

Review

Elevated Tropospheric Ozone Concentration Alters Soil CO₂ Emission: A Meta-Analysis

Enzhu Hu ¹, Zhimin Ren ¹, Sheng Xu ^{2,*} and Weiwei Zhang ^{3,*}

¹ Institute of Resources and Environmental Sciences, School of Metallurgy, Northeastern University, Shenyang 110819, China; huez@smm.neu.edu.cn (E.H.); 1801644@stu.neu.edu.cn (Z.R.)

² Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

³ Ministry of Education Key Lab for Eco-Restoration of Contaminated Environment, Shenyang University, Shenyang 110044, China

* Correspondence: xusheng@iae.ac.cn (S.X.); zww@syu.edu.cn (W.Z.)

Abstract: Elevated tropospheric ozone (O₃) concentration may substantially influence the below-ground processes of terrestrial ecosystems. Nevertheless, a comprehensive and quantitative understanding of O₃ impacts on soil CO₂ emission remains elusive, making the future sources or sinks of soil C uncertain. In this study, 77 pairs of observations (i.e., elevated O₃ concentration treatment versus control) extracted from 16 peer-reviewed studies were synthesized using meta-analysis. The results depicted that soil CO₂ efflux was significantly reduced under short-term O₃ exposure (≤ 1 year, $p < 0.05$), while it was increased under extended duration (> 1 year, $p < 0.05$). Particularly, soil CO₂ emission was stimulated in nonagricultural ecosystems, in the free-air CO₂ enrichment (FACE) experiment, and in the soils of lower pH. The effect sizes of soil CO₂ efflux were significantly positively correlated with experimental duration and were significantly negatively correlated with soil pH, respectively. The ozone effect on soil CO₂ efflux would be enhanced at warm temperatures and high precipitation. The duration of O₃ exposure was the fundamental factor in analyzing O₃ impacts on soil CO₂ emission.

Keywords: elevated O₃ concentration; meta-analysis; soil CO₂ emission; climate change



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1. Introduction

Ozone (O₃) in the troposphere is not just a greenhouse gas but also an air pollutant that is prejudicial to human health and injures vegetation [1,2]. Tropospheric O₃ concentrations (hereinafter referred to as [O₃]) have significantly increased by 1–2% per year in recent decades due to accelerated industrial development and intensive combustion of fossil fuels [3].

As a highly oxidizing phytotoxic pollutant, O₃ could inhibit plant growth and cause considerable reduction in crop yields and quality [4]. Moreover, elevated [O₃] may impact the C allocation to roots [5], alter the rhizodeposition [6], change the quantity and composition of root exudate [7], modify the nutrient and energy supply to soil microorganisms [8], influence the microbial community diversity [9,10], and accordingly, influence soil CO₂ emission flux [11,12].

The rising levels of atmospheric O₃ make it difficult to assess the future soil C source/sink function. A few studies have reported increased CO₂ efflux under elevated [O₃] [13–16], but there are also results to the contrary [11,17]. These inconsistencies could be attributed to the variations in ecosystem type [18], O₃ exposure duration [19], fumigation facility [15], and the complexity of soil and climate conditions [20].

The purposes of this study were (1) to assess the responses of soil CO₂ emission flux to elevated [O₃] using meta-analytic techniques and (2) to reveal the possible sources of variation.

2. Materials and Methods

2.1. Data Sources

All peer-reviewed literature on soil CO₂ emissions with reference to elevated O₃ concentration were extensively searched through Scopus (<https://www.scopus.com> (accessed on 19 February 2021)) and ISI Web of Science (<http://apps.webofknowledge.com> (accessed on 19 February 2021)) with the following key terms: (O₃ OR ozone) AND (soil OR rhizosphere* OR terrestrial OR land) AND (carbon dioxide OR respiration* OR CO₂) AND (elevate* OR impact* OR affect* OR effect* OR alter* OR respond* OR decrease* OR increase*). The relevant Chinese literature were searched through the China National Knowledge Infrastructure Database (CNKI) (<http://www.cnki.net/> (accessed on 19 February 2021)). The search produced a total of 293 publications in ISI, 244 publications in Scopus, and 279 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, 28 publications were identified for further screening.

Data in the published sources were screened according to the following criteria: (1) only field studies were included; (2) experiments of control (ambient [O₃]) and O₃ enrichment treatments were carried out at the same site, with similar microclimate, vegetation and initial soil conditions; (3) the means, standard deviation (SD), and replicate numbers (*n*) could be obtained; (4) the facilities of O₃ fumigation were open top chamber (OTC) or free-air O₃ concentration enrichment (FACE); (5) publications and their measurements were excluded if the data were reported more completely in another source. According to these criteria, 7 studies based on pot-plants and 5 studies of root respiration or emissions from soil-plant ecosystems were excluded. The final database consisted of 16 articles published between 2001 and 2019, including 13 papers indexed by both ISI and Scopus and 3 studies indexed by CNKI (Appendix A). The data were acquired from 10 experiments performed at 32°03' N to 62°39' N latitude and 88°12' W to 119°42' E longitude. The mean annual temperature (MAT) ranged from 4.9 °C to 22.5 °C, and the mean annual precipitation (MAP) ranged from 307 mm to 1200 mm. The average increment in [O₃] across all studies was 23 ± 10 nL/L.

In the present study, the cumulative or average soil CO₂ efflux over a year or across a growing season was included as a single data point [21,22]. Thus, if the original study put forward the temporal dynamics of the variable, the annual or growing-seasonal averaged mean (\bar{M}) and standard error (\bar{SE}) were calculated as [23]

$$\bar{M} = \sum_{i=1}^j M_i / j, \quad (1)$$

$$\bar{SE} = \sqrt{\frac{\sum_{i=1}^j SE_i^2 (n_i - 1) n_i}{\sum_{i=1}^j n_i \cdot \sum_{i=1}^j (n_i - 1)}}, \quad (2)$$

where *j* is the observation times with each year or growing season (≥ 2), and *M_i*, *SE_i*, and *n_i* represent mean, standard error, and sample size in the *i*th observation, respectively.

Soil CO₂ efflux data were considered to be independent if they referred to multiple O₃ levels or to different additional treatments within a single study [24]. Values from context or tables were directly obtained, and data from the figures were digitized and extracted using GetData Graph Digitizer version 2.24 (<http://getdata-graph-digitizer.com> (accessed on 26 March 2008)). The unidentified error bar was assumed to be standard error [25]. Unidentified replicate numbers were assumed to be the replication of the plot [25]. The detailed dataset could be found in the *Mendeley Data* (doi:10.17632/jxfkh54hkh.1).

To explain variations in the responses of soil CO₂ efflux to elevated [O₃], the following categorical variables were chosen: (1) O₃ level: low ($\leq 1.5 \times$ ambient [O₃]), high ($> 1.5 \times$ ambient [O₃]); (2) exposure duration: short (duration ≤ 1 year), medium (1 year < duration

≤ 5 year), long (duration > 5 year); (3) soil pH: $\text{pH} \leq 6.0$ and $\text{pH} > 6$; (4) ecosystem types: agricultural and nonagricultural; (5) fumigation methods: FACE and OTC. Meanwhile, information such as source of data, study site, latitude, longitude, soil texture, and climate was acquired directly from the selected papers or their references (Mendeley Data, doi:10.17632/jxfkh54hkh.1).

2.2. Analysis

The meta-analyses were performed using MetaWin 2.1 software (Sinauer Associates, Inc., Sunderland, MA, USA [26]). For each pair of observation, the effect size and variance were calculated as

$$\ln R = \ln(X_E/X_C), \quad (3)$$

$$v = \frac{SD_E^2}{n_E X_E^2} + \frac{SD_C^2}{n_C X_C^2}, \quad (4)$$

where $\ln R$ is the effect size; X_E and X_C are annual or growing-season averaged means in the experimental (elevated $[\text{O}_3]$) and control treatments, respectively; n_E and n_C are the replicate numbers; and SD_E and SD_C are the standard deviations.

Before proceeding with the weighted analyses, normal quantile plots and the frequency distributions of $\ln R$ (Figure 1) were plotted to check the data normality [27]. A weighted random-effects model was used to calculate the overall and grouped effect size [28]. The 95% bias-corrected confidence intervals (CIs) around the effect size were assessed using a bootstrapped resampling technique with 64,999 iterations. The response to elevated $[\text{O}_3]$ was considered significant if the 95% bootstrapped CIs did not overlap zero. The O_3 -induced percentage change was calculated as $(R-1) \times 100\%$ [24]. Positive percentage change indicates an increase in the soil CO_2 efflux in response to elevated $[\text{O}_3]$, and a negative value indicates a decrease.

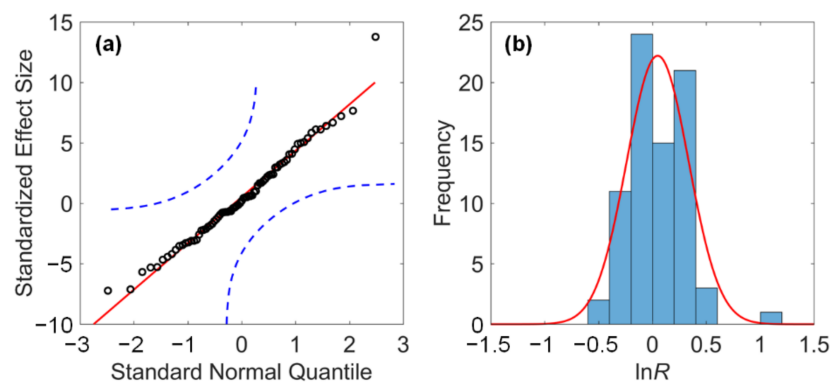


Figure 1. Normal quantile plots (a) and frequency distributions (b) of the natural logarithms of the response ratios for soil CO_2 efflux responses to elevated $[\text{O}_3]$. The red solid line in (a) is the diagonal reference line. The blue dashed lines in (a) show the Lilliefors confidence bounds. The red solid curve in (b) is a Gaussian fit to the frequency data.

The total heterogeneity of each variable (Q_T) was tested against a χ^2 -distribution with $n-1$ degrees of freedom (Table 1) [26]. It was also assessed using an I^2 index that quantifies the ratio of true variation caused by real differences between studies to total variation (true variation + sampling error) (Table 1) [29]. A positive I^2 value indicates true heterogeneity [30]. Afterwards, the categorical analysis was proceeded across all data by dividing the total heterogeneity (Q_T) into the within-group heterogeneity (Q_W) and between-group heterogeneity (Q_B). The dataset was then subdivided into different levels if the Q_B of categorical variables was significant, i.e., the randomized p -values < 0.05 (Table 1) [31]. Significant differences between two levels were reported if their 95% bootstrapped CIs did not overlap [32].

Table 1. The total heterogeneity (Q_T); percentage of heterogeneity due to true variation among effect sizes (I^2); and between-group heterogeneity (Q_B), estimated using resampling tests with 64,999 iterations to generate a randomized p -value for responses of soil CO₂ efflux to elevated O₃ relative to controls in a weighted random-effects meta-analysis with categorical structure.

Number of observations	77
Q_T	1131.41 ***
I^2	93
Q_B (O ₃ level)	0.43
Q_B (Exposure duration)	35.96 ***
Q_B (Soil pH)	28.46 ***
Q_B (Ecosystem)	12.49 **
Q_B (Fumigation method)	18.01 ***

p -values < 0.05 are considered significant. ** p < 0.01; *** p < 0.001.

A continuous meta-analysis model was employed to examine the relationship between $\ln R$ and environmental or forcing factors, such as duration of ozone exposure, soil pH, MAT, and MAP. Weighted regressions were used to test whether the slopes differed from zero using a parametric mixed model approach [26]. The regression relationship was considered significant if p < 0.05.

The possibility of publication bias within each group was firstly evaluated statistically with Kendall's tau rank correlation and Spearman's rho rank correlation between the standardized effect size and replicate number of each study (Table 2) [26]. Then, a Rosenthal's fail-safe number at $\alpha = 0.05$ was calculated (Table 2) [33]. A publication bias was confirmed only if all above methods showed positive results simultaneously (namely, if rank correlation tests were significant (p < 0.05) and Rosenthal's fail-safe number less than $5k + 10$, where k is the observation number).

Table 2. Kendall's tau and Spearman's rho rank correlation tests as well as Rosenthal's fail-safe numbers for assessing publication bias ^a.

Index	k	Kendall's Tau	Spearman's Rho	Rosenthal's Fail-Safe Number
Overall	77	0.56	0.54	NS ^b
O ₃ level	Low	0.44	0.49	NS
	High	0.80	0.89	NS
Exposure duration	Short	0.62	0.78	935.3
	Medium	0.87	0.83	751.1
	Long	0.88	0.92	732.1
Soil pH	pH ≤ 6	0.88	0.99	2520.9
	pH > 6	0.95	0.90	669.3
Ecosystem	Nonagricultural	0.89	0.99	1595.4
	Agricultural	0.73	0.64	NS
Fumigation method	FACE	0.64	0.73	2355.8
	OTC	0.06	0.03	NS

^a No publication bias if the p -values of rank correlation tests are >0.05 or if the Rosenthal's fail-safe numbers are greater than $(5k + 10)$. ^b NS means the response to e[O₃] was nonsignificant.

3. Results and Discussion

Although soil CO₂ efflux shows an increasing trend (4.6%) at elevated [O₃] compared with the control [O₃] across all studies (the grand mean change in Figure 2), it did not reach a significant level since its bias-corrected 95% bootstrapped Cis overlapped zero. However, the significant Q_T (p < 0.05) and positive I^2 values suggested heterogeneity among studies (Table 1). The Q_B values of most groups were significant, except for the O₃ level category (Table 1), which implied that the responses magnitudes of soil CO₂ efflux to elevated [O₃] were affected by O₃ experimental duration, ecosystem type, soil pH, and fumigation method.

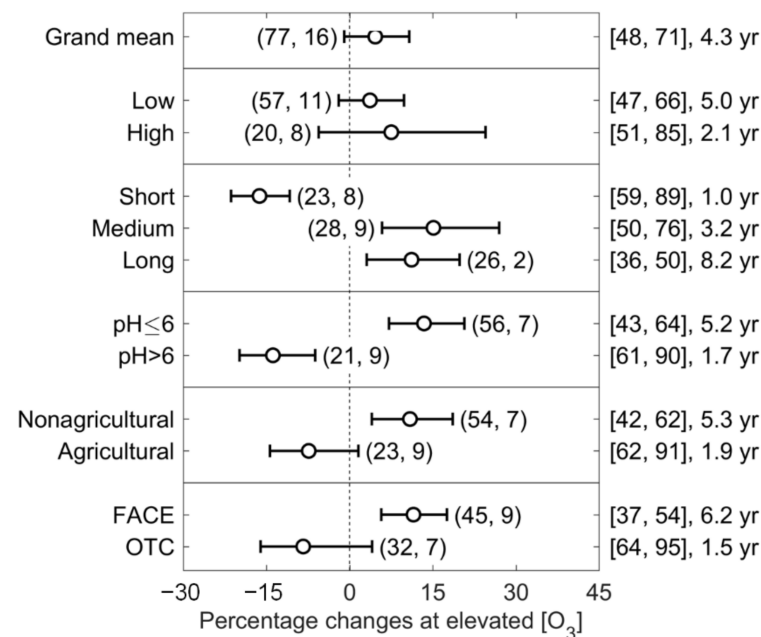


Figure 2. Overall and grouped cumulative responses with significant Q_B of soil CO_2 efflux to elevated $[\text{O}_3]$. Symbols represent the grand mean percentage change at elevated $[\text{O}_3]$ relative to control, and the bars show the 95% bias-corrected bootstrapped confidence intervals. The number of observations and articles are provided in parentheses. Mean control $[\text{O}_3]$ and elevated $[\text{O}_3]$ are given in brackets. The average fumigation durations are given on the right side.

3.1. Effect of O_3 Exposure Duration

As illustrated in Figure 2, there were significant differences in the responses of soil CO_2 efflux to elevated $[\text{O}_3]$ among three O_3 exposure duration levels. Statistically significant increases in soil CO_2 efflux were found in both medium (15.1%) and long (11.2%) O_3 fumigation ($p < 0.05$), while the opposite was observed in the short-term exposure (−16.3%, $p < 0.05$). The weighted meta-regression analysis showed a significantly positive correlation between $\ln R$ and O_3 exposure duration ($p < 0.01$, Figure 3a), which also showed that prolonged O_3 exposure could stimulate soil CO_2 emission.

Elevated $[\text{O}_3]$ is known to influence the transport of photosynthates from leaves to roots, reduce the quantity of root residue and exudates, and decrease belowground carbon allocation from root exudates to soil labile carbon pools [34]. Therefore, soil carbon contents generally decreased under elevated $[\text{O}_3]$ [11]. This may explain the decreased soil CO_2 efflux under short O_3 fumigation (≤ 1 year) since soil organic matter is the main carbon source for soil respiration and the energy source of microbial activity [35]. However, the responses of belowground processes to elevated $[\text{O}_3]$ is much less and slower than that of aboveground processes. Short-term O_3 exposure may give a false estimate of the O_3 effects, and longer-term studies would be more meaningful when the experimental results are interpreted by cumulative status [36]. Pregitzer et al. [13] reported an increase in fine-root biomass over a long-time O_3 exposure. The greater root biomass increased the autotrophic root component of soil respiration, and the root detritus increased the carbon source of soil microorganisms, hence improving the soil heterotrophic respiration. This may be the possible reason for the enhanced soil CO_2 efflux under prolonged O_3 exposure.

3.2. Effect of Soil pH

As shown in Figure 2, the pre-existing soil pH significantly affected the magnitude and even direction of soil CO_2 responses to elevated $[\text{O}_3]$: the O_3 effect was positive in soils of $\text{pH} \leq 6.0$ (13.4%), whereas it was significantly negative in soils of $\text{pH} > 6$ (−13.8%). There was also a significantly negative correlation between $\ln R$ and soil pH ($p < 0.001$, Figure 3b). O_3 exposure duration was longer in the group of soil $\text{pH} \leq 6.0$ (5.2 years) than that in the

group of soil pH > 6.0 (1.7 years), indicating that exposure duration may influence the results of soil pH categorization. In other words, the regression relationship between soil CO₂ efflux and soil pH may be essentially related to the regression relationships between soil CO₂ efflux and O₃ exposure duration.

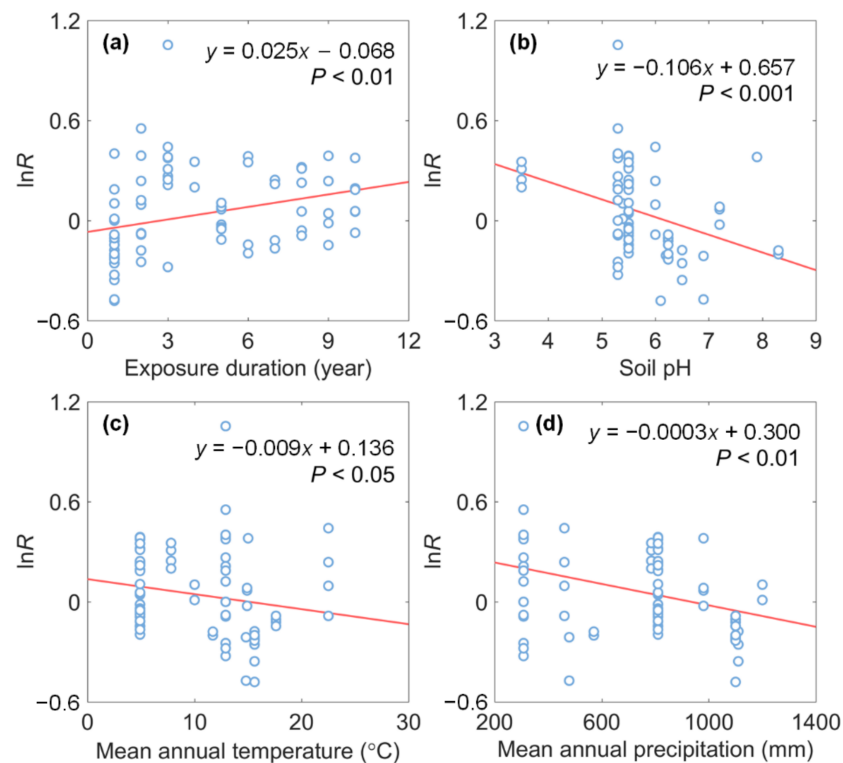


Figure 3. Weighted meta-regression between the effect sizes (the natural logarithms of the response ratios, $\ln R$, vertical axis) of response variables and environmental factors: (a) exposure duration, (b) soil pH, (c) MAT and (d) MAP, respectively. Solid line denotes weighted meta-regression.

3.3. Difference between Agricultural and Nonagricultural Ecosystems

In terms of ecosystem type, soil CO₂ efflux in response to elevated [O₃] was increased by 10.9% in nonagricultural soil and tended to decrease in agricultural soil (Figure 2). The O₃ exposure duration for nonagricultural ecosystems (5.3 years) was generally longer than that for agricultural land (1.9 years). Extended O₃ exposure duration may be beneficial to soil CO₂ emissions. Moreover, crops on agricultural land are mostly annual plants. The reduced crop residue consequently decreased soil organic matter under O₃ exposure, which may lead to a decrease in soil CO₂ efflux (Figure 2) [14]. However, plants in nonagricultural ecosystems, such as forest and grassland, are mostly perennials and include both tolerant and sensitive species or genotypes. Over a relatively long time of O₃ exposure, the tolerant species become more able to live and occupy the living space of sensitive species, resulting in a relatively increased total root biomass [13]. Greater root biomass would be conducive to soil autotrophic and heterotrophic soil respiration [13]. Consequently, the nonagricultural soil CO₂ efflux increased and responded more significantly to elevated [O₃] than the agricultural soil.

3.4. Effect of O₃ Fumigation Method

There was a significant difference on O₃-induced percentage change of soil CO₂ efflux between fumigation methods. Elevated [O₃] strongly increased soil CO₂ efflux by 11.5% in FACE experiments but did not induce significant changes in OTC experiments. The duration of O₃ exposure in FACE experiments (6.2 years) was generally longer than that in

OTC experiments (1.5 years), and longer O₃ fumigation was intended to exacerbate the O₃ effect.

3.5. Effect of Climate Conditions

Weighted meta-regression analyses in Figure 3c,d showed that that the effect size of soil CO₂ efflux was significantly negatively correlated with MAT ($p < 0.05$) and MAP ($p < 0.01$), respectively. This means the O₃ effect on soil CO₂ efflux was more detrimental (i.e., more negative effect sizes) in higher temperature and more precipitation areas.

Previous studies have reported contrasting effects of warming and O₃ on plant physiology, rhizosphere chemical environment, and microbial communities [37,38]. However, the results of weighted meta-regression analyses in the present study revealed that the negative responses of soil CO₂ efflux to elevated [O₃] were generally enhanced in warmer areas. In addition, some studies showed that moderate drought might alleviate the O₃ effects on photosynthesis, physiology, stomata characteristics, fine-root dynamics, and soil respiration [39]. In other words, the detrimental response of soil CO₂ efflux to elevated [O₃] would be enhanced at higher precipitation conditions, which is consistent with the negative meta-regression results in the present study.

4. Conclusions

Understanding the effects of elevated [O₃] on soil CO₂ emission is of great importance for assessing future soil C source/sink status since soil plays an important role in the global C budget. The present meta-analysis revealed that O₃ exposure duration was an important factor that controlled the O₃ effect on soil CO₂ emission. Soil CO₂ efflux was significantly reduced in short-term (≤ 1 year) O₃ exposure but increased in prolonged (> 1 year) duration. The response magnitudes of soil CO₂ efflux to elevated [O₃] were exacerbated in warm areas with high precipitation. The significantly stimulated soil CO₂ emission in nonagricultural ecosystems, in the FACE experiment, and in the soils of $\text{pH} \leq 6.0$ was associated with a longer experimental duration. Such responses need to be validated in further studies since the confounding effects among classes reduces the effectiveness of meta-analysis in generalizing across studies.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

The list of 16 papers from which the data were extracted for this meta-analysis.

1. Pregitzer, K.S.; Burton, A.J.; King, J.S.; Zak, D.R. Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO₂ and tropospheric O₃. *New Phytol.* **2008**, *180*, 153–161, doi:10.1111/j.1469-8137.2008.02564.x.
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