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The role of carbon, nitrogen, and phosphorus in leaf decomposition mediated by aquatic fungi

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ABSTRACT

The role of carbon, nitrogen, and phosphorus in leaf decomposition mediated by aquatic fungi

The aquatic microbial decomposition of leaf litter has been the subject of many field studies throughout the world. However, field experiments cannot always separate the effects of the multiple biotic and abiotic factors involved in the process. In this laboratory experiment, we controlled the abiotic factors and the fungal decomposer community during decomposition of alder, oak and eucalypt leaf litter in order to determine if variation in leaf carbon, nitrogen and phosphorus (CNP) ratios during decomposition was similar among the three species. Initial CNP values differed among the three species with alder being the richest (C:N = 16, C:P = 903, N:P = 57) and oak being the poorest (C:N = 55, C:P = 1779, N:P = 32) species. In all leaf species, nitrogen was immobilized during decomposition (final < initial C:N ratios), while phosphorus was released (final > initial C:P ratios). Final CNP values were lowest in alder (C:N = 11, C:P = 2495, N:P = 224) but there was a change in the ranking of oak and eucalypt regarding nutrient contents. Leaf species were similar regarding the variation in C:N (final/initial = 0.7 to 0.8) but C:P and N:P increased more in eucalypt and oak than in alder (final/initial C:P = 5.9, 3.9 and 2.8, final/initial N:P = 7.5, 4.7, 3.9, respectively for eucalypt, oak and alder). The lowest decrease in P of alder leaves may explain the highest mass loss observed in this species, most probably due to a higher fungal colonization despite the controlled fungal decomposer community. In conclusion, CNP ratio in leaves seems to determine the fungal-mediated mass loss of leaf litter.

Key words: CNP, leaf litter, fungal decomposition

RESUMEN

El papel del carbono, nitrógeno y fósforo en la descomposición de hojarasca mediada por hongos acuáticos

La descomposición microbiana acuática de la hojarasca ha sido objeto de muchos estudios de campo en todo el mundo. Sin embargo, en los experimentos de campo es imposible separar los efectos de los múltiples factores bióticos y abióticos involucrados en el proceso. En este experimento de laboratorio, controlamos los factores abióticos y la comunidad de hifomicetos acuáticos durante la descomposicion de hojarasca de aliso, roble y eucalipto para determinar si la variación en los cocientes de carbono, nitrógeno y fósforo (CNP) durante la descomposición fue similar entre los tres especies. Los valores iniciales de CNP difirieron entre las tres especies con el aliso siendo el más rico (C:N = 16, C:P = 903, N:P = 57) y el roble siendo el más pobre (C:N = 55, C:P = 1779, N:P = 32). En todas las especies foliares, el nitrógeno fue inmovilizado durante la descomposición (C:N final < inicial), mientras que el fósforo fue liberado (C:P final > inicial). Los valores finales de CNP fueron los más bajos en aliso (C:N = 11, C:P = 2495, N:P = 224) pero hubo un cambio en el ranking de roble y eucalipto con respecto a los contenidos de nutrientes. Las especies foliares fueron similares con respecto a la variación en C:N (final/inicial = 0.7 a 0.8) pero C:P y N:P aumentaron más en eucalipto y roble que en aliso (final/inicial C:P = 5.9, 3.9 y 2.8, final/inicial N:P = 7.5, 4.7 y 3.9, respectivamente para eucalipto, roble y aliso). La disminución más baja en P de las hojas del aliso puede explicar la pérdida de masa más alta observada en esta especie, lo más probablemen-

te debido a una colonización de hongos más alta a pesar de la comunidad fúngica controlada. En conclusión, los cocientes CNP en las hojas parecen determinar su destino durante la descomposición.

Palabras clave: *CNP, hojarasca, descomposición fúngica*

INTRODUCTION

In many headwater streams, sunlight cannot reach the water's surface due to shading by the forest canopy, thus limiting photosynthesis in the water. Therefore, most headwater streams rely on autumn leaf fall to supply much of the carbon needed to support the aquatic foodweb throughout the year (Fisher & Likens, 1973; Vannote *et al.*, 1980). In the water, the leaves leach out nutrients that flow downstream providing nourishment along the way. Microorganisms colonize and decompose the leaves and aquatic shredders process them thus facilitating the flow of energy through the system. Fungi dominate the microbial communities associated with decomposing leaf litter in streams (Gulis & Suberkropp, 2003), constituting more than 63 % of total microbial biomass (Baldy *et al.*, 2002).

Leaf decomposition proceeds at different paces depending on the physic-chemical characteristics of the stream but also on physic-chemical characteristics of the leaf species. For a given stream, softer, nutrient-rich leaves decompose faster than hard, nutrient-poor leaves due to higher fungal colonization (Gessner & Chauvet, 1994; Canhoto & Graça, 1995; Canhoto & Graça,1996). As a consequence, consumption by leaf-shredding invertebrates increases (Kaushik & Hynes, 1971), and the effects of physical abrasion due to the flowing water are more pronounced (Abelho, 2008). The chemical characteristics of the leaves typically change during decomposition, with a consistent increase in nitrogen and a concomitant decrease in C:N ratios attributable to N immobilization in microbial biomass (Bärlocher, 2016). The increase in N content during decomposition is usually more

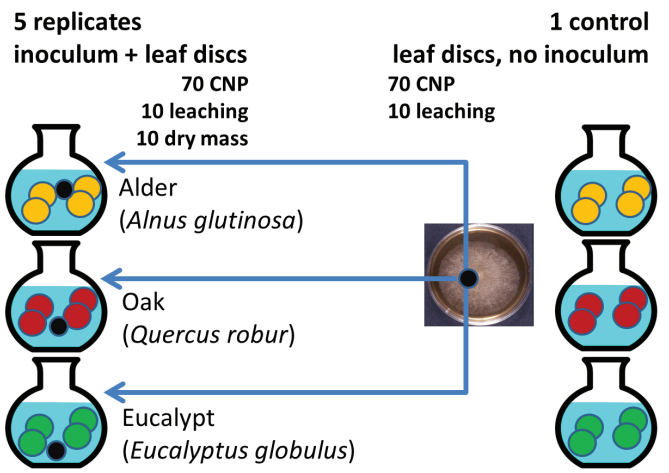

Figure 1. Experimental design. Five replicates per species with leaf discs and mycelium plugs of 6 aquatic hyphomycete species were used to assess mass loss due to fungal degradation after correcting for mass loss due to leaching. One control per leaf species was used to assess mass loss due to other factors in order to correct mass loss values of the 5 replicates. *Diseño experimental del experimento de microcosmos. Se usaron cinco réplicas por especie con discos de hojas y tapones de micelio de 6 especies de hifomicetos acuáticos para evaluar la pérdida de masa debido a la degradación fúngica después de corregir la pérdida de masa debido a la lixiviación. Se usó un control por especie de hoja para evaluar la pérdida de masa debido a otros factores con el fin de corregir los valores de pérdida de masa de las 5 repeticiones.*

pronounced in N-rich, soft than in N-poor, hard leaves. Is this a direct consequence of the initial properties of the leaf liter? Will the different paces of decomposition and the different changes in quality of N-poor and N-rich leaves still occur with similar fungal colonization?

In this microcosm laboratory experiment we controlled the extrinsic abiotic factors and the composition of the aquatic hyphomycete community during decomposition of alder (*Alnus glutinosa*), oak (*Quercus robur*) and eucalypt (*Eucalyptus globulus*) in order to assess how initial CNP values of leaf litter influence fungal-mediated mass loss.

MATERIALS AND METHODS

Experimental setup

Leaves of alder (*Alnus glutinosa* (L.) Gaertn.), eucalypt (*Eucalyptus globulus* Labill.) and oak (*Quercus robur* L.) were collected from single trees before abscission, dried and stored in the dark at room temperature until needed. Pure cultures of six aquatic hyphomycetes species in agar plates were provided by C. Canhoto: *Articulospora tetracladia*, *Tetracladium marchalianum*, *Tricladium chaetocladium*, *Clavariopsis aquatica*, *Anguilospora filiformis* and *Tetrachaetum elegans*.

A total of 530 leaf discs were cut (cork borer Ø = 8 mm) avoiding the main veins, from the leaves of each species, and oven-dried (60 °C; 48h). Two sets of 10 leaf discs needled together were weighed to the nearest 0.1 mg to assess mass loss due to leaching and mass loss during decomposition. The other leaf discs were used to determine carbon, nitrogen and phosphorus. The leaf discs were placed in an Erlenmeyer filled with 500 mL of distilled water and sterilized (autoclave 121 °C; 15 min) to remove any microbial colonization and to promote leaching.

After sterilization, one set of 10 leaf discs from each replicate was oven-dried (60 °C; 48h) and weighed to determine mass loss due to leaching. The other leaf discs were transferred to 500 mL Erlenmeyers with 160 mL of a mineral nutrient solution (100 mg $CaCl_2.2H_2O$, 10 mg $MgSO_4.7H_2O$, 0.5 g 3-morpholino propane sulfonic acid (MOPS), 0.55 mg K_2HPO_4 and 10 mg KNO_3 per liter of sterile distilled water; pH = 7; Chauvet & Suberkropp, 1988). Each leaf species was replicated five times in individual Erlenmeyers (Fig. 1) inoculated with one plug of the edge of the mycelium of each aquatic hyphomycete species (cork borer Ø = 9 mm). A sixth Erlenmeyer with leaves but no fungal inoculum was used as the control to assess mass loss due to other factors. The Erlenmeyers were incubated on an orbital shaker (100 rpm, 20 °C ± 1°C) for 20 days. The mycelium plugs were removed after one week and the medium was replaced weekly.

After 20 days, the set of 10 leaf discs was oven-dried (60 °C; 48h) and weighed to the nearest 0.1 mg to assess final dry mass. The remaining leaf discs from each Erlenmeyer were placed in individual zip lock bags, freeze-dried and stored at -20 °C for quantification of carbon, nitrogen and phosphorus.

C, N and P of leaf discs

Initial and final carbon, nitrogen and phosphorus content of the leaf material was quantified by standard laboratory procedures at Escola Superior Agrária, Instituto Politécnico de Coimbra. Shortly, C was determined by combustion at 590 °C followed by infra-red detection (Leco®, 1997). Nitrogen was determined by acid digestion (Kjeldahl method), distillation and quantification through titration (Bremner, 1965). Phosphorus was determined by dry mineralization (480-500 °C) followed by atomic absorption spectrometry quantification (Lucas & Sequeira, 1976; Ribas *et al.*, 1988).

Data analysis

The mass loss due to leaching was used to correct the final dry mass in the replicates and the controls. The percentage of each nutrient in the leaf litter was divided by its molar mass to obtain the content in moles and calculate the molar mass ratios C:N, C:P and N:P.

The effect of leaf identity on the response variables was tested by one-way ANOVA, and Tukey's HSD test was used for pairwise comparisons when differences among leaf species were

significant (Zar, 1996). Final nutrient contents were compared with average initial values by t-test for single means (test of means against reference constant). Whenever necessary, data was transformed to attain homogeneity of variances; percentages were transformed with the arcsine function while all other data was transformed with the natural logarithm (Zar, 1996). All analyses were performed with the software STATISTICA 13.0 with significance set at $p = 0.05$.

RESULTS

All leaf species leached a significant amount of mass during sterilization. Dry mass remaining after leaching was 68-71 % in alder and 73-76 % in oak and eucalypt (Fig. 2, top). After correcting for leaching, there was no mass loss in the controls and all mass loss was thus considered to be due to fungal degradation. Alder final dry mass was significantly lower than oak and eucalypt final dry mass (Fig. 2, bottom).

Initial carbon, nitrogen and phosphorus contents, as well as their molar ratios, varied significantly among leaf species ($F > 10.9$, $p < 0.04$), with alder richest and oak poorest in nitrogen and phosphorus (Table 1). Concomitantly, C:N and C:P ratios were lowest in alder and highest in oak, while N:P ratios were lowest in eucalypt and highest in alder (Table 1).

Except for carbon in alder and oak leaf litter, all nutrients changed significantly during decomposition ($t > 3.4$, $p < 0.05$); nitrogen was immobilized (i.e., final < initial values), while phosphorus was released (i.e., final > initial values) in all leaf species with higher final values in alder than oak or eucalypt (Fig. 3). Final C:N ratios differed significantly among the three leaf species while C:P ratios were significantly different between alder and the other two species ($F > 10.1$, $p < 0.002$; Fig. 3). There was no significant effect of leaf litter on final N:P ratios ($F = 1.6$, $p = 0.2$; Fig. 3). The ratios of final to initial values were in general higher in eucalypt followed by oak, while alder showed the smallest ratios between final and initial values (Fig. 3). There was a significant effect of leaf species on final to initial ratios of C content, C:P and N:P of leaf litter ($F > 5.5$, $p < 0.03$; Table 1).

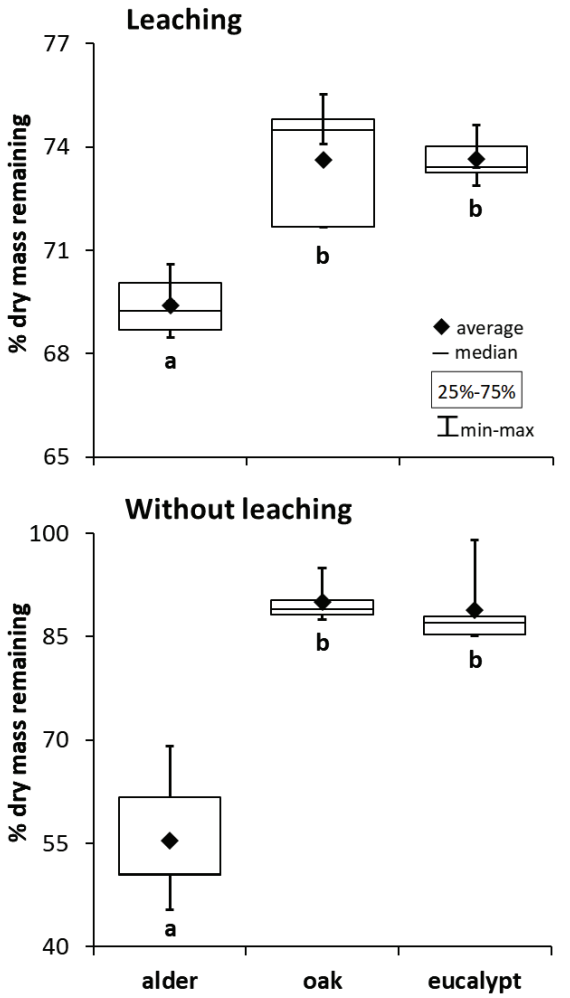

Figure 2. Dry mass remaining after leaching (top) and dry mass remaining after correcting for leaching (bottom). Different letters indicate significant differences among leaf species after Tukey HSD. *Pérdida de masa debido a lixiviación (arriba) y masa seca restante después de corregir la lixiviación (abajo). Diferentes letras indican diferencias significativas entre las especies de hojas después de Tukey HSD.*

DISCUSSION

This experiment exposed leaf litter to the same initial conditions. Further alterations of the incubation medium due to soluble compounds was reduced to a minimum due to the artificial leaching of the leaves and to the changing of the medium during the experiment. Moreover, correcting mass loss due to leaching allowed isolating the effects of fungal degradation on mass loss.

Table 1. Initial values (i) and final/initial ratios (f/i) of carbon (C), nitrogen (N), phosphorus (P), C:N, C:P and N:P of alder (A), oak (O) and eucalypt (E) leaves. Values are ranges (ni = 2 and nf = 5). Species with different superscripts are significantly different after Tukey HSD. Asterisks indicate significant differences between initial and final values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). *Valores iniciales (i) y relaciones final/inicial (f/i) de carbono (C), nitrógeno (N), fósforo (P), C: N, C: P y C: P de hojas de aliso (A), roble (O) y eucalipto (E). Los valores son rangos (ni = 2 y nf = 5). Las especies con diferentes superíndices son significativamente diferentes después de Tukey HSD. Los asteriscos indican diferencias significativas entre los valores iniciales y finales (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).*

	Alder	Oak	Eucalypt
C_i	47-49 ^{ab}	41-46 ^{b}	54-55 ^{a}
N_i	3.5-3.6 ^{a}	0.9-1.0 ^{b}	1.4-1.4 ^{c}
P_i	0.14-0.14 ^{a}	0.06-0.06 ^{b}	0.10-0.12 ^{c}
$C:N_i$	16-16 ^{a}	50-61 ^{b}	45-47 ^{b}
$C:P_i$	870-936 ^{a}	1696-1861 ^{b}	1196-1371 ^{a}
$N:P_i$	56-58 ^{a}	31-34 ^{b}	27-29 ^{b}
C_f/C_i	0.9-1.0 ^{a}	1.0-1.2 ^{b}	1.1-1.1 ^{b} ****
N_f/N_i	1.0-1.5 ^{a} *	1.2-1.4 ^{a} **	1.3-1.4 ^{a} ****
P_f/P_i	0.3-0.7 ^{a} ***	0.2-0.7 ^{a} **	0.2-0.2 ^{a} ****
$C:N_f/C:N_i$	0.6-0.9 ^{a} ***	0.7-0.9 ^{a} *	0.8-0.8 ^{a} ****
$C:P_f/C:P_i$	1.2-3.6 ^{a} *	1.6-5.9 ^{a} *	4.7-6.5 ^{b} **
$N:P_f/N:P_i$	1.9-5.0 ^{a} **	1.7-7.2 ^{a} *	5.9-8.2 ^{b} **

Similarly to field studies (Canhoto & Graça, 1996; Abelho, 2008), mass remaining of alder at the end of the experiment was significantly lower than either eucalypt or oak. This shows that, even under controlled experimental conditions, aquatic hyphomycetes have differential colonization and/or different activity among leaf litter.

Aquatic hyphomycetes may use nutrients from the leaves and/or from the water (Suberkropp & Chauvet, 1995). Since the incubation medium was relatively poor in nutrients (Chauvet & Suberkropp, 1988), fungi most probably had to use the leaf nutrients for growth (Suberkropp & Chauvet, 1995); in this case, the aquatic hyphomycetes growing on oak and eucalypt would be nutrient-limited in comparison to the ones growing on alder (Danger & Chauvet, 2013).

While initial nutrient content reflects the characteristics of the leaf species, final values include the fungal biomass associated with decomposing litter. Some fungal species can reduce their N-content when facing low N resources (Levi & Cowling, 1969) and store P in excess (Beever & Burns, 1981). As aquatic hyphomycetes are clearly not homeostatic (Danger & Chauvet, 2013) it is possible that the mycelium associated with the poor-N leaves had less amounts of N. In fact, the final N content of the controls with no fungal innoculum (0.93 % in oak, 1.54 % in eucalypt and 1.71 % in alder) was lower than initial content in all leaf species showing that, in the absence of fungal colonization, nitrogen is lost due to leaching. Thus, the difference between initial and final nutrient contents can be attributed to fungal colonization in the three leaf species. Alder was initially richer in nitrogen and remained richer at the end of the experiment, but the greatest changes occurred in the poorer oak and eucalypt leaves.

In this experiment, final C:P ratios of decomposing leaf litter attained very high values, ranging, on average, 2495 in alder to 7516 in eucalypt. While part of the increase in the C:P ratios was due to the loss of soluble phosphorus during leaching, fungal colonization may have also contributed to the increase in C:P ratios. Fungi have the ability to uptake and store excess phosphorus in their biomass (Gulis *et al.*, 2017). Danger & Chauvet (2013) showed that fungal

mycelium of *Lemonniera terrestris*, *Articulospora tetracladia* and *Tricladium chaetocladium* can reach C:P ratios of 1166 to 1499. Thus, fungal colonization might have contributed to the high C:P ratios obtained in the present experiment.

Alder had the highest N and P content and remained so at the end of the experiment. It also showed the smallest differences between initial and final values, while oak and eucalypt leaves showed the highest difference between initial and final values. The small loss of P in alder leaves may explain the highest mass loss observed in this species, most probably due to a higher and/or differential fungal colonization despite the controlled fungal decomposer community. García-Palacios *et al.* (2016) reported that

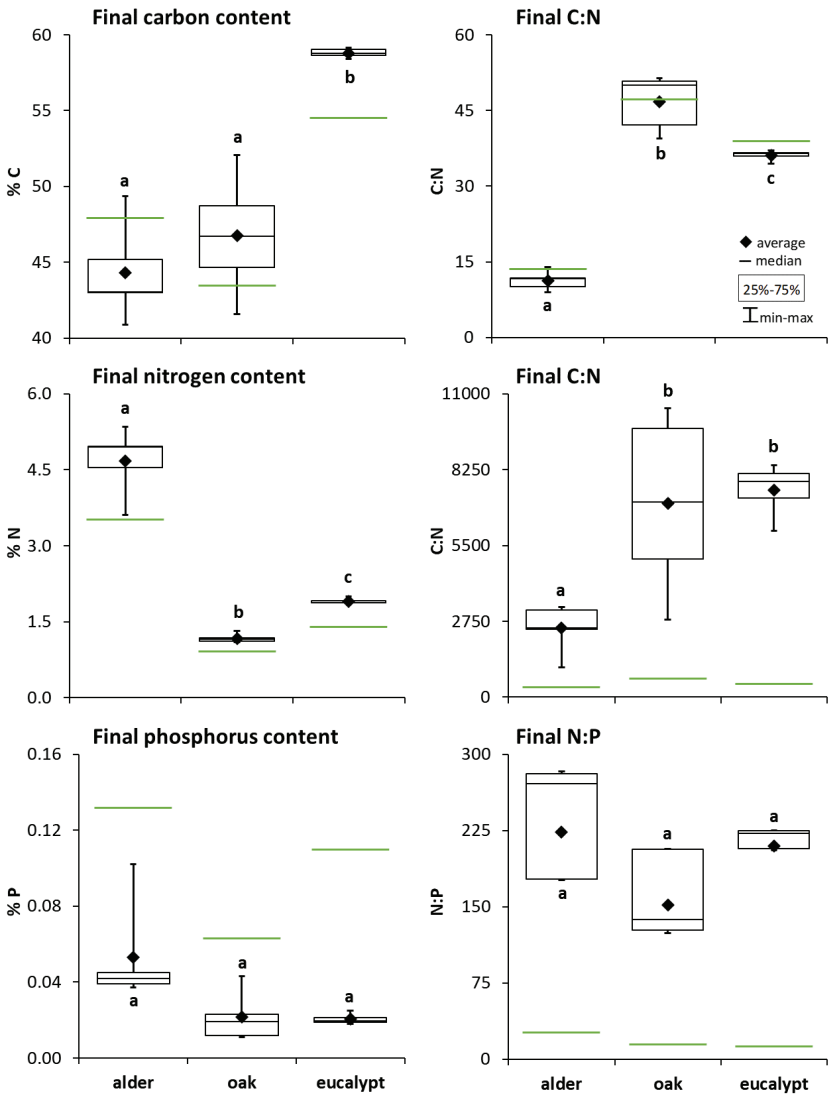

Figure 3. Final carbon, nitrogen and phosphorus contents (left) and final C:N, C:P and N:P ratios (right) of alder, oak and eucalypt leaves. Different letters indicate significant differences among leaf species after Tukey HSD. The horizontal lines show the initial values for comparison purposes. *Contenido final de carbono, nitrógeno y fósforo (izquierda) y proporción final C: N, C: P y N: P (derecha) de hojas de aliso, roble y eucalipto. Diferentes letras indican diferencias significativas entre las especies de hojas después de Tukey HSD. Las líneas horizontales muestran los valores iniciales para comparación.*

decomposition in streams is driven by litter N concentrations but also by the complexity of the decomposer community.It is possible that leaf litter was differentially colonized by early (*Anguilospora filiformis*, *Tetrachaetum elegans* and *Tricladium chaetocladium*) and late (*Articulospora tetracladia*, *Clavariopsis aquatica*, and *Tetracladium marchalianum*) colonizer species (Cornut *et al.*, 2015). In an experiment using a controlled inoculum of aquatic hyphomycetes, Cornut *et al.* (2015) found that leaf litter conditioned by different fungal assemblages resulted in different N and P concentration on leaves, thus altering elemental composition of leaf litter.

Despite the differences among species regarding the variations in C, N, and P values, and their ratios during decomposition, alder decomposed faster than the other leaves suggesting that initial CNP content of leaves determines the fungal-mediated mass loss of leaf litter. However, to fully understand the influence of fungal colonization on CNP changes during decomposition, the fungal community should be assessed in future work.

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