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

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Abstract

Priming effects in soil science describe the influence of labile carbon inputs on rates of microbial soil organic matter mineralisation, which can either increase (positive priming) or decrease (negative priming). While both positive and negative priming effects occur in natural ecosystems, the latter is less documented in the peer-reviewed literature and the overall impact of priming effects on the carbon balance of vegetated ecosystems remains elusive. Here, we highlight three aspects which need to be discussed to ensure (rhizosphere) priming effects are correctly perceived in their ecological context and measured at appropriate scales: (i) We emphasize the importance of evaluating net C balances because usually experimental C inputs exceed C losses meaning even positive priming doesn't cause net C-loss; (ii) We caution against publication bias, which forces overrepresentation of positive priming effects, neglects negative or no priming, and potentially misguides conclusions about C loss; and (iii) We highlight the need to distinguish between general priming effects and rhizosphere-specific priming, which differ in their scale and driving factors, and hence require different methodological approaches. Future research should explore potential discrepancies between laboratory and field studies and examine the role of rhizosphere priming in nutrient cycling and plant nutrition.

More nuance and context in (rhizosphere) priming papers is needed

Rhizosphere priming effects refer to the changes in soil microbial activity and nutrient cycling caused by root exudates from plants. The labile carbon compounds in exudates can either stimulate microbial growth and metabolism, leading to increased mineralization of soil organic matter (positive priming), or decrease microbial soil mineralisation when microbes assimilate primarily plant-derived carbon (negative priming) (Kuzyakov et al., 2000; Blagodatskaya et al., 2011; Dijkstra et al., 2013). Both positive and negative priming effects are commonly reported in the literature, and they are not mutually exclusive in ecosystems (Bastida et al. 2019; Feng & Zhu, 2021; Michel et al. 2024). In

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many studies, observations include both positive and negative priming either depending on experimental condition, or sometimes substrate amendments also result in mixed positive, negative and/or no priming within one unique modality (Chen at al. 2014; Qiao at al. 2016; Heitkötter at al. 2017; Hicks at al. 2019; Michel et al., 2022). Individual priming effects are mostly short-term phenomena, but continuously occur in the rhizosphere of living plants, where active root exudation provides energy-rich labile carbon to soil microbes, while rhizodeposition also supplies more complex substances like cellulose to the soil (Canarini et al. 2019; Villarino et al. 2021). While it is increasingly recognised that priming effects are an important mechanism to regulate plant nutrition, the impact of priming effects on the overall carbon balance remains controversial (Dijkstra et al., 2013; Zhu et al. 2014; Holz et al., 2023, Pausch et al., 2024). Here, we highlight three aspects which need to be discussed to ensure (rhizosphere) priming effects are correctly perceived in their ecological context and measured at appropriate scales to avoid a one-sided narrative distorted towards carbon loss caused by positive priming.

(i) The first aspect is that there is little empirical evidence for net C losses from priming as in most studies, including those reporting exclusively positive priming effects, the experimentally added quantities of carbon to the study system exceed the amounts lost in basal and primed respiration.

(ii) The second aspect is that publication bias is critical, with studies tending to overrepresent positive priming and inferring C loss without empirical evidence.

(iii) The third aspect is a lack of distinction between priming effects (PE) and rhizosphere priming effects (RPE) which are measured at different scales, have different drivers and therefore differ in their ecological interpretability.

i) Even positive priming effects seldom cause net carbon loss

Many studies focus on carbon losses from (positive) priming effects, which has been the historic narrative in priming literature (e.g. Löhnis, 1926; Jenkinson et al. 1985). Yet, more recent studies with a more comprehensive view on carbon budgets revealed that there is little evidence for net carbon loss from priming effects (Qiao et al., 2014; Liang et al., 2018; Siles et al., 2022; Qin et al., 2024; Chen et al. 2025). For example, a recent meta-analysis evaluating the impact of priming effects derived from crop residues and their interaction with nitrogen inputs concluded that there was no carbon loss despite the positive priming reported (Qin et al. 2024; Figure 1a). This finding aligns with assessments in many soil incubation studies which demonstrate a net carbon balance in favour of C sequestration because in these experiments the carbon inputs from labile substrates usually exceed the carbon outputs from basal and primed respiration by at least one order of magnitude (Qiao et al., 2014; Cardinael et al., 2015; Liang et al., 2018; Schiedung et al., 2023; Qin et al., 2024). In accordance with these observations in lab incubations, several studies upscaling priming effects over longer time scales and to areas of several hectares also indicate that priming effects may not change

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overall C-budgets. For example, Schiedung et al. (2023) evaluated priming effects along a 20-year chronosequence of land inversion in New Zealand to identify the dependence of priming effects on root-derived C in topsoil and sub soils. Even though positive priming was reported, overall, carbon losses with 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 carbon dynamics of two wheat genotypes grown under two temperatures and found no net soil organic C loss or gain as C loss caused by higher RPE was counteracted by increased microbial growth/turnover. Similarly, 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 effect. Equalising priming with carbon loss is hence not a valid conclusion and to avoid misleading the reader, where possible studies should evaluate the experimental carbon inputs and outputs and report the net C balance.

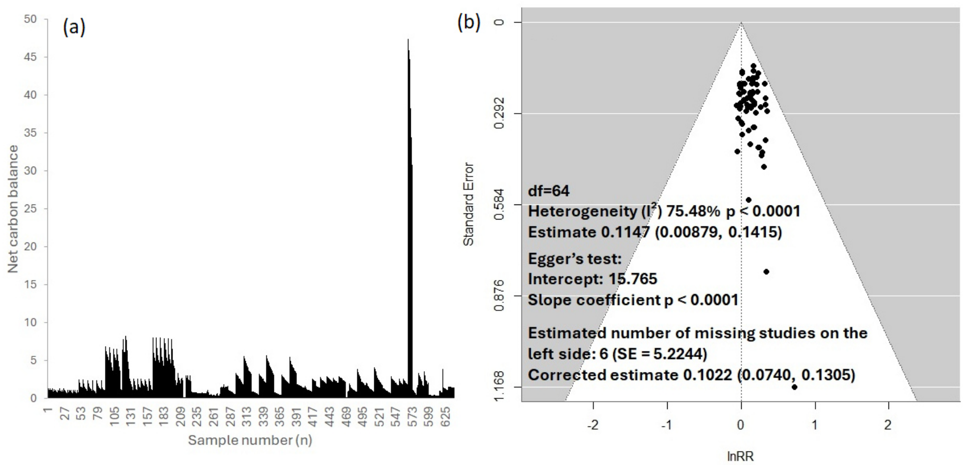

Figure 1 (a) Net carbon balance of the studies included in the meta-analysis of Qin et al. (2024) and (b) revised funnel plot of logged response ratio and standard errors of the priming studies included in Xu et al. (2024).

ii) Publication bias causes overrepresentation of positive priming in the literature

Another recent meta-analysis claimed positive priming effects were globally dominant, but also indicates the influence of publication bias (Xu et al., 2024). For some ecosystems such as tundra and wetlands, a priming estimate of +125% was obtained, but graphical analysis of the data distribution suggested these values are likely biased and excluding them dropped the priming estimate to 28(+4)%. As for a balanced scientific discourse and a strong statement about the global direction of priming effects careful evaluation of publication bias is imperative, especially in meta-analysis, the

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data was here subjected to a re-evaluation of potential publication bias (Figure 1b). Funnel plots are a common graphical tool in meta-analysis to visually assess the presence of publication bias and to check for the consistency of study results across different sample sizes (Viechtbauer, 2010; Cleophas et al., 2017; Shi & Lin, 2019). While different methods exist, usually the measure of the effect size (e.g., mean difference, odds ratio, etc.) from each individual study is plotted on the x-axis, and on the y-axis the standard error of the effect size or another measure of the precision of each study. The higher the standard error, the less precise the estimate. Funnel plots are then evaluated for symmetry: in the absence of bias, they should resemble an inverted funnel, with larger (more precise) studies at the top and smaller (less precise) studies scattered at the base. Asymmetry may suggest publication bias, such as an overrepresentation of small studies with large effects due to selective publication of positive findings. The triangle represents the 95% confidence interval, and studies outside this interval may indicate heterogeneity (I^2) or bias. Heterogeneity reflects inconsistent results caused by variations in study design, populations, interventions, or actual outcomes. Additionally, variability in methodological quality or publication bias, particularly if studies cluster at one end, can contribute to asymmetry, making it a key indicator of bias.

The original funnel plot by Xu et al. (2024) plots "percent change of priming effects" on the x-axis and "variance (vi)" on the y-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. report response ratio (RR), odds ratio (OR), etc.). Therefore, the percent change was back transformed to lnRR to redo the plot. On the y-axis, originally the variance (vi) was plotted. Variance, the square of the standard deviation, 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, a revised funnel plot was made using standard error (SE) on the y-axis and lnRR on the x-axis and then used Heterogeneity, Egger's test and the trim-and-fill method to identify potential asymmetry and bias in the meta-analysis. The analysis was performed using R (R Core team, 2024) with the additional package metafor (Viechtbauer, 2010). The revised funnel plot (Figure 1b) has a clear asymmetry towards the right (positive values) indicating publication bias in this direction. The I^2 of 75.48% (Q-test: $p < 0.0001$) indicates a moderate-to-high level of heterogeneity between the studies, which suggests variability in the effect sizes across the studies included in the meta-analysis. Potential sources of this variability are different methods amongst the studies or true publication bias. The pooled effect size at this stage is 0.1147 (CI_{95}: 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

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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_{95}: 0.0740, 0.1305, p-value < 0.0001). This corresponds to a percent change in PE of around 10.7%. It would be interesting to recalculate a global PE estimate from the primary research data of all underlying meta-analysis corrected for publication bias.

Positive priming effects are predominantly reported, but this analysis suggests that there is significant publication bias which may systematically suppresses studies reporting more moderate or even negative priming effects. Therefore, it seems very important to encourage the publication of studies observing no or negative priming to avoid reinforcing an already problematic bias towards positive PE, and in the worst case even falsely inferring C losses. To generate a complete understanding of priming effects, it is further necessary to discuss how common phenomena like confirmation, expectation, publication and positivity biases can impacted the way priming is presented in the peer-reviewed literature (Jennions & Møller, 2002; Oswald & Grosjean, 2004; Jeng 2006; Hoorens 2014).

iii) Methodological mismatch? Limited scalability of soil incubations and the need to differentiate priming effects from rhizosphere priming effects

'Priming effects (PE)' refer to interactions between soils, soil microbes and added substances, while 'rhizosphere priming effects (RPE)' more specifically describe the interactions between living plant roots, their exudation and other rhizodeposition, rhizosphere microbes and rhizosphere soils. It is important to distinguish between the two, because they differ in their driving factors and the scale of inference. Priming effects are caused by a static, sometimes repeated, source of substrate input, and usually measured in soil incubation. Rhizosphere priming effects describe changes in SOM mineralisation in the root zone, and are hence subject to dynamic changes in C and nutrient supply and demand, where the plant acts simultaneously as a sink for nutrients and water and a source of carbon. Hence, several plant physiological parameters like rate of photosynthesis and root exudation are also determinant for rhizosphere priming effects (Dijkstra et al. 2013; Yin et al. 2018; Tang et al. 2019). It is important to acknowledge the limitations in the scalability of isolated soil incubations to ecosystem processes given that carbon, nutrient and water pools and fluxes are different in the rhizosphere of living plants as compared to reductionist lab incubations. Moreover, soil incubations are usually conducted under standardised conditions of temperature and soil moisture, and usually soils are sieved before the incubation. Therefore, we have limited knowledge of priming effects in intact soils under variable environmental conditions, and cannot conclude about an impact of priming effects at ecosystem scale based on this data (Chen et al., 2023). Therefore, it is crucial for future studies to assess whether estimates of priming effect (PE) and mechanistic insights derived from soil incubations accurately reflect processes of rhizosphere priming effects (RPE) in natural ecosystems.

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Conclusion

Priming papers should as a rule evaluate the net C balance by juxtapositioning the quantities of primed C and added C to understand whether C has been lost from the system or not. Because often there is no net C loss from soil despite positive priming being reported. To reliably determine the direction of priming across several studies, publication bias needs to be evaluated very carefully, ideally at the level of first-order meta-analysis already. And prior to that, publication of negative or no priming effects should be encouraged. Future studies should also investigate potential discrepancies between soil incubations and field experiments and could address the potential to leverage rhizosphere priming effects to optimise plant nutrition.

Data availability: The data re-analysed here is available in the cited papers and respective supplementary material.

Author contribution: JM analysed the data and wrote the first draft. All authors critically evaluated the manuscript and approved the final version.

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