Title: Impacts of nitrogen addition on plant species richness and abundance: A global meta-analysis

Running head: Nitrogen addition reduces plant species richness and abundance.

Manuscript category: Meta-analysis
Abstract

Aim Experimental nitrogen (N) addition (fertilization) studies are commonly used to quantify the impacts of increased N inputs on plant biodiversity. However, as plant community responses can vary considerably among individual studies, there is a clear need to synthesize and generalize findings with meta-analytical approaches. Our goal was to quantify changes in species richness and abundance in plant communities in response to N addition across different environmental contexts, while controlling for different experimental designs.

Location Global

Time period Data range: 1985 – 2016; Publication years: 1990-2018

Major taxa studied Plants

Methods We performed a meta-analysis of 115 experiments reported in 85 studies assessing the effects of N addition on terrestrial natural and semi-natural plant communities. We quantified changes in plant biodiversity in relation to N addition using four metrics: species richness (SR), individual species abundance (IA), mean species abundance (MSA) and geometric mean abundance (GMA).

Results For all metrics, greater amounts of annual N addition resulted in larger declines in plant diversity. Additionally, MSA decreased more steeply with N that was applied in reduced (NH₄⁺) rather than oxidised (NO₃⁻) form. Species richness loss with increasing N amounts was found to be larger in warmer sites. Further, greater losses in species richness were found in sites with longer experimental duration, smaller plot sizes and lower soil cation exchange capacity (CEC). Finally, abundance reductions of individual species were larger for N-sensitive plant life-form types (legumes and non-vascular plants).

Main conclusions N enrichment decreases both species richness and abundance of plant communities in N-addition experiments, but the magnitude of the response differs among biodiversity metrics and with the environmental and experimental context. This underlines the importance of integrating multiple dimensions of biodiversity as well as relevant modifying factors into assessments of biodiversity responses to global environmental change.
**Keywords:** anthropogenic impacts, biodiversity, eutrophication, global change, GLOBIO, vegetation, soil acidification
Introduction

Nitrogen (N) deposition is among the main drivers of the loss of plant biodiversity in terrestrial ecosystems (Bobbink et al., 2010; Sala et al., 2000; Vellend et al., 2017). In the last century, enhanced emissions of nitrogenous compounds caused by agricultural and industrial activities have increased atmospheric nitrogen (N) deposition in natural and semi-natural ecosystems across the world (Erisman et al., 2013; Galloway et al., 2008), with concomitant consequences for the biodiversity of these ecosystems (Bobbink et al., 2010; Dise et al., 2011). Biodiversity is key for maintaining the functioning of ecosystems and the provision of ecosystem services (Cardinale et al., 2012; Hooper et al., 2005). Plant diversity, for example, enhances the ability of ecosystems to maintain multiple functions and processes, such as carbon sequestration, productivity, and the build-up of nutrient pools (Maestre et al., 2012). Apart from positive effects on ecosystem productivity, diversity also provides increased erosion control, resistance to invasive species and pest regulation (Quijas et al., 2012).

The responses of plant communities to N deposition vary depending on the environmental context (Simkin et al., 2016; Vellend et al., 2017; Perring et al., 2018). Modifying factors include the amount and duration of N deposition, which determine the cumulative N input over time (Bernhardt-Römermann et al., 2015; Duprè et al., 2010); soil pH and acid neutralizing capacity (Clark et al., 2007; Simkin et al., 2016); the chemical forms of N input (Stevens et al., 2011) and environmental conditions such as climate (Clark et al., 2007; Humbert, Dwyer, Andrey, & Arlettaz, 2016; Limpens et al., 2011) and vegetation types (Pardo et al., 2011; Simkin et al., 2016). Additionally, land use history might play a relevant role, as this may drive the composition and function of plant communities into different trajectories of change (Perring et al., 2018).

There are two main empirical approaches to study the impact of N on plant diversity (Hettelingh, Stevens, Posch, Bobbink, & de Vries, 2015). These approaches are experimental N addition studies, and observational studies investigating plant species diversity over a gradient of N deposition, either in time series analysis (e.g. Stevens, Duprè et al., 2010; Stevens, Thompson, Grime, Long, and Gowing, 2010) or over a spatial gradient (e.g. Jones et al., 2004; Duprè et al., 2010). Observational
Gradient studies can benefit from existing datasets (e.g. Simkin et al., 2016), but need to correct for confounding site factors and cannot prove causality (Dise et al., 2011). Experimental studies, on the other hand, allow for effects to be directly attributed to N addition. However, experimental studies typically assess relatively short-term responses only and often use higher levels of N addition compared to atmospheric deposition in the field. Furthermore, the results might be influenced by experimental design and local environmental conditions, which limits the possibilities for regional and global extrapolation (Hettelingh et al., 2015). The latter might be solved by setting up globally distributed experiments such as the Nutrient Network (Firn et al., 2011; Borer et al., 2014), but also by synthesizing multiple N-addition experiments with a meta-analysis, allowing to derive a more general quantitative response of plant species diversity to N enrichment.

Previous meta-analyses that addressed impacts of N on plant assemblages focused on species richness or biomass in specific ecosystems (i.e. Limpens et al., 2011; Humbert et al., 2016) or in specific geographic regions (i.e. Clark et al., 2007; Fu and Shen, 2016) or continents (i.e. De Schrijver et al., 2011; Soons et al., 2017). To our knowledge, a systematic meta-analysis covering multiple dimensions of biodiversity in multiple ecosystems across the globe is yet lacking. In addition to covering a large geographical extent, it is particularly important to consider metrics beyond species richness, such as measures of species abundance, as different aspects of biodiversity may respond differently to environmental change (Dornelas et al., 2014; Schipper et al., 2016; Winfree, Fox, Williams, Reilly, & Cariveau, 2015). In this study we synthesized a large number of N-addition studies worldwide, in order to reveal overall effects of N addition on various metrics of local plant biodiversity and explore the role of potential experimental (amount of yearly N applied, experimental duration, type of fertilizer, plot size) and environmental (temperature, precipitation, soil pH, soil cation exchange capacity, atmospheric N deposition) moderators (Figure 1a). We considered four metrics of biodiversity change to incorporate richness and abundance as two essential dimensions of biodiversity (Schipper et al., 2016) (Figure 1b): species richness (SR), individual species abundance (IA) (Benitez-López et al., 2017), mean species abundance (MSA) (Alkemade et al., 2009) and
geometric mean abundance (GMA) (Buckland, Magurran, Green, & Fewster, 2005; Buckland, Studeny, Magurran, Illian, & Newson, 2011). The metrics adopted cover different domains of the richness-abundance space and in our meta-analysis represent the changes observed between treatment and control plots (Figure 1b).

We expected local biodiversity to decrease with increasing yearly N addition amounts and experimental duration, reflecting the negative effect of cumulative N enrichment (De Schrijver et al., 2011; Humbert et al., 2016). We further hypothesized that larger negative impacts to N addition will occur in sites with low soil pH and low atmospheric N deposition, as plants growing in such conditions tend to be more adapted to low N availability (Bobbink et al., 2010; Simkin et al., 2016). We also expected that fertilizer types containing reduced N forms (NH$_4^+$) result in higher impacts on plant diversity than oxidised forms (NO$_3^-$), as reduced N tends to strongly acidify the soil and disadvantage the nutrient uptake of N-poor adapted species (van den Berg, Peters, Ashmore & Roelofs, 2008; Song et al., 2012). We further hypothesized that species losses would be larger in larger experimental plots, as these have higher chances of including rare species, which may also be more likely to go extinct in the treatment plots (Perring et al., 2018$^b$). Higher impacts were also expected in sites with low soil cation exchange capacity (CEC), as lower CEC indicates higher susceptibility to acidification in response to N addition (De Vries, Posch & Kämäri, 1989; Clark et al., 2007). We further hypothesized losses to be larger in experiments conducted under higher mean annual temperature and precipitation, because these conditions are expected to result in higher N mineralisation rates hence enhanced N availability following fertilization (Dise et al., 2011; Yang, Ryals, Cusack & Silver, 2017).

Methods

Selection of primary studies

In April 2018, we used the Scopus and Web of Science databases to collect primary studies. The search strings were composed of ‘OR’ and ‘AND’ statements combining terms related to N-addition
experiments and different dimensions of plant species diversity, for example (“nitrogen fertilization” OR “nitrogen addition”) AND (“abundance” OR “composition” OR “number” OR “richness”) (see the complete search strings in Supporting Information Appendix S1). We selected relevant studies based on title and abstract, and then scanned their full texts and supporting materials to extract data on N-addition experiments. Where factorial treatment combinations were present, we retained data from control and N addition plots alone to avoid confounding effects. Thus, we excluded data from plots where N addition was performed together with watering, temperature increase, litter removal, grazing, fire manipulation or where N was added in combination with other nutrients. We limited our selection to experiments conducted on natural or semi-natural vegetation excluding studies conducted on crops, mono-cultures or where species were artificially introduced in plots. Finally, we removed studies that reported the same data as other studies already included in our database. To avoid over-representation, we collected data on species richness and abundance change at the final year of each experiment.

Our literature search yielded a total of 2314 studies, of which we selected 85 relevant studies (published between March 1990 and January 2018) that reported data from 115 N-addition experiments performed between 1985 and 2016 in different geographical locations (Figure 2; Table S2.1, Appendix S2). Of the 85 studies, 48 reported data on species richness, 15 on individual species abundance, and 22 on both species richness and abundance (a list of the data sources is found in Appendix A). We extracted the number of species and species-specific abundance data separately from treatment and control plots and calculated the four biodiversity metrics as described in Table 1. Abundance data were extracted for each species reported in both the treatment and control plots, for a total of 403 taxa. The majority of these were identified to species level, but 32 were indicated with the genus name only. Thus, the total number of species in our dataset might be slightly overestimated. We recorded a total of 220 pairwise comparisons for SR. At the species level, we included 871 individual species abundance comparisons (IA), some across multiple N fertilization levels within the same experiment, which resulted in 89 observations for MSA and GMA. Nitrogen addition levels
ranged from 3.75 to 572 kg N ha\(^{-1}\) yr\(^{-1}\) in the species richness dataset (mean = 124.8 kg ha\(^{-1}\) yr\(^{-1}\); median = 92 kg ha\(^{-1}\) yr\(^{-1}\)), and from 7 to 480 kg N ha\(^{-1}\) yr\(^{-1}\) in the species abundance dataset (mean = 96.5 kg ha\(^{-1}\) yr\(^{-1}\); median = 70 kg ha\(^{-1}\) yr\(^{-1}\)).

**Calculation of the effect sizes**

We calculated four biodiversity metrics for the meta-analysis, including the species richness ratio (SR), individual species abundance ratio (IA), mean species abundance (MSA), and geometric mean abundance (GMA) (Table 1). Both SR and IA were obtained by log-transforming the ratio between the species richness and individual species abundance in each N-treatment plot and control plot, respectively (Hedges, Gurevitch, & Curtis, 1999). Some species had zero abundance in treatment plots, precluding log-transformation for IA calculation. Therefore, we transformed IA effect sizes using a modification of the transformation proposed by Smithson and Verkuilen (2006) to shrink the ratios and avoid zero values (Benítez-López et al., 2017) (eq.1):

\[
y_i = \frac{(y \times N + 0.5)}{N}
\]

(eq. 1)

where \(y\) is the ratio \((A_T / A_C)\) of individual species abundance in the treatment \((A_T)\) and control \((A_C)\) and \(N\) is the number of observations in the individual species abundance dataset \((N = 871)\). This resulted in a distribution of ratios \((y_i)\) slightly displaced towards larger values (before transformation: [0, 82.5], after transformation: [0.0006, 82.5006]). The new ratios were then log-transformed to obtain IA. Since ratios \(A_T / A_C\) cannot be calculated when abundance in the control is equal to 0, we decided to exclude species that were present only in the treatments from the calculation of the IA and GMA metrics, following the definitions and approaches applied in previous studies (Table 1).

We calculated MSA as the mean of the ratios of individual species abundance in each treatment versus the corresponding control (Alkemade et al., 2009; Benítez-López, Alkemade, & Verweij, 2010). Following the definition of MSA, the individual ratios were truncated at 1 for species with a higher abundance in the treatment group compared to the control group (Table 1). As MSA captures losses in abundance of species that are found in reference conditions (control plots) only, it cannot go beyond
the original abundance and richness (Figure 1b). Finally, GMA was calculated as the back-transformed mean of the log-transformed individual abundance ratios, without truncation (Buckland et al., 2011). The GMA metric (Buckland et al., 2005; Buckland et al., 2011) also combines abundance and species richness into one index but allows for gains in the abundance dimension (Figure 1b).

**Moderators**

Factors influencing plant community responses to N were selected *a priori* based on literature study (Figure 1a; Table S3.1, Appendix S3) and data availability. Nine moderators were considered in the analysis: 1) the annual amount of N added in the experiment (kg N ha\(^{-1}\) yr\(^{-1}\)); 2) the annual amount of background N deposition (kg N ha\(^{-1}\) yr\(^{-1}\)) (i.e. the amount of N deposited from the atmosphere, which is independent from the experimental N addition); 3) mean annual temperature (°C); 4) mean annual precipitation (mm yr\(^{-1}\)); 5) duration of the experiment (number of years of N addition); 6) the type of N fertilizer, categorized as fertilizers containing nitrate (NO\(_3\)) (i.e. ammonium nitrate or alkali nitrates) or fertilizers containing ammonium (NH\(_4\)) as the only source of N (i.e. urea, urine, ammonium sulphate and ammonium chloride) (see details in Table S4.1, Appendix S4); 7) plot size (m\(^2\)) (i.e. the area of vegetation surveyed to estimate richness or abundance in each experiment); 8) initial soil pH at the experimental sites (estimated before N addition); 9) soil cation exchange capacity (CEC) (cmol kg\(^{-1}\)). Additionally, we examined overall biodiversity responses among the ecosystem type where the study/experiment took place, with ecosystems categorized into five broad categories (temperate grasslands and heathlands, semi-arid ecosystems, bogs/peatlands, arctic/alpine ecosystems, and forests) (see details about grouping criteria in Table S4.2, Appendix S4). Further, we categorized each taxon into plant life-form types (herbaceous forbs, graminoids, legumes, ferns, woody plants and non-vascular plants; see Table S4.3, Appendix S4) and used this to assess possible differences in individual abundance response among different species groups.

We collected from each study the location (geographic coordinates), experimental setup (yearly amount of N addition, experimental duration, type of N fertilizer, plot size) and ecosystem type.
Because many studies did not report atmospheric N deposition levels, we collected these data from the global TM5 model for the year 2000 (Dentener et al., 2006). For the same reason, we extracted estimates of cation exchange capacity (CEC) and soil pH from the 250 m resolution global SoilGrids data (Hengl et al., 2014; Hengl et al., 2017), by averaging values provided for soil depths of 0-5, 5-15 and 15-30 cm. Data on temperature and precipitation were derived from the global Climate Research Unit database, which comprises series of monthly meteorological data on a 0.5° * 0.5° grid (New, Hulme, & Jones, 1999). For each observation we extracted data for the corresponding year and calculated the mean temperature and precipitation over the 12 monthly values.

**Data analysis**

We performed the meta-analysis using multilevel mixed-effect models to control for non-independence in the data due to multiple effect sizes per study and species (Nakagawa & Santos, 2012). We first fitted single meta-regression models using yearly N addition as the only moderator, in order to compare changes among the metrics for a given amount of N applied. Then, we fitted multiple meta-regression models by including other moderators as well as interaction terms between the amount of N addition and these other moderators. Except for mean annual temperature and soil pH, we log-transformed all continuous moderators, as the data showed strong positive skewness, and we scaled and centred all continuous variables. The only moderate correlation among moderators was between mean annual precipitation and soil pH (richness dataset $\rho=-0.75$; abundance dataset $\rho=-0.68$).

Based on this, we decided not to exclude any moderators upfront. We performed stepwise backward selection based on the Bayesian Information Criterion (BIC), whereby we excluded a moderator only in case it was also dropped from the interaction term. We estimated the amount of heterogeneity reduced in the best models selected and by each moderator using the omnibus Wald-type test of moderators (Benitez-López et al., 2017).

We accounted for the correlation in the true effects by using experiments as the random effect in the models. For the IA metric, we used a crossed random effect structure including both experiment and
species as random components. We nested the individual estimates within the experiment grouping-level in the random structure of the models to account for the possibility that the underlying true effects within experiments are not homogeneous (Konstantopoulos, 2011). We weighted the importance of the effect sizes of SR and IA by the inverse of the sampling variance (Hedges et al., 1999) (Table 1). Because of non-independence of the effect sizes, we computed the variance-covariance matrix based on Lajeunesse (2011). For SR and IA, the models were fitted with the \textit{rma.mv} function of the R package ‘metafor’ (Viechtbauer, 2010). Observations were weighted by the inverse of the sampling variance (Table 1), which we calculated from standard deviation directly from papers or through personal contact with the authors. We imputed missing standard deviations using the coefficient of variation from all complete cases with the R package ‘metagear’ (Lajeunesse, 2016).

Since MSA and GMA have a different structure compared to log-transformed response ratios and standard deviations are not reported for these derived metrics, we used the number of replicates in each experiment to weight the observations (Soons et al., 2017). We fitted multi-level linear mixed-effect models for MSA and GMA with the \textit{lme} function of the R package ‘nlme’ (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2017). Finally, we used null models to estimate the weighted mean pooled effect size, namely the overall amount of plant diversity change across all experiments, independently from the amount of N addition. Based on these models, we also investigated publication bias with visual estimation of the funnel plots (Nakagawa & Santos, 2012). We tested the significance of funnel plots asymmetry with the Egger’s test by fitting the residuals of the null model with observation precision (1/SE or the inverse of number of replicates) as a moderator (Møller & Jennions, 2001; Nakagawa & Santos, 2012). Results of null models and publication bias are reported in Appendix S5. All analyses were performed in the R environment (version 3.4.2) (R Core Team, 2017).

**Results**
We found all metrics of plant diversity to respond negatively to increasing yearly N addition (Figure 3). The single meta-regression models estimated different amounts of plant diversity loss per unit of N addition, depending on the metric considered. For example, with a yearly amount of 100 kg N ha\(^{-1}\) yr\(^{-1}\) the models indicated a relative loss in species richness by 17% and in individual abundance by 64%, whereas the MSA and GMA were estimated to be reduced by 34% and 36%, respectively, compared to the control plots. Only the GMA metric showed a non-linear relationship with yearly N amounts, indicating that a small amount of N addition may lead to an increase in abundance or evenness (Figure 3d).

The multiple meta-regression models showed that responses of plant biodiversity to N addition are influenced by various environmental and experimental covariates (Table 2; see Appendix S6 for detailed model outputs). Climatic moderators were found to influence the responses of the abundance metrics, indicating stronger declines in areas with greater mean annual precipitation (for IA and GMA) or higher mean annual temperature (for MSA). In addition, the lowest BIC model for SR retained a significant interaction between yearly N addition amounts and mean annual temperature (Table 2). Species richness decreased not only with yearly N addition amounts, but also with experimental duration, indicating cumulative effects over time. We also found that plot size was a relevant moderator for SR, with larger relative losses occurring in smaller plots. Additionally, we found that overall losses in SR were less pronounced in soils with higher cation exchange capacity (CEC). For instance, after a 5-year experiment with an addition level of 100 kg N ha\(^{-1}\) yr\(^{-1}\), the model estimates 10% of species richness loss for soils with a moderately high buffering capacity to acidification (CEC = 35 cmol kg\(^{-1}\)). However, estimated species richness loss drops to 30% if the same experiment (i.e. same duration and yearly N addition) is conducted on a poorly buffered soil (CEC = 8 cmol kg\(^{-1}\)). The best model for MSA retained a significant interaction between yearly N addition amount and fertilizer type, with stronger declines for N applied in a reduced form (NH\(_4^+\)in urea or ammonium sulphate) as compared to fertilizer containing oxidised N forms (NO\(_3^-\) in ammonium nitrate or alkali nitrates).
We did not find a significant interaction between N application and ecosystem type for any metric, indicating that the overall direction of biodiversity change with increasing yearly N addition was the same in all the ecosystem types considered (Figure 4). For plant life-form types, we did not find a significant interaction with N application either, i.e., all plant groups decreased with increasing N addition amounts. A single regression model with life-form types as moderator indicated the largest mean losses for the most N-sensitive groups (-85% for legumes; -75% for non-vascular plants; Figure 5). The responses of woody species and ferns showed larger variation and was not significantly different from zero.

Discussion

N dose-response relationships

The biodiversity loss observed was strongly driven by the amount of yearly N addition. The higher the N addition to the soil, the larger the negative impact on local plant diversity, reflecting that the coexistence of different species is promoted by nutrient limitation (Harpole et al., 2011; Soons et al., 2017). Growing accumulation of N in the soil increases soil acidification, which progressively determine abundance loss up to the complete extirpation of species adapted to N-poor conditions (Bobbink et al., 2010). In addition, eutrophication caused by N enrichment causes plant diversity losses through enhanced light competition (Hautier, Niklaus, & Hector, 2009). The negative relationships between plant biodiversity and the amount of N addition agree with the results of previous meta-analyses conducted on a large geographical extent across multiple ecosystems types (De Schrijver et al., 2016; Soons et al., 2017) and in mountain grasslands specifically (Humbert et al., 2016), although these studies did not consider species abundance. Abundance metrics and species richness were found to decrease at different rates as N addition increased. The largest declines were observed for IA, possibly because at the assemblage level extremely negative responses of some species (like the full extirpation occurring in the treatment plot) might be buffered by positive responses of other species in the same plot.
Experimental duration and cumulative N enrichment

For species richness, we found that experimental duration had a negative additive effect comparable in magnitude to the effect of the yearly N addition amount (Table 2), in accordance with the results of Humbert et al. (2016). This suggests that plant communities respond similarly to cumulative N application and cumulative atmospheric N deposition (Stevens et al., 2004; Duprè et al., 2010) and indicates that large diversity losses may occur even at low yearly N amounts when fertilization is protracted over a long-time period (Clark & Tilman, 2008). In the short term, species richness loss due to N application is likely to be buffered by species gain. However, species turnover tends to decline after several years of N addition (i.e. long experimental duration), when plant communities have become adapted to N inputs and populations of a few well-established N-tolerant species dominate the plots (Dise et al., 2011; Bobbink & Hettelingh, 2011). The absence of an effect of experimental duration on the responses of the species abundance metrics may reflect that these metrics do not capture effects of species replacement, because they include only species that were already present in the controls. Further, our models did not reveal a significant modifying influence of the background N deposition on the biodiversity responses (Table 2). This might reflect that background annual N deposition rates were too small (0.7-46.3 kg N ha⁻¹ yr⁻¹) compared to N amounts applied in the experiments. In addition, it may reflect that the data source used to retrieve the N deposition levels (50 * 50 km resolution) was not detailed enough to adequately capture the site-specific deposition rates.

Scale dependence

There is evidence that effects of experimental N addition on local species richness are scale-dependent. For example, Lan et al. (2015) found that proportional loss following N addition was significantly higher in larger plots (> 8 m²). Contrary to these findings, we did not find a significant interaction between the rate of species richness change and plot size and we found overall larger richness loss in smaller plot sizes (1 x 1 m or less) compared to larger ones (3 x 3 m or more). Possibly, in larger plots chances are bigger to survey a few remaining individuals of the same species,
decreasing the chance of full extirpation from the sampled area. Like our results, Perring et al. (2018) found that richness response ratios across 1814 survey-resurvey plots in European temperate forest understories were positively related to the plot size of the survey. This may reflect that chances to encounter the same species in two different plots increase with plot size.

As we studied effects on local or site-level biodiversity only, we cannot make inferences on the impacts of N on plant biodiversity at larger extents. Trends in local biodiversity have implications for changes in biodiversity at larger scales, but the mechanisms involved in these links are not yet fully understood (McGill, Dornelas, Gotelli & Magurran, 2015). Chase (2010) found that higher beta diversity (specifically spatial turnover) in more productive mesocosms yielded higher overall (gamma) diversity at greater nutrient levels. However, the extent to which such effects will also occur in response to atmospheric N deposition remains elusive, as atmospheric deposition levels are lower than typical experimental N addition doses and because responses may be confounded by influences of other environmental pressures. This may also explain why previous analyses of temporal changes in site-level plant diversity revealed no clear trends in species richness (Vellend et al. 2013; Vellend et al., 2017), despite increasing atmospheric N deposition levels occurring in the last century.

**Effect of N fertilizer type**

In our analysis, fertilizer type itself did not induce a significant response in any of the metrics considered, indicating similar overall impacts of the two types of N fertilizer. However, we found that MSA decreased more strongly when N was added as urea or ammonium nitrate (containing only \( \text{NH}_4^+ \)) rather than ammonium nitrate or alkali nitrate (fertilizers also containing \( \text{NO}_3^- \)). In general, differences in the chemical form of fertilizer applied are very often neglected in the experimental design of N addition studies (but see Dias, Malveiro, Martins-Loução, Sheppard, & Cruz, 2011; Song et al., 2012). Yet, evidence suggests that plant species occurring in the same community differ in their ability to take up \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) forms, implying that plant community composition and abundance may strongly depend on the partitioning of differentially available soil N forms (Kahmen, Renker, Unsicker, & Buchmann, 2006; McKane et al., 2002; Miller & Bowman, 2002). Various studies in
Northern Europe suggest that larger species losses are expected with increasing \(NH_4^+\) deposition due to increased acidification, especially in case of oligotrophic ecosystems that are sensitive to \(NH_4^+:NO_3^-\) increase, such as heathlands, bogs, and acidic grasslands (Kleijn, Bekker, Bobbink, Graaf, & Roelofs, 2008; Paulissen, van der Ven, Dees, & Bobbink, 2004), while acidification tends to be less severe when \(NO_3^-\) fertilizers are applied instead (van den Berg et al., 2008). Future nutrient addition experiments should account for the type of fertilizer applied to better elucidate such differences.

**Soil properties**

Soil acidification is one of the major processes to drive biodiversity loss following atmospheric N enrichment (Stevens et al., 2011). Yet, we did not find any evidence of soil pH modifying the relationship between local plant biodiversity and N addition, similar to the results of previous meta-analyses (De Schrijver et al., 2011; Humbert et al., 2016). Soil acidity follows a negative linear relationship with base saturation (exchangeable base cations) (Beery & Wilding, 1971). However, the drop in base saturation is independent of initial soil pH, but it is dependent on soil cation exchange capacity (CEC) when the soil pH ranges between 4-7 units, as in the case of our data (Helling, Chesters & Corey, 1964, De Vries et al., 1989; Ulrich, 1986). This may explain why we found that the response of species richness was not modified by initial soil pH, but instead was related to the soil CEC, which reflects the ability of the soil to buffer N-induced acidification. Thus, in sites with higher soil CEC, the negative impact of N addition through acidification is reduced by base cation exchange in the soil, resulting in a lower species loss compared to sites with low CEC. Similar to our findings, greater species loss has been associated with lower soil CEC across 23 N-addition experiments in North America (Clark et al. 2007). Likely, soil CEC may also explain the small species richness response observed in peatlands and bogs, where overall mean effect size was close to zero (Figure 4). These ecosystems had the highest soil CEC values in our data (32 ± 3 cmol kg\(^{-1}\)), reflecting the high organic matter content that characterizes peatland soils.

**Climate**
The best models selected for the abundance metrics retained main effects of the two climatic moderators (Table 2), suggesting that overall larger abundance losses occur in sites with higher mean annual temperature (for MSA) and precipitation (for IA and GMA). We also found evidence that the slope of the dose-response relationship for species richness is dependent on mean annual temperature at the site level, indicating that richness decreases more steeply with N dose in warmer sites. Similar outcomes have been reported for species richness of mountain grasslands (Humbert et al. 2016) and the abundance of *Sphagnum* mosses (Limpens et al. 2011), probably because N uptake tends to increase with temperature (Cross, Hood, Benstead, Huryn, & Nelson, 2015). In grasslands, higher temperature and precipitation have been found to amplify aboveground biomass growth in response to N addition (Shaw et al., 2002; Zavaleta, Shaw, Chiariello, Mooney, & Field, 2003). Similarly, in forests and tundra ecosystems, temperature has been shown to positively affect net primary productivity following N addition (LeBauer and Treseder 2008). This in turn negatively influences plant biodiversity, as increased biomass results in increased competition for light and in the loss of rare species (Soons et al., 2017). In addition, higher precipitation could also lead to increased N mineralisation (Yang et al. 2017) which, in the absence of increased N loss via leaching or gaseous emissions, could result in higher N availability and increased biodiversity loss. Although in general plant assemblage responses in our analysis were not very different among ecosystem types, the modifying role of temperature and precipitation highlight the importance to account for biogeographical and climatic gradients to assess the impacts of N enrichment on local plant diversity across large geographical extents.

*Individual responses of plant life-form types*

We found that abundance losses were particularly large for legumes and non-vascular plants (mosses and lichens). Indeed, both groups have been identified as the most sensitive to increased N inputs (Bobbink et al., 2010; Craine et al., 2002). Previous studies showed that vascular plants are known to outcompete mosses after N enrichment due to light competition (Malmer, Albinsson, Svensson, & Wallen, 2003; van der Wal, Pearce, & Brooker, 2005), with a substantial decline of nonvascular plants...
beyond 10-15 kg N ha⁻¹ yr⁻¹ (Bobbink et al., 2010). A large negative response of legumes was also expected, as increased soil N availability represents a disadvantage for N fixation (Craine et al., 2002).

Long-term fertilization studies conducted on multiple sites in the USA found substantial declines in N-fixers (Suding et al., 2005) and an overall large decline in total legume biomass was also detected in previous systematic reviews (Fu and Shen, 2016; Humbert et al., 2016). In addition, we found the abundance of individual graminoids decreased, on average, by half. This contradicts the general hypothesis that graminoids tend to become dominant following N enrichment (see e.g. Bobbink et al., 2010; Dise et al., 2011) and contrasts with previous meta-analyses of N addition studies that reported significant increases in total biomass of grasses and sedges (De Schrijver et al., 2011; Fu and Shen, 2016; Humbert et al., 2016). Such discrepancies with our results could reflect the fact that grass encroachment following N input usually comes about by one or few species only (Bobbink et al., 2010) while the rest of the graminoid species are progressively outcompeted in the treatment plots, resulting, on average, in a loss of graminoids’ individual abundance. Finally, the relatively small impacts on woody species might be due to longer persistence in vegetation thanks to their longer life span, which may exceed the typical duration of the experiments.

Further insight into the mechanisms behind community change with N enrichment, including individual abundance responses, may be provided by trait analyses (see e.g. La Pierre & Smith, 2015; Read, Henning, Classen & Sanders, 2018). However, analyses of changes in plant functional traits (at both within- and among-species levels) were out of scope of our meta-analysis and the primary studies analysed.

**Concluding remarks**

We showed the importance of minimizing N enrichment in terrestrial ecosystems to reduce local plant biodiversity loss. Compared to several previous studies that summarized the impacts of N-addition experiments on plant biodiversity, we improved our understanding of the responses of plant communities to N enrichment by including not only species richness but also abundance metrics, which showed stronger responses and have been unexplored in meta-analyses so far. Further, we shed
more light on the roles of different moderators influencing the response of species richness and abundance, thus showing how biodiversity loss is context-dependent and underlining the importance to integrate multiple dimensions of biodiversity into assessments of biodiversity responses to global environmental change.
References


Table 1: Summary table of the metrics and weights used to quantify biodiversity change in the meta-analysis.

<table>
<thead>
<tr>
<th>Effect size</th>
<th>Description</th>
<th>Calculation</th>
<th>Weight</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species richness (SR)</td>
<td>Log-transformed response ratio of mean species richness in the treatment (ST) and control (SC)</td>
<td>$SR = \ln \left( \frac{\bar{S}_T}{\bar{S}_C} \right)$</td>
<td>Inverse of the sampling variance</td>
<td>De Schrijver et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bernhardt-Römermann et al. (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Humbert et al. (2016)</td>
</tr>
<tr>
<td>Individual species abundance (IA)</td>
<td>Log-transformed response ratio of mean individual abundance of species in the treatment (AT) and control (AC) *</td>
<td>$IA = \ln \left( \frac{\bar{A}_T}{\bar{A}_C} \right)$</td>
<td>Inverse of the sampling variance</td>
<td>Benítez-López et al. (2017)</td>
</tr>
<tr>
<td>Mean species abundance (MSA)</td>
<td>Mean of the individual species abundance response ratios (truncated at 1 if AT &gt; AC). N is number of species in each observation.</td>
<td>$MSA = \frac{\sum_{\bar{A}_T &lt; \bar{A}_C} (\bar{A}_T - \bar{A}<em>C) + \sum</em>{\bar{A}_T \geq \bar{A}_C} 1}{N}$</td>
<td>Number of replicates</td>
<td>Alkemade et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Benítez-López et al. (2010)</td>
</tr>
<tr>
<td>Geometric mean abundance (GMA)</td>
<td>Mean of log-transformed response ratios of mean individual abundance. N is number of species in each observation.</td>
<td>$GMA = \exp \left( \frac{\sum (\ln(\bar{A}_T) - \ln(\bar{A}_C))}{N} \right)$</td>
<td>Number of replicates</td>
<td>Buckland et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Schipper et al. (2016)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Santini et al. (2017)</td>
</tr>
</tbody>
</table>

* Before log-transformation, the ratio was first transformed following Smithson and Verkuilen (2006) to shrink the data and avoid zero values in the treatment. See ‘Methods’
Table 2: Standardized coefficients (slope estimates) of terms retained in the best meta-regression models based on the Bayesian information criterion (BIC). Nadd = amount of yearly N addition; duration = duration of the experiment; CEC = cation exchange capacity; plot size = size of the plot; Nadd:MAT = interaction term between Nadd and MAT; Nadd:NO₃ / Nadd:NH₄ = interaction term (slope) of responses to Nadd depending on fertilizer used in the experiment (containing NO₃ or NH₄ only, respectively); MAP = mean annual precipitation; MAT = mean annual temperature. The omnibus test statistics ($Q_M$ and $P_Q$) indicate the amount of residual heterogeneity explained for each individual moderator and for the whole model. In case of an interaction, the omnibus test is reported for the interaction term only. See Appendix S6 for detailed model outputs.

<table>
<thead>
<tr>
<th>Effect size</th>
<th>Fixed effect (moderators)</th>
<th>Estimate</th>
<th>SE</th>
<th>Z-value</th>
<th>LCI</th>
<th>UCI</th>
<th>$P$-value</th>
<th>$Q_M$ (d.f.)</th>
<th>$P_Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species richness (SR)</strong></td>
<td>Nadd</td>
<td>-0.111</td>
<td>0.016</td>
<td>-6.855</td>
<td>-0.142</td>
<td>-0.079</td>
<td>&lt;.0001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>duration</td>
<td>-0.093</td>
<td>0.024</td>
<td>-3.909</td>
<td>-0.140</td>
<td>-0.046</td>
<td>&lt;.0001</td>
<td>15.7 (1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>CEC</td>
<td>0.076</td>
<td>0.023</td>
<td>3.237</td>
<td>0.030</td>
<td>0.122</td>
<td>0.001</td>
<td>10.5 (1)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>plot size</td>
<td>0.101</td>
<td>0.024</td>
<td>4.168</td>
<td>0.054</td>
<td>0.149</td>
<td>&lt;.0001</td>
<td>17.4 (1)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>MAT</td>
<td>-0.015</td>
<td>0.024</td>
<td>-0.610</td>
<td>-0.062</td>
<td>0.033</td>
<td>0.542</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Nadd:MAT</td>
<td>-0.049</td>
<td>0.019</td>
<td>-2.599</td>
<td>-0.085</td>
<td>-0.012</td>
<td>0.009</td>
<td>6.7 (1)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Individual species abundance (IA)</strong></td>
<td>Nadd</td>
<td>-0.275</td>
<td>0.081</td>
<td>-3.389</td>
<td>-0.434</td>
<td>-0.116</td>
<td>0.001</td>
<td>11.5 (1)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>-0.441</td>
<td>0.146</td>
<td>-3.011</td>
<td>-0.728</td>
<td>-0.154</td>
<td>0.002</td>
<td>9.1 (1)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Mean species abundance (MSA)</strong></td>
<td>Nadd:NO₃</td>
<td>-0.014</td>
<td>0.014</td>
<td>-0.958</td>
<td>-0.042</td>
<td>0.014</td>
<td>0.014</td>
<td>6.5 (1)</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Nadd:NH₄</td>
<td>-0.072</td>
<td>0.022</td>
<td>-2.552</td>
<td>-0.145</td>
<td>0.000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>MAT</td>
<td>-0.050</td>
<td>0.023</td>
<td>-2.314</td>
<td>-0.092</td>
<td>-0.008</td>
<td>0.025</td>
<td>5.2 (1)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>26.0 (2)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td><strong>Geometric mean abundance (GMA)</strong></td>
<td>Nadd</td>
<td>-0.103</td>
<td>0.037</td>
<td>-2.796</td>
<td>-0.175</td>
<td>-0.030</td>
<td>0.008</td>
<td>6.8 (1)</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>MAP</td>
<td>-0.181</td>
<td>0.059</td>
<td>-3.079</td>
<td>-0.295</td>
<td>-0.065</td>
<td>0.004</td>
<td>9.5 (1)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.3 (3)</td>
<td>&lt; .0001</td>
</tr>
</tbody>
</table>
Figure 1: Graphical representation of a) relationships between key factors (i.e. moderators; pink boxes) and fundamental processes (grey boxes) that trigger plant species responses in N-addition experiments. Solid arrows represent direct effects, while dashed arrows represent context-dependent effects (i.e. in the experiments, the extent of soil acidification and N mineralisation may be positively or negatively affected by soil fertility and climatic conditions, respectively), and b) the linkages between the changes in biodiversity metrics considered in this study. Richness and abundance represent the two dimensions of biodiversity affected by N addition, with ‘-’, ‘0’ and ‘+’ on the axes indicating loss, no change and increase, respectively. SR = species richness, IA = individual species abundance, MSA = mean species abundance, and GMA = geometric mean abundance.
Figure 2: Geographical distribution of the studies included in the meta-analysis. Studies included experiments reporting on species richness only (= red circles); abundance only (= blue squares); or both species richness and abundance (= green triangles). Point size depicts the number of observations available (i.e. the number of N addition level) from each experiment.
Figure 3: Effect of yearly N addition amount (kg N ha\(^{-1}\) yr\(^{-1}\)) on plant biodiversity metrics: a) species richness (SR); b) individual species abundance (IA); c) mean species abundance (MSA); and d) geometric mean abundance (GMA). Solid lines represent model predictions with log-transformed yearly N addition as moderator only, allowing for quadratic term inclusion when significantly improving the goodness of fit (the dotted lines represent the corresponding 95% CI bounds). The dashed lines indicate no biodiversity change compared to the control. Point size depicts observation weight.
**Figure 4:** Mean pooled biodiversity change (and 95% CI) per ecosystem type, expressed as percentage of change in N addition plots compared to control plots. Biodiversity change is quantified with species richness (SR), individual species abundance (IA), mean species abundance (MSA), and geometric mean abundance (GMA). Values are obtained by fitting the models without the intercept term, to estimate the mean pooled effect of each level. Significance level (*$P < 0.01$; **$P < 0.001$; ***$P < 0.0001$) and number of observation is provided for each estimate.
Figure 5: Individual species abundance ratios (and 95% CI) for forbs (F), graminoids (G), leguminosae (L), non-vascular plants (M), ferns (P) and woody species (W) (n = number of observations of each plant life-form type). Extremely negative effect sizes indicate the extirpation of species in the treatment plots. Diamonds represent overall weighted mean effect size estimate for each group (and 95% CI). Significance levels are provided for each mean estimate (**P < 0.001; ***P < 0.0001). The values are obtained by running the model without the intercept term to estimate the mean pooled effect of each level.
Appendix A – Data Sources


Kwak, J.


Vourlitis, G. L., & Pasquini, S. C. (2009). Experimental dry-season N deposition alters species composition in southern Californian mediterranean-type shrublands. *ECOLOGY*, 90(8), 2183–2189. [https://doi.org/10.1890/08-1121.1](https://doi.org/10.1890/08-1121.1)


Xu, X., Liu, H., Song, Z., Wang, W., Hu, G., & Qi, Z. Response of aboveground biomass and diversity to nitrogen addition along a degradation gradient in the Inner Mongolian steppe, China. *Scientific reports*, 5, 10284. [https://doi.org/10.1038/srep10284](https://doi.org/10.1038/srep10284)


Supporting Information

Appendix S1: Search string for primary studies collection

Appendix S2: Summary of primary studies included in the meta-analysis

Appendix S3: Influencing factors on plant diversity response to N addition

Appendix S4: Description of categorical variables (ecosystem types, fertilizers and plant life-form types)

Appendix S5: Mean pooled effect sizes and funnel plots of the null-model residuals

Appendix S6: Detailed model outputs of best meta-regression models