Effect of elevated tropospheric ozone on soil carbon and nitrogen: a meta-analysis

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Abstract

Elevated tropospheric ozone concentration ([O₃]) may substantially influence the belowground processes of the terrestrial ecosystem. Nevertheless, a comprehensive and quantitative understanding of the responses of soil C and N dynamics to elevated [O₃] remains elusive. In this study, the results of 41 peer-reviewed studies were synthesized using meta-analytic techniques, to quantify the impact of O₃ on ten variables associated with soil C and N, i.e. total C (TC, including soil organic C), total N (TN), dissolved organic C (DOC), ammonia N (NH₄⁺), nitrate N (NO₃⁻), microbial biomass C (MBC) and N (MBN), rates of nitrification (NTF) and denitrification (DNF), as well as C/N ratio. The results depicted that all these variables showed significant changes (P < 0.05) with [O₃] increased by 27.6 ± 18.7 nl l⁻¹ (mean ± SD), including decreases in TC, DOC, TN, NH₄⁺, MBC, MBN and NTF, and increases in C/N, NO₃⁻ and DNF. The effect sizes of TN, NTF, and DNF were significantly correlated with O₃ fumigation levels and experimental duration (P < 0.05). Soil pH and climate were essential in analyses of O₃ exposure. The altered soil C and N dynamics under elevated [O₃] may reduce its C sink capacity, and change soil N availability and thus, impact plant growth and enhance soil N losses.

1. Introduction

Tropospheric ozone (O₃) is an important greenhouse gas that contributes to global warming (Zhao et al. 2019), and is a pollutant that is detrimental to human health and damages vegetation (Krupa and Manning 1988, Jerrett et al. 2009). O₃ is known to impair plant growth by reducing the photosynthetic rate, causing decreased yield and quality of sensitive species (Mills et al. 2018, Ainsworth et al. 2020). In recent years, considerable attention has been paid to belowground ecosystem processes. Elevated [O₃] may impact the C allocation to roots (Andersen 2003), thereby altering rhizodeposition (Kou et al. 2014), changing the amount and composition of the root exudate (McCready and Andersen 2000), modifying the nutrient and energy supply to soil microorganisms (Feng et al. 2015), shifting the microbial diversity and community structure (Phillips et al. 2002, Min et al. 2015, Chen et al. 2019), and accordingly, influence soil C and N dynamics (Kanerva et al. 2007, Hu et al. 2018). The O₃-induced alternation in soil C and N status may influence C sequestration capability, greenhouse gas emissions, and ecosystem N losses, which might have feedback effects on climate change (Kanerva et al. 2006, Chen et al. 2015).

The effects and mechanisms of elevated [O₃] on belowground processes remains controversial despite
extensive research. For example, numerous studies found that the belowground C allocation increases under elevated \([O_3]\) (Nouchi et al 1995, McCrady and Andersen 2000, Duckmanton and Widdow 2018), whereas others obtained contrary results (Landolt et al 2000, Yoshida et al 2001, Andersen et al 2010). In addition, many studies showed that elevated \([O_3]\) remarkably decreased soil NH\(_4^+\) and NO\(_3^-\) contents, and thus reduced soil N availability (Holmes et al 2003, Kanerva et al 2006, Pereira et al 2011, Formánek et al 2014, He et al 2014, Kou et al 2014, Toet et al 2017), whereas others reported the opposite results (Chen et al 2015a, Wu et al 2016). Moreover, Mörsky et al (2008) found that elevated \([O_3]\) increased soil microbial biomass, whereas others showed that it was unaffected (Cheng et al 2011, Zhang et al 2014) or decreased (Phillips et al 2002, Kanerva et al 2008, Bao et al 2015). The factors which drove the discrepancies in findings across studies were still unclear.

This study aimed to assess the responses of soil C and N contents, N transformation processes, and microbial biomass to elevated \([O_3]\) by using meta-analytic techniques. The sources of variation were evaluated by addressing the following questions. (1) How do \(O_3\) fumigation level, experimental duration, and \(O_3\) fumigation methods affect the responses of soil C and N dynamics to elevated \([O_3]\)? (2) Do \(O_3\)-induced responses of soil C and N dynamics vary with various terrestrial ecosystems? (3) Do soil sampling types, soil pH, additional elevated \([CO_2]\), as well as geographical and climate conditions impact the responses of soil C and N dynamics to elevated \([O_3]\)?

2. Material and methods

2.1. Data sources

All peer-reviewed literature, including journal articles, degree dissertations, and monographs related to soil C and N status with reference to elevated \([O_3]\), were extensively searched through the ISI Web of Science (https://apps.webofknowledge.com) and the Scopus (www.scopus.com) by using the following key terms: \([O_3]\) OR ozone AND [soil OR rhizosphere* OR terrestrial OR land] AND [carbon OR TC OR “OC OR TN OR *nitr* OR ammon* OR mineraliz*].

The relevant Chinese literatures were searched in the China National Knowledge Infrastructure Database (CNKI, www.cnki.net/). This search produced a total of 579 publications in ISI, 533 publications in Scopus, and 507 publications in CNKI. After examining the relevance, eliminating the duplications in both ISI and Scopus, and cross-checking the reference lists to avoid missing relevant studies, 75 publications were identified for further screening.

The searched publications were carefully screened according to the following criteria. (i) The studies were conducted in the field, and reported \(O_3\) effects on at least one of the interesting variables, including total C (TC, including soil organic C), dissolved organic C (DOC), microbial biomass C (MBC), total N (TN), ammonia N (NH\(_4^+\)), nitrate N (NO\(_3^-\)), microbial biomass N (MBN), nitrification (NTF) rate, and denitrification (DNF) rate. (ii) The microclimate, vegetation, and initial soil conditions were the same between the control (ambient \(O_3\)) and \(O_3\) enrichment treatments. (iii) The means, standard deviations (SDs), and replicate numbers (\(n\)) of the target variables could be directly acquired or obtained from the corresponding authors. (iv) The \(O_3\) fumigation was performed over at least one growing season using open top chamber (OTC) or free-air \(O_3\) concentration enrichment (FACE) technology. (v) Published articles and their measurements were excluded if the data were reported more completely in another article. (vi) For studies on \(O_3\) and \(CO_2\) treatments with full factorial design (i.e. control, \(CO_2\) exposure, \(O_3\) exposure, and combined \(CO_2\) and \(O_3\) exposure), the data of \(CO_2\) exposure alone were not extracted. According to these criteria, 22 studies based on pot-plants, 2 studies on lab cultivations, and 2 studies on lysimeters were excluded. Four publications that only reported charcoal-filtered air as control were not used. Furthermore, four articles that reported data more completely in another article were eliminated. The final database consisted of 41 articles published between 2003 and 2020, including 28 English papers indexed by both ISI and Scopus and 13 Chinese literatures indexed by CNKI (supplementary material A available online at stacks.iop.org/ERL/17/043001/mmedia). The data were acquired from 17 experiments performed at 25°28′ N to 60°49′ N latitude and 88°14′ W to 123°24′ E longitude. The mean annual temperature (MAT) ranged from 1.1 °C to 26.6 °C, and the mean annual precipitation (MAP) ranged from 460 to 1280 mm. The average increment in \([O_3]\) across all studies was 27.6 ± 18.7 nl l\(^{-1}\). Note that studies in the Southern hemisphere were absent from the database (Hu et al 2021).

Values for each variable were extracted independently if they were obtained from different experiments, corresponded to multiple \(O_3\) levels or additional \([CO_2]\) exposure within a single study. To acquire as many observations as possible, values were assumed independently if they were obtained from the same experiment but measured on different dates, or when the soil samples were collected from distinct layers within the soil depth range of 0–25 cm. Similar assumptions have been widely held in previous studies (Wittig et al 2009, Li et al 2017, Feng et al 2018, Meng et al 2019). Results obtained from the same experiment were considered independent and included separately in the database if they were associated with different plant species or cultivars, N treatments, and warming or drought stresses (de Graaff et al 2006).

Values of each variable from text or tables were extracted directly. Data from figures were digitized.
The total heterogeneity of each variable was assessed using the statistic $Q_T$ and $I^2$ index (table S.1) (Rosenberg et al. 2000, Higgins and Thompson 2002, Huedo-Medina et al. 2006). Categorical analysis was performed for all data by dividing $Q_T$ into within-group heterogeneity ($Q_W$) and between-group heterogeneity ($Q_B$). The datasets were then grouped on the basis of the levels of the categorical variables with significant $Q_B$ (table S.1) (Wittig et al. 2009). The difference between the means of categories were considered to be significant if their bias-corrected 95% bootstrapped CIs did not overlap (Gurevitch and Hedges 1999, Ainsworth 2008).

Continuous models were applied to determine the relationships between lnR and environmental or forcing factors, such as $O_3$ fog duration, experimental duration, soil pH value, and latitude, MAP of the experimental site. A parametric mixed model approach was used to test whether the slopes of weighted regressions differed from zero (Rosenberg et al. 2000). The regressions were considered to be significant if $P < 0.05$.

Multiple methods were included in the test of publication bias, including funnel plots (figure S.3), Kendall's tau (table S.2) and Spearman's rho (table S.3) rank correlation tests, as well as Rosenthal's fail-safe number ($\alpha = 0.05$) (table S.4) (Rosenthal 1979). A publication bias was confirmed only if all three methods showed positive results simultaneously, namely, that the funnel plot clearly showed asymmetry (only for the overall data set), or the rank correlation test was significant ($P < 0.05$), and Rosenthal's fail-safe number appeared less than $5k + 10$, where $k$ is the observation number. For the data set with confirmed publication bias, the 'true' overall/grouped effect size and CIs were estimated using a 'Trim and Fill' approach (Jin et al. 2015).
5.8%, respectively, whereas it increased soil NO$_3^-$ by 7.0%. In addition, the changes on substrate NH$_4^+$ and NO$_3^-$ availability for nitrifying and denitrifying bacteria led to a decrease in soil NTF (7.5%) and an increase in soil DNF (5.0%), respectively. Elevated [O$_3$] significantly increased C/N by 3.1%. And there was a strong positive relationship between effect sizes of elevated [O$_3$] on soil TN, MBN, NTF, DNF, and C/N ratio. Symbols show the grand mean percentage changes under elevated [O$_3$] relative to the control, and the bars represent the bias-corrected 95% bootstrapped CIs. Number of observations and articles are provided in parentheses. Mean elevated [O$_3$] and control [O$_3$] (in brackets) are given on the right side.

### 3.2. Effects of O$_3$ fumigation level

The magnitude of O$_3$ effects on soil TN, MBN, NTF, and DNF was dependent on the O$_3$ exposure level due to their significant Q$_H$ values ($P < 0.05$; table S.1). As illustrated in figure 2, elevated [O$_3$] generally has the most pronounced effect at high O$_3$ level, and it has no significant effect on these four variables at moderate O$_3$ levels. Notably, Kendall’s tau and Spearman’s rho rank correlation tests for publication bias of TN were significant at moderate O$_3$ level ($P < 0.05$), while it has no significant changes at low O$_3$ level. The overlapped CIs suggested that no significant difference in TN existed among the three O$_3$ levels (figure 2).

In terms of MBN, the O$_3$-induced percentage decrease was significantly larger ($P < 0.05$) at high O$_3$ levels (23.5%) than at a low O$_3$ level (11.8%), and both were significantly different from zero ($P < 0.05$). Likewise, the reduction of NTF induced by elevated [O$_3$] was significantly larger ($P < 0.05$) at high [O$_3$] (16.1%) than at moderate [O$_3$] (5.4%). As for DNF, the effect of O$_3$ treatment was not significant at low and moderate O$_3$ levels, and a significant increase was noted at high O$_3$ level (12.3%; $P < 0.05$). No between-group heterogeneity was found for other response variables when the studies were grouped by O$_3$ exposure level (table S.1 and figure S.5).

Weighted meta-regression analyses showed that the level of elevated [O$_3$] was significantly negatively related with the effect sizes of TN and MBN ($P < 0.05$; table S.5). Moreover, there was a significant negative correlation between the effect size of NTF and O$_3$ exposure level ($P < 0.001$; table S.5), whereas the increasing [O$_3$] had a significant positive effect on the...
3.3. Effects of experimental duration
The temporal patterns of responses of soil NO$_3^−$, MBN, NTF, and DNF were investigated by grouping the data by experimental duration, because of their significant Q$_A$ values ($P < 0.05$). Soil MBN and NTF progressively decreased with increasing duration of exposure to elevated [O$_3$], whereas an inverse trend was observed for DNF (figure 3 and table S.5). Short O$_3$ exposure had no significant effect on MBN and DNF, whereas it marginally significantly increased soil NO$_3^−$ and NTF by about 15.0% and 6.0%, respectively. For the medium experimental duration, MBN and NTF declined moderately with average reductions of 17.2% and 14.2% ($P < 0.05$), respectively, whereas the changes of NO$_3^−$ were not significantly different from zero. Note that the DNF for medium experimental duration showed publication bias according to the results of Kendall’s tau and Spearman’s rho rank correlation tests, as well as Rosenthal’s fail-safe number (tables S.2 and S.4). After adjustments using the ‘Trim and Fill’ method, the ‘true’ effect size of DNF for medium O$_3$ exposure duration was still significantly increased (10.4%, $P < 0.05$). The O$_3$-induced percentage decline of MBN for long O$_3$ exposures (16.0%) were similar to that at medium exposures, whereas NTF was markedly (22.9%) decreased by O$_3$ exposure of $> 3$ years. The percentage increase in DNF after long O$_3$ exposure was approximately two times higher than that after medium exposure. As expected, the longer the duration of O$_3$ exposure, the more pronounced was the O$_3$ impact on these variables. The only exception was NO$_3^−$, which showed a similar change (16.3%, $P < 0.05$) under long O$_3$ exposure as that under short duration.

The heterogeneities on the responses of soil TN, NTF and DNF to different O$_3$ durations could be explained by the continuous model, according to the significant Q$_A$ values ($P < 0.05$). As expected, the longer the duration of O$_3$ exposure, the more pronounced were the O$_3$ impacts on NTF and DNF. The duration of ozone exposure did not induce significant differences in other response variables (tables S.1 and S.5; figure S.6). Soil TC content for medium experimental duration showed publication bias (tables S.2 and S.4), indicating that stronger O$_3$ effects were somewhat more likely to be published than weaker O$_3$ effects under such circumstance. After being adjusted using the ‘Trim and Fill’ method, the ‘true’ mean effect size of TC for medium experimental duration showed a significant decrease of 10.4% ($P < 0.05$; figure S.6).

3.4. Effects of O$_3$ fumigation methods
Of the total 41 articles, 24 and 17 reported the use of FACE and OTC, respectively. According to the results of heterogeneity analysis (table S.1), the fumigation method significantly affects the responses of MBC, MBN and NTF to elevated [O$_3$], but did not induce significant differences in other response variables. Of three variables with significant Q$_A$ values, MBC and MBN showed overlapped CIs between FACE and OTC ($P > 0.05$; figure S.7), while only NTF showed a significant larger decrease in FACE than that in OTC ($P < 0.05$; figure S.7).

3.5. Effects of soil sampling types
Soil MBC, MBN, NH$_4^+$, NO$_3^−$, NTF and DNF showed significant Q$_B$ values ($P < 0.05$) between rhizosphere soil and bulk soil (table S.1). For rhizosphere soil samples, elevated [O$_3$] significantly decreased MBC, MBN and NTF by 14.9%, 18.0% and 12.9%, respectively ($P < 0.05$); and increased soil NO$_3^−$ and DNF by 20.1% and 11.5%, respectively ($P < 0.05$); while it has no significant impact on soil NH$_4^+$ (figure 4). On the contrary, elevated [O$_3$] has no significant effect on these variables in bulk
soil, except it significantly reduced bulk soil NH$_4$$^+$ by 10.1% ($P < 0.05$; figure 4). The differences on the responses of soil MBC, MBN, NTF and DNF between rhizosphere and bulk soil were significant ($P < 0.05$) owing to their un-overlapped CIs (figure 4). No between-group heterogeneity was found on other response variables when the studies were grouped by soil sampling type (table S.1 and figure S.8).

### 3.6. Effects of soil pH

The O$_3$-induced impacts on soil C and N were potentially related to the pre-existing soil pH (table S.1). The responses of soil TC, TN, NH$_4$$^+$, and NTF to elevated [O$_3$] grouped by soil pH according to their significant Q$_0$ values ($P < 0.05$) are shown in figure 5.

A statistically significant decrease in TC under elevated [O$_3$] occurred in neutral soil (7.2%, $P < 0.05$), whereas a marginally significant decrease was noted in alkaline soil (1.9%), and no significant change was found in the acid soil. Soil TN content in response to elevated [O$_3$] was reduced by 4.3% (after being adjusted by the 'Trim and Fill' approach due to significant publication bias) and 6.1% in neutral and alkaline soils, respectively; and not affected significantly in acid soil (figure 5). Detailed meta-regression analysis showed that the O$_3$-induced effects on soil TN content were significantly and negatively related to soil pH ($P < 0.001$; table S.5), indicating that the negative O$_3$ effects on TN were exacerbated at higher soil pH.

In addition, lower soil pH caused stronger declines in soil NH$_4$$^+$ content under elevated [O$_3$] (figure 5). Soil NH$_4$$^+$ was remarkably decreased by 23.6% ($P < 0.05$) in acidic soil, but was not significantly affected in both neutral and alkaline soils. Indeed, the effect size of soil NH$_4$$^+$ was positively correlated with soil pH ($P < 0.001$; table S.5), which indicated that higher soil pH ameliorates the negative O$_3$ effects on soil NH$_4$$^+$ content.

Elevated [O$_3$] strongly decreased soil NTF by 5.7% and 20.6% in acid and neutral soils, respectively (figure 5), and this effect was significantly negatively related to soil pH ($P < 0.001$; table S.5).

Although statistically or marginally significant negative effects of elevated [O$_3$] were noted on soil MBN, no significant differences were observed among soil pH classes (figure S.9). However, the weighted meta-regression analysis showed a
significant negative relationship between the effect size of MBN and soil pH ($P < 0.01$; table S.5).

The number of studies for DNF in neutral and alkaline groups were not sufficient to conduct quantitative comparisons. However, the weighted meta-regression analysis showed a strong significant negative relationship between the effect size of O$_3$ on DNF and soil pH ($P < 0.01$; table S.5).

### 3.7. Other categorical analyses

Significant heterogeneities were found on TN and MBN in response to elevated [O$_3$] across terrestrial ecosystems ($P < 0.05$; table S.1). However, there were no significant differences among groups for TN owing to the overlapped CIs (figure S.10). On the contrary, soil MBN was significantly reduced by 17.9% in cropland, and was significantly different from that in grassland which tended to be increased ($P < 0.05$; figure S.10).

In addition, only TN, NH$_4^+$ and NO$_3^-$ have sufficient studies ($k > 10$) to conduct comparisons between solely O$_3$ stress and combined O$_3$ and CO$_2$ exposure. However, there was no between-group heterogeneity for either variable (table S.1). Thus, the magnitude of the responses to elevated [O$_3$] were independent of the additional [CO$_2$] exposure in the present analysis.

### 3.8. Effects of latitude and climate conditions

The results of weighted meta-regression analyses for response variables in correlation with latitude, MAT, and MAP are shown in table S.6. The effect sizes of soil TN, MBN, and DNF were significantly correlated with latitude ($P < 0.05$). Taking as an example the regressing results of MBN ($\ln(R(MBN)) = 1.02 \times 10^{-2} \times \text{Latitude} - 0.528$), the fitted effect size of MBN varied from $-0.269$ to $0.090$ within a latitude range of $25^\circ - 28^\circ$ towards the ground and preventing any appreciable penetration of O$_3$ into the soil (Turner et al 1974, Blum and Tingey 1977). Therefore, the effects of O$_3$ on soil C and N seem unlikely to be direct, but are likely to be mediated indirectly through altering the quantity and quality of plant C inputs and resource allocations (Andersen 2003, Kasurinen et al 2005, Kanerva et al 2006).

Previous meta-analytic studies revealed that chronic O$_3$ exposure decreased photosynthetic rates, Rubisco activity, stomatal conductance, and chlorophyll content, and eventually altered source-sink balance in sensitive plants (Andersen 2003, Valkama et al 2007, Feng et al 2008, Feng and Kobayashi 2009, Wittig et al 2009, Li et al 2017, Feng et al 2018). Although the observed responses vary depending on the species and exposure conditions, elevated [O$_3$] is known to seriously influence the transport of photosynthates from leaves to roots. In many cases, decreased root C allocation occurred rapidly and even before shoot responses could be observed (Andersen 2003). Decreased C allocation below ground altered rhizodeposition, reduced the quantity of root residue and exudates and thereby decreased C flux from root exudates to the soil labile C pool (Yoshida et al 2001, Andersen 2003, Jones et al 2009). Therefore, the contents of soil TC and DOC generally decreased under elevated [O$_3$] (figure 1). As a labile form of soil C, DOC principally consists of low-molecular-weight sugars, fulvic acids, and amino acids (McKeeague et al 1986, van Hees et al 2005). The greater reduction of DOC than that of TC may suggest a relative enrichment of recalcitrant organic C, such as high-molecular-weight phenolic compounds, in soil (Jones et al 2009). Since soil microorganisms are
generally C-limited (Anderson and Domsch 1978), the decrease in soil labile C availability may explain the reduced soil microbial biomass (figure 6) (de Graaff et al 2006).

The availability of soil TN, particularly NH$_4^+$ contents, were also reduced under elevated [O$_3$] (figure 1). The inhibition of soil N availability indicated a deficiency in available N for soil microbes under elevated [O$_3$]. The reduced soil NH$_4^+$ concentrations or MBN in response to elevated [O$_3$] could be attributed to the decreased belowground C inputs (Kanerva et al 2006, Bhatia et al 2011), or, in some cases, higher NTF and DNF rates (Pereira et al 2011). Indeed, the DNF rate was generally higher under elevated [O$_3$] (figure 1). Previous studies have shown that soil oxygen concentration, NO$_3^-$ availability, as well as organic C supply are the three most important factors that determine the DNF rate (Del Grosso et al 2000, Lu et al 2011). Since soil DOC was generally decreased under elevated [O$_3$], the acceleration of the DNF process may be attributed to the increased NO$_3^-$ availability (figure 6), and in some cases, decreased oxygen concentration due to the relative increases in the soil water content driven by the reduced stomatal conductance and transpiration rates under elevated [O$_3$] (McLaughlin et al 2007, Hu et al 2018). In contrast, the NTF rate was generally reduced by O$_3$ stress (figure 1). Soil NTF rates have shown to be mainly controlled by soil NH$_4^+$ availability (figure 6) (Norton 2008). Decreased substrate quantity and quality under elevated [O$_3$] may exert negative effects on soil NTF. In theory, the inhibited NTF rate and accelerated DNF rate would decrease soil NO$_3^-$ under elevated [O$_3$]. The marginally significant increase of soil NO$_3^-$ may be explained by the suppressed N demand for plant shoots due to reduced biomass (Broberg et al 2017). Moreover, the increased NO$_3^-$ may result in a high risk of soil N loss via leaching and runoff (Hu et al 2018).

4.2. Confounding O$_3$ effects among classes

The response magnitudes of most variables to elevated [O$_3$] were affected by O$_3$ levels, experimental duration, soil pH and sampling type, etc. However, these categorical factors may couple with each other. For example, [O$_3$] in the control and the elevated treatment for rhizosphere soil were generally higher than that for bulk soil (figure 4), which may lead to more significant responses of MBC, MBN, NH$_4^+$, NO$_3^-$, NTF and DNF in rhizosphere soil than that in bulk soil. In addition, MBN responded more significantly in cropland than that in grassland because of the much higher [O$_3$] in the former, and higher a O$_3$ level would exacerbate the detrimental effect of O$_3$ on soil MBN owing to the significant negative regression relationship ($P < 0.05$; table S.5).

Note that the O$_3$ effects on MBN and NTF were non-significant under a moderate O$_3$ level, while they significantly decreased at low and high O$_3$ levels. One possible reason is the relatively lower mean [O$_3$] of both control and experimental treatments for these response variables under a moderate O$_3$ level (figure 2). In another case, medium O$_3$ exposure had no significant effect on NO$_3^-$, while it significantly increased at short and long O$_3$ exposures. The relative smaller or even opposite responses of these variables at a moderate (medium) group than that at a low (short) or high (long) groups may also reflect the possibility of soil microbial acclimation to moderate doses of O$_3$ stress. Recently, Agathokleous et al (2019a, 2019b, 2019c) found that many determinants of plant yield followed hermetic-like biphasic dose-response relationships under elevated [O$_3$] stress. Since the effect of elevated [O$_3$] on soil processes are generally mediated indirectly through plants, it may also yield pleiotropic responses. Based on this assumption, the moderate doses of O$_3$ stress may upregulate adaptive responses that may enhance both microbial and biochemical resilience, while high doses may induce inhibitory responses to soil microbes (Agathokleous et al 2019a).

In general, autotrophic nitrifiers function better under neutral and/or slightly alkaline conditions, and NTF rates are often low in acidic soils (Chen et al 2013). In the present analysis, the responses of NTF in acid soil was significantly less negative than that in neutral soil according to their un-overlapped CIs ($P < 0.05$; figure 5). Note that the increment of [O$_3$] was higher in acid soils (42 nl l$^{-1}$) than that in neutral soils (19 nl l$^{-1}$). And higher O$_3$ level under acid condition should have exacerbated the detrimental effect of O$_3$ on NTF. The seemed paradoxical results may be explained by the longer experiment duration (average of 3.7 years) in neutral soil than that in acid soil (average of 1.9 years), since O$_3$ effect on NTF was significantly negatively related to experimental duration ($P < 0.001$; table S.5). In other words, the regression relationship between NTF and soil pH may be essentially related to the regression relationship between NTF and O$_3$ exposure duration.

The O$_3$-induced effect on NTF was more negative at alkaline condition (figure 5). It means the ability...
for oxidizing NH$_4^+$ to NO$_3^−$ was more restrained by elevated [O$_3$] at higher soil pH, which is conducive to the reserve of soil NH$_4^+$ (i.e. the substrate for NTF). In other words, the negative O$_3$ effects on soil NH$_4^+$ content was ameliorated at higher soil pH (figure 5). Moreover, the number of DNF data was not enough to conduct a categorical analysis at different soil pH levels. But its general increasing pattern (figure 1) may imply an increase in soil N$_2$O emissions under elevated [O$_3$]. Šimek and Cooper (2002) reported that heterotrophic denitrifiers function better at higher soil pH. Consequently, the total gaseous N loss via DNF would be higher in neutral and slightly alkaline soils than acidic ones. It may explain the exacerbated TN loss due to elevated [O$_3$] at higher soil pH.

In addition, the increase in [O$_3$] used in OTC experiments (40.0 nl l$^{-1}$) was generally higher than that used in FACE experiments (23.1 nl l$^{-1}$) for NTF, and higher [O$_3$] was supposed to exacerbate the detrimental effect of O$_3$. However, NTF decreased more in FACE than that in OTC (figure S.7), which may be attributed to the longer experiment duration in FACE studies. Consequently, extended duration may counteract the limitation of lower O$_3$ levels on soil NTF responses to elevated [O$_3$] in FACE and neutral soil conditions.

Since O$_3$ exposure level and duration may offset one another in explaining response variances among groups, a better way would be to combine them together as a cumulative dose variable. The commonly used dose-based indices are accumulated O$_3$ exposure indices (such as AOT40, SUM06 and W126) and flux-based index (e.g. POD$_4$). They all played significant roles in quantifying O$_3$ impacts on plant growth and soil properties (Zhang et al 2017, Hu et al 2019). However, the calculation of exposure indices requires hourly averaged [O$_3$] and effective fumigation time, and the calculation of POD$_4$ required further information including hourly data of photosynthetic photon flux density, temperature, water vapour pressure deficit and plant available water, etc. In the 41 papers of present meta-analysis, only 9 papers reported AOT40. So far, we cannot obtain the hourly data for the calculation of dose indices for the other 32 studies. Estimating the cumulative dose by multiplying daylight mean [O$_3$] and plant growing season is infeasible and will import immeasurable errors. Because [O$_3$] has obvious daily and seasonal variations, and O$_3$ fumigation usually only applied during the daylight 7–9 h except for rain, dew and fog. Moreover, the effect of elevated [O$_3$] on soil C and N varied with plant growing stages (Hu et al 2018). Once enough data has been gathered, an updated categorical and/or continuous meta-analysis based on dose index will be more efficient in revealing the variation mechanisms of O$_3$ impacts on soil C and N.

4.3. Effects of climate conditions on soil C and N responses to elevated [O$_3$]

Previous studies have reported contrasting effects of warming and O$_3$ on plant physiology, rhizosphere chemical environment, and microbial communities (Changey et al 2018, Qiu et al 2018). In addition, some studies showed that moderate drought might alleviate the O$_3$ effects on photosynthesis, physiology, stomata characteristics, fine-root dynamics, and soil respiration (Xu et al 2017). However, the results of weighted meta-regression analyses in the present study revealed that the responses of soil C and N dynamics to elevated [O$_3$] were generally enhanced at low latitudes—warmer areas with higher precipitation. Note that the lower the latitude is, the longer the annual growing season as well as the effective O$_3$ fumigation time would be. In the present study, the average O$_3$ exposure duration was estimated to be approximately 6 months per year for sites south of 40° N, while it was about 4 months for sites north of 40° N. This may partially explain the detrimental O$_3$ effect at low latitudes. Moreover, soil microbial activities associated with N cycling, such as NTF and DNF, were usually stimulated with increasing temperature (Saad and Conrad 1993). Moreover, increased MAT and MAP usually accelerated soil N leaching losses from terrestrial ecosystems (Jabloun et al 2015). Thus, a decrease in soil N availability due to increased temperature and precipitation may exacerbate the negative O$_3$ effect on soil N pool (Lu et al 2011).

5. Conclusions and outlook

Understanding the effects of elevated [O$_3$] on soil C and N is important, since soil is a major C source or sink, and soil N availability not only limits plant growth but also controls soil N losses. Our meta-analysis revealed that elevated [O$_3$] substantially influenced both C and N dynamics, and thus altered the ability of the soil to store and cycle these nutrients. In general, under elevated [O$_3$], soil TC, DOC, TN and NH$_4^+$ contents decreased, microbial growth and NTF rate was inhibited, while soil NO$_3^−$ content, hence, DNF rate were stimulated. Soil NTF and DNF were more responsive to elevated [O$_3$] under higher O$_3$ levels, extended fumigation durations, and higher soil pH values. The response magnitudes of most variables to elevated [O$_3$] were exacerbated at low latitudes—warm areas with high precipitation, while they were generally independent of the fumigation method, terrestrial ecosystem types, and additional elevated [CO$_2$].

Meta-analysis has a very important role in generalizing across studies. The moderators in the present study, including discrete factors and continuous variables, provided useful information and enriched the understanding about discrepancies of soil C and N responses to elevated [O$_3$]. However, some
confounding effects among classes may influence the robustness of the meta-analysis. For example, the greater $O_3$ effects on rhizosphere soil than on bulk soil may be ascribed to the higher $O_3$ levels in the former. And in some cases, longer experimental duration conduced the limitation of lower level $O_3$ impacts on soil NTF in FACE and neutral soil conditions. Moreover, some controversial issues including NTF and NO$_3^-$ at a moderate $O_3$ level (figures 2 and S.5), NH$_4^+$ at short and long duration groups (figure S.6) cannot be fully explained due to large measurement uncertainties. Dose indices combining the duration and level of $O_3$ exposure may explain the resulting impacts more compactly. However, there is insufficient dose data in current literature. The limitations of the present study require further investigation in the future. In addition, a more comprehensive meta-analysis on both aboveground and belowground response are required to validate the indirect mediated hypothesis of $O_3$ impacts on soils. As the research progresses, an increasing number of associated data will be published, making it possible to reveal the response routes and mechanisms of the whole plant-soil system to elevated $[O_3]$. 

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: http://doi.org/10.6084/m9.figshare.16993174.

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