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Title: Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide

Running title: N addition increases SOC over time globally

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Abstract

Soil organic carbon (SOC) is the largest carbon sink in terrestrial ecosystems and plays a critical role in mitigating climate change. Increasing reactive nitrogen (N) in ecosystems caused by anthropogenic N input substantially affects SOC dynamics. However, uncertainties remain concerning the effects of N addition on SOC in both organic and mineral soil layers over time at the global scale. Here, we analyzed a large empirical data set spanning 60 years across 369 sites worldwide to explore the temporal dynamics of SOC to N addition. We found that N addition significantly increased SOC across the globe by 4.2% (2.7–5.8%). SOC increases were amplified from short- to long-term N addition durations in both organic and mineral soil layers. The positive effects of N addition on SOC were independent of ecosystem types, mean annual temperature and precipitation. Our findings suggest that SOC increases largely resulted from the enhanced plant C input to soils coupled with reduced C loss from decomposition and amplification was associated with reduced microbial biomass and respiration under long-term N addition. Our study suggests that N addition will enhance SOC sequestration over time and contribute to future climate change mitigation.

Keywords Nitrogen addition, Soil organic carbon, Carbon inputs, Dissolved organic carbon, Soil respiration, Soil layer, Carbon sequestration

Introduction

The global nitrogen (N) cycle has been substantially altered by excessive reactive N (such as N fertilizer) input (Compton et al., 2011; Niu et al., 2016), and this N imbalance is set to continue to grow in the future (Galloway et al., 2008; Liu et al. 2013). Ecosystem carbon (C) cycling and its feedback to climate change can be altered by increasing N loading, given that C and N cycling are firmly coupled (Greaver et al., 2016; Gu et al., 2018; Reay et al., 2008). Soil stores more C than vegetation and the atmosphere combined (Carvalhais et al., 2014; Lehmann & Kleber, 2015), thus a slight alteration in soil organic carbon (SOC) caused by N addition could dramatically affect global climate change. An understanding of SOC response to ecosystem N imbalance is therefore critical to addressing future climate change. Although aboveground ecosystem C response to N addition has been effectively quantified (Schulte-Uebbing & de Vries, 2018; Stevens et al., 2015; Xia & Wan, 2008), changes in SOC remain uncertain given divergent results are observed among studies (Boot et al., 2016; Kazanski et al., 2019; Tian et al. 2019).

In general, soil C dynamics are determined by two dominant processes: C gains derived by plant growth and turnover and C loss through microbial decomposition (Houghton, 2007). A number of studies suggest that N addition has the potential to affect both processes (Janssens et al., 2010; Liu & Greaver 2010; Song et al., 2019; Xia & Wan, 2008). Soil N availability for plant growth in terrestrial ecosystems is typically limiting (Ågren et al., 2012; LeBauer & Treseder, 2008); a constraint that has been significantly alleviated by N addition (Niu et al., 2010). Earlier studies have shown that N addition stimulated plant growth, and thus increased both plant biomass production and C stock (Schulte-Uebbing & de Vries, 2018; Stevens et al., 2015; Xia & Wan, 2008). These increases could enhance plant above- and belowground litter, exudate C incorporation into soils, and benefit to SOC formation and accumulation. However, a recent controlled experiment suggested that N addition did not change plant C input into soils as plant growth can acclimate to N enrichment (Lu et al., 2018). Simultaneously, N addition could either affect microbial biomass and activity positively by increasing plant fresh C incorporation into soils or negatively by changing soil pH (Treseder, 2008). Whether N addition increases SOC depends on the balance between the response of C inputs and effluxes (Liu & Greaver 2010). On the one hand, external N loading to the ecosystem may increase plant C incorporation into the soil (Fornara & Tilman, 2012) and/or decrease soil respiration (Janssens

et al., 2010), resulting in a positive C balance and stimulating soil SOC retention. Alternatively, N addition could reduce or not affect SOC compared to control plots (Reay et al., 2008). For example, increasing reactive N limits C inputs and can increase heterotrophic respiration (Boot et al., 2016), which may trigger C input less than efflux to reduce net SOC. Therefore, more research is needed to explore N addition effects on SOC as well as its regulatory mechanism. Unfortunately, individual studies are unlikely to be able to identify the general effect of N addition on SOC due to the nature of soil ecological complexity and spatial heterogeneity (Culina et al., 2018). However, it may be possible that an analysis that synthesizes quantitative information from a large number of controlled field experiments can reveal the global-scale patterns of SOC changes in response to N addition.

SOC dynamics in response to N addition may be a long-term ecological process, as soil C formation often takes decades or longer (Carvalhais et al., 2014; Wiesmeier et al., 2019). However, current studies generally focus on SOC dynamics over short timescales (Liu & Greaver 2010; Lu et al. 2011), making it difficult to gain insights into long-term SOC changes resulting from N addition. Several recent studies have suggested that chronic N addition has had the potential to greatly increase reactive N accumulation resulting in N saturation in terrestrial ecosystems (Niu et al., 2016; Tian, Wang, Sun, & Niu 2016). This would limit both plant growth and fresh C input into soils. Alternatively, cumulative N input would change microbial community structure and activity (Wang et al., 2018), thus affecting soil C loss. The changes in plant C inputs and soil C losses could collectively regulate SOC dynamics in long-term N addition regimes, but the magnitude and direction remain unclear. Exploring the effect of long-term nitrogen addition on SOC will contribute a scientific understanding of global C cycling and thus help inform climate change policy worldwide. Therefore, it is necessary to characterize the long-term dynamics of SOC and explore its controlling mechanisms.

The changes of SOC in response to N addition should further consider soil depth, given that the responses in soil C pool to climate change are strongly depth-dependent (Balesdent et al., 2018). Earlier studies have suggested that N addition would only stimulate SOC in the organic soil layer (Liu & Greaver 2010). This increase may result from a reduction in decomposition activity of microorganisms with N addition, given that most C is found at or near soil surfaces rather than transformed into deeper soils (Zak et al., 2008). However, SOC sequestered in mineral or deeper soil layers not only results from aboveground fresh C input (e.g., dissolved organic C (DOC) leaching)

(Sokol & Bradford, 2019) but is also strongly associated with root C incorporation (Clemmensen et al., 2013; Fisher et al., 1994). A recent meta-analysis showed that N addition increased plant fine root turnover, and, in turn, enhanced plant belowground C mixed into soils (Li et al., 2015). We would expect a substantial increase in SOC response to N addition in mineral soil layers given that C input from roots is more efficient in forming stable soil C (Jackson et al., 2017; Sokol & Bradford, 2019). Additionally, about half of global SOC is stored below 30 cm of soil (Balesdent et al., 2018), and changes in this C pool caused by external N input can substantially affect the global C cycle. However, most studies on the dynamics of SOC in response to N addition have focused on the surface soil (e.g., 0-10 cm) (Tian et al., 2019). Thus, the dynamics of SOC in the deeper, mineral soil layer response to N addition remain uncertain. Hence, we test whether the responses in SOC to N addition change with mineral soil depths.

In this study, we compiled a global data set of 4,803 paired observations from 369 sites spanning 60 years to evaluate the dynamics of SOC in response to long-term N addition (Figure 1). Newly available longer-term data sets enable us to test whether N addition has long-term effects on SOC in both organic and mineral soil layers. We also partitioned our data set into three time-periods; < 3 , $3-12$ and ≥ 12 yr to compare differences in SOC response among studies varying in duration of N addition. We further examined the effects of both plant C inputs and soil C losses on SOC to determine the underlying mechanisms by which N addition affects SOC over time (Figure S1). In this case, plant C inputs were characterized by the combination of plant aboveground biomass, litterfall, root biomass, fine root biomass, and autotrophic respiration. We quantified C losses using soil respiration and heterotrophic respiration (see Methods for details of variable selection). The specific questions we address in this study included: (1) how does SOC respond to N addition over time? (2) how does SOC in both organic and mineral soil layers respond to N addition at the global scale? (3) how do plant C inputs and soil C losses regulate SOC dynamics under N addition through time?

Methods

Variable selection

We investigated changes in SOC in response to long-term N addition and how plant C inputs and soil C losses respond to external N loading regulating SOC dynamics. As a result, studies that

purposefully demonstrated the effect of N addition on SOC and/or its related variables were selected for our analyses. In some cases, values are reported for soil organic matter content; we converted it to SOC content using a conversion factor of 0.58 (Atwood et al., 2017). N addition, to our knowledge, stimulates plant growth — for example, increases aboveground biomass (AGB) (Schulte-Uebbing & de Vries, 2018; Song et al., 2019; Xia & Wan, 2008) and root biomass (RB) (Li et al., 2015; Song et al., 2019; Xia & Wan, 2008). These increases can enhance both plant litter (including litterfall (Song et al., 2019) and root necromass (Kou et al., 2018; Li et al., 2015)) and exudate transfer into the soil through the partial decomposition of soil biota (Cotrufo et al., 2015). Therefore, we selected AGB and litterfall to collectively quantify plant aboveground C inputs since litterfall is difficult to determine in some ecosystems (e.g., cropland). Root litter and exudates are also challenging to measure directly, so we represent belowground plant C inputs by total root biomass (RB), fine root biomass (FRB), and autotrophic respiration (Ra; reflects root metabolic activity (Tang et al., 2019)) combined. Dissolved organic carbon (DOC), which is a labile component of SOC that is generally attributed to plant-derived C inputs (Cotrufo et al., 2015; Sokol & Bradford, 2019), was included in our analyses. We indicate soil C losses with soil respiration (Rs) and heterotrophic respiration (Rh), although Rh is a substantial part of Rs. We included microbial biomass C (MBC) as it is strongly correlated with Rh (Zhang et al., 2018) as well as the degradation of SOC (Ogle, 2018). Soil pH that is directly affected by N addition, and thus restricts microbial activity influencing SOC retention (Janssens et al., 2010), was also selected for our analyses.

Data collection and compilation

Publications (e.g., peer-reviewed journal articles and dissertations) that reported SOC or its contributor responses to N addition (up to July 10, 2019) were identified using Web of Science, Google Scholar, and China National Knowledge Infrastructure (CNKI, <http://www.cnki.net>). The search string was: “(nitrogen addition OR nitrogen enrichment OR nitrogen deposition OR nitrogen supply OR nitrogen input OR nitrogen fertilization) and (soil organic carbon (SOC) OR dissolved organic carbon (DOC) OR microbial biomass carbon (MBC) OR litterfall OR root biomass (RB) OR plant biomass OR soil respiration (Rs) OR heterotrophic respiration (Rh) OR pH)”. To avoid possible selection bias, only primary studies satisfying the following four criteria were selected: (1) N addition experiment conducted directly in terrestrial ecosystems excluding laboratory incubation studies with

N addition. They must contain at least one of our chosen response variables. (2) Consistent biotic and abiotic conditions in N addition (treatment) and ambient (control) plots at the beginning of experiments (excluding experimental N addition). (3) For studies with experimental factors other than N addition, only treatment and control plot data were selected. (4) Means and variable replicates in control and N treatments could be extracted from the experimental context, tables, or digitized graphs. We only used once if the same data for a study appeared in several publications. When experimental data for consecutive years were reported, we only selected data in the latest year to adhere to the statistical assumption of independence among observations. We also recorded, as individual observations, publications where data included several experiments under different conditions, such as diverse ecosystems, N addition rates, and soil depths. If data appeared in graphs, we extracted using GetData Graph Digitizer v.2.24 (<http://getdata-graph-digitizer.com/>).

For each published study, mean values, sample size, and standard deviation (if reported) of selected variables were extracted. We also collected experimental variables described in publications. These include N addition rate ($\text{kg N ha}^{-1} \text{ yr}^{-1}$, the magnitude of N application per year), N addition duration (years, the number of years with N repeated applied), N form (the types of N fertilizer), and N application frequency (yr^{-1} , the number of N applications per year). If the rate of N addition was changed during the N addition experiment, we then calculated overall application dose using the amount of N fertilizer and experimental duration (total N application/duration). We used mixed N to represent N form if multiple N fertilizers were applied in different periods (see, for example, refs (Belay et al., 2002)). Other data collected from publications included: latitude, longitude, ecosystem types, mean annual temperature (MAT), mean annual precipitation (MAP), soil layers (organic versus mineral layer), and soil depth (the middle value of the sampling depth interval). Because within-study soil depth records for the organic layer were available for only a few of the 476 studies, we recorded soil depth only to be used for belowground variable analyses of the mineral layer. We downloaded values of MAT and MAP from the Worldclim database (<http://www.worldclim.org/>) using geographical location information (i.e., longitude and latitude) if the data were unavailable in the publications. This resulted in a broad range of MAT ($-10\sim 33^\circ\text{C}$; Figure S2a) and MAP ($72\sim 7400 \text{ mm yr}^{-1}$; Figure S2b) encompassed in our data set. To examine whether the response of SOC to N addition

was dependent on ecosystem, we classified sites into eight ecosystem types on the basis of the categories or dominant vegetation types reported in the publications (Figure 1).

Statistical analysis

To evaluate the effect sizes of N addition on SOC and its related variables, we calculated the natural log-transformed response ratio ($\ln(RR)$) from each observation as recommended (Hedges et al., 1999). In meta-analysis, the estimation of effect sizes and subsequent inferences, may depend on weighted methods of individual observations (Van Groenigen et al., 2011). Our data set show that sampling variance was not reported for 35 of the 476 studies. More importantly, the mean effect of selected variables would be determined predominantly by a few individual observations that were assigned extreme importance if weighted by sampling variances (Wu et al., 2011). Consequently, we used the number of replicates to calculate weighting factors (Wr) instead of the variance as follows (Pittelkow et al., 2015): $Wr = (N_t \times N_c) / (N_t + N_c)$, where N_t and N_c are the replication of observations in the N addition treatment and control, respectively.

We evaluated how SOC changed in response to N treatment for as long as 60 years based on the following approaches. First, we use a linear mixed-effects model: $\ln(RR) = \beta_0 + \beta_1 \times \ln(D) + \pi_{\text{Study}} + \varepsilon$ to test whether the overall effect differed from zero and whether experimental duration affected the $\ln(RR)$; where β_1 is coefficient, π_{Study} is the random effect of “Study” to explicitly account for the autocorrelation among observations within each study, and ε is sampling error. Our analyses used a restricted maximum likelihood algorithm (lme4 package (version 1.1-20) (Bates et al., 2015)), with each associated observation weighted by Wr . We also compared linear and logarithmic linear responses to examine the assumption of linearity between logarithmic response ratio and continuous predictors. We found that the model with logarithmic D results in lower or similar Akaike information criterion (AIC) values (Table S1). When predictor variables were scaled (z transformation), β_0 is the overall effect at the mean of $\ln(D)$ (Cohen et al., 2013).

We then fitted a full model with the random-factor “Study” weighted by Wr , to determine whether N addition rate, N form, and geographic factors (i.e., ecosystems, MAT, and MAP) affected the temporal response of SOC to N addition. The experimental duration was a first-order interaction with all predictors of putative in the full model. To prevent the model from overfitting, we use the ‘dredge’ function of the MuMIn 1.42.1 package (Bartoń, 2018) to select the parsimonious model

among all alternatives. For the most parsimonious models, SOC content and stock were affected by the interaction of N addition duration and rate, but not by N addition rate. The terms for geographic factors and N form were excluded (Table S2). To better illustrate the interactive effect between the duration and rate of N addition, we calculated rate-dependent experimental duration effects by N application levels of ≤ 50 , 50–100, and > 100 kg N ha⁻¹ yr⁻¹, respectively. The levels of N addition rate were the common categories used in previous meta-analysis (Song et al., 2019) of the effect of N addition on ecosystem function. A bootstrap procedure (4,999 iterations) was conducted to estimate the 95% confidence intervals.

Finally, we partitioned our total data set into N addition duration < 3 , 3–12 and ≥ 12 yr based on random forest analysis (randomForest 4.6-14 package (Liaw & Wiener, 2002)) (Figure S3), to compare the difference of changes in SOC response to different N treatment lengths. The divergent duration represents short (< 3 yr), medium (3–12 yr), and long-term (≥ 12 yr) N addition treatments. From short- to long-term N additions, we found that the responses of SOC content and stock showed a substantial and marginal increase, respectively (Table S3).

We also performed an analysis using a random-effect model with geographical factors as the only fixed effect, to examine further whether geographical factors had a significant effect on the SOC response ratio. The analysis confirmed that there is no divergence in the responses of SOC to N addition among experimental regions or ecosystems. We also examined whether SOC, in response to N addition, differed with N application frequency, soil layer (organic versus mineral) and soil mineral layer depth. We found that the soil layer affected SOC content, but did not interact with the duration or rate of N treatment.

To test the potential pathways determining the effect of N addition on SOC, we first examined whether the response of variables related to plant C inputs and soil C losses to external N loading was affected by the duration and rate of N addition. The dependent variables were natural-log transformed if the model with log transformed variables showed lower or similar AIC values in linearity assumption tests. Next, we modelled structural equations to determine how N additions affected SOC dynamics, but this analysis was limited by the number of paired variables in our data set. Here, we only found positive N addition effects on DOC *via* stimulating litterfall C inputs, and negative N addition effects on MBC *via* reducing soil pH. Then, we analyzed the relationship between the

response ratio of SOC and its related variables in the whole data set and portioned N addition duration using SMA regression (smatr version 3.5.2) (Warton et al., 2012). We focused these analyses on SOC content because the number of SOC content observations was approximately 3.6-fold more than SOC stock in our data set. All analyses were performed in R (version 3.5.1) (R Core Team, 2018).

Results

Responses of SOC content and stock to N addition

N addition significantly increased SOC content at the global scale, with longer duration (≥ 12 yr) N addition having the largest effect. Averaged across all ecosystems, climates and treatment lengths, N addition increased SOC content by 4.2% (95% confidence intervals, 2.7–5.8%, $p < 0.001$) (Figure 2a). This positive effect of N addition on SOC amplified with N treatment duration ($p < 0.05$, Figure 2 and Tables S2 and S3). Among different treatment durations, N addition increased SOC content at both medium (3–12 yr; $p < 0.001$) and long (≥ 12 yr; $p < 0.001$) durations, but not in short duration studies (< 3 yr; $p > 0.05$) (Figure 2a). N addition increased SOC stock ($p < 0.001$, Figure S4a) and this positive effect strengthened with N treatment length ($p < 0.05$, Figure S4b and Table S2). We found a positive correlation between SOC content and stock ($p < 0.05$, Figure S4c).

Furthermore, responses of SOC content and stock increased across timescales and were dependent on N addition rates with a more pronounced response at higher N addition rates (Figures 2b and S4b and Table S2).

N addition increased SOC in both organic and mineral layers (all $p < 0.05$, Figures 3a and S4d), a result that was consistent across diverse ecosystem types, climates and standing soil C contents (all $p > 0.05$, Table S4). Increases in SOC content in response to N addition were much higher in organic layers ($p < 0.001$, Figure 3a and Table S4) and enhanced in both soil layers across timescales (all $p < 0.05$) (Figure 3b, c). Our findings showed a consistent SOC stock response to N addition across both soil layers ($p > 0.05$, Figure S4d and Table S4). The mineral soil depth ranges of SOC data we compiled from 0 to 160 cm, and most of them are distributed at 0-50 cm (Figure S5). Although the response of SOC to climate change strongly depends on soil depth (Balesdent et al., 2018), the SOC changes in response to N addition we observed were independent of mineral soil depth (Figure S5 and Table S4). Among different soil depths, N addition significantly increased SOC content in 0-30 cm and 40-50 cm, and those increases are approximately 4.2% (Figure S5a). The SOC

response did not change among ecosystems, mean annual temperatures, or precipitation levels (all $p > 0.05$, Figure S6 and Table S4), despite the wide geographical range covered in this study.

C inputs in response to N addition

N addition increased aboveground plant C inputs across all studies. On average, N addition stimulated aboveground plant biomass and litterfall (all $p < 0.001$, Figure 4a). These positive effects did not change with experimental duration ($p > 0.05$) but increased with N addition rate ($p < 0.001$) (Figures 4a and S7). DOC, which is a labile component of SOC that depends strongly on plant C inputs (Sokol & Bradford, 2019), increased with N addition by an average of 6.7% ($p < 0.05$, Figure 4a). A positive correlation between litterfall and DOC response ratio was also observed (Figures S8a and S9a).

N addition increased total root biomass and autotrophic respiration ($p < 0.05$ and $p = 0.05$, respectively), but not fine root biomass ($p > 0.05$) (Figure 4b). Responses of total and fine root biomass did not differ among divergent N addition durations (all $p > 0.05$, Figure 4b and Table S3), despite total root biomass increased significantly ($p < 0.05$) and fine root biomass increased marginally ($p = 0.085$) over time (Figure S7a). Total root biomass tended to increase while fine root biomass tended to decrease with increasing N addition rates (all $p < 0.1$, Figure S7b). Additionally, the responses of SOC content and total root biomass were positively correlated across soil profiles (Figure S8b).

C losses from respiration in response to N addition

N addition substantially decreased heterotrophic respiration ($p < 0.001$), but not total soil respiration ($p > 0.05$) (Figure 4c). The responses of soil total and heterotrophic respiration to N addition decreased through time (all $p < 0.05$, Figure S7a). In comparison, soil total respiration decreased marginally, and soil heterotrophic respiration decreased significantly over the long-term ($p = 0.058$ and $p < 0.05$, respectively, Figure 4c). Furthermore, soil total and heterotrophic respiration substantially decreased with increasing of N addition rate (all $p < 0.05$, Figure S7b).

Microbial biomass C (MBC), which is an indicator of decomposer density and strongly related to heterotrophic respiration (Figure S10a), can easily be affected by altering soil pH (Figures. S9b and S10b). N addition reduced soil pH ($p < 0.001$), thereby significantly reducing MBC ($p < 0.001$, Figure 4c). The negative effect of N addition on MBC also increased with both N addition duration and rate

(all $p < 0.05$, Figures 4c and S7). In short-term studies, N addition did not affect MBC ($p > 0.05$, Figure 4c). In contrast, MBC declined significantly with N addition over the medium- and long-term (all $p < 0.05$, Figure 4c).

Discussion

N addition increased SOC over time

We quantified the positive effect of N addition on SOC with a comprehensive meta-analysis. Our results showed that globally, N addition increased SOC content by an average of 4.2% and SOC stock by 11.0% across organic and mineral layers. Importantly, SOC response to N addition increased with N addition duration, with a 432.9% greater response in long- compared to short-term studies ($p < 0.05$, Figure 2 and Table S3). Our results also suggested that the increase in N addition effect over time was dependent on the rate of N addition, with more pronounced responses in SOC content and stock observed at higher N addition rates. Several meta-analyses have tested the effect of N addition on SOC (Liu & Greaver 2010; Lu et al. 2011). However, here we compiled a larger dataset, and to our best knowledge, are the first to find that the positive effects of N addition on SOC across soil layers are increased over time. Furthermore, we explored the responses of plant C inputs and soil C losses to external N input and discovered several mechanisms that regulate how N addition affects the temporal dynamics of SOC.

First, changes in SOC in response to N addition increased over time, probably due to a combination of plant C inputs and soil C losses (Figure 5). Aboveground fresh C inputs are an essential source of SOC (Crow et al., 2009; Steffens et al., 2015). Our findings suggest that the contributions of plant aboveground C to SOC sequestration are consistent over 27 years. The positive correlation between litterfall and DOC response ratio provided further support for this finding. Plant root C inputs, that form stable soil organic matter much more efficiently than aboveground C inputs (Jackson et al., 2017; Sokol & Bradford, 2019), are another crucial soil C source (Clemmensen et al., 2013; Fornara & Tilman, 2012). Our results showed that fine root turnover increased and more root C was incorporated into the soil under N addition (Li et al., 2015), as N addition increased plant total root biomass but not fine root biomass. That changes in total and fine root biomass were not different for short to long-term N addition durations would suggest that the role of plant roots to SOC

accumulation was unchanged over time. This finding is supported by the positive correlation of SOC content and root biomass response. Autotrophic respiration reflects the metabolic activity of plant roots (Tang et al., 2019), that increased under N addition, but did not change with duration of N addition ($p > 0.05$, Figures 4b and S7a). This further suggests that external N inputs may increase root biomass, making a significant contribution to SOC accumulation (Fornara & Tilman, 2012).

Therefore, further studies to quantify the contribution of plant root C to SOC sequestration under N addition are necessary. Collectively, N addition could stimulate plant C incorporation, and results suggest that these positive SOC contributions did not decrease through time. Alternatively, soil C losses from respiration is an essential indicator of the degradation and stabilization of SOC (Janssens et al., 2010). Our findings suggested that soil C losses decreased as the duration of N addition increased (Zhou et al., 2014). Microorganisms degrade soil organic matter and are strongly associated with soil C loss (Ogle, 2018). N addition reduces MBC and heterotrophic respiration, thus increasing SOC, especially with more litterfall (Janssens et al., 2010; Liu & Greaver, 2010; Xia & Wan, 2008). Combining increases in plant C inputs and decreases in soil C losses suggest that N addition enhances and preserves SOC in the soil over time, with a significant increase of SOC in long-term N treatments.

A second regulatory mechanism of how N addition affects the temporal dynamics of SOC is the strong role of the rate of N addition. This is likely associated with changes in microorganisms and C losses in response to N addition. Our results showed that N addition stimulated plant aboveground C incorporated into the soil, and this increase was enhanced by increasing N addition rate. Plant total and fine root biomass showed different trends as N addition rate increased, suggesting that the incorporation of root C to the soil may increase with the rate of N addition (Fornara & Tilman, 2012). These results collectively show that the contributions of plant C inputs to SOC sequestration increased with N addition rate and remained unchanged over time. Furthermore, short-term N addition has less influence on microorganisms and soil C losses (all $p > 0.05$, Figure 4c), due to the buffering effect of soil on pH reduction and lagged microbial responses (Janssens et al., 2010). External labile C incorporation into the soil from plant growth and turnover could increase microbial biomass and activity (Bradford et al., 2013), which may increase SOC loss particularly at higher rates of N addition. This assertion is supported by Zhai et al. (2017), who found that soil C losses from

heterotrophic respiration were stimulated by short-term N addition and positively increased with N application rate. MBC and soil C losses, however, decreased with increasing duration and rate of N addition (Zhang et al., 2018), and plant C inputs increased with N addition rate. This likely resulted in more substantial increases in SOC when N addition rates were higher and longer-term.

Changes in SOC increased in both organic and mineral soils

We found, throughout the data we explored, that N addition stimulated SOC in both organic and mineral layers over time, lacking a saturating response and suggesting that persistent N addition could stimulate SOC across soil profiles. These results contrast with earlier meta-analyses (Liu & Greaver 2010), that found soil C increased only in the organic horizon with N addition/control comparisons. The difference may reflect the relatively short duration of N treatments in previous studies combined with the strong time-dependent effect of SOC response to N addition. Our results showed that changes in SOC content were greater in, but not limited to the soil organic layer as we also detected a smaller soil mineral layer response. Plant litterfall C may be preferentially transferred into the organic layer by decomposers (Sokol & Bradford, 2019) potentially resulting in SOC content-response to N addition that differs among soil layers. However, the changes in SOC stock we observed did not differ among soil organic and mineral layers. This was not surprising, given that SOC content is negatively correlated with soil bulk density (Yang et al., 2009) and SOC stock was determined by both variables (Carvalhais et al., 2014). It is therefore surprising that the response of SOC was unchanged with increasing soil depth in the mineral layer. This may result from biomass responses of total and fine roots that increased with the mineral depth (all $p < 0.05$), the consistent decrease of MBC in mineral horizons ($p > 0.05$) (Figure S11), and DOC leaching (Cotrufo et al., 2015; Sokol & Bradford, 2019). Since the changes in SOC were consistent as soil depth increased, our findings suggested that projecting SOC changes in response to N addition should consider soil depth (Balesdent et al., 2018). Global change soil research, to our knowledge, has largely focused on the effect of N addition on SOC in shallow soil layers (Deng et al., 2020). Estimates, only from shallow soils, may substantially underestimate changes of N-addition-driven SOC in the mineral soil layer. We emphasize that soil depth information should be incorporated to improve how the effects of long-term N addition on SOC are understood.

Consistent response of SOC across the globe

The data used in this meta-analysis were compiled from global N addition experiments covering a wide range of geographic variation. Our results showed that the response of SOC to N addition did not change across ecosystems, mean annual temperature or precipitation levels. In contrast to our findings, a recent meta-analysis showed that the responses of SOC to N addition changed among diverse ecosystems (Deng et al., 2020). However, the results of Deng et al. (2020) were concluded from 126 paired global observations suggesting that statistical differences may derive from unbalance ecosystem type sampling. Further data synthesis studies need to pay attention to the influence of the statistical limitations of the small number of studies on the results. Our global compilation of experimental data from 369 sites spanned the wide geographical range of MAT (-10~33°C) and MAP (72~7400 mm) is greater than the previous study (Deng et al., 2020). The new data set allows us to capture larger patterns in the dynamics of SOC to N addition. For this reason, we suggested that N addition will increase SOC across the globe. When analyzed by ecosystem types, the responses of SOC content increased in natural forests, plantations and croplands (all $p < 0.05$), marginally increased in shrublands ($p = 0.096$), but did not change in other ecosystems (Figure S6a). The lack of the N addition effects in the tundra, desert and wetland could be attributable to the small number of these studies (For example, only 2 tundra studies with measured SOC were compiled in our dataset (Figure S6 and Dataset S1)). The shorter treatment durations probably resulted in the lack of N addition effect in grasslands. Thus, we propose more long-term N addition experiments conducted in divergent ecosystem types to gain insights into SOC responses to N addition at the global scale.

Given that global stock of SOC is 2397 Pg (up to 1 m depth) (Carvalhais et al., 2014) and that N addition effects on SOC increase over time, we suggest that N treatments with a rate of 30 kg ha⁻¹ y⁻¹ have the potential to increase organic C in the soil by ~300 Pg over 20 years (average 15 Pg C yr⁻¹; estimates based on Figure S4b with N addition rate of 0–50 (mean ± S.D. = 32.8 ± 10.8) kg ha⁻¹ y⁻¹). It will be an enormous challenge to preserve SOC, especially in topsoil, as it is vulnerable to biogeochemical and physical disturbance (Jackson et al., 2017). Furthermore, it should be mentioned that several factors might affect the positive effect of N addition on SOC, including climate changes (Crowther et al., 2016; Pellegrini et al., 2018) and SOC saturation (Castellano et al., 2012), etc. For instance, a number of studies have found that warming stimulated SOC loss (Crowther et al., 2016).

Whether the negative effect of warming affects the response of SOC to nitrogen addition is still uncertain. Therefore, further studies on SOC dynamics in response to long-term N addition under changing climates are necessary for better understanding the feedbacks of ecosystem C to climate change.

In conclusion, our analyses of a new global empirical data set provides evidence for N addition substantially increasing SOC. SOC changes from our analyses highlight that present estimates of C sequestration induced by N addition (Schulte-Uebbing & de Vries, 2018; Stevens et al., 2015; Xia & Wan, 2008), which do not account for C dynamics in the soil horizon, significantly underestimate the influence of long-term N addition on terrestrial ecosystems. Our findings suggest that sustained N addition could increase SOC sequestration across organic and mineral soil layers over time in terrestrial ecosystems and play a critical role in alleviating the dramatic rise of atmospheric carbon dioxide in the future.

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Author contributions All authors contributed intellectual content, research assistance and / or manuscript preparation. XX conceived the idea and designed the study. CX collected and analyzed the data with help from XX, C J and HYHC. CX and XX wrote the manuscript with input from all authors.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Geographical distribution of study sites (n = 369) included in the meta-analysis. Colors represent the types of ecosystems, and dot shapes represent duration of manipulative N addition as indicated in the legend.

Figure 2. Effects of N addition on SOC content over time. a, Comparison of SOC content in N addition and control treatments across all studies, by length of N treatment. Values are mean ± 95% confidence intervals, with the number of observations shown above each N addition duration. b, Changes in the response of SOC content through time and by the rate of N addition. Black and colored lines indicate responses of mean and specific N addition rate intervals respectively, with bootstrapped 95% confidence intervals in shaded regions. See Tables S2 and S3 for statistical details.

Figure 3. Logarithmic response ratio of SOC content for two soil horizons (organic and mineral layers). a, Comparisons of SOC content in response to N addition between organic and mineral layers ($p < 0.001$). Dots and error bars represent means ± bootstrapped 95% confidence intervals for N

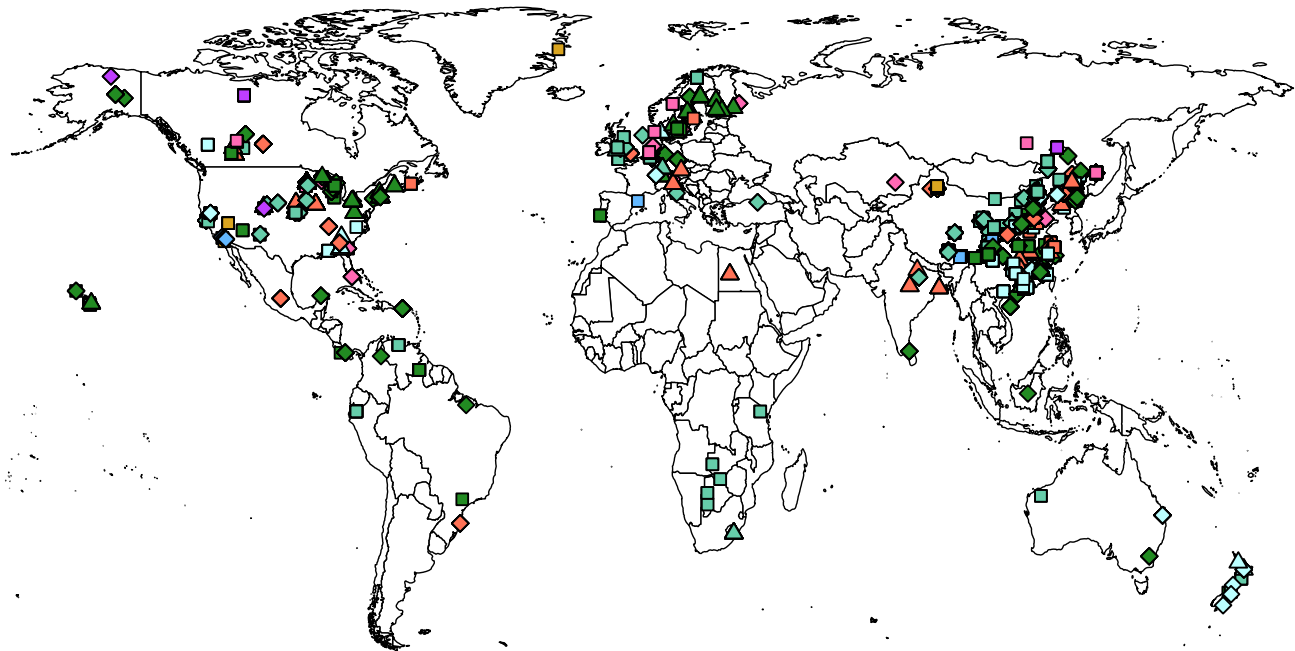
addition effect and experimental treatment duration respectively. Number of observations in each category appear below symbols. b and c, Linear regression of the response ratio of SOC content and N addition duration among organic layers (b; $p < 0.05$; the dot above the dashed line was not used in model fit) and mineral layers (c; $p < 0.05$).

Figure 4. Effects of N addition on SOC-related variables for all studies and partitioned by N addition duration. a, Comparisons of plant aboveground C input variables and dissolved organic C (DOC) response to N addition by N addition duration. b, Comparisons of belowground C input variable responses to N addition by treatment duration. c, Comparisons of soil C loss, microbial biomass C (MBC) and pH response to N addition in studies differing in duration. Values are means $\pm 95\%$ confidence intervals of logarithmic response ratios between N addition and control treatments, with the number of observations in each category shown. Plant aboveground biomass (AGB), total root biomass (RB), fine root biomass (FRB), soil respiration (Rs), heterotrophic respiration (Rh), and autotrophic respiration (Ra). See Table S3 for statistical comparisons.

Figure 5. Schematic illustrating mechanisms of N addition affecting SOC content in short- (blue), medium- (red) and long-term (orange) studies. The asterisk (*) indicates N addition effect is significant ($p < 0.05$), and abbreviations are the same as in Figure 4.

Graphical abstract

We analyzed a large empirical data set spanning 60 years across 369 sites worldwide to explore the temporal dynamics of SOC to N addition. N addition significantly increased SOC across the globe, and those increases were amplified from short- to long-term N addition durations in both organic and mineral soil layers. Our study suggests that N addition will enhance SOC sequestration over time and contribute to future climate change mitigation.



Duration

□ < 3 years

◇ 3 – 12 years

△ ≥ 12 years

Ecosystems

● Cropland

● Desert

● Grassland

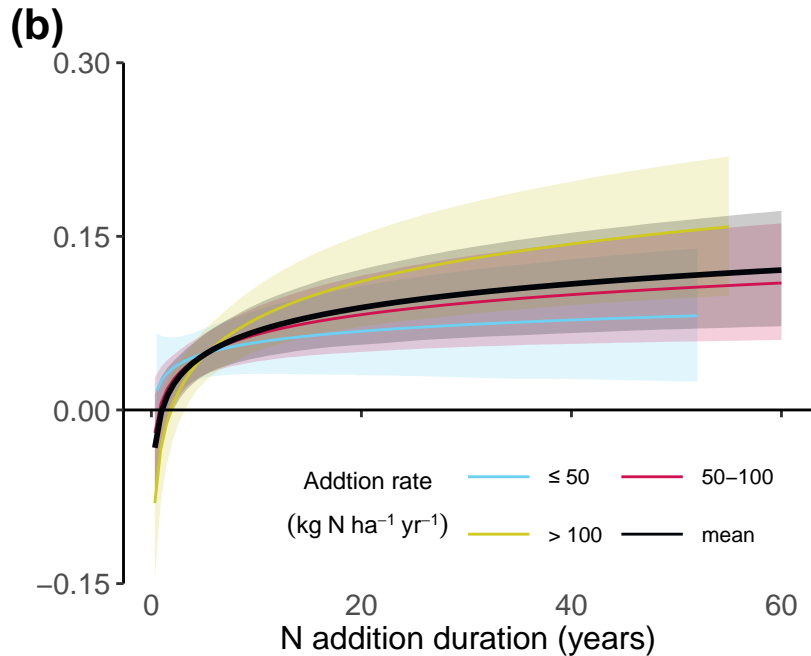
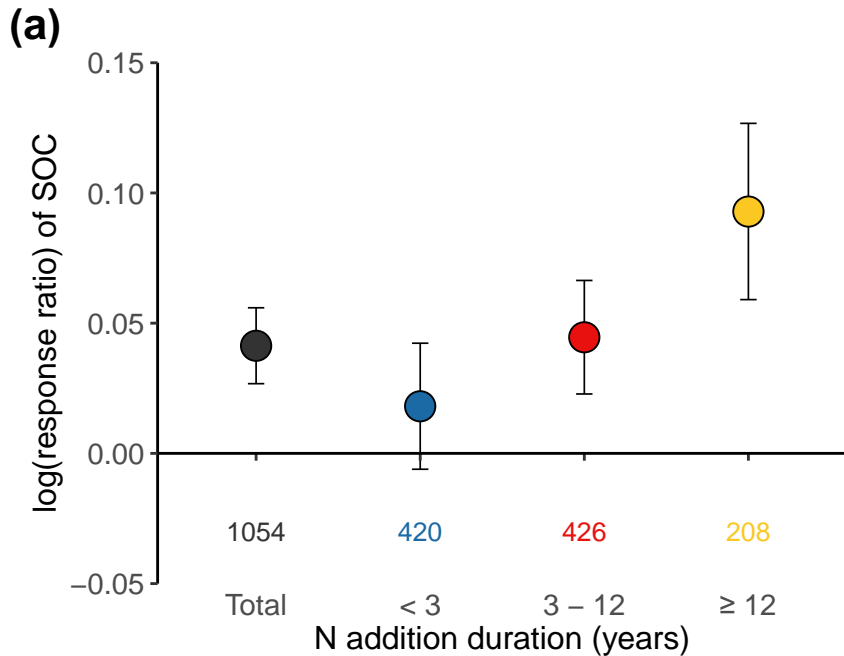
● Natural forest

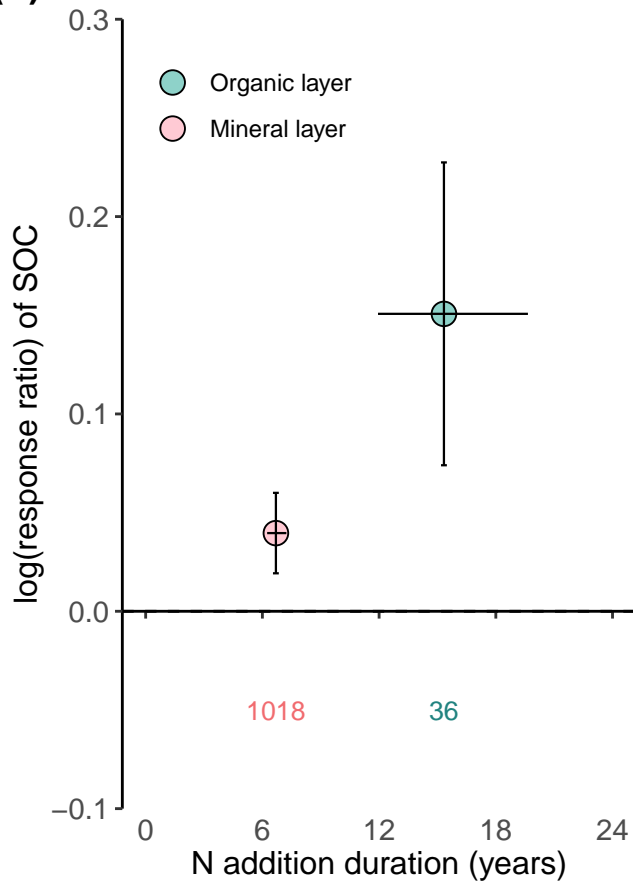
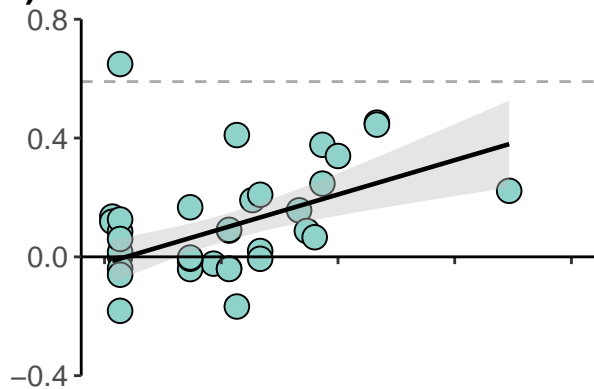
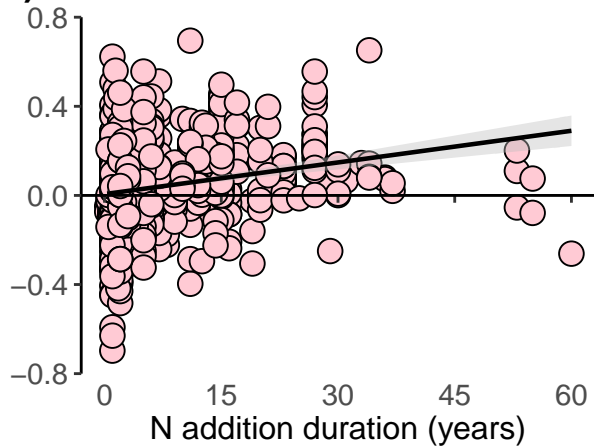
● Plantation

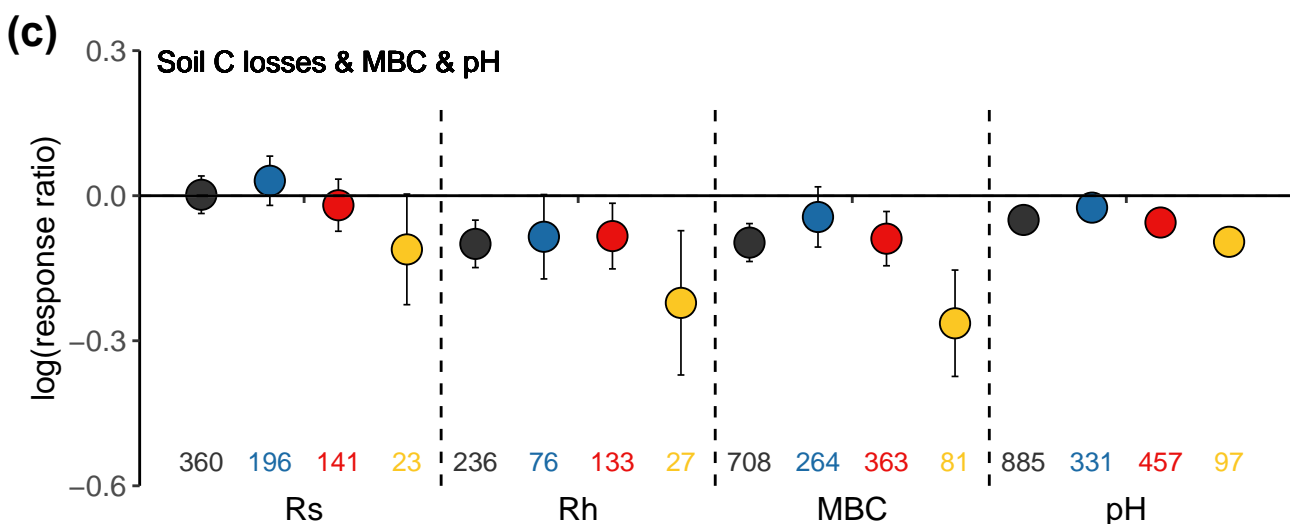
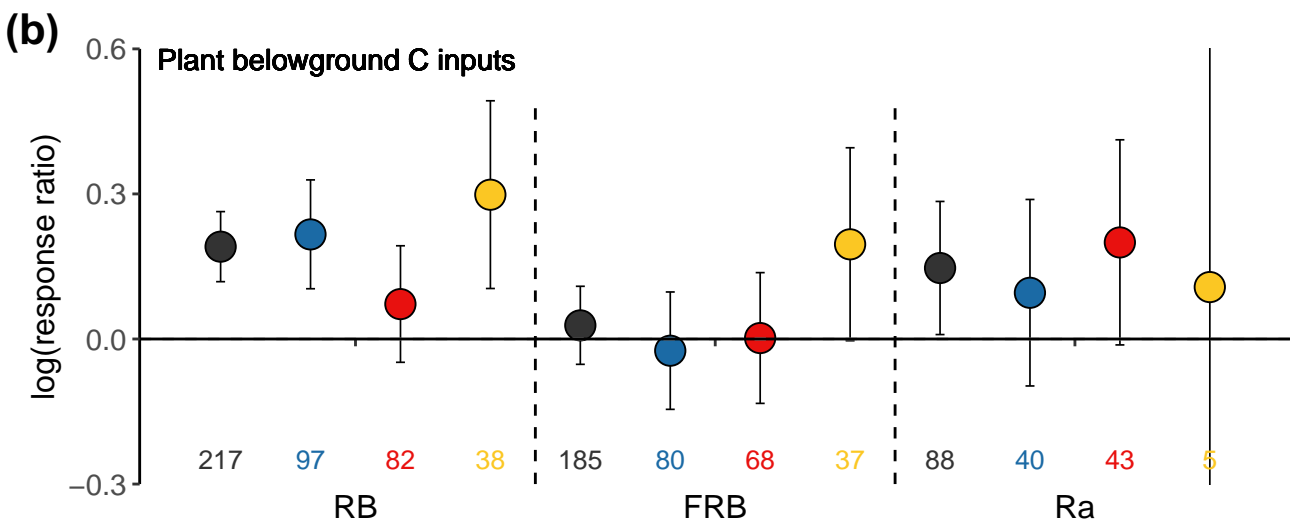
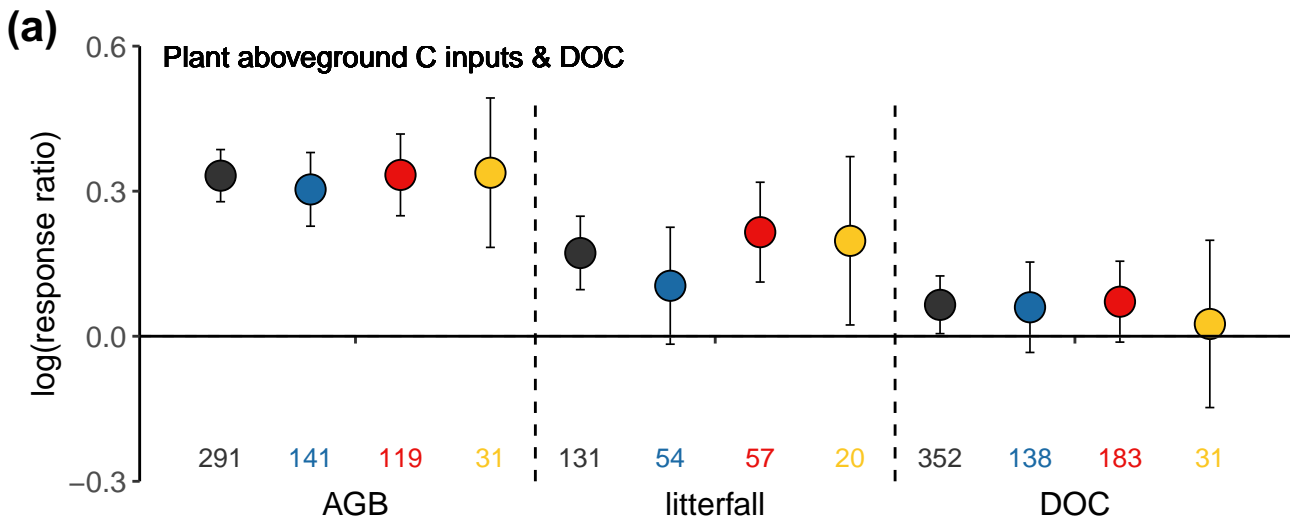
● Shrubland

● Tundra

● Wetland



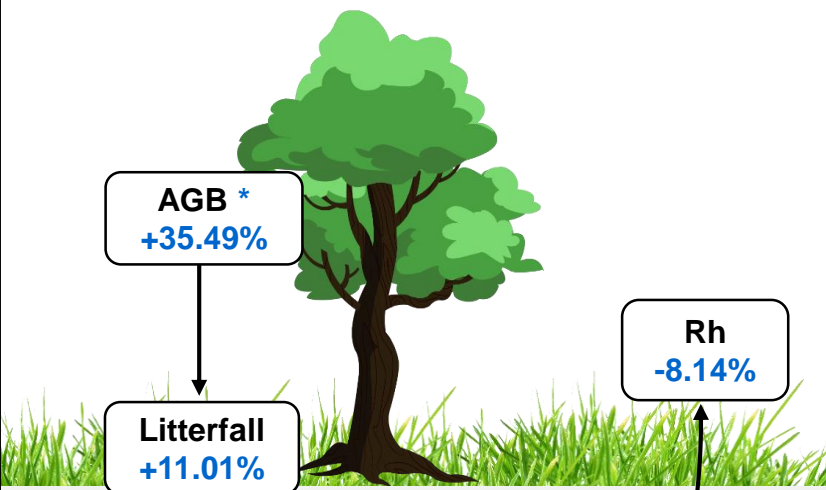
(a)**(b)****(c)**



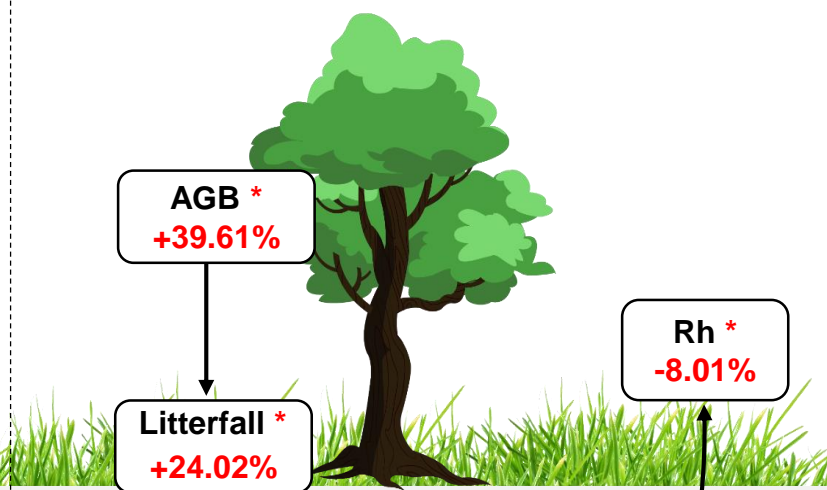
N addition duration (years)

- Total
- < 3
- 3 – 12
- ≥ 12

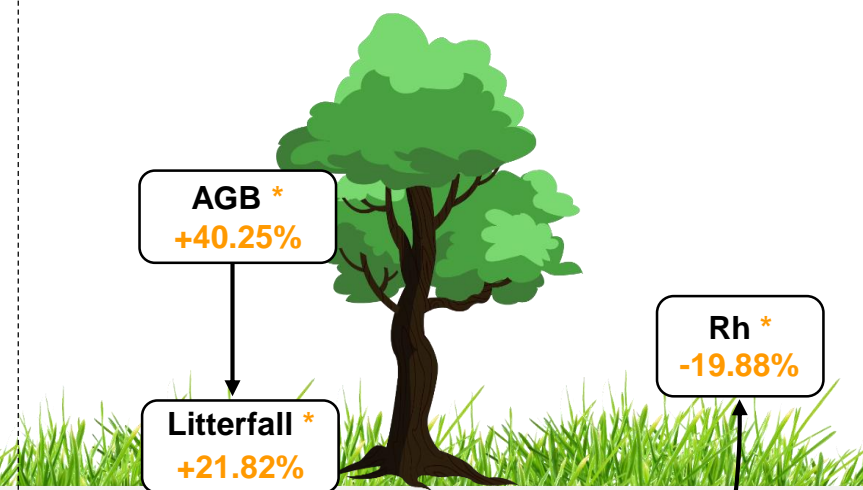
Nitrogen addition (duration < 3 years)



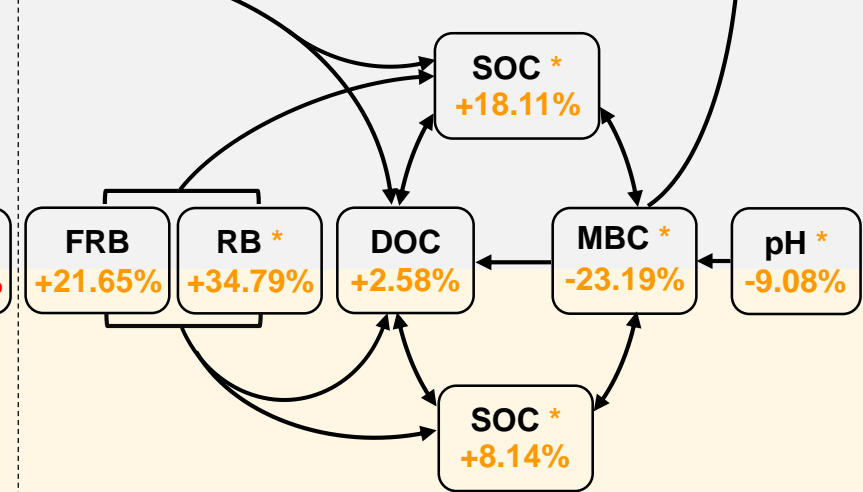
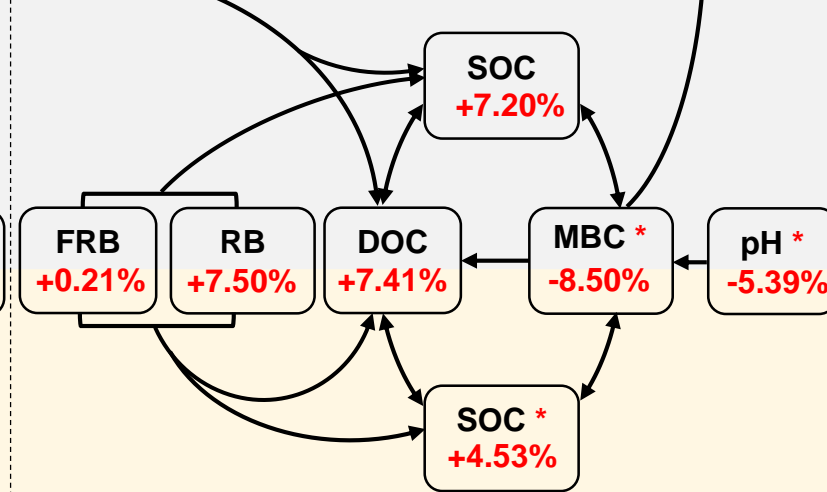
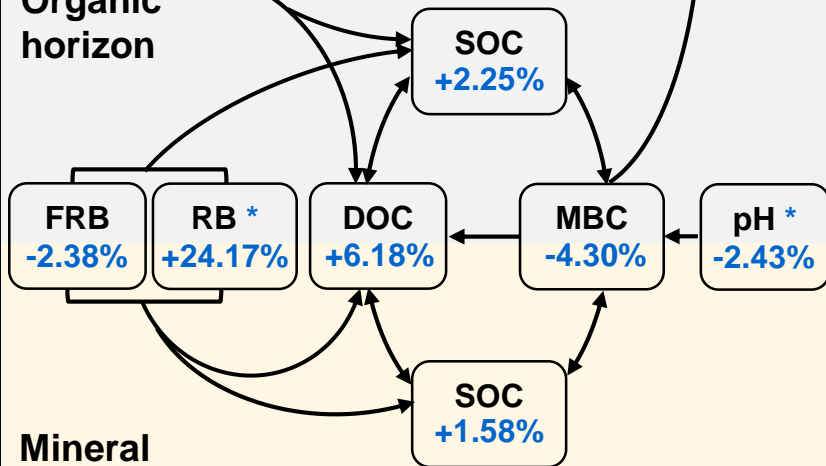
Nitrogen addition (3 years ≤ duration < 12 years)



Nitrogen addition (duration ≥ 12 years)



Organic horizon



Mineral horizon