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Relationship between site-specific nitrogen concentrations in mosses and measured wet bulk atmospheric nitrogen deposition across Europe

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Abstract

To assess the relationship between nitrogen concentrations in mosses and wet bulk nitrogen deposition or concentrations in precipitation, moss tissue and deposition were sampled within a distance of 1 km of each other in seven European countries. Relationships for various forms of nitrogen appeared to be asymptotic, with data for different countries being positioned at different locations along the asymptotic relationship and saturation occurring at a wet bulk nitrogen deposition of ca. 20 kg N ha^{-1} yr^{-1}. The asymptotic behaviour was more pronounced for ammonium-N than nitrate-N, with high ammonium deposition at German sites being most influential in providing evidence of the asymptotic behaviour. Within countries, relationships were only significant for Finland and Switzerland and were more or less linear. The results confirm previous relationships described for modelled total deposition. Nitrogen concentration in mosses can be applied to identify areas at risk of high nitrogen deposition at European scale.

Capsule: Nitrogen concentration in mosses shows saturation occurring at a measured wet bulk nitrogen deposition of ca. 20 kg N ha^{-1} yr^{-1}.

Keywords: biomonitoring; moss survey; bulk nitrogen deposition; ammonium; nitrate.

1. Introduction

For ectohydric moss species, the lack of a well-developed root system, vascular system and protective cuticle means that they receive and take up water, nutrients and contaminants mainly from atmospheric deposition (dry, wet and occult). Hence, such mosses have shown to be suitable indicators of atmospheric deposition of, for example, nitrogen (Harmens et al., 2011; Pitcairn et al., 2006; Salemaa et al., 2008; Solga et al., 2005; Zechmeister et al., 2008), heavy metals (Harmens et al., 2010; Harmens et al., 2012; Schröder et al., 2010b) and selected persistent organic pollutants (Foan et al., 2010, 2014; Harmens et al., 2013a). The moss monitoring technique provides a complementary, time-integrated measure of element deposition from the atmosphere to terrestrial systems. As it is easier and cheaper than conventional deposition analysis, a much higher sampling density can be achieved than with conventional deposition analysis. Hence, passive biomonitoring of atmospheric nitrogen deposition using mosses would allow the determination of the variation in atmospheric nitrogen deposition at a high spatial resolution, including in countries or areas where nitrogen deposition monitoring networks are absent.

For nitrogen, sometimes the relationship between atmospheric deposition rates and the concentration in mosses is weak (Stevens et al., 2011) or shown to be species-specific (Arroniz-Crespo et al., 2008; Salemaa et al., 2008). One possible explanation for the weak relationship between the deposition and accumulation of nitrogen is the regulation of tissue loads in mosses because nitrogen is known to play an important role in the metabolism of organisms (e.g., Koranda et al. 2007; Arróniz-Crespo et al. 2008), in contrast to for example non-essential heavy metals such as cadmium and lead. Such regulation may distort the patterns of nitrogen deposition identified by biomonitoring with terrestrial mosses. Schröder et al. (2010a) have shown that atmospheric nitrogen deposition, as modelled by the European Monitoring and Evaluation Programme (EMEP), is the primary factor determining total nitrogen concentrations in mosses. Harmens et al. (2011) observed an asymptotic relationship between the total nitrogen concentrations in mosses and EMEP modelled total nitrogen deposition (averaged per 50 km x 50 km grid) across Europe, with saturation (i.e. no further increasing nitrogen concentration in moss tissues with increasing nitrogen deposition) occurring at a total deposition rate of ca. 15 kg N ha^{-1} yr^{-1}. Whether such as relationship also holds when both the nitrogen concentration in moss and atmospheric wet nitrogen deposition are measured at nearby sites across Europe, is unknown.

Only a few studies have examined the relationship between the nitrogen concentration in mosses and measured (as opposed to modelled) nitrogen deposition in the immediate vicinity of the moss sampling sites (Skudnik et al, 2014; Solga et al., 2005; Thöni et al., 2008; Zechmeister et al., 2008), in monitoring studies not conducted in the immediate vicinity of local sources (e.g. Pitcairn et al., 2006). These studies were all conducted at the (sub-)national scale and such data is not available at the European scale. The strength and shape of the relationship observed in these (sub-)national studies varies between countries. For example, in Switzerland, a strong, significant (r^2 = 0.91) linear relationship was found between the total nitrogen concentration in mosses and measured site-specific wet bulk nitrogen deposition (Harmens et al., 2011; Thöni et al., 2008). Less strong but still significant linear relationships were also reported for North Rhine-Westphalia in Germany (Solga et al., 2005) and Austria (Zechmeister et al., 2008). Skudnik et al. (2014) showed a weak but significant linear-logaritmic relationship between the nitrogen concentration in mosses and atmospheric bulk nitrogen deposition. To investigate the strength and shape of the relationship at the European scale, data on nitrogen concentrations in mosses and measured wet bulk nitrogen deposition were collected in seven European countries. Only monitoring sites where the distance between the moss sampling site and the atmospheric deposition was less than 1 km were considered.

As different moss species were used in the current study, we also investigated whether moss species differ in their nitrogen concentration when sampled at the same sites, as this might confound the relationship between atmospheric nitrogen deposition and the nitrogen concentration in mosses (Arroniz-Crespo et al., 2008; Salemaa et al., 2008). Although there are other factors potentially confounding the relationship between atmospheric nitrogen deposition and its concentration in mosses, these were not investigated here but have been discussed previously in more detail (Harmens et al., 2011, Schröder et al., 2010a) and some are further discussed in the results and discussion section.

Despite the sometimes reported linear relationship between the nitrogen concentration in mosses and measured wet bulk nitrogen deposition at the (sub-) national scale (Harmens et al., 2011; Solga et al., 2005; Thöni et al., 2008), we hypothesise that the relationship will show an asymptotic behaviour at the European scale (conform Harmens et al., 2011, using modelled nitrogen deposition) when higher deposition rates are included. However, we expect less scatter in the underlying data than for modelled deposition (Harmens et al., 2011). We also tested whether the relationship is affected by nitrogen speciation in deposition and whether the strength of the relationship differs for nitrogen deposition or nitrogen concentration in precipitation.

2. Materials and methods

Sites

Mosses were collected between 1998 and 2012 at selected sites in seven European countries (Figure 1): Austria (AT), Switzerland (CH), the German Bundesland Niedersachsen (DE-NI), Spain (ES), Finland (FI), France (FR), and Slovenia (SI, although some of sites were in Austria and Italy close to the Slovenian border). For this study, moss data were only included from sites (97 in total) where the distance to the deposition monitoring site was less than 1 km (the maximum distance recorded was 900 m). At some sites (s) sampling was repeated in time, leading to 160 data points (p) for comparison (AT 26s, 26p; CH 18s, 33p; DE-NI 6s, 33p; FR 24s, 36p; SI 11s, 11p; FI 11s, 19p; ES 1s, 2p). At some forested sites the deposition was characterised as throughfall below the canopy of trees rather than bulk deposition only. This was the case for the majority of data points in Germany, all sites in France and the one site in Spain. Including throughfall for forested sites in Germany allowed the inclusion of high deposition data beyond the level that was included in the study described previously by Harmens et al. (2011).

Moss species and sample preparation

The main moss species sampled were *Pleurozium schreberi* (Willd. ex Brid.) Mitt. (*Ps*, at 44.4% of the sites) and *Hypnum cupressiforme* Hedw. (*Hc*, 36.3 %). Where neither of these could be found, other species were collected (19.4 %): *Hylocomium splendens* (Hedw.) Schimp. (*Hs*; 6.3%), *Pseudoscleropodium purum* (Hedw.) M.Fleisch. (*Pp*; 6.3%), *Thuidium tamariscinum* (Hedw.) Schimp. (*Tt*; 5.6%) or *Abietinella abietina* (Hedw.) M.Fleisch. (*Aa*; 1.3%; Figure 1). Moss sampling and preparation were conducted according to guidelines described in the ICP Vegetation moss monitoring manual (ICP Vegetation, 2010). Moss samples were either collected below the canopy of trees but not from stems (hence, exposed to throughfall deposition), or in open areas or forest clearings at least 3 m away from tree crowns (see Table 1 for details). Litter and other debris was removed from the mosses and green and brownish parts were separated for analysis (estimated 2 to 3 years' growth). After drying the mosses were ground to a powder for the determination of nitrogen.

Deposition sampling

Most countries collected precipitation using bulk samplers with open funnels, although France collected precipitation in gutters beneath the canopy of trees; Finland and Slovenia also used snow collectors during winter, i.e. bulk samplers designed for winter conditions (Table 1). Often, deposition was sampled according the manuals of the ICP Forests (see Table 1 for details). Precipitation was collected in two or four week intervals. Wet bulk nitrogen deposition (open field or throughfall) was determined from nitrogen concentration in the samples and the amount of precipitation. Where possible, the averages of three years of deposition data (year of moss sampling and the previous two years) were calculated to correspond with the estimated two to three years of moss growth and to allow for the variation in deposition between years. For Germany, 10 data points have deposition data from only one year and 11 data points have only averages of two years.

Nitrogen analysis

The nitrogen concentration in mosses was determined using the Kjeldahl method (Kjeldahl, 1883), a modified micro-Kjeldahl method (Kubin and Siira, 1980), or by elemental analysis following the Dumas method (Dumas, 1831; Table 1). Various methods were applied to determine the nitrogen concentration in precipitation and throughfall (see Table 1 for details). Nitrogen deposition in precipitation or throughfall was also calculated as the sum of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ as collected by the samplers and we will refer to this as ‘bulk nitrogen’ deposition. In addition, some countries (Finland and Germany) measured dissolved organic nitrogen (DON) or the total nitrogen concentration (France and Slovenia) in precipitation (96 data points for comparison). We will refer to this as ‘total bulk nitrogen’ deposition, either measured (France and Slovenia) or calculated from ‘bulk nitrogen’ plus organic nitrogen deposition (other countries). One should bear in mind that this is not total nitrogen deposition as the total dry deposition of nitrogen from aerosols and gas was not determined. In contrast to wet-only collectors, bulk samplers often contain a fraction of total dry deposition, so open bulk samplers do not only collect wet deposition (Thimonier, 1998, and reference therein).

Quality assurance

Participating laboratories, except for Germany, determined the nitrogen concentration in moss reference material M2 and M3 (Steinnes et al., 1997) for quality assurance purposes (Table

2). Generally, the results from participating laboratories agreed well with the recommended values (Harmens et al., 2010) for the nitrogen concentration in M2 and M3. In France, the laboratory practise differed between 2006 and 2011, resulting in higher nitrogen concentrations in the reference material M2 and M3 (Table 2). Hence, the 2011 data for France were adjusted to reduce variability in the French data due to inter-laboratory difference. In addition, some laboratories used other certified reference material to assure good quality data, whereas the German laboratory was accredited according to standards developed by the International Organization for Standardization (DIN EN ISO 17025). In many countries the deposition sampling was conducted according to protocols and procedure developed by International Cooperative Programmes and the determination of the concentration of different nitrogen forms in deposition was subject to ring tests, inter-laboratory calibration exercises and standards developed by the International Organization for Standardization (see table 1 for details).

Statistical analysis

Statistical analysis was conducted using the R statistical package (www.r-project.org). A test for differences between moss species was carried out by fitting a linear mixed model to moss nitrogen concentrations, taking species as a factor and site as a random effect. The routine lme of the R statistical package was used for this purpose. When the nitrogen concentration in mosses was plotted against the various forms of measured nitrogen deposition or concentration in precipitation, the moss concentrations were adjusted to allow for the variability between moss species. An asymptotic relationship has been fitted to the data using the R package non-linear least squares package gnls. The asymptotic relationship fitted to the data can be described by the following equation:

$$y = c + A \times \left(1 - \exp\left(-bx\right)\right)$$

y = nitrogen concentration in mosses;

c = intercept on the y-axis;

x = deposition or concentration in precipitation of various nitrogen forms;

A + *c* = the asymptote;

exp(-*bx*) represents the rate at which the asymptote is approached.

A non-linear mixed model was fitted to the data with parameter *b* being allowed to vary with site as a random effect. Clear statistical outliers in the data were omitted from the analysis.

3. Results and discussion

Interspecies variation in nitrogen concentration

Previous studies have shown that the relationship between atmospheric nitrogen deposition rates and the nitrogen concentration in mosses can be species-specific (Arroniz-Crespo et al., 2008; Salemaa et al., 2008). Hence, the sampling of different moss species in the current study might be a confounding factor and introduce ambiguity into the interpretation of the possible causes of variability in the nitrogen concentration in mosses. Although atmospheric nitrogen deposition was identified as the primary factor determining the total nitrogen concentration in mosses, the use of different mosses species in biomonitoring programmes across Europe also contributes to the spatial variation of nitrogen concentrations in mosses (Schröder et al., 2010a; Harmens et al., 2011).

In the current study, the nitrogen concentration in mosses was determined for different mosses species at a selection of sites (Figure 2). The analysis indicates significant differences (F=76.6; 4 and 125 df) between moss species. At the extremes of the range are *H. cupressiforme* (lowest) and *T. tamariscinum* (highest), showing a significant difference ($p < 0.0001$) of 2.15 mg N g^{-1} dry wt. Other species fall between *H. cupressiforme* and *T. tamariscinum*, with overlapping confidence intervals. For a single set of paired values, the analysis is equivalent to a paired t-test, showing significant differences ($p < 0.05$) for some paired species: *H. cupressiforme* contained less nitrogen than *T. tamariscinum* and *P. purum*, *P. schreberi* contained less nitrogen than *H. splendens* (Figure 2). For further analysis of the data (see below), *H. cupressiforme* was taken as a baseline species and responses of other species were linearly adjusted for bias with respect to *H. cupressiforme*. The maximum adjustment was -2.14 mg g^{-1} for *T. tamariscinum*. Plots of the nitrogen concentration in mosses by paired species (Figure 2) suggested that a simple bias adjustment was sufficient.

Relationship between nitrogen concentration in mosses and various forms of wet nitrogen deposition or concentrations in precipitation

Figure 3 and 4 show the relationship between the nitrogen concentration in mosses and the various forms of wet nitrogen deposition (NO_3^-, NH_4^+, sum of NO_3^- and NH_4^+ ('bulk nitrogen') and sum of NO_3^-, NH_4^+ and organic N ('total bulk nitrogen')) or concentrations in precipitation respectively. Following inspection of the data and preliminary model fitting, the parameter *c* (intercept on the y-axis) was set at 2 mg N g^{-1} dry weight, ensuring that the modelled data also showed a good fit at the lower range, representing the Finnish data. Parameter *c* is an approximation of the apparent nitrogen concentration in mosses in the absence of any nitrogen deposition. While there is the appearance of an asymptotic relationship, there is considerable scatter, with differing variability between countries, and data for different countries positioned at different locations along the asymptotic relationship. The model is therefore a first attempt to show the relationship between the nitrogen concentration in mosses and the various deposition and concentration in precipitation variables across Europe. It does not take full account of the correlations between some data points.

The lowest wet bulk nitrogen deposition rates were found in Finland (Figure 3), resulting in the lowest nitrogen concentrations in mosses (Poikolainen et al., 2009). The Finnish data are at the lower end of the relationship, more or less within the initial linear part. In Finland, the nitrogen concentration in mosses is strongly correlated ($p < 0.05$; F-test 1, 16 df) with all forms of nitrogen deposition and concentration in precipitation. The same is true for Switzerland ($p < 0.05$; F-test 1, 30 df), where the relationship between nitrogen concentration in mosses and wet bulk nitrogen deposition is more or less linear (Harmens et al., 2011, 2013b). Although the moss and deposition data for Austria, France and Slovenia are in a similar range as those for Switzerland, representing the middle range of the data across all countries, a nationwide analysis of the data shows a lot of scatter with no significant relationship ($p > 0.05$; F-test on 1 and appropriate df by country) between the nitrogen concentration in mosses and all forms of wet nitrogen deposition or concentration in precipitation. Especially the data for France are well-scattered regionally and not consistent with the overall asymptotic behaviour shown in the Europe-wide data.

The German data were restricted to a few sites in Niedersachen (North-West Germany), which were sampled in various years (Mohr, 1999; Mohr et al., 2009). The German throughfall data, associated with high nitrogen deposition and concentration in precipitation, are the only data that lie along the asymptotic part of the relationships shown in Figure 3 and 4. A few data points were available for Germany from non-throughfall sites and these points fall within the mid-range of the asymptotic curves. The inclusion of the German throughfall data allowed us to verify whether the asymptotic relationship observed in an earlier Europe-wide study with modelled total deposition data (Harmens et al., 2011) would also hold when using measured wet bulk deposition data, including bulk nitrogen deposition data above 20 kg N ha^{-1} yr^{-1}. Kluge et al. (2013) and Skudnik et al. (2014) found significantly higher nitrogen concentrations in mosses when exposed to throughfall in forests compared to exposure to atmospheric nitrogen deposition in open fields.

A priori, there was no reason to assume that the inclusion of throughfall nitrogen might make a qualitative difference to the relationship between nitrogen deposition and nitrogen concentration in mosses. However, nitrogen speciation in throughfall might differ from that in wet deposition due to canopy exchange processes, possibly affecting the ammonium-N to nitrate-N ratio (Draaijers et al., 1997; Adriaenssens et al., 2012) and the contribution of dissolved organic nitrogen (DON; Drápelová, 2012), potentially affecting the uptake of nitrogen in mosses (see below). Although mosses have a preference for ammonium uptake (see below), which might suppress the utilization of DON, the contribution of atmospheric DON to the nitrogen concentration in mosses could be significant (Liu et al., 2013). In addition, the microclimate in forest undergrowth is likely to differ from more exposed locations and such microclimate differences might affect the relationships studied here (Harmens et al., 2011). The data from the current study do not allow direct assessment of the impact of throughfall as at none of the sites a comparison was made between nitrogen concentrations in mosses sampled under the influence of tree canopies and mosses sampled in the open field.

Ammonium and nitrate deposition are generally of the same order, with the exception of the throughfall sites in Germany, where ammonium deposition exceeded nitrate by a factor of two to three. The German sites with high ammonium deposition rates are the most influential in providing evidence of asymptotic behaviour in the nitrogen concentration in mosses. That is to say, the nitrogen concentration in mosses does not appear to respond to increasing ammonium-N deposition of over 12 kg ha^{-1} yr^{-1}, and wet bulk N deposition of over 20 kg ha^{-1} yr^{-1} (Figure 3), or at ammonium-N concentration in precipitation of over 2 mg l^{-1} (Figure 4). In contrast, the asymptotic behaviour is very weak with respect to nitrate-N deposition or concentration in precipitation. The asymptotic behaviour with respect to ammonium-N is even more pronounced when precipitation concentrations are considered, because rainfall at the throughfall sites in Germany is relatively low (Figure 4). Saturation of nitrogen concentration in mosses at high ammonium deposition or concentration in precipitation might reflect a lower uptake efficiency at higher nitrogen exposure (Pitcairn et al., 2006; Wiedermann et al., 2009). Previous studies have reported a higher uptake of ammonium than nitrate in mosses (Forsum et al., 2006; Jauhiainen et al., 1998; Liu et al., 2013; Pearce et al., 2003; Soares and Pearson, 1997; Wiedermann et al., 2009), which is probably due to the high cation-exchange capacity common for mosses (Bates, 1992). Utilising NH_4^+ as a nitrogen source as opposed to NO_3^- is commonly regarded as being more energy efficient, achieving greater specific growth rates. NO_3^- assimilation in mosses was found to be negligible when the supply rate of reduced dissolved nitrogen (NH_4^+ plus DON) was significantly higher than that of NO_3^- (Liu et al., 2012). However, in the current study, the supply rate of NH_4^+ and NO_3^- was similar at most sites except in Germany, so it is unknown whether NO_3^- assimilation was low. If NO_3^- assimilation was low, the effect of NO_3^- deposition on the nitrogen concentration in mosses is likely to be overestimated.

The Akaike Information Criterion (AIC), an indicator of model fit, suggests that the best fit is obtained by the combined concentration of ammonium and nitrate in rainfall for data including all countries (Table 3). Analysis for Finland, France, Germany and Slovenia only indicates that there is no further improvement in fit using total nitrogen concentration in precipitation. In Germany and France, the average contribution of DON to the total wet bulk deposition ranged from 6 to 28% respectively, which is similar to the range reported for the Czech Republic (Drápelová, 2012).

Uncertainty in the contribution of other sources to the nitrogen concentration in mosses

The lack of data on other nitrogen sources potentially contributing to the nitrogen concentration in mosses is likely to contribute to the scatter in the data and the uncertainty of the relationships shown in Figure 3 and 4. In the current study, we only included nitrogen from wet bulk deposition as data on dry deposition was lacking for most sites (although some dry deposition will be included in wet bulk deposition samplers; Thimonier, 1998). Pitcairn et al. (2006) have shown that nitrogen concentration in mosses respond differently to wet and dry deposited nitrogen. For a 1 kg ha^{-1} yr^{-1} increase in nitrogen deposition, tissue nitrogen increased by 0. 1 mg g^{-1} at wet deposition sites but by 0.3 mg g^{-1} at sites dominated by dry deposited ammonia downwind of livestock (poultry and pig) farms in Scotland. Larger concentrations of nitrogen (up to 40 mg g^{-1}) occurred in mosses at sites where nitrogen deposition was dominated by dry deposited ammonia and where rainfall (and therefore leaching losses) was small, compared with sites where deposition was dominated by wet deposition (up to 16 mg g^{-1}). In the current study, the maximum nitrogen concentration in mosses was 25 mg g^{-1} at throughfall sites in the agriculturally intensive region of Germany, where dry nitrogen deposition is high. Thus, the critical nitrogen concentration of 20 mg g^{-1}, specified by Pitcairn et al. (1998) was exceeded considerably in Germany.

In addition to inorganic nitrogen, mosses also take up DON, hence analysis of DON should be included to account fully for nitrogen input to mosses from precipitation (Forsum et al., 2006). Several studies have reported the preferred uptake of ammonia, followed by DON or amino acids, over nitrate (Forsum et al., 2006; Hill et al., 2011; Liu et al., 2013; Wanek and Portl, 2008; Wiedermann et al., 2009) and in some cases amino acids may be the preferred source of nitrogen for certain moss species (Kielland, 1997; McKane et al., 1993). In the current study, the relationships shown in Figure 3 and 4 did not change much when DON (total bulk nitrogen deposition or total nitrogen precipitation) was included in addition to ammonia and nitrate.

Scatter in the data might also be caused by uptake of nitrogen from the soil (Ayres et al., 2006). Although Liu et al. (2013) reported that the uptake of nitrogen from the soil might contribute significantly (ca. 37%) to the nitrogen concentration in terricolous mosses, this is in contrast to other studies stating that mosses receive most of the nitrogen from deposition, leaching and throughfall (Kotanen, 2002; Li and Vitt, 1997; Rousk et al., 2013a; Turetsky, 2003). In the current study, mosses in forested areas were sampled from tree stumps where possible, where uptake of soil nitrogen is unlike to play a significant role.

At some lower nitrogen deposition sites with relatively high nitrogen concentration in mosses, cyanobacteria living in association with mosses could potentially be responsible for the high nitrogen concentration in mosses (Rousk et al., 2013b). However, the number of cyanobacteria cells was shown to decline significantly at nitrogen deposition rates of 5 kg ha^{-1} yr^{-1} or more compared to the background deposition rate of 2 kg ha^{-1} yr^{-1} (Gundale et al., 2011). In the current study, sites with relatively high nitrogen concentrations in mosses at low nitrogen deposition were found in Austria, where cyanobacterial associations were not observed, and in France, where the drier climate is not conducive to high cyanobacterial activity (Rousk et al., 2013b). A relatively high nitrogen concentration in mosses was also observed at one Finnish site, however, this is unlikely to be due to cyanobacterial fixation of nitrogen as at many other Finnish sites with lower nitrogen deposition rates the nitrogen concentration in mosses was much lower. Leppänen et al. (2013) showed that nitrogen fixation associated with mosses increased towards the north and was hardly observed in the south of Finland, where nitrogen deposition rates are higher.

Other factors that are likely to contribute to the scatter in the data (e.g. effects of nitrogen and microclimate on moss growth, surrounding vegetation type and land use) have been discussed in more detail elsewhere (Harmens et al., 2011; Schröder et al., 2010a). In the current study, the distance between the moss sampling sites and the deposition measurement sites varied between 1 – 900 m. In general, there is a high spatial and temporal variability in throughfall (Thimonier, 1998) and in wet deposition of nitrogen (Harmens et al., 2011 and references therein), especially in mountainous regions. Hence, a distance of up to 900 m between moss sampling and deposition measurement site could also contribute to the scatter in the data.

Conclusions

As previously described for modelled nitrogen deposition, the relationship between nitrogen concentration in mosses and measured (total) wet bulk deposition or concentration in precipitation across Europe is best described by an asymptotic relationship. The asymptotic relationship is much stronger for ammonia-N than for nitrate-N in bulk deposition or precipitation. Saturation appears to occur at wet bulk nitrogen deposition rates of ca. 20 kg ha^{-1} yr^{-1}. Up to such deposition rates, linear relationships have been observed in some countries (Finland and Switzerland) but not in others. Considerable scatter was observed in the relationship at the European level, although less than previously found with modelled total deposition (Harmens et al., 2011). The scatter in the data might potentially be reduced by repeating this study with:

- Both mosses and precipitation sampled at the same site, rather than up to 1 km apart, to minimise the influence of spatial variation in nitrogen deposition;
- Including analysis of ammonia and nitrogen dioxide measured with passive samplers as an indication of dry deposition and measurements of DON to calculate total nitrogen deposition;
- Further harmonising and improving the methodology of moss and deposition sampling, and chemical analysis, and minimise the potential uptake of nitrogen from soil;
- Measuring nitrogen concentration in mosses at more sites with high nitrogen deposition or concentration in precipitation.

The moss technique remains a valuable tool to identify areas at risk of high nitrogen deposition at a high spatial resolution in a cost-effective manner and appears to be a complementary tool for estimating wet bulk nitrogen deposition in low to medium nitrogen deposition areas. In addition, data for various years will allow analysis of temporal trends in atmospheric nitrogen deposition (Harmens et al., 2013b).

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Table 1. Overview of methods applied in selected European countries to determine nitrogen concentrations in mosses and bulk precipitation.

Country	Institute	Deposition or throughfall	Moss species*	Sample years	Analysis N in moss	Moss QA method	Sampler type	Analysis of N deposition	QA method deposition	Monitoring network	Reference
Austria (AT)	Umweltbundesamt Wien	Deposition	Aa, Hc, Hs, Ps	2005	Elemental analysis	Standards M2& M3; ÖNORM CEN/TS 15407	Bulk sampler	Chemoluminescens	Multiple sampling	ICP Forests (Smidt, 2007) National network (Leder et al., 2005)	Leder et al., 2005 Smidt, 2007 Zechmeister et al., 2008
Finland (FI)	Finnish Forest Research Institute (Metla)	Deposition	Ps	2009 & 2011	Modified Kjeldahl (Kubin and Siira, 1980)	Moss standards M2 & M3	Bulk sampler, incl. snow collector	NO_3-N: Ion chromatography (IC); NH_4-N, N_{tot}: Flow injection analysis	ICP Forests Manual (Clarke et al., 2010)	ICP Forests	Clarke et al., 2010 Kubin and Siira, 1980
France (FR)	Muséum national d'Histoire naturelle, Office National des Forêts	Throughfall	Hc, Hs, Pp, Tt	2006 & 2011	Elemental analysis	Moss standards M2 & M3, repeated sampling	Gutters beneath canopy	NO_3^-, NH_4^+: IC N total: chemoluminescence	ICP Forests Manual (Clarke et al., 2010) Ring test (Marchetto et al., 2009b)	RENECOFOR network (ICP Forests) BRAMM network (ICP Vegetation)	Clarke et al., 2010
Germany - Niedersachsen (DE-NI)	Landwirtschaftskammer Niedersachsen	Deposition and throughfall	Hc, Ps	1998-2010	Kjeldahl	Accredited DIN EN ISO 17025	Bulk sampler	Continous flow analyzer			
Slovenia** (SI)	Slovenian Forestry Institute	Deposition	Hc	2010	Elemental analysis	Standards M2 & M3	Bulk samplers, incl. snow collector	NO_3^-, NH_4^+: IC N total: UV-Vis Spectrophotometer	QC standards: Use of reference materials and ring tests (König et al., 2013)	ICP Forests intensive monitoring plots	Clarke et al., 2010 Hansen et al., 2013 König et al., 2013 Mosello et al., 2002 Smidt, 2007 Žlindra et al., 2011
Spain (ES)	University of Navarra	Throughfall	Hc	2010 & 2012	Elemental analysis	Standards M2 & M3	Bulk sampler	NO_3^-, NH_4^+: IC	Intercalibration ICP Waters; certified material	ICP Integrated Monitoring	Delgado et al., 2013
Switzerland (CH)	FUB - Research Group for Environmental Monitoring	Deposition	Hc, Ps	2005 & 2010	Kjeldahl	Standards M2 & M3, NIST-SRM 1515, repeated sampling	Bulk sampler	NO_3^-: IC NH_4^+: Flow injection analysis & Indophenolmethod (Spectrophotometer)	Reference material simulated rain: CRM 408 CEC bcr 1993; Ring test (Marchetto et al., 2009a)	ICP Forest (Thimonier et al., 2005) Swiss intercantonal research project	Leonardi and Flückiger, 1987 Marchetto et al., 2009a Thimonier et al., 2005 Thöni and Seitler, 2010

* Hs: *Hylocomium splendens,* Hc: *Hypnum cupressiforme,* Ps: *Pleurozium shreberi,* Pp: *Pseudoscleropodium purum,* Tt: *Thuidium tamariscinum.* Aa: *Abietinella abietina*

** A few moss samples were collected in Italy (1 site) and Austria (3 sites) near the Slovenian border, where deposition was sampled by the Ministry for Agriculture and Forestry Policies, CONECOFOR Service, National Forest Service and the Institut für Waldwachstum und Waldbau Waldschadenserfassung respectively.

Table 2. Nitrogen concentration (mg N g^{-1} dry weight; mean ± one standard deviation) in the moss standards M2 and M3 (Harmens et al., 2010). N is the number of repeated analyses of the standard; the value in parenthesis indicates the year of analysis for those countries who repeated the sampling with time.

Moss standard	**Recommended value**	**Austria**	**Switzerland (2010)**	**France**	**Finland**	**Slovenia**	**Spain**
M2 (mg N g^{-1} dry wt)	8.36 ± 0.62 (N = 10)	6.95 ± 0.28 (N=2)	7.81 ± 0.62 (N = 6)	8.32 ± 0.11 (N = 5) (2006) 9.05 ± 0.31 (N = 17) (2011)		8.27 ± 0.23 (N = 6)	8.80 ± 0.13 (N = 6)
M3 (mg N g^{-1} dry wt)	6.81 ± 0.52 (N = 8)	6.06 ± 0.29 (N=2)	6.93 ± 0.26 (N = 4)	6.57 ± 0.13 (N = 10) (2006) 7.48 ± 0.28 (N = 17) (2011)	6.82 ± 0.29 (N = 6) (2009) 6.66 ± 0.13 (N = 5) (2011)	6.72 ± 0.26 (N = 6)	7.30± 0.11 (N = 6)

Table 3. Parameters of the asymptotic relationship between nitrogen concentration in mosses and wet bulk deposition or concentration in precipitation for different nitrogen forms. The asymptotic relationship is described as y = c + A x (1 – exp(-bx); AIC = Akaike Information Criterion.

Bulk deposition/concentration variable	A	b	AIC*
NH_4-N deposition	20.5	0.1911	542.3
NO_3-N deposition	22.5	0.1843	635.9
NO_3-N + NH_4-N deposition	21.4	0.0919	517.3
Total N deposition**	21.3	0.0781	329.2
NH_4-N concentration	20.0	0.0017	487.2
NO_3-N concentration	22.0	0.0016	544.5
NO_3-N + NH_4-N concentration	20.7	0.0008	454.5
Total N concentration**	20.6	0.0006	288.1

* AIC for total N deposition and precipitation cannot be compared to other AIC due to different number of data involved.

** Finland, France, Germany and Slovenia only.

Figure legends

Figure 1. Sites where mosses and bulk precipitation were sampled for nitrogen analysis.

Figure 2. Deviation of the relationship between nitrogen concentration in paired moss species from the 1:1 relationship (solid line). Paired moss species where sampled at the same sites in one or more countries; n.s. = no significant difference between species.

Figure 3. Relationship between the deposition of different nitrogen forms in wet bulk deposition (mean of 3 years of deposition) and the nitrogen concentration in mosses. Moss and precipitation samples were collected less than 1 km apart in Austria (AT), Switzerland (CH), Germany – Niedersachen (DE-NI), Spain (ES), Finland (FI), France (FR) and Slovenia (SI). Total wet bulk nitrogen deposition (i.e. including dissolved organic nitrogen) was only determined in four countries (DE-NI, FI, FR, SI).

Figure 4. Relationship between the concentration of different nitrogen forms in precipitation (mean of 3 years of deposition) and the nitrogen concentration in mosses. Moss and precipitation samples were collected less than 1 km apart in Austria (AT), Switzerland (CH), Germany – Niedersachen (DE-NI), Spain (ES), Finland (FI), France (FR) and Slovenia (SI). Total bulk nitrogen concentration (i.e. including dissolved organic nitrogen) was only determined in four countries (DE-NI, FI, FR, SI).

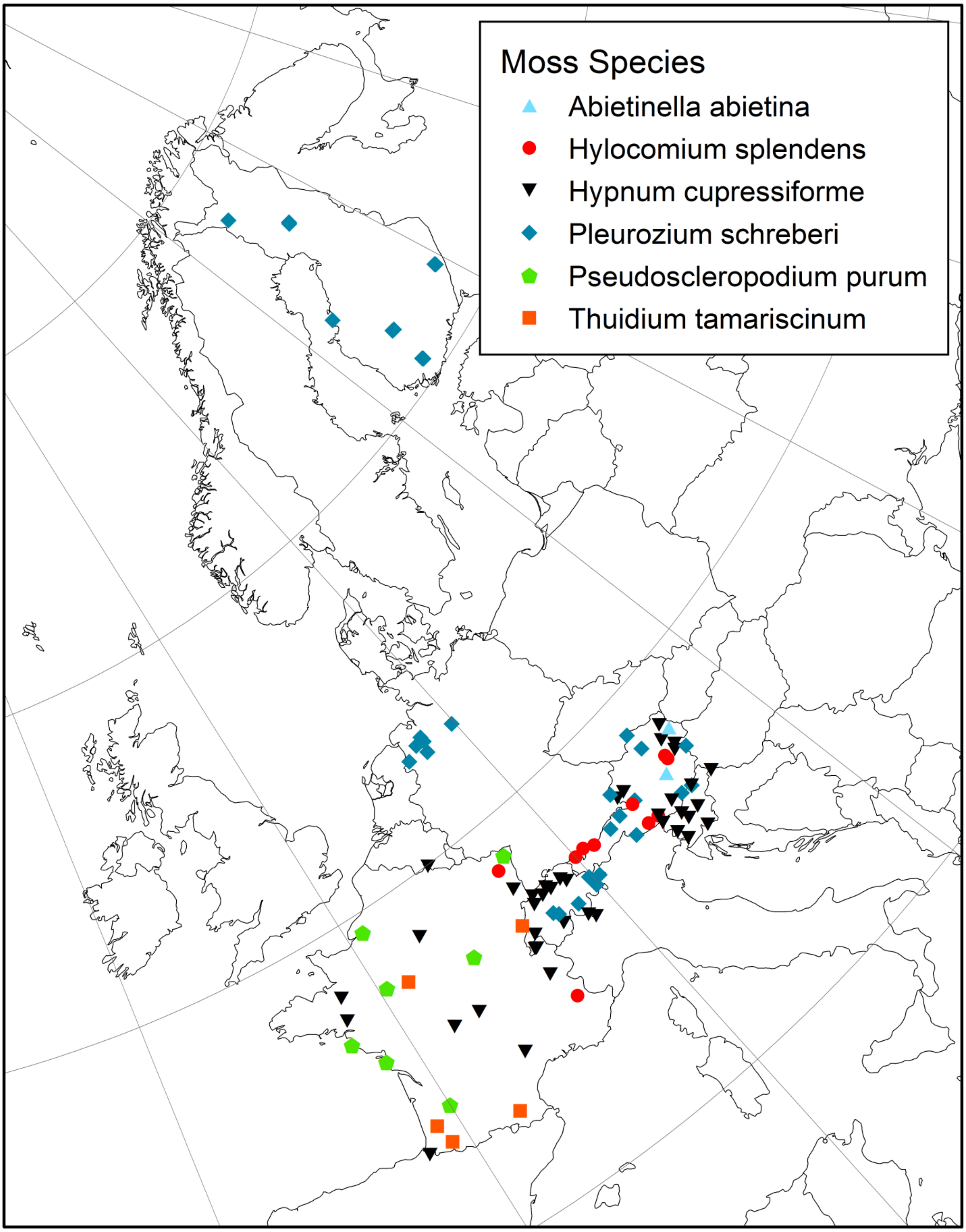
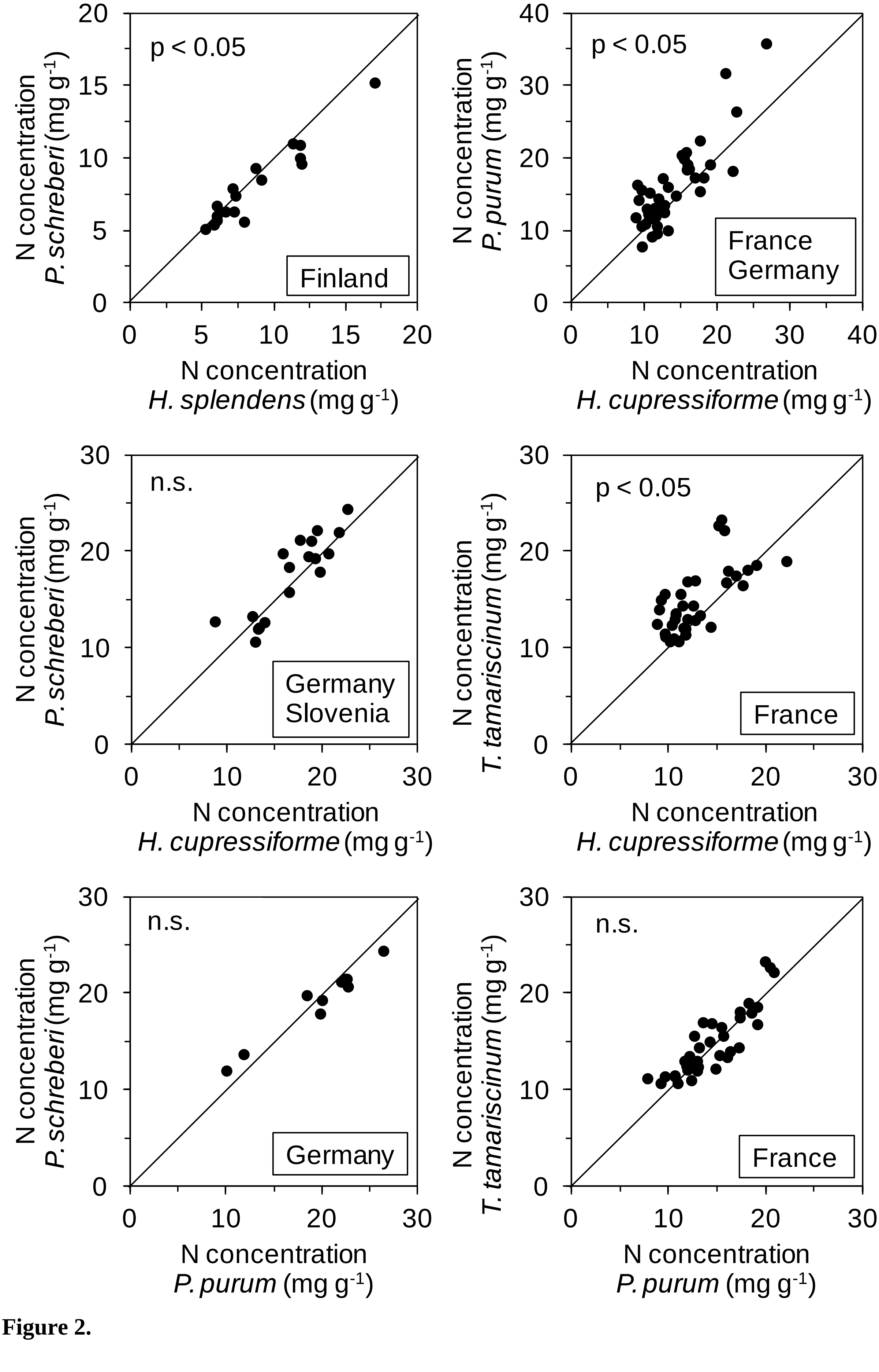

Moss Species
Abietinella abietina
Hylocomium splendens
Hypnum cupressiforme
Pleurozium schreberi
Pseudoscleropodium purum
Thuidium tamariscinum

Figure 1.



p < 0.05
Finland
N concentration P. schreberi (mg g-1)
N concentration H. splendens (mg g-1)
p < 0.05
France
Germany
N concentration P. purum (mg g-1)
N concentration H. cupressiforme (mg g-1)
n.s.
Germany
Slovenia
N concentration P. schreberi (mg g-1)
N concentration H. cupressiforme (mg g-1)
p < 0.05
France
N concentration T. tamariscinum (mg g-1)
N concentration H. cupressiforme (mg g-1)
n.s.
Germany
N concentration P. schreberi (mg g-1)
N concentration P. purum (mg g-1)
n.s.
France
N concentration T. tamariscinum (mg g-1)
N concentration P. purum (mg g-1)
0
5
10
15
20
30
40

Figure 2.

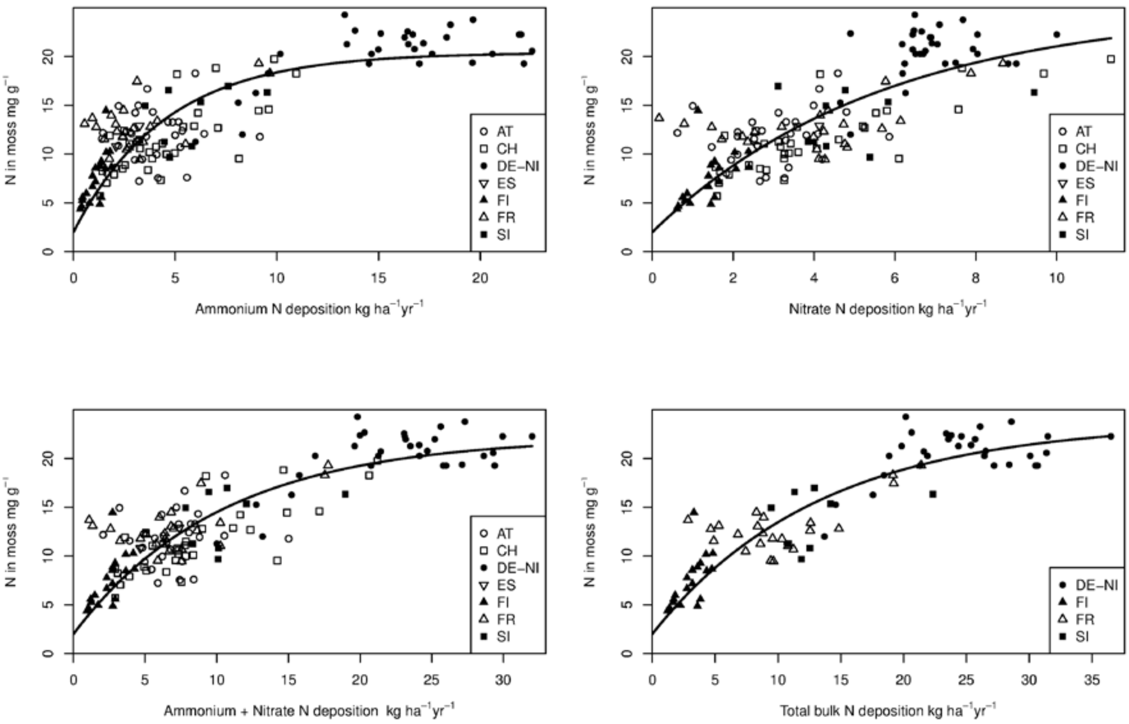


Figure 3.

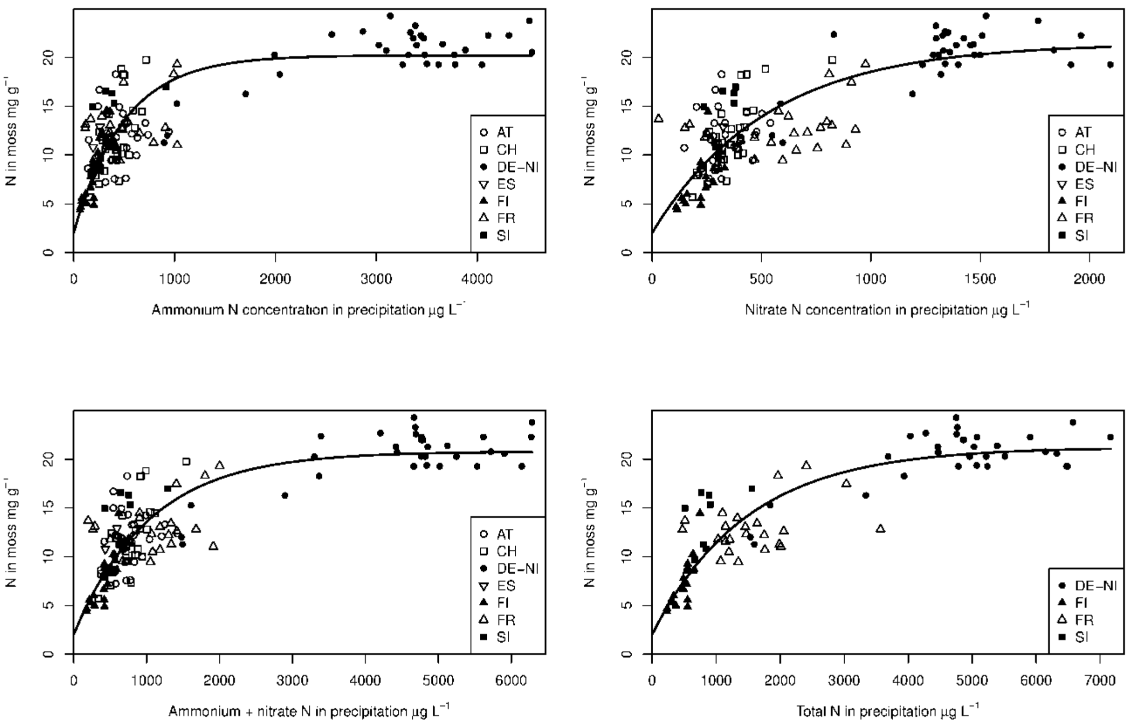


Figure 4.