

We would like to thank both reviewers for their comments, many of which were extremely useful and helped us improve the manuscript. On the few occasions that we disagreed with the reviewers we have argued our case and amended the text. We hope that our responses are satisfactory. We have acknowledged the reviewers constructive comments in the manuscript.

Reviewer 1

This study addresses the question of decomposability of organic matter in hydromorphic podzol soils from the Amazon basin. These soils are extremely interesting given their debated origin and vulnerability to environmental change. Through a set of incubations, the authors of this study found a strong response of C mineralization to oxygen and nitrogen additions, providing important information to improve our understanding of the main environmental controls that promote the stability of carbon in their Bh horizon.

In general, this is a very good study that merits publication in Biogeosciences. However, I do have a few major comments that prevent me to recommend publication in its current form.

Major comments

1. I strongly dislike the title of this study. The authors do not provide here any analysis of the explosiveness of this carbon reservoir and when such a 'bomb' explosion would occur. I know the term 'carbon time bomb' is used here metaphorically, but I do not find evidence in the results for such a metaphor. The study helps to understand the mechanisms for carbon stability in these soils, but this does not mean that there is a threat for a sudden increase in oxygen and nitrogen levels at 2 or 3 meters below the surface across the entire Amazon basin that would justify the metaphor of a carbon bomb. I think the title should emphasize the relevance of this study for understanding mechanisms, and do not trick readers with a suggestive title that is far from scientific rigor.

Response: we have proposed a new title that is more factual and less dramatic – “Vulnerability of soil organic carbon in Amazonian Podzols to changes in environmental conditions.”

2. Similarly, the analysis of the contribution of these soils to total respiration globally based on an extrapolation of the incubation results are out of place. Even though the authors took care to conduct the incubations with undisturbed cores, the treatment themselves are highly artificial. Again, the treatments are very useful to understand mechanisms of carbon stability in these soils, and this is the main contribution of the study. But I do not see the point to extrapolate those incubation results to the entire Amazon. I think the authors should stick with the main contribution of their results to scientific understanding and avoid speculative analyses.

Response: if the reviewer doesn't mind, we would like to keep this extrapolation. We have toned it down though, removed it from the abstract and removed parts of the discussion. Further, we have added a sentence along the lines of what the reviewers states about the artificial nature of the treatments. Accordingly, the last sentence of the abstract has been replaced with “The data suggest that the large pool of C in Amazonian Podzols may be vulnerable to increases in N and O₂ availability.”

The section of the results and discussion now reads - “In view of the very large quantities of organic C that are stored in the Bh horizon of the Amazonian Podzols (Montes et al., 2011), we

sought to estimate the annual CO₂ flux from these horizons to the atmosphere under the different conditions tested here. We first fitted a first order decay model with a single pool to the respiration data (Fig S4 – best fit based on the Akaike Information Criterion) and extrapolated the mineralisation curves to a year. Montes et al. (2011) estimated that 78.8 % of the 13 Pg C in the Podzol profiles is found in the Bh horizon (10.45 Pg C), which we used to estimate the potential total C fluxes from the Bh Horizon of the Amazonian Podzols (Fig. 4). The increase in CO₂ flux in the oxic treatment with N translates to an extra 0.41 Pg C yr⁻¹ being released to the atmosphere compared to the anoxic treatment ($P < 0.05$). The other two oxic treatments also resulted in increases in the amount of C released, but these differences were not statistically significant.

Global soil respiration estimates are subject to large uncertainties, due to the complex set of biogeochemical and biophysical processes that are involved. These uncertainties are one of the major causes of uncertainty in terrestrial ecosystem models (He et al., 2022). Nevertheless, a recent study has estimated global soil heterotrophic respiration to be 48.8 ± 0.9 Pg C yr⁻¹ (He et al., 2022). The potential increase in CO₂ flux from Amazonian Podzols could therefore be equivalent to 0.8% of global soil heterotrophic respiration. ”

3. I was very surprised by the results presented at line 169 on the respiration from the E horizon. My own analyses of carbon content and respiration from this horizon had shown no detectable amounts of carbon and respiration in this horizon. So, I found strange that the authors report higher mineralization rates here. However, Figure 1 shows that total C in the E horizon is zero or close to zero, which means that when you compute the specific mineralization rates dividing the values by a number close to zero, the values artificially increase. It is well known that when you divide any number by a value close to zero, the result is a very large number. Or in mathematical jargon, the limit goes to infinity. Therefore, the specific mineralization rates presented in Figure 2B for the E horizon are an artifact due to the division by a very small total C value. This should be corrected and removed from the discussion.

Response: here, we must respectfully disagree with the reviewer. The average total C value for the E horizon 2.24 mg/g soil. Whilst this value is certainly low, it is not 0. The very high total C content of the Oh horizon gives the impression that low values are lower than they actually are. The mineralisation rate per unit soil carbon is a useful indicator as it provides us with information on the decomposability of the organic matter present in the sample (Lomander et al., 1998; Fierer et al., 2003; Salomé et al., 2010) and, indeed, in two of these papers (Fierer et al., 2003; Salomé et al., 2010) the C content of the deep soil was lower, without the indicator being extremely high. In the case of this study, it is different. Here the indicator shows us that, despite there being very little C, it is readily mineralised. The C is far more readily mineralised in the E horizon than in the Bh horizon, which is interesting information as it suggests that the C input to the Bh horizon is labile. We propose to keep this information as we feel that it provides extra, useful information. We have, however, added a sentence to draw the reader's attention to the low amounts of C in the E horizon and the possibility that this may underly the high specific mineralisation rates - "These very high rates may be linked to the low total C content of the E horizon (2.3 mg g⁻¹ soil), however, others have not found such high specific mineralisation rates, even in deep soil where total C contents were lower than what was observed here (Fierer et al., 2003; Salomé et al., 2010). It is more likely therefore, that the high specific mineralisation rates suggest that the organic C in this horizon was highly labile and readily available. ”

Minor comments

- The authors cite the conceptual framework of Mayano et al a number of times to put the results in a larger context. This is very good, but I also would bring to the attention the conceptual framework of Davidson et al. (<https://doi.org/10.1111/gcb.12718>), which is very similar to that of Moyano, but expressed under a mathematical framework that is testable. I would recommend the authors to consider framing the study and results in the context of interactions between moisture and oxygen availability as in the DAMM model of Davidson. There are studies that have tested this framework experimentally manipulating oxygen and moisture levels simultaneously (<https://doi.org/10.5194/bg-14-703-2017>), and I think the present study adds important experimental support to the DAMM theoretical framework.

Response: we thank the reviewer for this very useful comment. We have used these two references to develop the discussion. However, we are not in a position to test the framework as in Sierra et al. because the experiment is not fully factorial. The treatments were all individual.

We have added the following to the discussion - “ Sierra et al. (2017) showed that moisture effects on decomposition rates are strongly modulated by O₂ availability. As the changes in moisture content in this experiment were small, then O₂ availability inside the cores may not have changed much, thus reducing any matric potential effect on decomposition.”

- Add the duration of the incubations to the caption of Figure 2.

Response: Done.

- I also found very interesting the results of the addition of the mixture of carbon compounds because they do not provide any evidence for the priming effect. In general, I see a bias in the literature that generally gives a lot of emphasis to results that support the priming effect, but when results are not consistent with the priming hypothesis they are not mentioned. I think it's important to also bring this to the attention for a more balanced discussion on the relevance of priming. For these soils, it seems that priming is not a relevant mechanism.

Response: the reviewer makes a good point. We should have discussed this more and have done so now in the results and discussion section – “There was a CO₂ pulse after both additions of the substrate cocktail, but this was due to the mineralisation of the substrate-C that was added rather than an increase in the mineralisation of Bh horizon organic C (Figs 3 & S3). The lack of a priming effect may be due to the low pH of the soil (Fig 1). The priming effect is more common in soils with pHs between 5.5 and 7.5 but tends to be lower at the pH values found here (Wang and Kuzyakov, 2024). Furthermore, it has been shown that soils that are characterised by high levels of mineral associated organic C, as is the case in the Bh horizons of Podzols (Schmidt et al., 2000; Doupoux et al., 2017), also tend to be less prone to the priming effect (Chen et al., 2019). It should also be noted that a month after the first addition of the substrate cocktail, only 22% of the added C was mineralised, and only 15% was mineralised slightly more than a month after the second addition (Fig S3). These mineralisation rates are lower than what is usually found. The mineralisation of glucose often exceeds 60% after a month's incubation (e.g. Hamer and Marschner, 2002), while that of pyruvate and vanillin can

exceed 30% (Chenu et al., 2025) and 20% (Juarez et al., 2013), respectively. These low mineralisation rates may also have been due to an N limitation, but this would have to be confirmed experimentally. Nitrogen limitation can arise due to microbial cells being unable to produce proteins, such as enzymes or membrane transport proteins, necessary for activity, as proteins are N rich molecules (Nunan et al., 2020).”

- In the methods section, you mentioned that the added carbon mixture was labelled with ¹³C. Did you obtain any insights from the analysis of ¹³C in respiration or in the remaining soils after the incubation?

Response: the reviewer is quite right in implying that we have under exploited these data. We have now added the following to the results and discussion (it is the same as the addition mention in our previous response) - “There was a CO₂ pulse after both additions of the substrate cocktail, but this was due to the mineralisation of the substrate-C that was added rather than an increase in the mineralisation of Bh horizon organic C (Figs 3 & S3). The lack of a priming effect may be due to the low pH of the soil (Fig 1). The priming effect is more common in soils with pHs between 5.5 and 7.5 but tends to be lower at the pH values found here (Wang and Kuzyakov, 2024). Furthermore, it has been shown that soils which are characterised by a high mineral associated organic C, as is the case in the Bh horizons of Podzols (Schmidt et al., 2000; Doupoux et al., 2017), also tend to be less prone to the priming effect (Chen et al., 2019). It should be noted, however, that a month after the first addition of the substrate cocktail, only 22% was mineralised, and only 15% was mineralised slightly more than a month after the second addition (Fig S3). These mineralisation rates are lower than what is usually found. The mineralisation of glucose often exceeds 60% after a month’s incubation (e.g. Hamer and Marschner, 2002), while that of pyruvate and vanillin can exceed 30% (Chenu et al., 2025) and 20% (Juarez et al., 2013), respectively. These low mineralisation rates may also have been due to an N limitation, but this was not confirmed experimentally. ”

Reviewer 2

This manuscript explores the potential for carbon mineralization across different soil horizons in Amazonian Podzols under varied incubation conditions (anoxic, oxygenated, oxygen + nitrogen, and oxygen + simple organic substrate). The idea is novel and highly relevant, especially given the global interest in tropical soil carbon dynamics and climate change. However, despite the interesting premise, the manuscript has serious structural and conceptual issues that must be addressed before it can be considered for publication. Firstly, the manuscript requires a thorough English language revision, as grammatical issues and awkward phrasing hinder readability and comprehension. The title is also problematic: it is provocative but unclear, and does not effectively convey the main focus or findings of the study. While the introduction provides a general overview of the topic, it suffers from repetition and lacks a clear statement of the hypothesis and objectives. The Materials and Methods section is particularly problematic. It lacks clarity in several areas, including sample numbers, experimental design, and site characterization. The Results and Discussion sections are underdeveloped and should be

clearly separated. The current discussion does not critically engage with the results or their broader implications for greenhouse gas emissions and tropical soil processes.

Response: we have proposed a new title that is more factual and less dramatic – “Vulnerability of soil organic carbon in Amazonian Podzols to changes in environmental conditions.”

Specific Comments

Introduction

The introduction should be revised to eliminate redundancy and improve logical flow. Most importantly, explicitly state the study's hypothesis and objectives at the end of the introduction.

Response: it is not clear to us what redundancy the reviewer is referring to and the logical flow is logical for us. However, we have added the hypotheses around which the study was design, which were indeed missing. We have added the following hypotheses to the introduction - “ The hypotheses were fourfold. The first was that reductions in moisture content from saturation to a matric potential of approximately -31.6 hPa result in increases in CO₂ emissions, due to increased O₂ availability in the pore space (Moyano et al., 2012; Sierra et al., 2017), but that further decreases in moisture content result in lower CO₂ emissions, due to reductions in the diffusion of C-substrates towards enzymes or the diffusion of enzymes towards insoluble C-substrates (Davidson et al., 2014). The second hypothesis was that anoxic conditions were responsible for the slow decomposition rates (Sierra et al., 2017; Davidson et al., 2014) and that decomposition is stimulated by increases in O₂ levels. he third hypothesis was that decomposition is N limited, as indicated by the characteristically high C:N ratios of Podzol Bh horizons (Montes et al., 2023) and, therefore, that the addition of mineral N stimulates decomposition. The fourth and final hypothesis was that the addition of readily available sources of C results in a priming effect (Fontaine et al., 2007) that releases CO₂ from Bh horizon organic matter.”.

We have also developed the paragraph on the priming effect to ensure that the ideas are clear to the reader. It now reads “As indicated above, the mineralisation of old, deep soil C can be stimulated by inputs of fresh, labile organic matter (Fontaine et al., 2007). This phenomenon is known as the “priming effect”. The priming effect is believed to arise due the inputs of fresh organic matter causing microbial communities to mine for N by decomposing organic matter or due to soil organic matter being co-metabolised during with the frsh inputs (Blagodatskaya & Kuzyakov, 2008). The death of plant biomass and its subsequent decomposition is likely release significant amounts of labile organic matter which could stimulate the mineralisation of the Bh horizon organic C.”

Materials and Methods

Geographic context: The Cabeça do Cachorro region is not widely known. Please include a simple map in the supplementary material to show the location.

Response: Done. A map indicating the region from which the samples were taken has been added to the supplementary materials. The map can be found in Fig S1 with the following legend - “Location of the studied profiles. Grey areas in the detailed map indicate hydromorphic podzol areas. Orange spot indicates area from where samples were taken.”

Sampling design: Clearly state the number of samples collected. From the current description, it appears to be 12 (3 sites × 4 horizons), but this should be explicitly confirmed.

Response: the reviewer is correct that the sampling was not as clearly described as it should have been. There were indeed 3 sites and 4 horizons. However, we took more than one core from each horizon at each site. There was one core taken from each site x horizon for each experimental treatment. We have added the following to the text - " One undisturbed sample was taken from each site x horizon per incubation treatment (see below) and for the establishment of moisture release characteristics, resulting in 10 undisturbed samples being taken from each horizon at each site, and a total of 120 samples. "

Site and soil description: Provide more information about the sampling site, including general environmental conditions and soil profile characteristics. For example, what are the typical depths or thicknesses of the soil horizons?

Response: We have added a description of the Podzol that was sampled - " The profiles at the three sites were typical Amazonian Podzols. They made up of a waterlogged O horizon of about 15cm, an E horizon that was also waterlogged and slightly less than a meter thick, a silt-loam Bh horizon that was slightly over a meter thick underneath which there was a C horizon. "

Visual documentation: Consider adding photos of the soil profiles and the incubation setup to the supplementary material, as these can enhance understanding of the experimental context.

Response: This is not possible. Undisturbed cores were sampled with a corer which had a shaft that could be extended to several meters. We did not open up any profiles for the sampling and therefore we do not have any photos.

Lines 120–122: This sentence is unclear and should be rephrased for clarity.

Response: The sentence has been rewritten as several sentences and is now as follows - "Two microcosm incubation experiments were set up in order to measure CO₂ emissions from samples in response to changes in environmental conditions. The first incubation measured the response to changes in moisture content and lasted 68 days. The second incubation measured changes in CO₂ emission in response to O₂, mineral N or substrate-C availability and lasted for 72 days. Both sets of incubations were carried out at 28°C in the dark. "

Replication: How many pots were used per treatment? What was the total number of incubation units?

Response: we have now indicated the replicate numbers in the M&M as follows - "There were three replicate microcosms for every treatment, resulting in a total of 60 microcosms for the first incubation and 12 microcosms for the second incubation. "

Matric potential selection: Justify why specific matric potentials were chosen for the incubations.

Response: we have added the following sentence - "The matric potentials were chosen in order to have a broad range of potentials from saturation (0 kPa) to the permanent wilting point (-1585 kPa). The range of matric potentials was centered on -31.6 kPa because respiration maxima are generally reached at approximately this potential (Moyano et al., 2012)."

Missing treatment rationale: Why was there no treatment combining oxygen + nitrogen + labile carbon? This would help distinguish the relative importance of N and C limitations.

Response: The sampling was an extremely difficult and expensive exercise that required 8 people in the Amazon forest for 10 days as well as a certain amount of preparation to ensure

that we could reach the Podzol soil that we sampled. The treatments that are presented in the manuscript are the maximum that we could test with the number of samples that we had. If we had had more samples we would have liked to test more. Treatment combinations, as the reviewer suggests, but also temperature for example. Nevertheless, we are able to conclude that the mineralisation of organic matter in the Bh horizon was N limited and not C limited without the combination. We have added the following to the statistical analysis paragraph - “Due to the difficulty and expense of the sampling exercise there were not a sufficient number of undisturbed cores for a fully factorial experimental design (i.e. there were no treatment combinations) and, therefore, treatment differences in the cumulative amount of CO₂ evolved from the soil samples during the incubations were tested by one-way ANOVA.”

Data analysis: Provide more information on the statistical methods used for analyzing treatment effects and comparing horizons.

Response: we have added some detail to the statistical section - “Due to the difficulty and expense of the sampling exercise there were not a sufficient number of undisturbed cores for a fully factorial experimental design (i.e. there were no treatment combinations) and, therefore, treatment differences in the cumulative amount of CO₂ evolved from the soil samples during the incubations were tested by one-way ANOVA. Differences in soil properties among horizons were also tested by one-way ANOVA. In order to estimate the annual CO₂ flux from the Bh horizon of Amazonian Podzols to the atmosphere under the different conditions tested here, a first-order decay model with one pool (Manzoni et al., 2012) was fitted to the cumulative CO₂ emission curves (Equation 1):

$$CO_2 = a(1 - e^{-\alpha t}) \quad (1)$$

where a is the pool of mineralisable C, α is the rate at which the organic C is mineralised and t is time. The model fitting was done using the nls command in R (R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>). ”

Modeling approach: Clarify what is meant by a “first-order decay model with one pool.” A brief explanation or reference is needed for non-specialist readers.

Response: we have added a citation. Please see the response to the previous comment.

Results and Discussion

The Results and Discussion sections should be clearly separated. Currently, the discussion is superficial and fails to adequately interpret the data.

Response: we would prefer not to separate the results and discussion. However, we have introduced sections and developed the discussion element along the lines suggested by both reviewers.

For example, the figure showing pH, total C, total N, and C/N ratio is informative, but the discussion of this data is limited to 3 paragraphs. Were there significant differences between horizons? Were statistical comparisons performed?

Response: we have added more detail about the differences among horizons, including the statistics, which had been omitted erroneously. As the profiles studied are typical of such Podzols, we have not discussed the data further, but have added some relevant citations to support the statement that they are typical. The first paragraph now reads as follows - “The soil

properties were typical of Amazonian Podzols (Montes et al., 2011; Sierra et al., 2013; Doupoux et al., 2017). The pH was acidic (<4.6) throughout the profile, without showing any significant differences among horizons (Fig. 1). The C and N contents ranged from 2 to 248 and from 0.1 to 9.3 mg g⁻¹ soil, respectively, with significantly higher values for both variables in the OH horizon (Fig. 1). The C/N ratios were all >20 but the Bh horizon showed by far the highest ratio at 53, which was significantly higher than in the other horizons. No other statistically significant differences were observed ”

Some key discussion points that should be expanded include:

E horizon properties and mineralization: Could the sandy texture and aeration of the E horizon be responsible for higher mineralization rates?

Response: This is a good point which we should have discussed. We have added the following to the text - “The high decomposability of the organic C in the E horizon may be due to the nature of the organic matter or due to the sandy texture of the horizon. In general, organic C is less persistent in sandy soils, possibly due to lower rates of mineral-associated organic matter formation (Haddix et al., 2020) or to more oxic conditions. The lack of difference in the mineralisation rates across matric potentials suggests that oxygen availability did not play a major role in this particular experiment, possibly because the cores were in oxic conditions within the microcosms. ”

Role of nitrogen: Lines 185–186 suggest nitrogen is more important than labile carbon under similar moisture conditions. This is an important point that should be explored in more depth, especially considering its implications for decomposition dynamics.

Response: we have developed this part of the discussion. We have added the following - “There was a CO₂ pulse after both additions of the substrate cocktail, but this was due to the mineralisation of the substrate-C that was added rather than an increase in the mineralisation of Bh horizon organic C (Figs 3 & S3). The lack of a priming effect may be due to the low pH of the soil (Fig 1). The priming effect is more common in soils with pHs between 5.5 and 7.5 but tends to be lower at the pH values found here (Wang and Kuzyakov, 2024). Furthermore, it has been shown that soils that are characterised by high levels of mineral associated organic C, as is the case in the Bh horizons of Podzols (Schmidt et al., 2000; Doupoux et al., 2017), also tend to be less prone to the priming effect (Chen et al., 2019). It should also be noted that a month after the first addition of the substrate cocktail, only 22% of the added C was mineralised, and only 15% was mineralised slightly more than a month after the second addition (Fig S3). These mineralisation rates are lower than what is usually found. The mineralisation of glucose often exceeds 60% after a month’s incubation (e.g. Hamer and Marschner, 2002), while that of pyruvate and vanillin can exceed 30% (Chenu et al., 2025) and 20% (Juarez et al., 2013), respectively. These low mineralisation rates may also have been due to an N limitation, but this would have to be confirmed experimentally. Nitrogen limitation can arise due to microbial cells being unable to produce proteins, such as enzymes or membrane transport proteins, necessary for activity, as proteins are N rich molecules (Nunan et al., 2020). ”

Depth of the Bh horizon: Despite potentially high carbon stocks, its location (~100 cm depth, as shown in Figure 1) may buffer its response to surface drying, thus limiting real-world emissions under changing moisture regimes.

Response: this is correct. We have reworked this part of the discussion in order to account for this comment and a comment on the artificial nature of the treatments from the other reviewer. It

now reads - "The first is that it is a laboratory study and, even though the samples were undisturbed, the experiment and the treatments are somewhat artificial. For example, the Bh horizons can often be found at depths greater than 1m (Doupoux et al., 2017) and the degree to which O₂ or N would reach it is uncertain. Ideally, an experiment testing similar treatments should be carried out in situ in order to determine the magnitude of the vulnerability of the organic C to N and O₂ availability, though this would be extremely difficult. "

Labile C and N inputs after dry periods: While pulses of labile C and N after dry periods can cause short-term emission spikes, these are often followed by stabilization or leaching. This should be discussed in the context of long-term carbon balance.

Response: due to the comments of the other reviewer, we have toned down and reduced the discussion about the effects of the treatments on the long term C balance. We propose not to discuss this point as it would add to the speculation that the first reviewer has asked to reduce.

Statistical analysis: Figures should clearly indicate statistical differences across horizons or treatments. This is essential for drawing valid conclusions.

Response: significant differences have now been indicated in the figures or in the figure legends

Figure captions: Improve figure captions to provide more context and description of the variables and treatments shown.

Response: we have added more information to the figure legends.