# Forest floor vegetation response to nitrogen deposition in Europe

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## Abstract

Chronic nitrogen (N) deposition is a threat to biodiversity that results from the eutrophication of ecosystems. We studied long-term monitoring data from 28 forest sites with a total of 1,335 permanent forest floor vegetation plots from northern Fennoscandia to southern Italy to analyse temporal trends in vascular plant species cover and diversity. We found that the cover of plant species which prefer nutrient-poor soils (oligotrophic species) decreased the more the measured N deposition exceeded the empirical critical load (CL) for eutrophication effects ($P = 0.002$). Although species preferring nutrient-rich sites (eutrophic species) did not experience a significantly increase in cover ($P = 0.440$), in comparison to oligotrophic species they had a marginally higher proportion among new occurring species ($P = 0.091$). The observed gradual replacement of oligotrophic species by eutrophic species as a response to N deposition seems to be a general pattern, as it was consistent on the European scale. Contrary to species cover changes, neither the decrease in species richness nor of homogeneity correlated with nitrogen CL exceedance (ExCL$_{emp}$N). We assume that the lack of diversity changes resulted from the restricted time period of our observations. Although existing habitat-specific empirical CL still hold some uncertainty, we exemplify that they are useful indicators for the sensitivity of forest floor vegetation to N deposition.

*Keywords:* air pollution, critical load, eutrophication, long-term ecological research, monitoring, plant species diversity

## Introduction

Elevated nitrogen (N) deposition from human sources exerts various effects on ecosystems (Erisman & De Vries, 2000; Galloway *et al.*, 2004; Manning *et al.*, 2006; Butterbach-Bahl & Gundersen, 2011; Sutton *et al.*, 2011). One severe negative effect is the threat to biodiversity that results from the eutrophication of sensitive ecosystems (Sala *et al.*, 2000). It is assumed that excess N favours a few plant species (Suding *et al.*, 2005) causing competitive exclusion and, in the long run, loss of less competitive species (Gilliam, 2006; Hautier *et al.*, 2009). Though our knowledge still is scarce, indirect impacts on faunal diversity are to be expected (Dise, 2011). As an example, certain butterfly species populations decrease because they depend on nutrient-poor habitats and are sensitive to N-induced stimulation of plant growth that causes microclimatic changes (Öckinger *et al.*, 2006; Wallis De Vries & Van Swaay, 2006). Another example is the decline of ant species that are associated with nutrient-poor grasslands (Pihlgren *et al.*, 2010).

In Europe, nitrogen compounds in precipitation have decreased since the year 1980, only minor reductions

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are however seen since the late-1990s (Tørseth *et al.*, 2012), and future reductions are not expected to remove the risk of eutrophication in Europe (Posch *et al.*, 2012). Many local to regional studies have shown that chronic N deposition leads to a shift in the plant species composition of the forest floor and eventually to diversity loss (Bobbink *et al.*, 1998, 2010; Gilliam, 2006; Seidling *et al.*, 2008; Van Dobben & De Vries, 2010; Bobbink & Hettelingh, 2011; Dise, 2011; Clark *et al.*, 2013). However, so far there is no unequivocal evidence that nitrogen deposition is a broad-scale driver behind the eutrophication signal in forest plant communities, as it is in other ecosystems such as grasslands (Dupré *et al.*, 2010; Stevens *et al.*, 2010) or heathlands (Bobbink *et al.*, 2010). The pivotal role of light availability for changes in the forest floor vegetation obviously confounds N effects. In a recent meta-analysis of experimental studies, decreasing plant species richness in forests, in contrast to other ecosystems, was not related to N deposition (De Schrijver *et al.*, 2011). Verheyen *et al.* (2012) analysed a large resurvey data set of deciduous forests in Europe where the shift in forest management caused a tree species change that was significantly correlated with the observed eutrophication signal in the forest floor vegetation but not N deposition per se. The explanatory variable in these studies was the average or the accumulated N deposition.

We advocate that the critical load (CL) approach, which is the key policy tool for the control of air pollution in Europe and increasingly also other parts of the world (Amann *et al.*, 2011; Pardo *et al.*, 2011a), is superior to direct deposition data when relating the effects of N deposition on forest floor vegetation. Owing to the coherent implementation of the CL approach in strategies for emission reductions under the United Nations Economic Commission for Europe (UNECE) Convention on Long-range Transboundary Air Pollution (LRTAP) and the EU National Emission Ceilings Directive, it will be used as the major EU indicator for the 2020 targets of the Convention of Biological Diversity (CBD) (EEA, 2012b). CL are deposition thresholds that are used to describe the sensitivity of ecosystems to airborne pollution. A CL is defined as 'a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge' (Nilsson & Grennfelt, 1988). The CL can be calculated with a mass balance approach or derived from empirical values. In comprehensive reviews of experimental and observational studies, empirical CL for eutrophication effects are available for ecosystems that occur in Europe and North America (De Vries *et al.*, 2010; Bobbink & Hettelingh, 2011; Pardo *et al.*, 2011b; Clark *et al.*, 2013). The higher the exceedance of the CL as compared with observed or modelled deposition, the higher the risk of biodiversity decline.

In this study, we assessed relationships between the CL exceedance and forest floor vegetation using long-term observations. The data comprise 28 sites with a total of 1,335 sampling units from northern Fennoscandia to southern Italy, covering a broad gradient in climate, deposition and forest types (Fig. 1; Table 1). Specifically, we ask the following questions:

- What kinds of temporal changes have occurred in the forest floor vegetation during the last two decades and which drivers are responsible for the changes? We analysed cover changes for 646 vascular plant species and related the results to Ellenberg indicator values. Ellenberg indicator values indicate species preferences along the nutrient (N), soil reaction (R), light (L), moisture (M) and temperature (T) gradients (Ellenberg *et al.*, 1992; Diekmann, 2003). In particular, we analysed whether species adapted to high nutrient availability (eutrophic species) have increased and those adapted to nutrient-poor conditions (oligotrophic species) have decreased in cover at sites that experienced an exceedance of the CL for eutrophication effects.

- Has diversity of forest floor vegetation changed over time? As species loss is often a very long-term process, we additionally analysed changes in compositional homogeneity (beta diversity). Again, we used Ellenberg indicator values and the CL exceedance as a means to reveal cause–effect relationships.

## Materials and methods

### Data

In total, 28 forest sites located along a climatic gradient through Europe with long-term vegetation monitoring data and measurements of N deposition were used. The majority of sites belong to the UNECE ICP Integrated Monitoring (http://www.syke.fi/nature/icpim) and ICP Forests programme (www.icp-forests.net) under the Convention on Long-Range Transboundary Air Pollution (CLRTAP), which is monitoring the effects of atmospheric deposition on ecosystems. Most of the sites are also part of the European long-term ecosystem research network (www.lter-europe.net). The sites cover the entire north-to-south gradient of Europe with a wide range of forest types from the boreal zone to the Mediterranean (Fig. 1). The size of the study sites range between 0.25 and 90 ha. The larger areas focus on the catchment scale studies. Forest vegetation of the study sites was recorded on permanent plots (hereafter called study plots) with different observation intervals (from annual to several years) and subsampling unit (hereafter called sampling units) size (Table 1).
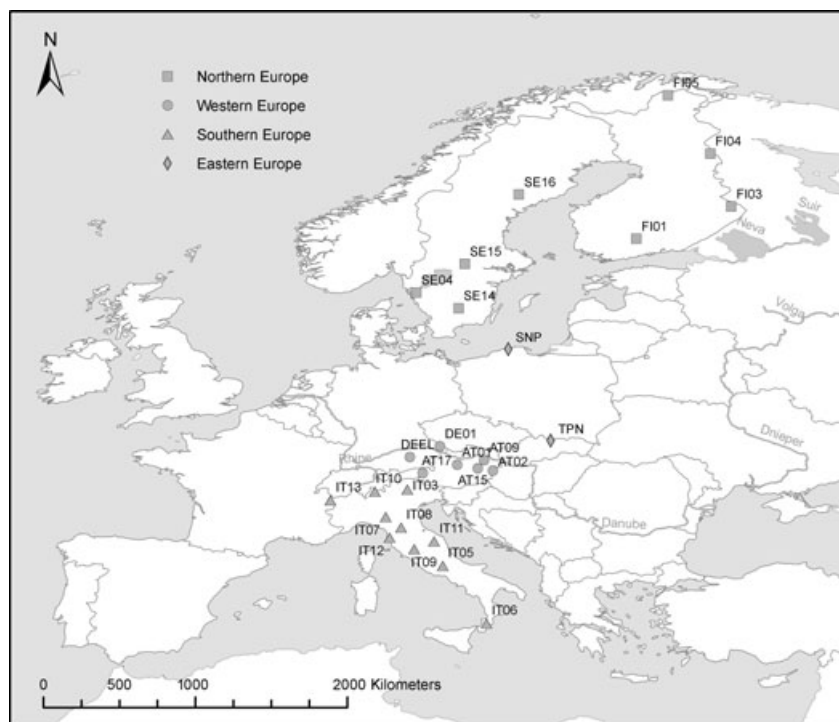
**Fig. 1** Distribution of the study sites in Europe.

Whereas study plots were selected subjectively to being representative of the study sites, sampling units were selected randomly or are distributed in a regular grid. In two study areas, sampling units were not part of a study plot but distributed in the entire catchments, and in three study areas sampling units are both part of a study plot and distributed in the entire catchment. In order to minimize the influence of tree harvesting or natural disturbances, we used only those sampling units that experienced less than 50% decrease in the tree layer cover between consecutive observations. For all analyses, we used the forest floor vascular plants only. Bryophytes and lichens were excluded because of their incompleteness in the entire data set and presumably high observer error. The cover of every vascular plant species was estimated in the percentage of the total sampling unit area or as ordinal abundance classes (Braun-Blanquet, 1951). The latter were transformed to their class means percentage of cover. In total, 646 species were recorded.

The exceedance of the CL for eutrophication effects (ExCL$_{emp}$N) was estimated as the measured throughfall N deposition minus the empirical CL for each site. We used the long-term average of the annual canopy throughfall deposition of inorganic N (Table 1). In addition, modelled inorganic N to deposition forests was used for 1995, a reference year, roughly in the middle of the time series with vegetation observations. Deposition estimates are available in a $50 \times 50$ km grid as derived from the EMEP/MSC-W unified atmospheric dispersion model that is used under the LRTAP Convention (Amann et al., 2011). At our study sites, throughfall deposition of inorganic N ranges from 0.6 to 20.2 kg N ha$^{-1}$ yr$^{-1}$, and

modelled deposition from 0.9 to 30.8 kg N ha$^{-1}$ yr$^{-1}$ (Table 1). The empirical CL are available for the forest types that have been classified according to the EUNIS (European Nature Information System) habitat system for Europe (Davies et al., 2004). Empirical CL were assigned according to the respective EUNIS habitat type with reference to the recommendations in Bobbink & Hettelingh (2011), which are based on extensive empirical studies and expert judgment on the effect of N addition on root growth and the nutrition of trees, on macrofungi and mycorrhiza and forest ground vegetation (Table 1).

*Statistical analyses*

The analyses compare long-term changes in the cover of vascular plant species, the number of species and the homogeneity (beta diversity) with ExCL$_{emp}$N of the forest sites. Additionally, Ellenberg indicator values for species optima along the nutrient (N), soil reaction (R), light (L), moisture (M) and temperature (T) gradients were used to investigate whether the observed changes were related to the environmental niche of the studied species. Low Ellenberg indicator values indicate plant species that prefer low nutrient availability (N value), acid soils (R value), low light availability (L value), dry sites (M value) and cold conditions (T value), while high Ellenberg values indicate the contrary (Ellenberg et al., 1992). In particular, we analysed whether species adapted to high nutrient availability have increased and those adapted to nutrient-poor conditions have decreased.

**Table 1** List of study sites with climatic and soil characteristics, nitrogen deposition, EUNIS habitat class, the empirical critical load for eutrophication effects ($CL_{emp}$), the number of study plots and sampling units and the time span of forest floor observations

| Country | Site name | Site code | Study area [ha] | T [°C] | P [mm] | Soil type(s) | Mean measured $N_{in}$ [kg ha$^{-1}$ yr$^{-1}$] | Modelled $N_{in}$ [kg ha$^{-1}$ yr$^{-1}$]* | EUNIS habitat classes | $CL_{emp}$ [kg ha$^{-1}$ yr$^{-1}$]† | Number of study plots/sampling units | Number of sampling units per catchment | First/last observation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Austria | LTER Zöbelboden | AT01 | 90 | 7.2 | 1618 | Lithic and Rendsic Leptosols/Chromic Cambisols/Hydromorphic Stagnosols | 16 (1994–2010) | 21.2 | Abies and Picea woodland (G3.1)/Mixed Abies – Picea – Fagus woodland (G4.6) | 10–15(20) | – | 167 × 100 m² | 1993/2010 |
| | Unterpullendorf | AT02 | 0.5 | 9.6 | 630 | Eutric Stagnic Vertic Cambisol | 8.8 (1996–2010) | 19.7 | Thermophilous deciduous woodland (G1.7) | 10–20 | 1/10 × 4 m² | – | 1996/2006 |
| | Klausen-Leopoldsdorf | AT09 | 0.5 | 8.2 | 804 | Endostagnic Endoskeletic Luvisol | 10.9 (1996–2010) | 20.1 | Fagus woodland (G1.6) | 10–20 | 1/10 × 4 m² | – | 1995/2006 |
| | Mürzzuschlag | AT15 | 0.5 | 6.0 | 933 | Eutric Calcaric Endoskeletic Cambisol | 7.9 (1996–2010) | 20.3 | Abies and Picea woodland (G3.1) | 10–15 | 1/10 × 4 m² | – | 1996/2006 |
| | Murau | AT16 | 0.5 | 5.0 | 918 | Hyperdystric Endoskeletic Cambisol | 2 (1996–2010) | 12 | Abies and Picea woodland (G3.1) | 10–15 | 1/10 × 4 m² | – | 1996/2006 |
| | Jochberg | AT17 | 0.5 | 5.7 | 1358 | Eutric Stagnic Episkeletic Fluvisol | 5.1 (1996–2010) | 20.6 | Abies and Picea woodland (G3.1) | 10–15 | 1/10 × 4 m² | – | 1996/2006 |
| Finland | Valkea-Kotinen | FI01 | 0.5 | 3.1 | 637 | Dystric Cambisols/Histosols | 1.5 (1995–2010) | 6 | Picea taiga woodland (G3.A)/Mixed taiga woodland with Betula (G4.2) | 5–8(10) | 2/24–36 × 0.25 m² | – | 1990/2009‡ |
| | Hietajärvi | FI03 | 0.5 | 2 | 622 | Haplic and Ferric Podzols/Fibric Histosols | 1.9 (1995–2010) | 3.6 | Picea taiga woodland (G3.A)/Mixed taiga woodland with Betula (G4.2) | 5–8 | 3/27–48 × 0.25 m² | – | 1990/2009‡ |
| | Pesosjärvi | FI04 | 0.5 | −0.5 | 477 | Podzols/Histosols | 1.1 (1995–2010) | 2.5 | Picea taiga woodland (G3.A)/Mixed taiga woodland with Betula (G4.2) | 5–8(10) | 3/18–36 × 0.25 m² | – | 1989/2009‡ |
| | Vuoskojärvi | FI05 | 0.5 | −1.9 | 358 | Podzols | 0.6 (1995–1997) | 0.9 | Picea taiga woodland (G3.A)/Mixed taiga woodland with Betula (G4.2) | 5–8(10) | 3/27–48 × 0.25 m² | – | 1989/2009‡ |
| Germany | Forellenbach | DE01 | 0.25 | 5.6 | 1279 | Dystric Cambisols | 9.9 (2001–2010) | 24.4 | Abies and Picea woodland (G3.1) | 10–20 | 1/16 × 4 m² | – | 1990/2000 |
| | Echinger Lohe | DEEL | 24 | 9 | 800 | Rendsic Leptosol | 19.7 (2004) | 30.8 | Meso– and eutrophic Quercus, Carpinus, Fraxinus, Acer, Tilia, Ulmus and related woodland (G1.A) | 15–20 | – | 8 × 225 m² | 1961/2003 |
| Italy | Passo Lavaze | IT03 | 0.5 | 6 | 922 | Haplic Podzols | 4.2 (1998–2011) | 26.7 | Abies and Picea woodland (G3.1) | 10–15 | 1/12 × 100 m² | – | 1999/2009 |
| | Selva Piana | IT05 | 0.5 | 10 | – | Humic Acrisols | 7.2 (1997–2011) | 17.8 | Fagus woodland (G1.6) | 10–20 | 1/12 × 100 m² | – | 1999/2009 |
| | Piano Limina | IT06 | 0.5 | 11 | 1850 | Haplic Podzols | 7.6 (1997–2011) | 9.7 | Fagus woodland (G1.6) | 10–20 | 1/12 × 100 m² | – | 1999/2009 |
| | Carrega | IT07 | 0.5 | 12 | 822 | Haplic Luvisols | 20.2 (1998–2009) | 30.6 | Acidophilous Quercus-dominated woodland (G1.8) | 10–15 | 1/12 × 100 m² | – | 1999/2009 |
| | Brasimone | IT08 | 0.5 | 10 | 1641 | Dystric Cambisols | 12.3 (1997–2006) | 28.9 | Fagus woodland (G1.6) | 10–20 | 1/12 × 100 m² | – | 1999/2009 |
| | Monte Rufeno | IT09 | 0.5 | 12 | 1048 | Dystric Cambisols | 7.2 (1997–2011) | 13.9 | Thermophilous deciduous woodland (G1.7) | 10–15 | 1/12 × 100 m² | – | 2000/2009 |
| | Val Masino | IT10 | 0.5 | 8 | 1336 | Humic Cambisols | 8.6 (1997–2011) | 18.2 | Abies and Picea woodland (G3.1) | 10–15 | 1/12 × 100 m² | – | 1999/2009 |

**Table 1** (continued)

| Country | Site name | Site code | Study area [ha] | T [°C] | P [mm] | Soil type(s) | EUNIS habitat classes | Mean measured $N_{in}$ [kg.ha$^{-1}$ yr$^{-1}$] | Modelled $N_{in}$ [kg.ha$^{-1}$ yr$^{-1}$]* | $CL_{emp}$ [kg.ha$^{-1}$ yr$^{-1}$]† | Number of study plots/sampling units | Number of sampling units per catchment | First/last observation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Roti | IT11 | 0.5 | 11 | – | Haplic Luvisols | Acidophilous Quercus–dominated woodland (G1.8) | 7.5 (1998–2011) | 16.1 | 10–15 | 1/12 × 100 m² | – | 1999/2009 |
| | Colognole | IT12 | 0.5 | 15 | 956 | Eutric Cambisols | Mediterranean evergreen Quercus woodland (G2.1) | 10.5 (1997–2011) | 16.7 | 3–7 | 1/12 × 100 m² | – | 1999/2009 |
| | La Thuile | IT13 | 0.5 | 6 | – | Lithic Leptosols | Abies and Picea woodland (G3.1) | 7.8 (2009–2011)§ | 19.6 | 10–15 | 1/12 × 100 m² | – | 1999/2010 |
| Poland | Słowiński National ark | SNP | 0.5 | 9 | 669 | Histo–Humic Gleysol | Pinus sylvestris woodland south of the taiga (G3.4) | 10.5 (1994–2010) | 13.7 | 5–15 | 1/100 × 25 m² | – | 1994/2011 |
| | Tatrzański National Park | TPN | 0.5 | 5.1 | 1100 | Calcaric Lithosol | Abies and Picea woodland (G3.1) | 16 (1999–2010) | 18.9 | 10–15 | 1/100 × 25 m² | – | 2001/2011 |
| Sweden | Gärdsjön F1 | SE04 | 3.7 | 6.7 | 1330 | Podzols | Abies and Picea woodland (G3.1) | 8.6 (1996–2009) | 13.8 | 10–15 | 2/32–33 × 0.25 m² | 20 × 100 m² | 1995/2007 |
| | Aneboda | SE14 | 19.6 | 5.8 | 880 | Podzols | Abies and Picea woodland (G3.1) | 2,5 (1996–2009) | 10.8 | 10–15 | 2/33 × 0.25 m² | – | 1982/2007 |
| | Kindlahöjden | SE15 | 19 | 4.2 | 970 | Podzols | Picea taiga woodland (G3.A) | 2.4 (1996–2009) | 8 | 5–10 | 2/32 × 0.25 m² | 48 × 100 m² | 1996/2011 |
| | Gammtratten | SE16 | 43 | 1.2 | 670 | Podzols | Picea taiga woodland (G3.A) | 1.3 (1999–2009) | 3 | 5–10 | 2/32 × 0.25 m² | 32 × 100 m² | 1994/2012 |

*Modelled inorganic N deposition in forests for 1995 as derived from the EMEP/MSC–W unified atmospheric dispersion model.
†Range of the empirical CL according to Bobbink & Hettelingh (2011).
‡From 1998 onwards 1/16 × 2 m².
§Only data on measured total nitrogen deposition is available for this study site.

*Cover changes of plant species.* We used the cover of each herb layer plant species in the study plots (the median of the cover values recorded in the respective sampling units) and the cover in the sampling units, which were recorded for the five catchment study sites (Table 1). For the detection of significant temporal changes of species cover, Generalized Linear Mixed Models (GLMM) with binomial error distribution were applied. GLMM have the ability to account for spatial dependence of the data while using a non-normal error distribution of the response. The percentage proportion of each single species (ranging between 0 and 100%) was the binomial response and the observation year was the independent variable (the fixed effect), which was nested within each study site (the random effect). Nesting was performed because we assume that changes in species cover within a study site are more similar than changes between study sites. GLMMs were performed by means of the *glmer* function in the R-package lme4 (Bates, 2011). The resulting fixed effect coefficients reflect the species-specific degrees of temporal cover changes. The random effect coefficients indicate the study site-specific deviations from the fixed effects, that is, the mean changes. The total change of a species at a study site is thus described by the sum of the fixed and the respective random effect. Hereafter, we call this the site-specific effect.

We compared the GLMM fixed effect coefficients with Ellenberg indicator values in order to assess broad-scale drivers of species changes, that is, trends that occur across Europe. For this analysis, we used ANOVA and subsequent Tukey tests for multiple comparisons. Only species that occur in more than five sites and had a significant fixed effect coefficient ($P < 0.05$) were taken into account. Vegetation changes that are due to $ExCL_{emp}N$ were addressed by the GLMM site-specific effect (sum of fixed and random effect coefficients) in the following way. Species with low (1–3) and high indicator values (7–9) were grouped. As an example, species with low Ellenberg N values 1–3 are bound to nutrient-poor sites; species with N values 7–9 prefer nutrient-rich sites. Then, we calculated a weighted mean site-specific effect for each site and group according to Eqn 1.

$$c_W = \frac{\sum_{i=1}^{n}(\beta_{fi} + \beta_{ri}) \times E_i}{\sum_{i=1}^{n} E_i}, \tag{1}$$

where $c_w$ is the weighted change coefficient, $i$ is the respective species of one such a group, $\beta_f$ is its fixed effect, $\beta_r$ is its site-specific random effect and E is its Ellenberg value which was transformed in order to upgrade species with extreme site preferences (original values 1, 2, 3 transformed to 3, 2, 1 and 7, 8, 9 to 1, 2, 3). Only those species that had a significant (AIC > 10) GLMM random coefficient were used. The relationship between $c_w$ and $ExCL_{emp}N$ was tested with an ordinary least square regression and *P*-values of the slope coefficient from a *t*-test statistic. In addition, the relationships between $c_w$ and measured N deposition, as well as between $c_w$ and $ExCL_{emp}N$, which was calculated with modelled N deposition, were tested. Apart from the relationships, deviations of $c_w$ from zero (no change in cover) in different parts of Europe (see Fig. 1) were tested with a two-sided Wilcoxon test.

*Changes in species richness and homogenization.* Trends in diversity were analysed using the number of vascular plant species and beta diversity. Species richness of each sampling unit was calculated as the number of all vascular plant species in the forest floor excluding those species that also occur in the tree or shrub layer, because the occurrence of many of these species depends on episodic fructification events. We tested the significance of the species number change between the first and the last record in each study plot with a paired Wilcoxon rank sum test. Overall changes in the species numbers and their relation with $ExCL_{emp}N$ were tested with a Linear Mixed Model (LMM), where the study plots were nested within study sites. We further tested if extant, newly occurring (colonizers) and extinct species of the study plots differed with regard to their respective Ellenberg values. The groups were compared with LMM where the median Ellenberg values of each study plot were nested in the study sites. The significance of the fixed effects of all LMMs was tested with a Likelihood ratio t-test statistic.

For the beta diversity we applied the Sørensen index, which is a monotonic transformation of gamma/alpha diversity (Jost, 2007). The differences in the index between the first and the last year of observation of each sampling unit were tested with a t-test after 100 iterations of resampling of 50% of all sampling units [R-package *betapart* (Baselga & Orme, 2012)]. The

relationship between the changes in the Sørensen index and $ExCL_{emp}N$ was tested with an LMM as described above.

## Results

### Overall trends of species cover

A total of 646 vascular plant species of the forest floor vegetation were studied. We restricted the analyses to species occurring in at least five sites, resulting in 486 species. When looking at general trends that occurred across all study sites where the respective species occurs (i.e. the fixed effect of the GLMMs that account for the mean trend), 36% of the 486 species showed a significant temporal change in cover ($P < 0.05$). Among those, 117 species decreased and 60 species increased in cover. The significant fixed effect coefficients (log transformed in order to achieve normality) were negatively correlated with the observation span (Pearson coefficient of $-0.30$, $P < 0.001$). This means that the longer the time trend, the lower the strength of the cover changes. The comparison of the change coefficients with Ellenberg indicator values did not reveal significant relationships (Fig. 2). However, species that prefer
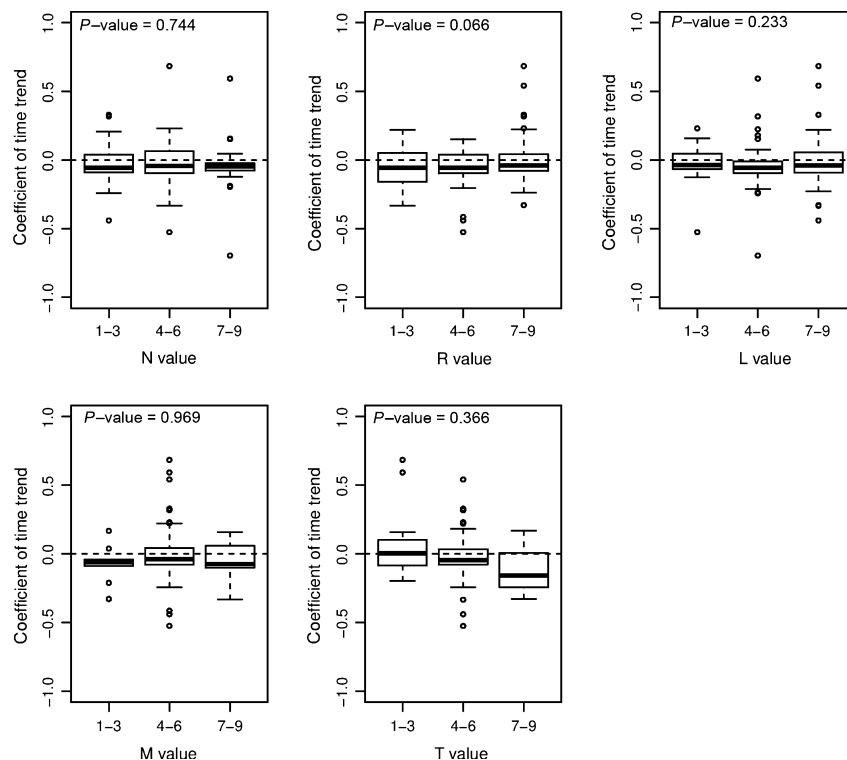


**Fig. 2** Relation between overall change of the forest floor plant species cover and Ellenberg indicator values for soil nutrients (N), soil reaction (R), light (L), moisture (M), and temperature (T). Species cover changes are shown as fixed effect coefficients with $P < 0.05$ derived from a Generalized Linear Mixed Model (positive values represent increasing cover, negative decreasing cover). All species with fewer than five observations were disregarded as well as those with indifferent or missing Ellenberg values resulting in 486 species. The differences of the group means were tested with an ANOVA *F*-Test (*P*-values are given in the upper-left corner).

base-rich soils showed a marginally less negative trend than species that prefer intermediate soil acidity ($P = 0.052$ with a Tukey multiple comparison test of the group means of an ANOVA, $P = 0.066$).

### Site-specific trends of species cover

The trend coefficient (random effect) of 170 species was significantly different between study sites (random effect AIC > 10, SOM Table 1). Contrary to the general trends, the site-specific changes of species cover showed a number of relationships with Ellenberg indicator values. First, the weighted change coefficient ($c_w$) of oligotrophic species (Ellenberg N values 1–3) was significantly negatively related to $ExCL_{emp}N$ ($R^2 = 0.32$, $P = 0.002$). Hence, most plant species that have their optimum under N limited conditions lost more cover in study sites with a higher $ExCL_{emp}N$ (Fig. 3). Only a

few oligotrophic species had no or an opposed relationship (SOM Fig. 1). Eutrophic plant species (N value 7–9) showed no relationship with $ExCL_{emp}N$ ($R^2 = 0.04$, $P = 0.440$). Secondly, acidophilous species decreased at sites with high $ExCL_{emp}N$ ($R^2 = 0.37$, $P = 0.001$). Species that prefer base-rich sites did not show any trend ($P = 0.916$). The third significant negative relationship with $ExCL_{emp}N$ was found for species that occur at dry sites ($R^2 = 0.37$, $P = 0.046$). Shade-tolerant species (Ellenberg L values 1–3) showed a marginally significant increasing trend with $ExCL_{emp}N$ ($R^2 = 0.13$, $P = 0.083$), and species preferring high solar radiation (L value 7–9) a marginally significant decreasing trend ($R^2 = 0.11$, $P = 0.097$). No relationship was found between Ellenberg temperature value and $ExCL_{emp}N$ ($P > 0.69$) (Fig. 3).

A number of trends in $c_w$, which help to detect confounding effects of climate change were analysed: the



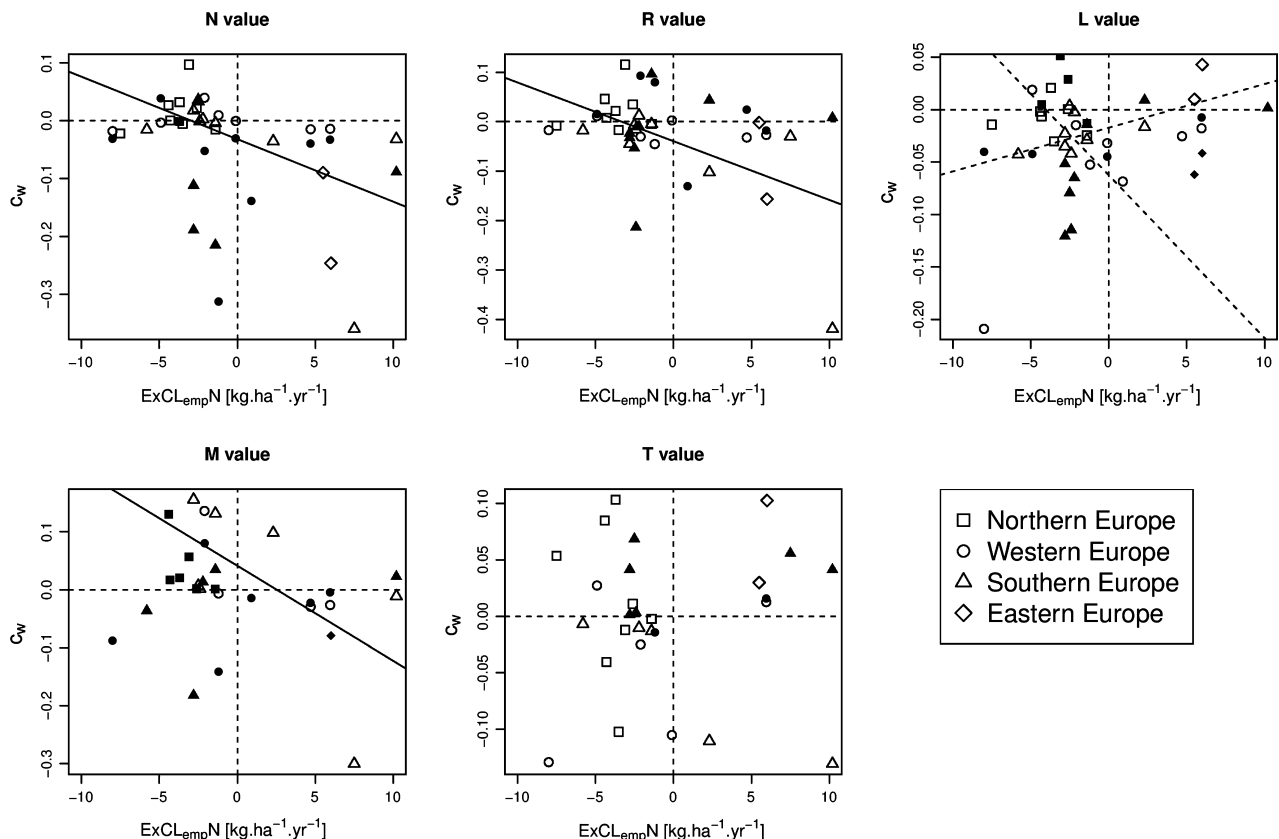**Fig. 3** Relationships between weighted averaged changes ($c_w$) of the cover of forest floor plant species groups and CL exceedance ($ExCL_{emp}N$). Species were grouped according to Ellenberg indicator values for nutrients (N), soil acidity (R), light (L), moisture (M) and temperature (T) (1–3: open symbols, 7–9: filled symbols). $ExCL_{emp}N$ is the difference between the canopy throughfall N deposition and the minimum empirical CL for nitrogen (negative values indicate no exceedance). Positive $c_w$ indicates increasing cover of species group, negative $c_w$ indicates decreasing cover (see Eqn 1). Significant linear regressions ($P < 0.05$, t-test) were found for oligotrophic species (N value 1–3), acidophilous species (R value 1–3) and species preferring dry sites (M value 1–3). Marginally significant linear regression ($P < 0.1$, t-test) was found for both light-demanding (L value 7–9) and shade-tolerant species (L value 1–3). The location of the sites in different parts of Europe is indicated by different symbols (see also Fig. 1).

cover of species preferring wet conditions increased in Northern Europe (mean $c_w$ = 0.12, $P$ = 0.016, two-sided Wilcoxon test). The cover of these species did not decrease in Southern Europe (mean $c_w$ = −0.493, $P$ = 0.219, two-sided Wilcoxon test). In Southern Europe, the cover of thermophilic species increased (mean $c_w$ = −0.091, $P$ = 0.008, two-sided Wilcoxon test), and the cover of cold-resistant plant species decreased, but only with marginal significance (mean $c_w$ = −0.054, $P$ = 0.063, two-sided Wilcoxon test) (see Fig. 3).

The relationships between $c_w$ and measured N deposition, as well as $c_w$ and $ExCL_{emp}N$, which is calculated with modelled N deposition, are all insignificant with only one exception. The $c_w$ of acidophilous species are significantly negative related with these indicators ($P$ < 0.002).

As for the overall temporal changes (the fixed effect coefficients), the site-specific trend (log transformed in order to achieve normality) was negatively correlated with the observation period length (Pearson coefficient of −0.1, $P$ < 0.003). This, again, means that the longer the time trend, the lower the strength of the temporal changes in cover.

### Diversity change

The total number of forest floor plant species per study site ranged between 8 and 227 (median = 38). The number of species increased at 10 study sites and decreased at 11 study sites, whereas 7 study sites experienced either no change in species diversity or the respective study plots showed both increases and decreases. The total number of forest floor plant species per study plot ranged between 2 and 60 (median = 14). A share of 14 (nine with a decrease) of the total of 41 study plots experienced a significant change in species number when tested with a paired Wilcoxon rank sum test ($P$ < 0.05). The change of the number of species across all study plots of the study areas was not significant (LMM fixed effect $P$ = 0.167). Three catchment sites (two with a decrease) of the total of five showed a significant change in species number. The observation period length of the study plots was positively correlated with species number change (Pearson $r$ = 0.52 for significant changes only).

The site-specific trends in species numbers, as derived from LMM, were not related to $ExCL_{emp}N$ ($R^2$ = −0.04, $P$ = 0.872). The plant species that occurred newly at the study plots had a significantly higher Ellenberg N value (median = 4) than those which became extinct or remained (median = 3) during the entire observation period ($P$ = 0.091). Species that remained had a significantly lower R value (median = 2) than the species that were lost or colonized (median = 4.5,

**Table 2** Comparison of Ellenberg indicator values for soil nutrients (N), soil acidity (R), light (L), moisture (M) and temperature (T) of newly occurring (colonized), lost and extant species in 41 study plots of the study sites. Significance of the difference between groups was tested with a Linear Mixed Model and a Likelihood ratio test (LR). Effects with a minimum $P$ < 0.1 are shown in bold

Median indicator value

| Ellenberg indicator | Colonized species | Lost species | Extant species | LR p |
|---|---|---|---|---|
| **N** | **4** | **3** | **3** | **0.091** |
| **R** | **4.5** | **4.5** | **2** | **0.049** |
| L | 5.5 | 5 | 5 | 0.103 |
| M | 5 | 5 | 5 | 0.778 |
| T | 4.75 | 5 | 5 | 0.390 |

$P$ = 0.049). No other Ellenberg values differed significantly (Table 2).

Apart from changes in species number, we analysed beta diversity, that is, the homogeneity of the forest floor vegetation. The mean Sørensen index of the study plots ranged between 0.241 and 0.906 (median = 0.586). The difference of the index between the last and the first observation year ranged between −0.235 and +0.272 (median = 0.009). We found a significant change in the beta diversity of the forest floor vegetation in 34 of 41 study plots ($P$ < 0.05, $t$-test). Significant homogenization occurred in 17 study plots. Among the five catchment scale samples, significant homogenization was detected in two study sites. Homogenization did not correlate with $ExCL_{emp}N$ ($R^2$ < 0.3, $P$ > 0.7).

### Discussion

We found no changes in the species composition of the forest floor vegetation that occurred across all sites in our European-wide data set, a result that corroborates other studies (De Schrijver *et al.*, 2011). This could be anticipated as we compare contrasting forest types that differ in their exposure and their sensitivity to airborne pollution. Our data set includes European-wide forest types that have a minimum empirical CL for N between 3 and 15 kg N ha$^{-1}$ yr$^{-1}$ (Bobbink & Hettelingh, 2011). With a canopy throughfall deposition of 0.6 to 20.2 kg N ha$^{-1}$ yr$^{-1}$, we are able to compare sites that experience chronic N deposition with those that do not. Thus, when we focused on CL exceedances and site-specific cover trends of individual species, trends became apparent and we were able to reveal a number of significant relationships. The cover of oligotrophic species decreased the higher the N deposition exceeded

the empirical CL. Although species which prefer nutrient-rich sites did not experience a significantly increasing cover, they had a higher proportion among newly occurring species than oligotrophic species. This is, to our knowledge, the first detection of an N deposition effect on the temporal trends in forest floor vegetation in a European-wide long-term monitoring data set. So far, the only broad-scale resurvey study that found a eutrophication signal in the temporal trends of forest floor vegetation showed that the underlying cause was a change in tree species composition and shifts towards a denser canopy cover rather than N deposition (Verheyen *et al.*, 2012). Elevated N availability for plants resulted from the increase of the proportions of trees with easily decomposable litter. The "N deposition signal" has been detected before in semi-natural grassland where N causes diversity loss across Europe (Dupré *et al.*, 2010; Stevens *et al.*, 2010). Here, we show that also in European forests oligotrophic species are decreasing in cover, although a decline in the species richness is not yet detectable.

Although we were able to show that oligotrophic species decreased disproportionately in sites with chronic N deposition, not all of these species decreased and forest floor vegetation did not change accordingly in all of these sites (SOM Fig. 1). Several additional factors combined with the specific life history strategies of the involved species also influenced vegetation change (Van Dobben & De Vries, 2010). The changes in species composition showed that oligotrophic species that decreased in cover mainly prefer dry and acidic soils. This could be expected as most oligotrophic forest species prefer acidic soils (Diekmann, 2003) and reach their optimum under dry conditions (Cornwell & Grubb, 2003). Owing to this dependency, we cannot judge how strong the role of soil recovery from acidification, which has occurred in many parts of Europe in recent decades (Kirk *et al.*, 2010; Jandl *et al.*, 2012; Akselsson *et al.*, 2013), was in increasing the eutrophication effect on acidophytic plant species. Species that tolerate dry conditions (Ellenberg value < 4) are seldom common species in European forests. Their competitive ability is limited in many forest types, rendering them susceptible to local extinction when eutrophic species increase their growth due to higher N availability (Bobbink *et al.*, 1998; Suding *et al.*, 2005). Furthermore, the sensitivity of plant species to drought stress can be elevated when N deposition is high (Dise, 2011). During the observation period, drought stress has occurred in Southern Europe owing to generally decreasing precipitation. In contrast, Northern Europe has experienced a precipitation increase (EEA, 2012a). Though we found no cover decrease of species preferring wet conditions in Southern Europe the cover of such species increased

in Northern Europe (see Fig. 3). Additionally, in Southern Europe, where climate warming was particularly strong (EEA, 2012a), the cover of thermophilic species increased whereas the cover of cold-resistant plant species decreased (Fig. 3). However, in general we think that our results should not be over interpreted with regard to climate effects owing to the small sample size and the response lag to temperature increases that was found in European forests (Bertrand *et al.*, 2011).

The pivotal role of light for changes in the forest floor vegetation often hampers the detection of other effects such as N deposition. A recent study of European broad-leaf forests showed that in recent decades, shade-tolerant species gained cover at the expense of light-demanding species (Verheyen *et al.*, 2012). They convincingly argue that the increase in wood biomass in European forests (Spiecker, 1999; Luyssaert *et al.*, 2010) caused this shading effect. We show, though only with marginal significance, that the cover of shade-tolerant species increased, whereas light-demanding species decreased the more the N deposition exceeded the CL. Apparently, the shading effect on plant species of the forest floor was more severe where N did not limit tree growth. Nevertheless, since we restricted our analyses to plots that experienced relatively weak change of the tree layer the confounding effect of light changes was obviously not very strong.

Although the changes in species cover in the forest floor vegetation were clearly related to CL exceedance, changes in diversity were not. The loss in species richness was not higher in forests that were exposed to a higher exceedance of the empirical CL. Accordingly, beta diversity did not decrease in these sites. When comparing the Ellenberg values of the species that remained with those that became extinct or colonized, we found that most of the species turnover (losses and gains) is ascribed to plant species that prefer well-buffered soils (high Ellenberg R values). Extant species prefer acid soils. We hypothesize that this is due to the generally higher species number and thus higher potential for turnover at sites with alkaline bedrock.

The homogenization hypothesis (Gilliam, 2006) states that in areas with high N deposition, the availability of N for plants is more homogeneously distributed in space, decreasing diversity in the long run. Homogenization of the forest floor vegetation was found in approximately half the sites but no relation with the exceedance of the CL was found. We assume that the observation period at most sites might have been too short to detect a possible eutrophication signal in the form of homogenization. At a much larger time scale of 70 years, homogenization of woodland plant communities in the United Kingdom was detected and related to eutrophication (Keith *et al.*, 2009). Nevertheless,

diversity loss due to soil N homogenizations might not occur in all forest types because species richness often peaks at intermediate ecosystem productivity. As an example, it has been shown that species richness cumulated at high nutrient availability in a deciduous forest in Germany due to a higher degree of niche differentiation (Bernhardt-Römermann *et al.*, 2010).

The length of the observation period correlated negatively with the trends in species cover, for both mean changes and site-specific trends. This is interesting because intuitively one would expect a larger change during a longer time period. With regard to air pollution, an explanation could be that in the sampling units where very long time series exist, changes occurred mostly for species that were already rare and in the first years (Nordin *et al.*, 2005; Gilliam, 2006). Changes in species richness are positively correlated with observation length, showing that species extinction and colonization are longer term processes than cover changes (Bowman *et al.*, 2006; Clark *et al.*, 2013). The highest variation of changes of species cover, that is, the site-specific trend coefficients, occurred at sites that have an intermediate CL exceedance. This result supports the finding of studies dealing with European grasslands, where the major changes occurred where N deposition was high but not excessively so (Stevens *et al.*, 2010). In regions where airborne N is chronically and highly excessive, vegetation may yet have changed to a degree that only minor changes are to be expected in future. However, past and future changes strongly depend on the forest management regime (Bernhardt-Römermann *et al.*, 2007).

We show that existing empirical CL are useful indicators of the sensitivity of forest floor vegetation to N loads. Similar conclusions were recently drawn with regard to nitrate leaching from soils (Holmberg *et al.*, 2013). It is recognized, however, that the empirical CL are a coarse measure of the sensitivity of ecosystems to nitrogen. The empirical CL are given for the comparatively broad EUNIS classes, which may contain several habitats and species (Bobbink & Hettelingh, 2011; Clark *et al.*, 2013). Furthermore, the lower limit of the CL has the same value for several EUNIS classes. A large effort has been made to improve these values but their reliability as absolute thresholds for deteriorating effects is still unsatisfactory (Bobbink & Hettelingh, 2011; Pardo *et al.*, 2011a; Payne *et al.*, 2013). To calculate the exceedance of the CL, canopy throughfall was used as a measure of N deposition reaching the forest floor. We use the difference between the canopy throughfall deposition and the empirical CL (minimum of the range) as an indicator of N effects. The respective zero value is not to be considered as an absolute threshold for N exceedance but as a relative value along the entire gradient from no exceedance to high exceedance. This is because canopy throughfall, which is available for most forest ecosystem studies, underestimates total deposition due to stemflow and direct uptake of N in the canopy (Butterbach-Bahl & Gundersen, 2011).

As an alternative to observed deposition, we also calculated CL exceedances with modelled deposition values. European models of N deposition that take dry and wet deposition into account predict values between 0.9 and 30.8 kg N ha$^{-1}$ yr$^{-1}$ for the studied sites (Table 1) and accordingly higher exceedances than we defined with canopy throughfall deposition (by approximately 30%). These deposition models are used in policy support together with the location of habitats in or out of protected areas to show the distribution of threats to biodiversity and the potential for improvement with emission reduction measures (De Vries *et al.*, 2010; Pardo *et al.*, 2011a; EEA, 2012b). We found that when applying this data, the eutrophication signal in forest floor vegetation, which we found by using the measured N deposition, cannot be detected due to the additional model error variation. In our data set, the relationship between modelled and measured N deposition has an $R^2 = 0.63$. Large scale deposition models are less accurate in topographically complex areas (Weathers *et al.*, 2000) and generally underestimate the impact of local sources (industry, agriculture). The total deposition to forest ecosystems is additionally affected by the structure and tree species composition (De Schrijver *et al.*, 2007). It is thus of utmost importance to measure N deposition together with biological either responses at concrete sites or to appropriately downscale these models for regional assessments (Van Dobben & De Vries, 2010).

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Results of Generalized Linear Mixed Model analysis for forest floor plant species. Only those species are listed that showed a different temporal trend of their cover in the study sites (random effect AIC > 10). The total change of a species at a study site is given as the site-specific effect (the sum of the fixed and the respective random effect). Nitrogen Critical Load exceedance (ExCL$_{emp}$N) and Ellenberg indicator values for nutrients (N), soil acidity (R), light (L), moisture (M) and temperature (T) are shown (0 indicates indifferent species). Species with low (1–3) and high indicator values (7–9) were grouped. As an example, species with low Ellenberg values 1–3 are bound to nutrient-poor sites (oligotrophic species); species with N values 7–9 prefer nutrient-rich sites (eutrophic species). ExCL$_{emp}$N is the difference between the canopy throughfall N deposition and the minimum empirical CL for nitrogen (negative values indicate no exceedance).

**Figure S1.** Relationships between the site-specific trend of the cover of oligotrophic plant species and the CL exceedance (ExCL$_{emp}$N). All plant species with an Ellenberg N value < 4 are shown that occurred in more than five study sites and had a significant random effect (AIC > 10). ExCL$_{emp}$N is the difference between the canopy throughfall N deposition and the minimum empirical CL for nitrogen (negative values indicate no exceedance). The site-specific trend of plant species cover was derived from a GLMM (the sum of fixed and random effect). $R^2$ of a least square regression and the *P*-value of the slope (*t*-test statistic) are given in the upper right corner of the plot (see SOM Table 1 for full species names).