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Temporal patterns of soil carbon emission in tropical forests under long-term nitrogen deposition

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Soil represents the largest terrestrial carbon pool, and it liberates massive amounts of carbon dioxide (CO_2) to the atmosphere via respiration, which can influence global carbon cycle. In recent decades, anthropogenic activities have dramatically increased the rates of atmospheric nitrogen (N) deposition worldwide, but our current understanding of soil respiration dynamics in anthropogenic N-deposition environments remains poor. Here we monitored soil CO_2 emission rates monthly following 9–13 years of N-addition treatments in three tropical forests in southern China. We found a three-phase pattern of soil CO_2 emission (insignificant changes–dramatic decline–insignificant changes) in three tropical forests and across three N-addition gradients. During the course of the experiments, N addition reduced a total cumulative amount of 6.53–9.06 $MgCO_2$ ha^{-1} with the efficiency of 5.80–13.13 $MgCO_2$ Mg N^{-1}. The mechanisms underlying the temporal patterns of soil respiration were related to the lack of plant and microbial responses (phase 1), the reduction in fine root and microbial biomass due to soil acidification (phase 2) and the reorganization of plant and microbial community (phase 3). These findings advance our understanding of soil respiration dynamics and support prediction of long-term soil C fluxes in tropical forests in the context of N deposition.

Elevation in atmospheric carbon dioxide (CO_2) concentrations, owing to anthropogenic activities (for example, fossil fuel combustion and land-use changes), becomes a scientific and political concern in the current world[1]. Both the Paris Agreement[2] and Intergovernmental Panel on Climate Change reports[3] emphasize the control of increased atmospheric CO_2 as an important step towards the goal of climate change mitigation, and meanwhile suggest the necessity of understanding the sources and sinks of atmospheric CO_2. Compared with the human-caused increases in atmospheric CO_2, annual C efflux into atmosphere from soil is much larger[4]. Soil represents the largest carbon (C) pool in terrestrial ecosystems, and at least half of terrestrial soil organic C lies in forests[5]. Importantly, global forest C fluxes are dominated by tropical and subtropical forests, which account for 78% of the total CO_2 emission and 55% of the total CO_2 uptake[6]. Hence, a small change in soil C fluxes of tropical/subtropical forests can lead to a significant change of atmospheric CO_2 concentrations.

Anthropogenic activities have enhanced not only atmospheric CO_2 but also deposition of reactive nitrogen (N). At the global scale,

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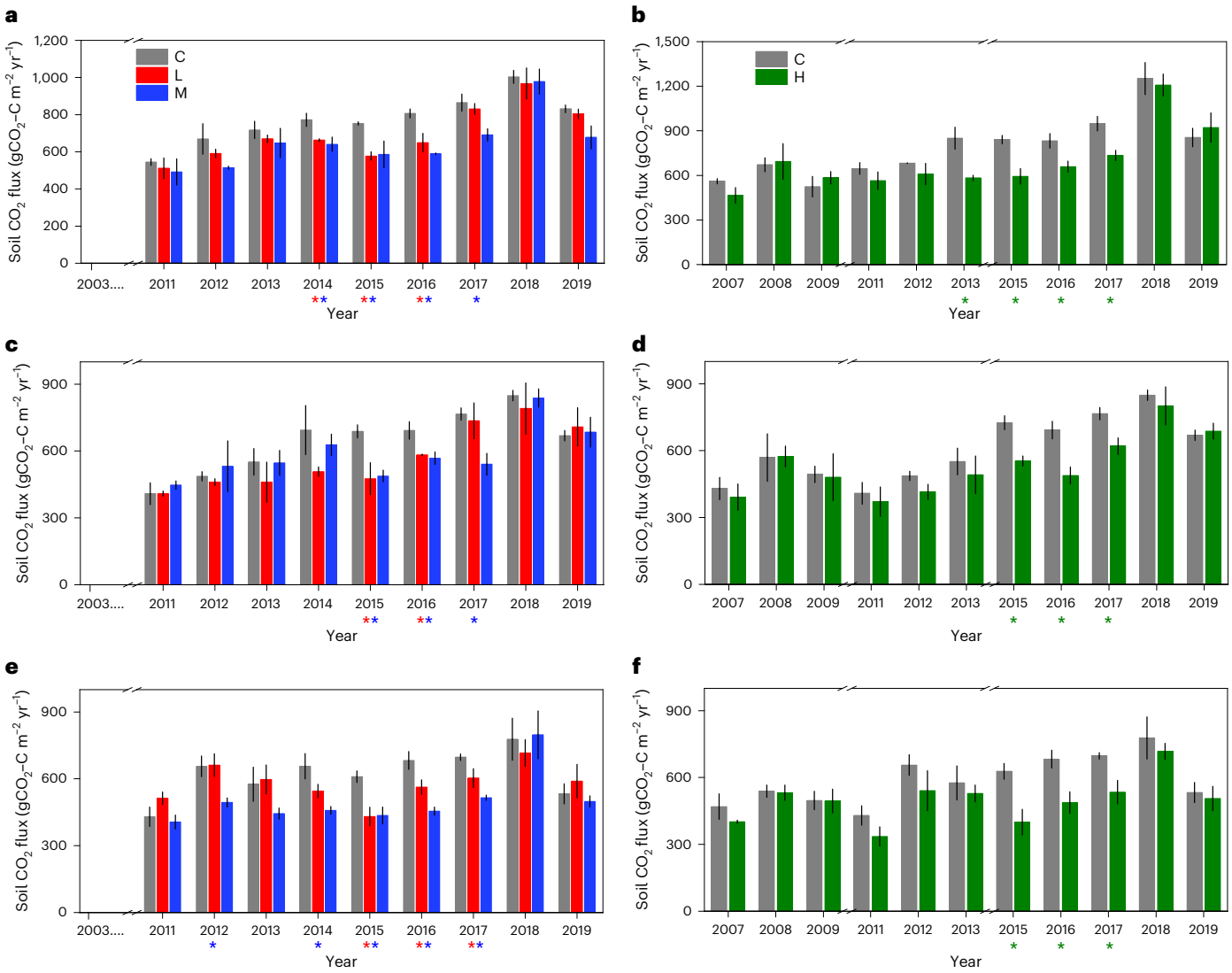

Fig. 1 | Effects of long-term N addition on annual soil respiration rates in the primary, secondary and planted forests. a,**c**,**e**, In Exp1, N treatments started from 2003, and annual soil CO_2 emission rates were monitored in the control, low N-addition and medium N-addition plots (0, 50 and 100 kg N ha^{-1} yr^{-1}, respectively) from 2011 to 2019 in the primary forest (**a**), secondary forest (**c**) and planted forest (**e**). **b**,**d**,**f**, In Exp2, N treatments started from 2007, and annual soil CO_2 emission rates were monitored in the control and high N-addition plots (0 and 150 kg N ha^{-1} yr^{-1}, respectively) from 2007 to 2019 in the primary forest (**b**), secondary forest (**d**) and planted forest (**f**). Columns and error bars represent means and standard errors ($n = 3$), respectively. The red, blue and green asterisks indicate the years in which significant difference ($P < 0.05$; one-way ANOVA followed by Tukey's honest significant difference (HSD) test for Exp1 and independent-sample t test for Exp2) is detected between the control and low N-addition, medium N-addition and high N-addition plots, respectively. C, control; L, low N addition; M, medium N addition; H, high N addition.

atmospheric N deposition is estimated to increase from 86.6 Tg N yr^{-1} in 1984 to 93.6 Tg N yr^{-1} in 2016[7], although N deposition in portions of North America and much of Europe has declined[8,9]. Currently, the average rates of N deposition are ~20 kg N ha^{-1} yr^{-1} in tropical and subtropical regions[7,10], and the rates have reached 30–50 kg N ha^{-1} yr^{-1} and stabilized in parts of Southeast Asia[11,12]. Nitrogen deposition alters soil respiration (CO_2 emission) via regulating activities of plants and soil microbes[13]. On one hand, because plant growth is limited by N in many natural ecosystems[14], N inputs stimulate plant growth and C sequestration in plant tissues and soils, which provide sufficient C sources supporting root and microbial respiration[13]. On the other hand, because sufficient N in plant tissues is necessary for respiration maintenance[15], N inputs commonly lead to fast litter decomposition[16]. However, N inputs can inhibit organic matter decomposition and soil respiration if microbial growth is not limited by N but other nutrients (for example, phosphorus (P))[17,18].

Our current understanding and evaluation of the N-deposition impacts on forest soil respiration are based mainly on short-term N-addition experiments[13,19,20]. These experiments reported negative, positive or no effects on soil respiration, which largely depend on the N demand of plants and microbes[17,21,22]. Global meta-analysis showed that there were 191 observations of soil respiration responses to N addition in forests, but ~80% of them were short term (for example, 0–3 years; Extended Data Fig. 1). A few studies had the longest periods of 7–9 years but monitored CO_2 fluxes in growing season only[23,24]. Due to the differences in N-addition rates, duration and forest types, these published studies cannot provide a comprehensive knowledge of how long-term N addition affects soil respiration. Empirical evidence showed that the responses of soil respiration to environmental changes (for example, warming and elevated CO_2) could vary with duration[25,26]. Thus, without long-term field-based research, the real patterns, magnitudes and mechanisms of soil respiration in response to N inputs cannot be

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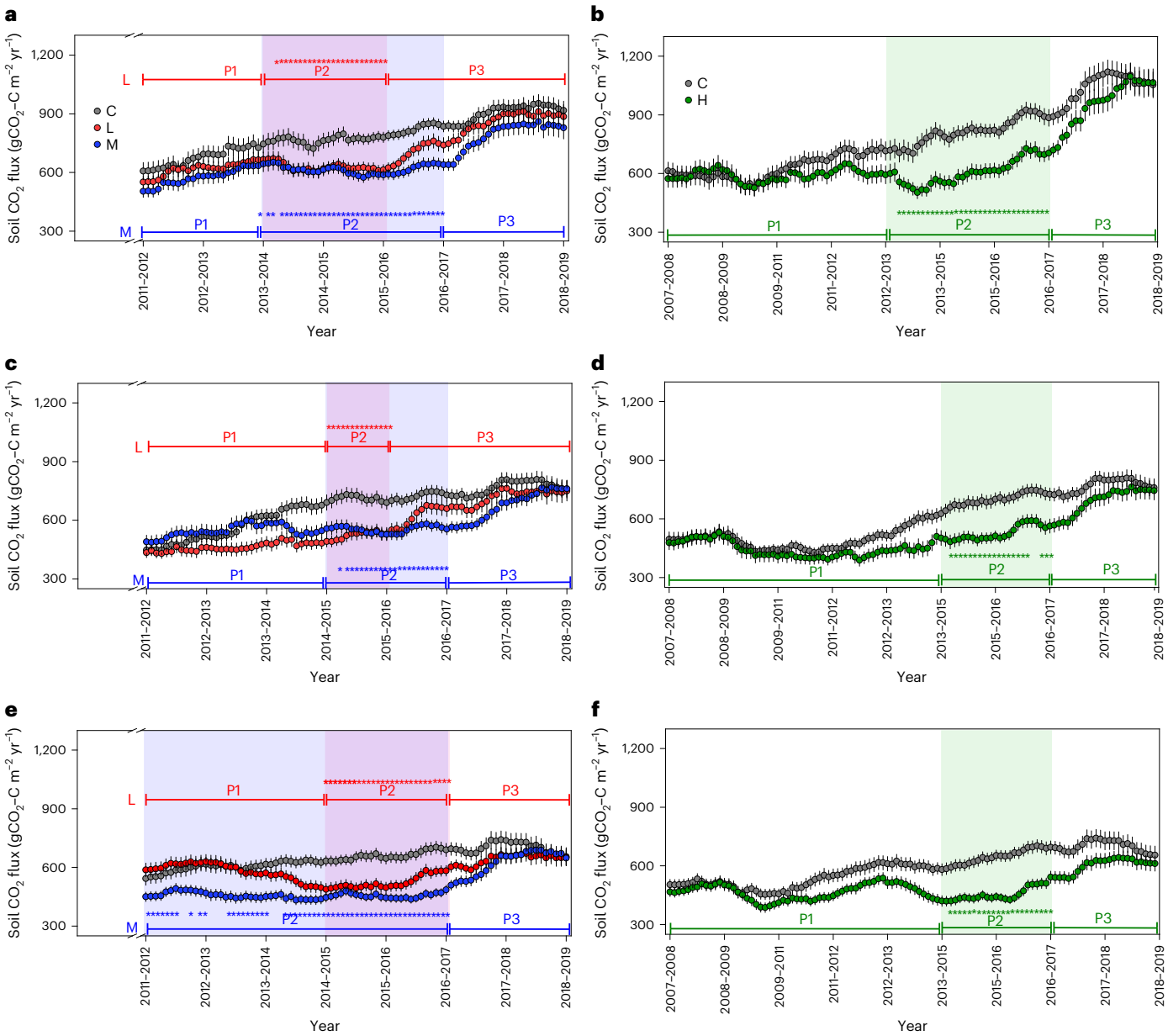

Fig. 2 | Temporal trends of soil respiration in the control and N-addition plots in the primary, secondary and planted forests based on the moving subset window analysis. a,**c**,**e**, Exp1: N treatments starting from 2003. Two-year rolling mean rates of soil CO_2 emission in the control (grey points), low N-addition (50 kg N ha^{-1} yr^{-1}; red points) and medium N-addition (100 kg N ha^{-1} yr^{-1}; blue points) plots with a movement of month from January 2011 to December 2019 in the primary forest (**a**), secondary forest (**c**) and planted forest (**e**). **b**,**d**,**f**, Exp2: N treatments starting from 2007. Two-year rolling mean rates of soil CO_2 emission in the control (0 kg ha^{-1} yr^{-1} N; grey points) and high N-addition (150 kg N ha^{-1} yr^{-1}; green points) plots with a movement of month from February 2007 to December 2019 in the primary forest (**b**), secondary forest (**d**) and planted forest (**f**). Solid circles (data points) and error bars represent two-year means and standard errors ($n = 3$), respectively. The red, blue and green asterisks indicate the data points for which significant difference ($P < 0.05$; one-way ANOVA followed by Tukey's HSD test for Exp1 and independent-sample t test for Exp2) is detected between the control and low N-addition, medium N-addition and high N-addition plots, respectively. Significant difference ($P < 0.05$) is detected only in phase 2. For the medium N-addition treatment in the planted forest, phase 1 (lack of CO_2 flux response) is too short (1 year; Fig. 4) to be detected via two-year rolling means. P1–P3, phases 1–3.

determined. Further, the evaluation and prediction of soil C emission and atmospheric CO_2 variation could be inaccurate in the context of chronic N deposition.

In this Article, we explore soil respiration in response to 9–13 years of N addition and its underlying mechanisms in three tropical forests. The study was conduced in Dinghushan biosphere reserve of southern China (112° 10′ E, 23° 10′ N), where the first long-term N-deposition research platform of Chinese forest ecosystem was established[27]. The reserve contains three forests: a primary forest, a secondary forest and a planted forest. Experimental treatments were initiated in July 2003 with three levels of N addition (each in three replicates) in each forest: control, low N and medium N (0, 50 and 100 kg N ha^{-1} yr^{-1}, respectively). Each plot (10 m × 20 m) was surrounded by a 10-m-wide buffer strip. To predict the accumulated effects of long-term N deposition, we established another pair of treatments (each in three replicates) nearby in February 2007: control and high N (0 and 150 kg N ha^{-1} yr^{-1}, respectively), with each plot (5 m × 5 m) surrounded by a 5-m-wide buffer strip. All the plots were laid out randomly (a completely randomized design; Supplementary Fig. 1). Using static chamber technique, we monitored soil CO_2 emission rates monthly in the control and N-addition plots

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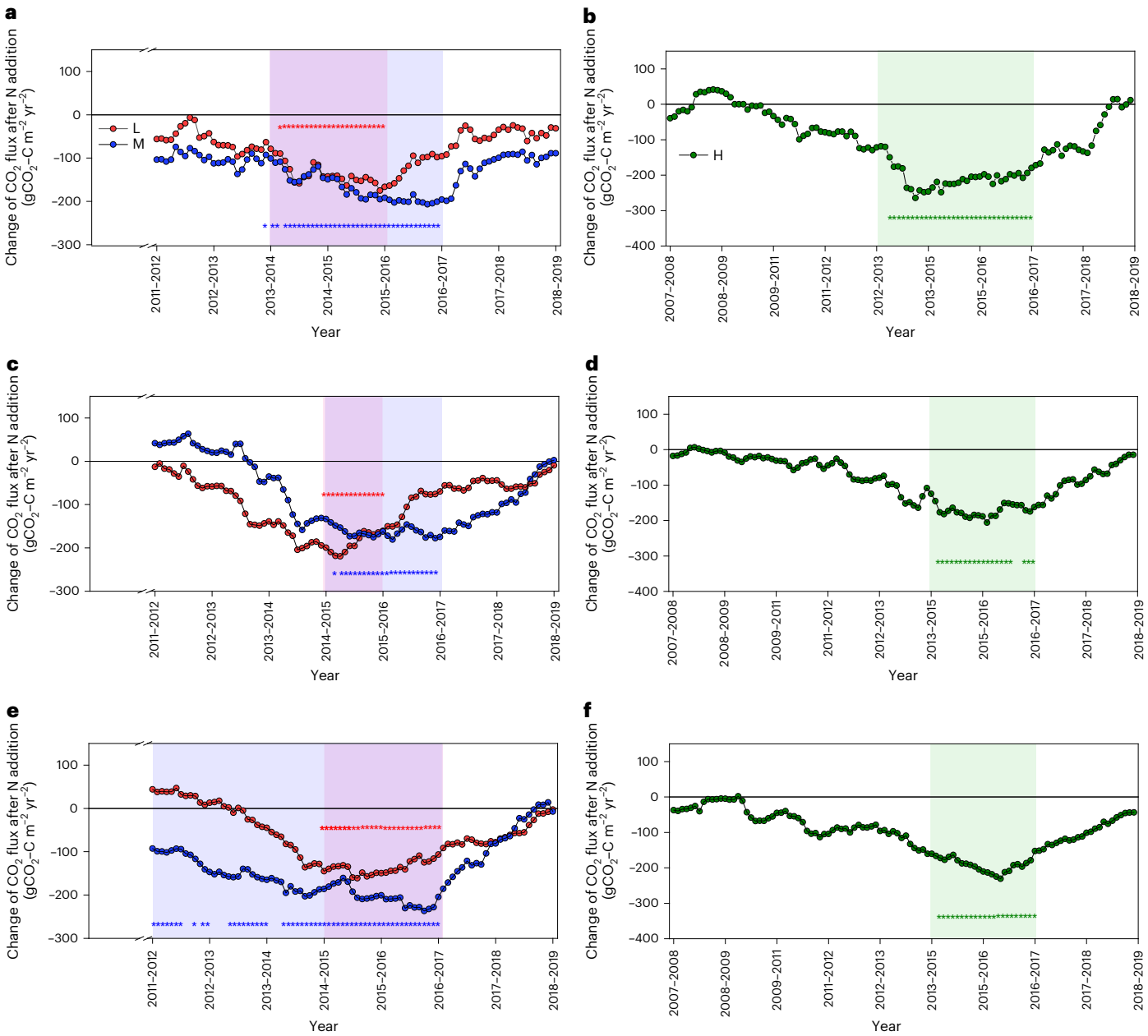

Fig. 3 | Temporal trends of changes of soil respiration after N addition in the primary, secondary and planted forests based on the moving subset window analysis. a,**c**,**e**, Exp1: N treatments starting from 2003. Two-year rolling mean changes of soil CO_2 emission in the low and medium N-addition (50 and 100 kg N ha^{-1} yr^{-1}, respectively) plots relative to the control plots (N treatment − control) with a movement of month from January 2011 to December 2019 in primary forest (**a**), secondary forest (**c**) and planted forest (**e**). **b**,**d**,**f**, Exp2: N treatments starting from 2007. Two-year rolling mean changes of soil CO_2 emission in the high N-addition (150 kg N ha^{-1} yr^{-1}) plots relative to the control (0 kg N ha^{-1} yr^{-1}) plots with a movement of month from February 2007 to December 2019 in primary forest (**b**), secondary forest (**d**) and planted forest (**f**). Solid circles (data points) represent the differences between treatments and control. The red, blue and green asterisks indicate the data points for which significant difference ($P < 0.05$; one-way analysis of variance followed by Tukey's HSD test for Exp1 and independent-sample t test for Exp2) is detected between the control and low N-addition, medium N-addition and high N-addition plots, respectively ($n = 3$).

(Supplementary Table 1). To explain the variation in soil respiration, we analysed C and N cycling processes of soils, plants and microbes (Supplementary Table 2).

Temporal patterns of soil respiration responses

The studied forests (the primary, secondary and planted forests) have experienced high rates of atmospheric N deposition (36–52 kg N ha^{-1} yr^{-1}) since 1990, and the forest soils exhibited leaching losses of inorganic and organic N[11]. Additional N inputs (for example, fertilization) reduced fine root biomass[17,28] and soil microbial biomass[29] in these forests. Thus, we expected that N addition would inhibit soil respiration. Our results of experiment 1 (Exp1: N treatments starting from 2003), however, showed that annual respiration rates in soils did not change under low and medium N treatments over the first few years we monitored (Fig. 1a,c,e). Until 2012–2015 (10th–13th years of N treatments), low and/or medium N addition reduced soil respiration rates, but this effect lasted for 3–6 years and disappeared in 2018. High N treatments also repeated but shortened these patterns. Our results of experiment 2 (Exp2: N treatments starting from 2007) showed that soil respiration was inhibited by high N treatments after 2013 or 2015 (seventh or

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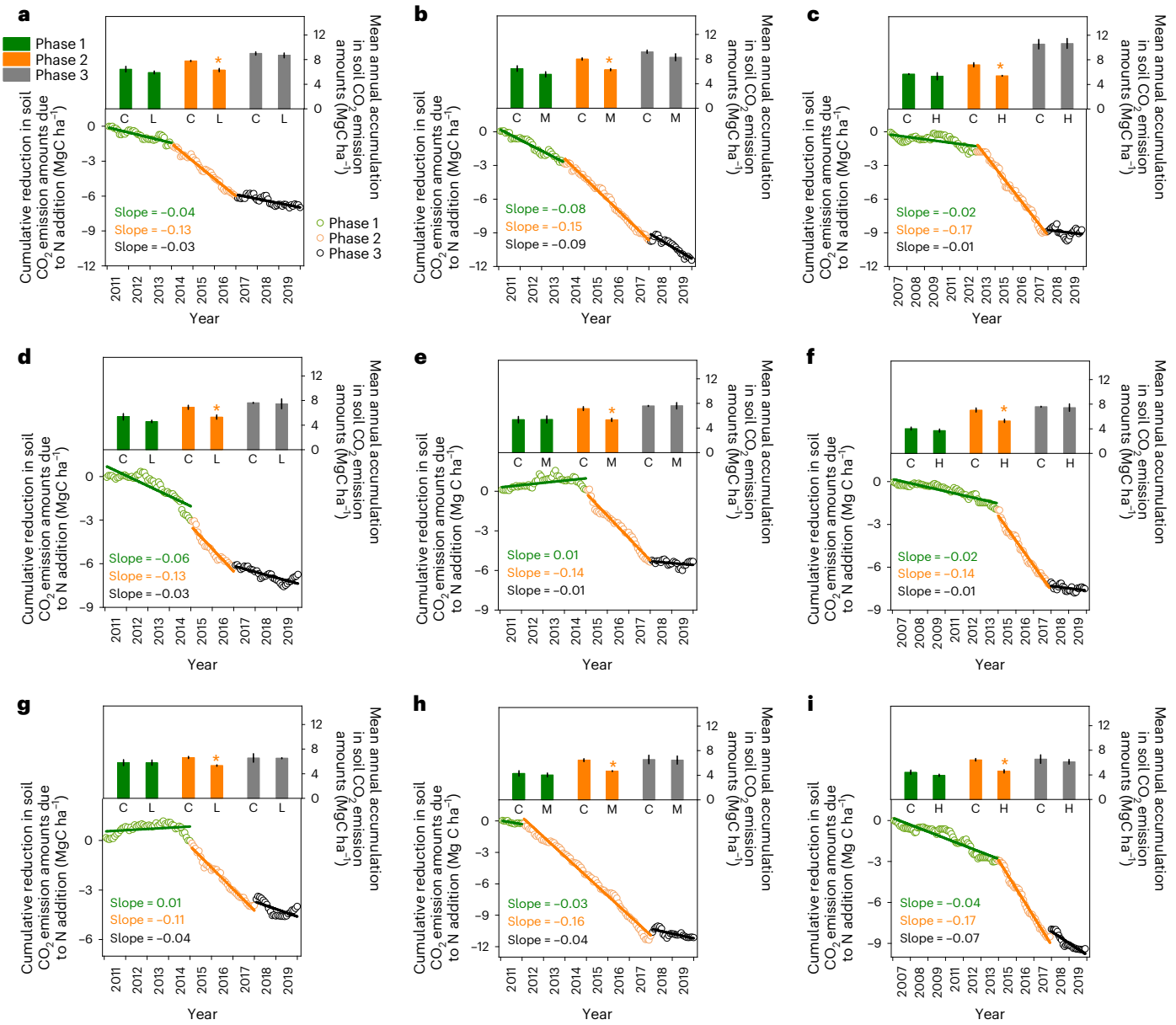

Fig. 4 | Effects of long-term N addition on annual and total cumulative amounts of soil CO_2 emission in the primary, secondary and planted forests. **a**,**b**,**d**,**e**,**g**,**h**, N treatments starting from 2003. Mean annual accumulation in soil CO_2 emission amounts in the control, low N-addition and medium N-addition (0, 50 and 100 kg N ha^{-1} yr^{-1}, respectively) plots (top) and the cumulative reduction in soil CO_2 emission amounts due to low (**a**,**d**,**g**) and medium (**b**,**e**,**h**) N addition (bottom) in phases 1–3 in primary forest (**a**,**b**), secondary forest (**d**,**e**) and planted forest (**g**,**h**). **c**,**f**,**i**, N treatments starting from 2007. Mean annual accumulation in soil CO_2 emission amounts in the control and high N-addition (0 and 150 kg N ha^{-1} yr^{-1}, respectively) plots (top) and the cumulative reduction in soil CO_2 emission amounts due to high N addition (bottom) in phases 1–3 in primary forest (**c**), secondary forest (**f**) and planted forest (**i**). Columns and error bars represent means and standard errors ($n = 3$), respectively. The asterisks indicate significant difference ($P < 0.05$; independent-sample t test) between the control and N-addition plots. Linear regression models were used to analyse the relationships between cumulative reduction in soil CO_2 emission amounts and time (month) with the slopes provided. The green, orange and grey columns (and lines) represent phases 1, 2 and 3 (in accordance with phases 1–3 in Fig. 2), respectively.

ninth year of N treatments), and the inhibitory effects disappeared in 2017 (Fig. 1b,d,f). The repeated-measures analysis of variance (ANOVA) showed interactive effects of N treatment and year ($P < 0.001$; Extended Data Fig. 2). These findings together indicate that the soil respiration responses to N addition vary with the treatments proceeding.

On the basis of the moving subset window analysis (24-month rolling mean of soil CO_2 fluxes; see details in Methods), we observed a clear temporal trend of CO_2 emission across duration and confirmed a three-phase pattern (insignificant changes–dramatic decline–insignificant changes; Figs. 2 and 3). In phase 1, although N addition tended to reduce soil respiration rates gradually, this effect is insignificant. After entering phase 2, soil respiration declined dramatically, and this phase lasted for 3–6 years depending on treatment level and forest type. In the next phase (phase 3), the inhibitory effect on soil respiration weakened gradually and became insignificant again. This phased variation in soil respiration responses helps us understand why some previous studies in tropical forests with a short duration (for example, <2 years) found no changes of soil respiration after N addition[30,31], but others with a longer duration (3–6 years) observed reduction[17,32].

The total cumulative reduction in CO_2 emission amounts (N treatments versus control) also exhibited three phases: slow or no reduction

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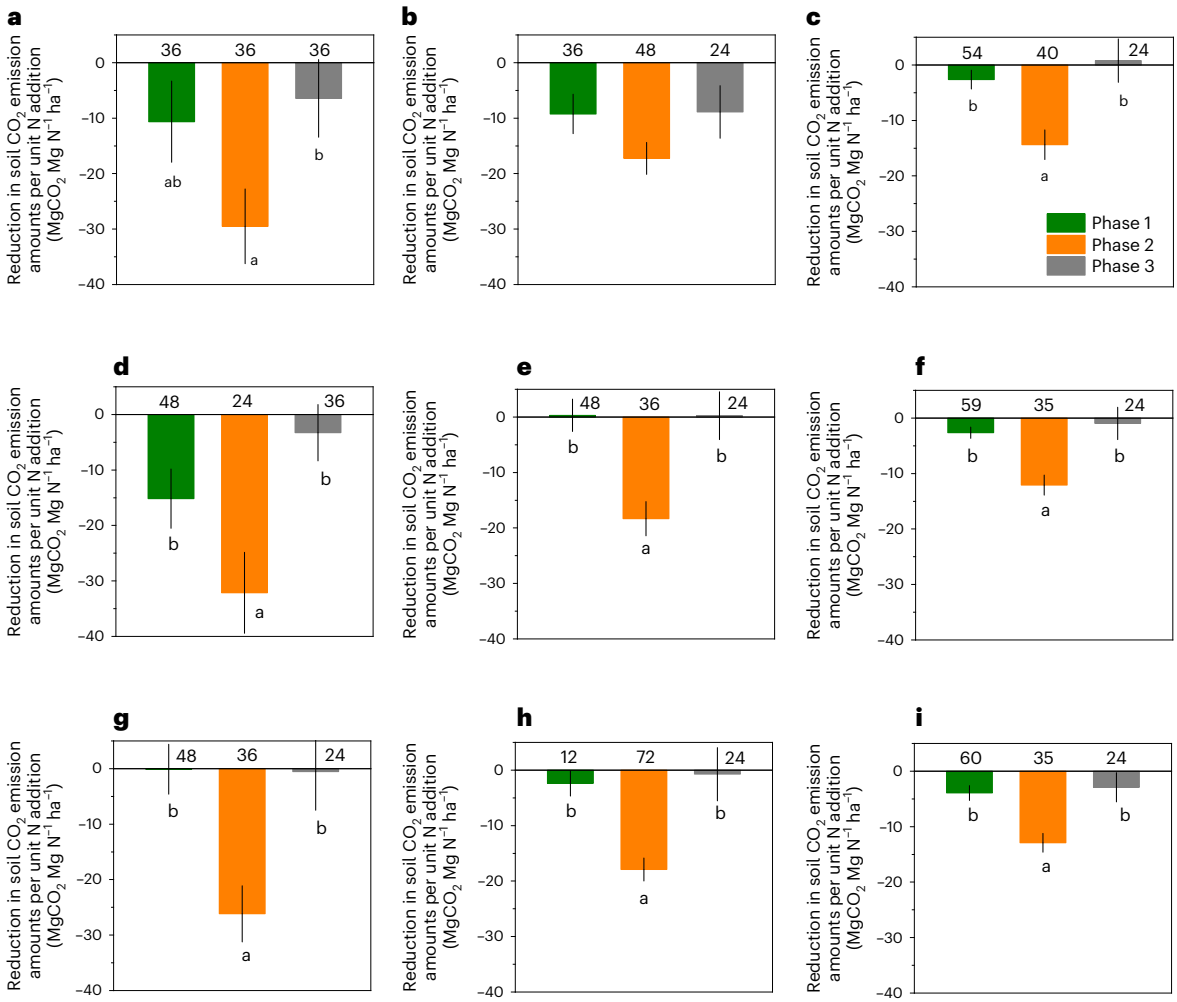

Fig. 5 | Efficiency of soil CO_2 emission reduced by N addition in phases 1–3. **a**,**b**,**d**,**e**,**g**,**h**, N treatments starting from 2003. Reduction in soil CO_2 emission amounts per unit N addition for low (**a**,**d**,**g**) and medium (**b**,**e**,**h**) N addition in the primary forest (**a**,**b**), secondary forest (**d**,**e**) and planted forest (**g**,**h**). **c**,**f**,**i**, N treatments starting from 2007. Reduction in soil CO_2 emission amounts per unit N addition for high N addition in the primary forest (**c**), secondary forest (**f**) and planted forest (**i**). Columns and error bars represent means and standard errors (the numbers shown in bar charts represent *n* values), respectively. The green, orange and grey colours represent phases 1, 2 and 3 (in accordance with phases 1–3 in Fig. 2), respectively. Different lowercase letters represent significant difference ($P < 0.05$; one-way analysis of variance followed by Tukey's HSD test) among three phases.

(slope = −0.08 to 0.01 in phase 1), fast reduction (slope = −0.17 to −0.11 in phase 2), and slow reduction again (slope = −0.09 to −0.01 in phase 3; linear regression model; Fig. 4, the bottom panels). The mean annual accumulation in CO_2 emission amounts declined in phase 2 ($P = 0.002–0.028$) but not in phase 1 or 3 ($P = 0.208–0.994$; Fig. 4, the top panels). Regardless of forest type and N-addition level, the efficiency of N-induced reduction in soil CO_2 emission (reduction in soil CO_2 emission amounts per unit N addition) in phase 2 was higher than those in phases 1 and 3 (Fig. 5). Together, these findings demonstrate that the extents of the negative N-addition impacts on soil CO_2 emission alter with duration, which contrasts with the previous conclusion that N addition had a minor effect on soil respiration in tropical forests based on meta-analyses[13,19].

The three phases of soil CO_2 emission occurred in all the studied forests (Fig. 2), indicating that difference in forest types does not affect the response patterns of soil respiration (no interactive effects of forest type and N treatment; $P = 0.46–0.82$; Extended Data Fig. 2). However, the decline phase (phase 2) occurred early in the primary forest under low and high N treatments and in the planted forest under medium N treatments (Figs. 1 and 2). This is possibly because the two forests had higher initial N (NH_4^+ and NO_3^-) concentrations in soils than the secondary forest[33]. Nevertheless, the cumulative reductions in CO_2 emission amounts (over the course of the experiments) were comparable among the primary, secondary and planted forests (9.06 (±1.30), 6.53 (±0.64) and 8.17 (±2.15) MgC ha^{-1}, respectively; $P = 0.516$, one-way ANOVA; Supplementary Fig. 2). This suggests that differences in forest types do not affect the total reduction amounts of soil CO_2 emission after N addition. Together with a recent finding that 11 years of N addition increased soil C pools by 5.48–16.47 MgC ha^{-1} in our primary forest[34], our results indicate that the reduced CO_2 emission (2.70–9.41 MgC ha^{-1}, an estimation with the same duration; Fig. 4) makes an important contribution to soil C sequestration. Given chronic high N deposition (36–52 kg N ha^{-1} yr^{-1}) in our forest sites[11,34], our findings support a previous observation that the studied primary forest (>400 years) could still accumulate organic C in soils, from 1.40% to 2.35% between 1979 and 2003[35].

Different N-addition rates did not affect the response patterns but the periods of soil respiration. Compared with low N treatments, medium N addition shortened the periods of phase 1 by three years in the planted forest, and high N addition shortened the periods by four–five years in all the forests (Fig. 1). Several previous meta-analyses reported that difference in N-addition rates had less impact on the

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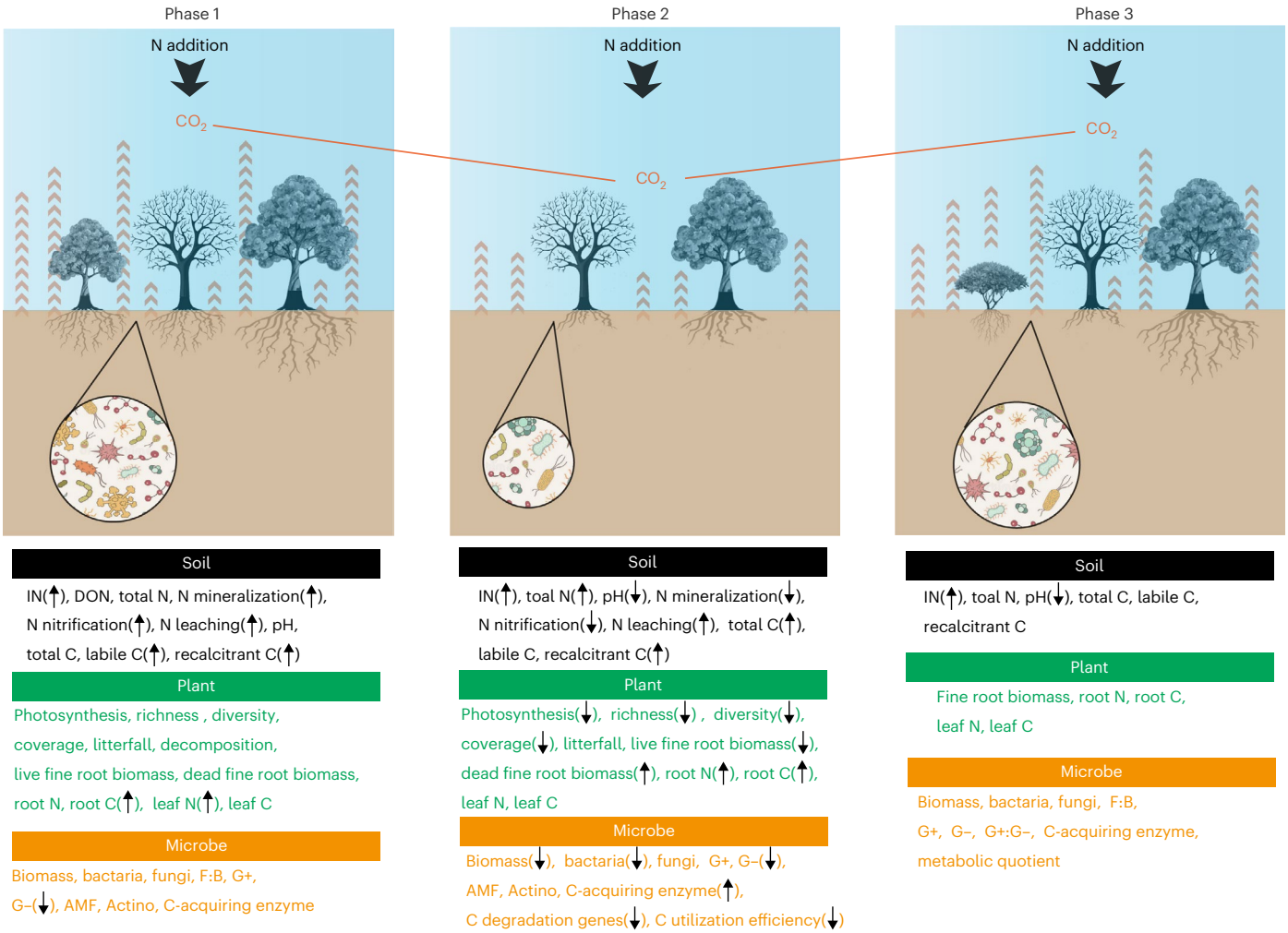

Fig. 6 | Conceptual framework of soil respiration response to long-term N addition in phases 1–3. The framework depicts how soil CO_2 emission changes with N addition as well as C and N cycling of soils, plants and microbes (based on 849 observations from the plots of the three studied forests; Supplementary Table 2). Up and down arrows represent significant positive and negative responses to N addition, and variables that lack arrows showed no significant response ($P < 0.05$; one-way analysis of variance followed by Tukey's HSD test). Asterisks represent the number of variables that were significantly changed by N addition. 'Soil labile C' includes particular organic C, readily oxidizable organic C, light-fraction C and dissolved organic C. 'Soil recalcitrant C' includes non-readily oxidizable organic C and heavy-fraction C. 'C-acquiring enzyme' includes cellobiohydrolases, α-glucosidase, β-1,4-glucosidase, β-glycosidases, β-xylosidases and polyphenol oxidase. IN, inorganic N; DON, dissolved organic N; F:B, fungi: bacteria; AMF, arbuscular mycorrhiza fungi; Actino, actinomycete.

extents of soil respiration responses[13,20]. Similarly, we found that the cumulative reduction in CO_2 emission amounts (over the course of the experiments) did not differ among low, medium and high N treatments ($P = 0.238$, one-way ANOVA; Supplementary Fig. 2). These results suggest that increased N-addition rates may help us predict the patterns of soil respiration responses in advance with a minor impact on the estimation of total CO_2 fluxes in our forest sites. Nevertheless, the efficiency of N-induced reduction in soil respiration declined from 13.13 to 5.80 $MgCO_2$ Mg N^{-1} across N-addition gradients (Fig. 5 and Supplementary Fig. 3). This is consistent with the efficiency of soil C sequestration that also declined from 10.49 to 8.54 MgC Mg N^{-1} with N-addition levels[34]. Hence, although tropical forest soils are capable of increasing C sequestration under N deposition[34,36], the efficiency of sequestration will decline if N-deposition rates increase in the future[7].

Mechanisms underlying the variation of soil respiration

Consistent with the estimated temporal trends of global soil respiration[37], the mean rates of soil respiration increased by 13.46–55.74% from 2007 to 2019 in our forest sites (Fig. 2). This could be partially related to global climate changes (for example, warming and the change of precipitation pattern) and the consequent variation in soil temperature and moisture (Extended Data Figs. 3 and 4). We observed increases in soil temperature (slope = 0.007–0.018, $P \leq 0.038$) and moisture (slope = 0.026–0.076, $P < 0.001$) over the past 9–13 years (linear regression model; Extended Data Figs. 5 and 6). In the control and N-addition plots, the two variables could account for 28–34% of the temporal increases in CO_2 emission ($P < 0.001$, three-dimensional surface model with polynomial regression; Extended Data Fig. 7), and soil temperature could explain more than soil moisture did (Extended Data Figs. 8–10).

To further explore the mechanisms underlying the soil respiration responses under N addition, we integrated C and N cycling processes of soils, plants and microbes (Supplementary Table 2) and developed a conceptual framework in phases 1–3 (Fig. 6). Phase 1 seems to be a period of less plant and microbial responses. In this phase, N addition changed mainly soil C and N processes, including the increases in soil inorganic N (NH_4^+ and NO_3^-) concentrations[38], mineralization and nitrification rates, N leaching rates[39] and labile and recalcitrant C concentrations[40]. Although the concentrations of root C and foliar N

began to increase[41] and the abundance of Gram-negative (G−) bacteria reduced[42], the microbial and plant communities were less affected overall.

After entering phase 2, the plant and microbial responses were intensive and negative. Apart from soil inorganic N concentrations and N leaching rates, total N concentrations were also enhanced[34]. Soil mineralization and nitrification rates began to decrease[43], indicating that soil N supply exceeded demand. In particular, soil pH decreased[34] and acidification intensified[44], which led to faster rates of root death as characterized by decrease in live fine root biomass and increase in dead fine root biomass[28]. Plant growth (for example, photosynthetic efficiency) and diversity (for example, richness, abundance and coverage) declined[45]. Similarly, the N-induced soil acidification inhibited microbial growth and reduced microbial biomass, particularly bacterial richness and G− abundance[29]. Microbial C-cycling processes, such as the efficiency of C (for example, sugar and amino acid) utilization and the intensity of C (for example, cellulose and chitin) degradation, slowed down[36]. Together, these lines of evidence indicate that both autotrophic (root) respiration and heterotrophic (microbial) respiration may have declined, leading to increases in soil C concentrations and soil C pools[34].

In the transition from phase 2 to phase 3, plant and microbial communities reorganized. On one hand, the appearance of new individuals of vine plants (for example, *Gnetum montanum* and *Calamus rhabdcladus*) replenished the vacant niches, and they became dominant species in the understory layer[45]. On the other hand, the death of fine roots and microbial residues provided sufficient liable and recalcitrant C for soil microbes, leading to increases in abundances of some unidentified bacteria[29] and C (for example, starch and hemicellulose) degradation genes[36]. Thus, in phase 3, the C and N concentrations of foliages and roots, fine root and microbial biomass, and microbial community structure were not changed by N addition, indicating stabilization of plant and microbial community. Soil respiration rates did not decrease any more, and soil total C concentrations did not increase in this phase.

Implications

Overall, there are several important findings and implications of our study. First, we provide the important line of evidence that 9–13 years of N addition resulted in three-phase patterns of soil respiration (insignificant changes–dramatic decline–insignificant changes) in three tropical forests and across three N-addition gradients. These patterns, similar to the phased variation of soil respiration feedback over 26-year warming treatment at Harvard forest[25], indicate that the responses of soil respiration to N-addition treatments also alter with duration. Compared with low and medium N treatments, high N addition shortens the response patterns of soil respiration, meaning that similar patterns require a longer time to observe by experiments using lower N-addition rates or under current N-deposition scenarios[7]. If so, many published large-scale studies based on short-term N-addition experiments may have drawn inaccurate conclusions. For example, some short-term experiments failing to capture the decline period (phase 2) may underrate the N-addition effects, while some failing to capture the stabilization period (phase 3) may overrate the effects. Our study showed that N addition reduced soil respiration rates by 9.59–15.14% over phases 1–3 (Supplementary Fig. 4), two- to fivefold higher than those (2.94–6.25% in tropical forests and 6.18–6.61% in global forests) estimated by previous large-scale studies[13,19,20].

Second, our study demonstrates that long-term N addition reduces soil CO_2 emission in multiple tropical forests. Even in many temperate and boreal forests, chronic N inputs and then accumulation will inhibit soil respiration eventually if soil N availability is improved or limitation of other nutrients (for example, P) occurs[18,46]. Using meta-analysis, we confirmed that N addition inhibited soil respiration in global forests (Extended Data Fig. 1d). Our results showed that 9–13 years of N addition reduced the cumulative CO_2 emission amounts by 6.53–9.06 MgC ha^{-1} with the efficiency of 5.80–13.13 $MgCO_2$ Mg N^{-1}. In the old-growth primary forest, if not considering the phased variation, N addition reduced soil CO_2 emission amounts by 0.84 MgC ha^{-1} yr^{-1} on average, equivalent to ~87% of net C sequestration in soils[34]. These lines of evidence support the previous notion that mature tropical forests can accelerate C sequestration in soils[34,35] and suggest that long-term N deposition may make an important contribution to soil C storage via inhibition on soil respiration. Hence, the temporal variation in soil C fluxes induced by N deposition should be incorporated into terrestrial C and N cycling models as well as estimation of terrestrial C pools in the future, which is important for the prediction of global climate change and the achievement of C neutrality goal.

Third, we proposed a conceptual framework to explain why the soil respiration responses to N input varied with duration based on 849 observations from the studied forest plots. The framework includes three phases: the lack of plant and microbial responses in phase 1, the reduction in fine root and microbial biomass due to soil acidification in phase 2 and the reorganization and stabilization of plant and microbial community in phase 3. This framework advances our understanding of how forest ecosystems respond and adapt to long-term N deposition, and it provides the important mechanisms supporting Earth system models of climate–biosphere feedbacks.

Last, our findings are based on a simulated N-deposition (fertilization) method. The timing and concentration of N entering ecosystems via fertilization may not be totally the same as N deposition. A recent study suggested that the responses of ecosystem structure and function (for example, above- and below-ground biomass, and C and N cycling) to a high frequency and low dose of fertilization are smaller than to single-pulse fertilization over one year[47]. Although our study has applied fertilization at a monthly or bimonthly frequency that is thought to approximate N deposition compared with single-pulse fertilization[47], we suggest more empirical studies with higher fertilization frequency or under natural N-deposition gradients for better evaluation, modelling and prediction of the N-deposition impacts in the future.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41561-022-01080-4.

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Methods

Site description

This study was conducted in Dinghushan biosphere reserve located in Guangdong Province of southern China (112° 10′ E, 23° 10′ N). The reserve has a total area of ~1,200 ha with three types of forests: a primary forest, a secondary forest and a planted forest. The primary forest, occupying ~20% of the reserve, has experienced more than 400 years of natural succession without human disturbance and is now dominated by multiple tree species, for example, *Castanopsis* (*C.*) *chinensis*, *Schima* (*S.*) *superba*, *Machilus chinensis* and *Cryptocarya chinensis*. The secondary and planted forests occupy about 50% and 20% of the reserve, respectively. Both forests originated from eroded sites and had been subjected to the stress of human activities for > 100 years. Until the 1930s, the two forests were clear-cut and planted with *Pinus* (*P.*) *massoniana*. The planted forest experienced human disturbance (for example, harvesting of understory litter) until the late 1990s and is dominated by *P. massoniana*. Although the secondary forest also experienced human disturbance, it was protected after the 1950s and invaded by native broadleaf species. The secondary forest is co-dominated by coniferous species (*P. massoniana*) and broadleaf species (for example, *C. chinensis* and *S. superba*), and it is considered as a transitional forest from the planted to primary forests[48].

The reserve has a typical humid monsoon climate. Mean annual temperature is 21 °C with the range from 12.6 °C (in January) to 28.0 °C (in July). Mean annual precipitation is 1,927 mm, 75% of which occurs from March to August and 6% from December to February. The studied forests have experienced high rates of atmospheric N deposition (36–52 kgN ha^{-1} yr^{-1}) since the 1990s[11,34,49]. Soils of the forests are lateritic red earth formed from sandstone with the pH of 3.7–4.1 (ref. [48]). From the planted to primary forests, soil organic matter increases from 2.73% to 5.35% (ref. [48]).

Experimental design

Experiment 1. The experimental design was referred to the European NITREX project[50,51] and that of Harvard Forest in North America[52]. The treatments were initiated in July 2003 with three levels of N addition (each in three replicates) in each forest: control (0 kg N ha^{-1} yr^{-1}), low N (50 kg N ha^{-1} yr^{-1}) and medium N (100 kg N ha^{-1} yr^{-1}). Each plot (10 m × 20 m) was surrounded by a 10-m-wide buffer strip. To exclude the interactive impacts from plant roots and leachate among the plots, we built concrete and plastic barriers in the soil around each plot. All the plots were laid out randomly in each forest (Supplementary Fig. 1). Solutions of NH_4NO_3 were sprayed on the forest floor monthly from July 2003 to December 2019. Fertilizer was mixed with 20 l of water for low and medium N-addition plots, and each control plot received an equivalent volume of water.

Experiment 2. We established another pair of treatments (each in three replicates) in each forest in February 2007: control (0 kg N ha^{-1} yr^{-1}) and high N (150 kg N ha^{-1} yr^{-1}). Each plot (5 m × 5 m) was surrounded by a 5-m-wide buffer strip. Plot size and fertilizer level were referred to those in a tropical forest in Costa Rica, where the responses of soil CO_2 fluxes to nutrient addition were studied[53]. Field plots and treatments were laid out randomly (Supplementary Fig. 1). Solutions of NH_4NO_3 were sprayed on the forest floor monthly from February 2007 to December 2019. Fertilizer was mixed with 5 l water for each N-addition plot. Each control plot received an equivalent volume of water correspondingly.

Due to limited space for manipulative experiments in the Dinghushan biosphere reserve, our study established only three replicated plots for each treatment, similar to other forest studies[24,54]. Despite the low number of replicated plots, the spatial variability of soil respiration in our study is comparable to those based on a higher number of sampling plots[25,26]. In addition, the preceding N-addition gradients are equal to ~1–3 times background N-deposition rates (~50 kg N ha^{-1} yr^{-1}) in our forest sites[11,34,49,55]. Although the rates of medium and high N addition are higher than the current rates of N deposition[56], they can be useful for predicting the impacts of elevated N deposition and the accumulated effects of chronic low rates of N deposition.

Soil respiration

The measurements of soil respiration began from January 2011 (after eight years of treatments for control, low N and medium N; experiment 1) and February 2007 (at the start of treatments for control and high N; experiment 2; see Supplementary Table 1 for details). Because three control plots in experiment 1 were close to those in experiment 2 in the secondary and planted forests, we merely measured soil respiration rates for the control plots of experiment 1 in the two forests. Soil respiration rates were measured for both control groups in experiment 1 and experiment 2 in the primary forest because they were far apart. Soil respiration rates were measured using static chamber methodology and analysed using gas chromatography[17]. Two chambers were installed in each plot at the start of the experiments. Each chamber contained an anchor ring and a removable cover chamber. Each anchor ring, a PVC pipe 25 cm in diameter and 16 cm high, was permanently anchored 5 cm into the soil. During gas sampling, a 35-cm-high cover chamber was attached tightly to the anchor ring using a water-filled groove for air sealing. A digital thermometer in the cover chamber was used to record air temperature, and a small fan (8 cm in diameter) was installed inside to ensure mixing the headspace. Gas samples were collected from each chamber in the morning from 9:00 to 10:00, during which soil CO_2 fluxes are close to the daily means[57]. Soil respiration rates were measured once per week for the growing season (from May to October) and fortnightly in other times. Gas samples were collected using a 100 ml plastic syringe at 0, 10, 20 and 30 min after chamber closure. The gas samples were analysed within 48 h using gas chromatography (Agilent 7890D, Agilent Co.). To minimize the effects of chamber closure on soil respiration, soil CO_2 fluxes were calculated on the basis of linear regression models of CO_2 concentrations against time using the data points from each chamber[17,57]. All the coefficients of determination (r^2) of the linear regression models were larger than 0.97 in the preliminary experiment.

Soil temperature and moisture

During each sampling, soil temperature and moisture were monitored at 5 cm below the surface of the soil in each chamber. Soil temperature was measured using a digital thermometer (Trime TDR, TES-1310, Ltd), and volumetric soil moisture (cm^3 H_2O cm^{-3} soil) was measured using an ADR probe (Pt-100, IMKO GmbH).

C and N cycling variables of soil, plants and microbes

To explore the mechanisms underlying soil respiration responses to N addition, we collected the literature (including articles and theses published from 2003 to 2020) that reported ecosystem C and N cycling processes in the studied forest plots. There are 45 relevant pieces of literature with a total of 849 observations. The dataset includes soil, plant and microbial C and N variables, as follows.

Soil. The dataset includes NH_4^+ concentration, NO_3^- concentration, dissolved organic N concentration, total N concentration, mineralization rate, nitrification rate, pH, total organic C concentration, labile organic C (particular organic C, readily oxidizable organic C, light-fraction C and dissolved organic C) concentration, recalcitrant organic C (non-readily oxidizable organic C and heavy-fraction C) concentration and organic C chemical composition (alkyl C, C-alkyl C, aromatic C and carbonyl C).

Plant. The dataset includes photosynthetic efficiency, community composition (richness, density and coverage), litterfall amount, litter decomposition rate, total fine root biomass, live fine root biomass, dead fine root biomass, root N concentration, root C concentration, leaf N concentration and leaf C concentration.

Microbe. The dataset includes microbial biomass, community composition (abundance of bacteria, fungi, G+ bacteria, G− bacteria, arbuscular mycorrhiza fungi and actinomycete (Actino)), C-acquiring enzyme (cellobiohydrolases, α-glucosidase, β-1,4-glucosidase, β-glycosidases, β-xylosidases, polyphenol oxidase and polyphenol oxidase) activity, C-degradation gene abundance for different organic compounds (starch, cellulose, hemicellulose, chitin and lignin), C mineralization rates and C utilization efficiency.

Statistical analyses

Data were tested for normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene's test) before ANOVA. A one-way ANOVA followed by Tukey's HSD test was used to determine the difference of soil CO_2 flux, soil temperature and soil moisture among the control, low N-addition and medium N-addition plots. A one-way ANOVA was used to compare the difference of the reduction in soil CO_2 emission (per unit N addition) among three phases. Repeated-measures ANOVA was used to determine the effects of sampling year, forest type and N-addition treatment on soil respiration. An independent-sample *t* test was used to compare the difference of soil CO_2 flux, soil temperature and soil moisture between the control and high N-addition plots. A linear regression model was used to explore the relationships of soil temperature and moisture against time. An exponential regression model (the best model with a higher fitting degree than other linear and nonlinear models) was selected to analyse the relationships between soil respiration rates and soil temperature. A logarithmic regression model (the best-fitting model) was selected to analyse the relationships between soil respiration rates and soil moisture. The three-dimensional surface model with polynomial regression was used to analyse the relationships among soil respiration rates, soil temperature and soil moisture.

A one-dimensional moving subset window analysis[58] was used to analyse the temporal trend of soil respiration responses in 2007 to 2019. Specifically, all the soil respiration data were arranged in ascending order of the month of measurement. The datasets were iteratively divided into subsets, each of which contained two years (24 months) of soil respiration data. The first subset contains the first 24 months of soil respiration data. The second and subsequent subsets were conducted by moving the data from the earliest month in the previous subset to the next month data with a total of 24 months. Consequently, the last subset contains the last 24 months of soil respiration data. Statistical analyses were performed using SPSS 19.0 (SPSS, Inc.).

Data availability

The data supporting the findings of this study are available from Figshare Digital Repository: https://figshare.com/articles/dataset/Dataset_Mianhai_Zheng_et_al_NG_2022/21291888.

Code availability

No custom code was used in this study.

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Author contributions

M.Z., J.M. and W.Z. conceived the original idea. M.Z., T.Z., X.L. S.W. and W.Z. performed the experiment. M.Z. collected soil, plant and microbial data and ran the statistical analyses. M.Z. wrote the first draft. M.Z., Y.L., J.L., Q.Y., J.H., Q.M., J.M. and W.Z. discussed and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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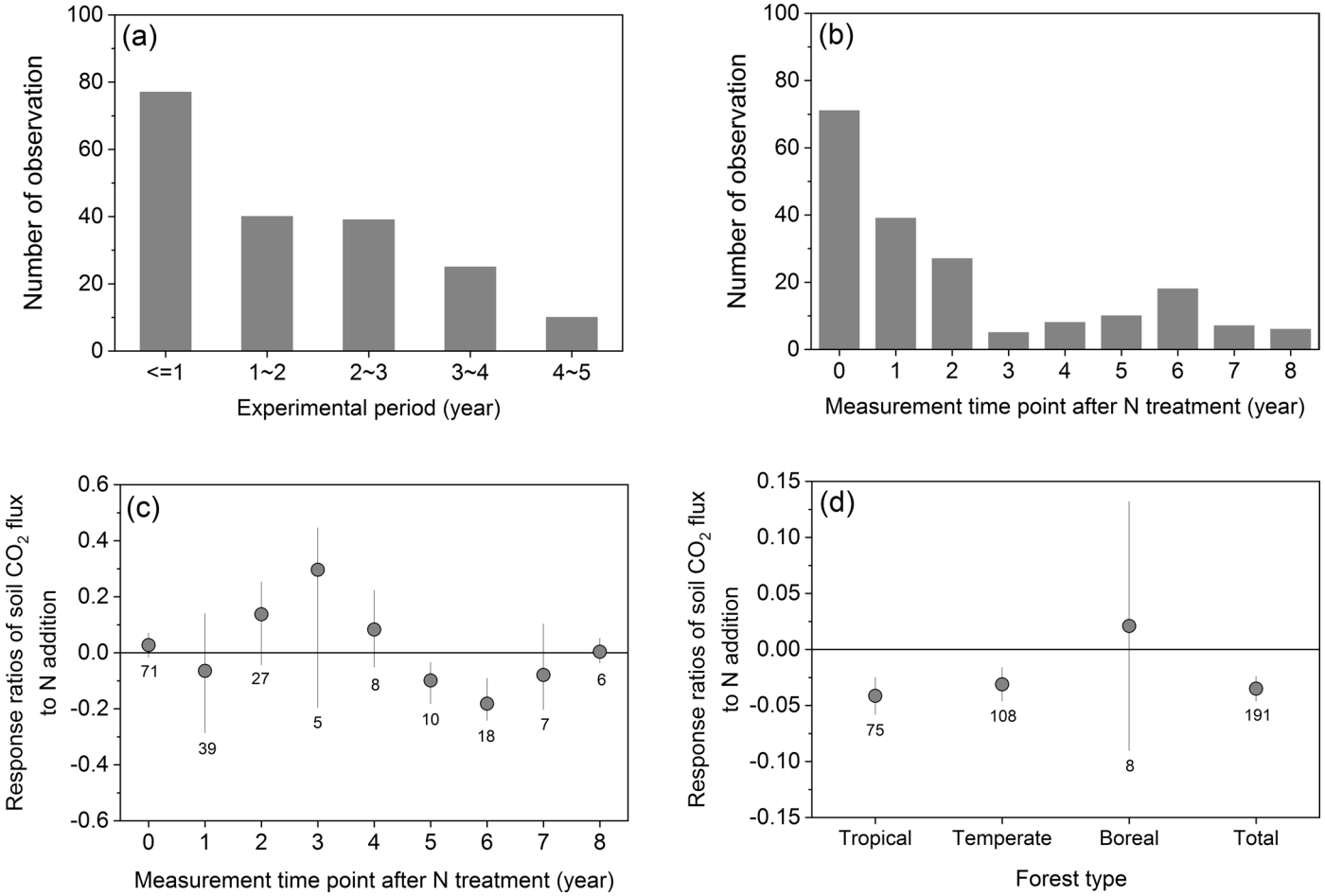

Extended Data Fig. 1 | Global meta-analysis of the N-addition effects on soil CO_2 flux in 62 forest sites. Distribution of the number of observation in different experimental periods **(a)**. Distribution of the number of observation in different measurement time point after N treatment **(b)**. Response ratios of soil CO_2 flux to N addition in different years after N-addition treatment **(c)**. Response ratios of soil CO_2 flux to N addition in different types of forests and the total response ratios **(d)**. Solid circles and error bars represent weighted mean response ratios and 95% confidence intervals, respectively. The numbers under the solid circles in (c, d) represent sample sizes (n values). Data were collected from the literatures published from 1995 to 2021.

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	Experiment 1			Experiment 2		
Source of variance	***df***	***F***	***P*-value**	***df***	***F***	***P*-value**
Between subjects						
Forest	2	20.92	**<0.001**	2	47.71	**<0.001**
N treatment	2	11.10	**0.001**	1	22.87	**<0.001**
Forest × N treatment	4	0.94	0.464	2	0.20	0.819
Within subjects						
Year	8	50.37	**<0.001**	10	37.24	**<0.001**
Year × Forest	16	2.86	**<0.001**	20	3.75	**<0.001**
Year × N treatment	16	3.14	**<0.001**	10	3.50	**<0.001**
Year × Forest × N treatment	32	0.66	0.917	20	0.38	0.992

Extended Data Fig. 2 | Statistical significance of repeated-measures analysis of variance for soil respiration measured in different years with the factors of forest type and N-addition treatment.

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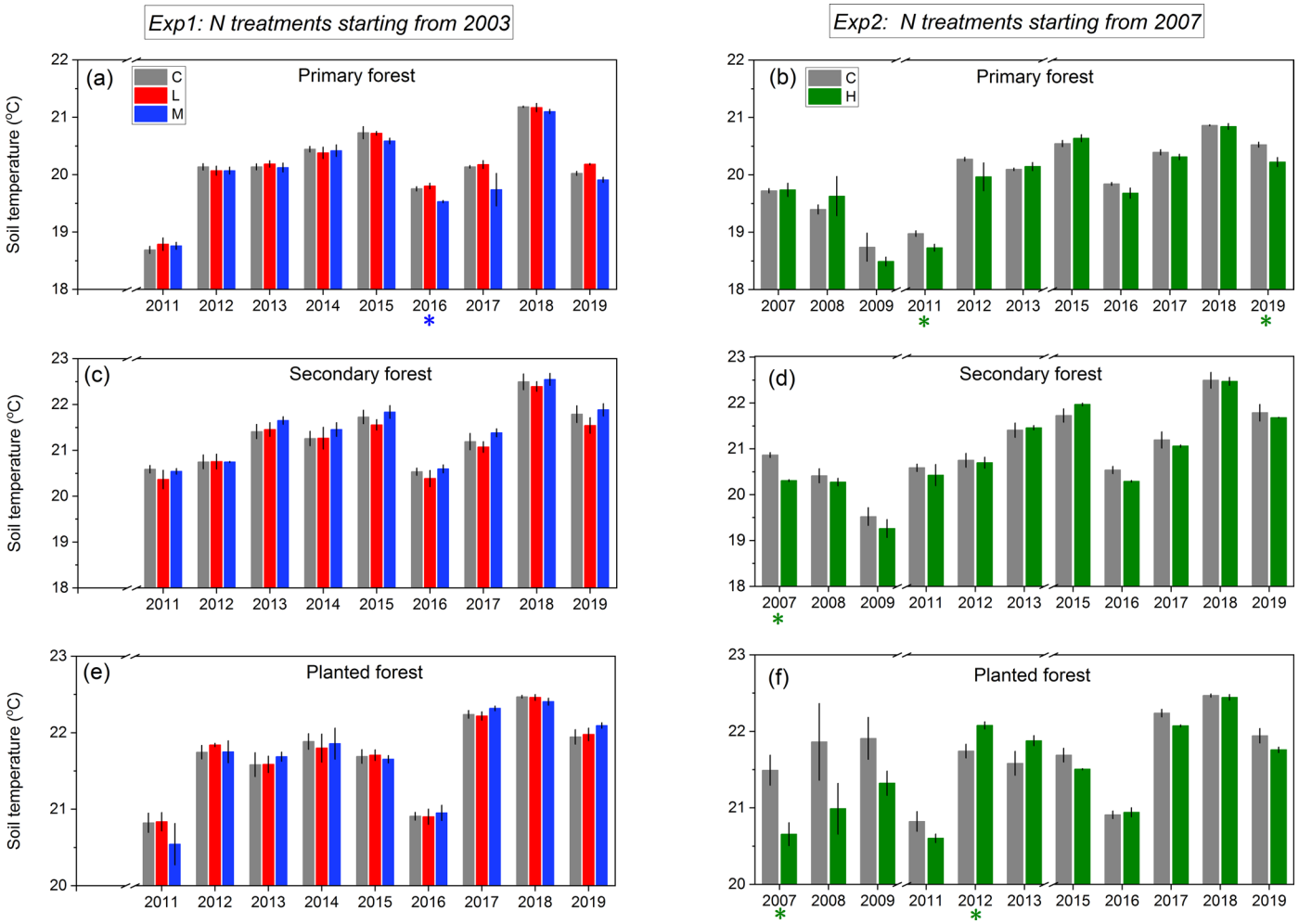

Extended Data Fig. 3 | Mean annual soil temperature in the control and N-addition plots of the primary forest (a-b), secondary forest (c-d), and planted forest (e-f). Columns and error bars represent means and standard errors (n = 3), respectively. The asterisks indicate the years in which statistical significance ($P < 0.05$; one-way analysis of variance followed by Tukey's HSD test for *Exp1* and independent-sample *t*-test for *Exp2*) is detected between the control and N-addition plots. C: control; L: low N addition; M: medium N addition; H: high N addition.

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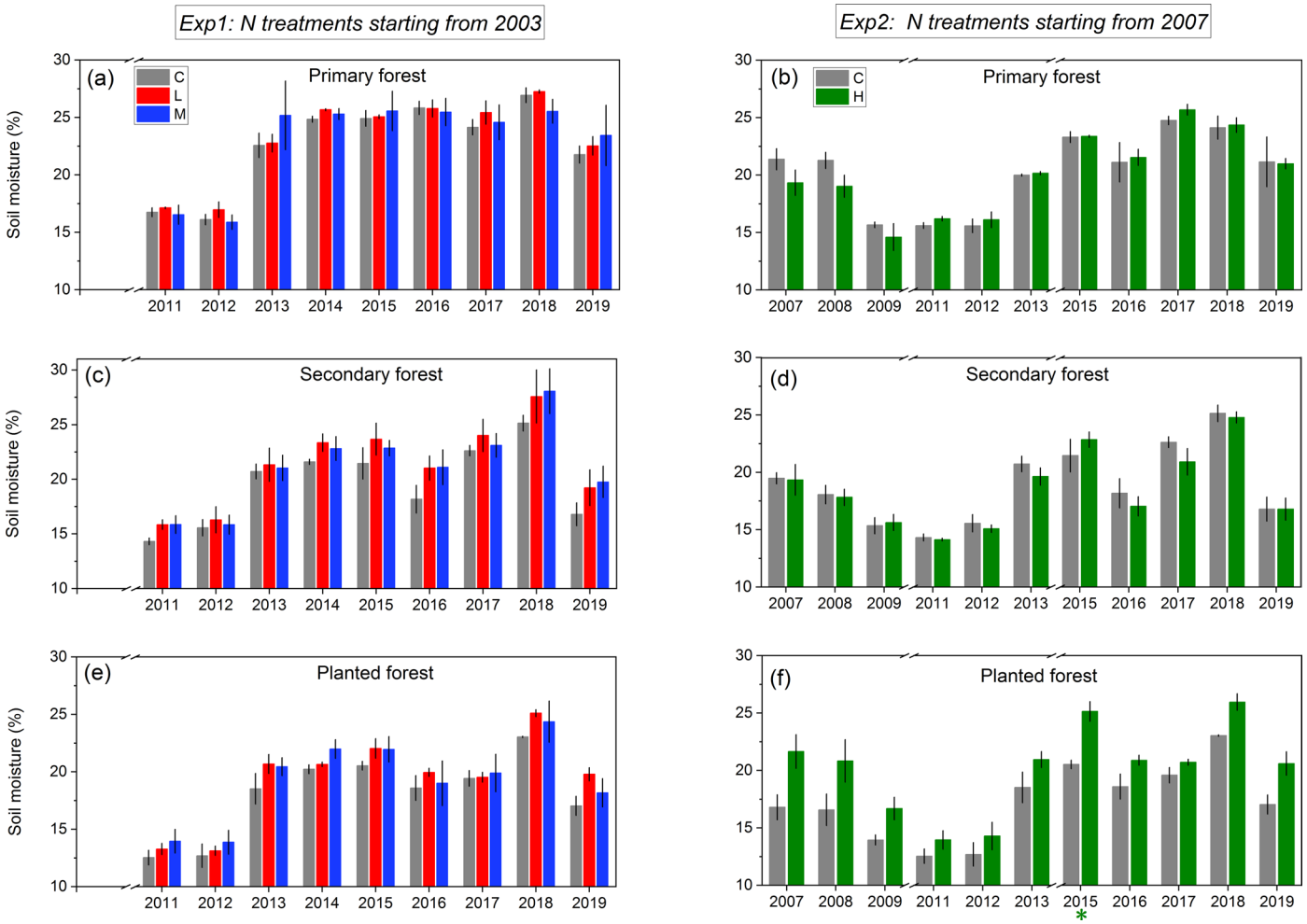

Extended Data Fig. 4 | Mean annual soil moisture in the control and N-addition plots of the primary forest (a-b), secondary forest (c-d), and planted forest (e-f). Columns and error bars represent means and standard errors (n = 3), respectively. The asterisks indicate the years in which statistical significance ($P < 0.05$; one-way analysis of variance followed by Tukey's HSD test for *Exp1* and independent-sample *t*-test for *Exp2*) is detected between the control and N-addition plots. C: control; L: low N addition; M: medium N addition; H: high N addition.

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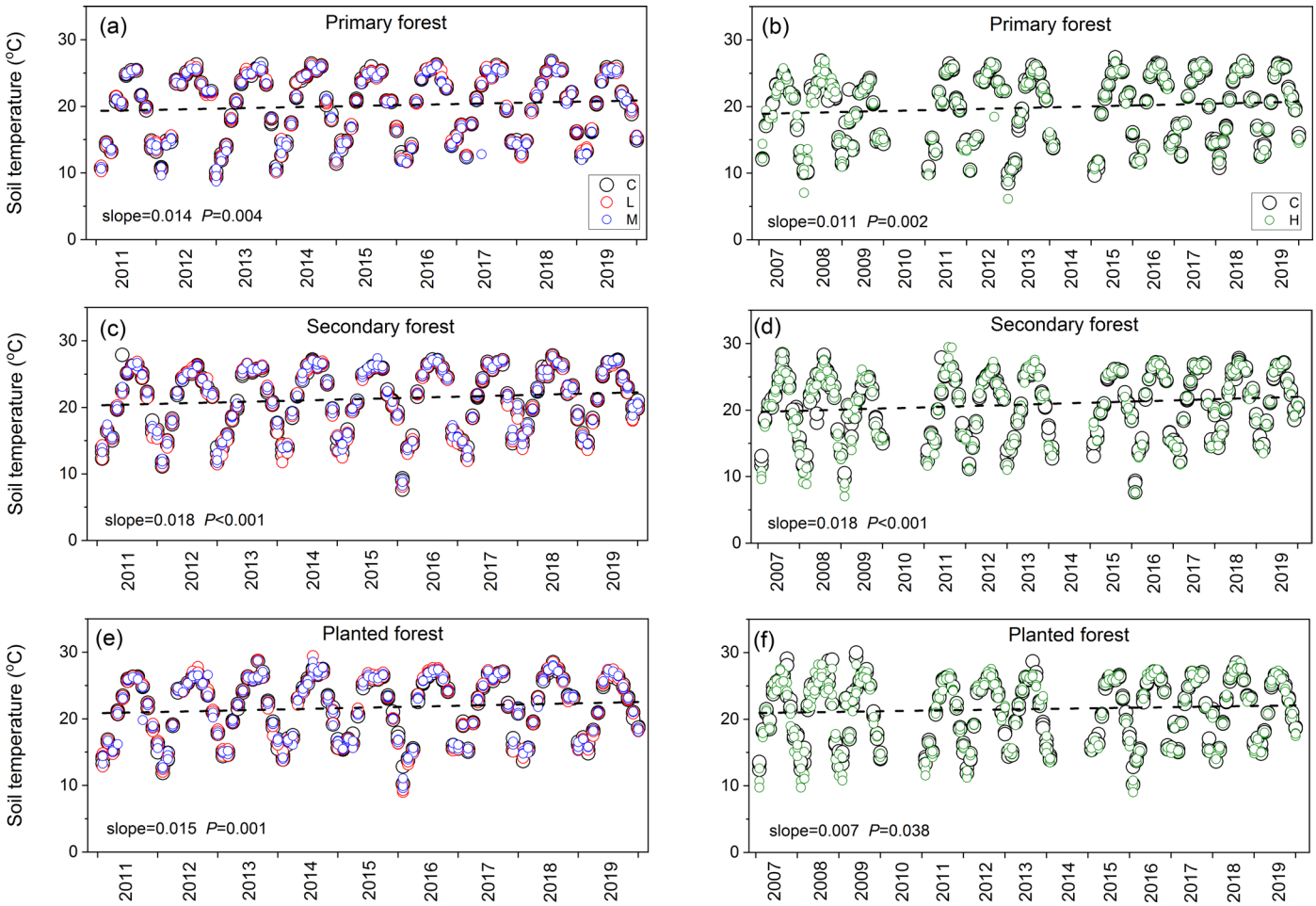

Extended Data Fig. 5 | The relationships between soil temperature and time in the primary, secondary, and planted forests. Linear regression models of soil temperature (in the control, low N-addition, and medium N-addition plots) against time from January 2011 to December 2019 (a, c, e). Linear regression models of soil temperature (in the control and high N-addition plots) against time from February 2007 to December 2019 (b, d, f). C: control; L: low N addition; M: medium N addition; H: high N addition.

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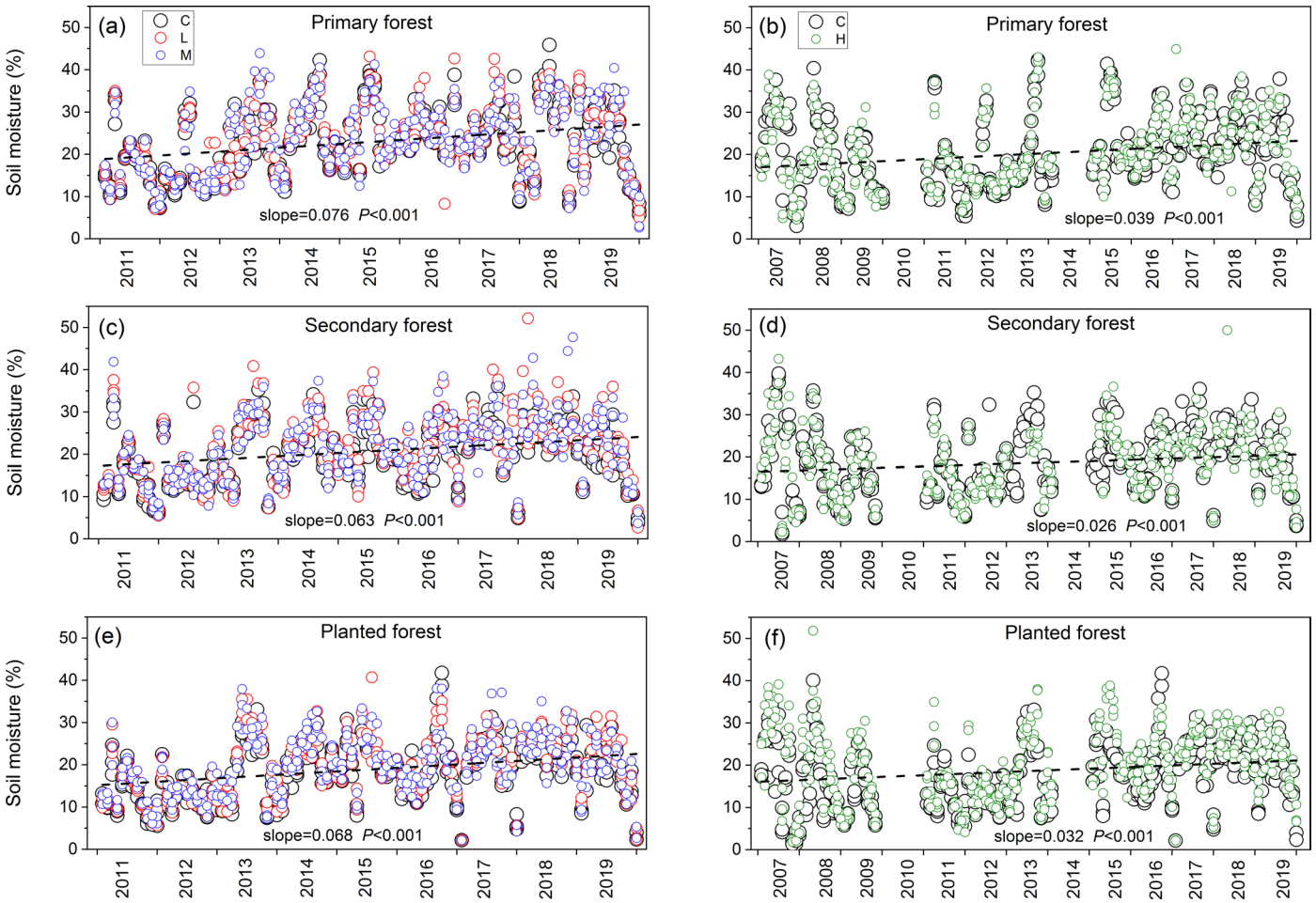

Extended Data Fig. 6 | The relationships between soil moisture and time in the primary, secondary, and planted forests. Linear regression models of soil moisture (in the control, low N-addition, and medium N-addition plots) against time from January 2011 to December 2019 (**a, c, e**). Linear regression models of soil moisture (in the control and high N-addition plots) against time from February 2007 to December 2019 (**b, d, f**). C: control; L: low N addition; M: medium N addition; H: high N addition.

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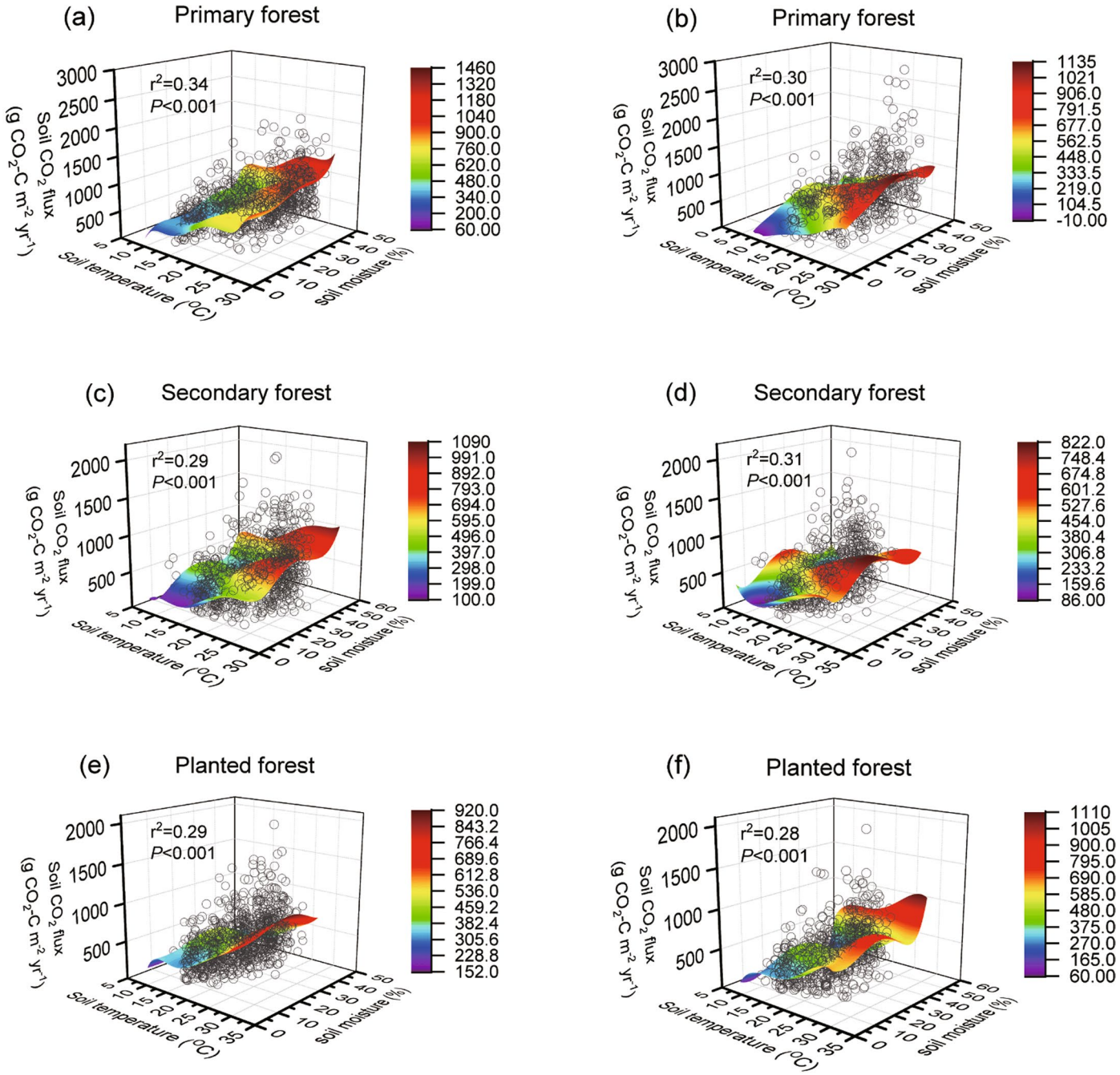

Extended Data Fig. 7 | Three-dimensional surface models of the relationships among soil respiration, soil temperature, and soil moisture in the three forests. Polynomial regression models of soil respiration against soil temperature and moisture (in combination of the control, low N-addition, and medium N-addition plots; Exp 1) from 2011 to 2019 (**a, c, e**). Polynomial regression models of soil respiration against soil temperature and moisture (in combination of the control and high N-addition plots: Exp 2) from 2007 to 2019 (**b, d, f**). C: control; L: low N addition; M: medium N addition; H: high N addition.

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Forest type	Experiment	Treatment	Formula	n	r^2	*P*-value
Primary forest	Exp 1	Control	Rs = exp(5.41+0.06×T)	324	0.35	<0.001
			Rs = 271.15×ln(M-3.30)	324	0.16	<0.001
		Low-N	Rs = exp(5.27+0.06×T)	324	0.29	<0.001
			Rs = 239.10×ln(M-2.75)	324	0.11	<0.001
		Medium-N	Rs = exp(5.23+0.06×T)	324	0.27	<0.001
			Rs = 241.03×ln(M-0.61)	324	0.09	<0.001
	Exp 2	Control	Rs = exp(5.28+0.07×T)	354	0.28	<0.001
			Rs = -529.75+437.74×ln(M+1.26)	354	0.18	<0.001
		High-N	Rs = exp(5.24+0.06×T)	354	0.20	<0.001
			Rs = -244.44+322.33×ln(M+1.05)	354	0.12	<0.001
Secondary forest	Exp 1	Control	Rs = exp(5.35+0.05×T)	324	0.20	<0.001
			Rs = -1674.51+699.27×ln(M+13.14)	324	0.20	<0.001
		Low-N	Rs = exp(4.92+0.07×T)	324	0.23	<0.001
			Rs = -524.66+339.60×ln(M+6.30)	324	0.08	<0.001
		Medium-N	Rs = exp(5.04+0.06×T)	324	0.23	<0.001
			Rs = -672.16+374.28×ln(M+8.67)	324	0.09	<0.001
	Exp 2	Control	Rs = exp(5.19+0.06×T)	378	0.20	<0.001
			Rs = -787.61+433.78×ln(M+6.88)	378	0.18	<0.001
		High-N	Rs = exp(4.79+0.07×T)	354	0.24	<0.001
			Rs = -318.17+284.04×ln(M+3.01)	354	0.11	<0.001
Planted forest	Exp 1	Control	Rs = exp(4.86+0.07×T)	324	0.35	<0.001
			Rs = -2860.06+876.88×ln(M+35.56)	324	0.13	<0.001
		Low-N	Rs = exp(4.95+0.06×T)	324	0.27	<0.001
			Rs = -673.19+358.47×ln(M+14.41)	324	0.07	<0.001
		Medium-N	Rs = exp(5.06+0.05×T)	324	0.20	<0.001
			Rs = -38165.87+6290.28×ln(M+488.07)	324	0.14	<0.001
	Exp 2	Control	Rs = exp(4.88+0.07×T)	381	0.29	<0.001
			Rs = -868.89+434.04×ln(M+12.49)	381	0.15	<0.001
		High-N	Rs = exp(5.07+0.05×T)	357	0.18	<0.001
			Rs = -183.73+207.90×ln(M+7.48)	357	0.08	<0.001

Extended Data Fig. 8 | The parameters and statistics of soil respiration to soil temperature (exponential regression models) and moisture (logarithmic regression models) in the control and N-addition plots in the studied forests. Experiment 1 (Exp 1): N treatments started from 2003, and soil respiration, temperature, and moisture were monitored from 2011 to 2019. Experiment 2 (Exp 2): N treatments started from 2007, and soil respiration, temperature, and moisture were monitored from 2007 to 2019. Rs: soil respiration; T: soil temperature; M: soil moisture.

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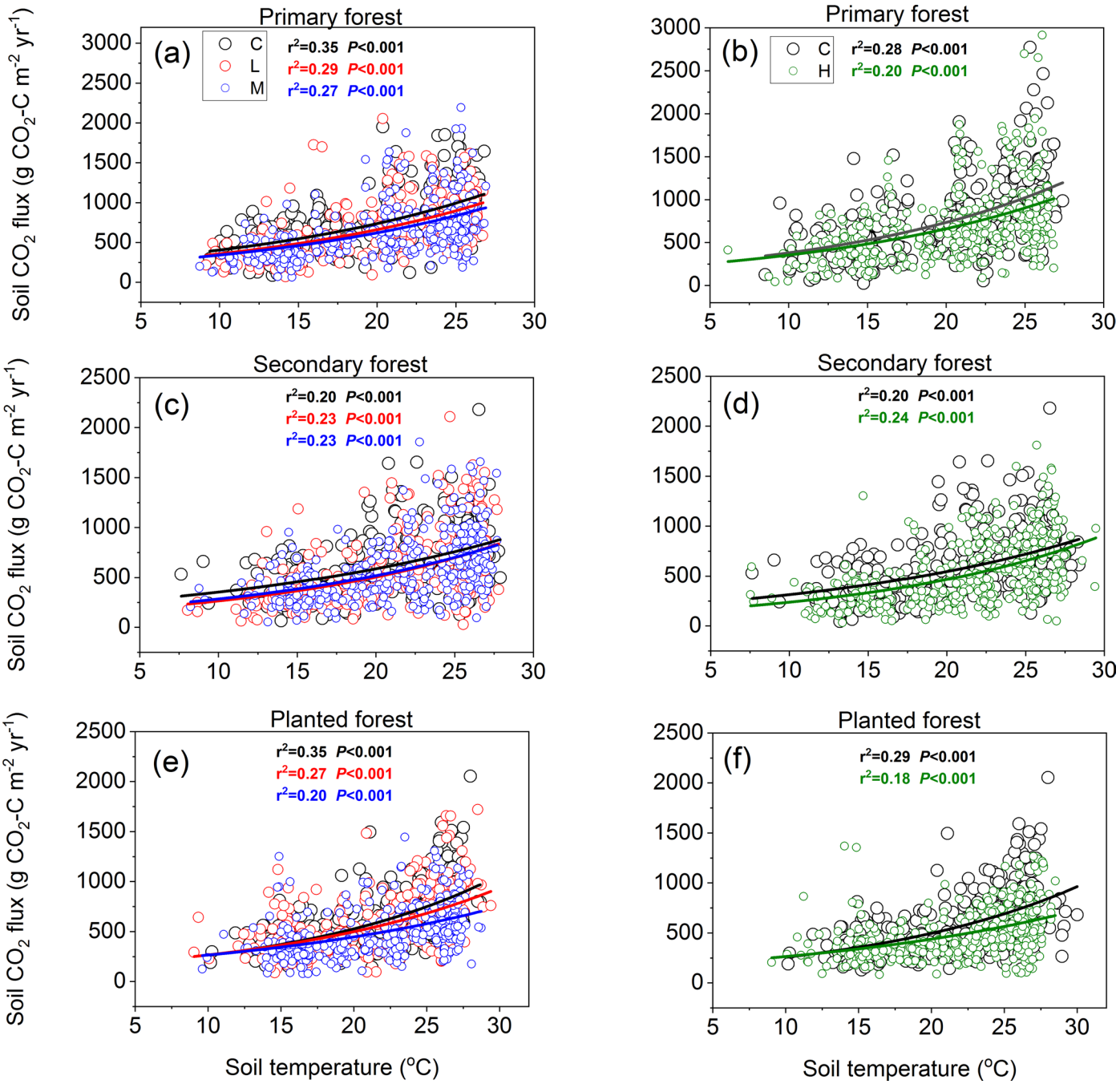
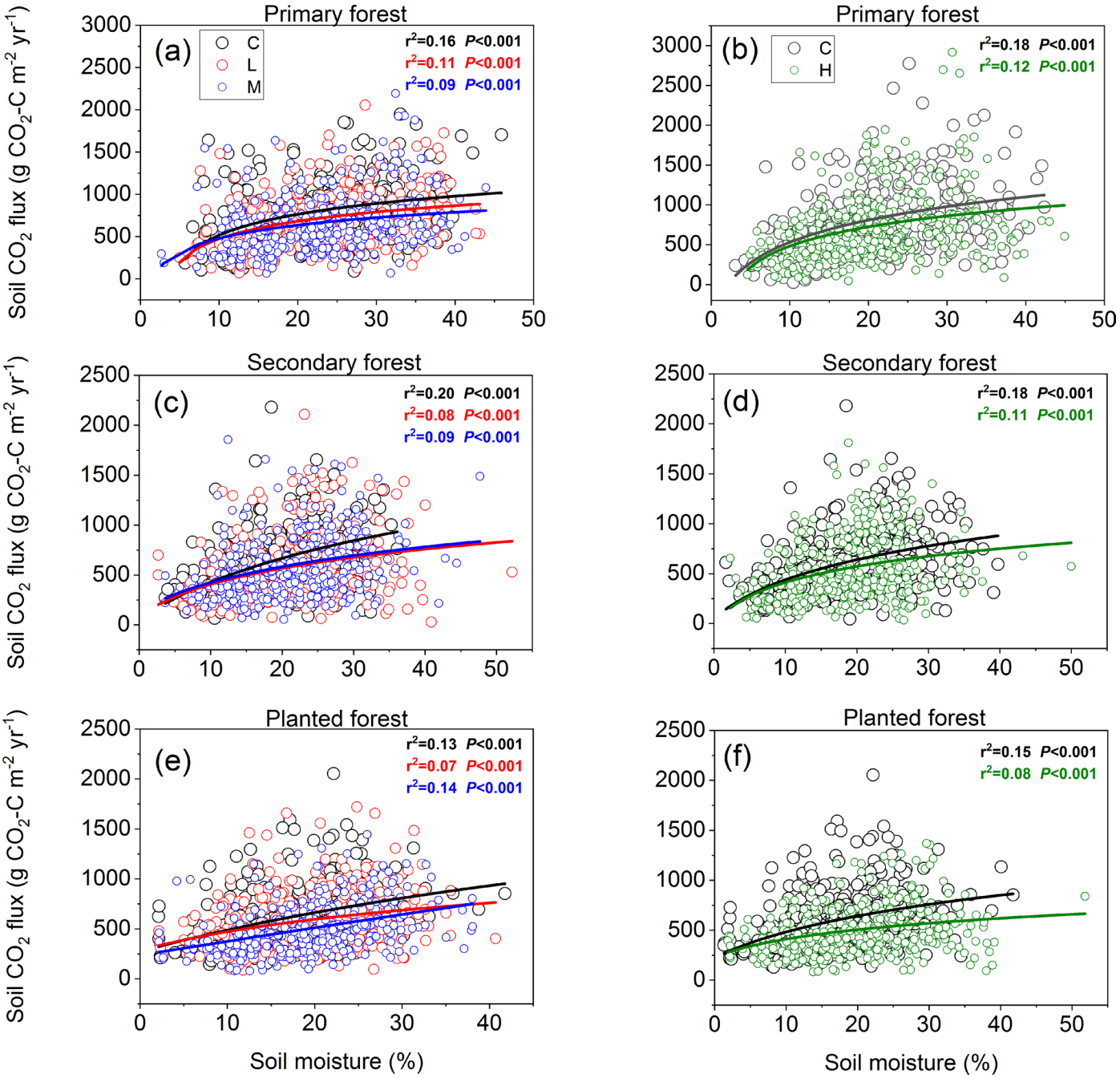


Extended Data Fig. 9 | Exponential regression models of soil respiration against soil temperature in the primary, secondary, and planted forests. Exponential regression models of soil CO_2 flux against soil temperature in the control, low N-addition, and medium N-addition plots in 2011 – 2019 (**a, c, e**). Exponential regression models of soil CO_2 flux against soil temperature in the control and high N-addition plots in 2007 – 2019 (**b, d, f**). C: control; L: low N addition; M: medium N addition; H: high N addition.

https://doi.org/10.1038/s41561-022-01080-4



Extended Data Fig. 10 | Logarithmic regression models of soil respiration against soil moisture in the primary, secondary, and planted forests. Logarithmic regression models of soil CO_2 flux against soil moisture in the control, low N-addition, and medium N-addition plots in 2011 – 2019 (**a, c, e**). Logarithmic regression models of soil CO_2 flux against soil moisture in the control and high N-addition plots in 2007 – 2019 (**b, d, f**). C: control; L: low N addition; M: medium N addition; H: high N addition.