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Original research paper

Mapping correlations between nitrogen concentrations in atmospheric deposition and mosses for natural landscapes in Europe

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Highlights

- Mosses can serve as bio-indicators of atmospheric nitrogen.
- Ecological land classes help relating exposure with effects assessments.
- Factors influencing N concentrations ranked including landscape characteristics.

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Mapping correlations between nitrogen concentrations in atmospheric deposition and mosses for natural landscapes in Europe

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Abstract

Recent investigations proved that nitrogen (N) concentrations in mosses are primarily determined by atmospheric deposition. The correlations are country- and N compound-specific and agree well with spatial patterns and temporal trends across Europe as a whole and in single European countries. This study investigates whether correlations between the

concentration of N in atmospheric deposition and mosses within the units of an ecological land classification of Europe can be established. To this end, N measurements from the 2005 European moss survey and modelled N atmospheric deposition in 2005 were intersected with a map of European landscapes. Then, considering minimum numbers of sampling sites required across Europe, in single European countries and within the landscapes of Europe and accounting for spatial auto-correlation, the correlations between the N concentration in mosses and corresponding deposition were calculated and mapped for each of those landscape units containing moss sampling sites. Using an example of one landscape with positive correlation and one landscape with no correlation between N concentrations in deposition and in mosses, influencing factors were ranked based on investigating the multivariate interactions between moss concentrations and, amongst others, atmospheric deposition, land use, elevation or moss species by classification and regression trees. From this study it could be concluded that the numbers of sampling sites within Europe and most participating countries as well as within most of the landscapes covering Europe are sufficient. Spatial patterns of correlations between the atmospheric N deposition and N concentration in mosses could be proven to vary across the landscapes of Europe. Where clear positive correlations between N concentrations in deposition and mosses exist in landscapes, multivariate ranking identifies the deposition as main influencing factor. In cases with no correlation between deposition and N concentrations in mosses, other factors such as e.g. moss species collected may be of importance. Therefore, mosses were proved to serve as biological indicators for atmospheric depositions and ecologically defined land classes could be identified as more complex indicators which allow relating exposure monitoring with effects assessment.

Keywords: Nitrogen deposition; bio-indication; ecological land classification; minimum number of sampling sites

1. Introduction

Nitrogen (N) is an essential plant nutrient. The N cycling in ecosystems is derived from biological N fixation, mineralization, and atmospheric deposition. Atmospheric deposition was a relatively unimportant N source until the beginning of the agricultural and industrial revolution with an increasing population and demands for food and energy. Since 1860, atmospheric deposition got more and more an important N source for ecosystems and can also be the dominant source. The shape of the effects of atmospheric N deposition depends on: duration, total amount, and N form of the deposition; sensitivity of plant species exposed to deposition; abiotic conditions in the ecosystem which can be influenced significantly by both past and present land use. Therefore, sensitivity to N deposition can vary between ecosystems or landscapes, respectively, as reviewed for the Global 200 priority ecoregions for conservation (Bobbink et al., 2010): Changes in species composition; direct toxicity of N gases and aerosols; long-term negative effects of increased ammonium and ammonia availability; soil-mediated effects of acidification; susceptibility to secondary stress and disturbance.

To avoid ecological damages due to atmospheric N deposition, the Gothenburg Protocol of the Convention on Long-range Transboundary Air Pollution (LRTAP) was developed with respect to the abatement of acidification, eutrophication and ground-level ozone. The implementation of the Gothenburg Protocol is monitored and evaluated by the European Monitoring and Evaluation Programme (EMEP), by collating emission data from parties, measuring air and precipitation quality and modelling atmospheric transport and deposition. Deposition of N is calculated from emission data compiled by EMEP by use of the EMEP

chemistry and atmospheric transport model and then verified against concentrations in air and precipitation. In 2005, 53 EMEP stations measured the concentration of N compounds in precipitation and wet deposition, whereas up to 41 stations reported air concentrations of N compounds (Fagerli and Hjellbrekke, 2007). Finally, the EMEP modelling results are mapped on grids of 50 km by 50 km.

Within the LRTAP Convention, the Working Group on Effects (WGE) provides information on the impacts of air pollutants on human health and the environment. The International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) has been coordinating the European moss survey since 2000. Within that survey, conducted every 5 years since 1990, naturally growing mosses are used as indicators of atmospheric deposition of pollutants. In 2005, mosses were sampled ca. 6000 sites in 28 countries and analyzed for heavy metals (Harmens et al., 2010) and, for the first time for N concentrations at ca. 3000 sites in 16 countries (Harmens et al., 2011). Compared to the EMEP monitoring network, the spatial resolution of the European moss survey in terms of extent, i.e. area covered by sampling sites, and grain, i.e. number of sampling sites, is much higher.

Although the N concentrations in mosses provide no direct quantitative measurement of atmospheric deposition, the moss survey data yield an indication of the spatial patterns and temporal trends of N deposition from the atmosphere to terrestrial systems (Harmens et al., 2011; Schröder et al., 2010b, 2011, 2012). Thus, for environmental impact assessments the moss survey data could help characterizing the N exposure of large areas, especially if they could be related with information on ecological characteristics of the receiving environmental systems. Factors other than atmospheric depositions also contribute to the variation of elemental concentrations in mosses (Holy et al., 2010; Schröder et al., 2008, 2010a,b). For nitrogen, these factors were discussed in more detail in Harmens et al. (2011) and Schröder et

al. (2010a). As these factors and their influence on the relationship between deposition and moss concentrations might be different for landscapes with different ecological characteristics, we hypothesise that the correlations between both N concentrations in depositions and mosses are landscape-specific. Therefore, the current study investigated the relationship between N concentrations in atmospheric deposition and in mosses for up to 40 ecologically defined land classes covering Europe. Our approach for Europe provides further detail to the global approach presented by Bobbink et al. (2010) for the G 200 ecoregions (Olson and Dienerstein, 2002).

2. Materials and methods

2.1. Moss sampling and chemical analyses

Mosses were sampled according to the guidelines described in the protocol for the 2005 European survey (ICP Vegetation, 2005). Since the sampling sites cover a broad range of ecologically different habitats, several carpet-forming moss species were collected (Fig. 1).

Figure 1: Geographical distribution of mosses sampled for N analyses. *Other species* containing those with each n < 20, i.e. *Br* - *Brachythecium rutabulum* (n = 12); *Brach sp.* - mosses of the genus *Brachythecium* (n = 14); *Hom sp.* mosses of the genus *Homalothecium* (n = 12); *Scler sp.* - mosses of the genus *Scleropodium* other than *Pp* (n = 2); *Ta* - *Thuidium abietinum*, (n = 2); *Tt* - *Thuidium tamariscinum* (n = 15). For full names cf. Fig. 6.

Although participants of the European moss survey generally aimed to only use the last two to three years' growth of moss material for nitrogen analysis, variations in environmental conditions between countries and years sometimes made it hard to identify years of growth accurately (Harmens et al., 2011). Each sampling site was located at least 300 m from main

125 roads and populated areas and at least 100 m from any road or single house. The majority of
126 mosses were sampled in forests (coniferous, broad-leaved or mixed), followed by 'moors and
127 heathland' and natural grassland. In forests, samples were collected as far as possible in small
128 open spaces to preclude any significant effect of canopy drip. Samples were generally dried
129 at room temperature and stored under those conditions until N analysis, although some
130 countries did refrigerate or deep-freeze the samples. For the determination of N, moss tissue
131 was dried at 40 °C and concentrations were determined according to either the Kjeldahl
132 method or via elemental analysis following the Dumas method; for details of methods used in
133 each country see Harmens et al. (2011). N concentrations are expressed as percentage N
134 based on dry weight and were only determined in the last 2-3 years' growth.
135 In 2005/6, a quality control exercise was conducted for assessing the analytical performance
136 of the participating laboratories (Harmens et al., 2011). Moss reference material M2 and M3
137 (Harmens et al., 2010; Steinnes et al., 1997) were distributed amongst participating
138 laboratories. In addition, some laboratories used other certified reference material for quality
139 assurance. For determination of the total nitrogen concentration in the reference material,
140 laboratories followed the same analytical procedure as used for the collected moss samples.
141 Generally, data obtained indicated good agreement between laboratories: The recommended
142 values for reference materials M2 and M3 showed a variation of 7.4 % and 7.6 %
143 respectively (Harmens et al., 2010). Only for one laboratory a correction factor was applied
144 to the total nitrogen concentration of the moss samples as the values for the moss standards
145 were outside the range of two standard deviations from the mean recommended value for the
146 reference material M2.
147 For this investigation we used the data on N concentrations in mosses sampled in 2005 at
148 2796 sites across Europe and spatially connected them with the modelled atmospheric
149 depositions of N (section 2.2) and the ecological land classes of Europe (section 2.3) within a

Geographic Information System (GIS). As the last two to three years of moss growth was selected for N determination, representing the accumulation of atmospheric depositions in mosses in up to three years previous to sampling (ICP Vegetation, 2005), average modelled N deposition data for the years 2003, 2004, and 2005 were included in the statistical analyses.

2.2. Monitoring and modelling atmospheric deposition

Using the EMEP transport model, atmospheric deposition of N (Simpson et al., 2012) was calculated from emission data compiled by EMEP. The modelled data were verified against measured concentrations in air and precipitation (Fagerli and Aas, 2008). In 2005, 53 EMEP stations measured the concentration of N compounds in precipitation and wet deposition, whereas up to 41 stations reported air concentrations of nitrogen compounds.

The modelled deposition data comprise uncertainties of data collected from emission inventories as well as from monitoring and modelling:

- The *uncertainty of emission data* is difficult to quantity since the national emission inventories do not provide respective information.
- The *uncertainty of modelling results* includes intrinsic model uncertainties, the overall model uncertainty and the comparison of modelled values with field observations. According to Simpson et al. (2012), the model performance compared to EMEP and other measurements is presented annually in EMEP validation reports (www.emep.int) or in papers as for instance in those from Fagerli and Aas (2008) and Simpson et al. (2006a, b) dealing with nitrogen compounds.
- To assure the *quality of monitoring data* measurements are validated through a quality assurance / quality control process involving the individual institutions responsible for the different sites and the EMEP-CCC as documented by the reports available in the Chemical Coordinating Centre EMEP series (www.emep.int). In addition to applied

reference methods and standard operation procedures, EMEP conducts laboratory- and field inter-comparison of most components defined by the monitoring programme. Field inter-comparisons are an important part of the quality assurance programme in EMEP to document the overall uncertainty in the methods used (Tørseth et al., 2012). The *uncertainty of monitoring data* includes the estimation of the uncertainty caused by analytical methods. While laboratory comparisons provided estimations of the accuracy of analytical methods, *overall measurement accuracy* was estimated by field campaigns.

From the above broad quality assurance framework could be concluded the difficulty to assess the uncertainty of atmospheric chemical transport models for deposition. This is mainly due to a lack of information about the quality of emission data and data on dry deposition. According to the EMEP data quality objective (EMEP / CCC 2001), the accuracy for the chemical analysis should be better than 10%, and that is met by most laboratories in the annual laboratory inter-comparison, often better than 5%. The uncertainty for the combined sampling and chemical analysis should be better than 15 -25%. There has been some field inter-comparison to assess the uncertainties in the overall measurements and they are in general within these objectives if the reference methods are used (wet only for precipitation and filterpack for air and aerosol). Notice that the above mentioned uncertainties refer to concentrations and not to deposition loads, because dry deposition is not measured in EMEP.

For wet-deposition, Simpson et al. (2006b) found that the EMEP model's wet-deposition of NO_3 and NH_4 were within 20-30% of values measured at sites within the ICP-Forests Level II programme, or 10-23% lower when compared to the EMEP/CCC network. For dry-deposition, Flechard et al. (2011) compared four different deposition-modules making use of data from 55 sites across Europe. This study found differences of the order of 2-3 between

the models, with estimates for particle deposition over forests showing especially large differences. Estimates of total deposition should of course be more robust than those of dry deposition, and analysis of the results of the EURODELTA ensemble study (7 chemical transport models) showed standard deviations between models of about 50-200 mg N / m^2 in regions where the ensemble mean was about 200-500 mg N /m^2 (Simpson et al., 2011). Given that airborne nitrogen species are usually reproduced within 30% though, and given the constraints of mass-balance, a first estimate of total deposition uncertainty might be around 30-50%.

2.3 Ecological Landscape Classification of Europe (ELCE)

The data on atmospheric deposition and their accumulation in mosses were linked to a map depicting the geographical distribution of ecological land classes across Europe. This map was calculated by means of Classification and Regression Trees (CART; Breimann et al., 1984) from 48 digital maps each depicting the spatial pattern of one of 48 ecologically relevant landscape characteristics covering climate, altitude, soil, and potential natural vegetation in Europe (Hornsmann et al., 2008). ELCE subdivides Europe into spatial units mapped on grids of about 20 km x 20 km. Data used for calculating the ELCE unit are data on the potential natural vegetation (PNV; Bohn et al., 2005), on altitude (Hastings et al., 1999) on soil texture (FAO, 1996) as well as on monthly averages on air temperature, sunshine duration, relative humidity and precipitation (New et al., 2002). The PNV was set as the target variable whereas the data on altitude, soil texture, and climate were chosen as predictors. CART allows the production of several levels of grain. In this investigation that is the numbers of ELCE units differentiated (Lam, 2004), depicting the spatial patterns of 200 ($ELCE_{200}$) to 40 ($ELCE_{40}$) units. In this investigation $ELCE_{40}$ was used. For further CART details we refer to section 2.6.

225

226 *2.4 Calculation of minimum number of sampling sites needed for reliable statistics*

227 Measurement values should be meaningful not only for one certain point in space and time.
228 Measurements taken in a geographically specified area should rather allow for
229 generalizations so that, e.g. their mean value is reliable with respect to variability and number
230 of measurements covering that region. The number of samples required is to be based on a
231 specified confidence interval of the mean of the variable considered (Nelson and Ward,
232 1981). Therefore, in this investigation the minimum number of sampling sites needed for
233 reliable statistics were calculated prior to the calculation of correlations between atmospheric
234 deposition of N and in the N concentration in mosses. Hox (2010) provides an overview of
235 sample size issues with regard to minimum sample sizes needed. our study, the minimum
236 number was computed for a) Europe in terms of the sum of the territories of countries which
237 participated in the moss survey 2005; b) each of the participating countries; c) each of the 40
238 ecological land classes of Europe covered by the survey network. For the countries (b) and
239 the land classes (c) both, the percentage countries and classes with missing monitoring sites
240 and the percentage of area covered were calculated. In contrast to countries, ELCE units are
241 not necessarily spatially contiguous. Therefore, the percentage was only calculated for those
242 parts of land classes covered by moss survey sampling sites buffered by the minimum auto-
243 correlation range of N.

244

245 *2.5 Correlations between modelled N deposition and measured concentrations in mosses*

246 As a widespread phenomenon in environmental systems, auto-correlation of a random
247 process is defined as the similarity of, or correlation between, values of a process at
248 neighbouring points in time or space. Positive autocorrelation means that the individual
249 observations contain information which is part of other temporal or spatial neighbouring

observations. Subsequently, the effective sample size will be lower than the number of realized observations. Thus, positive spatial auto-correlation enhances type I errors, so that parametric statistics such as Pearson correlation coefficients are declared significant when they should not be (Nelson and Ward, 1981). Therefore, Schröder et al. (2012) calculated spatial auto-correlations of both EMEP deposition data and moss data across Europe according to Dutilleul (1993).

Then, landscape-specific Spearman rank correlations between EMEP modelled atmospheric dry, wet and total deposition and concentrations in mosses for N were determined. In this investigation, Spearman rank correlation coefficients r_s were calculated because the measured concentrations mostly proved not to be normally distributed. Although this non-parametric correlation method is less powerful than parametric methods if the assumptions underlying the latter are met, it is less likely to give distorted results when the assumptions fail. The strength of correlation were classified as follows: r_s values $<|0.2|$ are very low, between |0.2| and |0.49| low, from |0.5| to |0.69| moderate, between |0.7| and |0.89| high and $\geq |0.9|$ very high (Schröder et al., 2010).

To assess the impact of using EMEP modelled data averaged over three years in comparison to modelled data for the year previous to moss sampling, correlations were also determined using only the EMEP modelled data for the year previous to moss sampling. Sampling-sites-specific N concentrations in mosses were averaged for each of the 50 km x 50 km EMEP grids containing the atmospheric N deposition values (Fig. 2) before correlations were calculated (Harmens et al., 2011). Moss data outside the mean ± 3 standard deviations were eliminated from the analysis leading to exclusion of 2-3% of the moss data.

Figure 2: Atmospheric N deposition modelled on a 50 km by 50 km grid (EMEP) and N concentrations in moss samples at individual sites

275

276 *2.6 Decision trees uncovering relations between N concentrations in mosses and potentially*
277 *influencing factors*

278 In this investigation, CART (Breiman et al., 1984) was not only used to compute a map
279 depicting the geographical distribution of ecologically defined land classes across Europe
280 (section 2.3). Additionally, CART was applied to detect correlations between the N
281 concentrations in mosses and sampling site-specific and regional characteristics, which
282 potentially could influence the concentration in mosses (Table 1).

283 CART does not make any assumptions regarding the distribution of the data and can use an
284 explanatory variable more than once, so it can work with multiple interrelated data. CART
285 can reveal hierarchical and nonlinear relationships among one dependent variable (N
286 concentration in mosses) and several describing variables (sampling sites and regional
287 characteristics) by subdividing a heterogeneous data set into more homogeneous subsets
288 (classes, groups, nodes) by a series of nested binary "if-then-else" splits. Each split
289 maximizes the homogeneity of the dependent variable. Each possible binary split for all
290 variables is evaluated recursively for the best class separation until homogeneous end points
291 (nodes) are reached. The predictor selected is the one for which the two new classes have the
292 greatest within-group similarity for the response variable. The two new classes are then
293 examined separately with respect to each of the predictor variables to see if they can be split
294 again. The resulting dendrogram can have multiple branches each of which represents a path
295 to a particular combination of independent variables defining variable subspaces.

296 CART results are easy to understand. Additionally, neither dependent nor independent
297 variables are assumed to follow any kind of statistical distribution. The variables can be a
298 mixture of categorical, interval, and continuous. CART is not at all affected by outliers,
299 collinearities or heteroscedasticity that affect parametric procedures. Outliers are isolated into

a node, and do not have any effect on splitting. CART is able to reveal interactions in the data set. The algorithm is invariant under monotone transformation of independent variables; that is, the transformation of explanatory variables to logarithms or squares or square roots has no effect on the tree produced.

3. Results and discussion

3.1 *Minimum number of sampling sites*

Table 1 contains the results of calculated minimum number of moss sampling sites for each of those 40 ELCE units which were covered by the European moss survey network 2005. In most cases the number of sampled sites exceeds the number of sites required. The minimum number of sampling sites required had failed in three out of 27 ECLE units (11.1%) with N determined in mosses: In these three land classes, 27 sites instead of 12, 6 instead of 2, and 8 instead of 4, should have been sampled, respectively.

Table 1: Moss species and minimum sample size needed for Europe, participating countries and ELCE units covered by the survey network with regard to mean and standard deviation of N concentrations in mosses 2005.

	N
Number of sites missing for adequate coverage of Europe	0
Area of Europe covered by countries with missing sites [km^2]	0
Number of ELCE units with missing sites	3 / 27 11.1%

Area covered by ELCE units with missing sites [km^2]	78563.0 3.3%
Number of countries with missing sites	0 / 16 0.0%
Area covered by countries with missing sites [km^2]	0 0%

317

318 The determination of minimum numbers of sampling sites needed for calculating reliable
319 mean values for Europe as a whole and for each of the 16 countries participating in the
320 nitrogen moss survey in 2005 revealed a similar picture as found for landscapes as spatial
321 reference system. The number of sampling sites could be proved to be adequate to estimate
322 reliable statistics on the N concentrations in mosses.
323 The results for the minimum sample size needed give reason to discuss whether the network
324 should be adjusted accordingly. Pesch and Schröder (2006) developed a methodology how to
325 optimize the moss monitoring network by example of Germany without reduction of
326 statistical power. Accordingly, the German moss survey network for 2005 was designed.
327 Hornsmann et al. (2008) complemented that approach for Europe by use of ELCE.

328

329 *3.2 Landscape-specific correlations between concentrations in atmospheric depositions and*
330 *in mosses*
331 Positive spatial auto-correlations could be proven and accounted for in the calculation of
332 statistical correlations between atmospheric deposition and concentration in mosses within
333 ELCE units (Fig.3). The results showed that the auto-correlation considerably reduces the
334 degrees of freedom. Despite this, the correlations remained statistically significant (Schröder
335 et al., 2012). Harmens et al. (2012) correlated metal deposition and concentrations in mosses
336 for single European countries. This is reasonable in terms of environmental policies but

should be added by correlation analyses within the spatial framework of ecologically defined land classes. Such spatial units are, contrary to species which are used to indicate single aspects of habitat quality including pollution, complex indicators comprehending the ecological coverage of land in terms of, e.g., soil, vegetation, elevation and climate (Aspinall and Pearson, 2000; Wallace et al., 2004). Figure 3 depicts the spatial structures of Spearman Rank correlations coefficients between concentrations of N in atmospheric deposition and mosses calculated and mapped for each of the $ELCE_{40}$ units.

Figure 3: Correlations of N concentrations in modelled atmospheric total deposition summed up for the years 2003-2005 and in mosses (2005) within ELCE units.

The spatial pattern of correlations between N deposition and N concentration in mosses (Figure 3) is characterized by clear clusters of values from |0.5| to |0.69| (16.1% of total area) in Great Britain and the Alps as well as in parts of central Europe and northern Scandinavia, indicated in yellow. Correlations of r_s > |0.7| (orange coloured) occur only to a small extent (0.9% of total area) in northern Europe, where N deposition was generally low. R_s values between |0.2| and |0.49| (27.7% of total area) were found in large areas of Finland and the Baltic States and to a lesser extent in parts of central Europe and Great Britain. Correlations < |0.2| (48.7% of total area) including negative values dominate landscapes in western, central and eastern Europe as well as parts of England, Scotland, southern Finland and the Baltic States.

The N concentrations in mosses over the last three years of growth were compared with the average EMEP modelled annual deposition for the three years previous to moss sampling in 2005, which provides a buffer for any annual variations that might occur. Considering the uncertainties in the EMEP modelled deposition data (section 2.2) and the potential limitations

362 and confounding factors in the use of mosses as monitors of atmospheric deposition (Aboal et
363 al., 2010; Harmens et al., 2011), the spatial patterns and temporal trends of both data sets
364 agree reasonably well for N. The landscape-specific results confirm that metal and N
365 concentrations in mosses can serve as a complementary method to determine spatial patterns
366 and temporal trends of N deposition.
367
368 *3.3 Uncovering interrelations between N concentration in mosses and influencing factors in*
369 *landscapes with strong and weak correlations between N deposition and N concentration in*
370 *mosses*
371 Previous analyses had indicated that total atmospheric deposition of N is the main factor
372 explaining the variation of N concentrations in mosses across Europe. However, other factors
373 potentially might also contribute to the spatial variation of N concentrations in mosses,
374 including, for example, the variation in moss species sampled, land use in the surrounding
375 area, altitude and distance to the sea (Harmens et al., 2011; Schröder et al., 2010). CART
376 calculations (section 2.6) were applied to explain quite different correlation values for N
377 concentrations in deposition and mosses, for example for ELCE units F_1.2 (r_s = 0.58) and
378 F_4.2 (r_s = 0.01) (Fig. 4), by uncovering the multivariate interactions between moss
379 concentrations and potentially influencing factors.
380
381 Figure 4: Geographical distribution of ELCE units F_1.2 and F_4.2 and moss sampling sites
382 across Europe.
383

384 The decision tree analysis identifies the following most powerful predictors for the N
385 concentration in mosses collected in ELCE unit F_1.2, covering parts of Great Britain and
386 France as well as several small areas across central Europe: total N deposition (Level 1),

387 moss species and population density (Level 2), wet total N and wet NO_x deposition,
388 percentage of urban areas in a radius of 5 km around sampling sites (Level 3), and percentage
389 of urban areas in 10 km and 25 km around sampling sites (Fig. 5). This CART model
390 explains 62% of the variance in data on 236 N measurements in mosses and proved them to
391 be mainly dominated by total N atmospheric deposition.
392
393 Figure 5: Decision Tree Analysis for N concentrations in modelled atmospheric total N
394 depositions summed up for the years 2003-2005 and mosses (2005) for ELCE unit F_1.2 (r_s =
395 0.58).

396
397 At sampling sites with deposition lower than 3675.3 µg m^{-2} a^{-1} the N concentration in *Ps*, *Hs*
398 and *Pp* is lower (0.83% of dry mass) than in *Rs*, *Hc*, *Sp* and *Tt* (1.09%) (full names of moss
399 species are listed in Figure 6). Each of both these CART subgroups is further subdivided by
400 the wet deposition of total N and NO_x. Another split is established for sampling sites with wet
401 deposition exceeding 194.39 µg m^{-2} a^{-1} NO_x. This sample is, finally, split into nodes 13 and
402 14 by the percentage of urban areas around the sampling sites. In mosses sampled at sites
403 with total N deposition higher than 3675.3 µg m^{-2} a^{-1} and a population density above 37.5
404 inhabitants per 1 sq km grid cell, the N concentrations (1.77%) are higher than mosses
405 collected at sites with lower population densities (N = 1.32%). The latter sub-sample is
406 further split by the percentage of urban areas in node 12 with a mean N concentration of
407 1.11% and node 11 with a mean N concentration amounting for 1.38%. The latter sub-sample
408 is split by the percentage of urban areas around the moss sampling sites into nodes 15 and 16
409 with mean N concentrations of 1.19% and 1.45%, respectively.
410 In ELCE unit F_4.2, mainly occurring in the Pyrenees, southern Great Britain and central
411 Europe, especially in the Alps as well as in the Carpathians and the Balkans, the correlation

between N concentrations in depositions and mosses is almost zero (r_s = 0.01). The low correlation might be explained by the fact that the majority of sampling sites fall within Central Europe where N deposition rates are high and saturation occurs of the N concentration in mosses (Harmens et al., 2011). The CART model calculated (Fig. 6) only explains 10% of the variance amongst the 369 measurements and identifies the moss species (Level 1) and the dry deposition of NO_x (Level 2) as the main predictors. In *Hs, Rs, Ps* and *Br* the N concentrations (1.24%) are lower than in *Hc, Ta, Pp, Dicr sp, Aa,* and *Tt* (1.45%). The latter sample is split by the dry deposition of NO_x below / equal or exceeding 738.41 µg / m^2 / a , yielding two CART-subgroups with N concentrations of 1.39% and 1.71% respectively.

Figure 6: Decision Tree Analysis for modelled atmospheric total N deposition averaged for the years 2003-2005 and mosses (2005) for ELCE unit F_4.2 (r_s = 0.01).
Aa *Abietinella abietina,* ***Br*** *Brachythecium rutabulum,* ***Dicr. Sp.*** *Dicranum,* ***Hc*** *Hypnum cupressiforme,* ***Hs*** *Hylocomium splendens,* ***Pp*** *Pseudoscleropodium purum,* ***Ps*** *Pleurozium schreberi,* ***Rs*** *Rhytidiadelphus squarrosus,* ***Sp*** *Scleropodium purum,* ***Ta*** *Thuidium abietinum,* ***Tt*** *Thuidium tamariscinum*

4. Conclusions

Substances emitted into the atmosphere such as N are removed at the Earth´s surface by atmospheric deposition and then accumulated in soils and plants. The partitioning between dry, occult and wet deposition depends on atmospheric concentrations of the respective element and landscape characteristics as for instance climate, land use, surface roughness. Unlike wet deposition, which is widely monitored in regional networks with wet-only or bulk precipitation collectors, measurements of dry N deposition are limited to some few sites and a

437 few days to a few months. Dry deposition monitoring networks across large areas such as
438 Europe are impracticable. Results from dry deposition modelling revealed that the differences
439 between models reached a factor 2–3 and exceeded the differences between monitoring sites
440 (Flechard et al., 2011. Thus, supplementary deposition monitoring techniques should be
441 applied which enable to collect dry, occult and wet depositions (Knappe et al. 2008) and to
442 cover large areas and different landscapes in a high spatial resolution.
443 From this study it can be concluded that the requirements mentioned above can be reached by
444 application of the moss technique. Mosses were proved to serve as biological indicators for
445 atmospheric depositions and ecologically defined land classes could be identified as more
446 complex indicators which allow relating exposure monitoring with effects assessment: For N
447 the correlations between concentrations in mosses and the EMEP modelled total atmospheric
448 deposition are landscape-specific and, in comparison with the landscape-specific correlations
449 for Cd, Hg and Pb (Schröder et al., 2013), substance-specific. Significant positive
450 correlations between atmospheric N deposition and the N concentration in mosses were found
451 for 13 out of 25 (= 52%) ELCE units. Non-significant or significant, low negative
452 correlations were found in landscapes where mosses were sampled in a relative small number
453 of EMEP grid squares. Correlations were generally not affected by using EMEP modelled
454 deposition data for the year previous to sampling or averaged over three years previous to
455 sampling of the mosses. For the majority of landscapes across Europe, the moss bio--
456 monitoring could be corroborated as a valid, complementary method for assessing spatial
457 patterns and temporal trends of atmospheric deposition of N across Europe. Atmospheric N
458 deposition and N concentration in mosses could be proven to differ considerably between
459 natural landscapes across Europe. In a following investigation, these results should be used to
460 detail estimations of critical loads of eutrophication exceedances which, in EU27, in 2000
461 and 2020 amount to 74% and 61%, respectively, under current legislation (Baseline

scenario). Under the Maximum Feasible Reduction scenario, the area at risk in EU27 could be 24% (Hettelingh et al., 2010). Thus, in parts of Europe, ecotoxicologically critical input levels are exceeded. Long-term exceedances of the critical N input rate can lead to an imbalance of nutrients and to changes in the species composition in sensitive ecosystems (Bobbink et al., 2010). For Natura2000 sites, 15% of the area in EU27 is at risk of significant change in bio-diversity in 2000 (Hettelingh et al., 2010) through high N inputs, which can increase the sensitivity of plants to climatic extremes and to biotic pests (Bobbink et al., 2010). These areas should be assessed in more detail by applying the approach presented in this investigation.

Furthermore, the first European N survey should be resumed and implemented as a long-term monitoring programme to enable detection of temporal trends. This would support the spatial modelling of atmospheric depositions and critical loads exceedances. Harmens et al. (2006) demonstrated the use of herbarium moss analyses for retrospective monitoring of long-term (ca. 1860 – ca. 2000) temporal trends of N concentration in mosses collected from Czech Republic, Finland, France and Switzerland. The study corroborated that before 1960 there were no changes in the total N concentration in mosses. However, after 1960 the total N concentration in mosses was increased in all countries, although significantly ($P < 0.05$) only in Switzerland. Total N deposition rates estimated by EMEP/M SC-West using the EMEP Unified model show broadly a similar trend: not much change in total N deposition rates up to 1960 (apart from the Czech Republic) and a clear rise since 1960 (Harmens et al., 2006).

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Figure 1: Geographical distribution of mosses sampled for N analyses. *Other species* containing those with n < 20, i.e. *Br* - *Brachythecium rutabulum* (n = 12); *Brach sp.* - mosses of the genus *Brachythecium* (n = 14); *Hom sp.* mosses of the genus *Homalothecium* (n = 12); *Scler sp.* - mosses of the genus *Scleropodium* other than *Pp* (n = 2); *Ta* - *Thuidium abietinum*, (n = 2); *Tt* - *Thuidium tamariscinum* (n = 15). For full names cf. Fig. 6.

Figure 2: Atmospheric N deposition modelled on a 50 km by 50 km grid (EMEP) and N concentrations in moss samples at individual sites

Figure 3: Correlations of N concentrations in modelled atmospheric total deposition summed up for the years 2003-2005 and in mosses (2005) within ELCE units.

662 Figure 4: Geographical distribution of ELCE units F_1.2 and F_4.2 and moss sampling sites
663 across Europe.
664
665 Figure 5: Decision Tree Analysis for N concentrations in modelled atmospheric total N
666 depositions summed up for the years 2003-2005 and mosses (2005) for ELCE unit F_1.2 (r_s =
667 0.58).
668
669 Figure 6: Decision Tree Analysis for modelled atmospheric total N deposition averaged for
670 the years 2003-2005 and mosses (2005) for ELCE unit F_4.2 (r_s = 0.01).
671 ***Aa*** *Abietinella abietina* (Hedw.) Fleisch., ***Br*** *Brachythecium rutabulum* (Hedw.) Schimp.,
672 ***Dicr. Sp.*** *Dicranum* species, ***Hc*** *Hypnum cupressiforme* Hedw., ***Hs*** *Hylocomium splendens*
673 (Hedw.) Schimp., ***Pp*** *Pseudoscleropodium purum* (Hedw.) Fleisch., ***Ps*** *Pleurozium schreberi*
674 (Brid.) Mitt., ***Rs*** *Rhytidiadelphus squarrosus* (Hedw.) Warnst., ***Ta*** *Thuidium abietinum*
675 (Hedw.) Schimp., ***Tt*** *Thuidium tamariscinum* (Hedw.) Schimp.
676

Figure(s)

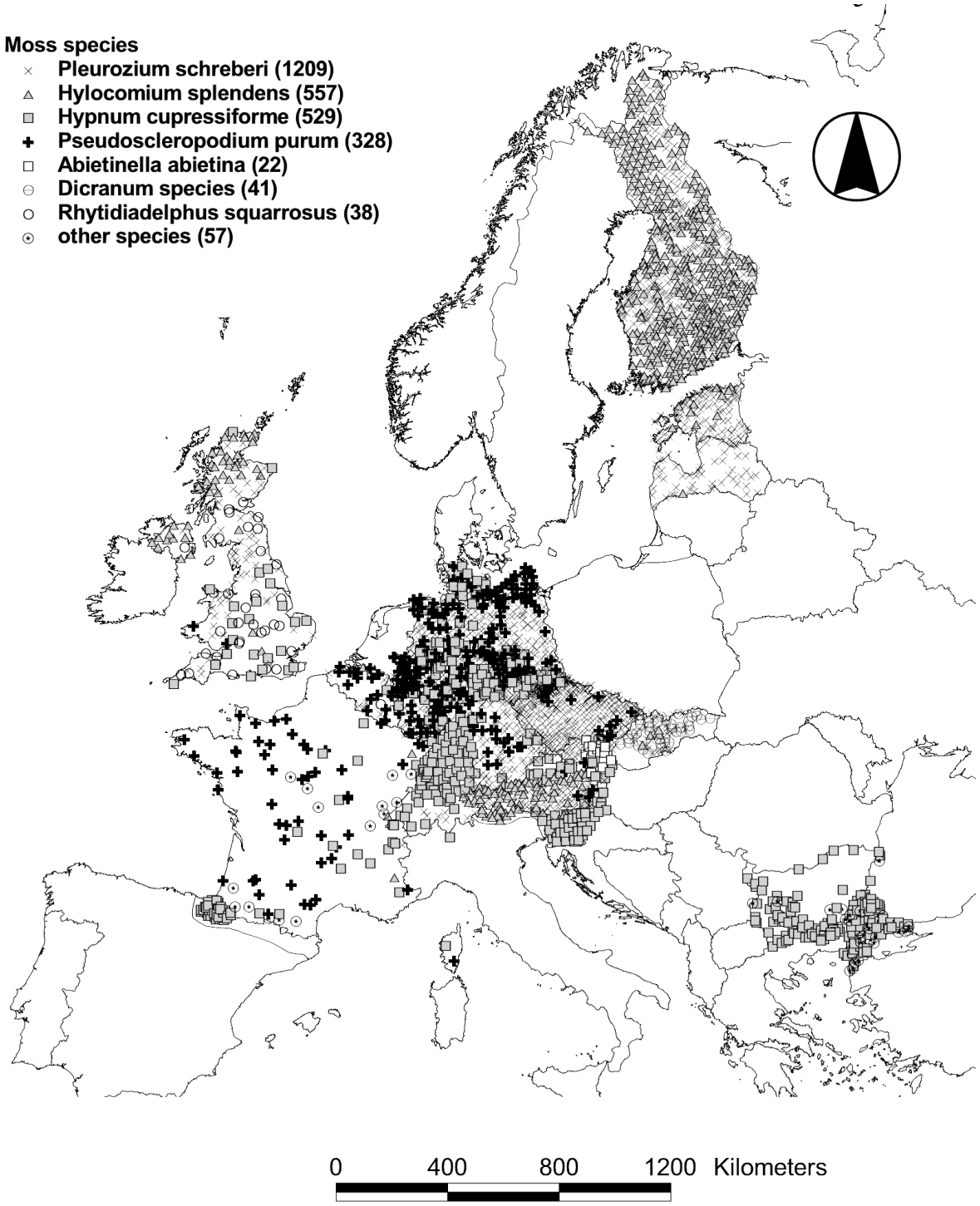


Figure 1: Geographical distribution of mosses sampled for N analyses *Other species* containing those with n < 20, i.e. *Br - Brachythecium rutabulum* (n = 12); *Brach sp.* - mosses of the genus *Brachythecium* (n = 14); *Hom sp.* mosses of the genus *Homalothecium* (n = 12); *Scler sp.* - mosses of the genus *Scleropodium* other than *Pp* (n = 2); *Ta - Thuidium abietinum*, (n = 2); *Tt - Thuidium tamariscinum* (n = 15). For full names cf. Fig. 6.

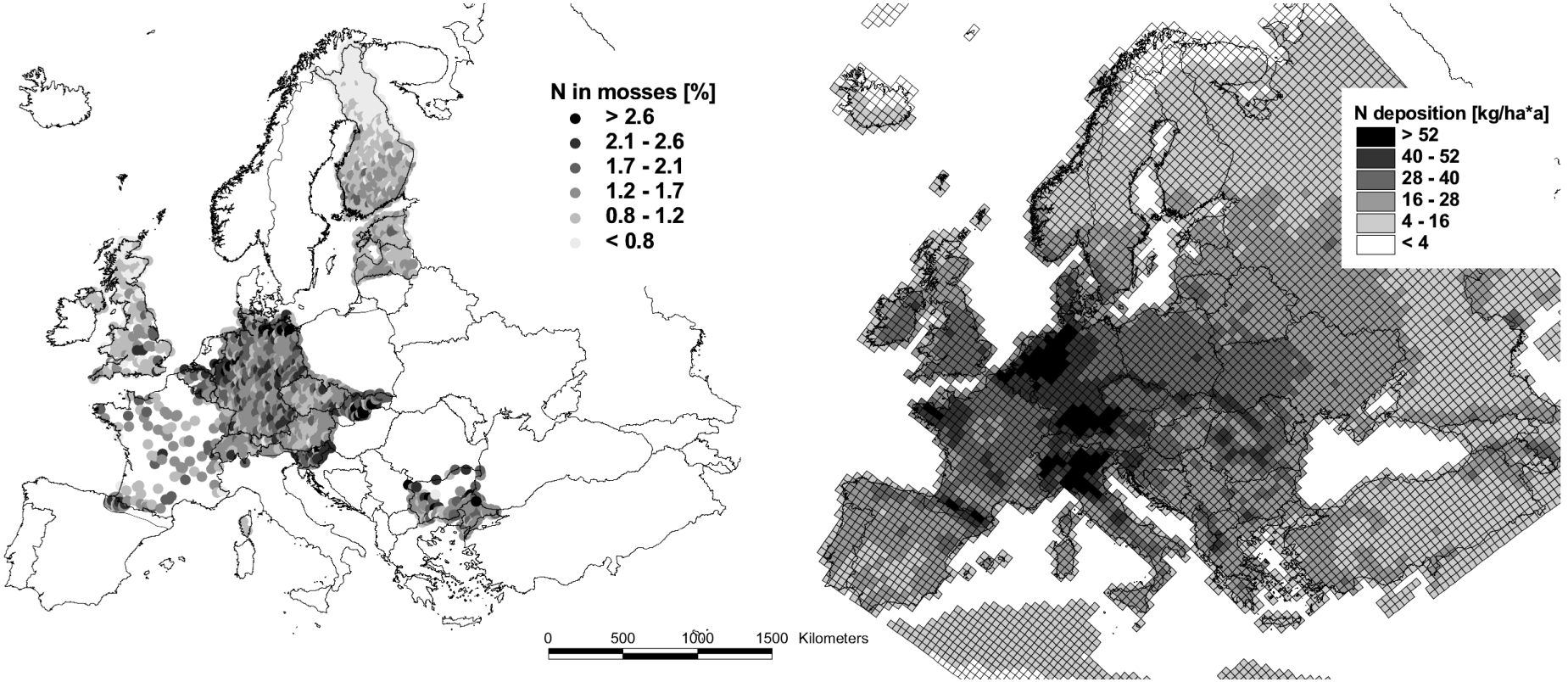


Figure 2: Atmospheric N concentrations in mosses samples at individual sites and N deposition modelled on a 50 km by 50 km grid (EMEP)

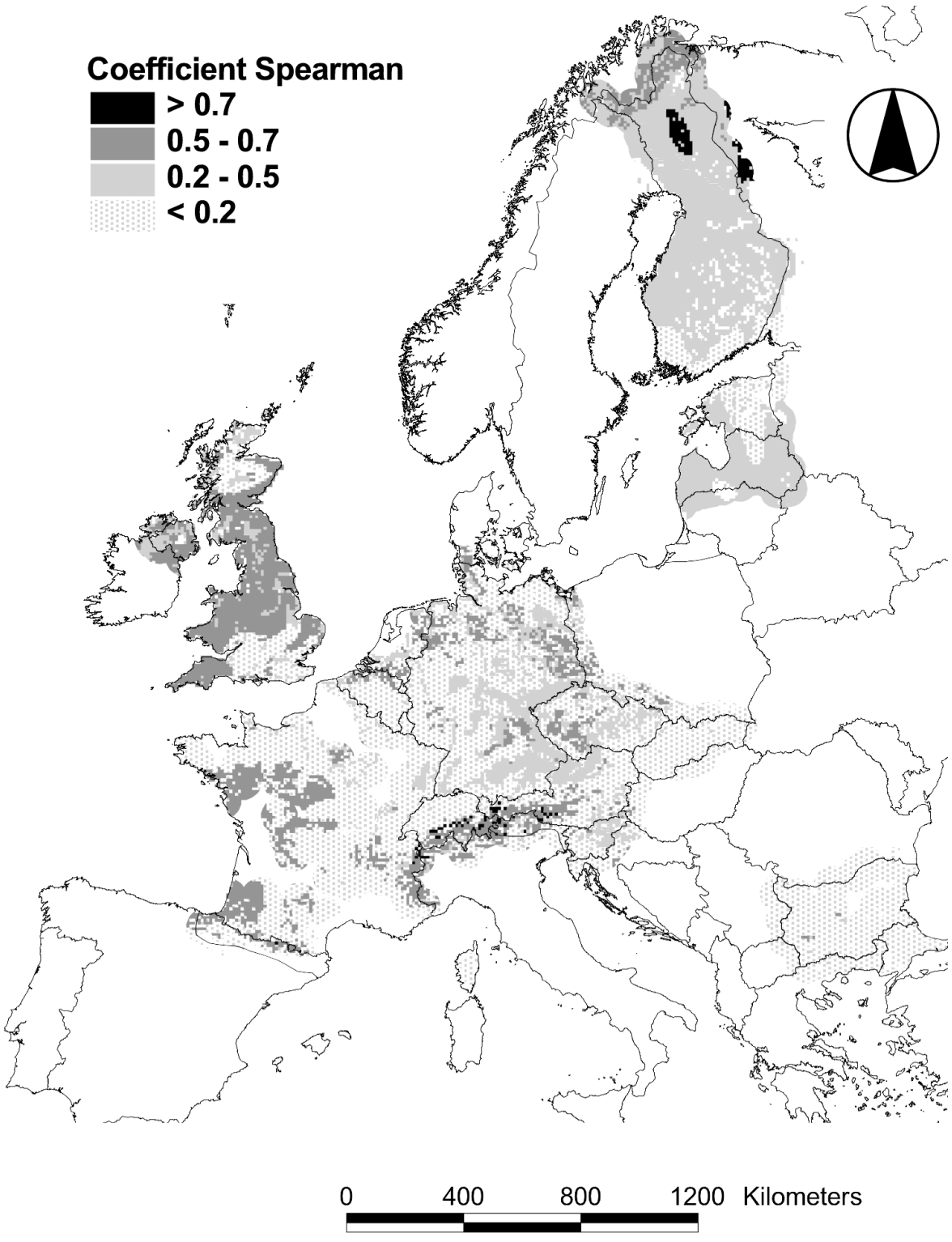


Figure 3: Correlations of N concentrations in modelled atmospheric total deposition summed up for the years 2003-2005 and in mosses (2005) within ELCE units.

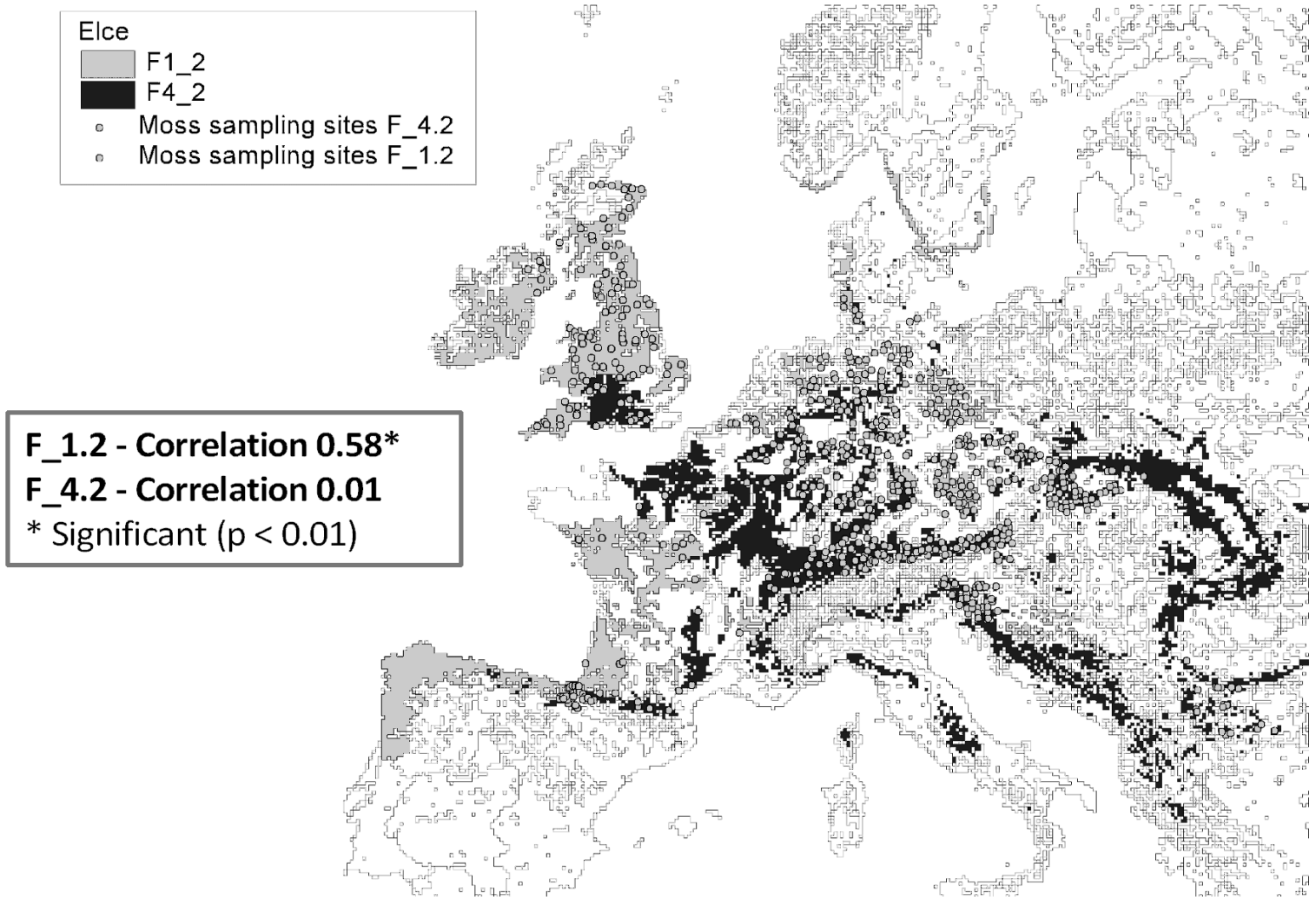


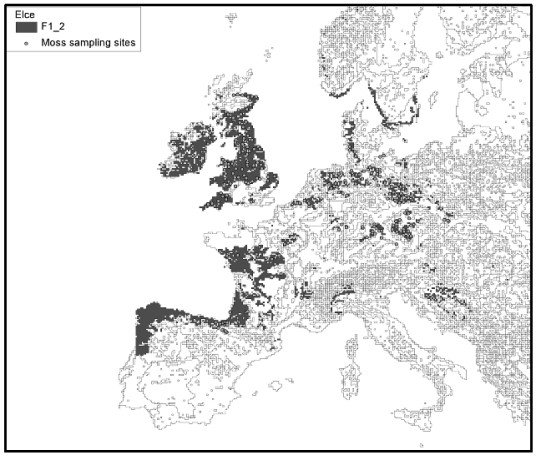
Figure 4: Geographical distribution of ELCE units F_1.2 and F_4.2 and moss sampling sites across Europe.

CART analysis for ELCE class F_1.2

N = 236

62 % explained variance

High correlation!



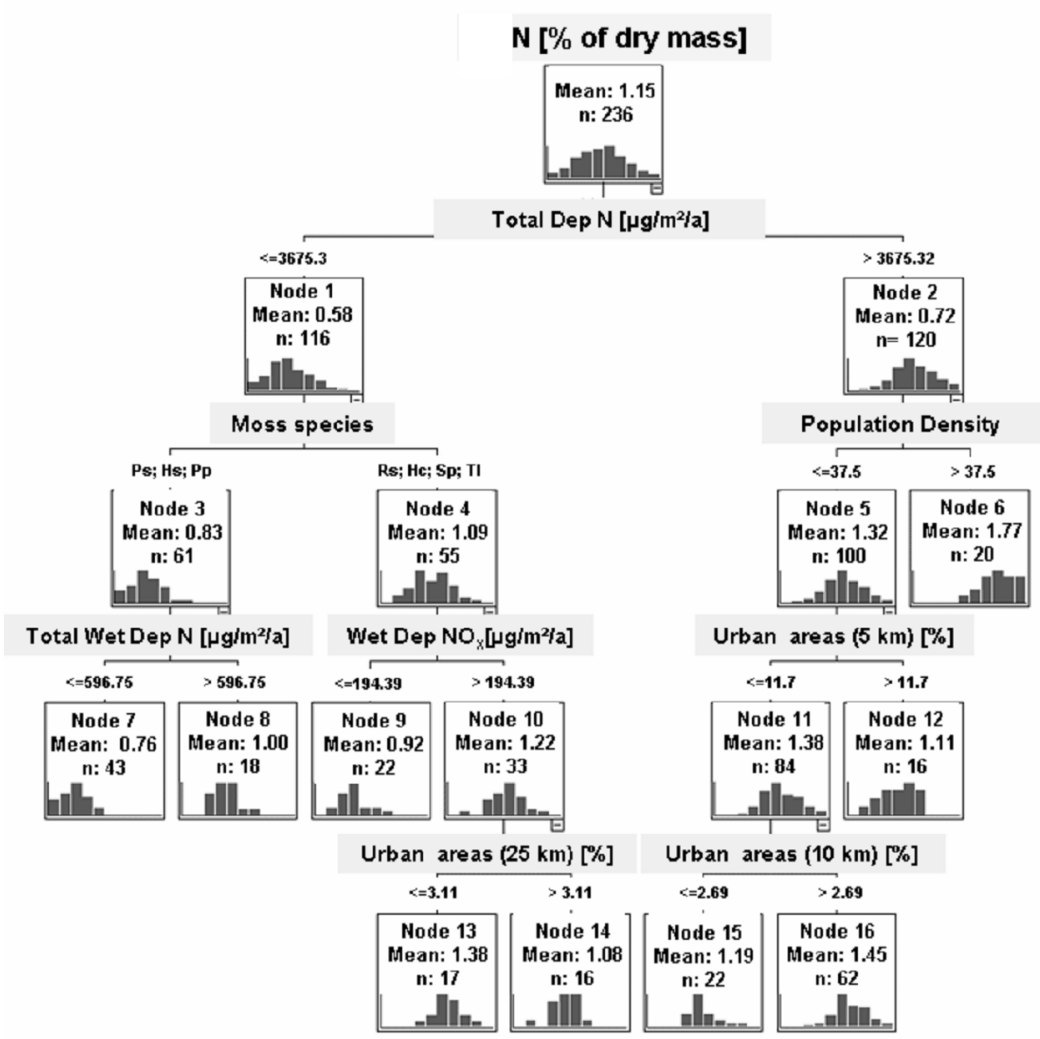


Figure 5: Decision Tree Analysis for N concentrations in modelled atmospheric total N depositions summed up for the years 2003-2005 and mosses (2005) for ELCE unit F_1.2 (r_s = 0.58).

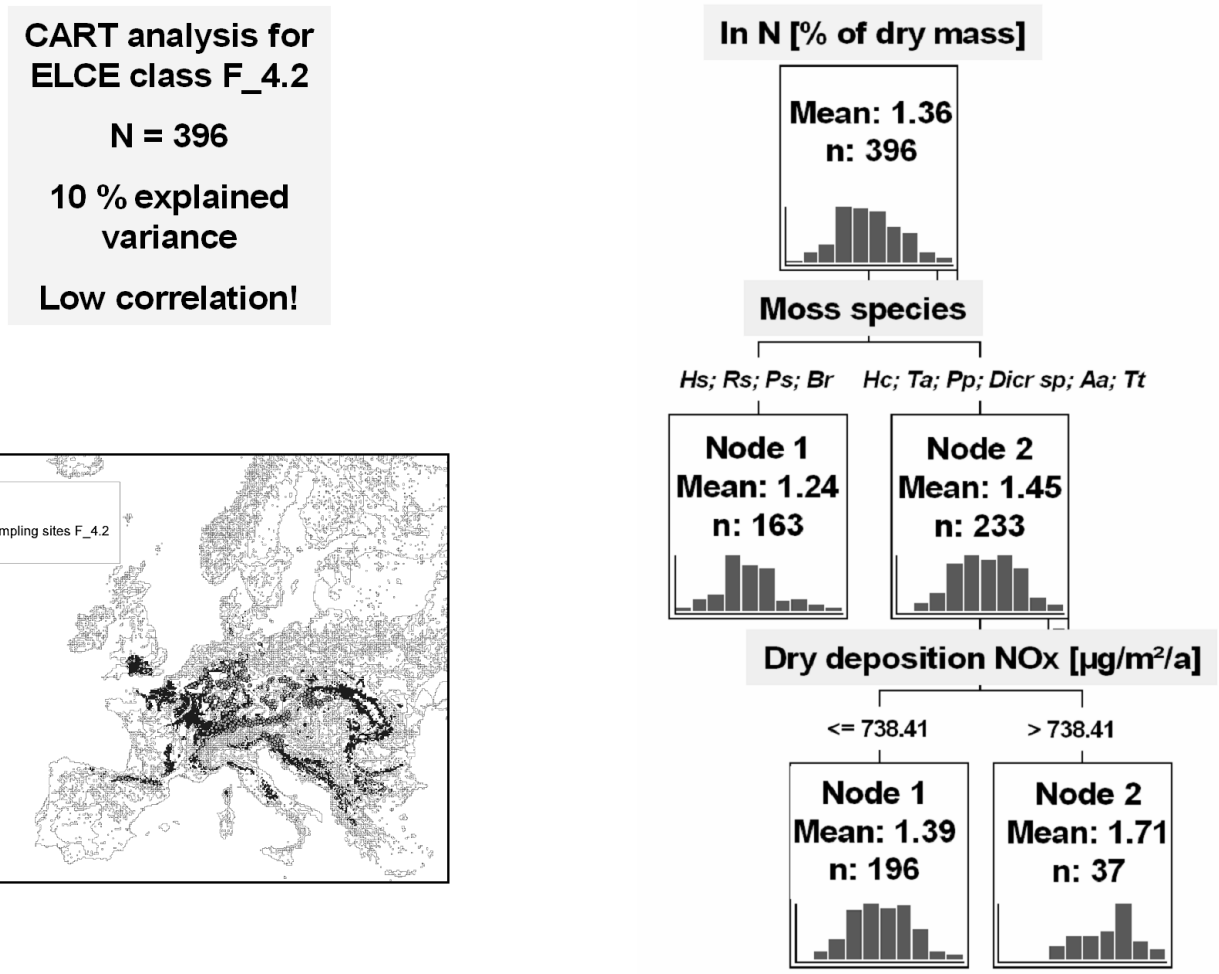


Figure 6: Decision Tree Analysis for modelled atmospheric total N deposition averaged for the years 2003-2005 and mosses (2005) for ELCE unit F_4.2 (r_s = 0.01) ***Aa*** *Abietinella abietina* (Hedw.) Fleisch., ***Br*** *Brachythecium rutabulum* (Hedw.) Schimp., ***Dicr. Sp.*** *Dicranum* species, ***Hc*** *Hypnum cupressiforme* Hedw., ***Hs*** *Hylocomium splendens* (Hedw.) Schimp., ***Pp*** *Pseudoscleropodium purum* (Hedw.) Fleisch., ***Ps*** *Pleurozium schreberi* (Brid.) Mitt., ***Rs*** *Rhytidiadelphus squarrosus* (Hedw.) Warnst., ***Ta*** *Thuidium abietinum* (Hedw.) Schimp., ***Tt*** *Thuidium tamariscinum* (Hedw.) Schimp.