Elevated CO\textsubscript{2} negates O\textsubscript{3} impacts on terrestrial carbon and nitrogen cycles

**Graphical abstract**

**Highlights**
- $e[O_3]$ decreases terrestrial C and N pools and fluxes
- $e[CO_2]$ increases terrestrial C and N pools and fluxes
- $e[CO_2]$ negates $e[O_3]$ impacts on terrestrial C and N pools and fluxes
- Evaluating terrestrial C and N feedbacks to concurrent climate changes is critical

**In brief**

Increases in tropospheric concentrations of O\textsubscript{3} and CO\textsubscript{2} substantially alter the functions of global terrestrial ecosystems and elements cycles. Here, we synthesize global datasets and find that increased O\textsubscript{3} concentration significantly decreases terrestrial net primary productivity and various carbon and nitrogen pools and fluxes, which are greatly stimulated by increased CO\textsubscript{2} concentration. Moreover, increases in CO\textsubscript{2} concentration negate or even overcompensate the detrimental effects of increased O\textsubscript{3} concentration on terrestrial ecosystem functions and carbon and nitrogen cycles.

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Elevated CO$_2$ negates O$_3$ impacts on terrestrial carbon and nitrogen cycles

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SUMMARY

Increasing tropospheric concentrations of ozone (e[O$_3$]) and carbon dioxide (e[CO$_2$]) profoundly perturb terrestrial ecosystem functions through carbon and nitrogen cycles, affecting beneficial services such as their capacity to combat climate change and provide food. However, the interactive effects of e[O$_3$] and e[CO$_2$] on these functions and services remain unclear. Here, we synthesize the results of 810 studies (9,109 observations), spanning boreal to tropical regions around the world, and show that e[O$_3$] significantly decreases global net primary productivity and food production as well as the capacity of ecosystems to store carbon and nitrogen, which are stimulated by e[CO$_2$]. More importantly, simultaneous increases in [CO$_2$] and [O$_3$] negate or even overcompensate the negative effects of e[O$_3$] on ecosystem functions and carbon and nitrogen cycles. Therefore, the negative effects of e[O$_3$] on terrestrial ecosystems would be overestimated if e[CO$_2$] impacts are not considered, stressing the need for evaluating terrestrial carbon and nitrogen feedbacks to concurrent changes in global atmospheric composition.
INTRODUCTION

Since the Industrial Revolution, anthropogenic activities have been changing global atmospheric composition (e.g., increasing ozone and carbon dioxide concentrations). This has profoundly disturbed terrestrial ecosystem functions that provide services vital to human life and health, such as combating climate change through sustaining ecosystem carbon storage and safeguarding food security. The increased emissions of nitrogen oxides (NOx) and volatile organic compounds (VOCs) in conjunction with fossil fuel burning have increased the global tropospheric ozone (O3) concentrations by 40% since preindustrial times. As a strong oxidant and air pollutant, O3 enters plant leaves through the stomata, generating reactive oxygen species and causing oxidative stress, which in turn reduces leaf photosynthesis, plant biomass, and crop yield. A previous study suggests that an elevated O3 concentration (e[O3]) significantly decreased tree photosynthesis by 12%, shoot biomass by 9%, and root biomass by 12%. This decrease in plant productivity may slow down the carbon (C) and nitrogen (N) cycling in terrestrial ecosystems.

Decreased aboveground (ANPP) and belowground net primary productivity (BNPP) under e[O3] result in lower C emissions through ecosystem respiration, and less soil C inputs through leaf and root litter, thereby likely decreasing soil organic C (SOC) and N stocks. Elevated O3 can also directly impose physiological stress on soil microorganisms and may decrease soil C turnover (e.g., SOC decomposition) and N transformation rates (e.g., mineralization, nitrification, and denitrification), which in turn down-regulates the size of soil labile C and N pools and emissions of greenhouse gases (GHGs) from soils (carbon dioxide [CO2], methane [CH4], and nitrous oxide [N2O]). Recent studies have examined the response of ecosystem C pools to e[O3] in forest ecosystems, but are largely limited to C components (e.g., ANPP and BNPP). So far, no study has comprehensively assessed the effects of e[O3] on various C and N pools in global terrestrial ecosystems, which limits our understanding of the impact of future [O3] on terrestrial C and N cycles.

In parallel to e[O3], atmospheric CO2 concentrations have increased by nearly 47% since preindustrial times. In contrast with e[O3], elevated CO2 concentrations (e[CO2]) stimulate plant photosynthesis, promote NPP and accelerate terrestrial C and N cycling. Recent meta-analyses indicate that e[CO2] significantly increased plant and soil C pools by 4%–23% in terrestrial ecosystems. These results raise the question of whether e[CO2] can overcome the negative effects of e[O3] on terrestrial C and N pools. We found that e[O3] and e[CO2] exerted opposite effects on the targeted C and N cycling variables, and the interactive effects of e[O3] and e[CO2] were mostly additive. Simultaneous increases in [CO2] and [O3] negated or even overcompensated the negative effects of e[O3] on terrestrial C and N pools and fluxes. These results highlight the importance of evaluating terrestrial C and N feedbacks to concurrent climate changes.

RESULTS AND DISCUSSION

Elevated O3 and CO2 concentrations

In total, 810 peer-reviewed publications (9,109 observations) reporting results from global terrestrial ecosystems, spanning boreal to tropical regions, were included in the meta-analysis (Figure S1). Most studies included in our dataset were conducted in the northern hemisphere; only 32 studies were conducted in the southern hemisphere. Across all studies included in our dataset, ambient atmospheric O3 and CO2 concentrations ranged between 15 and 50 ppbv (on average 36 ppbv) and 340–410 ppmv (on average 388 ppmv), respectively, while those of elevated O3 and CO2 concentrations ranged between 29 and 150 ppbv (on average 72 ppbv) and 400–880 ppmv (on average 615 ppmv). Models predict that the atmospheric [O3] and [CO2] will increase to 70–80 ppbv and 560–720 ppmv (RCP4.5–6.0) by 2100, respectively. Thus, the magnitudes of [O3] and [CO2] enrichment of the studies in our dataset are consistent with projections for the end of this century.

Effects of e[O3] on plant NPP and N pools

Averaged across all studies in our dataset, e[O3] significantly decreased leaf net photosynthetic rate (Pn) by 26.0% compared with ambient [O3] (Figures 1D and S6). The strong oxidative stress due to e[O3] may cause leaf cell damage and decrease the supply of carbohydrate precursors, which caused significantly decrease in stomatal conductance (gS), carboxylation efficiency (Vcmax), and leaf chlorophyll content (Chl) (Figure 1D). This explains the observed decreases in photosynthesis and
accelerated leaf senescence. As a result, the biomass of plant tissues (shoot, root, leaf, and stem) was significantly decreased by 15.1%–17.9% (Figures 1A and S5; Table S6). Root growth was more negatively affected by e[O3] than aboveground biomass as evidenced by the decreased root/shoot ratio (Figure S2). These results might be explained by the fact that leaves in the lower and upper canopies were the preferential sources of photosynthesis for root and shoot growth, respectively.34 As e[O3] accelerates the senescence of older (mature) leaves in the lower canopy, less C is allocated to roots, which explains the observed reductions of root/shoot ratio.7 Compared with the varied responses of leaf Pn resulting from the vertical gradient of light intensity, the decrease in canopy photosynthesis (Pc) may better reflect the damage of e[O3] on the capacity of plant assimilation of atmospheric CO2.3,35 Pc was not assessed in this study due to data deficiency. However, a previous study found that the Pc decreased significantly with increasing [O3] and the decreasing rate (percentage reduction per unit [O3] increase) was similar to the magnitude of decrease in plant aboveground biomass under e[O3].35

Across all studies, e[O3] significantly decreased crop yield by 25.6% (Figure 1A), which is within the range of reduction (23%–29%) reported in previous meta-analyses.36,37 Moreover, the decrease in crop yield increased significantly with the level of [O3] enrichment (Figure 3). Model simulations suggest that e[O3] decreased global crop yield by 227 Tg year−1 (1 Tg = 1012 g) during 2010–2012,38 accounting for 9% of global crop production. These results highlight the urgency to implement measures to lower tropospheric O3 concentrations, or at least to halt its increase, and to develop O3-tolerant crop cultivars to safeguard global food security.39

Beside plant biomass and crop yield, plant NPP and its components (ANPP, BNPP, leaf, and stem C pools) were also significantly reduced by 21.6%–28.6% by e[O3] (Figures 2A and S9) because of the decreased plant biomass and unaffected plant C concentrations (Figures 1A, 1G, and S7). Interestingly, e[O3] significantly increased N concentrations in plant components (except for leaf) (Figures 1J and S8) and decreased plant C/N ratios (Figure S2). This might be explained by the decreased plant biomass relative to the amount of available N4,5 or by N re-translocation from prematurely senescing leaves under e[O3].41 However, the increase in plant N concentrations was negligible compared with the magnitude of plant biomass reduction under e[O3], which led to significant decreases in various plant N pools (10.0%–14.0%) (Figures 2D and S10).

We extrapolated our results on ecosystem C and N pools and fluxes to the global scale by multiplying averaged area-scaled effect sizes with the corresponding total land area (see experimental procedures). We found that e[O3] significantly decreased plant NPP and total N pool (TNP) by 24.1 (95% confidence interval [CI], 11.5–36.7) Pg CO2-equivalent (CO2-eq) year−1 and 0.18 (95% CI, 0.03–0.32) Pg N year−1, respectively, in terrestrial ecosystems (Figures 2 and 6). The consistent decrease in plant NPP and N pools across all terrestrial ecosystems analyzed suggests that e[O3] significantly reduces the capacity of plant biomass to store C and N. More importantly, the magnitude of decrease in plant NPP and N pools increased significantly with the level of [O3] enrichment (Figure 3), suggesting that the losses in terrestrial plant C and N storage would be aggravated if [O3] continues to increase.5,42
Apart from \([O_3]\) concentration, climate conditions and ecosystem types also regulate the responses of plant NPP and N pools to e\([O_3]\) (see Note S1 for details). Decreases in plant NPP (ANPP and BNPP) and (leaf) N pools with e\([O_3]\) were significantly and positively correlated with mean annual temperature (MAT) and precipitation (MAP), suggesting that the plant productivity of subtropical and tropical terrestrial ecosystems may be hit hardest by elevated \([O_3]\). For ecosystems in cold and arid regions, plant photosynthesis and growth are relatively less likely to be inhibited by e\([O_3]\) because the physiological stresses from low temperature and water deficiency make plants less susceptible to the additional e\([O_3]\) stress. In contrast, when e\([O_3]\) becomes the primary stress in subtropical and tropical regions, reductions in plant photosynthesis and productivity are more severe. These findings corroborate simulations of the impact of e\([O_3]\) on global gross primary production (GPP), which showed that decreases in GPP in equatorial regions with higher MAT and MAP were greater than in North America and Asia. Thus, to minimize the damages of e\([O_3]\) on global C and N storages, there is an urgent need for air pollution control specifically in subtropical and tropical regions from the Middle East to India and East Asia where tropospheric \(O_3\) concentrations have strongly increased in recent decades.

Figure 2. Responses of C and N pools and fluxes to e\([O_3]\), e\([CO_2]\), and e\([O_3] \times e[CO_2]\)

Effects of elevated \(O_3\) (e\([O_3]\), on average 72 ppbv), elevated \(CO_2\) (e\([CO_2]\), on average 615 ppmv) and e\([O_3]\) (on average 67 ppbv) \times e\([CO_2]\) (on average 612 ppmv) on plant NPP components (A, B, and C) and N pools (D, E, and F), and soil C and N pools (G, H, and I) and greenhouse gas emissions (J, K, and L) in global terrestrial ecosystems. CH4 emissions represent the average emissions from paddy fields and wetlands. The number of experimental observations is in parentheses.

**Effects of e\([O_3]\) on soil C and N pools and GHG emissions**

As the largest C reservoir in terrestrial ecosystems, soils (0–1 m layer) store about 1,500 Pg (1 Pg = 10^15 g) of organic C, which is 3–4 times more than that stored in atmospheric and plant pools. SOC storage depends on the balance between soil C input (e.g., root and leaf litters) and output (soil respiration). e\([O_3]\) reduces soil C inputs from root exudates and leaf litters by decreasing plant root and leaf biomass (Figure 1A). In addition, the physiological stress caused by e\([O_3]\) might directly inhibit soil microbial activities, as evidenced by the decreases in soil microbial biomass (phospholipid-derived fatty acids, −12.2%) and the activities of C-degrading (ligninase to cellulase) enzymes (10.4%–28.0%) (Figure 2G; Table S7). This reduction in microbial activity and the associated microbial respiration may partly explain the decrease in soil respiration under e\([O_3]\) (10.4%) (Figure 2J), along with the determinant cause of decreased litter inputs and root biomass (root respiration). However, e\([O_3]\) reduces soil C inputs more strongly than soil respiration, which is consistent with a decrease in SOC and soil total N (TN) contents by 5.8% and 6.2% (Figure 2G), respectively, and a slight increase in soil C/N ratio by 1.3% (Figure S2). However, the responses of soil C and N pools to e\([O_3]\) vary with ecosystem type and e\([O_3]\) duration (see Note S2). Overall, on a global scale, e\([O_3]\) could potentially reduce SOC storage and TNP (0–20 cm layer) by 22.3 (95% CI, 7.5–37.2) Pg CO2-eq year\(^{-1}\) and 0.08 (95% CI, 0.35 to 0.19) Pg N year\(^{-1}\), suggesting that e\([O_3]\) largely damages the capacity of terrestrial ecosystems to store C and N in soils.

The various soil labile C and N pools also decreased under e\([O_3]\), but to a greater extent than SOC and TN (dissolved organic C [DOC], 15.2%; microbial biomass C [MBC], 15.5%; microbial biomass N [MBN], 22.9%; and NH\(_4^+\), 16.4%) (Figure 2). Soil labile C and N serve as metabolized substrates or energy sources for microbial organisms to produce GHGs (see also Note S3). For example, methanogenic archaea utilize organic C substrates (e.g., DOC) under anaerobic conditions to produce CH4, while NH\(_4^+\) and NO\(_3^−\) are the primary N substrates for
e\[CO_{2}\] production through heterotrophic nitrification and denitrification,\(^{51}\) with DOC acting as an energy source.\(^{52}\) Across all studies, the decreased availability of soil inorganic N and DOC under e\[O_3\] significantly reduced the rates of soil nitrification (−21.0%) and denitrification (−19.2%) (Table S8 and S9), thereby decreasing \(\text{N}_2\text{O}\) emissions (−21.1%, equivalent to 1.36 Pg \(\text{CO}_2\)-eq year\(^{-1}\); Figures 2J, 4, and S12). CH\(_4\) emissions from paddy fields and wetlands were also decreased by 19.2% (0.57 Pg \(\text{CO}_2\)-eq year\(^{-1}\); Figures 2J, 6, and S3), likely owing to a lower supply of organic C substrates.\(^{19,20}\) Previous studies postulated that e\[O_3\]-induced reductions in soil CH\(_4\) and \(\text{N}_2\text{O}\) emissions may negate the decreases in terrestrial net ecosystem production (NEP) and SOC storage.\(^{5,16,19}\) However, according to our analysis this was unlikely, as the decreases in soil CH\(_4\) and \(\text{N}_2\text{O}\) emissions under e\[O_3\] were smaller than the decreases in NEP (−12.1%) and SOC pool (−9.2%) (Figure 6). As a result, the overall source strength of global terrestrial ecosystems for GHGs under e\[O_3\] was increased by 20.4 Pg \(\text{CO}_2\)-eq year\(^{-1}\) (nGWP).

\[e\[CO_{2}\] \text{negates} e\[O_{3}\] \text{impacts on terrestrial C and N cycles}\]

In contrast with e\[O_3\], e\[CO_{2}\] generally increases plant photosynthesis and growth.\(^{24,27,29}\) Averaged across all studies in our dataset, e\[CO_{2}\] significantly stimulated plant photosynthesis by 32.6% (Figure 1E) and various plant tissue biomass by 19.4%–33.7% in global terrestrial ecosystems (Figure 1B). Plants exposed to e\[O_3\] showed reduced g\(_s\) (13.0%) (Figure 1E), which decreases O\(_3\) flux into plants and consequently its detrimental effects on plant photosynthesis.\(^{5,41,53}\) Our results showed that e\[O_3\] × e\[CO_{2}\] largely alleviated the decrease in \(V_{\text{cmax}}\) and leaf Chl under e\[O_3\] and significantly stimulated photosynthetic WUE (Figure 1F). As a result, plant photosynthesis was significantly increased by 16.7% under e\[O_3\] × e\[CO_{2}\], leading to an overall increase in the biomass of plant tissues (12.8%–20.8%) and crop yield (7.4%) (Figures 1C and S5). Also, the root/shoot ratio under e\[O_3\] × e\[CO_{2}\] remained unchanged compared with ambient conditions (Figure S2), which suggests that e\[CO_{2}\] enhanced the tolerance of plants to e\[O_3\] damage.\(^{5,53}\) As the stimulation in plant P\(_n\) increased significantly with the level of [\(\text{CO}_2\)] enrichment (Tables S1 and S10), the increases in e\[CO_{2}\] can overcome the decreased terrestrial plant productivity under e\[O_3\].

Plant NPP and its components (ANPP, BNPP, leaf, and stem C pools) were significantly increased by 19.8%–31.6% under e\[CO_{2}\] and by 17.3%–33.2% under e\[O_3\] × e\[CO_{2}\] (Figures 2B, 2C, and S9), because of the enhanced plant biomass and unaffected or stimulated plant C concentrations (Figure 1). In contrast to e\[O_3\], e\[CO_{2}\] and e\[O_3\] × e\[CO_{2}\] decreased plant N concentrations (Figures 1K and 1L), which resulted in an increase of plant C/N ratios (Figure S2). The negative effects of e\[CO_{2}\] on plant N concentrations were likely due to the growth dilution effect.\(^{54}\) The decrease in plant N concentrations was comparable in magnitude with the increase in plant biomass under e\[O_3\] × e\[CO_{2}\] and led to a slight but nonsignificant increase in various plant N pools (2.1%–10.7%). These N pools were significantly increased (5.4%–17.1%) under e\[CO_{2}\] alone (Figures 2E and 2F). Regarding the interactive effects of e\[O_3\] and e\[CO_{2}\], additive effects were observed for
NPP and TNP of terrestrial ecosystems under e\[CO2\] (31.3 Pg of e\[CO2\] on soil C and N pools may become more evident under by 0.2%–2.2% and 1.3%–4.3% under e\[O3\] long-term \[CO2\] elevation (Figure S4).

Globally, the increases in plant C and N pools (Figure 5). On a global scale, the increases in NPP and N pools by 7.1 Pg CO\(_2\)-eq year\(^{-1}\) and 0.011 Pg N year\(^{-1}\) under e\[CO2\] is positive, i.e., will result in a net gain in terrestrial plant C and N stocks. 8 Soil labile C (MBC and DOC) and N (MBN + N\(_\text{H}^+\)) contents were slightly increased by 0.3% and 3% under e\[CO2\] (Figure 6). These results further strengthen our argument that e\[CO2\] alleviates or even overcompensates the negative effects of e\[O3\] on global terrestrial ecosystems (Figure 7).

Our meta-analysis showed that, compared with ambient concentrations, elevated [O\(_3\)] significantly diminished the expected increase in the sink strength of terrestrial ecosystems for GHGs under e\[CO2\] alone by 81.5%. Nevertheless, global terrestrial ecosystems remain a C sink of about 5.7 Pg CO\(_2\)-eq year\(^{-1}\) and 0.15 Pg N CO\(_2\)-eq year\(^{-1}\), which are not fully compensated by the increases in soil CH\(_4\) and N\(_2\)O emissions. These results suggest that the negative effects of e\[O3\] on terrestrial ecosystems are overestimated if e\[CO2\] impacts are not considered.

Summary and the way forward

Our meta-analysis showed that, compared with ambient concentrations, elevated [O\(_3\)] significantly decreased plant productivity, and plant and soil C and N pools in global terrestrial ecosystems (Figure 7). However, the reductions in these C and N fluxes will be offset by the positive effects of e\[CO2\] on ecosystem C and N cycling, since the interactive effects of e\[O3\] and e\[CO2\] are mostly additive (Figure 5). Regardless of the levels of [O\(_3\)] and [CO\(_2\)] enrichment, elevated CO\(_2\) negates O\(_3\) impacts on terrestrial C and N cycles (Figures S20–S24; Note S3). These results did not necessarily indicate that e\[CO2\] is beneficial to ecosystem sustainability. Instead, we highlight that the complex interactive impacts of e\[O3\] and e\[CO2\] need to be considered when implementing
mitigation measures to curb the rising levels of CO₂ or O₃. Our findings further emphasize the importance of exploring the interactive effects of multiple global change drivers, to better understand how changes in the global atmospheric composition will affect the C and N cycles of terrestrial ecosystems. Moreover, the interactive effects of other global change factors (e.g., warming and drought) should also be explored, and the likely complicated responses of ecosystem processes to dynamically changing environmental factors need to be studied by well-designed multifactorial experiments, ⁴¹, ⁵⁶, ⁵⁷ to develop a more realistic view on future ecosystem functioning.

Most studies in our dataset were conducted in temperate regions, although the countries in the subtropics and tropics might be hit hardest by e[O₃]¹² and are more vulnerable to environmental changes owing to the importance of agriculture to their economies. Thus, there is an urgent need for more e[O₃] experiments in tropical regions. Overall, our study provides new insights into improving the prediction of C and N models in capturing the complex responses of terrestrial ecosystems to future atmospheric conditions.

**EXPERIMENTAL PROCEDURES**

**Resource availability**

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Longlong Xia (longlong.xia2@kit.edu).

**Materials availability**

This study did not generate new unique materials.

**Data and code availability**

The datasets generated during this study are available at https://doi.org/10.17632/4xft935wvy.1.

**Data compilation**

Peer-reviewed publications before July 2020 that reported the effects of e[O₃], e[CO₂], and e[O₃] x e[CO₂] on terrestrial C and N cycles were collected using the databases such as Web of Science, Scopus, Google Scholar, CABI Abstracts, Academic Search Complete (EBSCO), Current Contents Connect (ISI), China National Knowledge Infrastructure Database (CNKI), and China Wanfang Database. The keywords used in the search included “elevated O₃,” “elevated CO₂,” or “elevated O₃ and CO₂,” “plant photosynthesis (net Pₚ), gₛ, water use efficiency (WUE), the maximum carboxylation rate (Vₚₚₚₚmax) and leaf chlorophyll concentration (Chl),” “plant and its above- and belowground components biomass (shoot, root, leaf, and stem),” “root/shoot ratio,” “food

The natural log-transformed response ratio (ln RR) was used to quantify the effect size of the target variables for the control (ambient concentration) and treatment (e[O3], e[CO2], or e[O3] x e[CO2]) plots was calculated using the following equation:

\[ \ln \text{RR} = \ln \left( X_A / X_C \right) \] (Equation 1)

where \( X_A \) and \( X_C \) represent the mean of the treatment (e[O3], e[CO2], or e[O3] x e[CO2]) and control groups (ambient concentration) for the variable \( X \), respectively. The results are presented as the percentage changes (\( \% \)) of the control group (ambient concentration) for the variable \( X \) respectively. Positive percentage changes denote an increase due to e[O3], e[CO2], or e[O3] x e[CO2] whereas negative values indicate a decrease in the respective variables. The effect size was weighted by the inverse of its variance and the missing variance was estimated using the average coefficient of variation across the dataset.59

We performed a mixed-effects meta-analysis in R, using the meta.mv function in the metafor package which included "package" as a random effect.59

The effects of e[O3], e[CO2], or e[O3] x e[CO2] were considered significant if the 95% CIs did not overlap with zero. The means of the categorical variables (ecosystem types and fumigation methods) were considered significantly different from each other if their 95% CIs did not overlap. For each variable, total heterogeneity among the categorical group (Q1) was partitioned into within-group (Q2) and between-group (Q3). A significance of Q1 (p < 0.05) represents a significant difference between different levels of the categorical group (Tables S4 and S5). Meta-regression was conducted between lnRR of the target variables and climate parameters (MAT and MAP), soil properties (SOC and TN contents), and \( [O_3] \) and \( [CO_2] \) concentrations (Figure 3; Tables S1 and S2). Publication bias for the variables included in this meta-analysis was assessed using funnel plots.

Interactive effects

The interactive effects of e[O3] and e[CO2] on the variables were evaluated using Hedges' d. The interactive effect size (d) between e[O3] and e[CO2] was calculated using the following equation:

\[ d = \frac{(X_{AB} - X_A) - (X_B - X_C)}{2s} \times J(m) \] (Equation 2)

where \( X_{AB}, X_A, X_B, \) and \( X_C \) denote the mean of a variable in the control, e[O3], e[CO2], and their combination e[O3] x e[CO2], respectively; \( s \) and \( J(m) \) denote the pooled standard deviation and correction term for small sample bias, respectively, which were calculated using the following equations:

\[ s = \sqrt{\left( \frac{(n_a - 1)s_a^2 + (n_b - 1)s_b^2 + (n_c - 1)s_c^2 + (n_{ab} - 1)s_{ab}^2}{n_a + n_b + n_c - n_{ab} - 4} \right)} \] (Equation 3)

\[ J(m) = 1 - \frac{3}{4m - 1} \] (Equation 4)

where \( n_a, n_b, \) and \( n_c \) denote the sample size, and \( s_a, s_b, \) and \( s_c \) represent the standard deviation in the control e[O3], e[CO2], and their combination e[O3] x e[CO2], respectively; \( m \) is the degree of freedom (\( m = n_a + n_b + n_c - n_{ab} - 4 \)). We calculated the variance of \( d \) (\( V \)) using the following equation:

\[ V = \frac{1}{4} \left( \frac{d}{n_a} + \frac{1}{n_b} + \frac{1}{n_c} + \frac{1}{n_{ab}} + \frac{df}{2(n_a + n_b + n_c - n_{ab} - 4)} \right) \] (Equation 5)
The weighted mean \( d_i \) was calculated using the following equation:

\[
d_i = \frac{\sum_{l=1}^{L} \sum_{k=1}^{K} w_{lk} d_{lk}}{\sum_{l=1}^{L} \sum_{k=1}^{K} w_{lk}}
\]  

(Equation 6)

where \( l \) represents the group number, \( k \) is the number of comparisons in the \( i \)th group, and \( w \) is the weight that is the reciprocal of the variance \((1/\sigma^2)\). Weighted mean \( d_i \) and the 95% CIs were calculated using the “metaphor” package in R software. The interactive effects of \( e\left[O_3\right] \) and \( e\left[CO_2\right] \) on the variables were additive, synergistic, or antagonistic. The interactive effect was considered to be additive if the 95% CI overlapped with zero. For two-driver pairs whose individual effects were either both negative or had opposite directions, the interactive effect sizes less than zero were synergistic whereas those greater than zero were antagonistic.

Upscaling and uncertainty analysis

We scaled up the weighted and averaged results (area-scaled metrics, \( U \)-value) from this meta-analysis by multiplying them for target variables with the corresponding total land area:

\[
T = D \times A
\]  

(Equation 7)

where \( T \) is expressed in Pg CO₂-eq year\(^{-1}\) or Pg N year\(^{-1}\) and \( A \) is the total land area for wetlands (5.7 million km\(^2\)), rice paddies (1.3 million km\(^2\)), and upland (103 million km\(^2\)). When scaling up \( N_2O \) emissions, we distinguished between fertilized upland soils (i.e., 19 million km\(^2\) of fertilized grasslands and croplands minus 1.3 million km\(^2\) rice paddies) and nonfertilized upland soils (85.4 million km\(^2\)). \( U \) was weighted by the inverse of its variance. CO₂-eq emissions were calculated using the 100-year global warming potentials of 34 and 298 for CH₄ and N₂O, respectively.

For paired data, we determined the SOC and TN change rate (\( MU, g \, C, \text{or N kg}\(^{-1}\) year\(^{-1}\)) under atmospheric composition changes by plotting them versus the experimental duration, which was defined and calculated using the following equation:

\[
MU = (SOC_t - SOC_c) / t
\]  

(Equation 8)

where SOC\(_t\) and SOC\(_c\) represent the mean SOC (TN) change rate in treatment and control groups (ambient concentration), respectively. Furthermore, the SOC and TN pool (SOCl and TNL, \( t \) or C or N year\(^{-1}\) change rate) (0–20 cm layer) was estimated using the following equation:

\[
TU = (SOCt - SOCl) / t
\]  

(Equation 9)

Figure 7. A conceptual model summarizing terrestrial C and N feedbacks to \( e\left[O_3\right] \), \( e\left[CO_2\right] \) and \( e\left[CO_2\right] \times e\left[CO_2\right] \). Overall effects of elevated [O₃] (blue), elevated [CO₂] (red), and \( e\left[O_3\right] \times e\left[CO_2\right] \) (dark green) on plant C and N pools, soil C and N pools, and greenhouse gas emissions in terrestrial ecosystems. ANPP, above-ground net primary productivity; BNPP, below-ground net primary productivity; AGNP, above-ground N pool; BGNP, belowground N pool; SOC, soil organic C; DOC, dissolved organic C; MBC, microbial biomass C; MBN, microbial biomass N.
SOCp (TNp) = \sum_{i=1}^{m} A_i \times MU \times BD \times H \times 0.1 \quad \text{ (Equation 9)}

where $A_i$ is the habitat area (i.e., upland, wetland, and paddy field); BD is the bulk density (g cm$^{-3}$) and H is the depth of the soil layer (20 cm). Since the interactive effects of $e[O_3]$ and $e[CO_2]$ on plant and soil C and N pools, and GHG emissions were additive (Figure 6), the changes for various C and N variables under simultaneous $e[O_3]$ and $e[CO_2]$ were calculated by summing the values for $e[O_3]$ alone and $e[CO_2]$ alone (i.e., $e[O_3]$ and $e[CO_2]_{variable} = e[O_3]_{variable} + e[CO_2]_{variable}$) (Figure 6).

Uncertainties exist in our estimation of changes in C and N pools and GHG emissions on a global scale. First, the responses of GHG emissions, and soil C and N pools to $e[O_3]$ and $e[CO_2]$ may change over the experimental duration (Figure S4), which was not considered in the current estimation due to data limitation for long-term studies. Second, we assumed that the effects of $e[O_3]$ and $e[CO_2]$ mainly occur during the plant-growing season when converting seasonal fluxes to annual data, which may affect the upscaling results. Third, the responses of soil C and N pools and GHG emissions to $e[O_3]$ and $e[CO_2]$ may vary with climate conditions (e.g., temperature and precipitation) and soil properties (e.g., initial SOC content) (Figure 3 and S13), which was not assessed in the analysis. Fourth, the effects of $O_3$ are dose dependent and plants often exhibit threshold responses to cumulative doses of $O_3$ (Figures S14–S15). For example, staple crop species (e.g., rice, wheat, and corn) are less tolerant to $e[O_3]$ than tree species (e.g., spruce, silver fir, and pine) based on the AOT$_{40}$ index values (accumulated exposure over threshold of 40 ppbv) (Table S3). Our results further show that $e[O_3]$ levels of around 70 ppbv in combination with $e[CO_2]$ levels of around 550 ppmv increased the aboveground biomass of food crops (e.g., wheat, bean, and vegetables) at a similar or even greater magnitude than for tree species (e.g., pine and spruce) being exposed to a combination of $e[O_3]$ of about 70 ppbv and $e[CO_2]$ of about 700 ppmv (Figure S10). These results indicate that food crops respond more dramatically than tree species to concurrently changing $e[O_3]$ and $e[CO_2]$ levels. However, due to a lack of data, especially on the responses of plant/soil C and N pools to $e[O_3]$ and $e[CO_2]$ and the species effects (Data S7), we did not upscale the results for individual plant species (see Note S2 for details). This underscores the importance of the inclusion of these factors into future studies to reduce the uncertainties.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.öneear.2021.11.009.

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**AUTHOR CONTRIBUTIONS**

L.X. and K.B. designed the research. L.X. performed the data extraction and analysis with the support from S.L., R.K., K.B., D.C., Y.L., K.J.v.G., E.A.A., J.C., S.L., L.M., Y.Z., and L.X. wrote the first draft of the manuscript, with all authors contributing to the revisions.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**


