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Coinciding spring and autumn frosts have a limited impact on carbon fluxes in a grassland ecosystem

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Frosts, increasingly prevalent due to climate warming, can offset the carbon storage benefits of an extended growing season, potentially exacerbating climate warming. However, existing research primarily focus on species, with limited evidence on carbon fluxes at the ecosystem scale. Using a manipulative experiment simulating 7-day frosts in a temperate grassland, we find that ongoing frosts, whether in spring or autumn, have limited effects on gross ecosystem productivity, ecosystem respiration, and net ecosystem productivity during the frost measurement periods. However, frosts profoundly impact net ecosystem productivity over the entire growing season outside the frost measurement periods. Specifically, spring frosts significantly increase net ecosystem productivity, autumn frosts marginal decrease it, and the combined effect of both frosts neutralize net ecosystem productivity. The early-year (2018–2020) impacts of frosts on net ecosystem productivity may be driven by plant eco-physiological changes, whereas the late-year impacts (2021–2023) were attributed to shifts in plant community structure. Our findings suggest that frequent frosts in both seasons may not stimulate ecosystem carbon release in temperate grasslands. Understanding these patterns is crucial for predicting carbon balance and developing effective climate-change mitigation strategies in response to the future warmer climate.

Climate warming advances the onset of the green-up phase and delays the end of the yellowing phase in temperate and boreal regions, thereby increasing carbon sequestration and mitigating climate change[1–4]. Frost (canopy temperature < 0 °C) can occur during both the green-up and yellowing phases, since the timing of these phrases remains unchanged in temperate regions, the risk of vegetation exposure to frosts is expected to increase[5–8]. This hypothesis has been confirmed by observations in 43% of the northern hemisphere above the 30°N latitude, particularly notable in Europe, Asia, and North America[9,10]. Spring frosts, occurring when budlets are initiated but not yet fully expanded, pose a heightened risk of damage, ultimately leading to tissue injury and leaf loss[11–13]. In contrast, autumn frosts, which occur as plants approach senescence, can halt the seed-filling process and accelerate yellowing and defoliation, reducing plant germination capability in the following year[14,15]. Despite their differing impacts, frosts are considered critical factors that counteract the carbon sequestration benefits of a prolonged growing season[16,17], given the close linkage between vegetation dynamics and carbon fixation.

However, current frost-related studies primarily focused on plant fitness[12,18], phenological stages[19,20], and cold-resistance[21,22], with limited research on frost effects in carbon cycling[23]. Frost primarily damage plants at early phenological stages[24,25] with effects that can carry-over

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into the subsequent seasons[23]. The warmer spring air temperature may also increase plant vulnerability to cold spells[26,27]. In addition, frosts can injure shoot apical meristem, delay the onset of leaf-out and plant growth[28], and increase the risk of sterility and seed abortion[29]. Despite these negative impacts, frost damage severity varies depending on species[22,30], photosynthetic type[31], geographical locations[27,32], and differences between vegetative and reproductive tissues within species[33]. These studies, despite their numerousness, have predominantly centered on dominant tree species, neglecting the broader impacts of frosts at ecosystem scales. Additionally, research has predominantly addressed spring frosts, with little attention to autumn frosts[15,34]. This disparity may stem from that spring frosts, occurring when plant physiological activity is gradually increasing, have prolonged impacts on plant growth and fitness, whereas autumn frosts coincide with plant dormancy, resulting in more limited plant responses. As climate warming intensifies, frost remains an emerging topic with many unknowns, particularly regarding its impact on carbon cycling, which could exacerbate global warming[35]. Addressing these knowledge gaps is essential for understanding ecosystem responses.

Grassland ecosystems, the second largest in temperate regions following forests, play an important role in determining the carbon sequestration potential of temperate regions[36–38]. However, the studies focusing on frosts in grasslands are few[32,33], and even fewer for their effects on the carbon cycle. This is because that the data sources that most current frost-risk-related research relies on, such as climatic data with spectral phenology[6,20], phenocams observation[26], model estimate[17], and tree ring-based data[18,39], are useful for monitoring tall dominant trees[40] (e.g., beech forest) but unsuitable for discerning the identity and fitness of individual herbaceous species. Nevertheless, one annual herb exhibits a unique response to frost in terms of carbon cycling. Specifically, *Poa pratensis* subjected to spring frosts demonstrates increased drought tolerance in summer, rapid growth, and highest biomass, whereas autumn frosts have no influence on drought tolerance[14]. Consequently, grasslands, which are composed of diverse herbaceous species, may display a variety of species-specific response patterns to frosts[41,42], contributing to uncertainty in carbon cycle feedback at the community level.

In this work, we conduct a manipulative experiment that simulating frost events occurring in spring (around May 1st) and autumn (around October 1st) in a temperate grassland of Inner Mongolia. Frost treatments include spring (S) frost plots, autumn (A) frost plots, combined spring plus autumn (SA) frost plots and control (C). To simulate frost conditions, we attempt to lowered real-time ambient air temperature by 8 °C and maintained it for seven days and nights. Over the entire growing seasons, we measure and calculate ecosystem carbon fluxes including net primary production (NEP), ecosystem respiration (ER), and gross primary production (GEP) from 2017 to 2023. We find that spring frosts enhance NEP, whereas autumn frosts depress it. Alterations in vegetational community structure, particularly in the last three years of the study, can partly explain these observed changes. Our findings indicate that in temperate and boreal ecosystems, coinciding spring and autumn frosts may lead to frost-induced carbon fluxes smaller than anticipated.

Results and discussion

Frost simulations

Since the establishment of the experiment in autumn of 2017, frost simulations have been conducted 13 times, including 6 in spring and 7 in autumn (Fig. 1 and Supplementary Fig. 1). We found that during the 7-day frost simulation periods, the real-time air temperature (T_{air}) in the frost-treated plots was significantly lower than the ambient conditions (Fig. 1 and Supplementary Fig. 2), resulting in an average of 8.38 h/day of frost (7.17 hours/day in spring, 9.70 h/day in autumn *vs.* 2.37 h/day in ambient plots) with an average frost temperature of − 4.99 °C (− 5.54 °C and − 4.45 °C in spring and autumn *vs.* − 2.09 °C in ambient). The reduced T_{air} caused a significant decrease in soil temperature at soil depth of 20 cm by 2.46 °C (2.32 °C in spring and 2.60 °C in autumn) on average with greater reductions at night

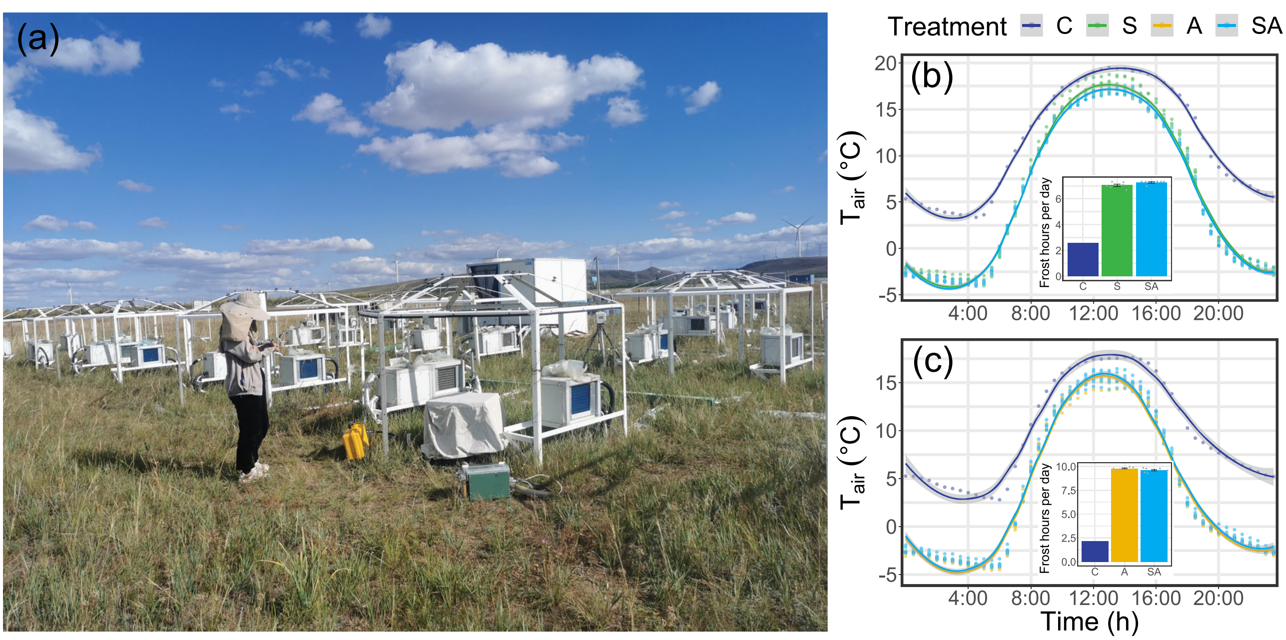

Fig. 1 | Frost simulation experiment (a) and its effects on air temperature (T_{air}) in spring (b) and autumn (c). a The figure showed the measurements for ecosystem carbon fluxes during the non-treatment period. The experiment was located at the Duolun Restoration Ecology Station on the Mongolian Plateau, China. **b**, **c** To achieve the effect of frost (< 0 °C), a refrigeration system was used to attempt to decrease the real-time T_{air} by approximately 8 °C, contributing to more frost hours per day in the treatment plots compared to the control (ambient) plot (inserts). Sample size $n = 5901$ in spring and $n = 6887$ in autumn. Data were presented as mean ± standard error across all sampling dates (biological replicates $n = 6$, gray point) for S, A, SA plots, and one control plot representing the ambient environment. C: the plots without frost, S: plots experiencing spring frosts, A: plots experiencing autumn frosts, SA: plots experiencing spring plus autumn frosts. Source data are provided as a Source Data file.

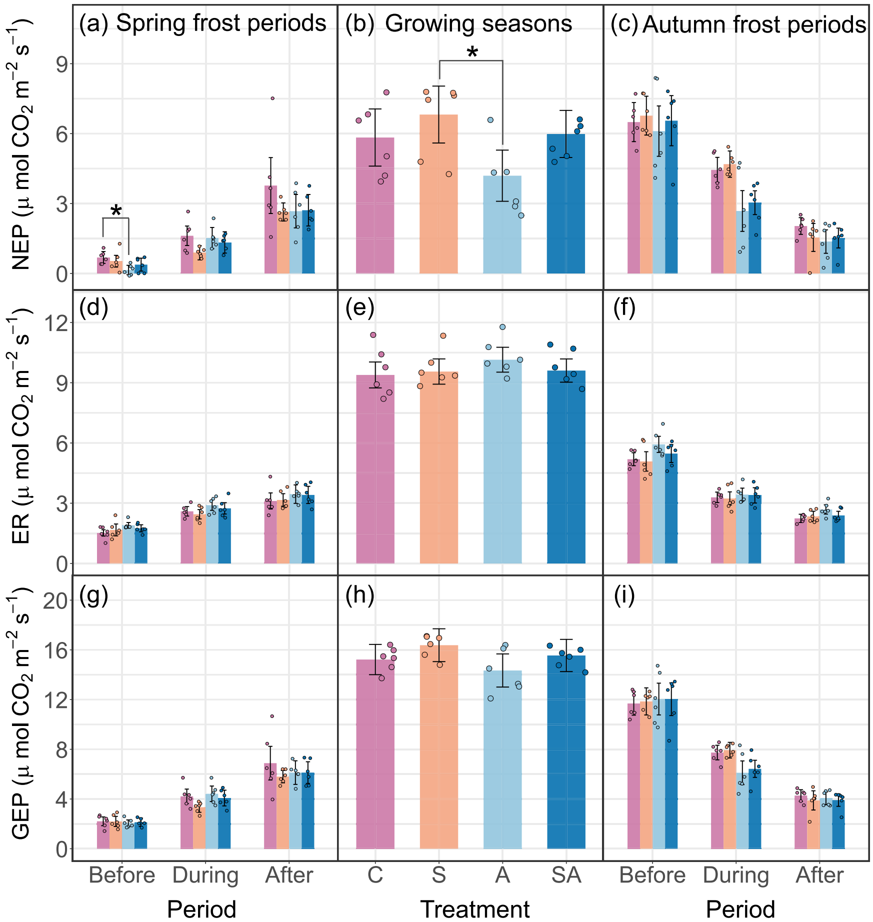

Fig. 2 | Effects of frosts on ecosystem carbon fluxes across from 2017 to 2023. The linear mixed-effect models illustrated the frosts effects on carbon fluxes, included net ecosystem productivity (NEP, **a**–**c**), ecosystem respiration (ER, **d**–**f**), and gross ecosystem productivity (GEP, **g**–**i**). Spring/autumn frost periods (left and right panels) extended for a total of 21 days that include 7 days before, 7 days during, and 7 days after the frost treatments, with 2-3 times repeated measurements within 7 days. The growing seasons (middle panel) in this study had 2-3 times measurements for each month and did not include the 21-days measurement periods. Parameter significance was assessed by *t* tests (two-tailed). Asterisks (*) indicated significant differences between treatments in pairwise comparisons at the $P < 0.05$ level without adjustment. The asterisks in Fig. 2a: t ratio = 2.78, $P = 0.05$; the asterisks in Fig. 2b: t ratio = 3.916, $P = 0.004$. Data were presented as mean ± standard error across all sampling date (biological replicates $n = 6$, colored point). Sample size $n = 2400$ for spring/autumn frost measurement periods, and $n = 1320$ for the growing seasons that exclude the 21-days measurement period. C: the plots without frost, S: plots experiencing spring frosts, A: plots experiencing autumn frosts, SA: plots experiencing spring plus autumn frosts. Source data are provided as a Source Data file.

(Supplementary Fig. 3). Furthermore, during non-frost periods, frosts had no effects on soil temperature (T_{soil}) and volumetric water content (VWC) at a depth of 10 cm (Supplementary Table 1). In addition, prior to the experiment, no significant differences in background vegetation, soil properties, or ecosystem carbon fluxes were observed between treatments and control plots (Supplementary Table 2).

Carbon fluxes responses to frosts during frost measurement periods

Frost measurement periods were defined as the 7-day frost treatment periods plus the 7-day before and after treatment. During these periods, neither spring nor autumn frosts significantly affected GEP or ER across any 7-day intervals (GEP: $F_{1,20} = 0$ ~ 2.787, all $P > 0.05$; ER: $F_{1,\ 20} = 0.032$ ~ 3.073, all $P > 0.05$, Supplementary Table 3).

However, the effects of frosts on NEP varied by season. Ongoing spring frosts did not significantly alter NEP across the 7-day frost periods from 2017 to 2023 ($F_{1,\ 20} = 0.2$, $P = 0.651$; see Supplementary Table 3). This was partly because NEP was only significantly decreased in 2019, while the tendencies between the control (C) and the spring treatments (S and SA) were inconsistent in other years (mainly for S comparing with C, Supplementary Fig. 4). In contrast, ongoing autumn frosts significantly reduced NEP ($F_{1,\ 20} = 7.86$, $P = 0.011$), with significant declines observed in 2018 and consistent downward tendencies in other years. Furthermore, the NEP reduction extended into the early green-up stages in the following growing seasons ($F_{1,\ 20} = 6.0$, $P = 0.023$; see Supplementary Table 3 and Supplementary Fig. 5).

Carbon fluxes responses to frosts during non-treatment periods

When considering the annual carbon budget, the contribution of frost during the frost measurement periods can be negligible due to their short durations (two 21-days per year). Consequently, the focus of the following research shifts to the growing seasons that excludes the frosts measurement periods in both spring and autumn in this study (i.e., non-treatment periods). We found that frosts affected NEP (spring frosts: $F_{1,\ 20} = 7.58$, $P = 0.012$; autumn frosts: $F_{1,\ 20} = 4.10$, $P = 0.057$) but had no impact on ER and GEP (Supplementary Table 1 and Fig. 2). This result indicated that frost effects on NEP may have lagged or carry-over into the non-treatment periods[23,43,44].

To further analyze NEP responses, we calculated the main effects both annually and across multiple years (2018–2023). Across all years, spring frosts significantly increased NEP, while autumn frosts marginally decreased it (Fig. 3a insert). At the annual scales, spring frosts generally had a positive effect on NEP, whereas autumn frosts tended to have a negative effect in most years (Fig. 3a), although these effects

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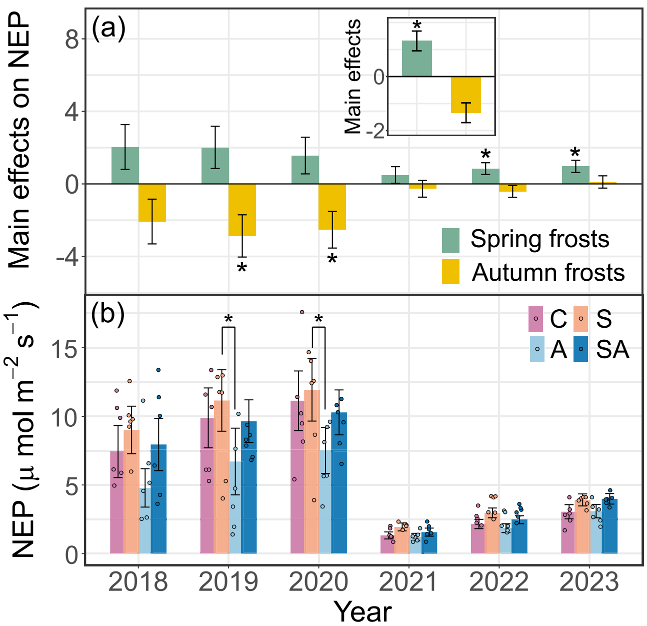

Fig. 3 | Effects of frosts on net primary productivity (NEP) during the non-treatment periods. a Main effects of frosts on net primary productivity (NEP, unit: μmol m^{-2} s^{-1}), (**b**) and the pairwise comparisons among treatment levels. In this study, the non-treatment periods refer to the growing seasons that excluded the frost measurement periods in both spring and autumn. Parameter significance was examined by F-test (Fig. 3a insert, two-tailed) and *t* tests (others, two-tailed). The main effect of autumn frosts on NEP across years were marginally significant ($P = 0.057$, yellow bar in Fig. 3a insert). In the upper panel, the main effect values and standard error were presented by the marginal means and the uncertainty of these marginal means, which are derived from a linear mixed effects model with sample size $n = 1320$. In the lower panel, data were presented as mean ± standard error across sampling dates for each year (biological replicates $n = 6$, colored point). The pairwise comparisons were also obtained by the same linear mixed-effects model with sample size $n = 1320$. Asterisks (*) indicated significant differences between treatments in pairwise comparisons at the $P < 0.05$ level without adjustment. C: the plots without frost, S: plots experiencing spring frosts, A: plots experiencing autumn frosts, SA: plots experiencing spring plus autumn frosts. Source data are provided as a Source Data file.

were not statistically significant in all years. This finding is in disagreement with previous research on frost, which generally suggests that frosts damage plant physiology[45,46], slow species growth[39,47], and reduce plant biomass[16,48]. Nonetheless, Zohner et al.[49] found that in beech and oak seedlings, despite initial reductions in leaf growth due to spring frost, subsequent summer growth exhibited significantly higher chlorophyll content and bud development, leading to the increased photosynthetic potential after the cessation of spring frost. This study is the few indicating a positive effect of spring frost, although similar with our finding, but at a species level. Consequently, our study provides evidence demonstrating that spring and autumn frosts have opposite effects on the carbon cycle in grasslands at a community scale.

In the subsequent analyses, we conducted pairwise comparisons for NEP among treatment levels. Across from 2017 to 2023, significant positive differences in NEP were observed between S and A plots (estimate for differences = 0.205, t ratio = 3.916, $P = 0.004$, Fig. 2b). In years where significant effects were detected, NEP in S plots was consistently higher than in A plots (2019: estimate for differences = 4.881, t ratio = 2.964, $P = 0.036$; 2020: estimate for differences = 4.090, t ratio = 2.862, $P = 0.044$, Fig. 3b). These results collectively indicated that the contrasting effects of spring and autumn frosts on NEP are primarily driven by responses in S and A plots.

Additive effects of spring and autumn frosts

We further found that, when spring and autumn frost treatments are combined, their effects (i.e., the increases by spring frosts and decreases by autumn frosts) may offset each other. Strong evidence for this lies in the insignificant interactions between spring and autumn frosts in a linear mixed-effects model for NEP (S × A: $F_{1,\ 20} = 0.31$, $P = 0.584$, Supplementary Table 1). This lack of interaction suggests an additive effect, where each factor contributes independently, resulting in a combined impact equal to the sum of their individual effects. Statistically, this means that observed values match the expected ones. In addition, we observed no significant differences in NEP between SA plots and C plots for each year (pairwise comparisons for SA - C: t ratio from 2018 to 2023 = − 2.251 ~ 0.673, all $P > 0.05$, Fig. 3b), suggesting that any additive effects from spring and autumn frosts may neutralize each other entirely.

To visualize these additive effects, we calculated the observed NEP in SA plots and compared it to the theoretical NEP values. Linear regression analysis revealed that the points from 2018 to 2020 slightly deviated from the 1:1 line, with the observed values exceeding the theoretical ones. However, for the later three years, data points aligned with the 1:1 line (Fig. 4a). Further monthly analyses for 2021–2023 showed that both absolute values and relative changes in cumulative NEP did not significantly differ from the 1:1 line (Absolute values: r statistic = − 0.30, df = 24, $P = 0.149$, estimate for slope = 0.942, 95% CIs for slope: [0.868, 1.023]; Relative changes: r statistic = 0.298, df = 24, $P = 0.139$, estimate for slope = 1.051, 95% CIs for slope: [0.983, 1.123], Fig. 4b). This confirms that spring and autumn frosts indeed have an additive effect on NEP in numerical terms for the later three years.

We conducted similar analyses for GEP and ER and found that spring and autumn frost events had no significant impact on either (GEP: spring frosts: $F_{1,\ 20} = 1.487$, $P = 0.237$, autumn frosts: $F_{1,\ 20} = 0.656$, $P = 0.427$; ER: spring frosts: $F_{1,\ 20} = 0.0002$, $P = 0.990$, autumn frosts: $F_{1,\ 20} = 0.283$, $P = 0.600$, Supplementary Table 1 and Supplementary Fig. 6) during the non-treatment period. Moreover, the actual combined effects of spring and autumn frost on either GEP and ER deviate numerically from the sum of their individual effects, respectively (relative changes for GEP: r statistic = 0.566, df = 24, $P = 0.003$, ER: r statistic = 0.603, df = 24, $P = 0.001$, Supplementary Figs. 7 and 8).

To date, research on additive effects remains relatively sparse, but such phenomena have been observed in the carbon cycle at various scales, including leaf-scale photosynthetic PSII electron flux and carbon assimilation[50], ecosystem-scale carbon fluxes[51], and terrestrial carbon storage[52]. Most of these effects are driven by climate change-related factors such as warming, precipitation shifts, and greenhouse gas emissions, while frost has been largely overlooked in this context. Our study extends the understanding of additive effects to frost interactions, offering important insights into their role in climate change responses.

The mechanism underlies the divergent impacts of frosts

To understand the mechanisms by which spring and autumn frosts drive NEP from the perspective of vegetation, we employed a piecewise structural equation model (SEM) to develop hypothesis models incorporating species richness, vegetational cover, and plant height at the community level. Species richness reflects photosynthetic potential arising from diverse vegetation, while vegetational cover and plant height collectively represent the total biomass involved in photosynthesis and respiration. During the early years of the experiment (2018–2020), no plant community metrics correlated with frost-induced changes in NEP. Although direct evidence for contrasting frost impacts was lacking in these years, field observations provided key insights. We noted that the dominant gramineous plants in this semi-arid grassland develop more tillers following spring frosts. If these

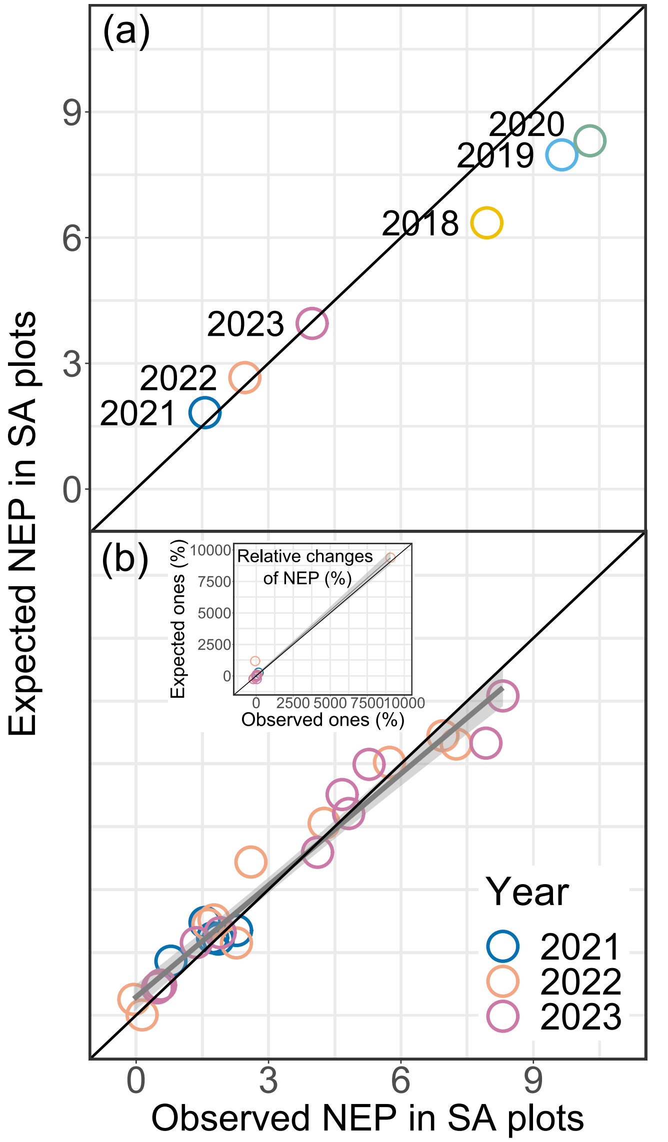

Fig. 4 | The linear correlations between expected and observed net ecosystem productivity (NEP) under the combined spring and autumn frosts. The non-treatment periods encompassed the years from 2018 to 2023 (**a**) and specifically from 2021 to 2023 (**b**). Expected NEP in SA plots were represented by the summed effects in S and A plots. In the right panel, the fitting line that was not significantly different from the 1:1 line in both absolute form (r statistic = − 0.30, df = 24, $P = 0.149$) and relative form (r statistic = 0.298, df = 24, $P = 0.139$, Insert). The r statistic uses a two-tailed test (two-tailed test). One point in the right panel and the inserts represented a mean value at each sampling date without biological replications. The sample size $n = 26$ for both right panel and insert. SA: plots experiencing spring plus autumn frosts. Source data are provided as a Source Data file.

newly emerged tillers exhibited elevated chlorophyll content and/or increased bud growth rates[49], it is plausible that spring frosts contributed to higher NEP.

In contrast, during the late years (2021–2023), species richness played a crucial role in shaping the contrasting impacts of frosts on NEP in a structural equation model (SEM, Goodness of fit: $\chi^2 = 2.766$, df = 2, $P = 0.251$) (Fig. 5). Specifically, spring frosts increased species richness (standardized path coefficient (β) = 0.227, $P = 0.037$), which subsequently stimulated NEP (β = 0.508, $P < 0.001$), resulting in an overall positive indirect effect on NEP. Conversely, autumn frosts decreased both species richness (β = − 0.485, $P < 0.001$) and grass cover (β = − 0.463, $P < 0.001$), resulting in an overall negative indirect

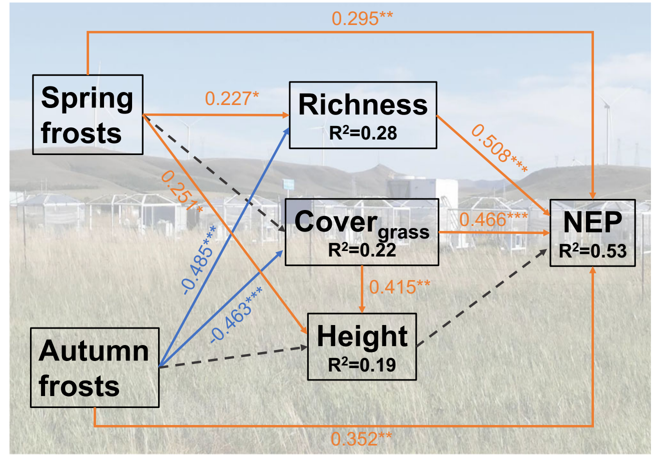

Fig. 5 | Piecewise structural equation model (SEM) showing how plant community indirectly affect net ecosystem production (NEP) during non-treatment period. Richness: species richness; $Cover_{grass}$: cover of grasses; Height: community-level plant height. Orange and blue arrows indicated the significant positive and negative relationships, respectively. Dash arrows referred to the insignificant linear relationships. The colored numbers near to arrows indicated the standard path coefficients, and black numbers (R^2) represented the proportion of variance explained for each dependent variable. Goodness of fit: $\chi^2 = 2.766$, df = 2, $P = 0.251$. Data was derived from the growing season spanning from 2021 to 2023, each NEP denoted the annual mean value with six replicates for each treatment, and each plant community variable denoted the month value at the peak of the growing season with six replicates for each treatment. Source data are provided as a Source Data file.

effect on NEP. Taken together, frost effects accounted for 53% of NEP variability through direct and indirect pathways.

Whether in the early or late three-year periods, the opposing impacts of spring and autumn frosts on NEP may be partly attributed to their timing of frosts. Spring frosts allow plants to have more time for gradual adjustment and adaptation, whereas autumn frosts occur closer to dormancy, likely exacerbating adverse effects[14].

In summary, our study provides robust evidence of the impacts of spring and autumn frosts on ecosystem carbon fluxes in a temperate grassland. It highlights the long-lasting and seasonally distinct effects of frost events. As climate change extends the growing season and potentially leads to concurrent spring and autumn frosts, their overall impact on ecosystem carbon budgets might be neutralized. These findings offer valuable insights into predicting the potential risks of frost-induced carbon emissions in a warming climate, emphasizing the need for additional frost experiments to further validate these results.

Despite the observed enhancement in carbon sequestration due to spring frosts, their broader ecological consequences should not be overlooked. Spring frosts can detrimentally affect plant reproductive success and production[53,54], disrupt food chains[55,56], reduce biodiversity[57], and weaken key ecosystem functions[7,20,33]. In addition, the simulated frosts in our experiment, which primarily reached temperatures around − 5 °C, were less intense than some extreme natural frost events. More severe spring frosts could alter the mechanisms driving carbon flux responses[42]. Given the emerging significance of frost events under climate change, we strongly advocate for the intensified research to investigate their impacts on community composition, structure, and broader ecosystem functions. A deep understanding of these dynamics is crucial for refining carbon cycle models and developing effective climate adaptation strategies.

Methods

Study site and frost simulation experiment

This study is conducted in the Duolun Restoration Ecology Station on the Inner Mongolia Plateau, China (42°02′N, 116°17′E, 1,324 m a.s.l.).

This region lies within the temperate continental monsoon climate zone with distinct seasons, characterized by dry, cold spring and autumn, and wet, hot summer. The mean annual temperature and annual precipitation (1954–2022) are 2.2 °C and 380 mm, respectively. The growing seasons typically commences around mid-April, coinciding with the greening up of most herbs, and ends by mid-October, when most herbs have yellowed. The vegetation is dominated by perennial grasses, such as *Stipa krylovii*, *Artemisia frigida*, *Agropyron cristatum*, *Artemisia pubescens,* and *Lespedeza davurica*. The soil type is identified as chestnut, with a higher proportion of sand and stones below a depth of 20 cm.

The frost simulation experiment was established in June 2017 in a semi-arid grassland. The timing for simulating spring and autumn frost events were determined based on long-term phenological observations of dominant species at the Duolun Restoration Ecology Station, which employ frost treatment after the majority of plants have flashed in spring and when the plants have started to senesce in autumn. We implemented a completely randomized design comprising four treatment levels and six replicates: spring frost (S), autumn frost (A), spring plus autumn frosts (SA), and controls (C). A pentagonal open top chamber (OTC, 2.26 m in diameter) was positioned within each plot (2.5 m × 2.5 m) with a 2-m buffer zone separating adjacent plots.

Frost treatment lasted for 7 days and was set to reduce the canopy T_{air} by 8 °C. A refrigeration system was used to cool the liquid (ethylene glycol) that filled in the pipes and then transported to each frost OTC in a closed loop. Electromagnetic valve actuator (EVA), installed on the inlet pipe nearby the OTC, automatically regulates the waterflow until the desired canopy temperature is attained within the OTC (More details see Supplementary Fig. 1). Real-time T_{air} and humidity monitored by HOBO probes (S-THB-M008, ONSET Inc, Massachusetts, USA) were placed at each frost OTC and a control OTC that without plastic plates. To avoid the warming effect caused by the plastic plates of OTCs, we initially installed only half of the plastic panels in each OTC in one day before the frost treatment began, and then added the rest once the refrigeration system was operating efficiently. On the day the frost treatment ended, we shut down the refrigeration system only after removing all the panels.

Notably, our frost treatment was employed by reducing the T_{air} in real-time by 8 °C. As a result, during most of the daytime, the T_{air} was lower than the ambient environment but did not reach freezing. Thus, frost and cooling occurred alternatively during the 7-day frost treatment period. This phenomenon is also very common in natural frost events. When frost events are approaching, a significant drop in T_{air} (cooling effect) is observed.

Ecosystem carbon fluxes and vegetation survey

Ecosystem carbon fluxes have been measured since 2017 via an infrared gas analyzer (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) equipped with a transparent chamber (0.5 m × 0.5 m × 0.5 m). During the measurements, the chamber was situated on the horizontal base of a square stainless-steel frame (0.5 m × 0.5 m and 2 cm in soil depth), which was inserted into the soil at the start of the experiment. Two electrical fans, fixed at two corners inside a transparent chamber, were employed to ensure continuous air mixing. An infrared gas analyzer recorded eight consecutive CO_2 concentrations at 10-second intervals, from which the CO_2 flux rate was calculated and termed as net ecosystem CO_2 exchange (NEE). After venting the chamber, CO_2 concentrations was measured again in the absence of light to calculate ecosystem respiration (ER). In this situation, the chamber was covered with an opaque cloth.

During the frost periods, ecosystem carbon fluxes was measured 2 or 3 times for 7 days before, 7 days during, and 7 days after the frost treatment, respectively. During the growing seasons other than frost periods, ecosystem fluxes exchange was measured 2 or 3 times in a month. Soil temperature (Ts) and moisture (volumetric water content, VWC) at soil depth of 10 cm were measured concurrently with carbon fluxes exchange measurements.

In this study, we used net ecosystem productivity (NEP, inverse of NEE) instead of NEE, as it specifically emphasizes vegetation carbon fixation. Positive NEP denotes a net ecosystem carbon sink, while negative NEP indicates a net ecosystem carbon source. Gross ecosystem productivity (GEP) was determined by the sum values of NEP and ER.

In addition, the vegetation surveys were conducted at the peak of each year from 2018 to 2023. The measurements include community richness, plant cover, and height by species.

Statistical analysis

Prior to analysis of variance, data were log-transformed if necessary. In addition, due to being significantly affected by interannual precipitation variability, particularly droughts since 2021, this dataset had large differences in variances between former (2018–2020) and later (2012–2023) three years. Therefore, we introduced an additional variable (Dr) to quantify interannual changes and incorporated it as a weight into the linear mixed-effects model (weights = varIdent(form = ~ 1 | Dr)). Linear mixed effect models (R: 'lme ()' in "nlme" package) were conducted to examine the effects of frosts on NEP, ER, and GEP under different temporal periods. During each 7 days of the frost periods (21 days) across all years from 2017 to 2023, date (format: yy/mm/dd), spring frosts (S) and autumn frosts (A) were considered as fixed factors (y ~ date × S × A, random = ~ 1|plot/year, weights = varIdent(form = ~ 1 | Dr)). During the growing seasons excluding frost periods across from 2018 to 2023, date (format: yy/mm/dd), spring frost (S) and autumn frost (A) were employed as three fixed factors (y ~ date × S × A, random = ~ 1|plot/year, weights = varIdent(form = ~ 1 | Dr)). Subsequently, the Tukey method was used to compare the significant differences between treatment levels based on linear mixed effect models (R: 'lsmeans ()' in "emmeans" package). In addition, linear mixed effect models were also used to determine the effects of frost on T_s and VWC during the growing seasons that excluding frost periods, respectively (y ~ date × S × A, random = ~ 1|plot/year, weights = varIdent(form = ~ 1 | Dr)).

To get the main effects of spring and autumn frosts on C fluxes, linear mixed-effect models were built (*y* ~ year × S × A, random = ~ 1|plot, weights = varIdent (form = ~ 1 | Dr)). Here, the fixed factor (Year) acted as a categorical variable. Based on these linear mixed-effect models, main effects were obtained by marginal means (R: 'lsmeans ()' in "emmeans" package). These marginal means included the mean values across from 2018 to 2023 and the mean values for each year, respectively. In addition, multiple comparisons between treatment levels for each year were also examined (R: 'lsmeans ()' in "emmeans" package).

The additive effects of spring and autumn frosts on NEP, ER, and GEP during the growing seasons were examined, since insignificant interactions were found in a linear mixed effect model for NEP (S × A: $P = 0.457$). In this study, additive effects were presented by two forms: expected values and expected relative changes in SA plots. Expected values in SA plots = $V_S + V_A - V_C$, where V refers to NEP, ER, or GEP. V_S refers to value in S plots. Similar, observed values = V_{SA}, where V_{SA} represents values observed in SA plots. Expected relative changes in SA plots = $((V_S - V_C)/ V_C) + ((V_A - V_C)/ V_C)$, observed relative changes in SA plot = $(V_{SA} - V_C)/ V_C$. Standardized major axis regressions were built (R: 'lmodel2 ()' in "lmodel2" package), and then used to test if the slopes significantly different from 1 (R: 'sma ()' in "smart" package). When the slopes in regression models did not different from 1 in both forms of expected values and expected relative changes, we considered that frosts indeed produced additive effects in numerical terms.

A piecewise structural equation model (SEM) was used to explore the contribution of vegetation regarding the effects of frosts on NEP (R: 'psem()' in the "piecewiseSEM" package). Given that frosts affect

NEP indirectly by altering plant photosynthesis and ecosystem respiration, we included the vegetational cover and plant height at the community level into the SEM hypothetical model, since these terms reflect the total amount of plants that are capable of photosynthesis and respiration. Species richness was also included because it can quantify the maximum photosynthetic potential arising from diverse plants. To match the plant variables in August each year, the repeated measurements of NEP were averaged to annual values. Linear mixed-effect models were used to build the piecewise SEM, spring frosts, autumn frosts, species richness, vegetational cover, and plant height at the community level were considered as fixed factors, the year, which nested within the plots, was incorporated as a random factor. After screening by goodness of fit (χ^2, df, *P-values*), we developed the final model.

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

Data availability
All the data that support the conclusion in this study have been deposited in figshare, https://doi.org/10.6084/m9.figshare.27452835. Source data are provided in this paper.

Code availability
R scripts that support the conclusion are publicly available in figshare, https://doi.org/10.6084/m9.figshare.27452835.

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Author contributions

J.H. invented the frost simulation device. S.W. and J.H. designed the frost simulation experiment. J.H. and C.T. collected the experimental data. J.H., D.H., and S.W. analyzed the data. J.H. write the draft of the manuscript. J.S. and J.R. and other authors gave the important suggestions to improving the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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