


RESEARCH ARTICLE

Atmospheric nitrogen deposition is related to plant biodiversity loss at multiple spatial scales

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Abstract

Due to various human activities, including intensive agriculture, traffic, and the burning of fossil fuels, in many parts of the world, current levels of reactive nitrogen emissions strongly exceed pre-industrial levels. Previous studies have shown that the atmospheric deposition of these excess nitrogen compounds onto semi-natural terrestrial environments has negative consequences for plant diversity. However, these previous studies mostly investigated biodiversity loss at local spatial scales, that is, at the scales of plots of typically a few square meters. Whether increased atmospheric nitrogen deposition also affects plant diversity at larger spatial scales remains unknown. Here, using grassland plant community data collected in 765 plots, across 153 different sites and 9 countries in northwestern Europe, we investigate whether relationships between atmospheric nitrogen deposition and plant biodiversity are scale-dependent. We found that high levels of atmospheric nitrogen deposition were associated with low levels of plant species richness at the plot scale but also at the scale of sites and regions. The presence of 39% of plant species was negatively associated with increasing levels of nitrogen deposition at large (site) scales, while only 1.5% of the species became more common with increasing nitrogen deposition, indicating that large-scale biodiversity changes were mostly driven by “loser” species, while “winner” species profiting from high N deposition were rare. Some of the “loser” species whose site presence was negatively associated with atmospheric nitrogen deposition are listed as “threatened” in at least some EU member states, suggesting that nitrogen deposition may be a key contributor to their threat status. Hence, reductions in reactive nitrogen emissions will likely benefit plant diversity not only at local but also at larger spatial scales.

KEYWORDS

acid grasslands, acidification, biodiversity, biotic homogenization, eutrophication, gamma diversity, landscape scale, nitrogen deposition, plant biodiversity, spatial scale

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1 | INTRODUCTION

Various human activities, including intensive agriculture, and the burning of fossil fuels by industry and traffic, have caused increased levels of reactive nitrogen (N) in the atmosphere, in the form of nitrogen oxides (NO_x) and ammonium aerosols (NH_x) (Duncan et al., 2016; Galloway et al., 2008). As a result, at a global scale, atmospheric deposition levels of N are currently around twice as high as a century ago (Galloway et al., 2004; Kanakidou et al., 2016). These elevated atmospheric N deposition levels are often considered a main environmental problem, as high N deposition is associated with decreased plant diversity (e.g., Bobbink et al., 1998; Bobbink et al., 2010; Maskell et al., 2010; Stevens et al., 2004).

Negative relationships between atmospheric N deposition and plant species richness have been widely observed, particularly in open, temperate ecosystems, including acid grasslands (e.g., Stevens et al., 2004; Stevens, Duprè, et al., 2010; Stevens, Thompson, et al., 2010) and heathlands (Southon et al., 2013), as well as various other ecosystems (Bobbink et al., 2010, 2022). Such negative relationships observed in nature are supported by experimental studies, where N additions in controlled settings have been observed to reduce plant species richness (Midolo et al., 2019). These negative effects are caused by various mechanisms (Stevens et al., 2018), particularly due to competitive exclusion following changes in resource ratios or reduced light availability (Eskelinen et al., 2022; Harpole et al., 2016; Hautier et al., 2009) but also due to soil acidification (Crawley et al., 2005). However, negative associations between plant species richness and N deposition levels have mostly been observed at “plot” scales of typically a few square meters, and less is known about the extent to which effects of N deposition on biodiversity are also significant at larger spatial scales.

A common approach to studying biodiversity across spatial scales is by partitioning biodiversity indicators, such as species richness, at large spatial scales (γ -diversity hereafter), into α -diversity, for example, the average number of species within smaller sampling areas, and β -diversity, which measures the spatial turnover in species compositions across sampling areas (Whittaker, 1960; McGill et al., 2015). While α - and γ -diversity are relatively straightforward to quantify, there are numerous metrics to quantify β -diversity, with different metrics capturing different components of species compositions (Anderson et al., 2011). On the one hand, multiplicative β -diversity indices, quantified by dividing γ -diversity by α -diversity, mostly focus on the joint presence of species. On the other hand, additive β -diversity indices, quantified by subtracting α -diversity from γ -diversity, are additionally based on joint absences of species and, therefore, should be particularly sensitive to scenarios where global change drivers consistently cause the same species to go extinct in local communities (Anderson et al., 2011). Hence, complementing the study of relationships between atmospheric N deposition and α -scale plant biodiversity (as done previously by, for example, Stevens et al., 2004; Bobbink et al., 2010; Maskell et al., 2010, typically at the scale of plots of a few square meters), with the joint study of so far underexplored relationships with plant β - and γ -diversity (at

site and regional scales) would inform about the scale at which plant biodiversity is most affected by atmospheric N deposition, as well as inform about potential processes causing biodiversity change.

We envision multiple alternative scenarios regarding how atmospheric N deposition alters biodiversity across spatial scales, including at the smallest scales of plots (of a few square meters), intermediate scales of sites (consisting of multiple plots within a 1-ha area), or regions (consisting of multiple sites dozens or hundreds of kilometers apart). First, losses in species richness at local spatial scales (α -diversity loss) could be dampened at larger scales (the γ -scale), if local biodiversity declines are moderate and random (Chase et al., 2019). In those cases, local losses of species following increased atmospheric N deposition would not reduce species richness at the γ -scale, as long as species locally lost at one site remain present elsewhere (Figure 1a,b). Furthermore, random species losses will reduce the similarity in species composition of local communities, thus resulting in increased β -diversity, irrespective of the type (additive versus multiplicative) of β -diversity (Püttker et al., 2014; Figure 1a,b). Alternatively, it is also possible that species richness loss is even stronger at the γ -scale than at the α -scale. For example, when at local scales, increased N availability causes some competitive species to become increasingly common and outcompete other species, it might be that at the local scale, the net effect on species richness is moderate, as species losses are partly compensated by the gains of competitive species. However, if both the “winners” and “losers” of increased N deposition are consistently the same species across local sites, then this causes homogenization in species composition across space, so that regional species losses are likely to be as strong, or even stronger, than local losses (Chase et al., 2019) (Figure 1a,c,d). In this scenario, both multiplicative- and additive-based metrics of β -diversity should decrease with increased N deposition (Figure 1c). In contrast, it might be that the “winners” of N deposition are not consistently the same species, but that the “losers,” that is, the species disappearing following increased N deposition, are consistently the same species. This might for example happen if consistently the same species disappear under high N deposition due to their low tolerance of soil acidification following eutrophication. In that case, additive, but not multiplicative, β -diversity should decrease with N deposition (Figure 1d).

Given the mechanisms by which N fertilization drives species loss at local spatial scales, which include non-random extinctions driven by, for example, competitive exclusion following eutrophication, as well as non-random extinctions driven by, for example, soil acidification (Bobbink et al., 2010; Stevens et al., 2018; Suding et al., 2005), it seems most likely that α -scale biodiversity loss following increased N deposition levels will eventually (possibly with some delay) also cause larger-scale (γ -scale) species loss. In line with this, Roth et al. (2015) observed a negative association between levels of atmospheric N deposition and plant richness at plots with a grain size of one square kilometer, and Seabloom et al. (2021) found proportionally similar reductions of smaller- and larger-scale plant biodiversity in response to nutrient addition. On the other hand, Midolo et al. (2019) found that experimental additions of N

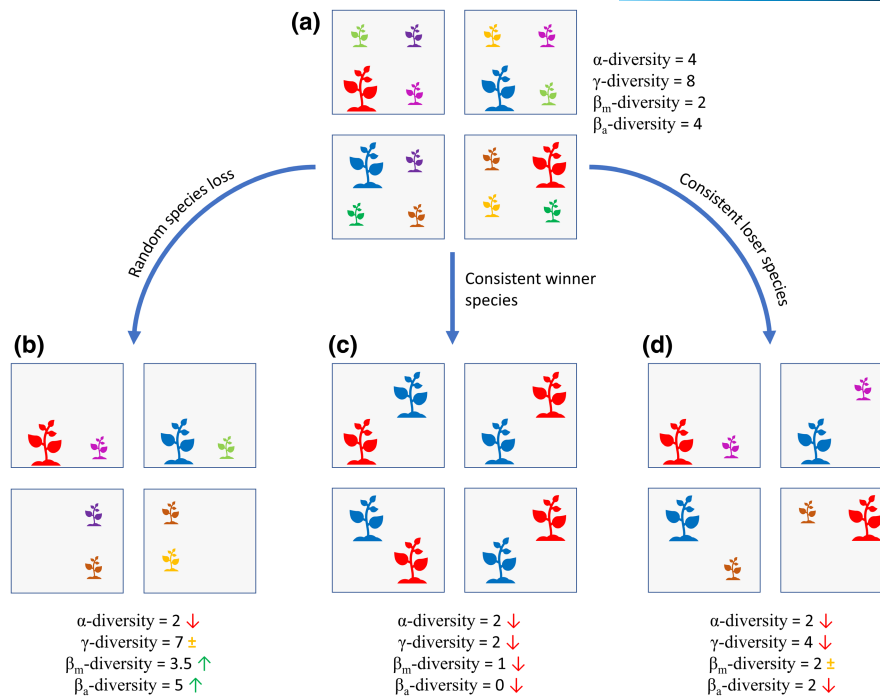


FIGURE 1 Hypothetical effects of N deposition on α -, β - and γ -diversity. Here, α -diversity represents the plot scale, while γ -diversity represents the site scale. An example of a starting situation (before increased levels of N deposition) of biodiversity is shown (a), as well as three alternative scenarios of biodiversity loss after increased levels of N deposition (b–d). (b) When N deposition causes random species losses, β -diversity is expected to increase, and γ -diversity loss is weak. In contrast (c), when consistently the same, fast-growing species profit from N fertilization, at the cost of other species, it is expected to lead to a decrease in both multiplicative (β_m) and additive (β_a) measures of β -diversity, as well as a decrease in γ -diversity. Last, (d) when there is consistency in the “losers” of increased N deposition, but when it is not consistently the same species that are “winners,” then N deposition is expected to lead to a decrease in additive measures of β -diversity, as well as a decrease in γ -diversity, while it would not alter multiplicative measures of β -diversity. For all scenarios, we expect effects at the site scale (here: γ -diversity) to be exacerbated at even larger (e.g., regional) spatial scales.

led to reductions in plant richness in small (1 m^2) plots, but not at slightly larger ($> \pm 10\text{ m}^2$) scales, although it is not known if these results would remain if the experiments had run longer, for example, >10 years). Effects of experimentally induced additions of N on grassland β -diversity also tend to be mixed, with some studies reporting decreases in measures of within-treatment plant β -diversity (Yang et al., 2022; Zhang et al., 2019), while other studies observed neutral effects (Chen et al., 2022; Hautier et al., 2020) or even increases (Harvey & MacDougall, 2018). However, it is important to note that the studies mentioned above mostly simulate in-site fertilization, as often applied in agricultural areas. Levels of atmospheric N deposition in natural areas are often significantly lower (e.g., in Europe mostly ranging from <2 to $40\text{ kg N ha}^{-1}\text{ year}^{-1}$) than fertilization levels on agricultural sites (on average around $60\text{ kg N ha}^{-1}\text{ year}^{-1}$ within the EU; Eurostat, 2015), even if these levels are still substantially higher than background N deposition rates in the absence of human activities. As such, one might expect that the effects of atmospheric N deposition on plant β -diversity might be weaker than the effects observed in fertilization experiments. On the other hand, most N fertilization experiments are relatively short-lived (ca 5–20 years), while “real world” human-caused increases in N deposition are often ongoing for several decades or more (Galloway et al., 2004), so their effects on plant β -diversity could be stronger than in controlled

experiments. In addition, most of the above studies only looked at a restricted range of scales of biodiversity, or only at single (either additive or multiplicative) measures of β -diversity.

In this study, we investigated how levels of atmospheric N deposition are related to plant biodiversity across multiple spatial scales (individual plots of $2 \times 2\text{ m}$, multiple plots with a 1-ha site, and regions that include multiple sites dozens or hundreds of kilometers apart), in semi-natural acidic grasslands of the *Violion caninae* alliance (Schwickerath, 1944) across nine different countries in Northwestern Europe. Furthermore, we determined the extent to which (non-random) species extinctions, or species gains, explain the scale-dependent responses of plant biodiversity to increasing levels of atmospheric N deposition. We hypothesized that (1) at both plot, site- and regional scales, species richness would be negatively associated with increased levels of N deposition, but that (2) these relationships would be particularly strong at larger spatial scales due to a few competitively superior species outcompeting other species following increased N deposition, resulting in negative relationships of both additive and multiplicative β -diversity with levels of atmospheric N deposition (Figure 1B). Last (3), we expect that this would also be reflected by individual species responses to atmospheric N deposition levels, with both a high number of plant species showing positive relationships between their site-scale occurrence and

atmospheric N deposition levels, as well as many species showing negative relationships.

2 | MATERIALS AND METHODS

2.1 | Study design

We re-analyzed vegetation data studied before by Stevens, Duprè, et al. (2010); Stevens, Thompson, et al. (2010); Stevens et al. (2011). In short, 153 *Violion caninae* sites, spread across nine European countries from the Atlantic biogeographic zone (Figure 2), were surveyed between 2002 and 2007. The sites were selected in such a way that they spanned a gradient in atmospheric N deposition, from 2.4 to 43.5 kg N ha⁻¹ year⁻¹. Care was taken to span these gradients both within and across countries as much as possible. All of the selected grasslands were unfertilized and managed by grazing or cutting, and mostly in nature reserves. Within each site, five randomly located plots of 2 × 2 m were set up. For more details on the design, refer to Stevens, Duprè, et al. (2010); Stevens, Thompson, et al. (2010); Stevens et al. (2011).

2.2 | Site data collection

Within each plot, all vascular plant species and bryophytes were identified and their cover was estimated using the Domin scale (Rich et al., 2005). This was done in the growing season (between May and September), in 2002/2003 (in the 68 plots from Great Britain), and in 2007 (plots from other countries). In addition, to account for other environmental factors when studying relationships between N deposition and plant biodiversity, we also used data on several geological and soil-related factors. First, in each site, the altitude, slope (in °), and soil depth, as well as management intensity (on a scale of 1–3, based on visual assessments of signs of grazing and mowing activities) was described. Furthermore, in each plot, a topsoil

(0–10 cm depth below the litter layer) sample was collected from two opposing corners using a trowel, and these were combined into a single sample. These soil samples were kept cool during transit to the laboratory, where they were air-dried. After drying, the levels of multiple soil factors were measured as follows: see “chemical measurements” section. For more details on the site measurements, refer to Stevens, Duprè, et al. (2010); Stevens, Thompson, et al. (2010); Stevens et al. (2011).

2.3 | Chemical measurements

Soil pH was measured with a pH probe in a 1:5 soil and deionized water mixture. Soil nitrate, ammonium, calcium, and aluminum concentrations were measured using two different methods. The soil samples from Great Britain collected in 2002 and 2003 were extracted with 1 M KCl, and then analyzed using an ion chromatograph. The soil samples that were collected in 2007 were shaken with 0.4 M NaCl, and were analyzed using an auto-analyzer. All extracts were determined using a 1:10 soil and extractant mixture. Aluminum, manganese, and calcium concentrations were determined using an ICP-MS. Phosphorus availability was quantified using a standard Olsen extraction and colorimetric determination (MAFF, 1986). Finally, total soil C and N content were quantified using a CN elemental analyzer by combustion and gas detection. For more details on the site measurements, refer to Stevens, Duprè, et al. (2010); Stevens, Thompson, et al. (2010); Stevens et al. (2011).

2.4 | Climate and N deposition levels

Meteorological data from the European Space Agency Monitoring Agriculture with Remote Sensing (MARS) unit was obtained (Monitoring Agricultural Resources, 2009). Based on these data, ten-year averages for mean annual potential transpiration from crop canopy, mean minimum daily temperature, mean maximum

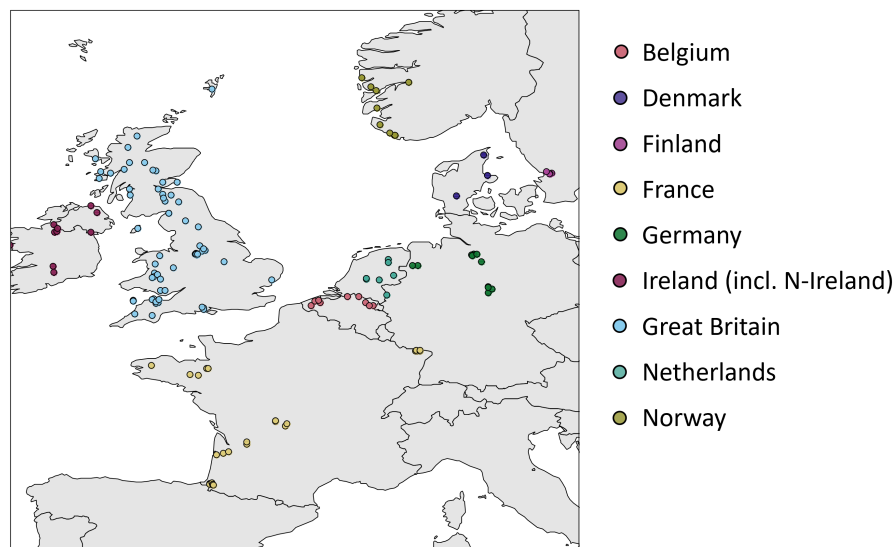


FIGURE 2 Map with locations of all studied sites. Different colors indicate sites from different countries. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

daily temperature and mean annual precipitation were quantified. The radiation index was calculated based on aspect, slope, and latitude following Oke (1987). Models were used to estimate total N, reduced N, oxidized N, and sulfur (SO_x) deposition for each site. For Germany (Gauger et al., 2002), the Netherlands (Asman & van Jaarsveld, 2002; Van Jaarsveld, 1995; Van Jaarsveld, 2004) and the United Kingdom (Smith et al., 2000; NEG-TAP, 2001) national models were used, whereas for the remaining countries, we used the European Monitoring and Evaluation Programme (EMEP)-based Integrated Deposition Model (IDEM) (Pieterse et al., 2007). For all of the models, three-year average deposition levels were calculated to provide an adequate estimate of longer-term N inputs. For more details, refer to Stevens, Duprè, et al. (2010); Stevens, Thompson, et al. (2010); Stevens et al. (2011).

2.5 | Data analysis

We first standardized all species names using the “Leipzig Catalogue of Vascular Plants” (Freiberg et al., 2020), and the “lcvp_search” function of the LCVP package (<https://github.com/ldiv-biodiversity/LCVP>) in R-4.2.1 (R Core Team, 2022). We then quantified plant α -, β - and γ -diversity at the plot-to-site scale. α -diversity was quantified by calculating the species richness within each plot, and then calculating the average species richness across all plots within a site. γ -diversity was quantified as the total number of species present among all plots within a site. Two metrics of β -diversity were quantified: (1) multiplicatively, as the γ -diversity divided by the α -diversity, whereby β -diversity can theoretically range between 1 (when γ -diversity does not exceed α -diversity) and 5 (when all five plots only contain unique species), and (2) following an additive approach, as the γ -diversity minus α -diversity.

We then quantified plant α -, β - and γ -diversity at the site-to-regional scale. To do so, we first divided all sites among 16 groups, separated by their range in atmospheric N deposition: $<6\text{kgNha}^{-1}\text{year}^{-1}$; from 6 up to but not including $8\text{kgNha}^{-1}\text{year}^{-1}$, and so on in 2kg-ranged groups up to $32\text{--}34\text{kgNha}^{-1}\text{year}^{-1}$, and then $>34\text{kgNha}^{-1}\text{year}^{-1}$. We chose these range levels, as (i) it allowed for having at least 3 sites within each level (a minimum number of sites is needed to quantify β - and γ -diversity), and (ii) because larger ranges (e.g., 5kg-ranged), would reduce the number of levels (in this case to 7 groups), compromising our statistical power. The groups contained between 3 and 22 different sites, and could include different countries.

We then calculated different biodiversity indicators for these groups of sites. Since γ -diversity, and as a consequence also β -diversity, increases with the number of sites included in the quantification, we standardized biodiversity quantification in such a way that it was always based on the same number of sites per group, namely on three sites (i.e., the minimum number of sites across all groups). This was done by selecting 100 times, for each group, three randomly assigned sites. For each random sample, we then quantified α -diversity as the average number of species per site. γ -diversity

was quantified as all species observed in at least one site within the group. Again, β -diversity was quantified in a multiplicative way, as the γ -diversity divided by the α -diversity, as well as in an additive way, as γ -diversity minus α -diversity. For each group of plots, we also calculated the average pairwise geographical distance between each combination of sites, as it is likely that this will influence levels of both β -diversity and γ -diversity (Keil et al., 2012). We then calculated average (across the 100 randomizations) α -, β - and γ -diversity values for each group of sites, as well as average values of geographical distance, and all other covariates. All biodiversity quantifications were done using the vegan package (Oksanen et al., 2022) using R-4.2.1 (R Core Team, 2022).

We used general linear models to analyze how, at the plot-to-site scale, α -, β - and γ -diversity were related to atmospheric N deposition. To do this, we ran general linear models, with either plot-to-site-based α -, β - and γ -diversity as the response variable, and the independent predictor variables were total atmospheric N deposition, as well as various covariates related to climate (mean annual temperature, mean annual precipitation, radiation index), geology (slope, altitude), soil conditions (soil depth, soil pH, soil aluminum, calcium, magnesium, manganese, nitrate, ammonium, phosphorus, total N and carbon concentrations, soil C:N ratio), SO_x deposition, and management (management intensity, mowing activities). We used a backward model selection procedure, whereby at each step, a non-significant ($p > .05$) predictor variable was removed until we ended up with a final model only containing significant variables. Given the focus on the effects of atmospheric N deposition in this study, we re-included N deposition to final models where it was otherwise absent due to non-significance: This was done for the model explaining multiplicative β -diversity. Although results are qualitatively the same, for completeness we also show the equivalent models without N deposition in Table S1. We compared these linear final models with equivalent linear mixed models, that had the same response variables and fixed factors, but that additionally included “country” as a random factor, to correct for regional differences in biodiversity indicators. Model comparisons indicated that those models without country included as a random factor always had the lowest Akaike Information Criterion (AIC) values, so these were used in further analyses. After having selected linear final models, we also ran, for each biodiversity metric, an equivalent model where non-linear effects on total N deposition were tested, as a negative exponential fit is often as good, or better, a predictor of plant diversity as a linear fit (e.g., Stevens, Duprè, et al., 2010; Stevens, Thompson, et al., 2010). Using the “nls” function in R-4.2.1 (R Core Team, 2022), we assessed 5 non-linear models. First we ran a logistic, sigmoid-shaped relationship between N deposition and biodiversity ($BD = c + \frac{d-c}{1 + \exp(-b(N-e))}$), where BD is the biodiversity indicator, c is a lower asymptote, d is a higher asymptote, b the slope at the inflection point where BD decreases/increases with atmospheric N, and e the N deposition (N) value where BD is halfway between c and d). Second, we ran a log-logistic, also sigmoid-shaped relationship, following $BD = c + \frac{d-c}{1 + \exp(-b(\log(N) - \log(e)))}$, with the same parameters as in the logistic model. Third, we ran an (also sigmoid-shaped, but a-symmetrical) Weibull function (type 1)

as: $BD = c + (d - c)\exp\{-\exp[-b(\log(N) - \log(e))]\}$. Here, the parameters have the same interpretation as above, except that e in this case is the abscissa of the inflection point. Fourth, we ran an (also sigmoid-shaped and asymmetric) Weibull function (type 2) as: $BD = c + (d - c)\{1 - \exp\{-\exp[b(\log(N) - \log(e))]\}\}$, with the same parameters as in the type 1 Weibull function. Last, we tested for an exponential biodiversity-N deposition relationship, modelled as: $BD = a(1 - k)^N$, where a is the BD values when $N=0$, and is the fraction of BD that is gained/lost per unit increase in N . In all these models, we also included, besides non-linear effects of N deposition, also linear effects of those covariates that were present in the final (i.e., after model selection), linear models. For an overview and justification of the different non-linear models we tested, we refer to the [Methods](#). We used AIC to assess which of these models representing different (non-)linear biodiversity-N deposition relationships were best fitting. Model assumptions (normality of residuals, homogeneity of variance) were assessed for final models, and always met.

Next, we analyzed how at the site-to-regional scale, α -, β - and γ -diversity were related to atmospheric N deposition. To do this, we ran general linear models, with either group-based α -, β - and γ -diversity values (averaged across 100 random draws) as the response variable. As at the site-to-regional scale, we had only 16 replicates, we could not include all factors as predictors in the models (to avoid depleting degrees of freedom). Therefore, we only included those factors that had significant effects on at least one of the plot-to-site-scale biodiversity indicators: atmospheric N deposition, as well as mean (across sites) values of slope, soil pH, soil Ca and Mg content. In addition, we included the mean pairwise spatial distance between sites as a covariate, as one would expect higher levels of β - and γ -diversity when different sites are further apart (Keil et al., 2012). Selection of final models, as well as comparing the fit of linear models with non-linear equivalents was done in the same way as for models for plot-to-site-based measures of biodiversity. All final models met the assumptions of normality and homogeneity of variance.

We also analyzed the responses (regarding the likelihood of presence at a site) of individual plant species to atmospheric N deposition. This was done for those 66 species (see [Table S3](#)) that were present in at least 15 sites. For each species, we modeled their response to atmospheric N deposition, as well as to the effects of those four environmental predictors that our biodiversity-related models identified as important in driving at least some biodiversity indicators: soil pH, soil Ca and Mg content, and slope. This analysis was done using logistic regression models, using the “glm” function in R-4.2.1 (R Core Team, 2022). We performed a backward model selection procedure, where non-significant ($p > 0.05$, Z-tests) covariates (other than atmospheric N deposition) were removed, till we ended up with a model including only significant predictors. We repeated the above models at the plot scale, where in addition, site was added as a random factor, using the “glmer” function of the “lme4” package (Bates et al., 2015) in R-4.2.1 (R Core Team, 2022).

As our measures of γ -diversity were based on five plots (at the site scale), or three sites (at the regional scale), it is possible that we underestimated diversity, in the sense that some species

were not observed at the site or regional scale, even if they were actually present. To address this issue, we also estimated “true” γ -diversity levels (i.e., the asymptote of observed richness-sample relationships), by extrapolating species richness levels based on the incidence-based frequency (i.e., number of plots [within a site] or sites [within a region]) of species, following Chao et al., 2014. We used the “ChaoRichness” function of the iNEXT package (Hsieh et al., 2016). Based on these “true” γ -diversity levels, we also quantified “true” multiplicative and additive β -diversity levels at both the plot-to-site and site-to-regional scale. After this, we repeated the main analyses on drivers of γ - and β -diversity described above, substituting observed diversity levels by the estimated true levels, to investigate whether our main results were sensitive to possible effects of undersampling. We performed these sensitivity analyses only for linear diversity-deposition relationships, as the main results showed that non-linear relationships were never parsimonious ([Table S2](#)).

3 | RESULTS

3.1 | Relationships between N deposition and plant biodiversity at the plot-to-site scale

We found that at the plot scale, there was a strong negative relationship between α -diversity and atmospheric N deposition, whereby each 1 kg increase of long-term N deposition per hectare per year was associated with a decrease of 0.229 species (standardized effect size: -0.375) ([Table 1](#), [Figure 3](#)). We found that γ -diversity showed a similar decrease with N deposition, decreasing with 0.361 (standardized effect size: -0.325) species for every 1 kg increase of long-term N deposition per hectare per year ([Table 1](#), [Figure 3](#)). In contrast, multiplicative-based β -diversity was not significantly related to N deposition, whereas additive-based β -diversity decreased significantly with atmospheric N deposition, by a rate of 0.132 species for 1 kg increase of long-term N deposition per hectare per year ([Table 1](#), [Figure 3](#)). In all cases, linear relationships between plot-to-site-scale biodiversity and atmospheric N deposition were better fitting than non-linear relationships ([Table S2](#)).

We also found that other factors besides atmospheric N deposition were significantly related to plot-to-site-scale biodiversity. Specifically, an increase of one unit in soil pH led on average to an increase of 6.360 in α -diversity, 12.211 in γ -diversity, and 5.851 in additive β -diversity ([Table 1](#)). Furthermore, with an increase of 1° in slope, there was on average an increase of 0.056 in α -diversity, 0.116 in γ -diversity and 0.060 in additive β -diversity ([Table 1](#)). While multiplicative β -diversity was not significantly related to atmospheric N deposition, it did increase by 0.184 with an increase of 1 g kg^{-1} soil Ca, while it decreased by -0.742 with an increase of 1 g kg^{-1} soil Mg ([Table 1](#)).

Compared to extrapolated site-scale γ -diversity, observed γ -diversity was 21% lower (average values for observed and extrapolated γ -diversity are 28.7 and 37.6, respectively). Sensitivity analyses showed that extrapolated γ -diversity, as well as extrapolated multiplicative and additive β -diversity responded in a similarly negative

TABLE 1 Outcomes of linear models, where α -diversity, multiplicative β -diversity, additive β -diversity, and γ -diversity were modeled in response to N deposition and other environmental factors, at both the site and regional scale.

Response	Predictors	Estimate \pm SE	Std. effect	T value	p value
Plot-to-site scale					
α -diversity	Intercept	-9.339 (4.276)		-2.184	0.0132
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.229 (0.042)	-0.375	-5.438	<0.0001
	Soil pH	6.360 (0.813)	0.576	7.824	<0.0001
	Slope (°)	0.056 (0.024)	0.156	2.378	0.0192
γ -diversity	Intercept	-21.724 (7.974)		-2.724	0.0076
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.361 (0.079)	-0.325	-4.589	<0.0001
	Soil pH	12.211 (1.516)	0.609	8.054	<0.0001
	Slope (°)	0.116 (0.044)	0.177	2.636	0.0097
β -diversity (m) ^a	Intercept	-1.811 (0.053)		34.196	<0.0001
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.00161 (0.00235)	-0.068	-0.683	0.4961
	Soil Ca (g kg ⁻¹)	0.184 (0.0510)	0.404	3.596	0.0005
	Soil Mg (g kg ⁻¹)	-0.742 (0.229)	-0.369	-3.240	0.0016
β -diversity (a)	Intercept	-12.385 (4.791)		-2.585	0.0111
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.132 (0.047)	-0.231	-2.785	0.0064
	Soil pH	5.851 (0.912)	0.570	6.423	<0.0001
	Slope (°)	0.060 (0.026)	0.179	2.265	0.0256
Site-to-regional scale					
α -diversity	Intercept	-5.80 (15.618)		-0.371	0.7165
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.367 (0.111)	-0.531	-3.315	0.0056
	Soil pH	8.879 (2.37)	0.457	2.855	0.0135
γ -diversity	Intercept	-41.996 (32.824)		-1.279	0.2234
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.655 (0.234)	-0.430	-2.814	0.0146
	Soil pH	24.314 (6.536)	0.568	3.720	0.0026
β -diversity (m) ^a	Intercept	1.538 (0.177)		8.707	<0.0001
	N deposition (kg ha ⁻¹ year ⁻¹)	0.0087 (0.0045)	0.646	1.927	0.0761
	Spatial distance (m)	5.70e-07 (1.73e-07)	1.104	3.294	0.0058
β -diversity (a) ^a	Intercept	-36.170 (19.335)		-1.871	0.0841
	N deposition (kg ha ⁻¹ year ⁻¹)	-0.288 (0.137)	-0.336	-2.099	0.0559
	Soil pH	15.435 (3.850)	0.643	4.009	0.0015

Abbreviation: Std. effect, standardized effect size.

^aModels labeled with an asterisk, deviate from finally selected models, in that N deposition was re-entered into the model despite not having significant effects. For associated models without N deposition (which showed qualitatively the same results), refer to [Table S1](#).

fashion to N deposition as observed γ -diversity and β -diversity indicators ([Table S3](#)).

Relationships between atmospheric N deposition and plant biodiversity at the site-to-regional scale.

At the site-to-regional scale, there was also a strong negative relationship between α -diversity and atmospheric N deposition, whereby each 1 kg increase of long-term N deposition per hectare per year was associated with a decrease of 0.367 species (standardized effect size: -0.531) ([Table 1](#), [Figure 4](#)). γ -diversity showed a similar decrease with N deposition, decreasing with 0.655 species (standardized effect size: -0.430) for every 1 kg increase of long-term N deposition per hectare per year ([Table 1](#), [Figure 4](#)). Hence, these results showed that the relative effects (quantified using

standardized effect size) of N deposition were somewhat stronger at site-to-regional scales (standardized effect sizes of -0.531 and -0.430 on α - and γ -diversity, respectively) than at plot-to-site scales (standardized effect sizes of -0.375 and -0.325 on α - and γ -diversity, respectively). In contrast, at the regional scale, neither multiplicative-based nor additive β -diversity were significantly related to N deposition, although there was a negative trend ($p = .0559$) for the relationship between additive β -diversity and atmospheric N deposition, where additive β -diversity decreased by 0.288 for each 1 kg increase of N per hectare per year ([Table 1](#), [Figure 4](#)). In all cases, linear relationships between site-to-regional-scale biodiversity and atmospheric N deposition were better fitting than non-linear relationships ([Table S2](#)).

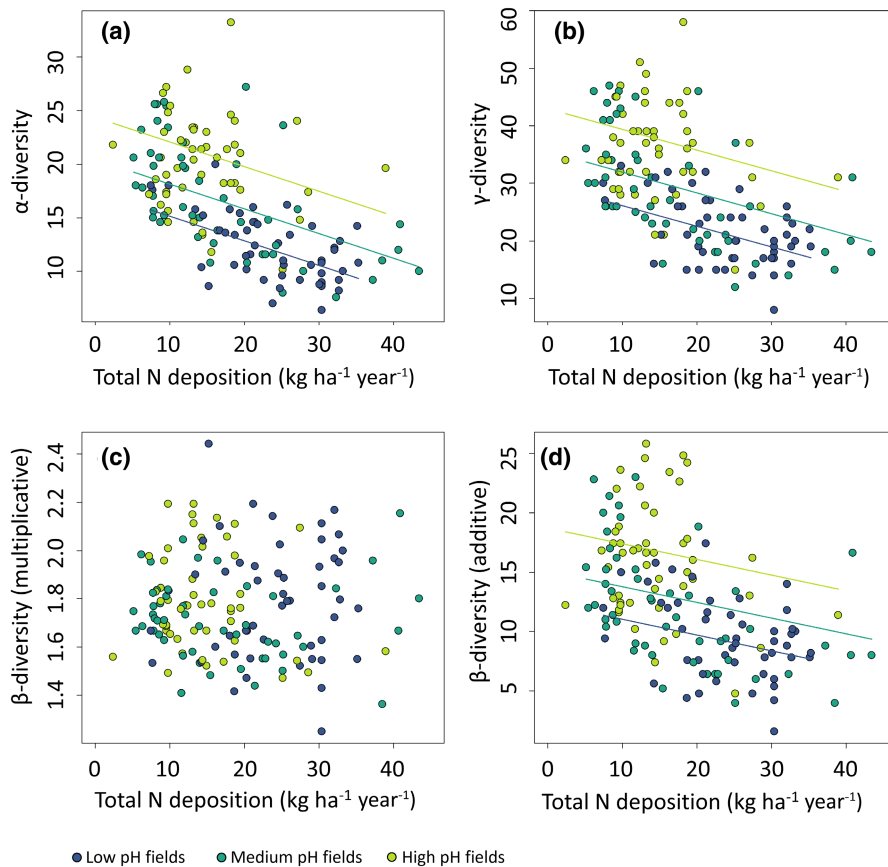


FIGURE 3 Relationships between α -diversity (a), γ -diversity (b), multiplicative β -diversity (c) and additive β -diversity (d) and N deposition, at the plot-to-site scale. Where relationships are significant, a trend line is shown (based on the models of Table 1). Colors indicate the different levels of pH: Dark blue = low pH (range: 3.688–4.360, i.e., 0–33 percentile), cyan = medium pH (range: 4.370–4.810, i.e., 34–67 percentile) and yellow = high pH (range: 4.822–5.720, i.e., 68–100 percentile).

We also found that other factors besides atmospheric N deposition were significantly related to variation in site-to-regional-scale biodiversity. Specifically, an increase of one unit in soil pH led on average to an increase of 8.879 in α -diversity, 24.314 in γ -diversity and 15.435 in additive β -diversity (Table 1). Furthermore, multiplicative β -diversity was also significantly positively associated to spatial distance among sites (Table 1).

Compared to extrapolated regional-scale γ -diversity, observed γ -diversity was 40% lower (average values for observed and extrapolated γ -diversity are 54.7 and 94.1, respectively). Sensitivity analyses showed that extrapolated multiplicative and additive β -diversity responded qualitatively similarly to N deposition as observed β -diversity indicators did, while the effect on γ -diversity turned from significantly negative ($p=0.015$) for observed γ -diversity to non-significantly negative ($p=0.224$) for extrapolated γ -diversity (Table S3).

3.2 | Relationships between individual species and atmospheric N deposition

Out of the 66 species for which we assessed relationships, we found that for 26 species (e.g., *Potentilla erecta*, *Campanula rotundifolia* and *Stachys officinalis*, for a full list and associated model results, see Table S4), their likelihood of occurrence at a site was negatively associated with high levels of atmospheric N deposition, while for only one species (*Galium saxatile*), its likelihood of occurrence was

positively associated with high levels of atmospheric N deposition (Figure 5). The presence of the remaining 39 species was not significantly related to atmospheric N deposition. At the plot-scale, similar relationships were found (Table S5).

4 | DISCUSSION

With this study, we investigated whether negative relationships between atmospheric N deposition and plant biodiversity, as frequently observed at local spatial scales (Bobbink et al., 1998, 2010; Maskell et al., 2010; Roth et al., 2015; Southon et al., 2013; Stevens et al., 2004), also occur at larger spatial scales. Overall, our results provide strong evidence that not only at small spatial grains (plot-scale) but also at the site and regional scale, high levels of atmospheric N deposition are associated with a decreased richness of plant species. These reductions in plant richness were mostly associated with decreased levels of additive, but not multiplicative β -diversity, mostly in line with the hypothesis that it is the consistent loss of some “loser species” (i.e., species that cannot cope with increased levels of atmospheric N deposition), rather than the gains of a few “winner” species replacing other species, that is driving overall patterns (Figure 1d).

Previous studies suggest that declines in plant biodiversity following increased levels of atmospheric N deposition can be driven by several processes, including increased aboveground or belowground competition following eutrophication (whereby abundant

FIGURE 4 Relationships between α -diversity (a), γ -diversity (b), multiplicative β -diversity (c) and distance-based β -diversity (d) and N deposition, at the site-to-regional scale. Where relationships are significant, a continuous trend line is shown, and when there is a trend ($0.05 < p < .1$) for a relationship, a dashed trend line is shown (based on the models of Table 1). Colors indicate the different levels of pH: Cyan=low pH (bottom 50%) and yellow=high pH (top 50%).

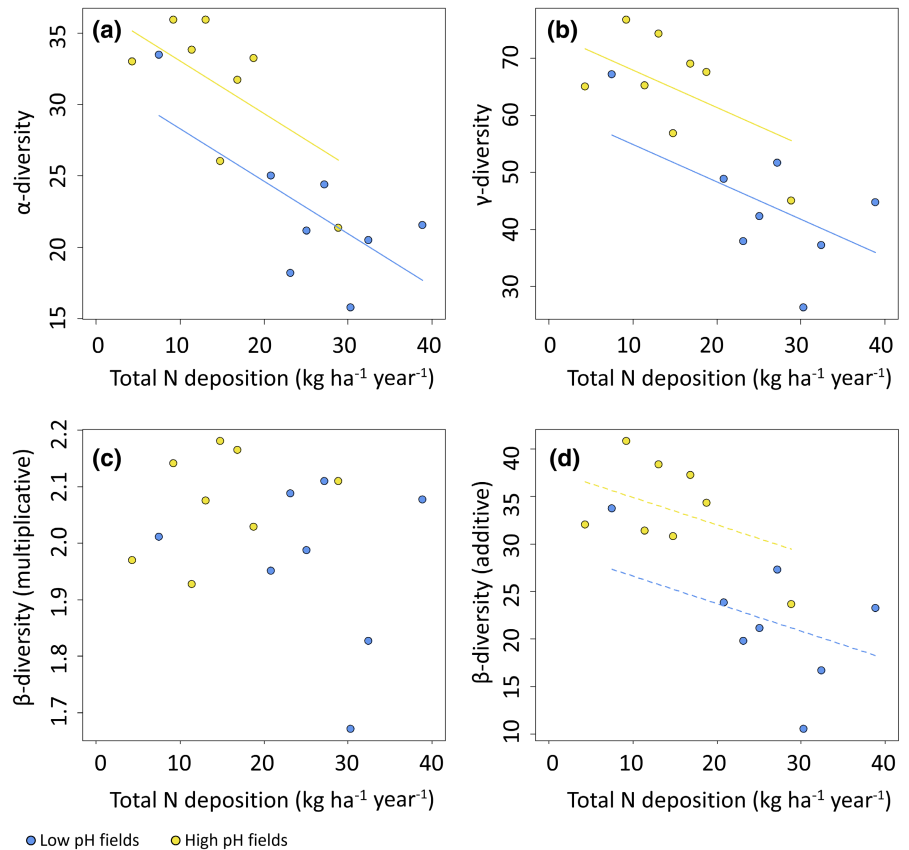
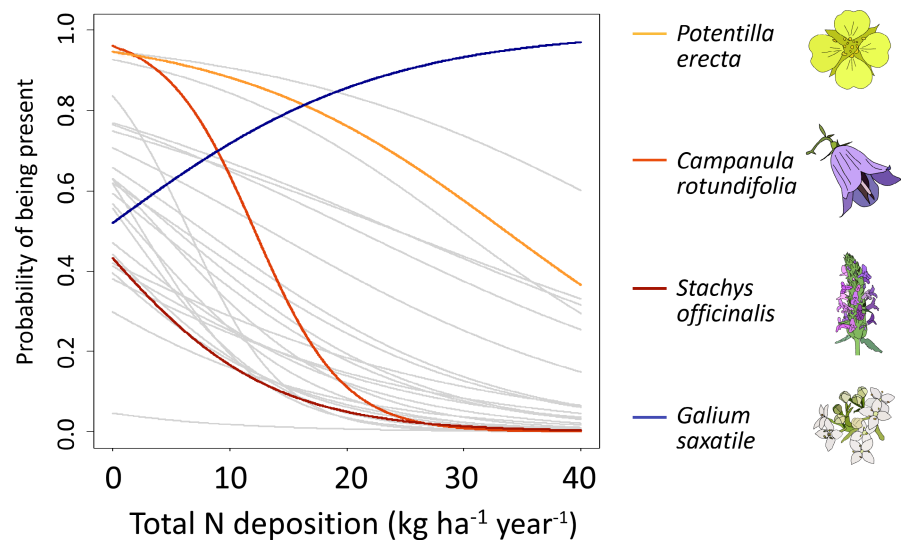


FIGURE 5 Modeled probability of presence of species as a function of the amount of total N deposition. The lines represent logistic model predictions, for those 27 species whose presence was significantly related to total N deposition. Lines of four species are highlighted in color, to exemplify three species (out of the 26 in total) that respond negatively to total N deposition, and the only species that responds positively.



species become increasingly dominant, at the cost of other species), and the inability of some plant species to cope with acidification (Bobbink et al., 1998; Stevens et al., 2018). While these processes mostly operate at local spatial scales, our study suggests that their effects also manifest at much larger spatial scales. This can happen when, for example, at multiple local sites, the same species disappear following increased levels of N deposition, and/or if at multiple sites, the same “winner” species that have a competitive advantage under increased levels of N deposition replace other, less competitive species. Two lines of evidence mostly point in the direction

that, while it is the same “loser” species that disappear, there are no “winner” species consistently replacing loser species across sites. First, we found that, at the site scale, additive, but not multiplicative, β -diversity decreased with increasing levels of atmospheric N deposition. At the regional scale, similarly strong relationships were found (see the standardized effect sizes), although the decrease of additive β -diversity with atmospheric N deposition was only marginally significant, possibly due to lower statistical power (sample sizes are 153 and 16 at the site scale and regional scale, respectively). Furthermore, at the regional scale, the decrease of γ -diversity with

atmospheric N deposition became non-significant when analyzing extrapolated γ -diversity that accounts for undersampling, suggesting that some N-sensitive species became so rare at high levels of N deposition that they were not observed in the regions we analyzed, even if they were in fact still present, albeit so rarely that they were often not observed. Multiplicative β -diversity is most sensitive to the joint presence of species, while additive β -diversity is also sensitive to the joint absences of species (Anderson et al., 2011) and, therefore, most likely to decrease when the same species disappear from multiple sites. Second, species-based analyses also showed that there was a high number (26) of species, including, for example, *Campanula rotundifolia*, *Festuca rubra*, *Hylocomium splendens*, *Potentilla erecta*, *Stachys officinalis* and *Succisa pratensis*, whose presence was negatively associated with high levels of atmospheric N deposition (see also Payne et al., 2013). There was also one species (*Galium saxatile*) whose presence was positively associated with atmospheric N deposition, indicating that our findings were closest to, but not 100% in line with, the “consistent loser species” scenario. Our results are largely in line with N addition experiments (Zhang et al., 2019; Lu et al., 2024; but see Hautier et al., 2020) where reductions in additive β -diversity in response to N addition were also shown. Furthermore, our results are in line with observational studies in European and North American forests, where herbaceous understorey communities homogenized over time, and the authors proposed that (N-driven) eutrophication was a major cause of this (Keith et al., 2009; Staude et al., 2020). Several possible mechanisms may underlie our results. First, it is possible that some species cannot tolerate N deposition-driven soil acidification and the associated leaching of base cations and increase in Al (de Graaf et al., 2009), and that it is consistently the same species that disappear from multiple sites as a result. In line with this, a previous study showed that acid-tolerant plant species were positively related to increased levels of atmospheric N deposition (Stevens, Duprè, et al., 2010; Stevens, Thompson, et al., 2010). Second, it is also possible that eutrophication causes increased below-or aboveground competition, and that always the same species are outcompeted. However, a previous study did not find evidence that plant species associated with high levels of N deposition are stronger competitors (Stevens, Duprè, et al., 2010; Stevens, Thompson, et al., 2010), perhaps because the outcomes of competition are more driven by the traits of “loser” species than by the traits of “winner” species. On the other hand, our analyses are based on species presence/absence patterns, and some species were present in the vast majority of sites (e.g., in 98% of all sites in the case of *Agrostis capillaris*), across the full range of observed N deposition levels. It is possible that increased dominance of such species in sites with higher N deposition levels could also have contributed to the loss of other, competitively weaker species, although testing this idea requires further study.

Our results showed that besides atmospheric N deposition, also other environmental factors were related to variation in plant biodiversity across spatial scales. For example, we found that soil pH was positively related to α -diversity, γ -diversity, and additive β -diversity, at both the site and regional scale. This largely confirms relationships

found in other studies that were conducted at both local (Ceulemans et al., 2012; Gaujour et al., 2012; Roem & Berendse, 2000) and larger (Dembicz et al., 2020) spatial scales and is also in line with experiments showing that nutrient and pH manipulation jointly affect local plant diversity in grasslands (Crawley et al., 2005). As this is an observational study, it is difficult to say whether these relationships between soil pH and different biodiversity indicators are independent of atmospheric N deposition. It is well established that atmospheric N deposition can lead to soil acidification (Ceulemans et al., 2012; Grennfelt & Hultberg, 1986; Vitousek et al., 1997), and in our study, we also observed a negative correlation between levels of atmospheric N deposition and soil pH. Hence, it is possible that the negative effect sizes of N deposition on biodiversity as estimated in our statistical models only partly capture the true effects of N deposition, with other effects including those through reductions in soil pH, so that overall effects of N deposition are even more negative for biodiversity. We also found that at the site scale, α -diversity, γ -diversity, and additive β -diversity were positively related to slope, so plant diversity was generally higher in more hilly areas. This is in line with previous findings (Kampmann et al., 2008), and possibly resulting from a higher heterogeneity in environmental conditions in hilly areas, or due to thinner soils, limiting nutrient availability and root growth, and therefore, competitive exclusion. We also found that at the site scale, multiplicative β -diversity was related to soil Ca^{2+} and Mg^{2+} concentrations. These base cations are important nutrients for plants, but are often in low supply in acid or acidified soils, where H^+ ions displace these soil-binding particles, which subsequently leach out. Given the acidifying effects of N deposition, the effects of soil Ca^{2+} and Mg^{2+} on multiplicative β -diversity might be ultimately driven by N deposition (Vitousek et al., 1997). Previous studies have shown positive relationships between soil-cation concentrations and plant biodiversity at the local scale (Zelnik & Čarni, 2013). Our study shows that soil Ca^{2+} is also positively related to β -diversity, possibly because a non-limiting supply of Ca^{2+} may allow the coexistence of some species that normally are not found together at local scales. However, to our surprise, soil Mg^{2+} was negatively related to multiplicative β -diversity, possibly indicating that areas with a high soil Mg^{2+} content also tend to be more environmentally homogeneous, although this requires further study.

By showing that in addition to local losses in biodiversity, the plant species richness at larger spatial scales is also negatively associated with increased levels of N deposition, an important implication of our study is that high levels of N emissions lead to increased risks of the disappearance of species not only at local scales but also at much larger spatial scales. Indeed, some of the plant species that we found to be negatively associated with atmospheric N levels are on red lists in EU countries where atmospheric N levels are high across the nation. This is for example the case for *Stachys officinalis* and *Succisa pratensis*, which are listed as “threatened” and “vulnerable,” respectively, in the Netherlands (<https://minlv.nederlandsesoorten.nl/>). It is possible that high levels of N emissions by agricultural activities, traffic and industry have contributed to the decline of these species, although it was beyond the scope of this study to reveal the

mechanisms that may have caused their negative associations with N deposition. While N emissions have declined somewhat in Europe in recent decades (Ackerman et al., 2018), emissions are still considered to be too high in most places across Europe (European Environment Agency, 2023; Hettelingh et al., 2015), possibly hampering recovery. Responses of individual species to N deposition are non-linear and show that in areas with very high levels of atmospheric N deposition (e.g., 30 kg N ha⁻¹ year⁻¹), moderate long-term deposition reductions of, for example, 5 kg N ha⁻¹ year⁻¹, might increase the likelihood of the reappearance of species such as *Campanula rotundifolia*. Stronger and more sustained deposition reductions are likely needed for species such as *Stachys officinalis* (Figure 5). Importantly, we have to be cautious regarding the ability of plant biodiversity to recover after reductions in atmospheric N deposition. While some previous studies have shown relatively rapid recovery of plant biodiversity after reductions in N emissions (Storkey et al., 2015), others studies only showed moderate recoveries at best (Berendse et al., 2021; Isbell et al., 2013; Stevens, 2016). Previous studies have shown that the effects of N deposition on plant diversity are particularly harmful when high N emissions have persisted for a long time, as N accumulates in soils (Soons et al., 2017), and because local seed banks may not contain certain species anymore (Basto et al., 2015). Hence, the longer high N emission levels have persisted, the longer it takes to get N out of ecosystems (passively, through leaching, or actively, through management). Therefore, recovery does likely not only depend on persistent reductions in N emissions but also on how quickly N stored in the soil and plants can be removed from the system, for example, by grassland management through haying (Tilman & Isbell, 2015). Given the findings of our study that atmospheric N deposition not only hampers local plant diversity but also larger-scale plant diversity, a key open question is whether policies and management activities that restore local-scale plant diversity can also reverse biotic homogenization and thereby larger-scale plant diversity. Until we know, reducing atmospheric N emissions, together with continuing or starting management activities that remove N from previously systems that have received too much N deposition, may be the most promising way to preserve or restore biodiversity.

AUTHOR CONTRIBUTIONS

Fons van der Plas: Conceptualization; formal analysis; visualization; writing – original draft; writing – review and editing. **Yann Hautier:** Conceptualization; writing – review and editing. **Tobias Ceulemans:** Conceptualization; writing – review and editing. **Didier Alard:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **Roland Bobbink:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **Martin Diekmann:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **Nancy B. Dise:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **Edu Dorland:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **Cecilia Dupré:** Funding acquisition; investigation; methodology; resources; writing – review and editing. **David Gowing:** Funding acquisition;

investigation; methodology; resources; writing – review and editing.

Carly Stevens: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; writing – review and editing.

ACKNOWLEDGEMENTS

We received funding from the European Science Foundation through the EURODIVERSITY-programme. National funds were provided by DfG (Germany), NERC (United Kingdom) and NWO (The Netherlands) and INRA, ADEME and Aquitaine Region (France). We thank everyone who assisted with field and laboratory work, as well as the conservation agencies and land owners who gave us permission working on their land.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data of this study, as well as the related R script, are publicly available on Figshare: see DOI <https://figshare.com/s/b378da518573e8436854>.

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SUPPORTING INFORMATION

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How to cite this article: van der Plas, F., Hautier, Y., Ceulemans, T., Alard, D., Bobbink, R., Diekmann, M., Dise, N. B., Dorland, E., Dupré, C., Gowing, D., & Stevens, C. (2024). Atmospheric nitrogen deposition is related to plant biodiversity loss at multiple spatial scales. *Global Change Biology*, 30, e17445. <https://doi.org/10.1111/gcb.17445>