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Negative to positive shifts in diversity effects on soil nitrogen over time

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Soil nitrogen (N) availability is of critical importance to the productivity of terrestrial ecosystems worldwide. Plant diversity continues to decline globally due to habitat conversion and degradation, but its influence on soil N remains uncertain. By conducting a global meta-analysis of 1,650 paired observations of soil N in plant species mixtures and monocultures from 149 studies, we show that, on average across observations, soil total N is 6.1% higher in species mixtures. This mixture effect on total N becomes more positive with the number of species in mixtures and with stand age. The mixture effects on net N mineralization rate and inorganic N concentrations shift from negative in young stands to positive in older stands with greater positive effects in more-diverse mixtures. These effects of mixtures were consistent among cropland, forest and grassland ecosystems and held across climate zones. Our results suggest that plant diversity conservation not only enhances the productivity of current vegetation but also increases soil N retention that will sustain the productivity of future vegetation.

Nitrogen (N) is an essential element for all living organisms and is often the main limiting nutrient for plant carbon assimilation in terrestrial ecosystems worldwide[1–3]. Consequently, changes in soil total N can alter the global carbon cycle, thereby impacting both global climate change and human food security[4]. Although some plants may possess a capacity to take up dissolved organic N in some environments, soil inorganic N (NH_4^+ and NO_3^-) is the major plant-available form for direct uptake through root systems[5]. Soil inorganic N is ultimately determined by the balance between inorganic N inputs via the microbial mineralization of organic N and outputs via the uptake of plants and soil microbes as well as via N leaching and gas emissions[6] (for example, N_2O, N_2, NO and NH_3) (Fig. 1). The net amount of inorganic N produced per unit of time (net N mineralization rate, the difference between gross N mineralization rate and gross N immobilization rate) is highly dependent on soil total N and plant–microbe interactions[7,8]. As local plant biodiversity (at patch, neighbourhood or stand scales) generally supports ecosystem function and is known to influence aspects of nitrogen cycling[6,8–12], and has declined globally due to land-use and related changes[13], its loss has been hypothesized to negatively impact soil N pools. However, support for this idea is uneven, as previous studies have reported positive[9,14], negligible[8] or even negative[15] effects of plant diversity on soil total N.

Plants play a decisive role in regulating soil N as they are the principal pathways through which N enters the soil. Plant diversity can increase plant productivity and N utilization while reducing soil N losses because of the differences of N uptake in space, time or the chemical forms among plant species within a community[11,16–18]. More-diverse plant communities can also increase the chance of having productive, dominant species with high N uptake[19]. Higher productivity and associated N retention induced by plant diversity increase both above- and below-ground litter inputs to soils and, in so doing, increase N return to the soil[14,17] (Fig. 1). Total soil inorganic N availability and relative abundance of soil inorganic N forms (NO_3^-, NH_4^+) are controlled by microbial organisms[7,12] (Fig. 1). Diverse plant communities can increase soil microbial biomass and activities because of a greater amount of plant-derived food and expanded microbial niches[20], and are more likely to enhance both mineralization and immobilization rates of soil N (refs. [21,22]) (Fig. 1). Furthermore, more efficient utilization of soil inorganic N in species-rich plant communities may reduce microbial nitrification rate[12], leading to a decreased NO_3^-/NH_4^+ ratio. However, the effects of plant diversity on soil N may change over time, similar to soil carbon and microbial communities[23,24]. Compared with those in monocultures, soil total and inorganic N in mixtures could be lower in young stands due to higher N uptakes from higher plant productivity with the positive effects of plant diversity on litter production and soil biota lagging temporally[25,26]; but they may become higher over time due to enhanced feedback of litter inputs, decomposition and soil biota as well as reduced N leaching[17,27].

Divergent empirical findings on the effects of plant diversity on soil N could also result from differences in ecosystem type, the presence of N-fixing plants in species mixtures, climate and soil depth (D) sampled. The responses of soil N to plant diversity may also vary contingent on ecosystem type (croplands, grasslands and forests) due to differences in the magnitudes of diversity–productivity relationships caused by dissimilarities in vegetation physiology, structure and lifespan[28]. The presence of N-fixing plants in species mixtures would also change soil microbial communities (for example, abundance of nitrifiers and denitrifiers) and in turn affect diversity effect on soil N cycling[12] (Fig. 1). Given the increased positive plant diversity effects on microbial biomass and respiration with decreasing temperatures[24] and the dominant role of soil-dwelling microorganisms in controlling soil N cycling[7], we expected that the effects of species mixtures on the soil microbial N mineralization rate and associated inorganic N would increase with reduced temperatures. We also expected a stronger effect of species mixtures on soil N in drier climates since the positive effects of plant diversity on biomass, which drives N cycling, tend to be more pronounced in reduced water availability[29]. The positive diversity effects on soil

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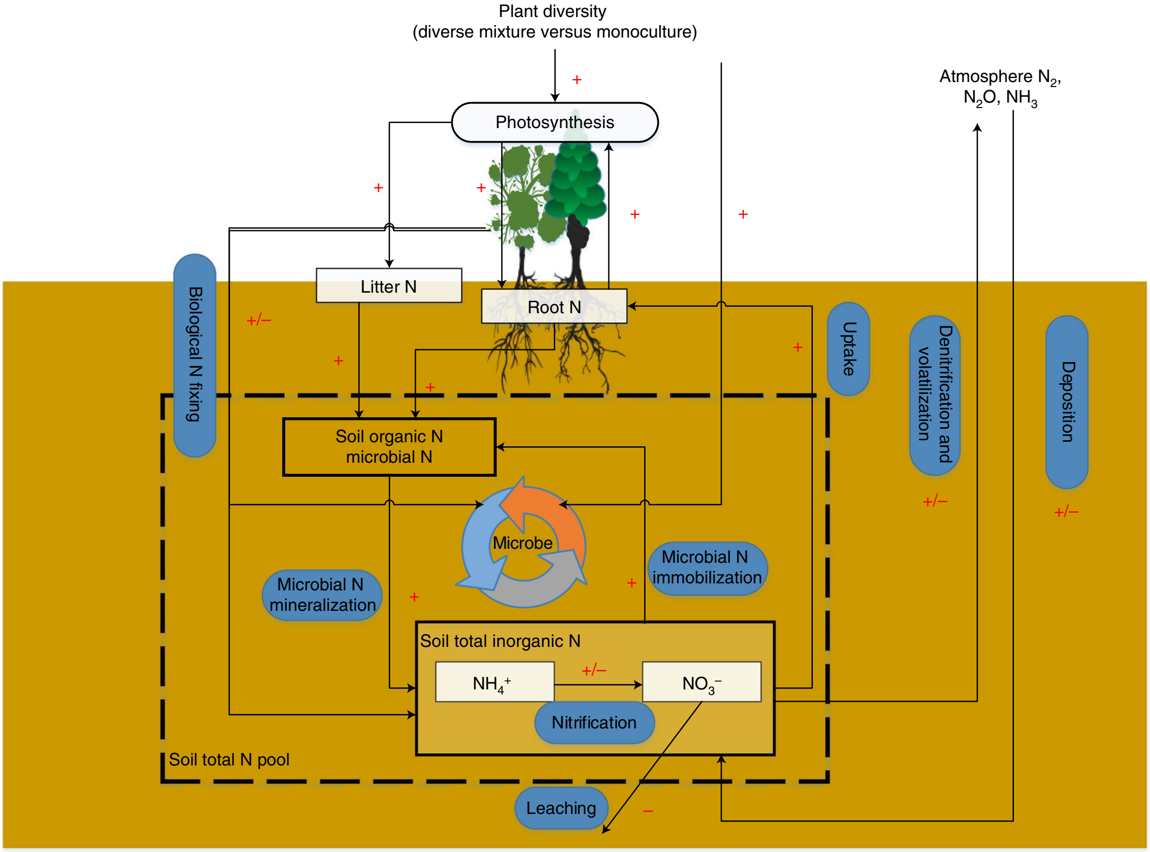

Fig. 1 | A conceptual diagram of the influence of plant diversity on the processes that control soil N. Rectangles represent the main biogenic forms of soil N; blue ovals indicate biogeochemical processes. Symbols '+', '−' and '+/−' represent expected positive, negative and unclear diversity effects on the processes, respectively.

total N may increase with *D* due to a stronger plant diversity effect on root litter inputs in deeper soils[25,30]. By contrast, soil inorganic N may be less in deeper soil layers in mixtures than in monocultures due to increased nutrient uptake from deeper soil layers[31].

We conducted a global meta-analysis with 1,650 paired observations of plant mixtures and constituent monocultures from 149 studies (131 experimental and 18 observational studies) (Supplementary Fig. 1). We quantified the effects of species mixtures as the natural log-transformed response ratio (lnRR) of the observed to the expected values of soil N variables, including total N, net N mineralization rate, total inorganic N, NH_4^+, NO_3^- and the ratio of NO_3^-/NH_4^+, in a mixture. The expected values in a mixture were calculated as the weighted average values of the constituent species in monocultures, in which weights represent the species proportions in the mixture (Methods). We hypothesized that (1) the effects of species mixtures on N would shift from negative to positive over time; (2) these effects would increase with the species richness in mixtures (SR), similar to those reported for above- and below-ground productivity[32,33], soil carbon[23], soil microbial biomass[24] and soil respiration[20]. We tested the preceding hypotheses for total N, net N mineralization rate, total inorganic N, NH_4^+, NO_3^- and the ratio of NO_3^-/NH_4^+ across a wide variety of ecosystem types and environmental conditions.

Results and discussion

As the responses of soil total N, net N mineralization rate, inorganic N, NO_3^-, NH_4^+ and NO_3^-/NH_4^+ to species mixtures, including the average responses and the effects of stand age (SA) and SR, generally held regardless of *D*, mean annual temperature (MAT), aridity index (AI) or ecosystem type (for example, croplands, grasslands and forests) as well as the presence or absence of N-fixing plants in species mixtures (Supplementary Tables 1 and 2), we present these for all data pooled unless otherwise stated. On average, across all sites, mixtures increased soil total N by 6.1% (95% confidence intervals (CIs), 3.4–8.8%), and the mixture effects increased with SA and SR, with more-pronounced positive effects in older, more-diverse mixtures (Fig. 2). Young species mixtures had lower soil total N compared with the expected values calculated from constituent monocultures, probably due to increased uptake of inorganic N through higher productivity while increased N mineralization lagging temporally[17,26]. Larger total soil N in older and more-diverse mixtures could be attributable in part to the substantially reduced N losses via leaching in old, species-rich plant communities[9,34]. The enhanced effect of plant mixtures on soil total N over time, coupled with those for plant productivity, soil carbon and soil microbial communities[17,23,24,32], suggests that plant diversity induces positive feedback to the ecosystem carbon and N cycles via increased carbon assimilation and reduced N leaching.

The mixture effect on soil net N mineralization rate shifted from negative to positive approximately seven years after stand establishment ($P < 0.001$), similarly among ecosystem types (Fig. 2a). The mixture effect on soil inorganic N also shifted from negative to positive over time, with an average effect of −6.9% (CI, −13.2% to −0.6%) due to most observations being in young stands (Fig. 2a). These age-dependent shifts of mixture effects indicate that the accumulated diversity effect on soil N retention over time has promoted N mineralization and subsequently increased inorganic N (refs. [8,10]). For both net N mineralization rate and

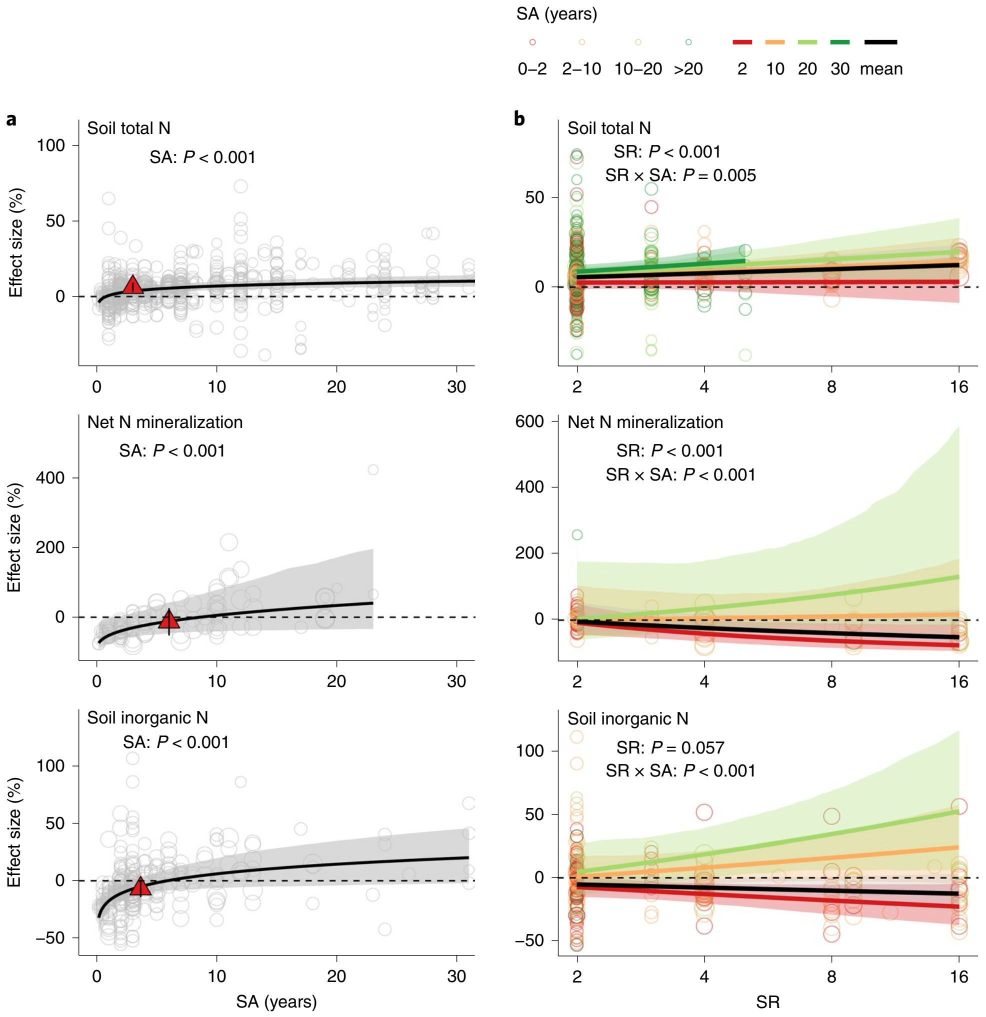

Fig. 2 | Comparison of soil total N, inorganic N and net N mineralization rate in species mixtures versus monocultures. a, In relation to stand age (SA). **b**, In relation to the species richness in mixtures (SR) and by SA intervals. Slope estimates in **a** and **b** are partial dependence, derived from the full model (Methods). The effects are quantified as the percentage changes in mixtures compared with the corresponding mean value of constituent monocultures. Red triangles and error bars represent the overall mean and its 95% CIs. Points represent the values predicted by partial regressions for each explanatory variable, with their sizes representing the relative weights of corresponding observations. The colours of the points indicate the different SAs of corresponding observations. Black and coloured lines represent the average and SA-specific responses, respectively, with their bootstrapped 95% CIs indicated by shading. The significance (P) is presented for each term tested with solid and dashed lines, respectively, for significant and insignificant interaction effects at $\alpha = 0.05$.

inorganic N, their decreases in young mixtures and increases in old mixtures compared with monocultures became more pronounced with increasing SR (Fig. 2b). The reduced net N mineralization rate in young, species-rich mixtures could have resulted from decreased plant N concentration with species diversity due to more efficient use of local resource pools[10,35]. Decreased plant N concentration in species mixture may create a high N demand for decomposers, resulting in large amounts of inorganic N having to be immobilized to increase the litter N concentration to the critical value required for net N release[36]. In addition, soil microbial N mineralization may respond to the increasing plant diversity with a delay of several years after the experiment was established, as observed for other soil microbial functions, such as microbial respiration and biomass[26,27]. The increased net N mineralization in older, species-rich mixtures suggests that gross N mineralization due to increased litter inputs and soil decomposers has increased more than N immobilization associated with decreased plant N concentration[8].

The mixture effects on soil NO_3^- and NH_4^+ also changed with SA, with effects on NO_3^- shifting from negative to null and on NH_4^+ from negative to positive over time, in line with findings of Oelmann et al.[8] (Fig. 3a). Moreover, the mixture effects on soil NO_3^- became more negative with increasing SR in both young and old stands, while the mixture effects on soil NH_4^+ increased with SR in old stands but not in young stands (Fig. 3b). The different responses of soil NO_3^- and NH_4^+ to species mixtures led to an average NO_3^-/NH_4^+ reduction of 22.9% (CI, −43.7% to −2.2%), with greater reductions in more-diverse stands, particularly at older ages (Fig. 3a,b). These results may have arisen from two non-excluding mechanisms. First, higher plant biomass of diverse mixtures might retain more soil moisture, which could increasingly suppress nitrification and, in turn, increase soil NH_4^+ availability[8]. Second, since increased soil organic matter in species mixtures[23] reduces NH_4^+ mobility and its availability to plants[5], species mixtures increase NO_3^- uptake, contributing to reduced NO_3^- leaching in species mixtures[34]. The increased soil NO_3^- and NH_4^+ in older, species-rich

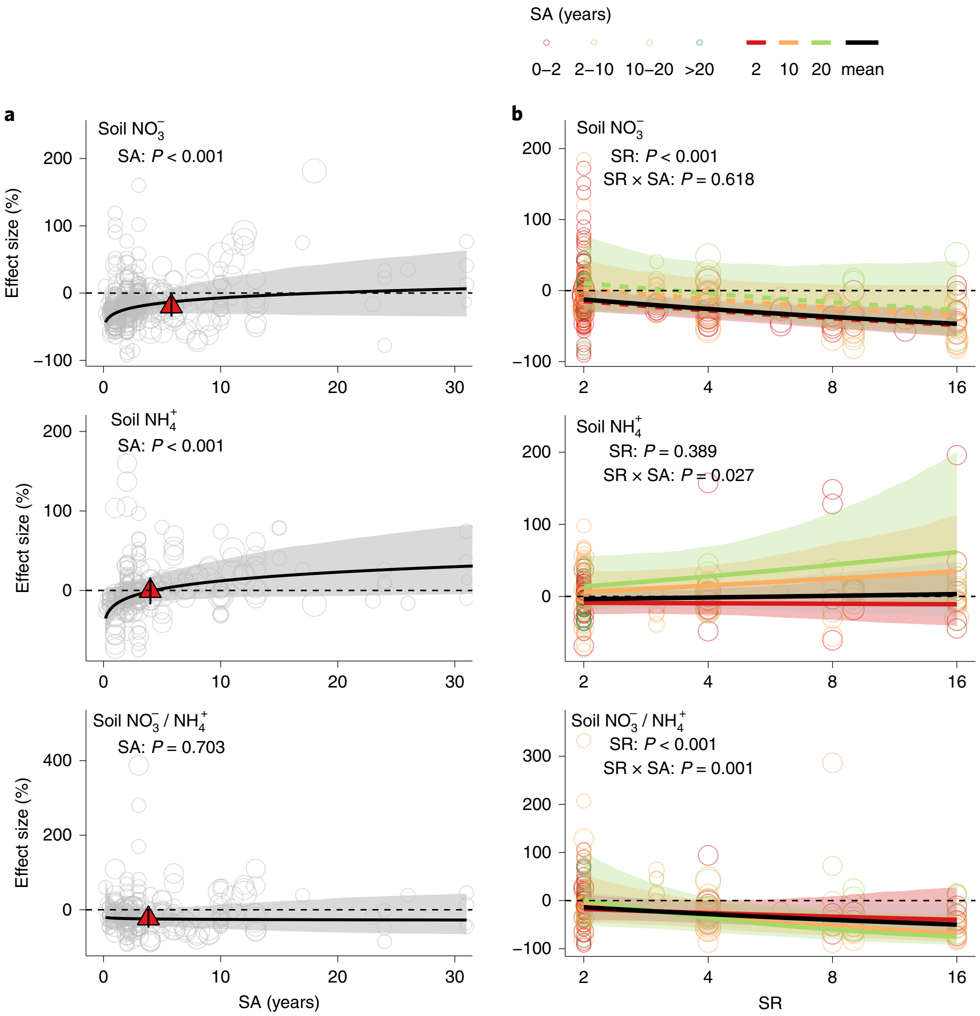

Fig. 3 | Comparison of soil NO$_3^-$, NH$_4^+$ and NO$_3^-$/NH$_4^+$ in species mixtures versus monocultures. a, In relation to stand age (SA). **b**, In relation to the species richness in mixtures (SR) and by SA intervals. Slope estimates in **a** and **b** are partial dependence, derived from the full model. The effects are quantified as the percentage changes in mixtures compared with the corresponding mean value of constituent monocultures. Red triangles and error bars represent the overall mean and its 95% CIs. Points represent the values predicted by partial regressions for each explanatory variable, with their sizes representing the relative weights of corresponding observations. The colours of the points indicate the different SAs of corresponding observations. Black and coloured lines represent the average and SA-specific responses, respectively, with their bootstrapped 95% CIs indicated by shading. The significance (*P*) is presented for each term tested with solid and dashed lines, respectively, for significant and insignificant interaction effects at $\alpha = 0.05$.

mixtures tracked the variation in total inorganic N and net N mineralization rate as well as those of soil microbial biomass and activity reported in a previous meta-analysis[24], suggesting increased litter inputs and soil decomposers may diminish the initial negative effect of plant diversity on soil NO$_3^-$ and NH$_4^+$ availability[6]. However, long-term diversity manipulation experiments in Jena found that soil NO$_3^-$ concentrations decreased with increasing richness, and this effect was consistent over time, which could be attributed to the reduced soil N availability due to frequent N removals from biomass harvests[27]. As N accumulation in groundwater and/or surface waters is one of the pressing environmental issues worldwide[37], conserving terrestrial plant diversity may help reduce NO$_3^-$ contamination in water.

Global conversion of species-rich natural forests and grasslands into single-species plantations and agricultural crops is a major cause of local biodiversity loss[13]. To better contextualize our results in the context of the proposed global restoration[38], we estimated the effects of 10%, 20%, 40% and 60% losses (that is, 100% SR at the establishment versus 90%, 80%, 60% and 40% at the establishment) from assemblages of 16 species (the highest SR level studied for both soil total N and inorganic N) on the basis of the estimated effects of the SR and SA (Fig. 2). We found that lower plant SR at establishment led to a detectable loss of soil total N and inorganic N in the second and fifth years, respectively. A 40% decrease in plant SR over a decade from conversion led to a loss of 2% and 5% in soil total N and inorganic N, respectively (Fig. 4). Given that global forests and grassland contain about 82 Pg of soil N (ref. [39]), a 40% decrease in SR in 10% of these areas over ten years could release 0.16 Pg of N from the soil (average 16 Tg yr^{-1} of N). This estimate of soil N loss with a 40% decrease in SR over 10% of the global forest and grassland area represents about 17% of global annual application of anthropogenic nitrogenous fertilizer (96 Tg yr^{-1} of N in 2010;[40]). This highlights that plant diversity conservation will have important and substantive positive impacts on soil N availability and associated ecosystem function[23,24,32], and perhaps more than might have been concluded on the basis of experiments on timescales of less than one decade.

Because of the importance of N-fixing plants for soil N accumulation and cycling[10,14], we examined whether the presence of

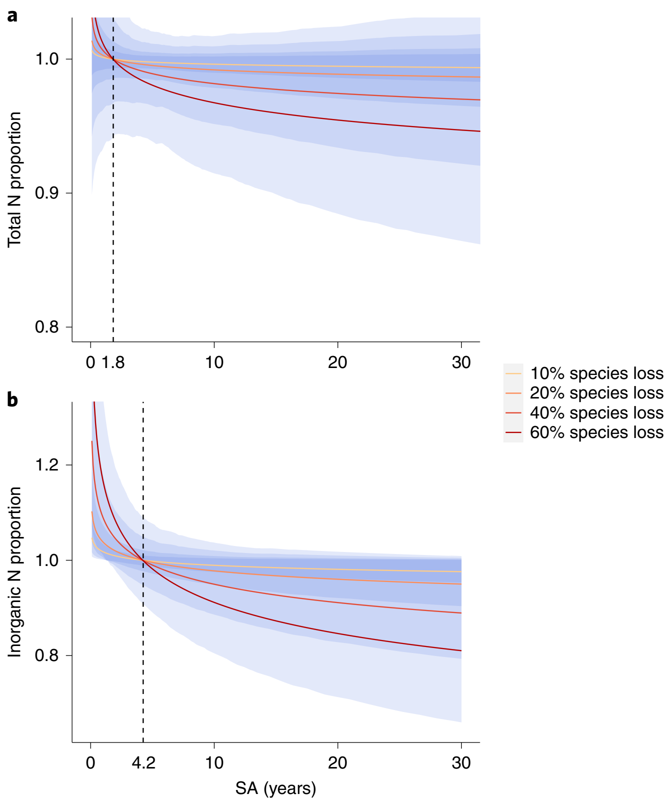

Fig. 4 | Predicted responses of soil total N and inorganic N to a range of plant species richness (SR) reductions at the establishment. a, Total N. **b**, Inorganic N. Coloured lines represent the SR reduction-specific responses with their bootstrapped 95% CIs indicated by shading. The dotted vertical lines represent the time when plant SR began to reduce the soil total N and inorganic N.

N-fixing plants in mixtures influences plant diversity effects on soil N. We included the presence (or absence) of N-fixing plants as a factor in our models to account for the effects of N-fixing plants. We found that presence or absence of N-fixing species in plant mixtures did not alter the species mixture effects on soil inorganic N, NH_4^+ and net N mineralization rate except soil NO_3^-, which responded more negatively in the mixtures with N-fixing plants ($P = 0.027$) (Supplementary Table 2 and Supplementary Fig. 2). This suggests that the positive effect of species mixtures on soil total N and negative effects on soil inorganic N are consistent among N-fixing and non-N-fixing plant communities. The stronger negative effect of species mixtures on soil NO_3^- in the mixtures with N-fixing plants was partly explained by the higher denitrification rate in mixtures with N-fixing plants[12]. The presence of N-fixing plants may accelerate the activity of denitrifiers and thereby decrease soil NO_3^- availability[12]. We also found that the presence or absence of N-fixing plants yielded qualitatively similar estimates for the effects of SR and SA (Supplementary Tables 2 and 3).

The responses of soil inorganic N, NO_3^-, NH_4^+ and net N mineralization rate to species mixtures were not altered by *D*, MAT, AI or ecosystem type (for example, croplands, grasslands and forests) (Supplementary Table 1 and Supplementary Fig. 3). This suggests that the effect of species mixtures on soil inorganic N is globally consistent across climates and ecosystem types, similar to the reported effects of species mixtures on above-ground and below-ground productivity[32,33], soil microbial biomass[24], soil respiration[20] and soil carbon[23]. However, when analysed by individual ecosystem types, plant mixture effects were not always similar between ecosystem types because of the differences in SR and SA (Supplementary Fig. 3). Therefore, the effects of ecosystem types without considering their difference in SR and SA should be interpreted with caution. In addition, we found the positive effect of species mixtures on soil total N increased with *D* (Supplementary Fig. 4a) because more N is reallocated to deeper soil layers from the topsoil in species mixtures due to deeper fine-root distribution in plant species mixtures compared with monocultures[31]. We also found that the positive species mixture effects on soil total N were more apparent in drier climates (Supplementary Fig. 4b), similar to diversity effects on productivity[41]. These water availability-dependent effects are attributable to the stronger plant diversity effect on productivity-induced litter production and soil biota abundance (for example, earthworms, collembola) in drier climates[41,42]. The higher litter production and soil biota could increase soil N retention via enhanced microbial immobilization sink for N (ref. [43]). Alternatively, soils in diverse communities may have higher moisture than those in monocultures[8], which could favour N denitrification and associated N loss in gaseous forms (for example, N_2, N_2O) in wetter sites[12].

Our meta-analysis demonstrates that the effects of plant species mixtures on soil N are strongly dependent on SA, shifting from negative in young stands to positive in older stands, with these age-dependent effects increasing with the species richness in mixtures. Our results highlight that global biodiversity loss, caused by land-use change such as the conversion of species-rich forests and grasslands into species-poor systems[13], not only decreases plant productivity[32,33] and soil carbon sequestration[23] but also reduces soil N availability for the future vegetation. Plant diversity conservation will sustain long-term soil N availability and reduce N pollution in the global hydrosphere.

Methods

Data collection. We systematically searched all peer-reviewed publications that were published before June 2020 that investigated the effects of plant diversity on soil total N, inorganic N and net N mineralization rate using the Web of Science (Core Collection; http://www.webofknowledge.com), Google Scholar (http://scholar.google.com) and the China National Knowledge Infrastructure (CNKI; https://www.cnki.net) with the search term 'soil nitrogen OR soil N OR nitrate OR ammonia OR no3 OR nh4 OR nitrogen mineralization AND plant diversity OR richness OR mixture OR pure OR polyculture OR monoculture OR overyielding'. We also searched for references within these papers. Our survey also included studies summarized in previously published diversity–ecosystem functioning meta-analyses[20,23,24]. The literature search was performed following the guidelines of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al.[44]; Supplementary Fig. 5).

We employed the following criteria to select the studies: (1) they were purposely designed to test the effects of plant diversity on soil total N (both total N content and stock), total inorganic N, NO_3^-, NH_4^+ and net N mineralization rate in mineral soils; (2) they had at least one species mixture treatment and constituent monocultures; (3) they had the same initial climatic and soil properties in the monoculture and mixture treatment plots. To better represent responses of soil N under natural conditions, we did not include greenhouse and mesocosms studies. In total, 141 publications met these criteria (Supplementary Fig. 5 and Supplementary Table 4). In ten publications, several experiments, each with independent controls, were conducted at different locations and were considered to be distinct studies. When different publications included the same data, we recorded the data only once. When a study included plant species mixtures of different numbers of species, we considered them as distinct observations. This resulted in 115 studies for soil total N (including both N content and stock), 50 studies for soil inorganic N, 26 studies for net N mineralization, 43 studies for soil NO_3^- and 41 studies for soil NH_4^+. Across all studies, the responses of soil N content and stock to species mixtures were statistically not significant (Supplementary Fig. 6).

For each site, we extracted the means, the number of replications and the standard deviations of soil total N, inorganic N, the net N mineralization rate, NO_3^- and NH_4^+, if reported. The net N mineralization rate data included both in situ covered-cylinder and laboratory-incubated measurements. Across all studies, the in situ covered-cylinder and laboratory-incubated measurements of mixture effects on net N mineralization rate did not differ significantly (Supplementary Fig. 7). We note, however, as all original studies in our metadata measured net N mineralization rates with plants and soils separated in what is a coupled system, there are uncertainties in the estimates of the true N mineralization rates in the field.

When an original study reported the results graphically, we used Plot Digitizer version 2.0 (ref. [45]) to extract data from the figures. We also extracted SR, functional-group richness (FR, number of plant functional groups), *D*, ecosystem type (for example, forest, grassland, cropland), latitude, longitude, MAT (°C), SA and soil pH measured in water (pH (H_2O), if reported) from original or cited papers, or cited data sources. Annual AI data were retrieved from the Consultative Group for International Agricultural Research Consortium for Spatial Information Global Aridity Index dataset[46] through the use of location information. The annual AI was calculated as the ratio of mean annual precipitation to mean annual potential evapotranspiration[47]. SA was recorded as the number of years since stand establishment after a stand-replacing disturbance in forests and the number of years between the initiation and measurements of the experiments in grasslands and croplands. The *D* was recorded as the midpoint of each soil-depth interval[48]. The species proportions in mixtures were based on the basal area or stem density in forests, coverage in croplands and sown seeds in grasslands. Plants in forests were classified into three functional groups (coniferous trees, leguminous broadleaved trees and nonleguminous broadleaved trees). Those of grasslands were classified into four functional groups (C_3 grasses, C_4 grasses, nonleguminous forbs and legumes), whereas those of croplands were classified into two functional groups (grains and legumes). The soil total N data included both experimental and observational environments (18 studies from 7 publications conducted in natural forests, Supplementary Table 4). We compared the estimates for the datasets with and without these natural forest studies and found that both datasets yielded qualitatively similar results (Supplementary Tables 3 and 5). Thus, we reported results based on the whole dataset.

Data analysis. The natural log-transformed response ratio (lnRR) was employed to quantify the effects of plant diversity following Hedges et al.[49]:

$$\ln RR = \ln(\bar{X}_t/\bar{X}_c) = \ln \bar{X}_t - \ln \bar{X}_c \tag{1}$$

where $\bar{X}_t$ and $\bar{X}_c$ are the observed values of a selected variable in the mixture and the expected value of the mixture in each study, respectively. We calculated $\bar{X}_c$ on the basis of weighted values of the constituent species in monocultures following Loreau and Hector[50]:

$$\bar{X}_c = \sum (p_i \times m_i) \tag{2}$$

where m_i is the observed value of the selected variable of the monoculture of species *i* and p_i is the proportion of species *i* density in the corresponding mixture. If a study had multiple richness levels in mixtures (for example, 1, 4, 8 and 16), lnRR was calculated for the SR levels 4, 8 and 16, respectively. When a study reported multiple types of mixtures (SR levels) and experimental years, $\bar{X}_t$ and $\bar{X}_c$ were calculated separately for each mixture type and experimental year.

In our dataset, standard deviation or the standard error was not reported in 42 of the 141 publications, and no single control group mean estimate was present in 81 of the remaining 99 publications with standard deviation or the standard error reported. Like in previous studies[33,51], we employed the number of replications for weighting:

$$W_r = (N_c \times N_t)/(N_c + N_t) \tag{3}$$

where W_r is the weight associated with each lnRR observation, and N_c and N_t are the number of replications in monocultures and corresponding mixtures, respectively.

Soil total N, total inorganic N, net N mineralization rate, NO_3^-, NH_4^+ and NO_3^-/NH_4^+ were considered as our response variables and analysed separately. To validate the linearity assumption for the continuous predictors, we first graphically plotted the lnRR versus individual predictors and identified logarithmic functions as an alternative to linear functions. We also statistically compared the linear and logarithmic functions with the predictor of interest as the fixed effect, and ‘study’ as the random effect, using the Akaike information criterion (AIC). We found that the logarithmic SR in mixtures, SA and linear *D* resulted in lower, or similar, AIC values (Supplementary Table 6). For consistency, we used logarithmic SR, SA and linear *D* in our final models. We used the following model to determine the effects of the ln(SR), ln(SA), *D* and environmental variables (*E*, that is, ecosystem type, MAT or AI) and their interactions with soil total N, total inorganic N, net N mineralization rate, NO_3^-, NH_4^+ and NO_3^-/NH_4^+:

$$\begin{aligned}\ln RR = {} & \beta_0 + \beta_1 \ln(SR) + \beta_2 \ln(SA) + \beta_3 D + \beta_4 E + \beta_5 \ln(SR) \times \ln(SA) \\ & + \beta_6 \ln(SR) \times D + \beta_7 \ln(SR) \times E + \beta_8 \ln(SA) \times D + \beta_9 \ln(SA) \times E \\ & + \beta_{10} D \times E + \beta_{11} \ln(SR) \times \ln(SA) \times D + \beta_{12} \ln(SR) \times \ln(SA) \times E \\ & + \beta_{13} \ln(SR) \times D \times E + \beta_{14} \ln(SA) \times D \times E + \beta_{15} \ln(SR) \times \ln(SA) \\ & \times D \times E + \pi_{study} + \varepsilon \end{aligned} \tag{4}$$

where β_i, π_{study} and ε are coefficients, the random effect factor of ‘study’ and sampling error, respectively. The random effect explicitly accounted for autocorrelation among observations within each study. We conducted the analysis using the restricted maximum likelihood estimation with the lme4 package[52]. We scaled all continuous predictors (observed values minus mean and divided by one standard deviation). When continuous predictors were scaled, β_0 is the overall mean lnRR at the mean ln(SR), ln(SA) and *D* (ref. [53]). The *E* in equation (4) were individually modelled for two reasons. First, these variables are inherently correlated, and simultaneous modelling would lead to strong multicollinearity[54]. Second, simultaneous modelling would include a large number of predictors, greater than the number of studies in our metadata.

To prevent overfitting[55], we selected the most parsimonious model among all alternatives instead of using stepwise multiple regression, which can be biased and has multiple shortcomings[56]. We applied the condition of keeping SR, SA and their interaction effect as they were intrinsic to the purpose of the study for assessing the effects of SR and SA in mixtures. Model selection was accomplished by using the ‘dredge’ function of the muMIn package[57], on the basis of the Akaike AIC. For soil total N and net N mineralization rate, there was more than one best model as the models with ΔAIC (AIC differences) ≤ 2 are considered equivalent[58] (Supplementary Table 7). However, these models contained similar terms, and we selected the models with the lowest AIC and highest weight for interpretation[59]. For the final models of soil net N mineralization rate, inorganic N, NO_3^-, NH_4^+ and NO_3^-/NH_4^+, all terms associated with *E* (equation (4)) were excluded. The model selection led to equation (5) for soil total N and equation (6) for soil total inorganic N, net N mineralization rate, NO_3^-, NH_4^+ and NO_3^-/NH_4^+ as the most parsimonious models, respectively:

$$\begin{aligned}\ln RR = {} & \beta_0 + \beta_1 \ln(SR) + \beta_2 \ln(SA) + \beta_3 D + \beta_4 AI \\ & + \beta_5 \ln(SR) \times \ln(SA) + \pi_{study} + \varepsilon \end{aligned} \tag{5}$$

$$\ln RR = \beta_0 + \beta_1 \ln(SR) + \beta_2 \ln(SA) + \beta_3 \ln(SR) \times \ln(SA) + \pi_{study} + \varepsilon \tag{6}$$

We analysed the potential for publication bias to influence our results using Egger’s regressions test for funnel plot asymmetry on mixed-effects models[60], with sample size as the predictor. Egger’s test was run on the main statistical tests we performed (response ratio across the entire dataset and then the response ratio including associated predictors in equations (5) and (6) as covariates). We did not find significant publication bias that might bias our results towards significant effects according to Egger’s regression (Supplementary Table 8). Collinearity among explanatory variables was examined by evaluating variance inflation factor (only models with all predictors having variance inflation factors <3 were accepted[61]), and no multicollinearity problem was found in the most parsimonious models (Supplementary Table 3). We applied Moran’s *I* test and the Durbin–Watson test on the residuals of equations (5) and (6) to assess whether our models could be affected by spatial and temporal autocorrelation. We implemented these tests by using the ‘ape’[62] and ‘DHARMa’[63] packages. We found that none of the regressions was affected by spatial or temporal autocorrelation (all $P > 0.05$, Supplementary Table 9).

To further examine the effects of *E*s, we conducted an analysis with each environment variable individually (ecosystem type, MAT or aridity index) as the only fixed factor and study as the random factor. The analysis confirmed that there was no difference in the responses of soil total N, total inorganic N, net N mineralization rate, NO_3^-, NH_4^+ and NO_3^-/NH_4^+ to mixtures among experimental systems (Supplementary Table 1). To disentangle the effects of SR and ecosystem types, we tested whether the response to SR differed with ecosystem type by conducting an analysis with the interaction of SR and ecosystem type [ln(SR) × *E*] as the only fixed factor and study as the random factor. We found the interaction term was insignificant for all soil N attributes studied (Supplementary Table 10). To examine the effects of soil quality on the response ratio to species mixtures, we conducted an analysis with the soil pH, a proxy for soil quality[64], as the only fixed factor and study as the random factor; we found the responses of soil total N, inorganic N, NO_3^-, NH_4^+ and net N mineralization rate to species mixtures were not altered with soil pH (Supplementary Table 1).

We used partial regressions (or predicted effects) to graphically demonstrate the effects of SA on soil N variables. Briefly, we used the residuals from modelling equations (5) and (6) plus the mean intercept coefficient (β_0) plus the coefficient (β_2) times SA. To graphically illustrate whether the effects of SR on lnRR differed with SA, we calculated SA-dependent SR effects as $\beta_0 + \beta_1 \ln(SR) + \beta_3 \ln(SR) \times \ln(SA)$ (or $\beta_5 \ln(SR) \times \ln(SA)$ for equation (5)) using the method described by Cohen et al.[53] at SAs of 2, 10, 20 and 30 years, which were the most common SA values in the original studies. For consistency, we chose an SA range of 0 to 30 years to present for all N variables, although the age range soil total N was much greater (Supplementary Fig. 8).

Since both SR and FR are important components of biodiversity[22], we also tested the effects of FR on lnRR by replacing the terms of SR in equations (5) and (6) with FR. We found qualitatively similar estimates and trends for SR and FR (Supplementary Tables 3 and 11). For simplicity, we reported the results of SR. In addition, to account for N-fixing plant effects, we added the absence and presence of N-fixing plants in mixtures as a factor in equations (5) and (6).

To provide a more easily interpretable illustration of the effects of plant diversity on total N and inorganic N over time, we compared outcomes when 16 species (maximum SR in the metadata) was reduced by 10%, 20%, 40% and 60% at the establishment over the course of 30 years for total N and inorganic N. We first defined the lnRR when the plant richness in mixtures was R_1 (no SR loss) and R_α (α% SR loss at the establishment)[24], respectively:

$$\mathrm{lnRR}_1 = \beta_0 + \beta_1 \ln(R_1) + \beta_2 \ln(A) + \beta_3 \ln(R_1) \times \ln(A) + \pi_{\mathrm{study}} + \varepsilon \tag{7}$$

$$\mathrm{lnRR}_\alpha = \beta_0 + \beta_1 \ln(R_\alpha) + \beta_2 \ln(A) + \beta_3 \ln(R_\alpha) \times \ln(A) + \pi_{\mathrm{study}} + \varepsilon \tag{8}$$

Then we compared the lnRR when the plant richness in mixtures was R_1 and R_α by (equation (8)) – (equation (7)):

$$\mathrm{lnRR}_\alpha - \mathrm{lnRR}_1 = (\beta_1 + \beta_3 \ln(A)) \times (\ln(R_\alpha) - \ln(R_1)) \tag{9}$$

$$\ln(\mathrm{RR}_\alpha/\mathrm{RR}_1) = \ln\left((R_\alpha/R_1)^{\beta_1+\beta_3\ln(A)}\right) \tag{10}$$

We assumed that the mean value of monocultures, X_c, did not vary with the number of monocultures of different species (that is, X_{c_α} was no different from X_{c_1}), In this framework, we sought to subtract the modelled effects of reduced richness from the modelled effects of full richness to examine how soil total N and inorganic N are impacted as plant diversity is reduced. Using equation (10) to estimate this loss in soil total N and inorganic N led to the following equation:

$$(X_{t\_\alpha}/X_{c\_\alpha})/(X_{t\_1}/X_{c\_1}) = \mathrm{RR}_\alpha/\mathrm{RR}_1 = (R_\alpha/R_1)^{\beta_1+\beta_3\ln(A)} \tag{11}$$

where P_α is the proportion of remaining soil total N and inorganic N under α% plant SR loss at the establishment for a period of T, and the other model terms were described in equation (6). On the basis of equation (11), we fitted curves for the decrease in soil total N and inorganic N over time when there was a 10%, 20%, 40% and 60% plant SR loss at the establishment.

Our analysis indicated that many of our models violated the assumption of normality on the basis of the Shapiro–Wilk test on model residuals. We thus bootstrapped the fitted coefficients by 1,000 iterations[65]. The coefficients were significant from zero at $\alpha = 0.05$ if the bootstrapped 95% CIs did not cover zero. The treatment effects were considered significant at $\alpha = 0.05$ if the 95% CIs of the estimated lnRR did not cover zero. The mean effect sizes between groups were significantly different if their 95% CIs did not overlap the other's mean. To facilitate interpretation, we transformed the lnRR and its corresponding CIs back to a percentage using $\left(e^{\overline{\ln \mathrm{RR}}} - 1\right) \times 100\%$. All statistical analyses were performed in R4.0.0[66].

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The source data underlying Figs. 1–4, Supplementary Figs. 1–8 and Supplementary Tables 1–11 are archived in Figshare (https://doi.org/10.6084/m9.figshare.11400552).

Code availability

The R scripts needed to reproduce the analysis is archived in Figshare (https://doi.org/10.6084/m9.figshare.11400552).

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Author contributions

X.C. and H.Y.H.C. designed research; X.C. collected data; X.C. performed the meta-analysis and wrote the first draft of the manuscript; and X.C., H.Y.H.C., E.B.S., C.C. and P.B.R. wrote interactively through multiple rounds of revisions.

Competing interests

The authors declare no competing interests.

Additional information

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Software and code

Policy information about availability of computer code

Data collection	Peer-reviewed publications that published prior to June 2020, which investigated the effects of plant diversity on soil total N, inorganic N, and net N mineralization rate were collected by searching the ISI Web of Science, Google Scholar, and the China National Knowledge Infrastructure . Plot Digitizer version 2.0 (Department of Physics at the University of South Alabama, Mobile, AL, USA) was used to extract data from the figures.
Data analysis	The data analysis was conducted in R 4.0.0 (R Core Team, 2020), using packages including 'data.table (version 1.11.4)', 'lme4 (version 1.1-17)', 'lmerTest (version 3.0-1)', 'ggplot2 (version 2.2.1)', 'maps (version 3.2.0)' and 'cowplot (version 0.9.2)'. Details were reported in statistical analysis section of the Methods.

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Ecological, evolutionary & environmental sciences study design

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Study description	We conducted a global meta-analysis with 1650 paired observations of plant monocultures and mixtures from 149 studies, to evaluate the responses of soil nitrogen to plant species mixtures. We investigated whether the plant mixture effects on the soil total nitrogen, net nitrogen mineralization rate and inorganic nitrogen concentrations were affected by plant species richness, stand age, ecosystem types, climatic factors and soil depth.
Research sample	The data for this study was obtained from 141 peer-reviewed publications that investigated the effects of plant diversity on soil nitrogen, using the Web of Science and Google Scholar, up to June 2020. The 141 publications were listed in Table S4 in Supplementary Materials.
Sampling strategy	We systematically searched all peer-reviewed publications that published prior to June 2020, which investigated the effects of plant diversity on soil total N, inorganic N, and net N mineralization rate using the Web of Science (Core Collection; http://www.webofknowledge.com), Google Scholar (http://scholar.google.com), and the China National Knowledge Infrastructure (CNKI; https://www.cnki.net) with the search term: “soil nitrogen OR soil N OR nitrate OR ammonia OR no3 OR nh4 OR nitrogen mineralization AND plant diversity OR richness OR mixture OR pure OR polyculture OR monoculture OR overyielding”, and also searched for references within these papers. Our survey also included studies summarized in previously published diversity-ecosystem functioning meta-analyses.
Data collection	Xinli Chen collected the data. For each study, we extracted the means, the number of replications, and standard deviations of soil total N, inorganic N, the net N mineralization rate, NO3- and NH4+, if reported. The net N mineralization rate data included both field- and laboratory-incubated measurements. When an original study reported the results graphically, we used Plot Digitizer version 2.0 (Department of Physics at the University of South Alabama, Mobile, AL, USA) to extract data from the figures. We also extracted species richness (SR, number of plant species), functional group richness (FR, number of plant functional groups), soil depth, ecosystem type (e.g., forest, grassland, cropland), latitude, longitude, mean annual temperature (MAT, °C), stand age from original or cited papers, or cited data sources. Annual aridity index data were retrieved from the CGIAR-CSI Global Aridity Index dataset39 through the use of location information. The annual aridity index was calculated as the ratio of mean annual precipitation to mean annual potential evapotranspiration. Stand age was recorded as the number of years since a stand-replacing disturbance in forests, and the number of years between the initiation and measurements of the experiments in grasslands and croplands. Soil depth was recorded as the midpoint of each soil depth interval. The species proportions in plant mixtures were based on the basal area or stem density in forests, coverage in croplands, and sown seeds in grasslands. Plants in forests were classified into three functional groups (coniferous trees, leguminous broadleaved trees, and nonleguminous broadleaved trees). Those of grasslands were classified into four functional groups (C3 grasses, C4 grasses, nonleguminous forbs, and legumes), whereas those of croplands were classified into two functional groups (grains and legumes).
Timing and spatial scale	The searching for publication and data collection continuously proceeded from November 8th, 2018 to June 1st, 2020. The timing scale of the observations in original studies ranged from 1992 to 2018. The observations were distributed in global terrestrial ecosystems.
Data exclusions	To better represent responses of soil N under natural conditions, we did not include greenhouse and mesocosms studies.
Reproducibility	All attempts to repeat the analysis and results were successful.
Randomization	The data were analyzed with a random effect model. Bootstrapping sampling was used.
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Methods

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