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Enhanced leaf turnover and nitrogen recycling sustain CO_2 fertilization effect on tree-ring growth

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Whether increased photosynthates under elevated atmospheric CO_2 could translate into sustained biomass accumulation in forest trees remains uncertain. Here we demonstrate how tree radial growth is closely linked to litterfall dynamics, which enhances nitrogen recycling to support a sustained effect of CO_2 fertilization on tree-ring growth. Our ten-year observations in two alpine treeline forests indicated that annual (or seasonal) stem radial increments generally had a positive relationship with the previous year's (or season's) litterfall and its associated nitrogen return and resorption. Annual tree-ring width, annual litterfall and annual nitrogen return and resorption all showed an increasing trend during 2007–2017, and most of the variations were explained by elevated atmospheric CO_2 rather than climate change. Similar patterns were found in the longer time series of tree-ring width index from 1986–2017. The regional representativeness of our observed patterns was confirmed by the literature data of six other tree species at 11 treeline sites over the Tibetan Plateau. Enhanced nitrogen recycling through increased litterfall under elevated atmospheric CO_2 supports a general increasing trend of tree-ring growth in recent decades, especially in cold and nitrogen-poor environments.

In boreal and alpine trees, it is highly debatable whether time series of tree-ring widths can provide a long-term record about responses of tree radial growth to elevated atmospheric CO_2 concentration (eCO_2). It has been reported that eCO_2 may have a significant enhancement effect[1–4] or alternatively just a minor effect[5,6] on tree-ring growth. It is still difficult to quantify the extent to which eCO_2 fertilization may explain the increasing trend of tree-ring widths in recent decades, because changes in air temperature and atmospheric CO_2 are highly synchronized and there is lack of suitable data to effectively tease apart the underlying physiological mechanisms[7–10]. In most cases there are increases in both litterfall[11–13] and tree-ring increments[14–17] under CO_2-enriched environments. However, it has been proposed that progressive nitrogen limitation under CO_2 enrichment may constrain the continuous response of tree-ring growth to eCO_2 unless nitrogen supply is enhanced from various sources[7,9,18]. Elevated CO_2 generally leads to increased photosynthesis[7,19,20], which is expected to accelerate leaf turnover and nitrogen recycling because increased photosynthetic rates are typically associated with reduced leaf lifespan across species and ecosystems[21,22]. It is unknown whether there is a close linkage between litterfall and tree-ring growth, and if litterfall-enhanced N recycling plays a role in sustaining the effect of CO_2 fertilization on tree-ring growth. Such knowledge is critical for predicting responses of forest carbon and nitrogen cycles to global change.

At alpine treelines, the early growing season prior to summer solstice is the most important period for tree radial growth and leaf physiology[23]. Seasonal stem increment and photosynthetic capacity tend to peak around summer solstice[24,25], which allows cold-limited trees sufficient time for completing secondary cell wall lignification before the winter. Low soil temperatures during the early growing season generally cause low soil nutrient availability, which is another major constraint on tree growth[26,27]. The seasonal signals in leaves[28,29] may regulate leaf turnover to improve plant nitrogen use efficiency through reabsorbing nitrogen from senesced leaves[30,31]. There is evidence that seasonal stem increment typically shows a lagged positive relationship with litterfall, suggesting a likely linkage between stem growth and leaf turnover in treeline forests[23]. In a steady-state canopy system, leaf mass productivity is equal to the rate of leaf mass loss, and nitrogen uptake rate in green leaves is equal to nitrogen loss rate by litterfall and leaching[30,31]. The litterfall-induced nitrogen return and resorption may play an important role in regulating responses of tree-ring growth to changing environmental conditions, which may contribute to a higher sensitivity to eCO_2 than to warming. Here we hypothesize that tree radial growth generally has a lagged positive relationship with litterfall and its induced nitrogen return and resorption, and their interannual variations are mainly explained by eCO_2 rather than by warming, suggesting a sustained effect of eCO_2 fertilization on tree-ring growth in recent decades.

To test the hypothesis, we conducted ten-year observations (2007–2017) of seasonal stem radial increment, canopy litterfall and its induced nitrogen return (N-ret) and resorption (N-res) as well as climate factors at two alpine treelines in southeast Tibet. We also investigated the variation in tree-ring width index over a longer time period (1986–2017) across the two treeline species (*Abies georgei* var. *smithii* and *Juniperus saltuaria*). The time-series data (1986–2017) of monthly climatic factors and atmospheric CO_2 concentration were obtained from the Nyingchi weather station in southeast Tibet (~10 km from our study sites) and the Mauna Loa Observatory, Hawaii, respectively. We aimed to examine three key issues: (1) whether seasonal and annual stem increments typically show a lagged positive relationship with litterfall, N-ret and N-res

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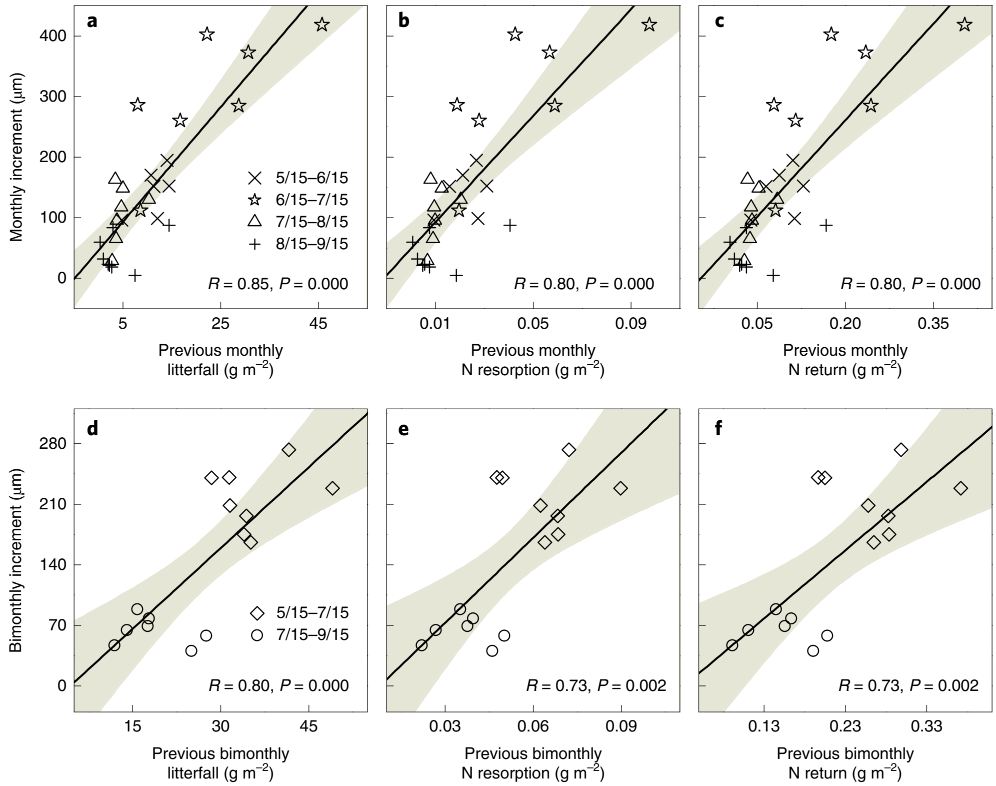

Fig. 1 | Relationships of seasonal stem increment to previous season's litterfall and its induced N recycling at two alpine treelines during 2007–2017. **a**–**c**, Monthly stem increments were positively correlated with previous monthly litterfall (**a**), N-res (**b**) and N-ret (**c**) in *A. georgei* var. *smithii* forest. **d**–**f**, Bimonthly stem increments were positively correlated with previous bimonthly litterfall (**d**), N-res (**e**) and N-ret (**f**) in *J. saltuaria* forest. The symbols are for seasonal stem increments. The relationships were tested using a simple linear model. The predicted mean (solid lines) is bounded by the 95% confidence intervals (shaded areas). The significance of correlation coefficient is estimated by two-tailed *t*-test with no adjustment for multiple comparisons.

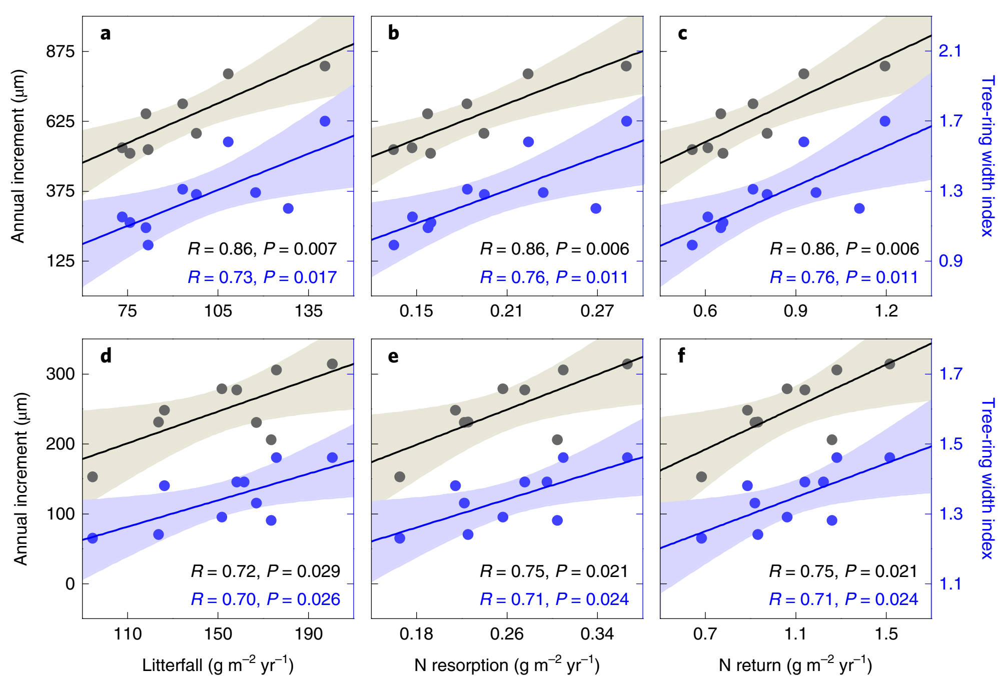

Fig. 2 | Relationships of annual stem increment to previous year's litterfall and its induced N recycling at two alpine treelines during 2007–2017. a–**c**, Annual stem increments were positively correlated with previous year's (from previous mid-June to current mid-June) litterfall (**a**), N-res (**b**) and N-ret (c) in *A. georgei* var. *smithii* forest. **d–f**, Annual stem increments were positively correlated with previous year's (from previous mid-June to current mid-June) litterfall (**d**), N-res (**e**) and N-ret (**f**) in *J. saltuaria* forest. Grey solid circles indicate annual stem increment by dendrometers and blue solid circles are for tree-ring width index by increment borers. The relationships were tested using a simple linear model. The predicted mean (solid lines) is bounded by the 95% confidence intervals (shaded areas). The significance of correlation coefficient is estimated by two-tailed *t*-test with no adjustment for multiple comparisons.

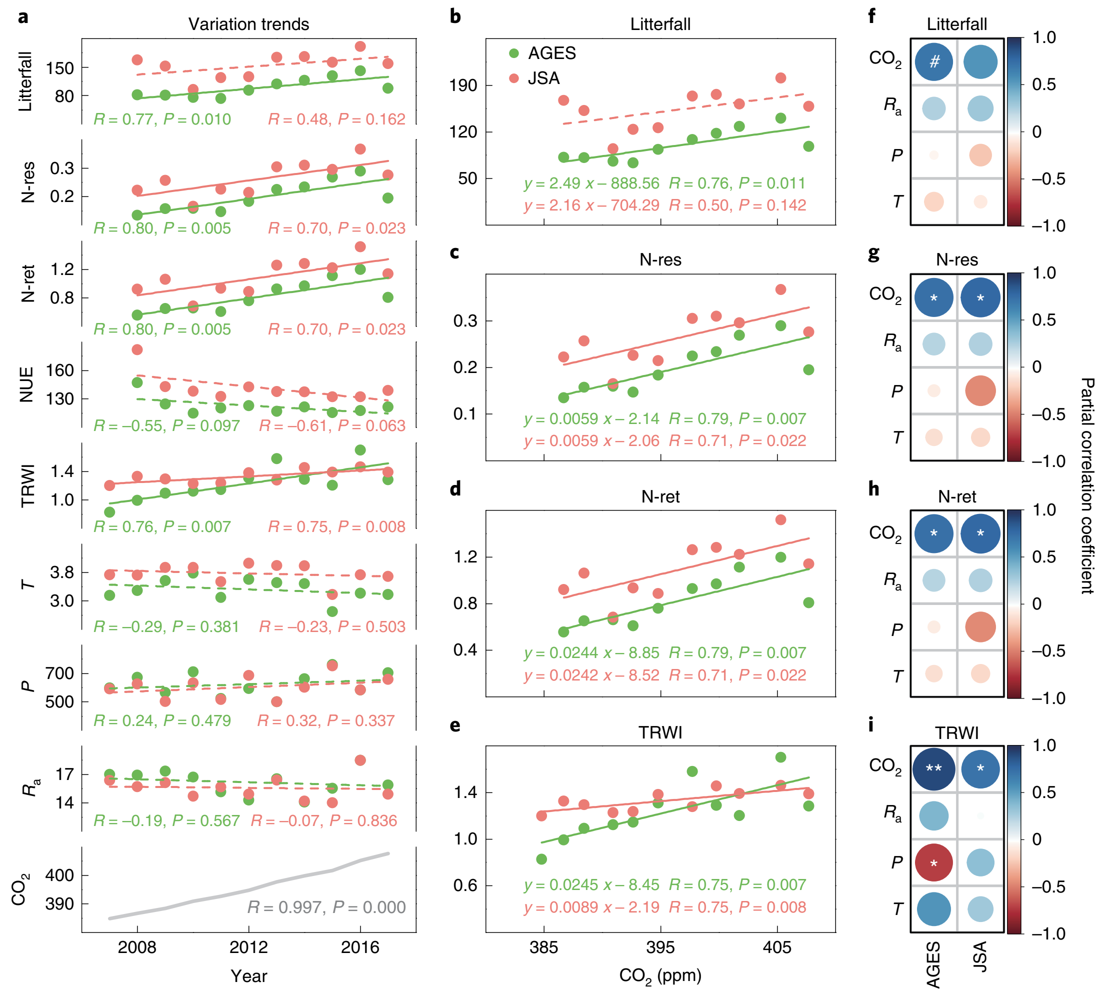

Fig. 3 | Relative contributions of climatic factors and atmospheric CO_2 to annual variations of litterfall, N recycling and tree-ring growth at two alpine treelines during 2007–2017. a, A simple linear model was used for testing variation trends in annual litterfall (g m^{-2} yr^{-1}), nitrogen resorption (N-res, g m^{-2} yr^{-1}), nitrogen return (N-ret, g m^{-2} yr^{-1}), nitrogen use efficiency (NUE, g DM g^{-1} N) and tree-ring width index (TRWI) for *A. georgei* var. *smithii* (AGES, green solid circles) and *J. saltuaria* (JSA, red solid circles), and the trends in climatic factors of growing season (May–August) mean minimum temperature (T, °C) and precipitation (P, mm) and solar radiation (R_a, MJ m^{-2} d^{-1}) observed at the two treelines, and in atmospheric CO_2 concentration (CO_2, ppm) from Mauna Loa Observatory, Hawaii (https://www.esrl.noaa.gov/gmd/ccgg/trends/). **b–e**, A simple linear model was used for testing relationships of litterfall (**b**), N-res (**c**), N-ret (**d**) and TRWI (**e**) to atmospheric CO_2 at both treelines. **f–i**, Partial correlation coefficients of multiple linear regressions for relationships of litterfall (**f**), N-res (**g**), N-ret (**h**) and TRWI (**i**) with climatic factors (T, P, R_a) and atmospheric CO_2 at both treelines; the partial correlation coefficients in different seasons and their exact *P* values are found in Supplementary Table 3. The significance of the correlation coefficient is estimated by two-tailed *t*-test with no adjustment for multiple comparisons. Significance level: $^{\#}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$.

across the two treeline stands; (2) whether annual tree-ring width, litterfall, N-ret and N-res all have a higher sensitivity to eCO_2 than to the variability of temperature, precipitation and solar radiation during 2007–2017; and if so, (3) whether similar patterns are found in the longer time series of tree-ring width index during 1986–2017. We also assessed whether climatic factors and atmospheric CO_2 have direct or indirect (via interactions with litterfall and N-ret/N-res) effects on tree radial growth using structural equation models. To test the regional representativeness of our observed patterns, we further examined the literature data of tree-ring widths for six other tree species at 11 treeline sites on the Tibetan Plateau (Supplementary Table 1).

Results

Relationship of stem increment with litterfall and N recycling. During 2007–2017, monthly stem increments in *A. georgei* var. *smithii* (Fig. 1a–c) or bimonthly stem increments in *J. saltuaria* (Fig. 1d–f) were positively correlated with previous monthly/bimonthly litterfall, N-res and N-ret. Also, annual stem increments and tree-ring width indices of *A. georgei* var. *smithii* (Fig. 2a–c) and *J. saltuaria* (Fig. 2d–f) generally showed a lagged positive relationship with annual litterfall, N-res and N-ret collected from previous mid-June to current mid-June (or from previous mid-May/mid-July to current mid-May/mid-July; Supplementary Table 2) during 2007–2017.

Response factors of TRWI, litterfall, N-res and N-ret. In response to the increase of atmospheric CO_2 and insignificant changes in temperature, precipitation and solar radiation during 2007–2017, tree-ring width index (TRWI), annual litterfall, N-res and N-ret in both treeline forests typically indicated a significant increasing trend (except for litterfall in *J. saltuaria*, Fig. 3a). While nitrogen use efficiency (NUE, defined as the inverse of weighted average leaf-litter nitrogen concentration) showed a slight decreasing trend

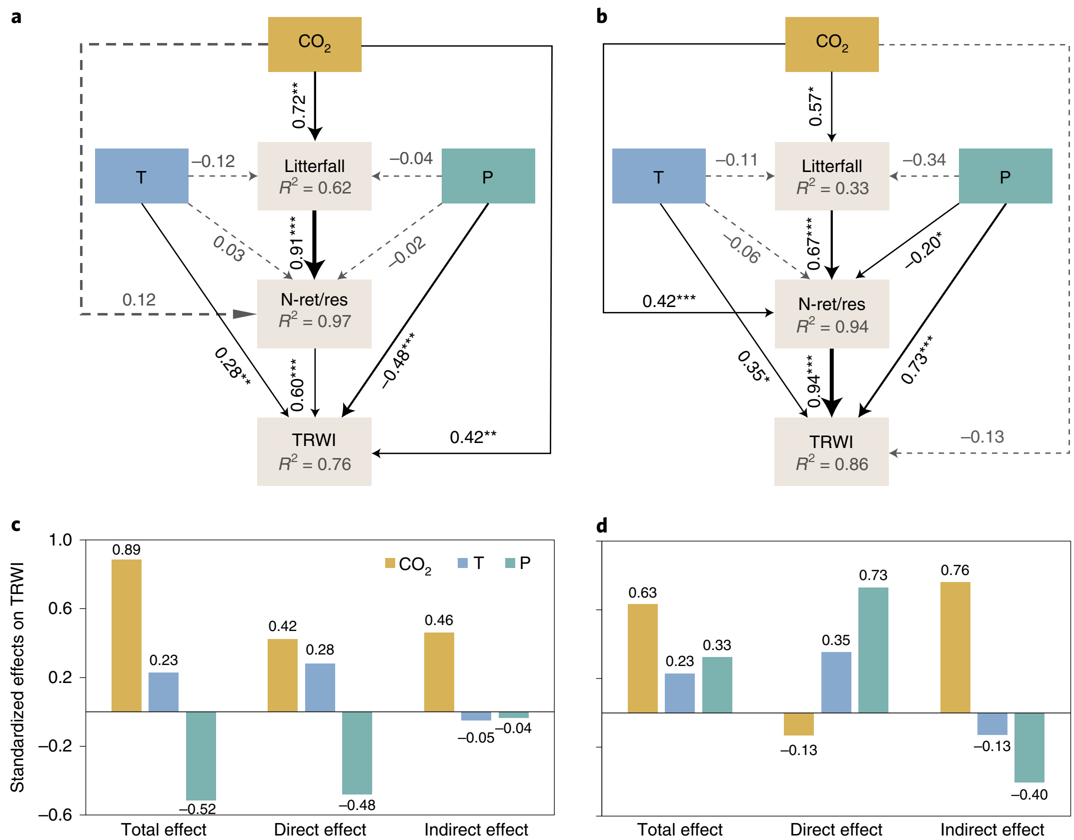

Fig. 4 | Structural equation models quantifying direct effects of climatic factors and atmospheric CO_2 and their indirect effects through interactions with litterfall and N-ret/N-res on tree-ring width index at two alpine treelines during 2007–2017. a,c, Standardized path coefficients (**a**) and the total, direct and indirect effects (**c**) of climatic factors and atmospheric CO_2 on TRWI in *A. georgei* var. *smithii* forest (χ^2 = 0.310, *P* = 0.578, CFI = 1.00, RMSEA = 0.000, AIC = 40.31). **b**,**d**, Standardized path coefficients (**b**) and the total, direct and indirect effects (**d**) of climatic factors and atmospheric CO_2 on TRWI in *J. saltuaria* forest (χ^2 = 0.507, *P* = 0.477, CFI = 1.00, RMSEA = 0.000, AIC = 40.51). Abbreviations of the measured variables are the same as in Fig. 3. Black solid arrows denote significant paths and arrow width indicates the strength of the relationship. Grey dashed arrows represent non-significant paths. The figures adjacent to arrows are for standardized path coefficients. R^2 value represents the proportion of variance explained for each dependent variable in the models. Significance level: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. The exact *P* values are found in Supplementary Table 4.

(P < 0.10, Fig. 3a), there were significant increasing trends in mean and minimum leaf-litter nitrogen concentrations of *A. georgei* var. *smithii* and in mean and maximum leaf-litter nitrogen concentrations of *J. saltuaria* (Supplementary Fig. 1a). In general, TRWI, annual litterfall, N-res and N-ret all increased with eCO_2 (except for litterfall in *J. saltuaria*, Fig. 3b–e). Partial correlation analysis of multiple regression models indicated that eCO_2 was the dominant driver of variations in tree-ring width index, annual litterfall, N-res and N-ret, compared with minor effects of temperature, precipitation and solar radiation at both treelines (Fig. 3f–i and Supplementary Table 3). Similar patterns were found for variation in leaf-litter nitrogen concentrations across both treeline forests (Supplementary Fig. 1b–g).

Structural equation modelling revealed clear, indirect effects of eCO_2 on tree radial growth via positive interactions with annual litterfall and nitrogen return/resorption in both treeline forests (Fig. 4a,b and Supplementary Table 4). For *A. georgei* var. *smithii*, there was also a significant direct effect of eCO_2 on tree radial growth (Fig. 4a), and the standardized direct eCO_2 effect was similar in magnitude to the sum of indirect effects (Fig. 4c). For *J. saltuaria*, there was no direct effect of eCO_2 on tree radial growth (Fig. 4b), and the indirect effects of eCO_2 far outweighed the direct effect (Fig. 4d). For climatic factors, direct effects on tree radial growth were larger than indirect effects. Growing season mean minimum temperature had a direct positive effect on tree-ring widths at both treelines (Fig. 4a,b). The direct precipitation effects on tree-ring width were negative in *A. georgei* var. *smithii* on the north-facing slope (Fig. 4a) but positive in *J. saltuaria* on the south-facing slope (Fig. 4b). In general, the total effect of eCO_2 was much higher than that of climate factors (Fig. 4c,d).

Over a longer time period (1986–2017), the TRWI and its ten-year moving averages generally showed significant increasing trends across the two treeline species in this study as well as six other tree species (at ten of 11 treeline sites) from the literature (Fig. 5a and Supplementary Fig. 2). Also, the long-term variations in tree-ring width indices (Fig. 5b) and their ten-year moving averages (Fig. 5c) of the eight treeline species (at 12 of 13 treeline sites) over the Tibetan Plateau were predominantly explained by eCO_2 rather than by climate change (more detailed information is found in Supplementary Table 5).

Discussion

Elevated CO_2 generally leads to increased photosynthesis and reduced transpiration, both resulting in increased forest water use efficiency[19]. However, uncertainty remains over whether the increased photosynthates could translate into sustained biomass accumulation in forest trees[7–9]. Under free-air CO_2 enrichment (FACE), growth rates of temperate tree species may increase (for example, *Populus*[15], *Pinus taeda*[16], *Larix decidua*[17]) or vary little (for example, hardwood forest trees[5], *Picea abies*[6], *Pinus uncinata*[17]). Carbon allocation to different

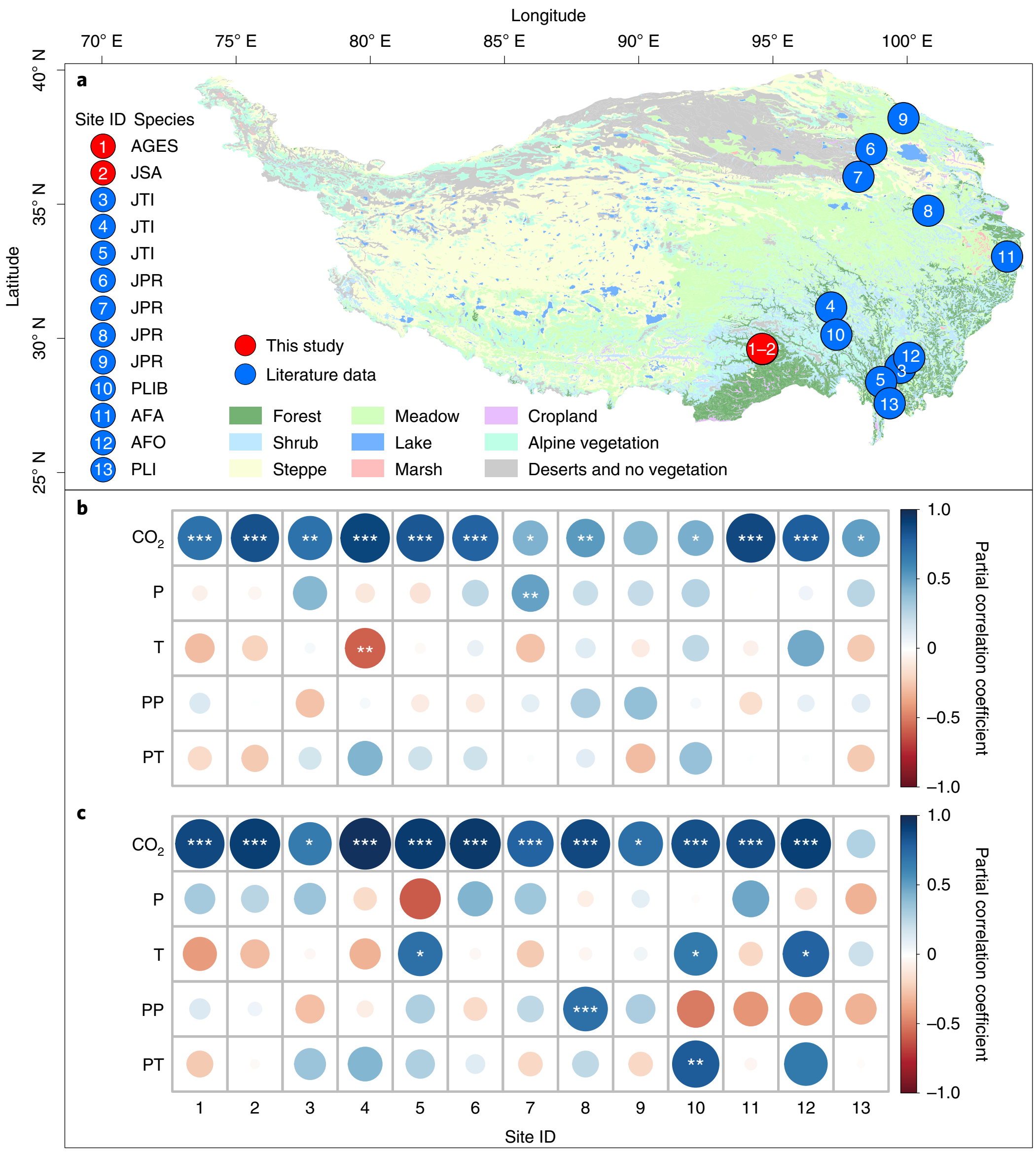

Fig. 5 | Relative contributions of climatic factors and atmospheric CO_2 to annual variations of tree-ring width index (since 1986) across 8 tree species and 13 treeline sites on the Tibetan Plateau. a, Spatial distribution of 13 tree-ring width chronologies. The background is the distribution map of vegetation types, which was drawn using ArcGIS 10.3 for Desktop with the vector data for Vegetation Atlas of China (1:1,000,000)[50], available free online at https://www.geodata.cn/. Species abbreviations: AGES, *Abies georgei* var. *smithii*; JSA, *Juniperus saltuaria*; JTI, *Juniperus tibetica*; JPR, *Juniperus przewalskii*; PLIB, *Picea likiangensis* var. *balfouriana*; AFA, *Abies faxoniana*; AFO, *Abies forrestii*; PLI, *Picea likiangensis*. **b**,**c**, Partial correlation coefficients of multiple linear regression for relationships of tree-ring width indices (**b**, TRWI) and their ten-year moving averages (**c**) to previous and current years' early season (May–June) mean minimum temperatures (PT, T) and precipitation (PP, P), and atmospheric CO_2 concentration (CO_2); the partial correlation coefficients between TRWI and climate factors in different seasons and their exact *P* values are found in Supplementary Tables 5 and 6. Detailed site information is found in Supplementary Table 1. The statistical significance is estimated by two-tailed *t*-test with no adjustment for multiple comparisons. Significance level: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

pools (for example, wood or fine root) plays an important role in determining carbon cycling and the capacity of carbon accumulation by trees[7,32]. In addition, it has been suggested that long-term exposure to enriched CO_2 may lead to photosynthetic acclimation because non-structural carbohydrates may accumulate in leaves and then leaf nitrogen and rubisco contents may decline, which diminishes or restrains the eCO_2 fertilization effect on plant growth[14]. Furthermore, the increase of photosynthetic rate under eCO_2 may increase the demand for nitrogen, leading to progressive nitrogen limitation on plant photosynthesis and growth[9,18]. However, such ecophysiological mechanisms revealed from FACE experiments do not offer an explanation for the increasing trend of tree-ring widths in recent decades observed in this study (Fig. 5 and Supplementary Fig. 2) and many previous reports (for example, *Populus tremuloides*[1], *Fitzroya cupressoides*[2], Norway spruce[3], red spruce[4]). Stable carbon and oxygen isotopic records from tree rings generally indicate that stimulated leaf photosynthesis, rather than reduced stomatal conductance, is primarily responsible for the recent general increase in tree water use efficiency under eCO_2, especially in areas with adequate water supply[33,34]. Even so, it is unclear whether and how eCO_2 may have continuously promoted the tree-ring growth of boreal and alpine trees in recent decades.

Leaf turnover and N recycling may play an important role in regulating tree growth response to eCO_2, but these possibilities have received little attention in previous studies. To conserve nitrogen resources, litterfall-induced N recycling is one of the most important strategies used by nearly all plant species[30]. Our ten-year observations revealed that elevated atmospheric CO_2 showed primarily an indirect enhancement effect on tree radial growth through increased litterfall and nitrogen return/resorption in the two treeline forests (Fig. 4). Annual nitrogen return by litterfall ranged from 0.56 to 1.52 g m^{-2} yr^{-1} in the two treeline forests (Fig. 3a), which was less than the nitrogen uptake rates measured in warm temperate forests (for example, 5.31 g m^{-2} yr^{-1} in aspen forests and 4.86 g m^{-2} yr^{-1} in pine forests[35]), probably due to low soil nitrogen availability at alpine treelines where temperature is much lower. The estimated nitrogen resorption (0.13–0.37 g m^{-2} yr^{-1}) was about 24% of the nitrogen return at both treelines. The litterfall-induced nitrogen return and resorption generally increased with eCO_2 (Fig. 3c,d), which was closely coupled with the increased stem radial growth in the two treeline forests (Figs. 1 and 2). In particular, the increased rates of annual nitrogen return by litterfall were ~2.43 g m^{-2} yr^{-1} per 100 ppm eCO_2 in both treeline forests (Fig. 3c,d), which was higher than the rates of annual nitrogen uptake by temperate trees under FACE environments (0.47–1.57 g m^{-2} yr^{-1} per 100 ppm eCO_2)[35]. The significant increasing trend of mean leaf-litter nitrogen concentrations in the recent decade (Supplementary Fig. 1a) suggests an increase of nitrogen uptake by trees with an equal amount to the nitrogen loss by litterfall[30,31]. The slightly decreased NUE (Fig. 3a) was not correlated with tree radial growth in both treeline forests ($P > 0.10$), which was consistent with previous reports[35]. Furthermore, tree-ring width index, annual litterfall, nitrogen return/resorption and leaf-litter nitrogen concentration all indicated a higher sensitivity to eCO_2 than to the variability of climatic factors (Figs. 3–5, Tables 3 and 4 and Supplementary Fig. 1), which is consistent with many previous studies[3,4]. Thus, the litterfall-enhanced nitrogen return and resorption can reduce the nitrogen limitation to plant growth under eCO_2. Such a mechanism provides a new explanation for the general increasing trend of tree radial growth in recent decades observed in this study (Fig. 3a, Fig. 5 and Supplementary Fig. 2) and previous reports[1–4].

Climate change impacts on tree radial growth varied with time periods and slope aspects (Figs. 3–5) because of variable patterns in precipitation change, snow cover and snow melting[23]. In water-limited forests, the decrease of stomatal conductance induced by eCO_2 can reduce water loss through transpiration and thus improve soil water availability[36]. However, severe stomatal closure due to intensified water stress caused by climate warming may also constrain leaf photosynthesis and subsequently tree growth[8,37]. This may explain the lack of response of tree radial growth to eCO_2 in some areas where annual precipitation is low[8,37] (site 9 in Fig. 5b). On the other hand, tree growth may vary little with eCO_2 when the increasing demand for nitrogen under rapid increase of atmospheric CO_2 exceeds the rate of N recycling from leaf turnover.

In temperate and boreal forests, $^{13}CO_2$ pulse-labelling experiments have revealed that earlywood, the determinant of annual tree-ring width, contains both photosynthates produced in the previous summer and autumn and in the current spring[38,39]. This may explain why annual stem increment (tree-ring width index) generally had a close relationship with the last year's litter production and its related nitrogen return and resorption processed from the onset of previous growing season to the beginning of the current growing season (Fig. 2 and Supplementary Table 2). These findings further support an active carbon storage strategy taken by trees in harsh environments, which allows trees to cope with the asynchrony of supply and demand for carbon in the early growing season and to prepare for unpredictable disturbances such as freezing damage[30,40]. The findings also provide a basis for interpreting the positive relationship between TRWI and previous year (and/or current year) photosynthetic productivity found in many earlier studies (for example, black spruce forest[41], Norway spruce forest[42], *Rhododendron* shrub[43], Howland forest[44]).

In evergreen conifers, reduced leaf area index by shedding older leaves in the early growing season can improve light penetration into the canopy and then increase soil temperature, resulting in increases of soil nitrogen availability and canopy photosynthetic productivity[31,45]. Nitrogen reabsorbed from senescent leaves and photosynthates produced by mature leaves are primarily used for new leaf growth, and the developing leaves become carbon sources for secondary growth of stem and root later, when they are half-grown[30,31,45]. For evergreen conifers, 50–80 days are needed to construct a full-grown leaf (that is, 25–40 days for a half-grown leaf)[21]. This may explain why monthly (or bimonthly) stem increment was positively correlated with previous monthly (or bimonthly) litterfall and nitrogen resorption/return, with a delay of one to two months (Fig. 1).

In conclusion, our data support the hypotheses that there is a close linkage between leaf turnover and tree-ring growth, and that litterfall-enhanced nitrogen return and resorption can reduce the nitrogen limitation to plant growth under elevated atmospheric CO_2. The findings offer a new explanation for the general increasing trend of tree-ring widths in recent decades that has been globally observed in temperate, boreal and alpine trees, and provide a new basis for detecting the long-term changes in forest litterfall and N recycling according to tree-ring width records. The revealed mechanisms for litterfall-accelerated N recycling and its link to the sustained effect of CO_2 fertilization on plant growth should be considered in terrestrial biogeochemical models.

Methods

Study sites. This study was conducted on the north-facing and south-facing slopes of a U-shaped valley at the peak of the Sergyemla Mountains (29° 36′ N, 94° 36′ E) in southeast Tibet. Dominant tree species of both treelines are *Abies georgei* var. *smithii* on the north-facing slope and *Juniperus saltuaria* on the south-facing slope. Along both slopes the vegetation changes from sub-alpine and treeline forests (tree height >4 m and canopy coverage >40%) to an open mosaic of alpine shrublands and grasslands. In August 2005, two long-term observing plots (50 × 50 m^2) were established in both treeline forests. The stand basal area and mean tree height (mean ± s.d.) were 39.7 m^2 ha^{-1} and 10.2 ± 1.0 m for *A. georgei* var. *smithii* and 39.8 m^2 ha^{-1} and 7.6 ± 0.5 m for *J. saltuaria*. Four automatic weather stations (HL20, Jauntering Inc., Taiwan) were installed in treeline forests of *A. georgei* var. *smithii* (4,320 m) and *J. saltuaria* (4,425 m) and above both treelines. During 2006–2017, annual mean air temperature was 0.6 °C and 1.1 °C in treeline forests of *A. georgei* var. *smithii* and *J. saltuaria*, respectively. The growing season precipitation (May–August) was similar between north-facing and south-facing slopes (769 mm and 745 mm, respectively). Daily mean soil volume moisture content during the growing season was typically >35% on both slopes. Spring soil warming date (when soil temperatures began to be continuously above 0 °C) was 20–30 days earlier on the south-facing slope than on the north-facing slope.

Litterfall and related nitrogen return and resorption. In each of the treeline forests, five 1.5 × 0.5 m^2 litterfall traps were randomly installed in mid-August 2006. Monthly litterfall (including dead leaves and twigs) was collected every mid-month from May to October, and the litterfall accumulated after the previous October was collected in mid-April or mid-May. The litterfall was separated into needles, twigs, cones, barks and others. The separated components were oven-dried at 70 °C for approximately 48 hours and then weighed. As the N recycling largely occurs from senescent leaves, the data of needle litterfall in *A. georgei* var. *smithii* and scale-leaf twig litterfall in *J. saltuaria* were used for all analyses in this study. The leaf-litter nitrogen concentration was analysed by the Kjeldahl method.

Given that leaf mass production is equal to leaf mass loss (litterfall), and nitrogen uptake in green leaves is approximate to nitrogen return by litterfall in a steady-state canopy system[30,31], the monthly nitrogen return (N-ret, g m^{-2}) was calculated as:

$$\text{N-ret} = N_{\text{litter}} \times M_{\text{litter}}/100 \quad (1)$$

where N_{litter} is the leaf-litter nitrogen concentration (%) and M_{litter} is the dry mass of monthly leaf litterfall (g m^{-2}). Also, the monthly nitrogen resorption (N-res, g m^{-2}) was calculated as:

$$\text{N-res} = \left(N_{\text{green}} - N_{\text{litter}}\right) \times M_{\text{litter}}/100 \quad (2)$$

where N_{green} is the nitrogen concentration of green leaves (%). Because of difficulty in seasonal destructive sampling of canopy leaves in long-term observations, N_{green}

was estimated from N_{litter} using a global empirical model ($N_{litter} = 0.60N_{green}$)[46] with a mass loss correction factor (MLCF, that is, the ratio of leaf litter mass to green leaf mass)[47]:

$$N_{green} = (N_{litter}/MLCF)/0.60 \quad (3)$$

To correct the intrinsic underestimation of nitrogen resorption due to the mass loss and the change of measurement basis during leaf senescence, the MLCF of 0.745 for conifers was used as in Vergutz et al.[47]. To validate equation (3), we measured nitrogen concentrations of green leaves from the canopy and dead leaves from litter traps for each of five individual trees at *A. georgei* var. *smithii* treeline once every mid-month during May and September 2017. The estimated N_{green} using equation (3) was highly correlated with the observed values, being close to a one-to-one relationship ($R^2 = 0.36$, $P < 0.001$, slope = 0.88; Supplementary Fig. 3).

Assuming that carbohydrates used for tree-ring growth are mainly from the reserves stored since the previous growing season and the concurrent photosynthates[38,39], annual litterfall and annual nitrogen resorption and return were calculated as the sum of seasonal measurements from previous mid-June to current mid-June during 2007–2017. NUE was estimated as the inverse of the monthly litterfall-weighted average leaf-litter nitrogen concentration because the ratio of biomass production to nitrogen uptake is equal to the ratio of litterfall to nitrogen loss[30,31].

Stem radial increment with dendrometers. In August 2005, eight mature and healthy trees at each of the two treeline sites were selected and mounted with automatic dendrometers of diameter and circumference (including 2 DD and 6 DC dendrometers, Ecomatik, Munich, Germany) at breast height to continuously monitor stem radial growth. The monitored trees of *A. georgei* var. *smithii* and *J. saltuaria* had an average height of 9.6 ± 1.8 m and 8.6 ± 0.9 m, diameter at breast height (DBH) of 37.2 ± 14.0 cm and 22.4 ± 3.1 cm, and stem age of 188 ± 61 yr and 207 ± 24 yr, respectively. Raw data were recorded hourly by a HL20 data logger (Jauntering Inc.). The data measured by DD and DC types of dendrometers were divided by 2 and 2π, respectively, to obtain the stem increment in radius. Because the data loggers were broken in 2007, we examined the data recorded during 2008–2017 in this study. Also, the data of 2014–2015 for *A. georgei* var. *smithii* and 2015 for *J. saltuaria* were lost because of sensor failure. Thus, there were eight and nine years of dendrometer data for *A. georgei* var. *smithii* and *J. saltuaria*, respectively.

Weekly stem radial increment was calculated as the difference of daily maximum values between the consecutive seventh day and the first day for each tree, year and species according to the method described by Rossi et al.[24]. Monthly stem increment was calculated as the sum of weekly increments within a month consistent with the sampling time of monthly litterfall. For the slow-growing *J. saltuaria*, bimonthly stem increment was calculated as the sum of two consecutive monthly increments. Annual stem increment was calculated as the sum of weekly increments over the growing season. Luo et al.[23] demonstrated that annual stem increments measured by dendrometers positively correlated well with the tree-ring widths of monitored trees, indicating that tree-ring growth signals can be extracted from dendrometer data. The average stem increment weighted by DBH of each monitored tree was related to the stand-level measurement of litterfall. Detailed information on the calculation method and the definition of onset and ending dates of stem radial increment is found in Luo et al.[23].

Tree-ring width chronologies and related climate data. In October 2016, two tree-ring cores at breast height were sampled with an increment borer (Haglöf, Sweden) for each of 30 trees (including the eight monitored trees with dendrometers) in each of both treeline species. In October 2017, we further collected additional two cores for each of the eight monitored trees with dendrometers. The cores were processed following standard dendrochronological practices and annual ring width was measured with a resolution of 0.01 mm. After performing the cross-dating check by using the COFECHA program[48], the raw data of the 76 tree-ring width series were transformed into indices by fitting a negative exponential function using the ARSTAN program[49].

The ten-year climate data (2007–2017) of monthly mean minimum temperature, precipitation and solar radiation were obtained from the automatic weather stations at the two treelines in the Sergyemla Mountains. The climate data for a longer time period (1986–2017) were obtained from Nyingchi weather station at 3,000 m, ~10 km from our study sites. The time-series data (1986–2017) of atmospheric CO_2 concentration were obtained from Mauna Loa Observatory, Hawaii (https://www.esrl.noaa.gov/gmd/ccgg/trends/).

To test the generality of our observed data in the Sergyemla Mountains, we further collected the literature data of tree-ring width chronologies for six other species at 11 treeline sites over the Tibetan Plateau, which were obtained from the published papers and the International Tree-Ring Data Bank, available online at the website (https://www.ncdc.noaa.gov/data-access/paleoclimatology-data/datasets/tree-ring) (Supplementary Table 1). The time-series data of monthly mean minimum temperature and monthly precipitations at weather stations near the 11 sampling sites were obtained from the China Meteorological Data Service Centre (http://data.cma.cn/data/detail/dataCode/A.0012.0001).

Data analysis. A simple linear model was used for examining the variation trends in annual litterfall, N-ret, N-res, NUE and TRWI, as well as climatic factors (growing season mean minimum temperature, precipitation and solar radiation) and atmospheric CO_2 concentration, and for testing the relationships of seasonal/annual stem increment with seasonal/annual litterfall and its associated nitrogen resorption and return during 2007–2017.

Partial correlation analysis of multiple linear regression was used for assessing the relative importance of the current year's seasonal mean minimum temperature (T), precipitation (P), solar radiation (R_a) and atmospheric CO_2 (CO_2), as well as the previous year's seasonal mean minimum temperature (PT), precipitation (PP), solar radiation (PR_a) and atmospheric CO_2 (PCO_2) in determining the variations of annual litterfall, N-ret, N-res and TRWI in the two treeline forests during 2007–2017. Climatic variables and atmospheric CO_2 in different periods, including the early season (May–June, April–June), summer (June–August), the whole growing season (May–August, April–September) and the year, were inputted into the above regression models, respectively. To verify our ten-year observed data, partial correlation analysis was further performed to examine whether the tree-ring width chronologies in recent decades (since 1986) across eight tree species and 13 treeline sites on the Tibetan Plateau collected from this study and the literature also show similar patterns associated with climatic factors and atmospheric CO_2. We further calculated ten-year moving averages of the 30-year data for tree-ring width indices, climatic factors and atmospheric CO_2 concentration across the eight tree species and the 13 treeline sites, and then performed the partial correlation analysis.

In addition, structural equation modelling was used to quantify direct effects of climatic factors and atmospheric CO_2 and their indirect effects through interactions with litterfall and nitrogen return/resorption on TRWI during 2007–2017. Based on the theoretical knowledge of the major factors controlling annual variation of TRWI, an a priori conceptual model was developed to relate climatic factors (T, P), atmospheric CO_2, annual litterfall and N-ret/N-res to TRWI. Maximum-likelihood estimation was used to obtain the path coefficients. The χ^2 goodness-of-fit test ($P < 0.05$ indicated a poor fit), comparative fit index (CFI), root mean square error of approximation (RMSEA) and Akaike information criterion (AIC) were used to test whether the model was a reasonable explanation of the observed pattern. Structural equation models were constructed by the AMOS v.24.0 package of the SPSS statistical software (SPSS Inc.).

All statistical analyses were performed using SPSS v.18.0 (SPSS Inc.). The map was drawn by ArcGIS v.10.3 for Desktop (Environmental Systems Research Institute, Inc.). All significant differences were taken at $P < 0.05$.

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

Data availability

The tree-ring width chronologies collected from the International Tree-Ring Data Bank are available at https://www.ncdc.noaa.gov/data-access/paleoclimatology-data/datasets/tree-ring. The time-series data of atmospheric CO_2 concentration were obtained from https://www.esrl.noaa.gov/gmd/ccgg/trends/. The time-series data of monthly climatic factors at weather stations were obtained from http://data.cma.cn/data/detail/dataCode/A.0012.0001. All observed data in this study are provided in this article and its associated supplementary information.

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Author contributions

T.L. designed the experiment. Y.G., L.Z., L.Y. and W.S. conducted the experiment. Y.G., L.Z., T.L., Y.P., I.J.W. and Y.L. analysed the data. Y.G., L.Z. and T.L. wrote the manuscript. Y.P., I.J.W. and Y.L. revised the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used.
Data analysis	All statistical analyses were performed using the SPSS 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The map was drawn by ArcGIS 10.3 for Desktop (Environmental Systems Research Institute, Inc., RedLands, California, USA).

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Ecological, evolutionary & environmental sciences study design

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Study description	We demonstrated how tree-ring width growth is closely linked to litterfall dynamics and how litterfall-enhanced nitrogen recycling supports a sustained effect of CO2 fertilization on tree-ring growth, based on our 10-year observed data at two alpine treeline sites.
Research sample	This study was conducted on the north-facing and south-facing slopes of a U-shaped valley at the peak of the Sergyemla Mountains (29°36′N, 94°36′E) in southeast Tibet. Dominant tree species of both treelines are Abies georgei var. smithii on the north-facing slope and Juniperus saltuaria on the south-facing slope. Along both slopes the vegetation changes from sub-alpine and treeline forests (tree height > 4 m and canopy coverage >40%) to open mosaic of alpine shrublands and grasslands. In August 2005, two long-term observing plots (50 × 50 m) were established in both treeline forests.
Sampling strategy	We conducted 10-year observations (2007-2017) of seasonal stem radial increment, canopy litterfall and its induced nitrogen return and resorption, as well as climate factors at two alpine treelines in southeast Tibet. We also investigated the variation of tree-ring width growth over a longer time period (1986-2017) across the two treeline species (Abies georgei var. smithii and Juniperus saltuaria). The time series data (1986-2017) of monthly climatic factors and atmospheric CO2 concentration were obtained from the Nyingchi weather station in southeast Tibet (ca. 10 km from our study sites) and the Mauna Loa Observatory, Hawaii, respectively.
Data collection	Four automatic weather stations were installed in the two treeline forests and above both treelines (hourly records). In each of both treeline forests, five 1.5m×0.5m litterfall traps were randomly installed to monthly collect the litterfall. Eight mature, healthy trees at each of the two treeline sites were selected and mounted with automatic dendrometers at breast height to continuously monitor stem radial growth (hourly records). In 2016-2017, 2 tree-ring cores at breast height were sampled with an increment borer for each of 30 trees (including the 8 monitored trees with dendrometers) in each of both treeline species, and annual ring width was measured with a resolution of 0.01 mm.
Timing and spatial scale	We aim to examine three key issues: 1) whether seasonal and annual stem increments typically show a lagged positive relationship to litterfall, N-return and N-resorption across the two treeline stands; 2) whether annual tree-ring width, litterfall, N-return and N-resorption all have a higher sensitivity to eCO2 than to the variability of temperature, precipitation and solar radiation during 2007-2017; and if so, 3) whether similar patterns are found in longer time series of tree-ring width index during 1986-2017. To test the scalability of our observed patterns, we further examined the literature data of tree-ring width for 6 other tree species at 11 treeline sites on the Tibetan Plateau.
Data exclusions	No data were excluded from the analyses.
Reproducibility	Annual tree-ring width, annual litterfall, and annual nitrogen return and resorption all showed an increasing trend during 2007-2017, and most of the variations were explained by elevated atmospheric CO2 rather than climate change. Similar patterns were found in longer time series of tree-ring width index during 1986-2017. The scalability of our observed patterns was confirmed by the literature data of 6 other tree species at 11 treeline sites over the Tibetan Plateau.
Randomization	In each of both treeline forests, five 1.5m×0.5m litterfall traps were randomly installed to monthly collect the litterfall. The 8 monitored trees with dendrometers at each of the two treeline sites were selected to represent different sizes of tree diameter.
Blinding	Blinding was not relevant to our long-term located observations and measurements with automatic instruments.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Two alpine treeline forests at 4300-4400 m, with annual mean air temperature of 0.6 °C - 1.1 °C, the world highest timberline.
Location	At the peak of the Sergyemla Mountains (29°36′N, 94°36′E, 4300-4400 m) in southeast Tibet, China.
Access & import/export	We are free to make 10-year observations/measurements of stem radial increment, canopy litterfall and its induced nitrogen return and resorption in two alpine treeline forests on the Tibetan Plateau.
Disturbance	No disturbance

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

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☒	☐ Animals and other organisms
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Methods

n/a	Involved in the study
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