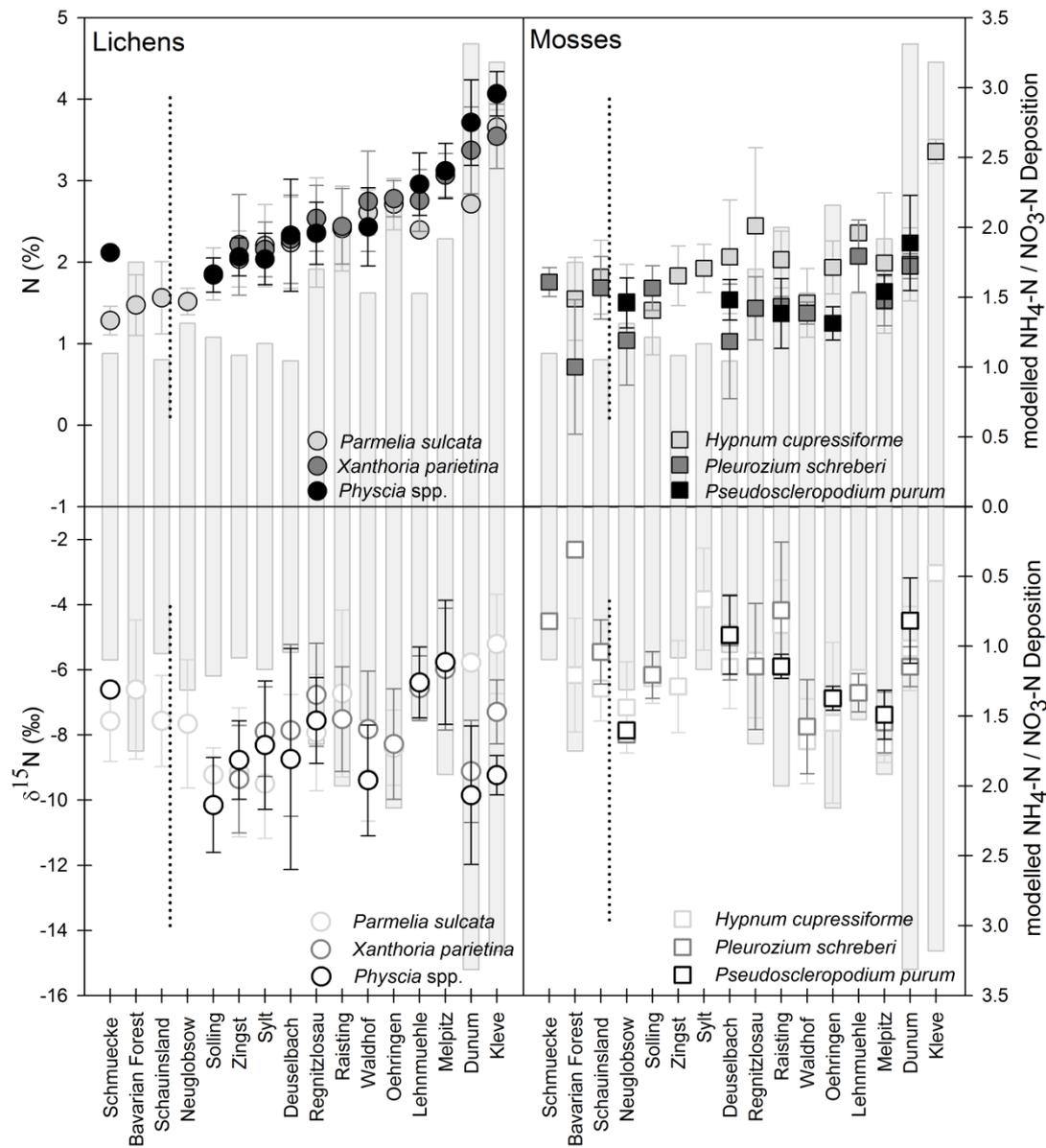
#### 3.1 Species- and spatial-specific accumulative responses to N

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. 4.3). The N content in lichen tissue ranged between 0.93% (*P. sulcata*, Bavarian Forest) and 4.69% (*Physcia* spp., Dunum), with an average of  $2.48\% \pm 0.79$  ( $n = 326$ ). 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. 4.2).

Tissue N content of all moss samples varied from 0.81% (*H. cupressiforme*, Bavarian Forest) to 3.64% (*H. cupressiforme*, Regnitzlosau). The average is  $1.77\% \pm 0.52$  ( $n = 153$ ). 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 according to the various N affected site categories ( $P < 0.001$ ) (Fig. 4.2).



**Figure 4.3** Average N concentrations and  $\delta^{15}\text{N}$  values of the lichen species (*P. sulcata*, *X. parietina* and *Physcia* spp.) and moss species (*H. cupressiforme*, *P. schreberi* and *P. purum*) (Mean  $\pm$ 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  $> 1000 \text{ mm yr}^{-1}$  (Schmuecke, Bavarian Forest and Schauinsland).

In the bark samples, an average of  $0.91\% \pm 0.25$  ( $n = 187$ ) 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. 4.4). A comparison of the mean values of the bark N content revealed a significant difference ( $P < 0.001$ ) between both moderate

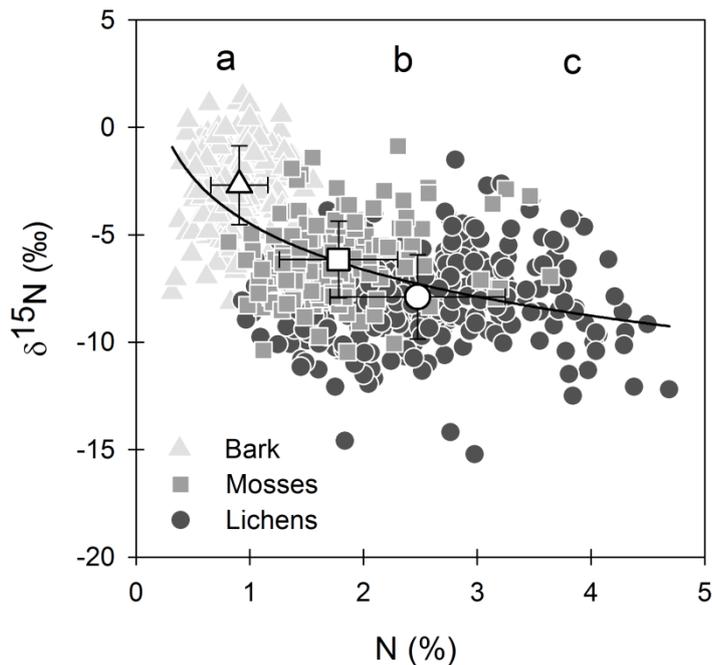
and highly agriculture affected areas on the one hand, and the coastal areas on the other. The forestry dominated areas ranked between these two groups.

### 3.2 Species- and spatial-specific natural abundance of $\delta^{15}\text{N}$

Within the taxonomic group, interspecific differences with respect to  $\delta^{15}\text{N}$  were only found at Kleve site within the lichens. *Physcia* spp. showed highly negative  $\delta^{15}\text{N}$  values, followed by *X. parietina* and finally *P. sulcata* which showed less negative  $\delta^{15}\text{N}$  values ( $P < 0.05$ ) (Fig. 4.3). The most negative  $\delta^{15}\text{N}$  signatures were detected in lichen samples. These  $\delta^{15}\text{N}$  signatures ranged between  $-15.2\text{‰}$  (*Physcia* spp., Deuselbach) to  $-1.5\text{‰}$  (*P. sulcata*, Raisting) ( $n = 326$ ). The measured  $\delta^{15}\text{N}$  values of the individual lichen species ranged from  $-12.1\text{‰}$  to  $-1.5\text{‰}$  for *P. sulcata* ( $n = 134$ ) and from  $-14.2\text{‰}$  to  $-2.6\text{‰}$  for *X. parietina* ( $n = 100$ ). The  $\delta^{15}\text{N}$  signatures of *Physcia* spp. ( $n = 92$ ) varied from  $-15.2\text{‰}$  to  $-3.2\text{‰}$ . There were no significant differences between the lichen species  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values).

The moss  $\delta^{15}\text{N}$  values varied from  $-10.5\text{‰}$  (*H. cupressiforme*, Oehringen) to  $-0.9\text{‰}$  (*H. cupressiforme*, Kleve) ( $n = 153$ ). Considering the  $\delta^{15}\text{N}$  signatures of the moss species separately, the largest range was found in *H. cupressiforme* ( $-10.5\text{‰}$  to  $-0.9\text{‰}$ ;  $n = 74$ ), followed by *P. schreberi* with a range from  $-8.8\text{‰}$  to  $-1.9\text{‰}$  ( $n = 51$ ) and finally *P. purum* which varied from  $-8.3\text{‰}$  to  $-3.6\text{‰}$  ( $n = 28$ ) (Fig. 4.3). The  $\delta^{15}\text{N}$  signatures between the moss species did not differ from each other. Additionally no differences were detected regarding the N affected site categories and the respective  $\delta^{15}\text{N}$  signatures in mosses.

From all the biomonitors tested, the bark samples showed the weakest negative  $\delta^{15}\text{N}$  signatures. The  $\delta^{15}\text{N}$  values ranged from  $-8.1\text{‰}$  (*Betula pendula*, Neuglobsow) to  $1.5\text{‰}$  (*Acer pseudoplatanus*, Lehmuehle), with an average of  $-2.7\text{‰} \pm 1.9$  ( $n = 187$ ) (Fig. 4.4). No differences were found concerning the various site categories.

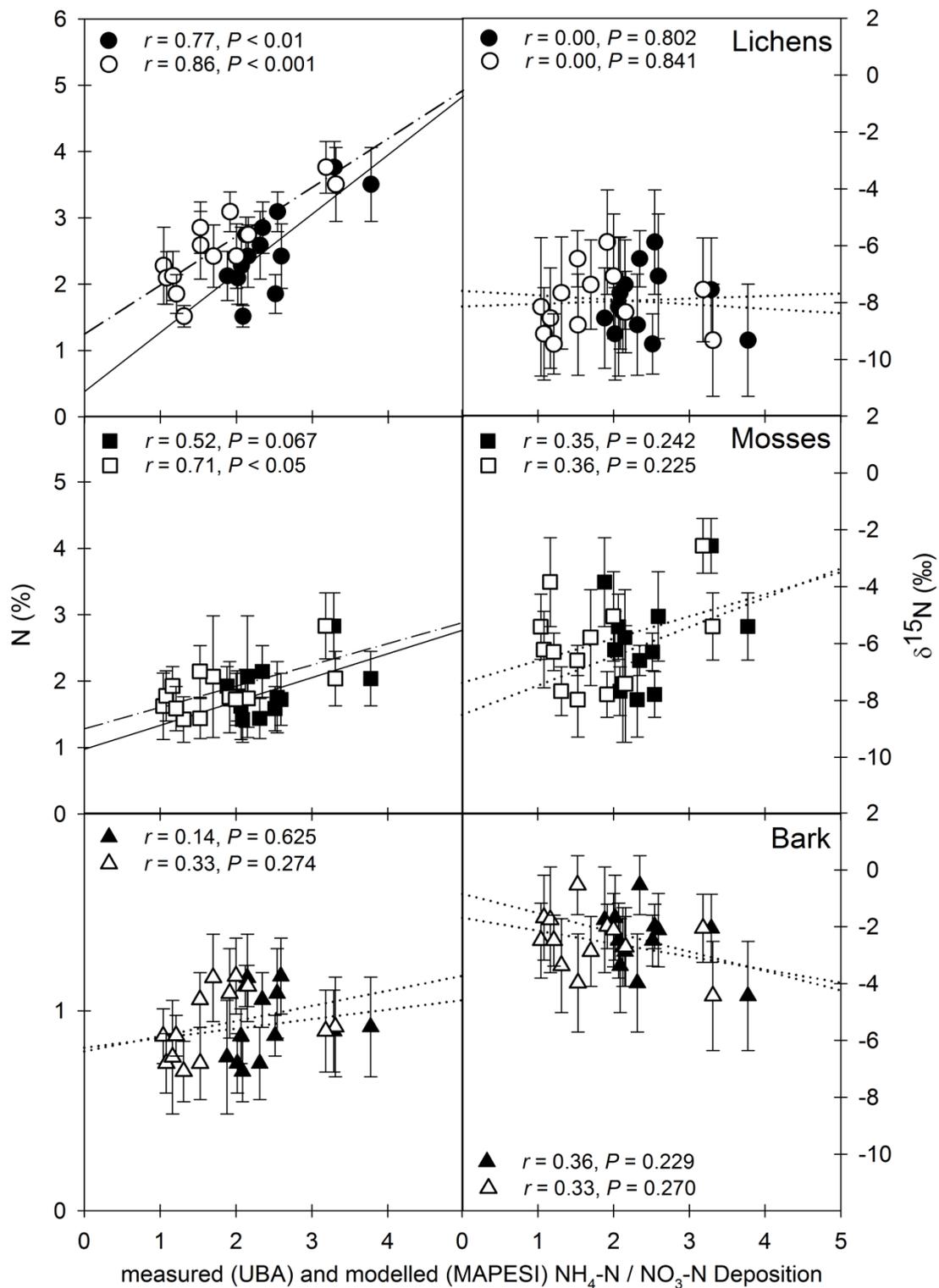


**Figure 4.4** Total N and  $\delta^{15}\text{N}$  values of all lichen ( $n = 326$ ), moss ( $n = 153$ ) and bark samples ( $n = 187$ ) and the means  $\pm$   $SD$  (white symbols). Different letters indicate significant statistical difference at level  $P < 0.001$ . Logarithmic regression ( $r = 0.56$ ,  $P < 0.0001$ ) for all data is shown.

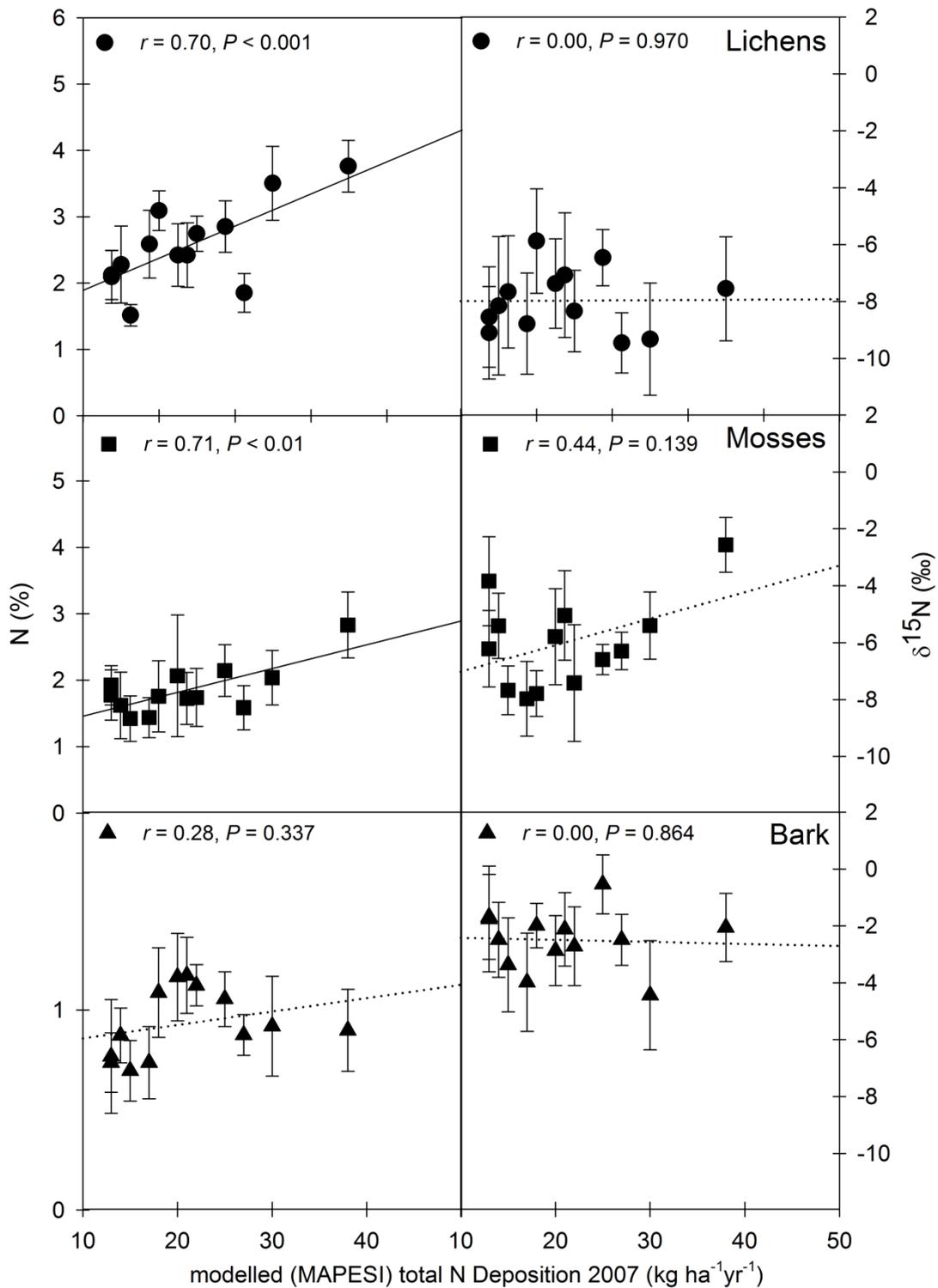
### 3.3 Relationship between tissue N, $\delta^{15}\text{N}$ patterns and N deposition data

In the assessments below, only sites with precipitation  $< 1000 \text{ mm yr}^{-1}$  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  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  ( $r = 0.86$ ,  $P < 0.001$ ). 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  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  deposition data ( $r = 0.71$ ,  $P < 0.05$ ) (Fig. 4.5; Fig. 4.6). A non-significant trend was found with measured on-site wet-only N deposition data ( $r = 0.52$ ,  $P = 0.067$ ) (Fig. 4.5). Bark samples only showed a positive trend between N content and both reduced N compounds and total N deposition, modelled by MAPESI (Fig. 4.5; Fig. 4.6).

No significant correlation was found between  $\delta^{15}\text{N}$  signatures and atmospheric enrichment of agriculture-related reduced N deposition. There was only a non-significant positive tendency between  $^{15}\text{N}$  enriched moss data and measured and modelled N deposition data (Fig. 4.5; Fig. 4.6). Bark samples show a non-significant negative trend (Fig. 4.5).



**Figure 4.5** Total nitrogen concentration and  $\delta^{15}\text{N}$  values of lichens (circles), mosses (squares) and bark samples (triangles) (Mean  $\pm$   $SD$ ) of the study sites ( $n = 13$ ) plotted against measured wet-only deposition ratio of  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  (UBA) (black symbols and black solid line) and MAPESI modelled total deposition quotient of  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  (white symbols and dash dot line).



**Figure 4.6** Total nitrogen concentration and  $\delta^{15}\text{N}$  values of lichens (circles), mosses (squares) and bark samples (triangles) (Mean  $\pm$  *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.

## 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%  $\pm$ 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 experiment has its highest induced NH<sub>3</sub> 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. 4.4). 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 (Tab. 4.2).

**Table 4.2** 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 <sup>a,b</sup>	hydrophilic layer of polysaccharides (cellulose)	hydrophilic layer of polysaccharides (cutin: cellulose, pectin, wax)
	no stomata, no cuticle <sup>b,c</sup>	no gametophyte stomata, no cuticle <sup>d</sup>	
	rhizines <sup>a</sup>	rhizoids <sup>e</sup>	
N sources	atmospheric sources <sup>f</sup>	atmospheric sources + soil pool <sup>g</sup>	atmospheric sources + soil pool <sup>h</sup>
N deposition type	wet + dry deposition <sup>f</sup>	wet + dry deposition <sup>i</sup>	wet + dry deposition <sup>h</sup>
N uptake	$\text{NH}_4^+$ , $\text{NH}_3$ , $\text{NO}_3^-$ <sup>c,j,k,l</sup>	$\text{NH}_3$ , $\text{NH}_4^+$ , $\text{NO}_3^-$ <sup>m,n,o</sup>	$\text{NH}_4^+$ , $\text{NH}_3$ , $\text{NO}_3^-$ <sup>h</sup>
	$\left. \begin{array}{l} \text{NH}_3 \\ \text{NH}_4^+ \\ \text{NO}_3^- \end{array} \right\}$ active	active	passive

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

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.

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. 4.2). The identified areas with highest N contents (Kleve, Dunum, Melpitz and Lehmuehle) 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 ( $\geq 1.6\%$ ) 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 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  $\text{NH}_4\text{-N}/\text{NO}_3\text{-N}$  values (Fig. 4.5). Furthermore, the tissue N concentration in lichens and mosses were significantly correlated with modelled total N deposition (Fig. 4.6). 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  $\text{NH}_4^+$  and  $\text{NO}_3^-$  simultaneously under natural N deposition conditions (Crittenden, 1998; Johansson et al., 2010). Studies have also revealed that  $\text{NH}_4^+$  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. 4.5).

#### *4.2 Patterns of $\delta^{15}\text{N}$ 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  $\text{NH}_x$  (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  $\delta^{15}\text{N}$  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  $^{15}\text{N}$ , varying from  $-15.2\text{‰}$  to  $1.5\text{‰}$ . The few positive  $\delta^{15}\text{N}$  values are all among the bark samples. N pollution dominated by N oxides ( $\text{NO}_y$ ) mostly lead to positive  $\delta^{15}\text{N}$  values (enrichment in  $^{15}\text{N}$ ), while pollution based on agriculture which is associated with  $\text{NH}_4^+$ , yield negative isotopic values (depletion in  $^{15}\text{N}$ ) (Freyer, 1978; Heaton et al., 1997).

The measured  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  in plant material next to agricultural  $\text{NH}_3$  sources are enriched in  $^{15}\text{N}$  and measured  $\delta^{15}\text{N}$  values downwind from a source exhibit less  $^{15}\text{N}$  (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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values in mosses collected in urban areas (average of  $+3.7\text{‰}$ ) and rural areas (average of  $-7.8\text{‰}$ ).

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  $\delta^{15}\text{N}$  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  $^{15}\text{N}$  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  $14\text{‰}$  within lichens and nearly  $10\text{‰}$  within the mosses and bark samples, suggest that these biomonitors are highly sensitive to different types of atmospheric N pollution. The wide range 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values in the nitrophytic lichens *X. parietina* and *Physcia* spp. (Fig. 4.3). 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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  values (Fig. 4.5). Other studies document better correlations between moss N chemistry and dry deposited  $\text{NH}_x$  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  $\text{NH}_4^+$  and  $\text{NO}_2$  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  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$  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. 357,100 km<sup>2</sup> (Statistische Ämter des Bundes und der Länder, 2013), the additional use of biomonitors should be considered.

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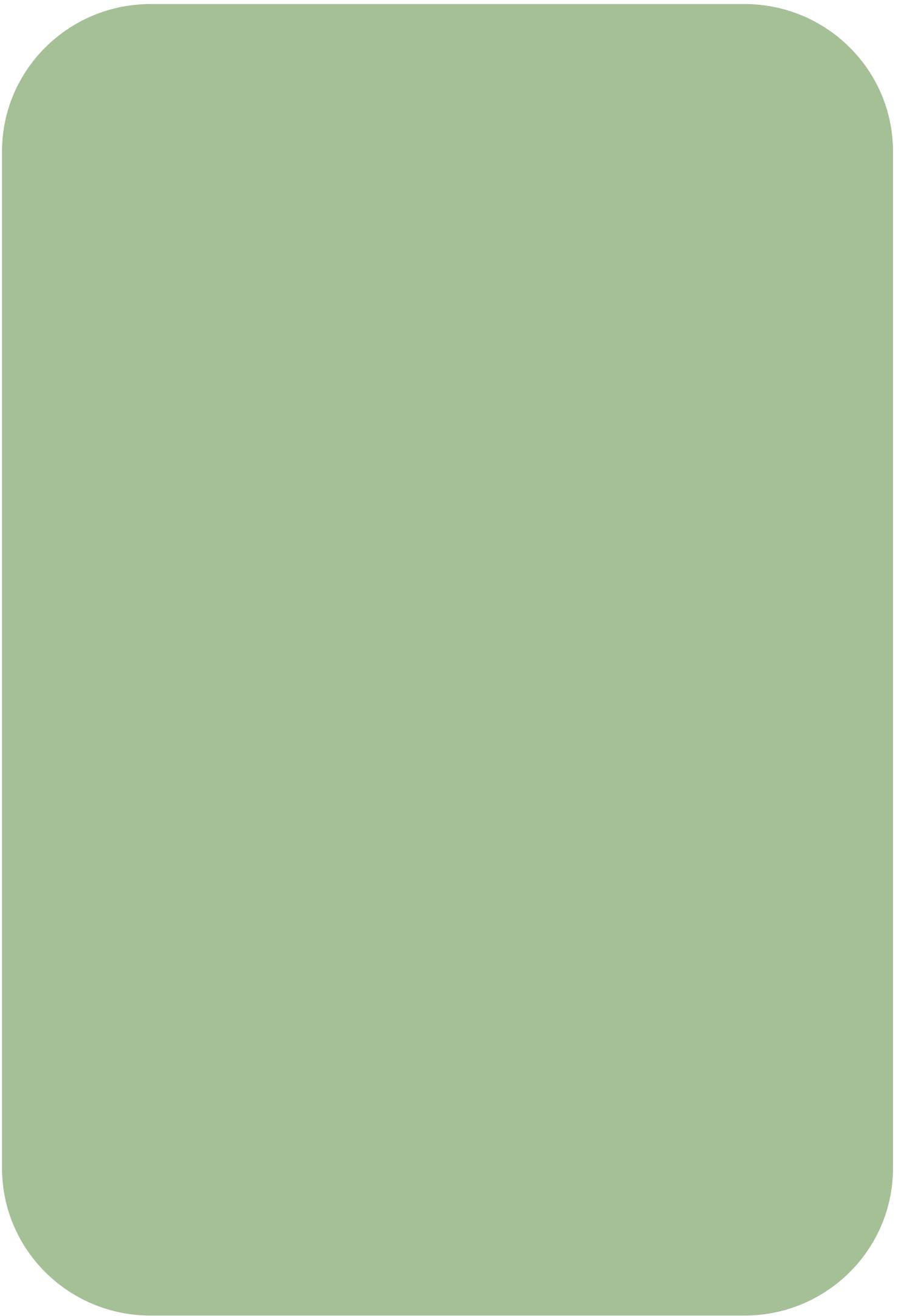
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# Chapter 5

Synthesis – The upshot

SH Boltersdorf

The previous chapters have contributed to the development and confirmation of the use of biomonitors, especially epiphytic lichens. The key challenge of the present thesis was to assess N deposition rates and define N sources with the help of total tissue N content and  $\delta^{15}\text{N}$  patterns in different lichen species. The research objectives were formulated to pursue the main aim, namely to verify the results and the use of the applied monitoring technique with lichens. Each of the formulated objectives was treated in a journal publication.

In general, the investigated lichen species vary in their distribution in the study region due to the spatial variation of N sources and N deposition rates (Chapter 2, Fig. 2.1). Interspecific differences related to different N deposition rates and sources on-site have been found within (Chapter 2, Fig. 2.3; Chapter 4, Fig. 4.3) and between (Chapter 2, Fig. 2.3, Fig. 2.5; Chapter 4, Fig. 4.2, Fig. 4.3, Fig. 4.4) each investigated taxonomic group (lichens, mosses and trees) concerning N contents and  $\delta^{15}\text{N}$  ratios. Eventually, intraspecific differences within each investigated species occurred as a result of various spatial variations of N impact (Chapter 2, Fig. 2.3; Chapter 3, Fig. 3.2; Chapter 4, Fig. 4.3).

The results are summarised in the following with special consideration of the overall research intention and the specific formulated research objective. These results are shown for the first time for the studied species in the study area.

i. *Specifying and assessing a suitable monitoring technique – identifying relationships between the tissue N chemistry (N%,  $\delta^{15}\text{N}$ ) of different monitor organisms (lichens and tree bark) and their N environment*

The study in Chapter 2 revealed that an increase in agriculture-derived N in ecosystems is responsible for different detected N contents and isotopic abundances of  $^{15}\text{N}$  in different epiphytic lichen species, tree bark and wet deposition samples. Species-specific N concentrations in lichen tissues were also found along a gradient of N deposition rates, which are related to areas with different levels of agricultural influence (Chapter 2, Fig. 2.3). Furthermore, an overview of the distribution and the potential use of different lichen species as appropriate monitoring organisms in the western part of Germany is provided. The nitrophytic lichen species *X. parietina*, *P. adscendens* and *P. tenella* were identified as particularly suitable organisms for biomonitoring of N. They are most able to reflect the atmospheric influence of intensive agriculture on-site, compared to other investigated lichen species, bark samples and wet deposition samples (Chapter 2, Fig. 2.6, Tab. 2.1). A linkage between N concentration in lichen tissues and N deposition data was found with the help of the lichen monitoring method for the first time along the gradient in the study region (Chapter 2, Fig. 2.6; Tab. 2.1). The results of this survey formed the basis

for an area-wide and grid-based implementation of the monitoring method on a broad spatial scale.

*ii. Implementation of a regional monitoring framework – verifying and mapping the N impact*

The results expanded the knowledge of the ability of nitrophytic lichens to reflect N loads and N sources only by tissue N content and tissue  $\delta^{15}\text{N}$  values (Chapter 3). Until now, this kind of investigation was mainly accomplished with mosses on a local (Kluge et al., 2013; Solga et al., 2005; 2006) and national scale (Pesch et al., 2007) in Germany, although identifying the prevailing atmospheric N source with the help of stable isotopes received less attention. The present study showed that nitrophytic lichens are able to reflect the quantity and quality of agriculture-derived N deposition along an N gradient on a regional scale (Chapter 3, Fig. 3.2). Compared to model-based maps, which document the N impacts in the north-western part of Germany (De Vries et al. 2011; Gauger et al. 2001), lichens provide a similar picture of N deposition patterns in the same region. Moreover, by consulting diverse N-related data for this study, such as livestock units per administrative district (Kreins 2013),  $\text{NH}_3$  emission density per administrative district (Rösemann et al., 2011), deposition type- (Wellbrock et al., 2005) and specific landscape categories led to a confirmation and reinforcement of the validity of the lichen monitoring system to identify N loads and N sources (Chapter 3, Fig. 3.5). Lichens accurately represent the atmospheric N deposition rates as well as the predominant source of N emission on-site.

Although it is known that lichens have similar uptake rates of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  at ecologically relevant N doses (Johansson et al. 2010), they will prefer  $\text{NH}_4^+$  uptake if artificially high N doses occur (Dahlman et al., 2004; Hauck, 2010). In the present investigation, lichens mostly showed a depletion in  $^{15}\text{N}$  (detection of mainly  $\text{NH}_y$  compounds in the atmosphere). Simultaneously, less negative  $\delta^{15}\text{N}$  ratios were detected in lichens along the Rhine River and in the Rhine-Ruhr metropolitan region by geostatistical analysis (Chapter 3, Fig. 3.2) suggesting that the prevalent N oxides from traffic and industrial emissions are responsible for the enrichment in  $^{15}\text{N}$  and correspondingly less negative  $\delta^{15}\text{N}$  values in the lichen tissues. This has also been demonstrated by Gerdol et al. (2002) and Pearson et al. (2000). Changing isotopic signatures in the studied lichens are also linked to changing altitudes (Chapter 3, Fig. 3.4). Lowland regions are highly affected by wet deposition leading to strong negative  $\delta^{15}\text{N}$  values, whereas occult/dry deposition in the uplands causes less negative  $\delta^{15}\text{N}$  values. This dependence was proven and affirmed by PLS-R analysis (Chapter 3, Fig. 3.6) and is in line with results of Zechmeister et al. (2008) who found a similar relationship in moss

species along an altitudinal transect in a nationwide study in Austria. PLS-R analysis also revealed a connection between  $\text{NH}_3$  density per  $\text{km}^2$  and livestock unit per  $\text{km}^2$  and the tissue N content in the lichen species (Chapter 3, Fig. 3.6).

The applied method was transferred to a national scale and the results were compared to other biomonitoring techniques as well as to measured and modelled N deposition data in Chapter 4. In this regard, the research issue – *iii. Critical appraisal of three biomonitoring techniques – comparative analysis of a widely applicable method based on measured and modelled data of the national N deposition assessment programme; improving the ability to quantitatively relate bioindicator responses to N deposition* was investigated. Data derived from UBA deposition measurement stations were chosen as reference for this survey. UBA-data are also used as a relevant basis for national and Europe-wide N deposition monitoring programmes. The detected N concentration ranges of all investigated biomonitors (lichens, mosses and tree bark) were able to reflect the high dimension of anthropogenic N pollution in Germany. Beside isotopic N signatures, total tissue N contents in lichens also allows an identification of N deposition originating from intensive agriculture and indicate the degree of N deposition in Germany (Chapter 4, Fig. 4.5). Compared to mosses, lichens reflect the N loads of different land use affected areas significantly better (Chapter 4, Fig. 4.2), although only moss monitoring has frequently been used in local, national and European studies (Bragazza et al., 2005; Foan et al., 2014; Harmens et al., 2011, 2013; Kluge et al., 2013; Leith et al., 2005; Pesch et al., 2007; 2008; Pitcairn et al., 2006, Schröder et al., 2010, 2011; Zechmeister et al., 2008). Regions with high agricultural influence could be clearly distinguished from less affected regions with the help of tissue N content in lichens. The study also shows that dealing with different biomonitors is a difficult task due to their variety of N responses (Chapter 4, Fig. 4.3, Fig. 4.4, Tab. 4.2). The specific receptor surfaces of the indicators and therefore their different strategies of N uptake are responsible for the different tissue N concentrations in each organism group. The isotopic signatures could potentially be used as a complementary instrument to assist in interpreting the prevailing N source on-site, especially in combination with data of the N content in plants or N deposition data. In Chapter 4 it has firstly been shown that  $\delta^{15}\text{N}$  values depend on the N source and the specific N transformations in each organism. Secondly, the  $\delta^{15}\text{N}$  values in the used biological monitors revealed that high agriculture related N deposition rates ( $\text{NH}_y$ ) are responsible for the increased N loads at the investigated deposition sites in Germany.

Overall, the presented results contribute substantially to the understanding in lichen research and highlight the possibility of using epiphytic lichens as a cost-efficient

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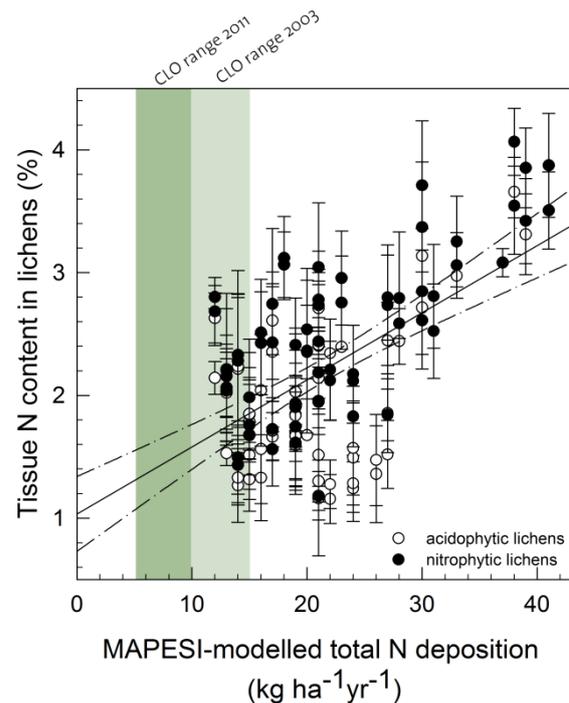
alternative compared to physico-chemical measurements to assess differences in  $\delta^{15}\text{N}$  and tissue N content among different N sources and doses on a regional and supra-regional scale. Monitoring the atmospheric N deposition by common monitoring stations is severely restricted in spatial extent and type. Lichens are powerful indicators due to their species-specific strong absorption and efficient incorporation of N. Therefore, lichens can at least support on-site N deposition measuring instruments by identifying the quality and quantity of the predominant N deposition rate and source. Monitoring with lichens is advantageous in heterogeneous areas with diverse local wind conditions and topographic variations. Because of errors in common deposition measurements, their low ability to determinable diffuse  $\text{NH}_3$  deposition and the wide dispersal of nitrogenous air pollution over long distances, an assessment of the time-integrated N impact on ecosystems with lichens would improve common N deposition measurements. Based on the temporarily and patchy installed deposition measurement devices in Germany, the N load has not been determined and assessed comprehensively on a wide spatial coverage and in differently structured areas so far.

The present surveys provide an overview for species-specific ranges of tissue N contents in lichens, mosses and tree bark and at the same time, offer a basis for indicating regions as already impacted, moderately or less affected by N. Key challenges in the research field of N bioindication and -monitoring are to indicate direct and site-specific evidence of conditions of high exposure to atmospheric  $\text{N}_r$  impacts and to provide an easy and practical method at low-cost compared to physical monitoring techniques for N deposition assessment (Sutton et al., 2004a), which are also often exposed to theft and vandalism (Nimis & Purvis, 2002). Especially due to the poor regional coverage of physical monitoring stations, which are also subject to frequent measuring faults, a quantitative method to reliably estimate the degree of exposure to N is required, and herein the applied lichen monitoring method provides an appropriate alternative. The biomonitoring method gives a broad indication of whether the N depositions are at low or high levels by comparing results with other equivalent monitoring studies (Chapter 2 + Chapter 4).

The presented lichen monitoring technique offers a practical site-based approach for assessing N concentrations, deposition rates and impacts almost everywhere. The current study contributes to the scientific development of N biomonitoring methods from an operational perspective. Measuring total tissue N is undoubtedly the most robust method in terms of evidence and published work relating to both N deposition and tissue N concentration under natural conditions (Sutton et al., 2004b). Moreover, lichens

represent a wide range of tissue N concentrations and are therefore able to reflect and to respond to small changes in N deposition in the environment.

The measurement of N accumulation can additionally be used to refine local estimates of the exceedance of critical loads (CLOs) by the definition of critical tissue N content (Sutton et al., 2004b). An empirical CLO range for lichens and algae in temperate and boreal forests is set at 5 to 10 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Bobbink & Hettelingh, 2011). Formerly, the CLO range was set at 10 to 15 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Achermann & Bobbink, 2003). In lichen-rich tundra habitats, the CLO range is set as low as 3 to 5 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Bobbink and Hettelingh, 2011). Using a linear regression (Fig. 5.1) between MAPESI (Modelling of Air Pollutants and EcoSystem Impact) - modelled total N deposition data and tissue N content in different lichen species at the study sites (Chapter 2 + Chapter 4) the total N deposition could be deduced. It was found that 87% of all investigated study sites (Chapter 2 – Chapter 4) exceed the CLO<sub>max 2011</sub> (CLO<sub>max2003</sub> = 75%). The 13% (CLO<sub>range 2003</sub> = 25%) of study sites currently within the CLO range of 2011 includes mainly sites in the low mountain ranges and in the so-called ‘background areas’ at high altitudes like at sites Schauinsland, Bavarian Forest and Schmuecke. Fenn et al. (2008) revealed that a CLO of 3.1 kg N ha<sup>-1</sup>yr<sup>-1</sup> protects lichen communities from a shift into a more nitrophytic composition. Taking into account this CLO range, only 2% of the investigated sites were not affected by N-related composition shifts. Considering all lichen species used in this thesis, it could be worked out that a lichen tissue N content of ~1.6% indicates that the CLO<sub>max</sub> of 10 kg ha<sup>-1</sup>yr<sup>-1</sup> has already been reached.



**Figure 5.1** Linear increase in tissue N content of *H. physodes*, *P. sulcata* (acidophytic lichens) and *X. parietina* and *Physcia* spp. (nitrophytic lichens) (Mean  $\pm$  SD per lichen species and study site) in response to MAPESI modelled total N deposition (2007) (UBA sites (Chapter 3), and study sites of Rhineland-Palatinate, North Rhine-Westphalia and Lower Saxony (Chapter 2)). Dash-dot lines present 95% confidence intervals, solid line shows regression line:  $y = 0.0547x + 1.0345$ ,  $r = 0.590$ ,  $P < 0.001$ ,  $n = 125$ . CLO range 2003: 10-15 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Achermann & Bobbink, 2003) and CLO range 2011: 5-10 kg N ha<sup>-1</sup>yr<sup>-1</sup> (Bobbink & Hettelingh, 2011) for lichens and algae in temperate and boreal forests.

Modelled data are used for many issues in the field of administrative approvals, emission reports and ecotoxicology, and they are subject to continuous developments and improvement processes. In addition to the model MAPESI, an advanced model version called Pollutant Input and EcosysTem Impact (PINETI) has recently developed. Based on this model, the total N deposition is approximately 30% less than was calculated by MAPESI-2007 (Wichink Kruit, 2014). Consequentially, only 77% of the present study sites will exceed the  $CLO_{\max 2011}$  of  $10 \text{ kg ha}^{-1}\text{yr}^{-1}$ , compared to 87% based on MAPESI-modelled total N deposition.

Generally, it remains debatable which determined N deposition should form the basis for policy-making decisions. Therefore, additional comparisons with measured total bulk N deposition rates on-site are urgently needed and should be increasingly used in future studies for evaluations. Particularly, in terms of the implementation of the lichen monitoring technique, a closer linkage and an ongoing comparison with recent measured and modelled N deposition data are needed to validate the application. Moreover, future research should focus on deeper and detailed explanation and understanding of the N uptake of lichens. Biomonitors are able to respond to several different forms of N. Therefore, a determination of the source and the prevailing form of N in the environment only based on bioindicator measurements is often difficult (Sutton et al., 2004a), except if isotope research is used and comparative data are available (Fogel et al., 2008; Franzen-Reuter, 2004; Lee et al., 2009; Russow et al., 2004; Skinner et al., 2006; Tozer et al., 2005, Zechmeister et al., 2008). Not many studies have been conducted using  $^{15}\text{N}$  abundances in lichens and especially studies on  $\text{NH}_3$  diffusion uptake by lichens are missing. Experiments with different deposition collectors (wet, dry and gaseous collectors) and simultaneous analyses of the chemical compositions, explicit the isotopic composition, in the deposition samplers on-site have to be accomplished for a prospective development of the assessment of N deposition rates. Additionally, interactions between substrate and epiphytic lichens need to be investigated more precisely, to exclude or to quantify more exactly further possible influences on the lichen N uptake through the respective substrate surface.

There is also a high demand for research regarding deciduous trees and their tree bark characteristics, like tree bark structure and composition (e.g. bark roughness, pH, conductivity and possible biochemical conversion of  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  on the bark surface by microorganisms) and their influence on lichen community and their nutrient uptake. Especially lichen growth and the temporal/seasonal variations of N accumulation in lichens should be subject to further studies to confirm the explanatory power of

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interpreting the annual atmospheric N deposition patterns and the respective N content in lichen thalli. There is still a lack of detailed knowledge about possible impacts of stem flow and throughfall (crown deposition) on epiphytic lichens. The effect of the complexity of eddies around trees and crown structure on atmospheric N deposition need to be investigated much more thoroughly in the context of lichen research. The potential future research approaches and surveys should be conducted in forest stands as well as in open fields.

Based on studies by Hallingbäck (1991,1992) and Van Herk et al. (2003), it is known that lichens are clearly the most sensitive group in the vegetation with respect to direct toxicity of  $\text{NH}_3$ , so that this group can also be considered as early warning systems for further agriculture-related N effects on terrestrial ecosystems (Bobbink & Hettelingh, 2011). The presented conceptual framework provides a long-term ecological monitoring method which is able to assess the human-accelerated environmental change, especially by integrating different kinds of monitoring methods and data. Changes and valuable insights into ecosystem structure, ecological processes and ecosystem services can be detected using long-term ecological monitoring methods. It would not be possible to understand the altering status of the life support system of the planet, without long-term information derived from monitoring systems (Lindenmayer & Likens, 2010).

Coupling ecological monitoring with citizen science provides a wealth and mayor source of data, which can be used to assess the environmental condition in many different regions. An example of this can be found in Seed et al. (2013), the UK OPAL (Open Air Laboratories) air survey, in which site-specific levels of air quality derived from lichen indices on a national-scale were investigated. Simultaneously, education and engagement for the community into scientific research would be supported (Bonney et al., 2009, Dickinson et al., 2010, Seed et al., 2013).

It should be considered in the future to incorporate citizen science in the field of monitoring, because finally it should be noted:

‘Monitoring sustainability will only be possible if the monitoring system is itself sustainable’ (Watson & Novelly, 2004).

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'The major problems of the world are the result of the difference between the way nature works and the way people think.'

Gregory Bateson [1904-1980]

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‘Success is not final, failure is not fatal: it is the courage to continue that counts’

Sir Winston Churchill [1874-1965]

... and many dear fellows who surrounded one, if the courage is temporarily absent.

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# Curriculum Vitae

Stefanie Helene Boltersdorf

Environmental Scientist

Date of birth                      June 30<sup>th</sup>, 1980  
Place of birth                      Andernach, Rhineland-Palatinate, Germany

## Education

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07/08 – 03/14                      *Doctoral Studies*, University of Trier  
Doctoral thesis: ‘A critical appraisal of accumulative biomonitors to assess and to map sources and rates of atmospheric nitrogen deposition on different regional scales in Germany.’

10/00 – 04/07                      *Applied Environmental Sciences*, University of Trier  
Focus: Structure and process analysis of the ecosystem and environmental analytics;  
Major subjects: Geobotany/Soil Science; Minor subjects:  
Cartography/Physical Geography /Regional Developing and Planning  
Diploma thesis: ‘Akkumulation luftgetragener Stoffe in Flechten.’

08/91 – 06/00                      University-entrance diploma, Geschwister-Scholl-Gymnasium Daun

07/87 – 06/91                      Primary school, Grundschule Ahrbrück

## Professional Practical Experience

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since 09/11                      Freelancer, HYDROSOL-Hellebrand & Müller GbR, Trier  
Phytosociologically consulting with field surveys (habitat type mapping)

04/09 – 02/13                      Assistant lecturer, University of Trier  
Department Geobotany  
Lectures, exercise courses und field exercises

01/08 – 02/13                      Research Assistant/Tutor, University of Trier  
Department Geobotany  
Tutorials ‘Grundlagen der Physische Geografie I – Vegetationsgeografie’  
and Exercise course: ‘Bioindikation ausgewählter Luftschadstoffe’

09/10                                  Freelancer, Office for conservation and work safety, Trier  
Biomonitoring (pedological mapping and chemical / physical analyses) in the course of a mountain authorization procedure in North Rhine-Westphalia

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- 03/08 – 06/08 Freelancer, University of Trier/State Agency for Environmental and Occupational Safety of the Saarland, Saarbrücken  
Data processing of pedological data and deposition data, building a database and website of the project ‘Forstliches Umweltmonitoring des Saarlandes‘
- 10/07 Internship, City Park Services, Auckland (New Zealand)  
Gardening in winter gardens and parks
- 08/06 Internship, Office for conservation and work safety, Trier  
Biomonitoring (pedological mapping and chemical / physical analyses) in the course of a mountain authorization procedure in North Rhine-Westphalia
- 01/03 – 12/05 Student research assistant/tutor, University of Trier  
Department Cartography  
Presentation of teaching content, homework help and correction of exercises: ‘Kartographische Informationsverarbeitung I/II‘, assistance/preparations of courses and field courses, system demonstrations of cartographic data processing, computer user support
- 05/05 – 07/05 Internship, Helmholtz Centre for Environmental Research (UFZ), Leipzig  
Department Computational Landscape Ecology (CLE)  
Simulation of floods and evaluation of the hydrodynamic 2D model TrimR2D (BMBF-Project: "3Z GRIMEX extreme floods in urban areas 2D model TrimR2D), Ground-Truth-Mapping
- 04/05 Internship, Landscape architecture office BGHplan, Trier  
Participation in the preparation of an accompanying landscape conservation plan, preparation of environmental reports for local development plans
- 08/04 – 10/04 Internship, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf (Switzerland)  
Department Forest Ecology  
Involved in ‘Treeline trees in a CO<sub>2</sub>- enriched world’ (Davos) and ‘The Swiss Canopy Crane Project in Hofstetten’(Jura), i.a.  
Field work, measuring soil respiration, biomass determination, chemical soil analyses
- 02/02 – 05/10 Temporary employee, Health food shop ‘Naturkost Zwiebel‘, Trier

## Teaching Activity

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Grundlagen der Geobotanik (lecture)  
Globale ökologische Veränderungen (lecture)  
Globale ökologische Veränderungen (seminar)  
Mikroskopierkurs Pflanzenanatomie (exercise course)

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Modellspezies und Testverfahren/Bioindikation (Flechten)  
(exercise course)  
Geobotanische – Standortkundliche Labor- und Geländeübung/Applied  
Vegetation Science (exercise course)

## Grants

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01/2013 'Mary Kingsley-funds' University of Trier – grant for congress participation  
10/2011 'Research fund' University of Trier – grant for congress participation  
07/08 – 06/11 'Doctoral Scholarship' of the German Environmental Foundation (DBU), Osnabrueck

## Abroad experiences/field trips

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02/10 – 04/10 Field work in the western part of Germany (25 × 25 km grid-based sampling) for the dissertation project  
09/08 – 02/09 Field work in the federal states Saarland/Rhineland-Palatinate/North Rhine-Westphalia and Lower Saxony and around Germany (relating to UBA air network) (Germany) for the dissertation project  
04/07 – 10/07 New Zealand, study trip  
06/06 – 08/2006 Field work in the federal states Rhineland-Palatinate and North Rhine-Westphalia (Muensterland) (Germany) for the diploma project  
08/04 Canada, Manitoba – Excursion in the fields of Physical Geography and Soil Science, University of Trier/Natural Resources Institute, University of Manitoba (Canada), Pedological and geomorphological focus  
08/02 Germany, Northern Alps – Excursion in Geobotany, University of Trier  
Phytosociological focus

## Other

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since 02. 2011 Juror at 'Jugend forscht' (Biology)  
12/05 Participation in an advanced training course for qualification to the Geopark Ranger (Natur- und Geopark Vulkaneifel GmbH Daun)

## Publications

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Peer-reviewed Journal Articles

**Boltersdorf SH, Werner W** (2014). Lichens as a useful mapping tool? – An approach to assess atmospheric N loads in Germany by total N content and stable isotope signature. *Environmental Monitoring and Assessment* 186 (8), 4767 – 4778. DOI: 10.1007/s10661-014-3736-3

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**Boltersdorf SH**, Pesch R, Werner W (2014). Comparative use of lichens, mosses and tree bark to evaluate nitrogen deposition in Germany. *Environmental Pollution* 189, 43 – 53. DOI: 10.1016/j.envpol.2014.02.017

**Boltersdorf S**, Werner W (2013). Source attribution of agriculture related deposition by using total nitrogen and  $\delta^{15}\text{N}$  in epiphytic lichen tissue, bark and deposition water samples in Germany. *Isotopes in Environmental and Health Studies* 49 (2), 197 – 218. DOI: 10.1080/10256016.2013.748051

## Abstracts

**Boltersdorf SH** (2011). Assessing the quality and quantity of N deposition by total N content and the stable isotope signatures in lichen tissue. In: *Geophysical Research Abstracts*, Vol. 13, EGU2011, p. 2251. <http://meetingorganizer.copernicus.org/EGU2011/EGU2011-2251.pdf>

**Boltersdorf SH** (2011). Do epiphytic lichens reflect agriculture-related depositions? In: *Geophysical Research Abstracts*, Vol. 13, EGU2011, p. 2124. <http://meetingorganizer.copernicus.org/EGU2011/EGU2011-2124.pdf>

**Boltersdorf SH**, Werner W (2010). Characterisation of nitrogen deposition and source attribution by using N-concentrations and  $\delta^{15}\text{N}$ -signatures of epiphytic lichens (*Xanthoria parietina* and *Hypogymnia physodes*) in Germany. In: *The future of biodiversity: Genes, Species, Ecosystems. Verhandlungen der Gesellschaft für Ökologie, Open topics. Book of abstracts*, eds. V Wolters, J Groh, F Peter, R Waldhardt. Gesellschaft für Ökologie, Berlin, Bd. 40, p. 432

**Boltersdorf SH**, Werner W (2009). Nitrogen concentrations and  $\delta^{15}\text{N}$  ratios in epiphytic lichens in correlation to nitrogen deposition rates in Germany. In: *GFÖ 2009 Dimensions of ecology. From global change to molecular ecology C1 Stable Isotopes in Ecology. Book of abstracts*, eds. S Holzheu, B Thies. Bayreuther Forum Ökologie (BFÖ), BayCEER, Bayreuth, Vol. 115, p. 101

**Boltersdorf SH**, Werner W (2008). Estimation of nitrogen deposition rates on a regional level using nitrogen concentrations of the epiphytic lichen *Xanthoria parietina*. In: *EURECO-GFOE 2008 Proceedings, Verhandlungen der Gesellschaft für Ökologie, Nitrogen enrichment in terrestrial ecosystems*, eds. J Stadler, F Schöppe, M Frenzel, Bd. 38, p. 205

## Talks

**Boltersdorf SH**, Pesch R, Schröder W, Werner W (2013). Germany-wide comparison of spatial distribution of nitrogen concentrations and stable isotope signatures of bryophytes and lichens. 26<sup>th</sup> Task Force Meeting - UNECE ICP Vegetation; Halmstad, Sweden. 1/2013

**Boltersdorf SH** (2011). Mapping agriculture-related deposition by using total nitrogen and  $\delta^{15}\text{N}$  signatures in epiphytic lichen tissue. Annual conference of German Association of Stable Isotope Research (GASIR); Villigen, Switzerland. 10/2011

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**Boltersdorf SH** (2011). Assessing the quality and quantity of N deposition by total N content and the stable isotope signatures in lichen tissue. European Geosciences Union (EGU), General Assembly; Vienna, Austria. 4/2011

**Boltersdorf SH, Werner W** (2011). Regional variation in the nitrogen content and  $\delta^{15}\text{N}$  signatures of lichens in relation to atmospheric nitrogen deposition. 24<sup>th</sup> Task Force Meeting - UNECE ICP Vegetation; Rapperswil-Jona, Switzerland. 1/2011

Werner W, Schultze J, **Boltersdorf SH** (2011). Patterns of wet deposition types and element concentration in mosses in Germany. 24<sup>th</sup> Task Force Meeting - UNECE ICP Vegetation; Rapperswil-Jona, Switzerland. 1/2011

**Boltersdorf SH** (2010). Stabile Isotopenmuster in epiphytischen Flechten zur Bewertung der N-Deposition in der BRD – Ergebnisse und Fazit. 102<sup>nd</sup> Scholar Seminar of German Environmental Foundation (DBU); Ostritz, Germany. 12/2010

**Boltersdorf SH, Werner W** (2009). Nitrogen concentrations and  $\delta^{15}\text{N}$  ratios in epiphytic lichens in correlation to nitrogen deposition rates in Germany. 39<sup>th</sup> annual conference of the Ecological Society of Germany, Switzerland and Austria; Bayreuth, Germany. 9/2009

**Boltersdorf SH** (2009). Stabile Isotopenmuster in epiphytischen Flechten zur Bewertung der N-Deposition in der BRD - Ergebnisse. 94<sup>th</sup> Scholar Seminar of German Environmental Foundation (DBU); Papenburg, Germany. 08/2009

Werner W, **Boltersdorf SH** (2009). Nitrogen concentrations and  $\delta^{15}\text{N}$ -ratios in epiphytic lichen *Xanthoria parietina* in correlation to nitrogen depositions rates and  $\delta^{15}\text{N}$ -ratios of colonized tree bark. 22<sup>nd</sup> Task Force Meeting - UNECE ICP Vegetation; Braunschweig, Germany. 2/2009

**Boltersdorf SH** (2008). Stabile Isotopenmuster in epiphytischen Flechten zur Bewertung der N-Deposition in der BRD - Untersuchungsdesign und Forschungshypothesen. 88th Scholar Seminar of German Environmental Foundation (DBU); Volkenroda, Germany. 11/2008

**Boltersdorf SH, Werner W** (2008). Estimation of nitrogen deposition rates on a regional level using nitrogen concentrations of the epiphytic lichen *Xanthoria parietina*. EURECO-GFOE 2008 "Biodiversity in an Ecosystem Context"; Ecological Society of Germany, Switzerland and Austria; Leipzig, Germany. 9/2008

## Posters

**Boltersdorf SH, Pesch R, Schröder W, Werner W** (2014). Physiological responses: Do lichens, mosses and tree bark react with different carbon and nitrogen isotope patterns along a nitrogen deposition gradient? 27<sup>th</sup> Task Force Meeting – UNECE ICP Vegetation; Paris, France. 1/2014

**Boltersdorf SH, Werner W** (2012). Trends in N quantity and  $\delta^{15}\text{N}$  signatures of different N deposition types in Germany indicated by lichens and bark samples. 25<sup>th</sup> Task Force Meeting - UNECE ICP Vegetation; Brescia, Italy. 2/2012

**Boltersdorf SH** (2011). Do epiphytic lichens reflect agriculture-related depositions? European Geosciences Union (EGU), General Assembly, Vienna, Austria. 4/2011

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**Boltersdorf SH** (2010). Assessing the nitrogen deposition in Germany with epiphytic lichen stable isotope signatures - an overview. International Research Day. International Post graduation Centre-University of Trier; Trier. Germany 12/2010

**Boltersdorf SH** (2010). Source attribution by comparing  $\delta^{15}\text{N}$ -signatures in epiphytic lichens along a gradient of different nitrogen emission sources. Annual conference of German Association of Stable Isotope Research (GASIR); Cologne, Germany. 10/2010

**Boltersdorf SH, Werner W** (2010). Characterisation of nitrogen deposition and source attribution by using N-concentrations and  $\delta^{15}\text{N}$ -signatures of epiphytic lichens (*Xanthoria parietina* and *Hypogymnia physodes*) in Germany. 40<sup>th</sup> annual conference of the Ecological Society of Germany, Switzerland and Austria; Giessen, Germany. 9/2010

**Boltersdorf SH, Schultze, J, Werner, W** (2010). Comparison of nitrogen content and  $\delta^{15}\text{N}$ -signatures in mosses (*Hypnum cupressiforme*) and lichens (*Parmelia sulcata*) with nitrogen deposition rates of Germany. 23<sup>rd</sup> Task Force Meeting - UNECE ICP Vegetation; Brüssel, Belgium. 2/2010

#### Non-Scientific Publications [photography]

**Boltersdorf S** (2013). Chin up!...when the water's up to your neck, in Schüler G, Bích Hông, V T, Schobel, S, Bidaud, C (Eds): The suffering of trees and people in a changing climate. How photographers perceive the impacts of climate change. Creativ WERBUNG, Kaiserslautern, Germany, pp. 256.

**Boltersdorf S, Mynott S** (2013). Wonderings and weathering – Honeycomb weathering. Imageo on Mondays. GeoLog – official blog of the European Geosciences Union. Online 18/02/2013. <http://geolog.egu.eu/2013/02/18/imageo-on-mondays-wonderings-and-weathering/>

#### Other [unpublished]

**Boltersdorf SH** (2011). Abschätzung von Stickstoffdepositionen mit Hilfe der epiphytisch wachsenden Flechten *Xanthoria parietina*, *Physcia adscendens*, *Parmelia sulcata* und *Hypogymnia physodes* und ihrer stabilen Isotopenmuster. DBU-project, Final Report, pp. 88, unpublished

**Boltersdorf SH** (2007). Akkumulation luftgetragener Stoffe in Flechten. Diploma thesis, pp. 154, unpublished

# Erklärung

Hiermit erkläre ich, dass mir die derzeitige Promotionsordnung bekannt ist und dass ich die vorliegende Dissertation selbständig verfasst habe.

Ich habe für die Arbeit keine anderen als die angegebenen Quellen und Hilfsmittel genutzt und die Ergebnisse anderer Beteiligter sowie anderer Autoren wurden als solche kenntlich gemacht.

Desweiteren erkläre ich, dass bisher kein Promotionsverfahren beantragt wurde und dass die vorliegende Dissertation weder im Ganzen noch in Teilen als Prüfungsarbeit bei einer anderen Fakultät oder Hochschule eingereicht wurde.

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(Unterschrift)

