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Progressive shifts in soil respiration response to long-term nitrogen enrichment across global forests and grasslands

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Soil stores the largest pool of actively cycling carbon (C) on land, and its dynamics under global changes are critical for projecting climate feedbacks. Global nitrogen (N) deposition has more than doubled since the 1860s; however, the temporal dynamics of soil C emissions under prolonged N deposition are poorly understood. Here, using a global time-series dataset compiled from 38 long-term experiments in forests and grasslands, we find that the response of soil respiration (Rs) to N addition systematically shifts from a transient increase persisting for approximately 2 y to a sustained suppression over time (mean effect = −6%; 95% CI = [−10%, −3%]). Rs in forests and grasslands followed consistent nonlinear trajectories, characterized by a rapid initial decline in the magnitude of the response, which progressively leveled off. Notably, suppression of Rs emerged when cumulative N inputs exceeded a threshold of ~100 kg N ha^{-1}. Consequently, the suppression was both stronger and occurred more rapidly under high-level N additions (>50 kg N ha^{-1} y^{-1}). These temporal dynamics were associated with N-induced soil acidification and related changes in microbial properties. Overall, these findings indicate that accurate prediction of land–atmosphere C fluxes under global change requires models to capture the reported temporal dynamics.

soil respiration | carbon cycling | temporal trajectory | global change ecology

Significance

Understanding how soil respiration (Rs) responds to sustained nitrogen loading is critical for constraining carbon–climate feedbacks. However, current assessments largely overlook the potential for substantial temporal shifts in these responses. Here, we address this gap by synthesizing time-series Rs records from long-term manipulation experiments worldwide. We reveal a nonlinear temporal trajectory in Rs responses to nitrogen addition, transitioning from short-term stimulation to long-term suppression. Nitrogen addition rate did not significantly accelerate this temporal shift, but higher nitrogen inputs produced stronger overall suppression. Our findings extend the prevailing spatial-oriented analyses by showing that nitrogen enrichment effects on soil carbon fluxes depend strongly on exposure duration and cumulative nitrogen input.

Anthropogenic activities have more than doubled global nitrogen (N) deposition since the 1860s (1), and global N deposition rates remain stubbornly high at 92.7 Tg N in 2020 (2). Through the close coupling between N and carbon (C) cycle, this elevated N input profoundly alters the ecosystem C fluxes (3, 4), particularly as N limitation widely constrains terrestrial plant production (5–7). Consequently, increased N deposition has led to substantial additional C sequestration in plant biomass (8–10), contributing approximately 9 to 20% of the land C sinks in recent decades (8, 11, 12). While the stimulation of plant growth is well documented, the concurrent change in soil respiration (Rs), a dominant soil to atmosphere CO_2 flux generated by root and microbial respiration that dwarfs anthropogenic C emissions (13), may be more consequential for the long-term terrestrial carbon balance. The direction and magnitude of changes in Rs varies markedly with N addition (14). Such changes in Rs have considerable implications for soil C, one of the Earth's most substantial biogeochemically active C pools (15).

Although syntheses of N addition experiments have not reached a consistent conclusion (14), a prevalent finding is an overall suppression of Rs (16–19), suggesting a potential increase of soil C stocks under increased N deposition (20, 21). However, the temporal dynamics and long-term effects of N-deposition on this crucial C flux remain unknown, particularly at the global scale. This is a major problem because N deposition is a persistent and pervasive environmental impact that could have nonlinear effects on Rs over time. Accurate future projections of land-atmosphere C-flux therefore depend on a better understanding of how Rs responds to persistent N-addition over longer timeframes. One plausible temporal Rs dynamic is a shift from an initial positive to a negative response over time. Short-term N addition can increase Rs due to enhanced plant growth and soil microbial activity. Such a positive short-term Rs response has been observed across multiple biomes (22–28). Short-term N addition increased Rs in N limited temperate and boreal forests (22–24, 26–28). In addition, experimental N addition can also stimulate Rs in phosphorus-limited tropical forests, primarily through a positive effect on fine root biomass (25, 29). Over time, several biogeochemical and biological processes triggered by excessive N inputs progressively change in ways that tend to reduce Rs over longer experimental durations (18, 30, 31).

N-addition typically leads to soil acidification (32), and these negative effects on soil pH become more pronounced over time, and with higher N addition rates (32, 33), which typically reduce Rs in the long-term (33, 34). Moreover, a global synthesis found a greater

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pH reduction in grassland than in forest under comparable N inputs (32), suggesting biome-dependent soil acidification responses to N fertilization. This variation in the trajectory of soil acidification suggests that the timing and progression of any acidification-mediated effects on Rs may also differ across biomes. Soil acidification may suppress soil microbial activity and hence lead to a rapid reduction in soil respiration (35). This suppression may be driven in part by a concurrent decline in soil microbial biomass (27, 34), a negative effect that intensifies with higher N addition rates and longer experimental duration (36). Additionally, long-term plant growth responses to N-addition, and associated adjustment in plant biomass allocation over time can also moderate Rs responses (37, 38), especially as an ecosystem approaches N saturation (39).

Critically, the rate of N addition can modify these temporal dynamics. In a boreal forest, for instance, the Rs response shifted from stimulation to suppression over time, and this temporal reversal was accelerated under higher N addition rates (27). Collectively, these lines of evidence suggest that the response of Rs to N additions can shift from an initial positive to a long-term negative effect, and that the rate at which N is added is a critical regulator of the pace of this temporal dynamic. Shifts in soil microbial community composition and function due to N-addition could underpin the temporal dynamics in Rs (40), particularly, if the microbial community acclimates to longer-term N addition, thereby alleviating the negative Rs response. For instance, a long-term N addition experiment in subtropical forests suggested an eventual reversal in negative Rs response over a decade of N addition (34). However, a critical question is whether the reduction in Rs follows a linear temporal trend as some previous syntheses suggest (30, 31), or a nonlinear trend, where it levels-off over time due to potential acclimation. This distinction is key to assessing the sustainability of any soil C accrual due to N addition. To date, a cross-biome global quantitative synthesis of the temporal trajectories of Rs under increased N deposition is still lacking.

To address this gap, we collected time series data from long-term field N addition experiments worldwide (Fig. 1 *A* and *B*) to examine how the response of Rs to N fertilization evolves over time. Specifically, we tested three hypotheses that 1) the effects of long-term N addition on soil respiration would shift from positive to negative over time, and this shift would occur more rapidly at higher N addition rates because soil acidification, microbial

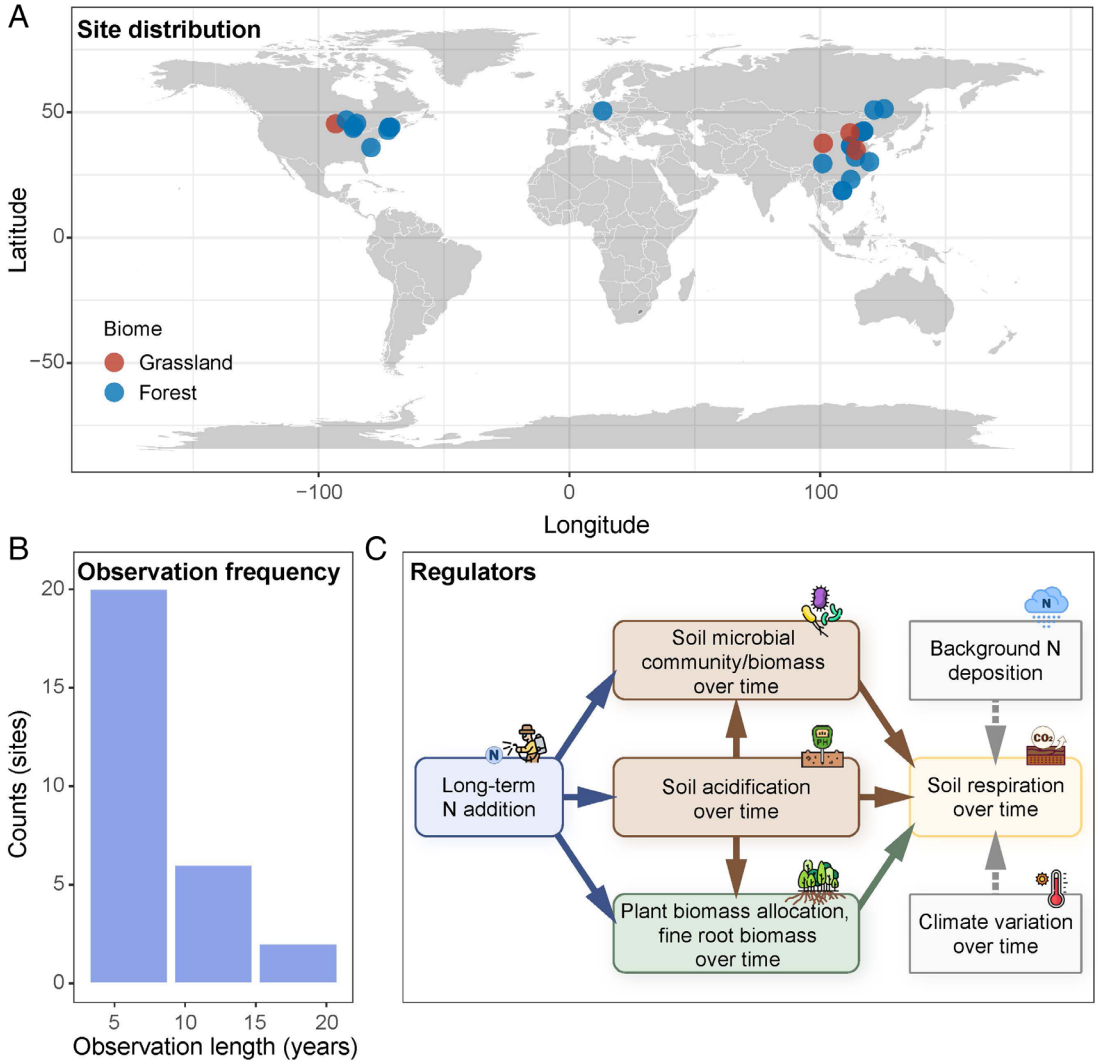


Fig. 1. Location of long-term N addition experiments with time-series soil respiration records and the potential key processes through which N addition regulates soil respiration dynamics. (*A*) Distribution of field experiment sites conducted in grassland (including alpine grassland and temperate grassland) and forest (including boreal forest, temperate forest, and tropical/ subtropical forest). (*B*) Frequency of soil respiration measurement duration. (*C*) Possible regulators underlying direct and indirect effects contributing to the temporal shifts in response of soil respiration to long-term N addition. Solid lines indicate pathways related to N addition effects and dashed lines represent confounding mediators.

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community responses, and shifts in plant belowground biomass allocation emerge faster (Fig. 1*C*); 2) the temporal dynamics of Rs under prolonged N addition would follow a nonlinear trajectory, with a rapid initial decrease that levels off over time owing to ecosystem acclimation; and 3) site specific factors, such as temporal climate variations and ambient environmental conditions (including background N deposition), may moderate these Rs dynamics (Fig. 1*C*).

Results and Discussion

Positive to Negative Shift in Rs Response to N Addition over Time. The dataset contained time series records of 508 site-year effect sizes for soil respiration, compiled from 38 field sites (Fig. 1*A* and *SI Appendix*, Table S1). These sites covered two major terrestrial biomes: forest and grassland; the former included 31 sites from boreal, temperate, and tropical/subtropical forests, whereas the latter contained seven sites from temperate and alpine grasslands. The N addition rate ranged from 8 to 300 kg N ha^{-1} y^{-1} with a median of 50 kg N ha^{-1} y^{-1} (*SI Appendix*, Fig. S1) and the experimental duration of Rs measurement ranged from 5 to 20 y (Fig. 1*B*). The temporal depth of these repeated Rs measurements, across diverse ecosystems, allowed us to test how the response of Rs to experimental N addition changes over time, across broad spatial scales.

Overall, the response of Rs to N addition (RR_{Rs}, natural log response ratio) decreased nonlinearly with experimental duration (Fig. 2*A* and *SI Appendix*, Table S2). The RR_{Rs} shifted from a transient initial positive response to negative with the natural logarithm of experimental duration (β = −0.05, 95% CI = [−0.09, −0.02]) (Fig. 2*A* and *SI Appendix*, Table S3). The temporal reduction was faster initially then leveled off, supporting our hypothesis of a nonlinear dynamic with ecosystem acclimation. This finding was in contrast with some previous syntheses showing linear decreases in Rs responses to N addition over time (30, 31). We found no significant interaction between ecosystem type and experimental duration (*SI Appendix*, Fig. S2); and a similar temporal trend in both forest and grassland (*SI Appendix*, Table S3) implies a rather general temporal pattern.

Our multilevel meta-analysis revealed an overall 6% reduction (95% CI = [−10%, −3%]) in Rs under N addition across all sites and durations (Fig. 2*B*). This estimate falls within the broad spectrum of results from previous syntheses, which range from positive responses of 2 to 6% (30, 31, 41) to negative effects of −10% and −20% (16, 18), or an insignificant response (21, 42, 43). Discrepancies among earlier studies have been attributed to differences in the scope of data and ecosystem coverage. In this study, Rs in forest showed an overall 8% reduction (95% CI = [−11.72%, −3.54%]) with long-term N addition, while that in grassland exhibited no net change (Fig. 2*C*, 95% CI = [−9.51, 8.35%]). Previous findings also revealed greater negative responses of Rs to N-addition in forest than grassland (30, 42). Critically, methodological variations in handling data may be a further key contributor to the apparent discrepancy. Some syntheses used only the most recent measurement from multiyear experiments (18, 21, 33, 44, 45) whereas others averaged responses across the available years within a study, which typically covered a shorter and more variable duration (16, 30). Nonetheless, these discrepancies underscore that the temporal dynamics of N-addition effects on Rs require greater attention.

Effect of N Addition Rates on Rs Responses over Time. Many N fertilization experiments generally add N at rates that are much higher than natural local N deposition rates (26, 30), and we present the study in the context of anthropogenically raised rates. We assessed whether the temporal shift in RR_{Rs} depended on N dosage. We categorized N addition levels into low and high dosages based on whether they were lower or higher than the median rate (50 kg N ha^{-1} y^{-1}). The results indicated no significant difference in the temporal change in RR_{Rs} at the low and high N

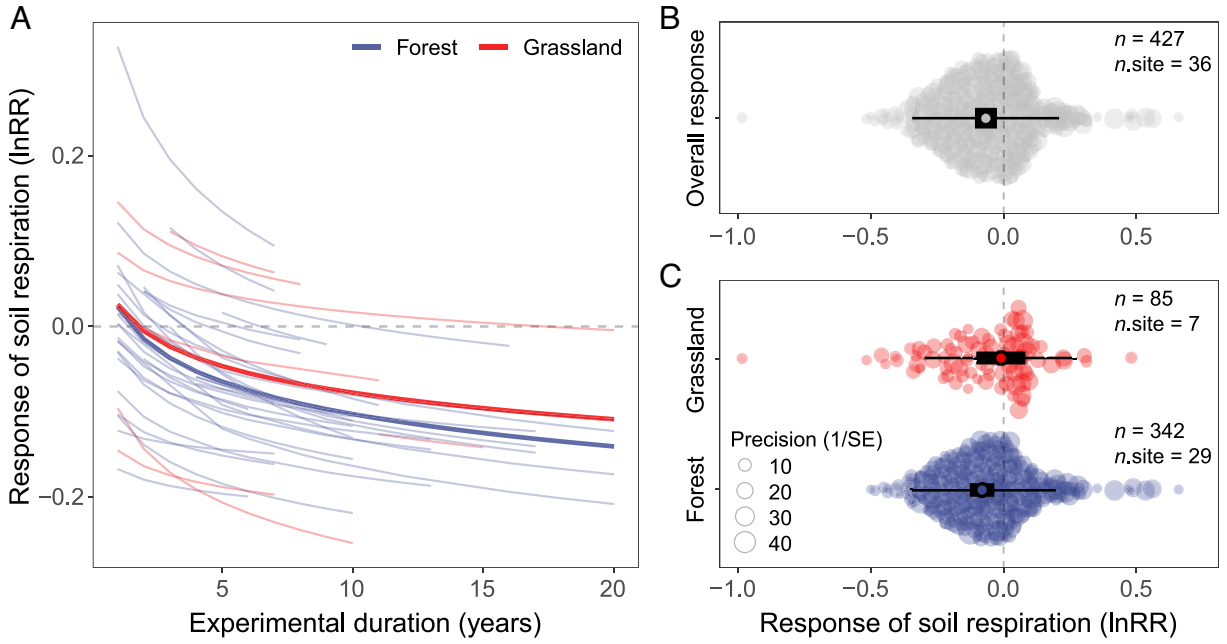


Fig. 2. Temporal dynamics in overall response of soil respiration to long-term experimental N addition. (*A*) Different sites of grassland and forest are depicted as thin red and blue lines, while overall model fitting for grassland and forest were shown in thick colored lines. (*B*) Overall response of soil respiration to experimental N addition quantified as differences in Rs between N addition and control treatment using the natural log response ratio, ln(N addition/control). (*C*) Overall response of soil respiration to long-term N addition in grassland and forest. Average response and black trunk represent 95% CI, while thick line represents 95% prediction interval. Each circle is an effect size and is scaled by the inverse sampling error variance. *n* and *n*.site represent number of observations and experimental sites, respectively.

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addition rates (Fig. 3), with rates of −0.04 y^{-1} (95% CI = [−0.07, −0.02]) and −0.07 y^{-1} (95% CI = [−0.12, −0.02]) for the two N addition levels, respectively (Fig. 3 *A* and *B*). However, a negative response did appear earlier under high-level N addition (>50 kg N ha^{-1} y^{-1}) than low-level N addition (Fig. 3 *A* and *B*). Reverse prediction suggested that low-level N did not reduce Rs within 3 y (95% CI = [0.96, 7.00]), while it was reduced in less than 1.5 y (95% CI = [0.43, 4.69]) at high-level N addition. When analyzed in a meta regression, we also found a positive to negative shift in RR_{Rs} under low-level N addition, while high-level N addition almost instantly reduced Rs (*SI Appendix*, Fig. S3). Together, these results indicate that high N inputs trigger the onset of suppression more rapidly, while the pace of decline is similar.

We then compared the overall mean effects of low- and high-level N additions. The mean negative effect of high-level N addition was greater than that of low-level N addition (Fig. 3*C*), with high-level N addition reducing Rs by 11% (95% CI = [−15%, −8%]), while low-level N addition showed no significant effect (overall −3%, 95% CI= [−7%, 1%]). Pairwise contrasts based on the multilevel meta-regression model showed that the mean effect differed significantly (estimate = −0.09, SE = 0.01, t = −6.86, $P < 0.01$) between the two N levels. When analyzed as a continuous variable, response of Rs decreased logarithmically with N addition rates (estimate = -0.09 kg N ha^{-1} y^{-1}, 95% CI = [−0.10, −0.07], *SI Appendix*, Fig. S4). This was consistent with some previous studies that also showed rate-dependent responses of Rs to N addition (18, 30), but was in contrast with other previous findings where Rs responses were not dependent on N-addition rates (21, 31, 42). Importantly, although our categorical analysis was based on the median (50 kg N ha^{-1} y^{-1}) of the data and the low-level N was higher than the global average N deposition rates of ~10 kg N ha^{-1} y^{-1} (2), this threshold remains relevant to many N-deposition hotspots globally that still experience rates exceeding 50 kg N ha^{-1} y^{-1}.

Effects of Cumulative N Inputs on Rs Response. Leveraging both the duration of the experiment and N addition rates, we further assessed the changes in the response of Rs to cumulative N input (N addition rate × duration). Meta regression confirmed that the RR_{Rs} shifted from a positive to a negative response, and decreased logarithmically, with cumulative N addition (Fig. 4*A*). The model at the individual site level also indicated significant negative relationships for over 39% (15/38) of the sites (*SI Appendix*, Fig. S5). Remarkably, the slope of these significant site-level relationships did not vary systematically with background N deposition, soil pH, mean annual precipitation (MAP), or mean annual temperature (MAT; *SI Appendix*, Fig. S6), which suggests highly consistent responses across different environmental conditions. Inverse prediction of linear mixed models suggested that N addition did not reduce Rs when cumulative N addition was below 97 (95 % CI = [45, 208]) kg N ha^{-1}. This observation with time series data is different from a regular meta-analysis that showed a linear trend between cumulative N addition and Rs (43). Our results therefore support the concept of ecosystem acclimation to N-addition and suggest that the Rs response to chronic N addition may attenuate and stabilize over time.

In fact, with sustained N inputs, the negative response of Rs to N addition may not only stabilize but could even reverse over time, leading to an alleviation of the suppression (34). This outcome was detected in a decade-long study in a tropical forest, which documented a phased Rs response where the negative effect diminished with continued N addition (34). To further test for acclimation, we examined whether the dose–response relationship of Rs to N addition itself changed over time. We found a statistically marginal interaction between N addition rates and experimental duration (P = 0.10, *SI Appendix*, Table S4). Specifically, the effect of N addition rates on RR_{Rs} became less negative with increasing experimental duration (Fig. 4*B*), suggesting that the reduction in Rs due to increasing N rates was alleviated over time. Together, these multiple lines of evidence indicated a clear nonlinear response of soil respiration to long-term experimental N addition, which is consistent with ecosystem acclimation.

Possible Regulators Underlying Temporal Shift in Rs Response to N Addition. The observed nonlinear temporal trend, whereby RR_{Rs} underwent a brief but rapid suppression before entering a prolonged leveling-off period, suggests a fundamental restructuring of the belowground system. Such a system-level response implies concurrent shifts in key ecosystem properties: soil acidification (33, 34), soil microbial biomass (18, 27, 31, 34, 41), and plant belowground carbon allocation and substrate availability (31, 34, 41). To explore the contribution of these factors, we matched available time series data for soil pH (17 sites, n = 193), microbial

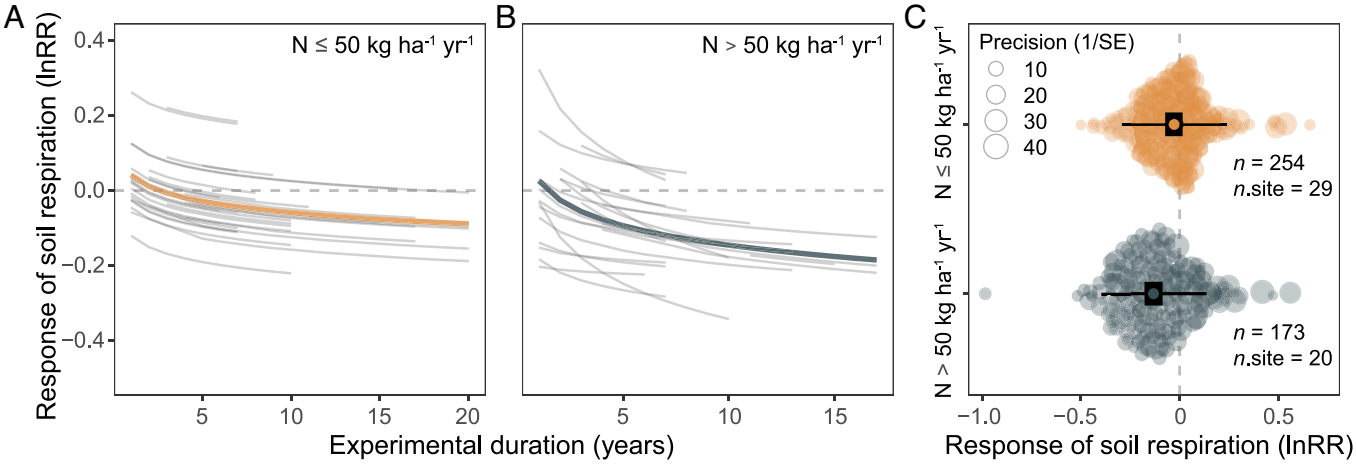


Fig. 3. Effects of N addition rates on the response of soil respiration to long-term experimental N addition. Temporal dynamics of soil respiration response to experimental N addition under (*A*) low and (*B*) high-level N addition, and the distinction was based on the median (50 kg N ha^{-1} y^{-1}) of the N addition levels. Different sites are depicted as thin gray lines, while overall model fitting for different N addition rates is shown in thick colored lines. (*C*) Meta-analysis showing the response of soil respiration to low- and high-level N addition. Each circle is an effect size and is scaled by the inverse sampling error variance. *n* and *n.site* represent number of observations and experimental sites, respectively.

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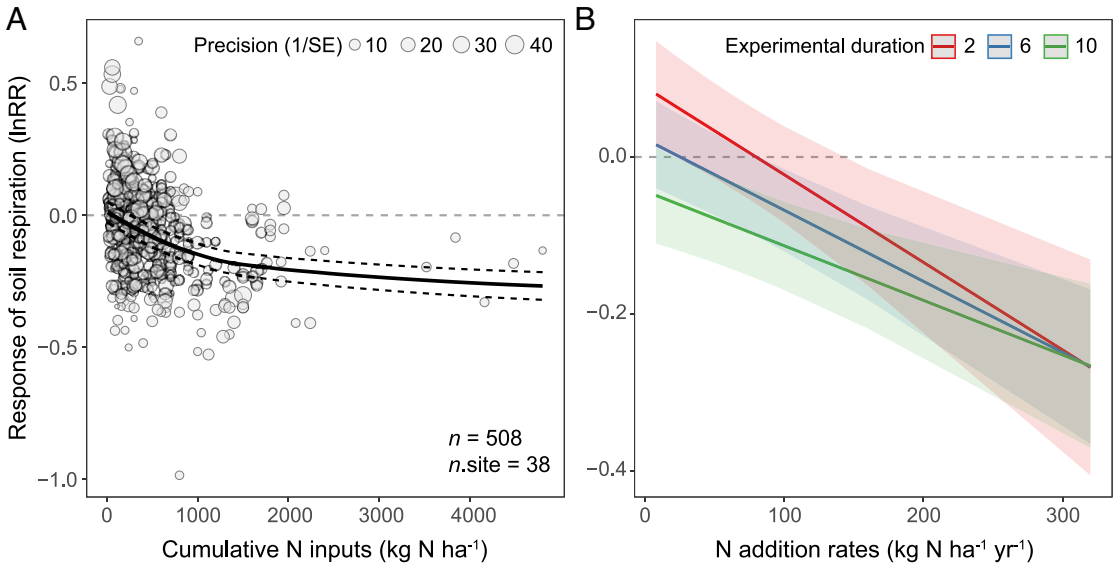


Fig. 4. Response of soil respiration to cumulative N addition. (*A*) Changes in the response ratio of soil respiration with cumulative N inputs, which is calculated by multiplying experimental duration and N addition rates. Each circle is an effect size and is scaled by the inverse sampling error variance. *n* and *n.site* represent number of observations and experimental sites, respectively. (*B*) Changes in the effect of N addition rates on Rs over time. Red, blue, and green lines represent the 25% (2 y), 50% (6 y), and 75% (10 y) quantile of experimental duration and the shaded area represents the 95% CI.

biomass (9 sites, n = 103), and fine root biomass (7 sites, n = 56) from our dataset.

Our result showed an overall negative effect of N-addition on soil pH (RR_{pH}), which was reduced by 0.21 (95% CI = [−0.33, −0.08]), and intensified with cumulative N addition (*SI Appendix*, Figs. S7 and S8). This is consistent with previous findings reporting a negative response of pH to N addition (32, 33) and increased acidification over time (33). Crucially, the finding that high N rates accelerate the temporal transition supports a rate-dependent saturation hypothesis, by which higher N inputs deplete soil buffering capacity and labile substrates more rapidly (39). However, acidification alone only partly explains the temporal dynamic of Rs response to N addition (Fig. 5*A* and *SI Appendix*, Table S5, marginal R^2 = 0.16).

Changes in soil microbial biomass (RR_{MBC}) provided an additional explanatory link between N addition and Rs. While previous studies reported conflicting results on microbial responses to N addition (31, 36), our analysis showed a shift in the microbial biomass response from positive to negative with increasing cumulative N input (*SI Appendix*, Fig. S8), closely mirroring the trajectory of Rs (Fig. 5*B* and *SI Appendix*, Table S6, marginal R^2 = 0.26). This suggests that initial stimulation of Rs was likely driven by a priming effect, where N addition alleviated microbial N-limitation (24, 46) and stimulated the decomposition of fresh plant C (25, 47). The subsequent suppression and attenuation reflect the long-term inhibition of soil microbial biomass and activity, potentially due to soil acidification effects on microbial activity (40, 48, 49). There was also no significant interaction between RR_{MBC} and biome, indicating no evidence that the effect differed between forests and grasslands.

Belowground plant inputs (fine root biomass, RR_{root}) showed an unexpected overall increase with N addition (*SI Appendix*, Fig. S7), which was in contrast to findings from a previous data synthesis (50). RR_{root} also decreased with cumulative N input,

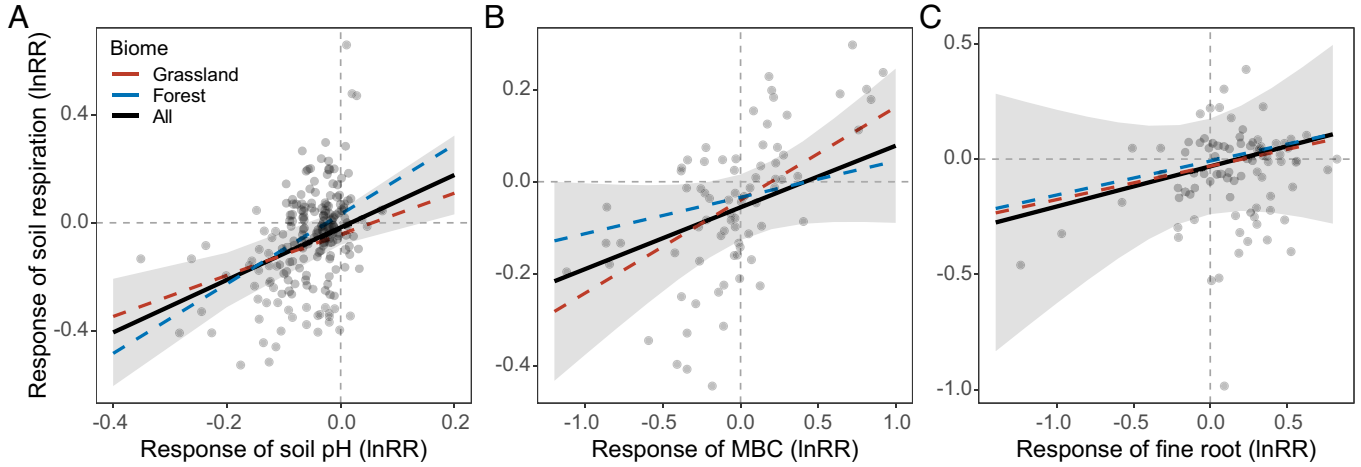


Fig. 5. Possible regulators of temporal dynamics of Rs response to experimental N addition. Moderating effects of soil pH (*A*), soil microbial biomass (MBC, *B*), and fine root biomass (*C*) on the response of Rs to N addition. None of the interaction terms involving the response ratio of regulators and biome were significant (*SI Appendix*, Tables S4–S6), indicating that the effects of these factors on soil respiration did not differ significantly across biomes. We therefore visualized the marginal effect of the response ratio of regulators on soil respiration as a solid black line with 95% CI, while predicted trends for each biome were shown as dashed lines.

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consistent with a previous finding that root production responds more negatively with longer experimental durations (50). A meta-analysis showed that the response of root respiration also attenuated with experimental duration (51), which might contribute to the temporal dynamics of soil respiration under N addition. Results of mixed effect model regression showed that RR of fine root biomass correlated weakly with RR_{Rs} (Fig. 5*C* and *SI Appendix*, Table S7, marginal R^2 = 0.06). In synthesis, while acidification acts as the initial trigger underpinning rapid changes in Rs due to N addition, the temporal dynamics of the soil microbial community then appear to take over as the dominant driver governing the trajectory of Rs responses over longer timeframes. The observed temporal pattern thus may reflect ecosystem-level acclimation.

Other factors, such as microbial community composition and P limitation, may also play an important role in mediating Rs response to N addition over time, but are challenging to quantify and consequently not measured in many long-term experiments. With N addition, microbial community composition will shift toward dominance by copiotrophic taxa (40, 52, 53). This shift can affect microbial functioning via changes in enzyme activity (48, 53) and microbial C use efficiency (i.e., the efficiency in converting organic C to microbial biomass), with consequences for soil C stocks. Second, global P limitation of plant growth has been increasing since the 1900s (54), and N addition might lead to progressive P limitation of ecosystems (55). Accordingly, it has been shown that N addition decreased Rs, while adding N and P together resulted in an increased Rs response over time (45).

Limitations and Implications. Although our dataset is unique in its temporal depth, studies that measured Rs over a decade account for only one third of these field sites, and longer-term response of Rs to experimental N addition might further modify the temporal pattern of Rs responses. For instance, long-term N addition in tropical forests revealed a phased response of Rs and a reversal phase over a decade of observation (34). Piecewise regression of the data revealed evidence of reversal effect at a tipping point around 13 y (*SI Appendix*, Fig. S9). Since long-term measurements were still limited, we argue that this potential temporal reversal should be confirmed with more longer-term studies. Second, different components of Rs, such as autotrophic (R_a) and heterotrophic respiration (R_h), may respond distinctively to long term N addition (30). A previous synthesis found that N addition generally suppressed R_h (14), but the response was context dependent, with negative effects more frequently observed in forest and grassland (16, 18, 30). The response also showed temporal shifts, where R_h mirrored the temporal dynamics of Rs observed in this study, i.e., showing a positive to negative shift over time (18). However, frequently used trenching methods are somewhat problematic because they not only affect R_a, but also plant C allocation to mycorrhizae, and rhizodeposition to heterotrophic decomposers. Third, the relative contribution of different regulators in shaping temporal dynamics of Rs cannot be perfectly quantified as strict time-series data for these variables were not available for most of the sites. Furthermore, site-specific confounding variables may also influence temporal dynamics of Rs responses to N addition (30). To account for this, we fitted a fixed effect OLS model with site-by-year fixed effects to control for unobserved heterogeneity across site-year combinations. The analysis showed a comparable rate of decline in RR_{Rs} with cumulative N input (*SI Appendix*, Table S8, 95% CI = [−0.12, −0.05]), suggesting that our main findings are robust.

In conclusion, time-series data from long-term N addition experiment reveal a positive to negative shift of Rs response over time. This temporal pattern indicates progressive acclimation and has important implications for soil C cycling under sustained nitrogen inputs. First, models of soil C dynamics should incorporate this temporal dynamic to improve predictions of soil C dynamics under persistent N addition rather than assuming a constant response. Second, the initial positive response shifted to a negative response even under low-level N addition, suggesting that the stimulatory effect was transient. However, given that global N deposition will likely decrease over the coming decades (2), this C benefit may be compromised (56). Overall, our findings demonstrate that reliable projections of future biogeochemical cycles under persistent global change require models to incorporate the mechanistic processes needed to reproduce the temporal dynamics documented in this study.

Materials and Methods

Database Compilation. To obtain time series records of Rs under N addition, we first identified experimental N addition sites from published meta-analyses. Unlike procedures adopted in previous meta-analyses, we used a site-based rather than publication-based compilation approach (*SI Appendix*, Fig. S10). To this end, we first established a database that included field sites with reports of Rs responses to N addition. To include as many field sites as possible, we searched for existing meta-analyses in November 2023 with the key words "soil respiration" and "meta" from Web of Science, which returned over 120 papers.

We compiled the database of time series data in two steps. First, we screened these meta-analyses and identified 21 regular meta-analyses involving N addition effect on Rs and further identified field experiments that fulfilled the following criteria: 1) measurement on Rs had to be conducted in the field in natural ecosystems (including forest plantation), and Rs should be reported as either annual mean flux or cumulative emission. 2) manipulation experiments had to include a N addition only treatment. Any N addition treatments that incorporated other elements were excluded to avoid potential confounding effects. According to these criteria, we were able to locate 228 field sites with Rs measurements (*SI Appendix*, Fig. S11). Second, we screened these field sites to identify experimental sites that recorded Rs over at least 5 y. Screening was done using multiple methods including cross reference search of publication and thesis published by leading and corresponding authors from Web of Science, Google scholar, Researchgate, China National Knowledge Infrastructure (CNKI), official and personal websites with keywords "respiration" and "nitrogen deposition." Eventually, we identified 38 field sites (Fig. 1) that met these criteria. We used a similar approach to collect soil pH, soil microbial biomass, and fine root biomass data to account for drivers of potential temporal shift in Rs. Given that very few sites collected 5 y' worth of data for these variables, we also retained those that reported these variables for at least 2 y, provided that these measurements included the early stage (within 3 y) and a later stage at the specific experimental site.

For each study site, the mean, sample size, and SD of Rs, soil pH, soil microbial biomass (MBC), and fine root biomass were obtained from the relevant tables or extracted from figures with Get Data Graph Digitizer 2.22. Data retained for soil pH, MBC, and root biomass were from surface soil layer. We also extracted the time series climate data (including mean annual temperature and annual precipitation) from the study site if possible. When the meteorological data from sites were unavailable based on their location, we obtained the data from WorldClim2 (https://www.worldclim.org) using site locations. Background N deposition rate of the study sites was also recorded from the literature published from these sites to account for potential confounding effects.

Statistical Analysis. We explained our data analysis in a flow chart (*SI Appendix*, Fig. S12). Specifically, to quantify the effects of experimental N addition on Rs, we used the log response ratio as the effect size measure, which was defined as natural logarithm of the ratio of the treatment group to the control group. Linear mixed models were fitted to assess the temporal changes in response ratio of Rs, and we accounted for temporal autocorrelation in the model using R package "*nlme*." Specifically, we constructed a full model including duration

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by N addition level interactions and duration by ecosystem type interactions. We then performed a backward model selection process by comparing model with a random intercept and a random slope to test if duration effect varied by sites. We compared a model with an autoregressive order 1 (corCAR1) correlation structure nested within each site-by-N-rate combination against a model with only nested random effects (to account for potential dependency across N levels within a site). The AR1 structure significantly improved model fit, so this specification was retained. We expected that the temporal trajectory would be gradual, nonlinear pattern than linear shifts with ecosystem acclimation. To assess this temporal shift of Rs over time, we did a model comparison of mixed effect models fitted with linear and logarithm terms. Models were assessed with Akaike information criterion (AIC), Bayesian information criterion (BIC), and rmse (*SI Appendix*, Table S3), and the model of logarithm term performed better, so we modeled the response ratio as a function of log-transformed experimental duration. We also modeled the response ratio as a function of log-transformed cumulative N addition (calculated as N addition rate × duration).

We determined the critical transition thresholds, defined as the time or cumulative N dose at which the N effect shifts from stimulative to suppressive, using an inverse prediction approach. These "zero-crossing points" were mathematically derived by solving the fitted regression equations for the point where the predicted response ratio equaled zero. To quantify the uncertainty of these thresholds while respecting the non-negative and right-skewed nature of temporal and dosage data, we calculated asymmetric 95% CI. Specifically, we estimated the SE of the thresholds on the logarithmic scale using the Delta method (via the "*msm*" R package), constructed the CIs on the log scale, and then back-transformed them to the original scale.

We did a multilevel meta-analytic model with a (sampling) variance–covariance matrix following the recommended procedures (57). Response ratio and its sampling variances were defined as follows.

$$lnRR = ln\left(\frac{Rs_{trt}}{Rs_{ck}}\right),$$

$$Var\left(lnRR\right) = \frac{{S_{trt}}^2}{n_{trt}{Rs_{trt}}^2} + \frac{{S_{ck}}^2}{n_{ck}{Rs_{ck}}^2},$$

where Rs_{trt} and Rs_{ck} are the mean of Rs under N addition and the control groups, respectively; n_{trt} and n_{ck} are the corresponding replications, S_{trt} and S_{ck} are the SD. Effect sizes were weighted by the inverse of their sampling variances. We also used the log-response ratio to assess the effect of N addition on MBC and root biomass, while calculated the response of soil pH to N addition as a standardized mean difference, rather than the log-response ratio, since it is more straightforward and easier to comprehend. This measure of response ratio was back transformed as percent change as $\left(e^{lnRR}-1\right)\times 100$.

We accounted for two types of dependence in these models. 1) multiple lnRR measurements in a site, and 2) correlation among sampling errors resulting from multiple measurements over time. The model specification included study site and observation level random effects, as well as sampling variance–covariance matrix. Intercept only model was fitted to estimate the overall response ratio of N addition, and models with moderators were fitted to assess the effect of N addition rates and the change in Rs with experimental duration and cumulative N inputs. The model was fitted using a restricted maximum likelihood (REML) estimator with the "*metafor*" R package and was visualized with the "*orchaRd*" R package. Publication bias was visually assessed using funnel plots of model residuals (*SI Appendix*, Fig. S13).

Linear mixed models were fitted to assess the association between the response ratio of Rs and response ratios of potential regulators (including soil pH, soil microbial biomass and fine root biomass). We did a separate analysis for each potential regulator and fitted a linear mixed-effects model including all two-way and three-way interactions among response ratios of the regulators, experimental duration, and biome, with experimental site as a random intercept and a continuous autoregressive correlation structure to account for temporal dependence associated with repeated measurements. The model was fitted using REML estimator. The marginal R^2 was used to quantify the proportion of heterogeneity explained by the regulators with R package "*Sjplot*" (58). We also ran a separate linear regression at the site level to assess the relationship between RR_{Rs} and cumulative N inputs, and assessed whether the slopes changed with confounding variables including climate, background N deposition rates and soil pH. Since it was challenging to include all of the confounding variables; we also ran a fixed effect with ordinary least squares (OLS) to account for unobserved heterogeneity across sites and years (59). SE were clustered at the site level to correct for within-site autocorrelation.

Data, Materials, and Software Availability. Data and code data have been deposited in figshare [https://doi.org/10.6084/m9.figshare.33193161 (60) and https://doi.org/10.6084/m9.figshare.32445852 (61)]. All other data are included in the article and/or *SI Appendix*.

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