

Vulnerability of soil organic carbon in Amazonian Podsols to changes in environmental conditions.

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32 **Abstract**

33 It has recently been shown that the C stocks in Amazonian podzols are very large. They are much larger than was
34 previously thought, particularly in the Bh horizon, which has been estimated to contain in excess of 10Pg C for
35 Amazonia alone. It is predicted that changes in the regional climate will result in a drier soil moisture regime, which
36 may affect the C dynamics in these generally waterlogged soils. In order to determine the vulnerability to
37 decomposition of the organic C contained in the Amazonian podzols as a result of environmental changes, we
38 established a series of incubation experiments in which the effects of different environmental factors were measured.
39 The direct effect of drier soil moisture regimes was tested by incubating undisturbed cores from the Bh horizon at a
40 range of matric potentials. Contrary to what is usually found in soils, no significant difference in mineralisation was
41 found among matric potentials, suggesting that other factors control microbial mineralisation of this organic C. In a
42 second series of incubations, the effect of nitrogen additions, of anoxic conditions and of labile C substrate additions
43 were also tested on undisturbed cores of the Bh horizon. Samples incubated under oxic conditions produced more than
44 twice as much CO₂ as samples incubated under anoxic conditions, whilst the mineralisation rates of samples incubated
45 under oxic conditions with the addition of N increased more than four-fold relative to the anoxic samples. The addition
46 of labile C did not have a significant effect on C mineralisation. The data suggest that the large pool of C in Amazonian
47 Podzols may be vulnerable to increases in N and O₂ availability.

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50 **1. Introduction**

51 Hydromorphic Podzols are widespread in equatorial regions (Bernoux et al., 2002; Quesada et al., 2011). They
52 are characterised by a deep sandy horizon on top of clayey horizons. A small portion of the dissolved organic matter
53 from the upper organic horizon moves freely through the sandy horizon and accumulates at the transition with the clay
54 horizon below, forming a deep Bh horizon that can reach thicknesses of several meters (Montes et al., 2011; Sierra et
55 al., 2013; Doupoux et al., 2017). In the Amazon basin, the majority of the dissolved organic carbon is transferred to the
56 Amazon river via a perched water-table in the sandy E horizon above the impermeable Bh horizon (Doupoux et al.,
57 2017). Despite the C loss to the river network, the C stocks in the Amazonian Podzols have been estimated to exceed
58 13Pg C, the majority of which is contained in the deep Bh horizons (Montes et al., 2011). This C represents a significant

59 portion of the C stored in the Amazon basin; the total woody biomass of Amazonian forests having been estimated to be
60 between 121 and 126 Pg C (Malhi et al., 2006). Furthermore, ^{14}C dating of the Bh horizon C suggests that the Bh C is
61 very old, reaching ages of up to 25 thousand years (Doupoux et al., 2017). The Bh horizons are also characterised by
62 organic matter with C/N ratios that can be exceptionally high, with values sometimes exceeding 80 (Montes et al.,
63 2023), and by the fact that they are cemented and relatively impermeable (Sierra et al., 2013; Montes et al., 2023). The
64 E horizon above it is therefore generally waterlogged, thus preventing oxygen from penetrating down the soil profile to
65 the Bh horizon.

66 The vulnerability of this C to changes in environmental conditions is still poorly characterised. The ^{14}C ages in
67 the Bh horizons suggest that it is very stable and resistant to decomposition. However, this does not mean that it is not
68 vulnerable to decomposition if environmental conditions change. Old soil C can be mineralised quite rapidly, as was
69 shown, for example, by Fontaine et al. (2007): the simple addition of cellulose to subsoil samples of a Cambisol
70 stimulated the mineralisation of old organic C with an apparent ^{14}C age of 2500 years, [a phenomenon known as the](#)
71 [“priming effect”](#). The decomposition of old, millennial organic C was shown to be as responsive to warming as fast
72 cycling C in high latitude soils (Vaughn and Torn, 2019). These data suggest therefore that old organic C may not be
73 intrinsically resistant to decomposition, but rather that it is not decomposed under the prevailing conditions.

74 Regional climate models, which downscale global climate projections to regions of interest, all predict reductions
75 in precipitation levels and longer or more frequent periods of drought in the Amazon region (Avila-Diaz et al., 2020)
76 and, indeed, the Amazon experienced record-breaking droughts in 2023 and 2024 (Marengo, 2024). A significant
77 potential consequence of prolonged periods of drought is that the perched water table above the Bh horizon could dry
78 out, thus leading to its aeration. It is well established that decomposition rates in aerobic conditions are far greater than
79 those in anoxic conditions (Linn and Doran, 1984, Moyano et al., 2012), so we might expect significant increases in Bh
80 horizon mineralisation rates subsequent to such changes in conditions.

81 The projected climate changes, compounded by deforestation and fires, may also lead to changes in the structure
82 and composition of the forest (Esquivel-Muelbert et al., 2019; Flores et al., 2024) and, ultimately, to forest dieback
83 (Boulton et al., 2022; Flores et al., 2024). There are already precursor signs of resilience loss in the Amazon forest
84 related to reductions in mean annual precipitation and human land-use (Boulton et al., 2022). Forests on hydromorphic
85 podzols are particularly vulnerable to dieback due to their shallow rooting depth, which is probably a consequence of
86 the lack of nutrients in the E horizon, due to its very low exchange capacity, as well as the mostly waterlogged
87 conditions. According to Sierra et al. (2013), the high bulk density of this E horizon below the organic horizon may also
88 play a role. Such dramatic changes in forest dynamics are known to alter element cycling and potentially result in

89 nutrient losses, including N, from the vegetation and the surface organic horizon to the soil horizons below (Xiong et
90 al., 2011). The very high C/N ratio of the Bh horizon organic matter suggests that the mineralisation of this organic C is
91 constrained by N availability and increases in N flux from overlaying horizons, due to the decomposition of dead
92 biomass or increased N deposition (Galloway et al., 2008), may unlock the Bh horizon C. The death of plant biomass
93 and its subsequent decomposition is also likely to release significant amounts of labile organic matter which could
94 stimulate the mineralisation of the Bh horizon organic matter through a priming effect.

95 ~~As indicated above, the mineralisation of old, deep soil C can be stimulated by inputs of fresh, labile organic
96 matter (Fontaine et al., 2007). This phenomenon is known as the “priming effect”. The priming effect is believed to
97 arise due the inputs of fresh organic matter causing microbial communities to mine for N by decomposing organic
98 matter or due to soil organic matter being co-metabolised during with the frsh inputs (Blagodatskaya & Kuzyakov,
99 2008). The death of plant biomass and its subsequent decomposition is likely to release significant amounts of labile
100 organic matter which could stimulate the mineralisation of the Bh horizon organic C through a priming effect.~~

101 Previous studies have suggested that large quantities of C could be released to the atmosphere from these Bh
102 horizons under certain conditions (Sierra et al., 2013; Montes et al., 2023). However, in these studies the structural
103 integrity of samples was not preserved, which is likely to have allowed far greater oxygenation of the samples than
104 would occur under natural circumstances. Furthermore, the disruption of the physical structure of soils is known to
105 stimulate the mineralisation of organic C (Rovira and Greacen 1957; Salomé et al., 2010).

106 Despite the Bh carbon pool beng very large, its sensitivity to individual environmental drivers remains poorly
107 constrained under realistic physical conditions. Here, we incubated undisturbed soil cores in order to obtain more
108 realistic estimations of the vulnerability of organic C in hydromorphic Podzols, particularly that in the Bh horizon, to a
109 range of potential future disruptions to the present environmental conditions. The conditions tested were changes in
110 moisture status, and in O₂ and N availability. We also tested the effect of the addition of a cocktail of labile organic
111 molecules that sought to mimic the arrival of soluble, labile organic matter from the soil surface.

112 The hypotheses were fourfold. The first was that reductions in moisture content from saturation to a matric
113 potential of approximately -31.6 hPa would result in increases in CO₂ emissions, due to increased O₂ availability in the
114 pore space (Moyano et al., 2012; Sierra et al., 2017), but that further decreases in moisture content would result in lower
115 CO₂ emissions, due to reductions in the diffusion of C-substrates towards enzymes or the diffusion of enzymes towards
116 insoluble C-substrates (Davidson et al., 2014). The second hypothesis was that anoxic conditions were responsible for
117 the slow decomposition rates (Sierra et al., 2017; Davidson et al., 2014) and that decomposition would be stimulated by
118 increases in O₂ levels. The third hypothesis was that decomposition is N limited, as indicated by the characteristically

119 high C:N ratios of Podzol Bh horizons (Montes et al., 2023) and, therefore, that the addition of mineral N would
120 stimulate decomposition. The fourth and final hypothesis was that the addition of readily available sources of C would
121 result in a priming effect (Fontaine et al., 2007) that releases CO₂ from Bh horizon organic matter.

122 **2. Materials and methods**

123 Samples were taken from three sites circa one hundred meters apart in the region of Cabeça do Cachorro,
124 Amazonas state, Brazil, near the town of São Gabriel da Cachoeira (Fig S1). The profiles at the three sites were typical
125 Amazonian Podzols. They were made up of a waterlogged O horizon of about 15cm, an E horizon that was also
126 waterlogged and slightly less than a meter thick, a silt-loam Bh horizon that was slightly over a meter thick underneath
127 which there was a C horizon. Bulk soil and undisturbed soil cores were collected from the OH, E, Bh and C horizons to
128 a depth of 3m using an augur inserted into a metal tube that was used to prevent the sandy, waterlogged E horizon from
129 collapsing into the bore hole. This sampling procedure was necessary due to the perched water table above the Bh
130 horizon. The samples were placed in medical sample containers and closed. They were thus maintained in anoxic
131 conditions prior to use. One undisturbed sample was taken from each site x horizon per incubation treatment (see
132 below) and for the establishment of moisture release characteristics, resulting in 10 undisturbed samples being taken
133 from each horizon at each site, and a total of 120 samples. Total C and N contents of the soils were determined by
134 elemental analysis, and pH measurements were carried out in a 1:5 soil:water mixture.

135 Soil moisture retention curves were established on three replicate samples from each horizon using a suction table
136 and ceramic pressure plates (Eijkelkamp). All samples used in the subsequent incubation experiments were adjusted to
137 the desired matric potential using a suction table and pressure plates.

138 Two microcosm incubation experiments were set up in order to measure CO₂ emissions from samples in response
139 to changes in environmental conditions. The first incubation measured the response to differences in moisture content in
140 O, E, Bh and C horizons. This incubation lasted 68 days. The second incubation measured changes in CO₂ emission in
141 response to O₂, mineral N or substrate-C availability in the Bh horizon and lasted for 72 days. Both sets of incubations
142 were carried out at 28°C in the dark. There were three replicate microcosms for every treatment, resulting in a total of
143 60 microcosms (4 horizons x 5 moisture contents x 3 replicates) for the first incubation and 12 microcosms (4
144 treatments x 3 replicates) for the second incubation. In both incubation experiments the samples were placed on sample
145 holders in 1 L air-tight jars that were sealed with rubber gaskets and firmly closed with spring-lock catches. The glass
146 lids of the jars were fitted with a septum that allowed for headspace sampling. The concentration of CO₂ in the
147 headspace of sample microcosms was analysed on 18 and 23 occasions during the first and second incubations,
148 respectively. The headspace of the microcosms was flushed with CO₂-free air at regular intervals to ensure that the

149 conditions did not become anoxic, except for the anoxic treatment in the second incubation experiment (see below). The
150 moisture content of the samples was adjusted gravimetrically when necessary. The CO₂ concentrations in the microcosm
151 headspaces were determined by gas chromatography (Agilent 3000A) and the isotopic signature of the CO₂ by gas
152 chromatography coupled to an Isochrome III isotope ratio mass spectrometer (Micromass-GVI Optima). The amount of
153 soil organic C and ¹³C-labelled substrate mineralised in the samples that received a cocktail of ¹³C-labelled substrates
154 (see below) was determined by isotopic mass balance (e.g. Ruamps et al., 2011).

155 In the first incubation experiment the three replicate samples from each horizon were incubated at matric
156 potentials of 0, -5, -31.6, -316 or -1585 kPa. The matric potentials were chosen in order to have a broad range of
157 potentials from saturation (0 kPa) to the permanent wilting point (-1585 kPa). The range of matric potentials was
158 centered on -31.6 kPa because respiration maxima are generally reached at approximately this potential (Moyano et al.,
159 2012). In the second experiment a series of treatments were imposed in order to determine whether microbial
160 decomposition of the Bh horizon organic matter was limited by O₂, N or energy availability. The samples were first pre-
161 incubated for two weeks under oxic conditions in order to ensure that there were no differences in soil respiration
162 among the samples chosen for the different treatments. After the pre-incubation, four treatments were imposed: an
163 anoxic treatment, an O₂ treatment, an O₂ + N treatment and an O₂ + simple organic substrate treatment.

164 The anoxic treatment was established by replacing the microcosm headspace with N₂. In the oxic treatments
165 samples were incubated in the presence of ambient O₂ levels, alone or with the addition of N (1.6 mg g⁻¹ soil, in order to
166 bring the C/N ratio of the soil to approximately 20) or a cocktail of substrates (15 mg g⁻¹ soil C). The cocktail was made
167 up of ¹³C-labelled 30% glucose, 50% vanillin and 20% pyruvic acid. These proportions were chosen to approximate the
168 soil solution of forest soils (Kaiser et al., 2001). On the 34th day of incubation, a second addition of N (half the amount
169 previously added) and of the substrate cocktail was carried out. The other two treatments received water. The matric
170 potential of the samples was set at -1585 kPa.

171 Due to the difficulty and expense of the sampling exercise, there were not a sufficient number of undisturbed
172 cores for a fully factorial experimental design (i.e. there were no treatment combinations) and, therefore, treatment and
173 horizon differences in the cumulative amount of CO₂ evolved from the soil samples during the incubations were tested
174 by one-way ANOVA. Data were log-transformed prior to analysis, where necessary. Differences in soil properties
175 among horizons were also tested by one-way ANOVA. In order to estimate the annual CO₂ flux from the Bh horizon of
176 Amazonian Podzols to the atmosphere under the different conditions tested here, a first-order decay model with one
177 pool (Manzoni et al., 2012) was fitted to the cumulative CO₂ emission curves (Equation 1):

$$178 \quad CO_2 = a(1 - e^{-at}) \quad (1)$$

179 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
180 was done using the nls command in R (R Core Team (2022). R: A language and environment for statistical computing.
181 R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>).

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184 3. Results and discussion

185 The soil properties were typical of Amazonian Podzols (Montes et al., 2011; Sierra et al., 2013; Doupoux et al.,
186 2017). The pH was acidic (<4.6) throughout the profile, without showing any significant differences among horizons
187 (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
188 higher values for both variables in the OH horizon (Fig. 1). The C/N ratios were all >20 but the Bh horizon showed by
189 far the highest ratio at 53, which was significantly higher than in the other horizons. No other statistically significant
190 differences were observed.

191 3.1 Effect of moisture status and horizon

192 We first tested the effects of changes in moisture status on the mineralisation rates of the organic C in each of the
193 OH, E, Bh and C horizons. This was achieved by adjusting the matric potential of the samples (based on the water
194 retention curves, Fig S2) and measuring CO₂ emissions during a subsequent incubation at 28°C and under aerobic
195 conditions. There were no clear trends with matric potential in any of the horizons (Fig. 2), contrary to what might be
196 expected (Moyano et al., 2012; Sierra et al., 2017). The first hypothesis was, thus, rejected. Whilst this is surprising, the
197 water retention curves (Fig S2) show that sample moisture content varied little as a function of matric potential,
198 suggesting that the saturation of the pore network changed little and that the environmental conditions of microbial
199 decomposers in the samples were relatively unaffected. Sierra et al. (2017) showed that moisture effects on
200 decomposition rates are strongly modulated by O₂ availability. As the changes in moisture content in this experiment
201 were small, O₂ availability inside the cores may not have changed much, thus reducing any matric potential effect on
202 decomposition.

203 Overall, the amounts of C mineralised from the Oh horizon were an order of magnitude higher ($P<0.001$) than
204 from the other horizons (Fig. 2A), reflecting the higher organic C content of this horizon. However, the high variability
205 in the data meant that the differences among horizons within each matric potential were not always significant. At the
206 matric potentials of -5 and -31.6 kPa significant differences were not found with the E and Bh horizons, respectively,
207 and neither of these horizons showed a significant difference with the OH horizon at -316kPa.

208 | The specific mineralisation rates (the amount of CO₂ produced per unit organic C) were significantly ($P < 0.001$)
209 higher in the E horizon (Fig. 2B). Here also, the high variability in the data meant that specific respiration rates in the E
210 horizon within a matric potential were not significantly different from the other horizons. The specific mineralisation
211 rate is indicative of the mineralisability of the organic C (Fierer et al., 2003; Salomé et al., 2010). With the exception of
212 the E horizon, the specific mineralisation rates were generally in the range of what has been found elsewhere in
213 incubations of approximately two months (usually between 1-3%, e.g. Salomé et al., 2010; Autret et al., 2020; Kan et
214 al., 2020), although the rate of the Bh horizon was at the lower end of this range. The specific mineralisation rates in the
215 E horizon were an order of magnitude higher however. These very high rates may be linked to the low total C content of
216 the E horizon (2.3 mg g⁻¹ soil), ~~however,~~ although others have not found such high specific mineralisation rates, even in
217 deep soil where total C contents were lower than what was observed here (Fierer et al., 2003; Salomé et al., 2010). It is
218 more likely therefore, that the high specific mineralisation rates suggest that the organic C in this horizon was highly
219 labile and readily available. The high decomposability of the organic C in the E horizon may be due to the nature of the
220 organic matter or due to the sandy texture of the horizon. In general, organic C is less persistent in sandy soils, possibly
221 due to lower rates of mineral-associated organic matter formation (Haddix et al., 2020) or to more oxic conditions.

222 | The lack of difference in the mineralisation rates across matric potentials suggests that oxygen availability did not
223 play a major role in this particular experiment, possibly because the cores were in oxic conditions within the
224 microcosms. The fact that the organic C in the E horizon was more mineralisable than in the Bh horizon (Fig. 2B)
225 suggests that the specific conditions in the Bh horizon had a strong limiting effect on the mineralisation, as the C in the
226 Bh horizon was likely transferred from the E horizon above it. The second series of incubations was carried out in order
227 to investigate the causes of the relatively low specific mineralisation rates in the Bh horizon.

228 3.2 Effects of N, C and O₂ availability

229 The second incubation experiment was carried out to test whether the decomposition of the organic matter in the
230 Bh horizon was N, O₂ or energy limited. The undisturbed samples were incubated for two weeks under oxic conditions
231 and at 28°C prior to initiating the treatments, in order to ensure that there were no differences among the samples
232 selected for each treatment (Fig 3A). There was a rapid divergence in mineralisation rates following the initiation of the
233 treatments and, by the end of the incubation, there were significant differences ($P < 0.001$) in the amounts of CO₂
234 released from the samples subjected to the different treatments (Fig 3B). The samples that received N under oxic
235 conditions mineralised approximately twice as much organic C as the oxic control ($P < 0.01$) and the samples that
236 received a cocktail of simple substrates ($P < 0.05$), and in excess of four times as much C as the samples incubated under
237 anoxic conditions ($P < 0.001$; Figs 3 & S3 for O₂ contents in all treatments). No significant differences were observed

238 between the oxic treatments with or without the addition of the simple substrate cocktail, even though the substrate
239 cocktail was rapidly mineralised after both additions (Fig S4). Both oxic treatments without N mineralised significantly
240 ($P < 0.01$) more than the anoxic treatment. These data suggest that the mineralisation of the Bh horizon organic C is
241 constrained by N and O₂ availability ([hypotheses 2 and 3](#)) rather than by energetic deficiencies in the organic matter.
242 The very large C/N ratio of the Bh horizon organic matter (Fig 1) tends to confirm that low N availability to soil
243 microbial decomposers is a major limiting factor of organic matter decomposition in these soils. An analysis of the ¹⁴C-
244 age of the organic C in other Amazonian Podzols showed that there was a negative relationship between the N content
245 ~~of the of the organic matter~~ and ~~the~~ the ¹⁴C-age of the organic ~~C~~matter (Montes et al., 2023), confirming that N
246 limitation is a major factor in the dynamics of C in such soils.

247 Although the CO₂ emissions in the in the anoxic treatment were significantly lower than the other treatments,
248 they were not negligible (Fig. 3). Fairbairn et al. (2023) have suggested that CO₂ emissions can remain high under
249 anoxic conditions due to the anaerobic degradation of soil organic matter, via processes such as fermentation or
250 anaerobic respiration. It is likely that the microbial communities in this soil were adapted to anoxic conditions, due to
251 the water saturation conditions, and would therefore have had the capacity for anaerobic degradation. Nevertheless, the
252 the CO₂ emission levels were significantly lower than in the oxic treatments, confirming the work of Sierra et al.
253 (2017), who showed that the O₂ levels impose a significant limit on CO₂ emissions.

254 There was a CO₂ pulse after both additions of the substrate cocktail, but this was due to the mineralisation of the
255 substrate-C that was added rather than an increase in the mineralisation of Bh horizon organic C (Figs 3 & S4), meaning
256 that hypothesis 4 was rejected. The lack of a priming effect may be due to the low pH of the soil (Fig 1). The priming
257 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
258 and Kuzyakov, 2024). Furthermore, it has been shown that soils that are characterised by high levels of mineral
259 associated organic C, as is the case in the Bh horizons of Podzols (Schmidt et al., 2000; Douppoux et al., 2017), also tend
260 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
261 the substrate cocktail, only 22% of the added C was mineralised, and only 15% was mineralised slightly more than a
262 month after the second addition (Fig S4). These mineralisation rates are lower than what is usually found. The
263 mineralisation of glucose often exceeds 60% after a month's incubation (e.g. Hamer and Marschner, 2002), while that
264 of pyruvate and vanillin can exceed 30% (Chenu et al., 2025) and 20% (Juarez et al., 2013), respectively. These low
265 mineralisation rates may also have been due to an N limitation, but this would have to be confirmed experimentally.
266 Nitrogen limitation can arise due to microbial cells being unable to produce proteins, such as enzymes or membrane
267 transport proteins, necessary for activity, as proteins are N rich molecules (Nunan et al., 2020).

268 In view of the very large quantities of organic C that are stored in the Bh horizon of the Amazonian Podzols
269 (Montes et al., 2011), we sought to estimate the annual CO₂ flux from these horizons to the atmosphere under the
270 different conditions tested here. We first fitted a first order decay model with a single pool to the respiration data (Fig
271 S5 – best fit based on the Akaike Information Criterion) and extrapolated the mineralisation curves to a year. Montes et
272 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
273 used to estimate the potential total C fluxes from the Bh Horizon of the Amazonian Podzols (Fig. 4). The increase in
274 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
275 the anoxic treatment ($P < 0.001$). The other two oxic treatments also resulted in significant increases in the amount of C
276 released ($P < 0.01$).

277 Global soil respiration estimates are subject to large uncertainties, due to the complex set of biogeochemical and
278 biophysical processes that are involved. These uncertainties are one of the major causes of uncertainty in terrestrial
279 ecosystem models (He et al., 2022). Nevertheless, a recent study has estimated global soil heterotrophic respiration to
280 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
281 equivalent to 0.8% of global soil heterotrophic respiration.

282 There are a number of uncertainties associated with the estimates put forward in this study. The first is that it is a
283 laboratory study and, even though the samples were undisturbed, the experiment and the treatments are somewhat
284 artificial. For example, the Bh horizons can often be found at depths greater than 1m (Doupoux et al., 2017) and the
285 degree to which O₂ or N would reach it is uncertain. Ideally, an experiment testing similar treatments should be carried
286 out *in situ* in order to determine the magnitude of the vulnerability of the organic C to N and O₂ availability, though this
287 would be extremely difficult.

288 The second uncertainty is the use of a first order decay model with only one pool to extrapolate to yearly CO₂
289 carbon fluxes. The use of single pool models has been criticised in the past because it can mask the presence of smaller
290 C pools with faster turnover times (Davidson et al., 2000). This criticism is less relevant here because the pool is small
291 and even if it did respond differently to the treatments, it would not change the overall trend of the results. Furthermore,
292 one might expect this smaller pool to respond more rapidly to N availability and, if anything, increase the observed N
293 effect. Nitrogen additions are often associated with an increase in enzymes that catalyse carbohydrate hydrolysis but a
294 decrease in oxidative enzymes that catalyse the breakdown of polyphenols (Moorhead and Sinsabaugh, 2006), thus
295 increasing the decomposition rates of the fast-cycling C pool and slowing down the decomposition of the slow-cycling
296 C pool. Although, the decomposition of fast-cycling C pools is likely less sensitive to variations in O₂ levels than that of

297 slow-cycling C pools (Lin et al., 2021), the smaller size of the pool is unlikely to change the overall conclusions, as
298 suggested above.

299 The third uncertainty lies in the duration of the incubations (72 days), which may not have been long enough to
300 detect the response of the slower-cycling C pools in these soils. However, the parameters obtained from the first order
301 decay model suggest that the treatments increased the size of the mineralisable pool rather than the rate at which the
302 pool was mineralised (Table S1). This suggests that the treatments increased the amount of C that was readily
303 mineralisable in the samples and, therefore, not detecting the slow C pool's response may not be problematic as this
304 change was detected. Furthermore, Montes et al. (2023) measured CO₂ emissions from Bh horizon samples of
305 Amazonian Podzols and found that the fast pool always accounted for < 0.5% soil C, far less than in any of the
306 treatments here, other than the anoxic treatment.

307 A fourth uncertainty associated with the study is that the amount of N arriving in the Bh horizons annually may
308 be lower than what was added here, despite the fact that future N deposition projections indicate significant increases in
309 reactive N deposition (Galloway et al., 2004) and forest dieback might also result in higher soil N concentrations
310 (Xiong et al., 2011).

311 The CO₂ flux from the Amazonian Podzols may be further enhanced by increases in average temperature that will
312 occur with climate change, due to the very high C/N ratio of the Bh horizon: CO₂ fluxes from soils with high C/N ratios
313 show a positive response to temperature (Karhu et al., 2014). Overall, ~~the these data~~significant increases in CO₂
314 emissions under oxic and N amended treatments suggest that the organic C contained in equatorial Podzols is
315 vulnerable to ~~increases in~~ N and O₂ availability and ~~that~~ significant amounts of CO₂ could be released to the
316 atmosphere, ~~and~~ thus exacerbate atmospheric CO₂ levels.

317 1. Data availability

318 The data that support the findings of this study are openly available in Zenodo at
319 <https://zenodo.org/records/15824641>.

320

321 **Author contribution**

322 Naoise Nunan: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing – original
323 draft, Writing – review & editing, Funding acquisition.

324 Claire Chenu: Conceptualization, Investigation, Writing – review & editing.

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333 The authors declare that they have no conflict of interest

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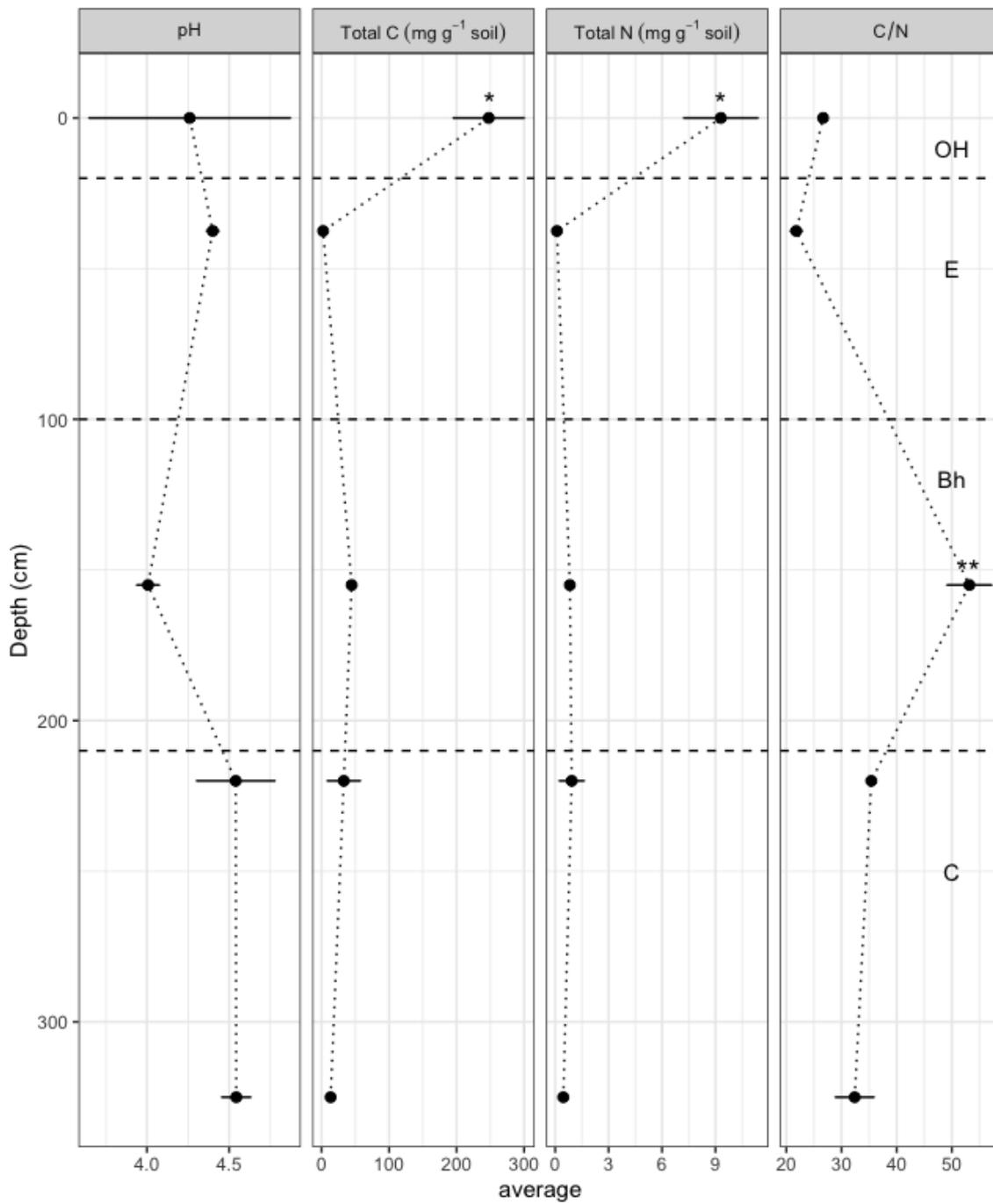
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Figures



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494 Figure 1 Properties of different horizons of the Podzols. The bars indicate the standard deviation of
 495 the mean where it is larger than the size of the symbols. Stars indicate horizons with values that are
 496 significantly (* < 0.05, ** < 0.01) higher than in other horizons.

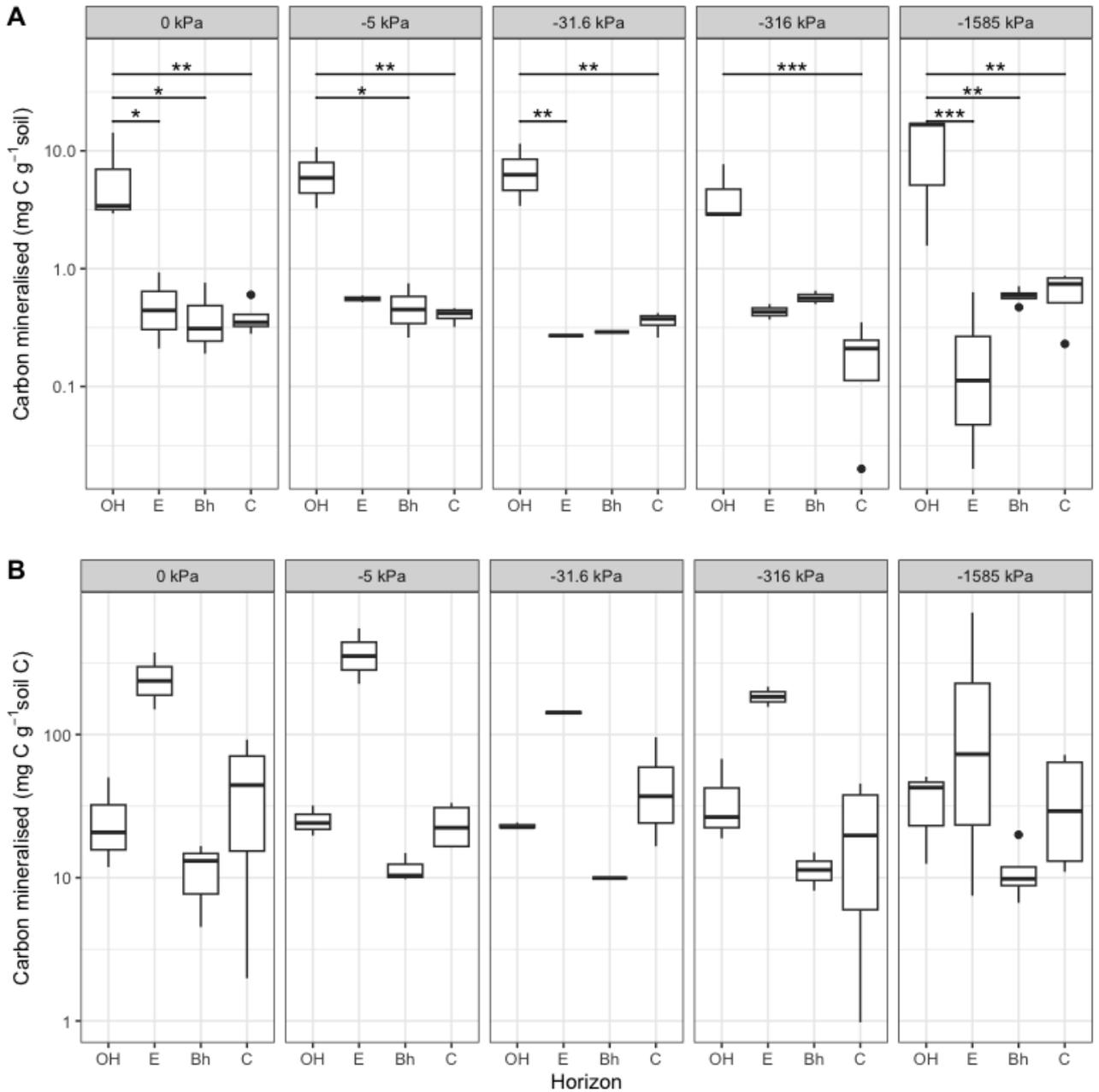
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503 Figure 2 Total C mineralisation in the different Podzol horizons at different matric potentials
504 during the 68 day incubation. Mineralisation is expressed per g soil (A) and per g soil C (B). Note
505 that the y-axes are in log scale. Across all matric potentials, the carbon mineralised (per g soil) from
506 the OH horizon was significantly ($P<0.001$) higher than in all other horizons. Differences within
507 each matric potential are shown in the graph (* <0.05 , ** <0.01 , *** <0.001). The carbon mineralised

508 per g soil C from the E horizon was significantly ($P<0.001$) higher than in all other horizons, but
509 showed no significant differences within each matric potential.

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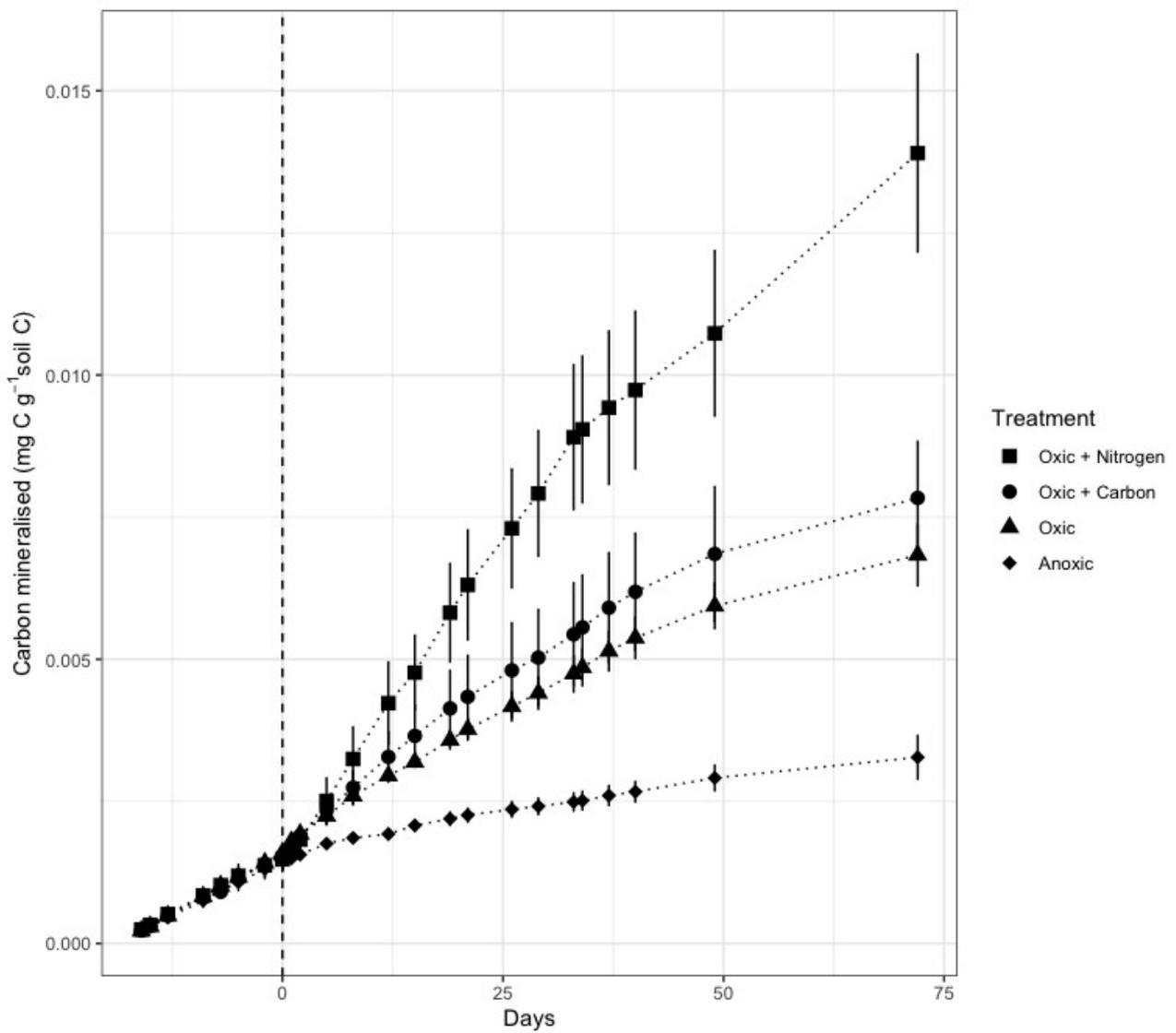
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517 Figure 3. Soil organic C mineralisation in Podzol Bh horizons prior to and after imposition of
518 treatments (dashed line indicates day at which treatments commenced). Bars indicate standard error

519 of the mean where error is larger than the size of the symbols. At the end of the incubation, the
520 amount of C mineralised in the oxic+N treatment was significantly higher than the oxic and the
521 oxic+C treatments ($P<0.05$) as well as the anoxic treatment ($P<0.001$). Both oxic treatments
522 without N were also significantly ($P<0.01$) higher than the anoxic treatment.

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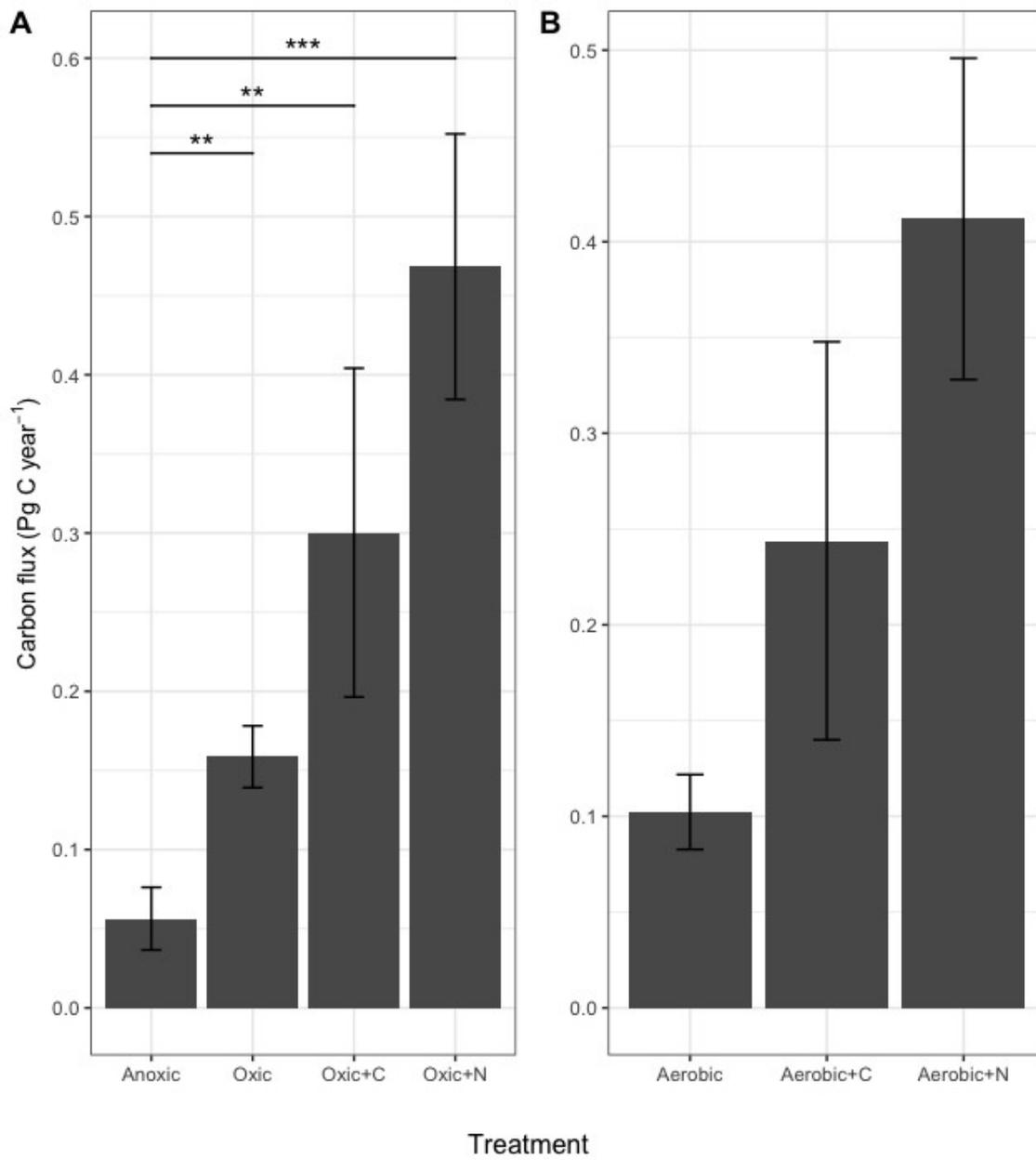
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533 Figure 4 Estimated annual carbon flux from Bh horizons to the atmosphere (A) and estimated
 534 increase in C flux to the atmosphere if the present anoxic conditions were to change (B). The bars
 535 indicate the standard error of the mean. The oxic+N and the oxic+C treatments resulted in
 536 significantly (**<0.01) more C flux than the anoxic treatment.

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