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Article

Decomposition Dynamics of *Typha angustifolia* under Aerobic Conditions

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The study of the *Typha* species has gained attention in tropical areas due to their rapid growth, nutrient release, and contribution to detritus in lakes. Analysis of the degradation of *Typha angustifolia* has shown that the first stage of decomposition is marked primarily by the release of soluble compounds, indicating that microorganisms have a minor influence at this stage. In subsequent stages, microorganisms act by degrading and consuming phenolic compounds and phosphorus. The remaining compounds form humic substances and are retained in the detritus. Thus, microorganisms significantly degrade organic matter, resulting in a two-fold increase in decomposition. Different forms of phosphorus could be detected in the remaining mass by ^{31}P NMR (nuclear magnetic resonance). By comparing the ^{31}P NMR data between the samples with and without the antibiotic, we can better understand the slow decomposition process of *Typha angustifolia*.

Keywords: *Typha angustifolia*, macrophyte, decomposition, phosphorus determination, ^{31}P NMR, polyphenol

Introduction

The decomposition of aquatic macrophytes plays an important role in nutrient cycling as well as carbon and energy flows in aquatic ecosystems.[1-3] In contrast, plants with slow decomposition processes increase autochthonous production of organic matter, clogging rates in surface-flow wetlands, and consequently terrestrialization in free-water surface wetlands.[4]

Typha angustifolia L. (Typhaceae) is a perennial, lignocellulosic, vascular plant that is widely distributed in Brazil, where it is known as "Taboa". Although *T. angustifolia* constitutes a major source of organic matter in aquatic ecosystems, its high growth rate is frequently associated with the depletion of dissolved oxygen and the death of fish.[4,5] Indeed, this plant is considered a weed in Brazil and other countries. Additionally, dead *T. angustifolia* biomass undergoes a long decomposition process, forming detritus[6] that leads to wetland terrestrialization. Understanding the decomposition process of *T. angustifolia* is important to evaluate the effects of this plant on the environment.

Important factors such as temperature, pH, nutrient content in the vegetal tissue, and the presence of microorganisms can influence the decomposition of aquatic macrophytes. The decomposition of these plants has been well studied and can be divided into the following three distinct stages: (*i*) leaching, which involves the release of soluble biomass during the first few days; (*ii*) catabolism, which comprises a period of colonization and microbial decomposition that converts complex organic compounds to simple and small molecules; and (*iii*) fragmentation, which is caused by mechanical and invertebrate motion.[7-10]

To determine the mechanism of degradation, comparative studies with and without microorganisms are needed to assess in which stage of degradation the presence of microorganisms is most important.[11]

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In several ecosystems, the availability of nutrient resources depends on the microbial decomposition of the detritus.[11-13] The main changes in nutrient forms occur during the catabolism stage of the decomposition process, with formation of phenolic compounds, humic substances, and phosphorous derivatives. Microorganisms incorporate inorganic nutrients from senescent biomass and exert an overall impact on both the internal wetland nutrient dynamics and overall nutrient sequestration, both of which are important functions of wetlands in the landscape.[4,10]

In this context, this work shows the influence of microorganisms on the decomposition mechanism of *Typha angustifolia* L., highlighting changes in the biomass, total polyphenols, phosphorus released by senescent plants, and different forms of phosphorus in the remaining mass.

Experimental

Experimental design for the decomposition

Samples of *T. angustifolia* were collected from a man-made lagoon. The plants were extensively washed with water to remove coarse materials, dried at 40 °C, and fragmented (the fragments were ca. 0.3-0.5 cm in size). Only the aerial parts (culms and leaves) were used. The water used in the assays was collected from the same lagoon; it was filtered and used immediately to set up the incubations. Sixty-four decomposition chambers were prepared in the laboratory using disposable plastic cups (500 mL). In each chamber, 3.0 g (dry weight) of plant fragments were added to 300 mL of lagoon water. The experiment was performed at room temperature (27.3 ± 2.6 °C), and two treatment conditions were established: with and without chloramphenicol antibiotic. The dose used in each cup was 18 mg *per* week. At the end of 90 days, 234 mg of chloramphenicol was added to each chamber containing samples treated with antibiotics to suppress bacterial growth.[11,14] The decomposition experiment was conducted for 120 days, and samples from each treatment were randomly collected at eight sampling times: 1, 3, 7, 15, 30, 60, 90 and 120 days.

The remaining mass and ash content

The plant detritus was filtered, oven-dried at 105 °C for 24 h and allowed to stand until a constant weight was achieved when measuring the remaining dry mass.

The detritus decomposition was exponentially modeled as proposed by Gamage and Asaeda:[11]

$$W_t = W_0 e^{-kt} \quad (1)$$

where W_t is the dry weight at time t, W_0 is the initial dry weight and k is the decay rate constant. By using this model, we assumed that the decomposition process follows first-order kinetics and that the decomposition rate constant at time t is proportional to the weight at time t. The decay rate was calculated using the equation 1.

The ash content was determined using a gravimetric method following calcination of the plant debris (600 °C, 4 h). The elemental composition of the ash was identified qualitatively by X-ray fluorescence. The analyses were performed in an energy-dispersive X-ray fluorescence (EDXRF) spectrometer using rhodium tubes with a voltage ranging from 0 to 40 keV and a collection time of 250 s.

Polyphenol and phosphorus content

Standard colorimetric analysis was used to determine the total phosphorus content[15] and the total polyphenol content[16] in the leachate samples. For determination of total phosphorus and total polyphenols, external standard curves using KH_2PO_4 and gallic acid, respectively, were generated to determine the best fit. The analyses were performed in triplicate.

An analysis of variance (paired sample *t*-test, $p < 0.05$) was used to compare the changes in mass as well as ash, phosphorus, and polyphenol contents between the two treatments tested at each sampling time.

Phosphorus determination by ^{31}P NMR

^{31}P NMR (nuclear magnetic resonance) studies of the dynamic forms of phosphorus that arise during the decomposition were based on procedures previously described by Cade-Menun *et al.*[17] and Cade-Menun.[18] The detritus at each sampling time (3.0 g at 15, 60, 90 and 120 days) was extracted with 150 mL of a 0.25 mol L^{-1} NaOH solution for 20 minutes. After filtration, the solution was immediately lyophilized, and 100 mg of the residue was dissolved in deuterated water (D_2O) for ^{31}P NMR analyses. The spectra were recorded on a Bruker Avance III 500 (11.75 T) spectrometer operated at 202 MHz (^{31}P) and equipped with a 5-mm broadband probe head (BBO) with a Z-gradient.

The ^{31}P NMR experiments were performed at 25 °C using the one-pulse sequence ("zg" in Bruker language). A total of 3072 free-induction decays (FIDs) were collected as 65,536 data points for a spectral width of 40,760 Hz with a relaxation delay of 2 s and an acquisition time of 0.8 s. The spectral processing was performed with 65,536 points and manual phase correction; an exponential weighting factor corresponding to a line broadening of 20 Hz was

applied. Phosphoric acid (H_3PO_4, 85%) was used as an external standard.

Results

The remaining mass and ash content

Figure 1a shows the remaining biomass over the incubation period for the treatments with and without chloramphenicol. The mass loss was very rapid in both treatments, with 28.45% (without antibiotic) and 21.65% (with antibiotic) of the dry mass removed during the first week.

However, leaching from the sample non treated with chloramphenicol (44.18%) occurred substantially faster than leaching from the sample containing the antibiotic (23.66%) during the first fifteen days ($p = 0.0053$). This trend continued at 120 days; the leaching rates from the samples without and with chloramphenicol were 74.82 and 53.28%, respectively ($p = 0.0047$).

The global rates of decomposition (k) for the samples treated without and with the antibiotic were 0.0149 and 0.00643 day^{-1}, respectively. The decomposition process was twice as fast in the presence of microorganisms.

The *in natura* plant ash content, which is composed of inorganic materials including metals, was 13.03%. The ash content of the decomposed material was 4.57 ± 0.54% and was similar for both treatments and at all of the time points during decomposition (Figure 1b). Following decomposition, the ash was composed of inorganic compounds including the insoluble metals K, Mg, Sr, Fe, Al, Ca, and Cu, as detected by X-ray fluorescence spectroscopy.

Total polyphenolic and phosphorus contents

The polyphenolic contents during the decomposition period are shown in Figure 1c. There was a rapid decrease in the polyphenolic content until the 7th day of the treatment without the antibiotic. Subsequently, small increases were observed. However, in the treatment with the antibiotic, there were differences at all of the decomposition stages. Specifically, a decrease in the polyphenolic content was

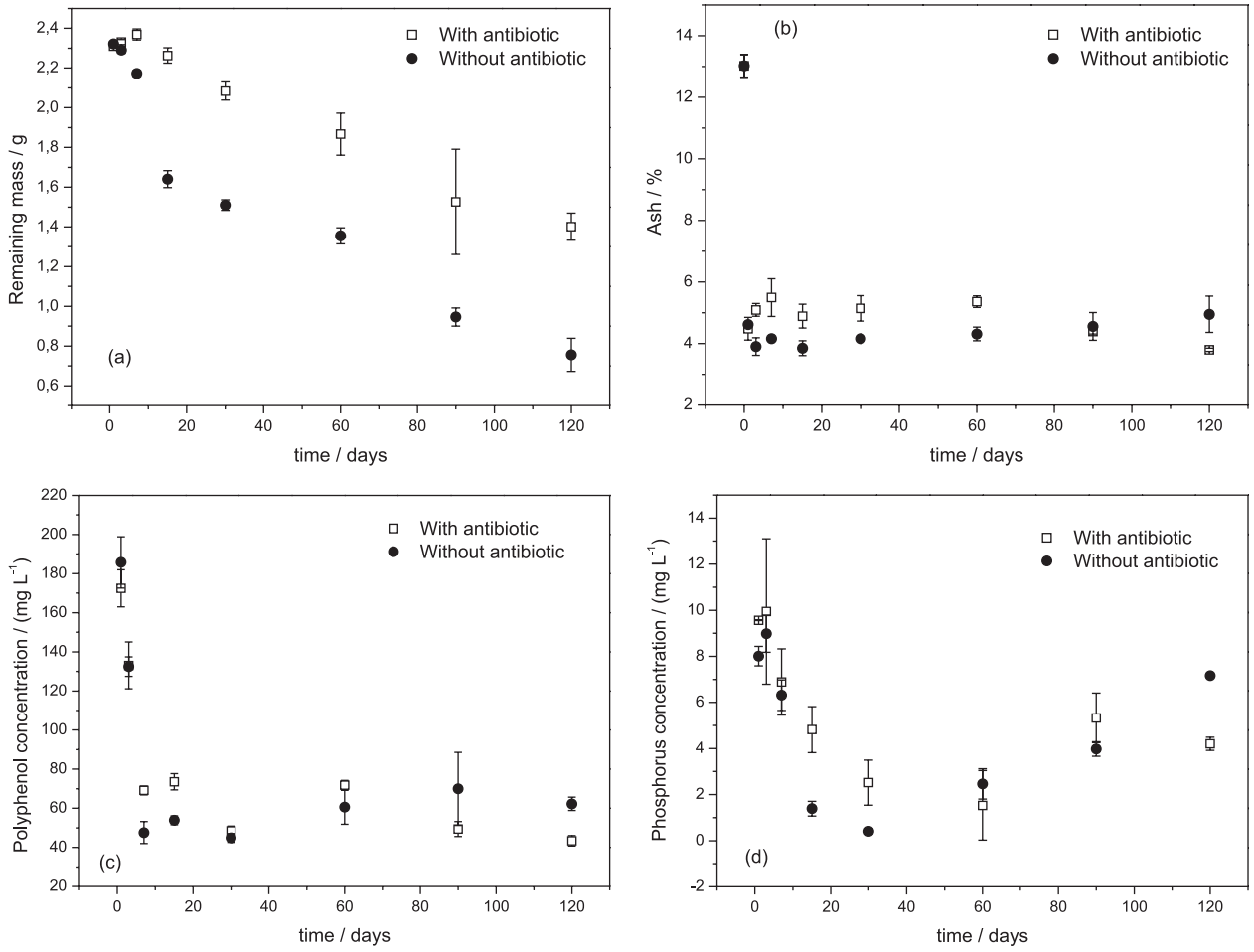

Figure 1. (a) Remaining dry mass; (b) ash percentage; (c) polyphenolic content; (d) phosphorus content in *T. angustifolia* during the decomposition process for samples treated with and without chloramphenicol. Vertical lines indicate the standard error.

observed for the first 30 days; this decrease was followed by an increase until the 60th day and another subsequent decrease.

The results from both treatments showed that the phosphorus content decreased until day 30 (Figure 1d). However, the decrease was faster in the samples treated without the antibiotic. After this period, there was a gradual increase in the phosphorus content in the samples treated without the antibiotic, while the phosphorus content gradually decreased until 60 days and subsequently increased until 90 days in the samples treated with the antibiotic.

Phosphorus determination by ^{31}P NMR

Orthophosphate and phosphomonoesters, such as myo-inositol, hexakisphosphate, scyllo-inositol hexakisphosphate, α-glycerophosphate, β-glycerophosphate, choline phosphate, guanosine-2'-monophosphate, and adenosine 2',5',[19,20] are usually identified by ^{31}P NMR at δ 6-7 and 4-6, respectively.[20] The ^{31}P NMR spectra obtained at different time points during degradation are shown in Figure 2 either without (Figure 2a) or with (Figure 2b) chloramphenicol. The signals at δ 6.11, 4.93, 4.45-4.57, 4.26, and −0.50 were assigned to orthophosphate, β-glycerophosphate, adenosine 2',5', choline phosphate, and nucleotide phosphate, respectively, according to the literature.[17,20-22] The ^{31}P NMR spectra of the plant *in natura* showed the following three signals: (*i*) an intense peak assigned to orthophosphate (δ 6.11); (*ii*) another peak assigned to adenosine 2',5' (δ 4.50); and (*iii*) an overlapped small peak assigned to choline phosphate (δ 4.26).

The signal for orthophosphate observed on the NMR spectra of both treatments showed a similar decrease until 60 days of degradation (Figure 2a). This change can be confirmed by a reduction in the relative proportion of the integral values for the orthophosphate signal in the spectra using the *in natura* sample as a reference (Table 1). After 60 days, the samples non treated with chloramphenicol showed a greater reduction in orthophosphate. The low-intensity signal for nucleotide phosphate, which is the dominant form of the phosphodiester, was observed at the initial and final degradation periods. Up till 60 days, an increase in adenosine 2',5' and the appearance of the β-glycerophosphate signal were observed (Figure 2a), increasing the relative proportion of the integral values for the phosphomonoester group and decreasing the ratio between the orthophosphate/phosphomonoester areas (Table 1). The choline phosphate signal intensity showed random changes along the degradation time (Figure 2a). During the final process (120 days), there was a little decrease in the intensity of the phosphomonoester group signal and an abrupt decrease in the orthophosphate signal, maintaining a small value for the ratio between orthophosphate/phosphomonoester (Table 1).

For the samples treated with antibiotic, the orthophosphate signal decreased at the beginning of the degradation process and remained constant thereafter (Figure 2b and Table 1). For the phosphomonoester group, the adenosine 2',5' and β-glycerophosphate signals increased at the beginning and then showed a slight decrease, while the choline phosphate signal remained almost constant. These changes in the signals can also be confirmed by the relative proportion of the phosphomonoester group and the ratio between orthophosphate/phosphomonoester (Table 1). Additionally, a weak signal for nucleotide phosphate appeared at 120 days (Figure 2b).

Discussion

Both treatments showed exponential decay with a progressive decrease in the content of the remaining mass. However, the decomposition was twice as fast in the presence of microorganisms.

The mass loss during the initial leaching process (first 72 hours) indicates that the influence of bacteria

Table 1. Relative proportion of the integral values for orthophosphate (Pi) and phosphomonoester group (PMN) in the ^{31}P NMR signals derived from the material extracted from the remaining mass of *T. angustifolia* at several degradation time points

Degradation time / days	Without antibiotic			With antibiotic		
	Pi[a]	PMN[b]	Pi/PMN[c]	Pi[a]	PMN[b]	Pi/PMN[c]
In natura	1[d]	1[d]	2.29	1[d]	1[d]	2.29
15	0.31	1.14	0.63	0.31	1.41	0.51
60	0.32	2.00	0.29	0.34	1.51	0.52
90	0.24	1.08	0.50	0.33	1.24	0.62
120	0.07	1.10	0.15	0.45	1.33	0.78

[a]Pi: orthophosphate; [b]PMN: phosphomonoester group; [c]Pi/PMN is the ratio of the areas of Pi to PMN at each degradation time; [d]Pi and PMN in *in natura* degradation time was used as reference to calibrate the integrals.

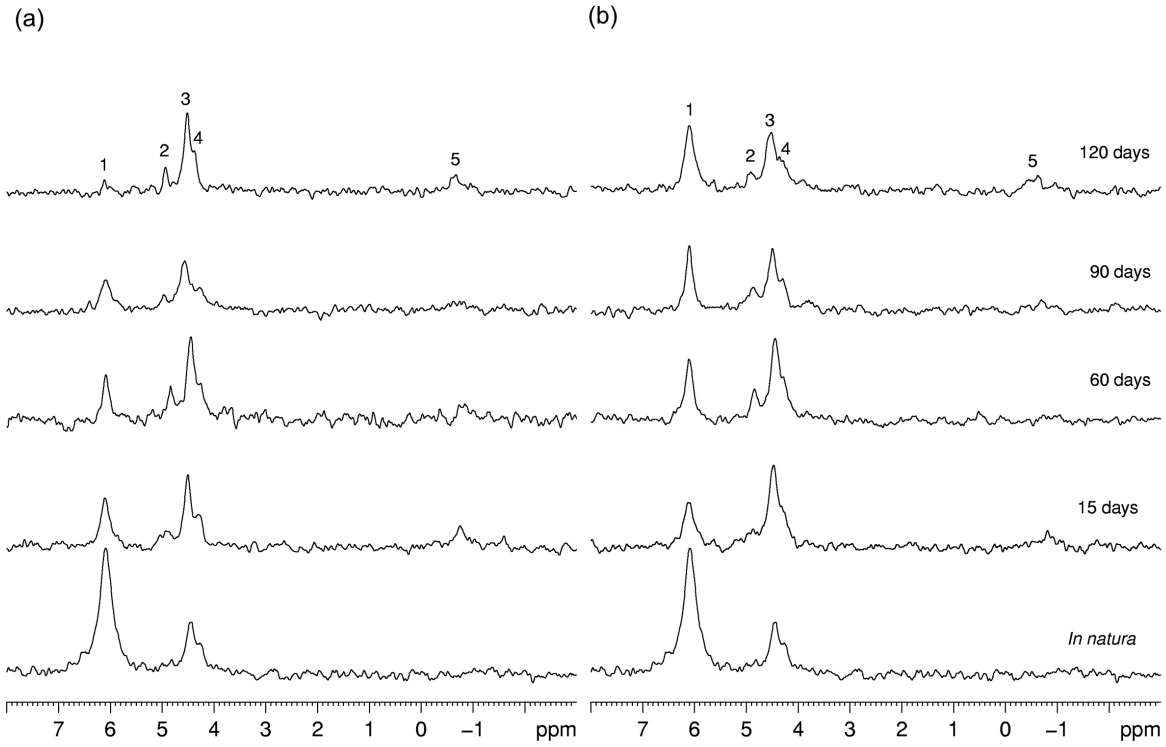

Figure 2. ^{31}P NMR spectra of the material extracted with 0.25 mol L^{-1} NaOH from the remaining mass of *T. angustifolia* in the treatments (a) without and (b) with chloramphenicol at different time points during the decomposition process (*in natura* and 15, 60, 90, and 120 days). 1, orthophosphate; 2, adenosine 2',5'; 3, choline phosphate; 4, β-glycerophosphate; and 5, nucleotide phosphate.

was minimal during this period.[11,23] The mass loss during this time is associated with the release of cytoplasmic fractions and hydrosoluble compounds that were present in the macrophyte.[24,25] The release of phosphorus during the initial decomposition period is linked to the loss of soluble phosphorus that had accumulated in plant tissue vacuoles.

The second stage of decomposition is catabolism. For the samples treated with the antibiotic, the degradation process was slow due to the absence of microorganisms. The rapid decomposition of the macrophyte in the treatment without the antibiotic is evidence of the action of microorganisms during the catabolism of the detritus. Polyphenols and phosphorus are released during the catabolism period through the degradation of lignocellulosic tissue and phosphorus-containing compounds present in internal tissues.

The early decay of polyphenols is due to leaching processes and mineralization of the detritus; later, decay is caused by oxidation of the refractory fractions. Depolymerization of methoxylated phenols occurs during the lignin degradation process. Microorganisms also degrade lignified structures in the plant tissues, releasing polyphenols into the environment. The small increases in the polyphenolic content that were observed are due to the degradation of most of the plant internal tissues. The released polyphenols are used as an energy source for the microorganisms or are converted to humic substances. In the absence of microorganisms, partially modified lignin can be incorporated into humic substances,[26] which have a high molecular weight and complex structures. In the presence of microorganisms, polyphenols (e.g., phenolic acids) can bind to one another or to other molecules and be converted into quinones through oxidation. Quinones can react with ammonia or nitrogen compounds to form humic substances, which can explain the decrease in polyphenols at 60 days of decomposition. Aromatic monomers derived from lignin also contribute to the formation of humic substances.

The presence of phosphorus in the aquatic environment enhances the development of microbial decomposers, increasing the production of hydrolytic enzymes that speed up the decomposition process.[27] The microorganisms consume phosphorus as an energy source, thus decreasing its content in water. Subsequently, they convert the organic phosphorus present in the plant tissue to inorganic phosphorus, and it is returned to the water column.

A previous study[28,29] on aquatic macrophytes demonstrated that there were high amounts of dissolved oxygen and a high pH during the decomposition

period, most likely due to atmospheric diffusion and the consumption/degradation of organic acids produced at the beginning of the decomposition process. Humic substances can complex with orthophosphate in the presence of Fe^{III} at low pH levels, though the mechanisms are not well known. The increase in the available phosphorus (orthophosphate) concentration during humification can indicate the presence of high pH and dissolved oxygen, decreasing the assimilation of orthophosphate.[29,30] Therefore, we can presume that the increase in phosphorus at 60 days was most likely due to large amounts of dissolved oxygen and a high pH, which prevented interactions between the humic substances, Fe^{III}, and phosphorus.

The predominance of orthophosphate observed during the initial degradation period (Figure 2) was in accordance with the observations reported by Cheesman *et al*.[10] The orthophosphate released to the water column during leaching can be incorporated into organic matter during the catabolism period, as observed by the increase of phosphomonoesters between days 30 and 60 (Table 1). At later time points in the degradation process, phosphomonoesters are consumed by microorganisms, reducing their content in the plant. The signals that indicate the presence of phosphomonoesters are also derived from the hydrolysis of phospholipids,[31] which are almost completely degraded 24 hours after the basic extraction process. Extraction with hydroxides results in RNA (ribonucleic acid) decomposition and generates a signal in the area around δ 0.00, e.g., the nucleotide phosphate assigned at δ –0.50 (Figure 2).

The inhibition of microorganism proliferation resulted in changes in macrophyte decomposition dynamics and the liberation of phosphorus. Degradation occurred via leaching, and catabolism can be correlated with the relative proportion of orthophosphate and phosphomonoesters at the various degradation times (Table 1). For the samples treated with the antibiotic, an increase in phosphomonoesters due to the hydrolysis of phospholipids occurred initially. However, due to the absence of microorganism action, the reduction in phosphomonoesters during the final degradation period was not as pronounced as it was in the samples without the antibiotic. This finding suggests that bacteria present in the samples control the decomposition, especially with respect to orthophosphate consumption.

Conclusions

The global decomposition rate (k) of *Typha angustifolia* in the presence of microorganisms is twice faster than decomposition rate of this organic matter without microorganisms. The microorganisms act on the organic matter, degrading lignocellulosic compounds and releasing polyphenols, which are rapidly converted to humic substances. They also quickly consume the available phosphorus and convert orthophosphate to different forms of phosphomonoesters. The orthophosphate released to the water column during leaching can be incorporated into the organic matter during the catabolism period, increasing the phosphomonoester content. However, the complete degradation of orthophosphate in the presence of microorganisms only occurs after 90 days, indicating that *Typha angustifolia* degradation is slow even in the presence of microorganisms.

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