Meta-analysis shows plant diversity enhances grassland carbon and nitrogen cycles

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Abstract

This study is a global meta-analysis of the effects of grassland plant species richness on aboveground and belowground carbon and nitrogen dynamics. Observations from 73 studies in grasslands totaling 1385 paired observations of plant mixtures and monocultures were compiled. Response variables included nine carbon and six nitrogen processes to plant diversity, examined the effects of experimental types and age on the responses, and predicted the carbon and nitrogen balance following different biodiversity loss scenario in grasslands. We found that carbon and nitrogen functions significantly enhanced in plant mixtures, but varied with experimental types. Most of the attributes was significantly correlated with species richness and experimental age, the relationship between species richness and carbon and nitrogen processes was interacted with experimental duration in the field experiments, except for soil respiration, fungal biomass, and soil nitrate nitrogen. Importantly, our results showed that the declines in soil carbon and nitrogen pool accelerated following plant diversity loss. Our meta-analysis revealed that the plant diversity has ubiquitous impacts on carbon and nitrogen cycles in grasslands, likely driven by complementarity effects of plant diversity on plant productivity and biomass, underlined interactive effects of plant diversity, experimental types and age, and climate on carbon and nitrogen processes, and suggested that the reduction in carbon and nitrogen stocks in grasslands will be larger following biodiversity loss in the future.

Introduction

Grasslands play a critical role in global carbon and nitrogen cycles (Scurlock & Hall, 1998; Suttie *et al.* 2005). Increasing grassland plant diversity can have positive effects on a wide range of ecosystem functions including plant productivity (Tilman *et al.* 2001; Ravenek *et al.* 2014; Oram *et al.* 2018; Prieto *et al.* 2015), soil carbon and nitrogen storage (Lange *et al.*2015; Weisser *et al.* 2017; Leloup*et al.* 2018; Yang *et al.*2019), and soil respiration (Chen *et al.* 2019). Declines in grassland plant diversity through grazing mismanagement, land conversion, and climate change will thus likely change many aspects of the carbon and nitrogen cycles of grassland ecosystems (Reich *et al.* 2012; Isbell *et al.* 2013; Hautier *et al.* 2018).

Numerous studies have shown close relationships between plant diversity and carbon and nitrogen cycling processes in grasslands (Hector *et al.* 2010; Oelmann*et al.* 2011; Mueller *et al.*2013; Craven *et al.* 2016). Above- and belowground biomass, soil organic carbon, and soil respiration have all been observed to increase significantly with species richness (Tilman *et al.* 2001; Cowles *et al.* 2016; Chen & Chen 2019; Yang *et al.* 2019) including a number of meta-analyses showing that these are very general patterns (Weisser *et al.* 2017; Chen*et al.* 2019; Wang *et al.* 2020). We expect, therefore, that carbon cycles in grasslands will be changed significantly under biodiversity loss scenarios. Given that the nitrogen cycle is closely coupled with carbon cycling (Zaehle 2013; Zaehle *et al.* 2014), we expect similar impacts on nitrogen cycling processes (Schmid *et al.*2009; Weisser *et al.* 2017; Fanin*et al.* 2018).

Numerous field and controlled environment experiments have examined plant diversity impacts on the provision of ecosystem services (Fridley 2002; Prieto *et al.* 2015; Craven *et al.* 2016). Plant diversity impacts on carbon and nitrogen cycles in these experiments may be sensitive to experimental type (i.e. field versus controlled environment) (Roscher *et al.* 2004; Thompson *et al.* 2005; Malchair *et al.* 2010; Kreyling *et al.* 2017), experimental duration (Chen *et al.* 2019; Chen *et al.* 2020), and, in the case of field experiments, environmental conditions (Duffy *et al.*2017; Chen *et al.* 2019). For example, after controlling for environmental covariates, increases in biomass with biodiversity are stronger than has previously been documented in experiments (Duffy*et al.* 2017). Interactions between plant diversity and experimental duration on carbon processes have also been reported. For example, Tilman *et al.* (2001) found that the diversity effects on productivity can become progressively stronger due to the enhancement of complementarity effects with time. Recent meta-analyses have found that the positive effects of plant diversity on soil organic carbon, microbial biomass, and microbial respiration are also enhanced with experimental duration (Chen *et al.* 2019; Chen *et al.* 2020). Finally, a long-term grassland experiment suggests that the close relationships between the carbon and nitrogen cycles (Zaehle *et al.* 2014) may enhance the effects of plant diversity on nitrogen processes with time (Mueller *et al.* 2013). Synthesis studies of these diversity experiments have not, however, explored either the effects of plant diversity across multiple carbon and nitrogen processes, or the interactive effects of plant diversity and experimental type (field and greenhouse experiments) and duration.

Here we compile data from 73 studies examining the effects of plant diversity in grasslands (Fig. 1). These studies report on nine ecosystem carbon processes (aboveground biomass, belowground biomass, total biomass, soil carbon pool, soil respiration, heterotrophic respiration, microbial biomass, bacterial biomass, and fungal biomass) and six nitrogen processes (aboveground nitrogen pool, soil nitrogen pool, soil ammonium nitrogen, soil nitrate nitrogen, soil nitrogen leaching, and soil nitrogen mineralization). We specifically test 1) how grassland plant mixtures impact carbon and nitrogen processes relative to monocultures, 2) whether diversity effects differ between field and greenhouse experiments, and 3) how plant diversity impacts change with experimental age in field experiments.

Material and methods

Data collection

We searched for all peer-reviewed publications that investigated the effects of plant diversity on carbon and nitrogen attributes in grasslands using the ISI Web of Science (isiknowledge.com) and China National Knowledge Infrastructure (CNKI, www.cnki.net) databases from 1980-2019. Keyword combinations such as diversity/mixture, grassland/pasture/meadow, carbon/C, nitrogen/N, respiration, and richness were used for the search. The following criteria were applied to select studies: 1) experiments had at least one pair of data comparing monoculture vs. mixture, 2) the species in monoculture were included in the mixtures at the same temporal and spatial scale, 3) response variables (means and measures of variance) were reported for at least one carbon or nitrogen ecosystem function: aboveground biomass (AGB), belowground biomass (BGB), total biomass (TB), soil carbon pool (SCP, %), soil respiration (Rs), heterotrophic respiration (Rh), microbial biomass (MB), bacterial biomass (BB), fungal biomass (FB), aboveground nitrogen pool (ANP), soil nitrogen pool (SNP, %), soil ammonium nitrogen (SAN), soil nitrate nitrogen (SNN), soil nitrogen leaching (SNL), soil nitrogen mineralization (SNM), and 4) the diversity level (species richness), experimental duration, and experimental type (field or greenhouse) were clearly described. Means, standard deviations/errors and samples size were extracted when necessary from digitized graphs using GetData Graph Digitizer (ver. 2.24, < www.getdata-graph-digitizer.com/ >). Sample sizes corresponding to each observation was derived based on the number of independent experimental units. The data were extracted from the Worldclim database at http://worldclim.org/ using the location information (e.g. latitude and longitude). Experimental age was divided into two durations: 1 (1– 4 years) and 4 (>4 years). In total, 73 studies, reporting 15 attributes, and 1385 observations about the effects of plant diversity on carbon and nitrogen processes were selected (Table S1).

Data analysis

We followed the methods of Hedges et al. (1999) and Gurevitch et al. (2018) to evaluate the changes in carbon and nitrogen attributes in plant mixtures. The log response ratio (*lnRR* , natural log of the ratio of the mean value of monocultures plots to that in mixtures) was calculated as below:

$$lnRR = \ln\left(\frac{x_t}{x_c}\right) = \ln\left(x_t\right) - ln(x_c)(1)$$

where x_t and x_c are means of the variable in all mixtures and monocultures plots respectively. Random-effects models were used to estimate weighted average response ratios via the *rma* function in the *metafor* package (Cooper*et al.* 2009) in R 3.5.1 (R Core Team, 2018). Weights for studies were estimates by sampling variance (Hedges *et al.* 1999) and the between-sampling variability:

$$w_v = \left(\frac{s_t^2}{n_t x_t^2} + \frac{s_c^2}{n_c x_c^2} + \tau^2\right)^{-1}(2)$$

where S_t, n_t, S_c and n_c are the standard deviation and sample size for the mixtures and monocultures respectively, and τ^2 the total amount of heterogeneity.

For each attribute, we tested the impacts of species richness (SR), experimental age (EA), and climate using the following model:

$$\lambda\nu PP = \beta_0 + \beta_1 \times \lambda\nu\Sigma P + \beta_2 \times EA + \beta_3 \times \varsigma\lambda\iota\mu\alpha\tau\epsilon + \beta_4 \times \lambda\nu\Sigma P \times EA + \beta_5 \times \lambda\nu\Sigma P \times \varsigma\lambda\iota\mu\alpha\tau\epsilon + \epsilon \ (3)$$

where β is the coefficient to be estimated; *e* is sampling error. We conducted the analyses using restricted maximum likelihood estimation by the *rma* function with w_v as the weight for each corresponding observation (Johnson and Omland 2004; Bates *et al.*2015). For ease of interpretation, *lnRR* and the corresponding 95% confidence intervals (CIs) were transformed to a percentage change between monocultures and mixtures as (e^{lnRR}-1) ×100%. To illustrate the effects of plant diversity on soil carbon and nitrogen pool with time, we compared the *lnRR* when the plant diversity in mixtures was R_1 (all species present) and R_α (α% lower species richness) using the following equation (Chen *et al* . 2019):

$$P_\alpha = (R_1/R_\alpha)^{\beta_1+\beta_4 T} \ (4)$$

where Π_a is the proportion of remaining soil carbon and nitrogen pool under α% lower species richness in a period of*T*. Other model terms were as described for Equation (3). Based on Equation (4), we fitted curves to estimate the potential decrease in soil carbon and nitrogen pool over time under scenarios of 10, 20, 40, and 80% decreases in species richness. We limited the forecasting to 30 years as that is the age of the longest field biodiversity experiment at Cedar Creek Natural History Area, Minnesota, USA (Tilman *et al.*2006).

Results

Average changes of ecosystem functions in plant mixtures

In field experiments, we observed significant increases in mixtures relative to monocultures for carbon processes including aboveground biomass (AGB, 64.6%), belowground biomass (BGB, 45.0%), total biomass (TB, 123.1%), soil carbon pool (SCP, 13.2%), soil respiration (Rs, 10.6%), heterotrophic respiration (Rh, 18.9%), microbial biomass (MB, 16.7%), fungal biomass (FB, 16.4%), and bacterial biomass (30.0%) (BB, Fig. 2). We observed similar increases for nitrogen processes including aboveground nitrogen pool (ANP, 26.5%), soil nitrogen pool (SNP, 7.8%), and soil ammonium nitrogen (SAN, 27.0%). Decreases occurred in soil nitrate nitrogen (SNN, -40.4%), soil nitrogen mineralization (SNM, -52.3%), and soil nitrogen leaching (SNL, -76.0%) (Fig. 2).

In greenhouse experiments, we similarly observed significant increases in mixtures relative to monocultures for measures including AGB (60.1%), BGB (53.1%), TB (49.6%), Rs (16.4%), and SNP (5.6%) (Fig. 2). The remaining attributes showed no significant differences.

The effects of plant diversity, experimental age, and climate

Carbon and nitrogen attributes increased linearly with experimental age in the field experiments, with the exceptions of FB and SNN (Fig. 3, Table S2), and increased logarithmically with species richness except for FB, SAN, SNN, and SNM (Fig. 4, Table S3). Importantly, significant interactions between plant diversity and experimental age were found for AGB, BGB, TB, SCP, Rh, MB, BB, ANP, SNP, SAN, and SNM (Table S4), indicating stronger species diversity effects on those variables in longer-term experiments (Fig. 4, Table S5). In greenhouse experiments, AGB, BGB, FB, BB, and ANP increased logarithmically with species richness (Fig. 5, Table S3). Few significant interactions between plant diversity and climate (mean annual temperature and precipitation) were observed, with only BGB, FB, and BB impacted (Fig. 6).

Predicted responses of soil carbon and nitrogen pool

We utilized the differences in species richness and experimental age effects in the field experiments to predict the impacts of long-term diversity declines (Fig. 7). A 10% decrease in species richness (from 100 to 90%) over one year reduced SCP and SNP by 0.5 and 1.1% respectively (Fig. 7). An 80% decrease in species richness (from 100 to 20%) over one year led to 2.3 and 4.8% reductions in SCP and SNP respectively (Fig. 7). The declines in SCP and SNP in response to the decrease in species richness became amplified with time. For example, a 10% decrease in species richness (from 100 to 90%) over five years led to a 2.4 and 5.2% reduction in SCP and SNP, respectively (Fig. 7).

Discussion

Many studies have explored the responses of carbon and nitrogen processes to increasing plant diversity from both experiments and meta-analysis (Tilman *et al.* 2001; Roscher *et al.* 2004; Ma and Chen 2016; Chen *et al.* 2019; Chen *et al.* 2020). Three aspects of our study distinguish it from previous syntheses studies. First, our study is the first to compare across a large number of carbon and nitrogen processes. Second, our study attempts to reconcile the effects of experimental type by exploring the differences between field and greenhouse experiments. Third and most importantly, we show that there are important interactions between plant diversity and experimental age in field experiments, with the effects of plant diversity becoming more pronounced with time.

Carbon and nitrogen cycle changed significantly in plant mixtures

We generally found support for the hypothesis that more diverse plant mixtures positively affect the provision of many carbon and nitrogen ecosystem services in grasslands. Overyielding in aboveground biomass (AGB), belowground biomass (BGB), and total biomass (TB) in plant mixtures has been commonly observed due to complementary plant interactions (Tilman *et al.* 1997; Ma & Chen 2016; Wang *et al.* 2020); the strong general effects of diversity found here demonstrate that these mechanisms are broadly important in grassland ecosystems. Further, we observed positive relationships between the soil carbon pool (SCP) response and BGB, suggesting that the higher SCP frequently observed in plant mixtures can be caused by complementary in BGB (Fig. S1a). Higher microbial biomass (MB), fungal and bacterial biomass, and heterotrophic respiration (Rh) in plant mixtures are likely also attributable to the effects of higher productivity on the carbon and nutrient inputs to the soil ecosystem (Bartelt-Ryser *et al.*2005; Eisenhauer *et al.* 2010). Soil respiration (Rs) combines Rh and root metabolism (Luo & Zhou 2008), so there is likely both Rh and plant BGB impacts in mixtures on Rs.

Positive plant diversity effects were also observed for nitrogen attributes. These can be attributed to the positive relationship between aboveground nitrogen pool (ANP) and AGB (Fig. S1b), and again demonstrate the general importance of diversity complementary effects in grasslands. Positive relationships were found between soil nitrogen stock (SNP) and SCP (Fig. S1c), and thus the higher SNP in plant mixtures may be induced by overyielding in above- and belowground biomass. Larger carbon accumulations in soils could provide more exchange sites for ammonium (Mueller *et al.* 2013), which may cause the observed higher soil ammonium under plant mixtures. Soil nitrate and nitrogen leaching in plant mixtures was lower than monocultures probably due to greater N uptake capacity in a diverse mixtures (Mueller *et al.* 2013; Leimer *et al.* 2016). Stoichiometric theory shows that net N mineralization was positively related to root N concentration (Manzoni *et al.* 2008), and reduction in root N concentration (Mueller *et al.* 2013) may lead to a decrease in soil nitrogen mineralization in plant mixtures.

Divergent response between field and greenhouse experiments

Our comparison of field and greenhouse experiments demonstrates that diversity effects were generally stronger in field experiments than in the greenhouse. Duration and experimental size constraints in greenhouse experiments likely explain much of the difference. The maximum diversity for individual measures in the greenhouse was 3-16 species compared to 16-60 in the field. Similarly, greenhouse experiments older than 3 years were rare (Fig. S2) while many field experiments had run for a decade or more. Given the experimental age by diversity interactions identified in the field experiments (discussed below), it is not surprising that field experiments show stronger effects than greenhouse studies. Mechanisms important in longer experiments that would not be detected in the greenhouse could include the accumulation of dead plant material with time (Bartelt-Ryser *et al.* 2005; Eisenhauer *et al.* 2010; Chen *et al.* 2019). Further, we found no significant diversity – climate interactions, suggesting that the stronger responses in field experiments was not induced by the greater range of climate conditions in the field.

Species richness - experimental age interactions

We show that the effects of plant diversity on carbon and nitrogen processes increased with experimental age in the field experiments. Increases in the magnitude of complementary effects with time have been observed (Cardinale *et al.* 2007; Ravenek *et al.* 2014; Wang *et al.* 2020). The likely mechanism behind these time effects is the accumulation of plant biomass and soil carbon with time (Khlifa *et al.* 2017). Positive interactions between plant diversity and experimental age on ANP and SNP may also be caused by the enhancement of complementary effects with time. However, There is a time lag in microbial processes response to changes in plant communities due to the accumulation of dead plant materials was needed before their response (Bartelt-Ryser *et al.* 2005; Eisenhauer *et al.* 2010), and thus the effects of plant diversity on microbial biomass, respiration, soil nitrate, and soil nitrogen mineralization, which are likely caused by the accumulation of primary production (Janssens *et al.* 2010; Mueller *et al.* 2013), was more pronounced at longer experimental duration.

Long-term impacts of diversity loss in grasslands

Our model shows that a 10% (from 100 to 90%) decrease in plant species richness could cause a small decline in SCP and SNP over one year, but cumulatively much larger declines are likely. The diversity of many plant communities worldwide is thought to be declining due to factors including global warming (Tilman & Lehman 2001; Tylianakis *et al.* 2008). The negative effects of plant diversity loss on carbon storage may generate positive feedbacks that could accelerate global warming. Nitrogen limitation of primary production in grasslands (Fay*et al.* 2015; Wieder *et al.* 2015), the positive interactive effects of plant diversity loss and time on SNP could aggravate the deficiency of nitrogen in grasslands, which substantially exacerbate the shortage of forage production.

Conclusions

Our meta-analysis provides comprehensive evidence that plant diversity increases have a positive effect on multiple carbon and nitrogen processes in grasslands. We show that the effects of plant diversity on carbon and nitrogen cycles are enhanced in field experiments relative to greenhouse studies, and that the effects increase with experimental duration. Prediction models based on the field experiments showed soil carbon and nitrogen release in grasslands following plant diversity loss was faster with time, which indicate that global warming and forage production shortage might be exacerbate with continuous plant diversity loss.

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Data availability statements

The data that support the findings of this study will be openly available at the Dryad Digital Repository.

References

Bates, D., Mächler, M., Bolker, B., Walker, S., (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software,*67, 1–48.

Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H, Balser, T. (2005). Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. Perspect. *Plant Ecology and Evolution* , 7, 27–49.

Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., et al. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity, *Proceedings of the national academy of sciences USA, 104* (46), 18123-18128.

Chen, X., and Chen, HYH. (2019). Plant diversity loss reduces soil respiration across terrestrial ecosystems. *Global Change Biology,*25, 1482-1492.

Chen, C., Chen, HYH., Chen, X., Huang, Z. (2019) Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nature Communications,* 10, 1332.

Chen, X., Chen, H., Chen, C., Ma, Z., Searle, E. B., Yu, Z., Huang, Z. (2020) Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis. *Biological Reviews,* 95, 167-183.

Cooper, H., Hedges, L. V., Valentine, J. C. (Eds.) (2009). The handbook of research synthesis and meta-analysis (2nd ed.). New York: Russell Sage Foundation.

Cowles, J. M., Wragg, P. D., Wright, A. J., Powers, J. S., Tilman, D. (2016). Shifting grassland plant community structure drives positive interactive effects of warming and diversity on aboveground net primary productivity. *Global Change Biology, 22* (2), 741-749.

Craven, D., Isbell, F., Manning, P., Connolly, J., Bruelheide, H., Ebeling, A., Eisenhauer, N. (2016). Plant diversity effects on grassland productivity are robust to both nutrient enrichment and drought. *Philosophical transactions - Royal Society. Biological sciences, 371* (1694).

Duffy, J. E., Godwin, C., M. Cardinale, B. J., (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* , 549, 261–264.

Eisenhauer, N., Beler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., et al. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology* , 91, 485–496.

Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M., et al. (2015). Grassland productivity limited by multiple nutrients. *Nature Plants* , 1, 1-5.

Fridley, J. D. (2002). Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia* , 132(2), 271-277.

Gurevitch, J., Koricheva, J., Nakagawa, S., Stewart, G. (2018). Meta-analysis and the science of research synthesis. *Nature, 555* (7695), 175-182.

Hautier, Y., Isbell, F., Borer, E. T., Seabloom, E. W., Harpole, W. S., Lind, E. M., Hector, A. (2018). Local loss and spatial homogenization of plant diversity reduce ecosystem multifunctionality. *Nature Ecology and Evolution, 2* (1), 50-56.

Hector, A., Hautier, Y., Saner, P., Wacker, L., Bagchi, R., Joshi, J., Caldeira, M. C. (2010). General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. *Ecology, 91* (8), 2213-2220.

Hedges, L., Gurevitch, J., Curtis, P. (1999). The meta-analysis of response ratios in experimental ecology.*Ecology, 80* (4), 1150-1156.

Isbell, F., Tilman, D., Polasky, S., Binder, S., Hawthorne, P. (2013). Low biodiversity state persists two decades after cessation of nutrient enrichment. *Ecology Letters, 16* (4), 454-460.

Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., et al. (2010). Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* , 3(5), 315-322.

Johnson, J. B., Omland, K. S. (2004). Model selection in ecology and evolution. *Trends Ecology and Evolution* , 19, 101–108.

Khlifa, R., Paquette, A., Messier, C., Reich, P. B. Munson, A. D. (2017). Do temperate tree species diversity and identity influence soil microbial community function and composition? Ecology and Evolution, 7, 7965–7974.

Kreyling, J. , Dengler, Jürgen, Walter, J. , Velev, N. , Ugurlu, E. , Sopotlieva, D. , et al. (2017). Species richness effects on grassland recovery from drought depend on community productivity in a multisite experiment. *Ecology Letters* , 20(11), 1405-1413.

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Gerd, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage.*Nature Communications, 6* , 6707.

Lefcheck, J. S., Byrnes, J. E. K., Isbell, F., Gamfeldt, L., Griffin, J. N., Eisenhauer, N., et al. (2015). Biodiversity enhances ecosystem multifunctionality across trophic levels and habitats. *Nature Communications* , 6, 6936.

Leimer, S., Oelmann, Y., Eisenhauer, N., Milcu, A., Wilcke, W. (2016). Mechanisms behind plant diversity effects on inorganic and organic n leaching from temperate grassland. *Biogeochemistry* , 1-15.

Leloup, J., Baude, M., Nunan, N., Meriguet, J., Dajoz, I., Le Roux, X., et al. (2018). Unravelling the effects of plant species diversity and aboveground litter input on soil bacterial communities. *Geoderma* , 317, 1-7.

Luo, Y., Zhou, X. (Eds.) (2008). Soil respiration and the environment.*Elsevier* .

Ma, Z., Chen, H. Y. H. (2016). Effects of species diversity on fine root productivity in diverse ecosystems: a global meta-analysis. *Global Ecology and Biogeography, 25* (11), 1387-1396.

Malchair, S., De Boeck, H. J., Lemmens, C. M. H. M., Merckx, R., Nijs, I., Ceulemans, R., Carnol, M. (2010). Do climate warming and plant species richness affect potential nitrification, basal respiration and ammonia-oxidizing bacteria in experimental grasslands?. *Soil Biology and Biochemistry* , 42(11), 1944-1951.

Manzoni, S., Jackson, R. B., Trofymow, J. A., Porporato, A. (2008). The global stoichiometry of litter nitrogen mineralization. *Science* , 321, 684–686.

Mueller, K. E., Hobbie, S. E., Tilman, D., Reich, B. P. (2013). Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Global Change Biology, 19* (4), 1249-1261.

Mueller, K. E., Hobbie, S. E., Oleksyn, J., Reich, P. B., Eissenstat, D. M. (2012). Do evergreen and deciduous trees have different effects on net N mineralization in soil?*Ecology, 93* (6), 1463-1472.

Nicolas, F., Michael, J., Gundale, Mark, et al. (2018). Consistent effects of biodiversity loss on multifunctionality across contrasting ecosystems. *Nature Ecology and Evolution,* 1, 1-13.

Oelmann, Y., Nina, B., Gleixner, B., Habekost, M., Roscher, C., Rosenkranz, S., et al. (2011). Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment, *Global Biogeochemistry Cycles* , 25, GB2014.

Oram, N. J., Ravenek, J. M., Barry, K. E., Weigelt, A., Chen, H., Gessler, A., Cahill, J. (2018). Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. *Journal of Ecology, 106* (1), 265-277.

Prieto, I., Violle, C., Barre, P., Durand, J. L., Ghesquiere, M., Litrico, I. (2015). Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. *Nature Plants, 1* (4), 15033.

R Core Team 2018 R: A Language and Environment for Statistical Computing (Vienna: R Foundation for Statistical Computing).

Ravenek, J. M., Bessler, H., Engels, C., Scherer-Lorenzen, M., Gessler, A., Gockele, A., Mommer, L. (2014). Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. *Oikos, 123* (12), 1528-1536.

Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. B., Eisenhauer, N. (2012). Impacts of biodiversity loss escalate through time as redundancy fades.*Science, 336* (6081), 589-592.

Roscher, C., Schumacher, J., Baade, J., Wilcke, W., Gleixner, G., Weisser, W. W., et al. (2004). The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. *Basic and Applied Ecology* , 5, 107–121.

Schmid, B., Balvanera, P., Cardinale, B. J., Godbold, J., Pfisterer, A. B., Raffaelli, D., et al. (2009). Consequences of species loss for ecosystem functioning: meta-analyses of data from biodiversity experiments. *Concurrency and Computation Practice and Experience* , 26(18), 2856–2879.

Scurlock, J., Hall, D. (1998). The global carbon sink: a grassland perspective. *Global Change Biology, 4* (2), 229-233.

Suttie, J., Reynolds, S., Batello, C., (Eds.). (2005). Grasslands of the World. *Food & Agriculture Organization* .

Tilman, D., Lehman, C. (2001). Human-caused environmental change: Impacts on plant diversity and evolution. *Proceedings of the national academy of sciences USA* , 98(10), 5433–5440.

Tilman, D., Lehman, C., Thomson, K. T. (1997). Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the national academy of sciences USA* , 94(5), 1857-1861.

Tilman, D., Reich, P. B., Knops, J. M. H. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* , 7093, 629-632.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., Lehman, C. (2001). Diversity and productivity in a long-term grassland experiment.*Science, 294* (5543), 843-845.

Thompson, K., Askew, A. P., Grime, J. P., Willis, N. P. D. J. (2005). Biodiversity, ecosystem function and plant traits in mature and immature plant communities. *Functional Ecology* , 19(2), 355-358.

Tylianakis, J. M., Didham, R. K., Bascompte, J., Wardle, D. A. (2008). Global change and species interactions in terrestrial ecosystems.*Ecology Letters* , 11(12), 1351–1363.

van Groenigen, K. J., Osenberg, C. W., Hungate, B. A. (2011). Increased soil emissions of potent greenhouse gases under increased atmospheric CO_2. *Nature, 475* (7355), 214-216.

Weisser, W. W., Roscher, C., Meyer, S. T., Ebeling, A., Luo, G., Allan, E., Eisenhauer, N. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology, 23* , 1-73.

Wieder, W. R., Cleveland, C. C., Smith, W. K., Todd-Brown, K. (2015). Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience* , 8(6), 441-444.

Yang, S., Liu, W., Qiao, C., Wang, J., Deng, M., Zhang, B., Liu, L. (2019). The decline in plant biodiversity slows down soil carbon turnover under increasing nitrogen deposition in a temperate steppe. *Functional ecology* , 33,1362–1372.

Yang, Y., Tilman, D., Furey, G., Lehman, C. (2019). Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nature Communications, 10* (1).

Zaehle, S. E. M. (2013). Terrestrial nitrogen-carbon cycle interactions at the global scale. *Philosophical transactions - Royal Society. Biological sciences* , 368(1621), 20130125.

Zaehle, S. E. M. B., De Kauwe, M. G., Walker, A. P, Dietze, M. C, Hickler, T, et al. (2014). Evaluation of 11 terrestrial carbon-nitrogen cycle models against observations from two temperate Free-Air CO_2 Enrichment studies. *New Phytologist, 202* (3), 803-822.

Zhang, Y., Chen, H. Y. H., Reich, P. B. (2012). Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. *Journal of Ecology, 100* (3), 742-749.

Figure Legends

Figure 1 Global distribution of grassland study sites used in the meta-analysis. Red circles and blue triangles indicate study sites in the field and greenhouse experiments. Source data are provided as a Source Data file.

Figure 2 Comparison of carbon and nitrogen attributes between plant mixtures and monocultures in grasslands. The effects represent the increase or decrease (%) of a given attribute compared to the corresponding mean of monocultures at the mean plant diversity in mixtures (see Methods). Values are mean ± 95% confidence intervals of the percentage effects between the plant mixtures and monocultures. The number of observations for each attribute is displayed in parentheses. Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; TB, total biomass; SCP, soil carbon pool; Rs, soil respiration; Rh, heterotrophic respiration; MB, microbial biomass; FB, fungal biomass; BB, bacterial biomass; ANP, aboveground nitrogen pool; SNP, soil nitrogen pool; SAN, soil ammonium nitrogen; SNN, soil nitrate nitrogen; SNM, soil nitrogen mineralization; SNL, soil nitrogen leaching. Different colors represent different experimental types.

Figure 3 Relationships between experimental age and the response ratios (RR) for carbon and nitrogen attributes in the field experiments. Plots show aboveground biomass (a), belowground biomass (b), total biomass (c), soil carbon pool (d), soil respiration (e), heterotrophic respiration (f), microbial biomass (g), fungal biomass (h), bacterial biomass (i), aboveground nitrogen pool (j), soil nitrogen pool (k), soil ammonium nitrogen (l), soil nitrate nitrogen (m), and soil nitrogen mineralization (n). The size of the bubble is the relative weight of the response ratio ($lnRR$) in the random-effects meta-regression. Larger bubbles indicate study outcomes that contributed a greater overall weight in random-effects meta-regression. Solid lines represent significant relationships. Shaded areas show 95% confidence intervals.

Figure 4 Interactions between species richness in plant mixtures and experimental duration in field experiments. Plots show aboveground biomass (a), belowground biomass (b), total biomass (c), soil carbon pool (d), soil respiration (e), heterotrophic respiration (f), microbial biomass (g), fungal biomass (h), bacterial biomass (i), aboveground nitrogen pool (j), soil nitrogen pool (k), soil ammonium nitrogen (l), soil nitrate nitrogen (m), and soil nitrogen mineralization (n). Blue, green, and red lines indicate experimental durations of 1 year, 4 years, and mean experimental duration across all observations; points with different shapes indicate experimental durations. The size of the bubble is the relative weight of the response ratio ($lnRR$) in the random-effects meta-regression. Larger bubbles indicate study outcomes that contributed a greater overall weight in the random-effects meta-regression. Shaded areas show 95% confidence interval of the fit.

Figure 5 Relationships between species richness and the response ratios (RR) in the greenhouse experiments. Plots show the aboveground biomass (a), belowground biomass (b), total biomass (c), soil carbon pool (d), soil respiration (e), heterotrophic respiration (f), microbial biomass (g), fungal biomass (h), bacterial biomass (i), aboveground nitrogen pool (j), soil nitrogen pool (k), soil ammonium nitrogen (l), soil nitrate nitrogen (m), and soil nitrogen mineralization (n). The size of the bubble is the relative weight of the response ratio ($lnRR$) in the random-effects meta-regression. Larger bubbles indicate study outcomes that contributed a greater overall weight in random-effects meta-regression. Solid lines represent significant relationships. Shaded areas show 95% confidence interval of the fit.

Figure 6 Interactions between species richness in plant mixtures and climate in the field experiments (β_5). Values are mean ± 95% confidence intervals. Plots show mean annual temperature (a) and precipitation (b). Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; TB, total biomass; SCP, soil carbon pool; Rs, soil respiration; Rh, heterotrophic respiration; MB, microbial biomass; FB, fungal biomass; BB, bacterial biomass; ANP, aboveground nitrogen pool; SNP, soil nitrogen pool; SAN, soil ammonium nitrogen; SNN, soil nitrate nitrogen; SNM, soil nitrogen mineralization. Data for TB comes from only one study site thus the interactive effects of species richness and climate cannot be tested.

Figure 7 Predicted responses of soil carbon (a) and nitrogen (b) pools to a range of plant species richness reduction in grasslands through field experiments. Plots show the plant species richness reduction at 10%, 20%, 40%, and 80%. Lines with different colors represent different species richness levels.

Figures

Figure 1

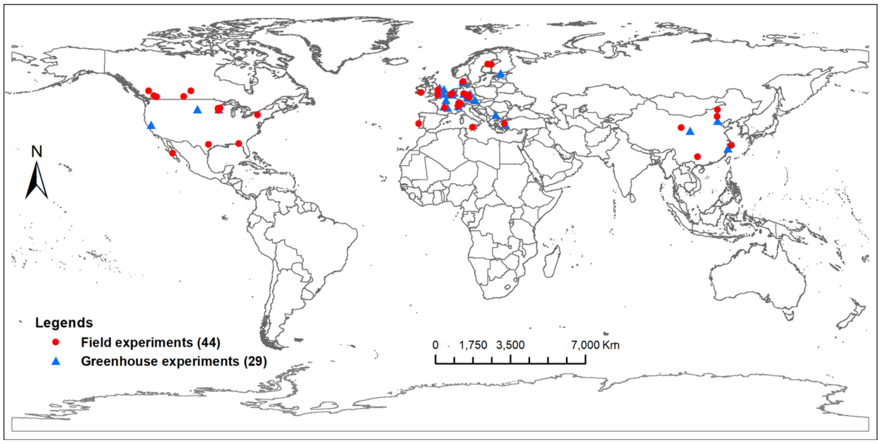


Figure 2

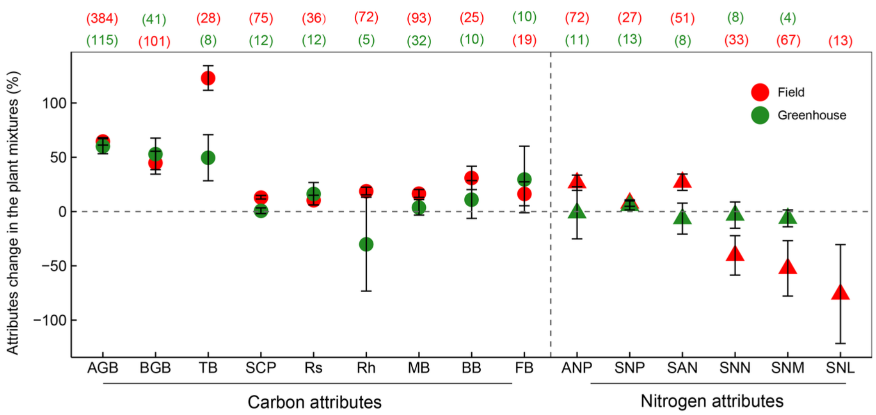


Figure 3

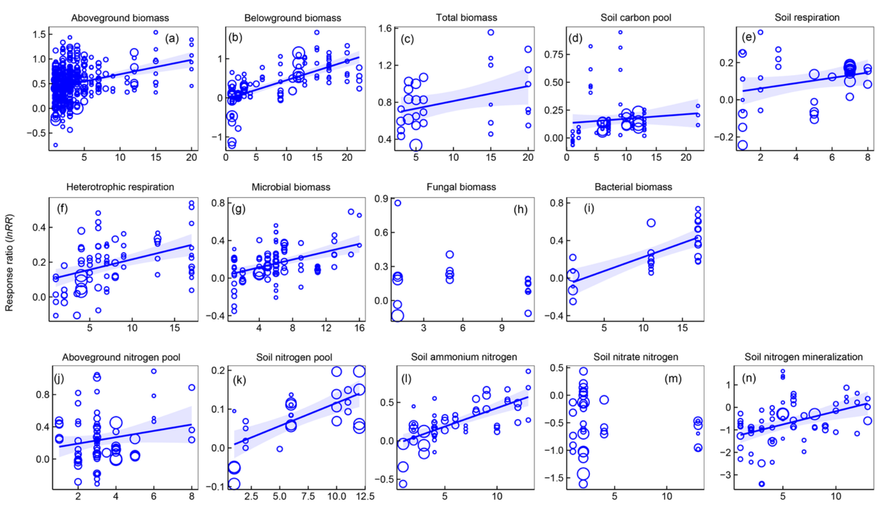


Figure 4

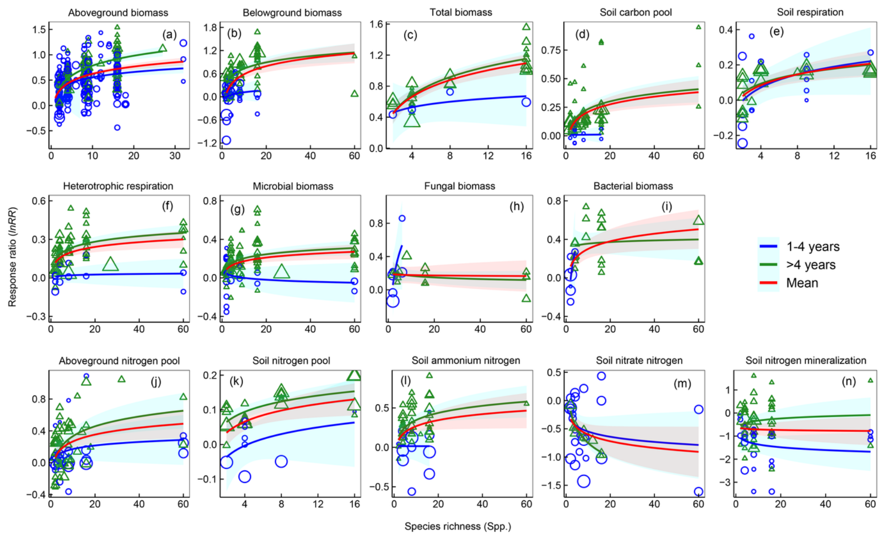


Figure 5

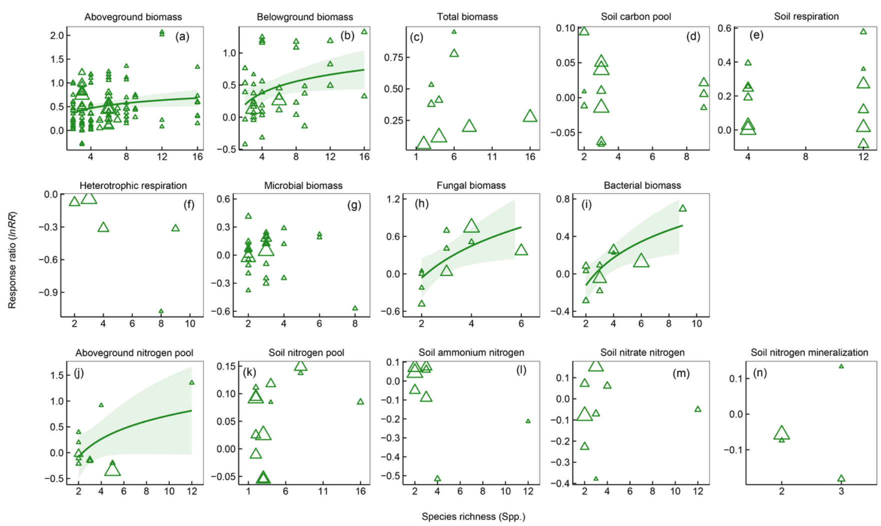


Figure 6

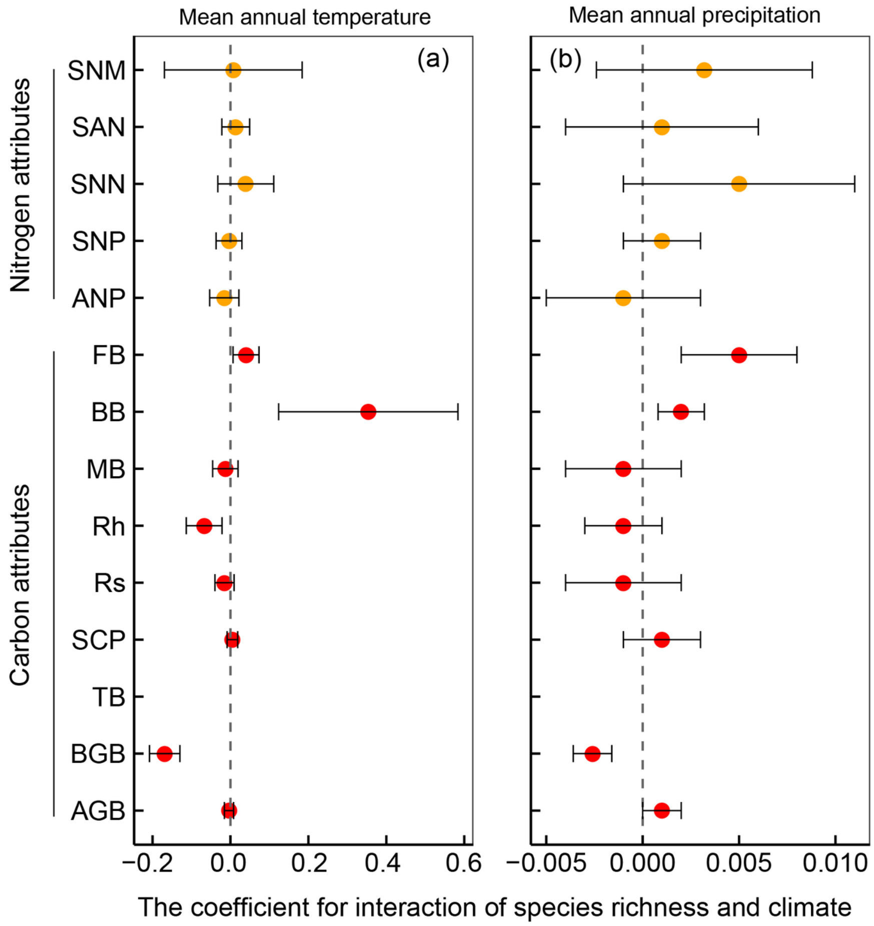
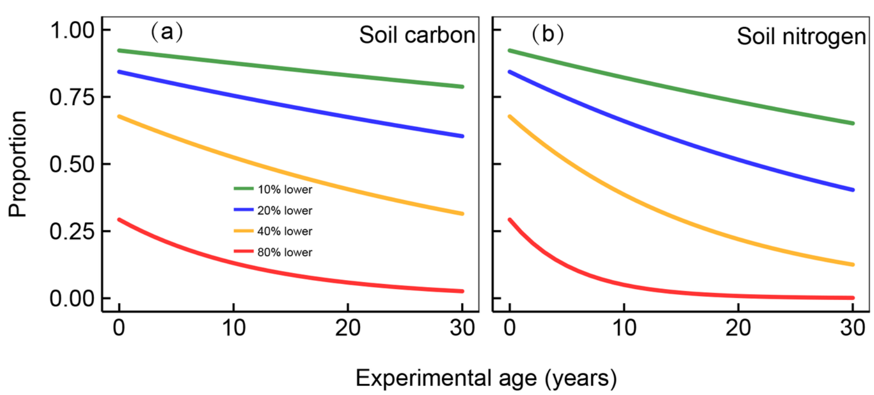
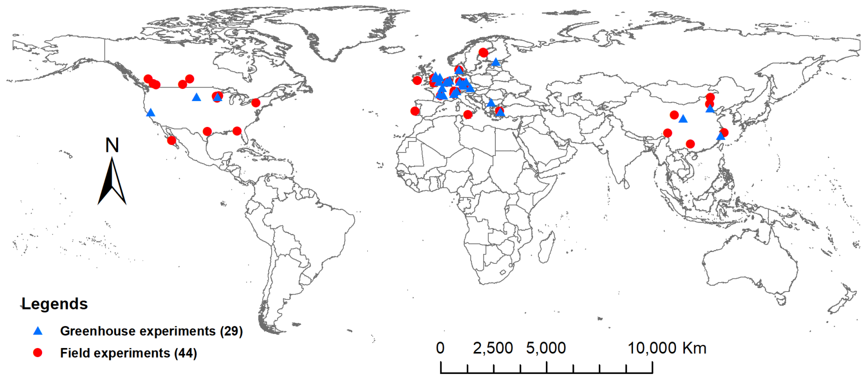
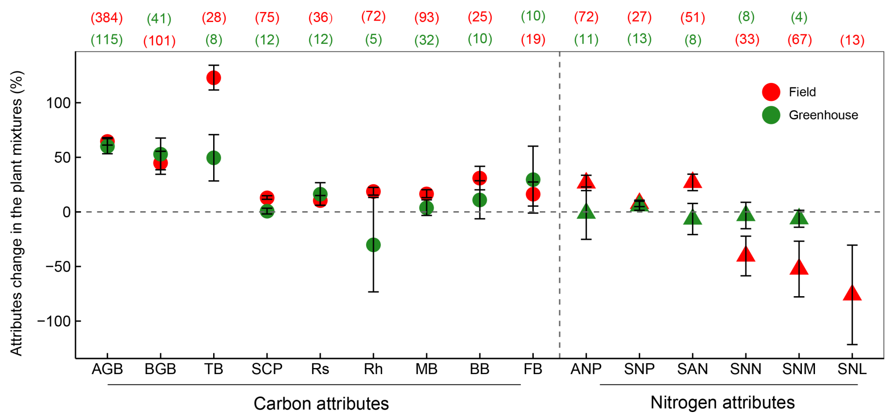
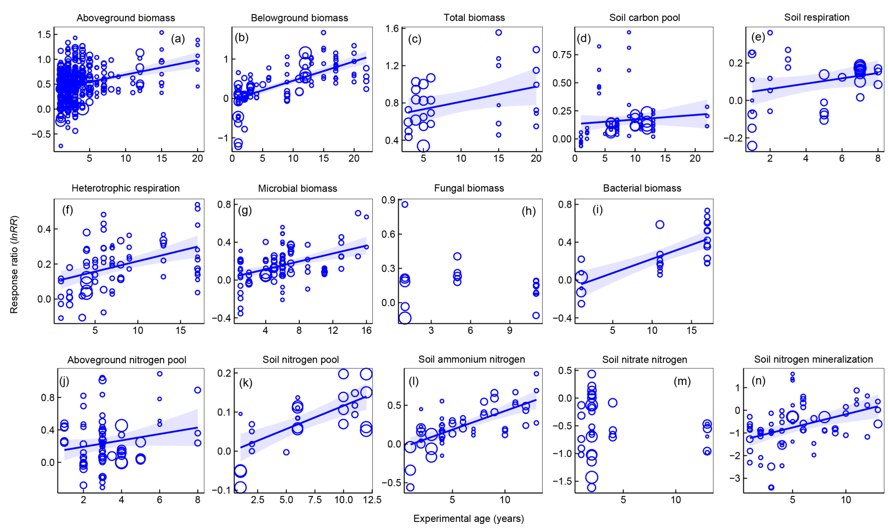

Mean annual temperature
Mean annual precipitation
(a)
(b)
Nitrogen attributes
Carbon attributes
SNM
SAN
SNN
SNP
ANP
FB
BB
MB
Rh
Rs
SCP
TB
BGB
AGB
−0.2 0.0 0.2 0.4 0.6
−0.005 0.000 0.005 0.010
The coefficient for interaction of species richness and climate

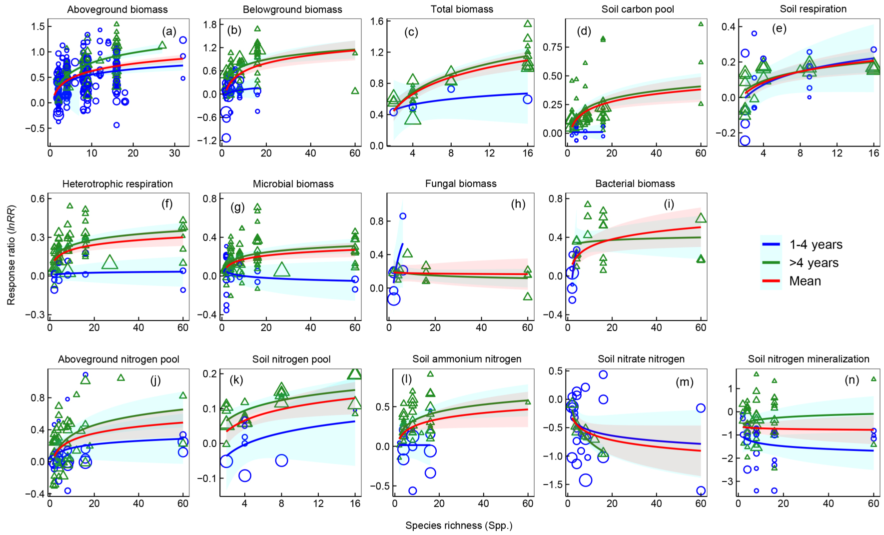
Figure 7


(a)
Soil carbon
(b)
Soil nitrogen
Proportion
1.00
0.75
0.50
0.25
0.00
10% lower
20% lower
40% lower
80% lower
0 10 20 30
Experimental age (years)

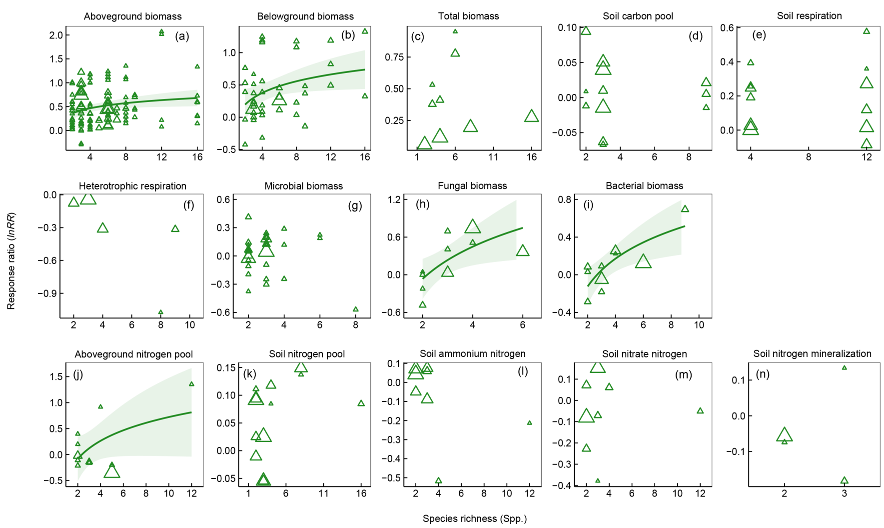

N
Legends
Greenhouse experiments (29)
Field experiments (44)
0 2,500 5,000 10,000 Km


(384) (41) (28) (75) (36) (72) (93) (25) (10) (72) (27) (51) (8) (4)
(115) (101) (8) (12) (12) (5) (32) (10) (19) (11) (13) (8) (33) (67) (13)
Field
Greenhouse
Attributes change in the plant mixtures (%)
100
50
0
−50
−100
AGB BGB TB SCP Rs Rh MB BB FB ANP SNP SAN SNN SNM SNL
Carbon attributes
Nitrogen attributes

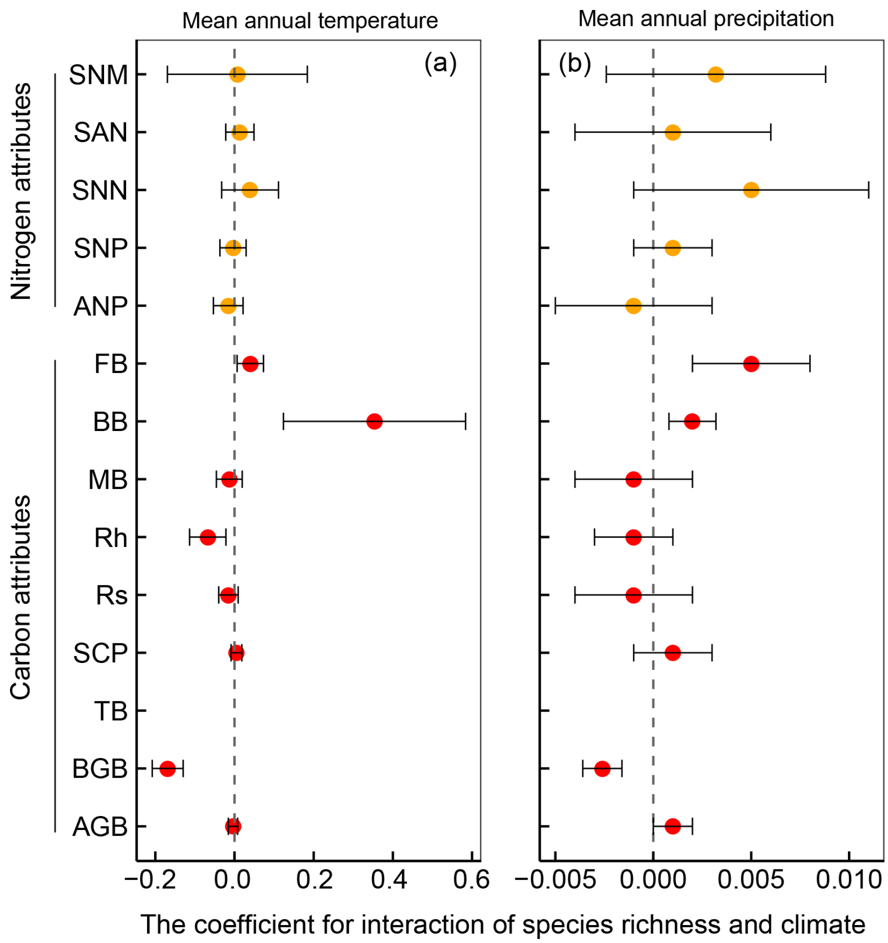

Aboveground biomass (a)
Belowground biomass (b)
Total biomass (c)
Soil carbon pool (d)
Soil respiration (e)
Heterotrophic respiration (f)
Microbial biomass (g)
Fungal biomass (h)
Bacterial biomass (i)
Aboveground nitrogen pool (j)
Soil nitrogen pool (k)
Soil ammonium nitrogen (l)
Soil nitrate nitrogen (m)
Soil nitrogen mineralization (n)
Response ratio (lnRR)
Experimental age (years)



Aboveground biomass
(a)
Belowground biomass
(b)
Total biomass
(c)
Soil carbon pool
(d)
Soil respiration
(e)
Heterotrophic respiration
(f)
Microbial biomass
(g)
Fungal biomass
(h)
Bacterial biomass
(i)
1-4 years
>4 years
Mean
Aboveground nitrogen pool
(j)
Soil nitrogen pool
(k)
Soil ammonium nitrogen
(l)
Soil nitrate nitrogen
(m)
Soil nitrogen mineralization
(n)
Response ratio (lnRR)
Species richness (Spp.)



Aboveground biomass
(a)
Belowground biomass
(b)
Total biomass
(c)
Soil carbon pool
(d)
Soil respiration
(e)
Heterotrophic respiration
(f)
Microbial biomass
(g)
Fungal biomass
(h)
Bacterial biomass
(i)
Aboveground nitrogen pool
(j)
Soil nitrogen pool
(k)
Soil ammonium nitrogen
(l)
Soil nitrate nitrogen
(m)
Soil nitrogen mineralization
(n)
Response ratio (lnRR)
Species richness (Spp.)


Mean annual temperature
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Nitrogen attributes
Carbon attributes
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0.000
0.005
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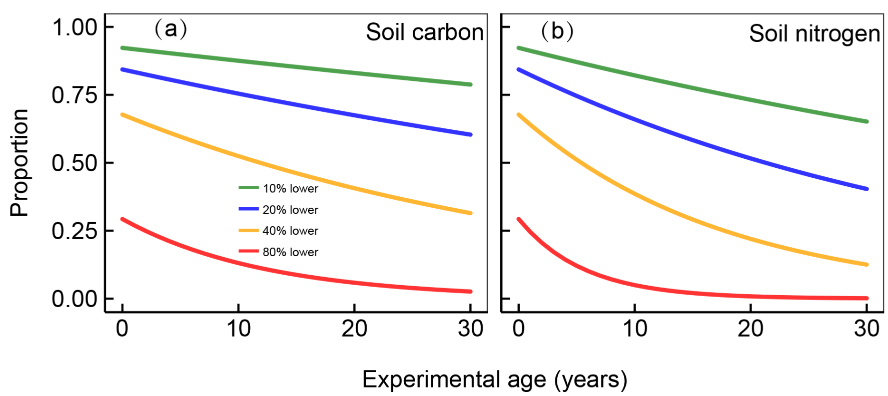
The coefficient for interaction of species richness and climate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