Letter to editor in response to

# Positive soil priming effects are the rule at a global scale

Xu, S., Delgado-Baquerizo, M., Kuzyakov, Y., Wu, Y., Liu, L., Yang, Y., Li, Y., Yu, Y., Zhu, B., & Yao, H. (2024). *Global Change Biology*, 30, e17502. https://doi.org/10.1111/gcb.17502.

### What if publication bias is the rule and net carbon loss from priming the exception?

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Two aspects need to be discussed to clarify the ecological context of (rhizosphere) priming effects.

## (1) No net carbon loss

Priming studies should as a rule evaluate the net carbon balance by juxtapositioning the quantities of primed carbon against the amounts of experimentally added carbon. Because in most studies, including those reporting exclusively positive priming effects, the net carbon balance is positive, meaning that there is no net carbon loss despite positive priming being reported (Liang et al., 2018; Qin et al., 2024, Fig.1a). This applies for both soil and field experiments. For example, Schiedung et al. (2023) evaluated priming effects along a 20-year chronosequence of land inversion to identify the dependence of priming effects on root-derived C in top and sub soils. Overall, carbon losses from priming never exceeded new root-derived carbon inputs. Similar observations were made by Yin et al. (2019) who studied rhizosphere priming effects and microbial biomass dynamics of two wheat genotypes under two temperatures and found no net soil organic carbon loss or gain as carbon loss caused by higher priming was counteracted by increased microbial growth/turnover. And Cardinael et al. (2015) used a 52-year long field experiment where SOC stocks of fallow fields were compared to SOC stocks of fields regularly receiving fresh or composted straw to show that no significant difference in SOC stocks dynamics occurred over the 52 years, suggesting no long-term impact of priming. It is further worth mentioning that ten of the 12 meta-analyses compiled in Xu et al. report data exclusively from soil incubations. Soil incubations are a reductionist method for studying priming in the absence of living plants, usually conducted with sieved soils under standardised temperature and soil moisture conditions. They don't account for plant nutrient and water uptake and mostly don't investigate the diversity of organic carbon inputs in terrestrial ecosystems, nor their diurnal and seasonal variation. This limits the scalability of priming effects observed in soil incubations to ecosystem processes (Chen et al., 2023).

#### (2) Overrepresentation of positive priming in the literature

A common graphical tool to assess study biases and (in)consistency of study results across different sample sizes are funnel plots (Viechtbauer, 2010; Shi & Lin, 2019). The funnel plot provided by Xu et al. (SupMat S2) plots "percent change" on the x-axis. A funnel plot is meant to show the distribution of effect sizes across studies. Transforming the effect size on the x-axis into percent change distorts the comparison, especially as the studies are not all reporting the same type of effect (e.g. RR, OR). On the y-axis, Xu et al. plotted variance (vi). Variance measures variability within a study but doesn't directly reflect the precision of the effect size estimate. Larger sample sizes reduce standard errors (SE), which explicitly measure precision, even if variance remains large. Funnel plots use SE on the

y-axis because it directly reflects how precisely each study estimates the true effect. Using variance does not give the same insight because variance does not correlate as directly with estimation precision. Therefore, we redid the plot using standard error (SE) on the y-axis and lnRR on the x-axis; and then used Heterogeneity (I<sup>2</sup>), Egger's test and the trim-and-fill method to identify potential unbalance (Viechtbauer, 2010, Fig.1b). The revised funnel plot has a clear asymmetry towards the right (positive values) indicating publication bias in this direction. The  $l^2$  of 75.48% (Q-test: p < 0.0001) indicates a moderate-to-high level of heterogeneity between the studies, suggesting variability in the effect sizes across the studies, which can be caused by different methods amongst the studies and/or publication bias. The pooled effect size at this stage is 0.1147 (CI<sub>95</sub>: 0.0879, 0.1415) and statistically significant (p < 0.0001). The trim-and-fill method was then used to further evaluate publication bias by estimating and adding missing studies to improve the symmetry of the effect size distribution (Shi et al., 2019). The estimated number of missing studies on the left side to achieve symmetry was n=6 (SE = 5.2244) and imputing them theoretically provided a new estimated effect size (log-transformed response ratio) of 0.1022 (CI<sub>9</sub>: 0.0740, 0.1305, p-value < 0.0001). This corresponds to a percent change in PE of around 10.7%, which is much lower than the values given by the authors. It is also critical that the abstract mentions a PE of (+125%), without mentioning that this high value is likely an outlier, and the corrected PE estimate is (+28%). Such selective presentation of data reinforces the already problematic bias towards positive PE and systematically suppresses studies reporting more moderate or even negative priming effects. To generate a complete understanding of priming effects, it is necessary to discuss how we can overcome common phenomena like confirmation, expectation and positivity biases and their impact on the way priming is presented in the peer-reviewed literature (Oswald & Grosjean, 2004; Jeng 2006).

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**Figure 1 (a)** Net carbon balance of the studies included in the meta-analysis of Qin et al. (2024) and **(b)** revised funnel plot of logged response ratio and standard errors of the priming studies included in Xu et al. (2024)