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Effects of ashes from grassland burning in the Paraná River floodplain on aquatic microcrustaceans

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Abstract. The burning of grasslands is a widespread practice in many landscapes, yet it has complex and multifaceted effects on natural ecosystems. In regions such as the Middle Paraná River floodplain, the frequency and intensity of wildfires have increased, primarily due to livestock production and related land-use activities. However, there is limited information on the potential effects of ash on aquatic biota. This study aims to evaluate the impact of ash produced from the burning of two distinct grasslands (grassland A, dominated by *Panicum* sp., and grassland B, dominated by *Cinnagrostis* sp.) in the Middle Paraná River floodplain on the Cladocera species *Ceriodaphnia reticulata.* Our findings reveal negative effects of ash from both grasslands on survival. In turn, while reproduction decreased in treatments with ash from grassland A, it significantly increased in treatments with ash from grassland B. Furthermore, survival rates were primarily influenced by the total ash particles, whereas reproduction was affected by both total ash particles and the fine fraction. Additional research is needed to better understand which fire characteristics influence the toxicity of ash to biota. These insights are critical for improving and refining integrated fire management practices.

[Keywords: zooplankton, fire, aquatic ecosystem, experiment]

Resumen. Efecto de las cenizas generadas en quemas de pastizales de la llanura de inundación del río Paraná sobre microcrustáceos acuáticos. La quema de pastizales es una práctica generalizada en muchos paisajes, pero tiene numerosos efectos complejos sobre los ecosistemas naturales. En regiones como la llanura de inundación del río Paraná Medio, la frecuencia y la intensidad de los incendios han aumentado debido, sobre todo, a la producción ganadera y otros usos del suelo relacionados. Hoy en día hay poca información disponible sobre los efectos potenciales de las cenizas en la biota acuática. En este estudio evaluamos el impacto de las cenizas generadas por la quema de dos pastizales distintos (pastizal A, dominado por *Panicum* sp., y pastizal B, dominado por *Cinnagrostis* sp.) en la llanura de inundación del río Paraná Medio, sobre el cladócero *Ceriodaphnia reticulata*. Los resultados mostraron efectos negativos de las cenizas de ambos pastizales sobre la supervivencia. Por otro lado, mientras que la reproducción disminuyó en los tratamientos con cenizas del pastizal A, aumentó significativamente en los tratamientos con cenizas del pastizal B. Asimismo, las tasas de supervivencia se vieron principalmente afectadas por el total de partículas de cenizas, mientras que la reproducción fue influenciada tanto por el total de partículas como por la fracción fina. Se requiere más investigación para determinar qué características del fuego influyen en la toxicidad de las cenizas sobre la biota. Estos aspectos son fundamentales para mejorar y refinar las prácticas de manejo integrado del fuego.

[Palabras clave: zooplancton, fuego, ecosistema acuático, experimento]

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Introduction

To manipulate vegetation and wildlife, fire has been used as a tool for millions of years (Bilbao et al. 2019; Bilbao et al. 2022; Millán Devera et al. 2022). Although fire is still employed in many regions as a traditional practice, social pressures have led to changes in land use, migrations and increased fire activity around the world (Myers 2006). The lack of proper fire planning and management results in significant uncontrolled fires that deteriorate the environment both functionally and structurally, with dramatic economic and social consequences.

The La Plata River Basin, and particularly, the Middle Paraná River is primarily composed of floodplains, reeds and grasslands. It is considered a wetland mosaic, characterized by lands that are periodically inundated by water and soils that are permanently or semi-permanently saturated. Both the climatic and, especially, the hydrological regimes define the structural and functional properties of these systems. Consequently, during periods of pronounced drought, the ecoregion became weaker and more vulnerable to anthropogenic perturbations, and particularly to intentional or accidental fires.

The introduction of livestock to the islands is a local practice aimed at fattening cattle because the animals can graze on good-quality grasslands and have access to high-quality water (Kandus and Morandeira 2020). In recent years, this practice has intensified due to the expansion of the agricultural frontier, which reduced the areas traditionally used for livestock and forced their relocation to riverine wetlands (Kandus et al. 2010; Sica et al. 2016). The controlled burning of grasslands is a widespread agricultural practice aimed at enhancing pasture quality, managing parasites, and mitigating the risk of uncontrolled wildfires. Between 2020 and 2023, ~89087 fire outbreaks were recorded in the Delta and Paraná islands, resulting in an average of 300000 ha/year affected (Ministry of Environment and Sustainable Development of Argentina 2023). These issues were exacerbated by extreme drought, lack of rainfall and historically low river levels, which led to the disappearance of streams and small rivers that typically acted as firebreaks (Draghi 2020).

Despite extensive research on the effects of fire on vegetation and changes in soil properties and structure, there is limited information on its impact on the quality of surrounding water bodies. Ashes produced during grassland fires can be incorporated to the aquatic systems through the wind and runoff (Montico et al. 2023). Uncontrolled vegetation fires produce large amounts of ash and promote wind and water erosion due to the lack of vegetation cover. This can facilitate the migration of particulate and dissolved elements —including heavy metals, organic carbon and nutrients associated to the vegetation ashes— into aquatic environments (Brown et al. 2013; Dahm et al. 2015). An increase in hydrometric levels can further contribute to the introduction of ashes into water systems, as rising water can flood areas with burnt vegetation. This process alters water quality and affects both direct and indirectly the communities of aquatic organisms, such as macroinvertebrates (Earl and Blinn 2003), amphibians (Pilliod et al. 2003), fish (Spencer et al. 2003; Kirsch et al. 2024) and microscopic organisms, like phytoplankton (Charette and Prepas 2003; Machado et al. 2008; Campos et al. 2012) and zooplankton (Brito et al. 2017).

Zooplankton individuals in rivers and streams are valuable indicators of water quality due to their small size and high sensitivity to environmental changes. This community consists of invertebrate organisms with varying sizes (ranging from a few micrometers to two millimeters in length) and shapes, which live suspended in the water and play a key role in the trophic network (Carpenter et al. 2001). They feed on particulate organic matter, microalgae and protozoa; in turn, are consumed by larger organisms such as fish and macroinvertebrates. Because zooplankton primarily feed through filtration, they incorporate significant amounts of particulate organic matter from the water column, contributing to the nutrients recycling process. This characteristic makes zooplankton fundamental to the matter and energy flows in aquatic environments (McQueen et al. 1989). Any changes in their structure or abundance can lead to both bottom-up and top-down effects within the trophic network, thereby altering ecosystem dynamics (Carpenter et al. 1988; Faithfull et al. 2011).

The presence of ashes in water can have negative effects on zooplankton in general, and on cladocerans in particular. This is because their antennae or swimming legs (i.e., setae or cilia that aid in filtration) are structurally fragile and can be seriously damaged when they are feeding from the water column. Additionally, the accumulation of ashes on their bodies (e.g., carapace, intestine) can alter their behavior, slow down their movements due to the impact on their swimming legs, or increase their body weight confining them to the lower level of the water column (Artells et al. 2013; Cole et al. 2013). If feeding is inhibited, other vital functions (i.e., physiology, behavior, reproduction) can be seriously affected (Balseiro et al. 2014).

Moreover, the evidence from synthetic materials suggests that the effect of ashes may depend on the size of the particles (Kim et al. 2010; Liu et al. 2019; Gutierrez et al. 2021) apart from the vegetation type. In this research, *Ceriodaphnia reticulata* (Jurine 1820) was selected as model species to assess the potential impacts of ashes on aquatic biota due to its common presence in the studied area.

In summary, our aim was to evaluate the effect of ashes from grassland burning in the floodplain of the Paraná River on individual and population metrics of this species. Specifically, our hypothesis is that ash particles negatively affect both individual and population parameters by decreasing survival and fecundity. Our results can contribute to enhance knowledge on the toxicological effects of ashes on the aquatic biota and support proposals for integrated fire management practices.

Materials and Methods

Ashes collection

The ashes were collected immediately after two fire events occurred in May (F1) and July (F2) 2021 in two different sites located in the floodplain of the Middle Paraná River (Figure 1). The first location, next to the Riacho Santa Fe, was dominated by *Panicum* sp. (hereafter referred to as grassland A), while the second location —next to the Paraná River— was dominated by *Cinnagrostis* sp. (grassland B).

Test organism

Ceriodaphnia reticulata specimens were initially collected using a planktonic net (50 μm) from shallow lakes adjacent to the Paraná River. They were then transferred to experimental tanks at the National Institute of Limonology (CONICET-UNL, Argentina). These tanks contain a diverse community of aquatic organisms native to the region, along with sediment and aquatic plants, creating a more natural environment. This setup facilitates gradual acclimatization and supports a stable zooplankton population through resistant stages present in the sediment. Subsequently, *C. reticulata* adult specimens were isolated and acclimated under controlled laboratory conditions (photoperiod: 16 h light-8 h darkness; temperature: 21±2 °C). These organisms were maintained in dechlorinated, aerated tap water and fed with *Tetradesmus obliquus* algae every other day at a density of ~1.4 cells/mL. Before the experiments, one mother from this 'stock' culture was isolated, and a new culture was started with its neonates. Therefore, all the experiments were conducted using clones derived from the same initial mother, which were reared under identical laboratory conditions. Tap water mean physical and chemical characteristics were pH: 7.1, conductivity: 1020 μS/cm, total hardness: 180 mg/L $CaCO_3$, alkalinity 120 mg/L $CaCO_3$, 39 mg/L Ca^{++}, 20 mg/L Mg^{++}, 146 mg/L HCO_3^-.

Experimental design

To analyze the effects of ashes from the two different fires on *C. reticulata*, five treatments were considered: culture water (control), culture water containing grassland A ashes (AA), culture water containing filtered grassland A ashes (FAA), culture water containing grassland B ashes (BA) and culture water containing filtered grassland B ashes (FBA). The control and treatments AA and BA were prepared simultaneously, and were maintained under controlled laboratory conditions (photoperiod: 16 h light-8 h darkness; temperature: 21±2 °C) with aerators to ensure adequate oxygenation.

The treatments with ashes (i.e., mother media AA and BA) were prepared in 10 L trays by adding 23 g of ashes (A or B) to 7 L of water media, between 12 and 24 h before the start of the assays, resulting in a concentration of 3.3 g/L. The concentration was chosen based on previous research (Brito et al. 2017), which established 3 g/L as the minimum concentration that did not cause lethal effects on three aquatic species from different trophic and functional levels (*Ceriodaphnia dubia*, *Danio rerio* and *Biomphalaria glabrata*).

The filtered treatments (FAA and FBA) were designed to analyze the effects caused by the finest ash particles and dissolved material in comparison with the whole ash sample. These treatments were prepared by filtering

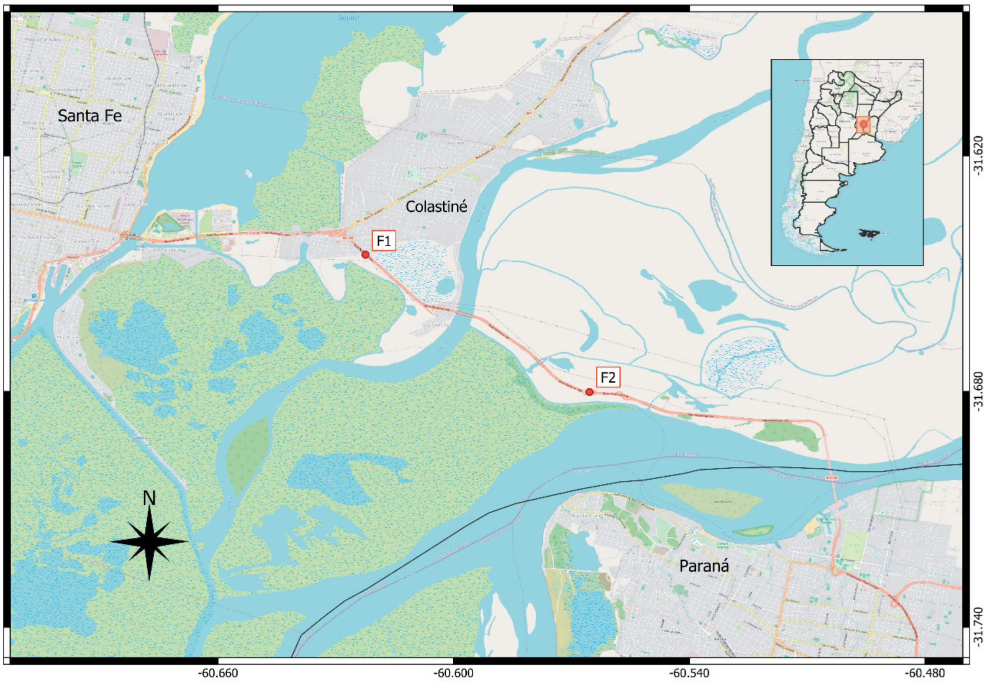

Figure 1. Map of the location of the two fire events: F1 (31°38'42.6'' S - 60°37'20.7'' W) and F2 (31°40'48.4'' S - 60°33'55.5'' W).

Figura 1. Mapa que muestra la ubicación de los dos eventos de quema: F1 (31°38'42.6'' S - 60°37'20.7'' O) y F2 (31°40'48.4'' S - 60°33'55.5'' O).

ash media with a 60 μm mesh each time media was needed.

To gather information about physical and chemical characteristics of the control, AA and BA media, we measured temperature, conductivity, pH and dissolved oxygen daily for 15 days using a HANNA multiparametric probe. In addition, 25 mL water samples from each media were collected on days 1, 7 and 15, and filtered through nitrocellulose membrane filters (0.45 μm pore size) to determine concentrations of total phenolic compounds using Folin-Ciocalteu reagent and gallic acid as standard (Box 1983), soluble reactive phosphorus (SRP) by the ascorbic acid method, nitrate plus nitrite nitrogen (NO_3^-+NO_2^-) by reduction of NO_3^- with hydrazine sulfate and subsequent determination of NO_2^- through diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride and total ammonia nitrogen (NH_3+NH_4^+) by the indophenol blue method. Chromophoric dissolved organic matter (CDOM) was spectrophotometrically evaluated at 440 and 700 nm using 1-cm quartz cuvettes (Helms et al. 2008). The 700 nm absorbance was subtracted from the value at 440 nm to correct offsets. Absorption coefficient (m^{-1}) at 440 nm (A_{440}) was calculated according to Kirk (1994) from the corrected absorbance at this wavelength and used as a CDOM concentration proxy (Helms et al. 2008).

Nutrient analyses were conducted only on the original (unfiltered) AA and BA media (mother media), not on the filtered treatments. Since the analysis method focused only on dissolved compounds, an additional filtration step with a pore size finer than that used to prepare FAA and FBA was applied. This process effectively removes particulate matter. Thus, we considered that nutrient concentrations in the originally prepared AA and BA media were equivalent to those in the FAA and FBA treatments.

Experimental procedure

To start the experiments, 25 mL of each media (control, AA, BA, and their respective filtered versions, FAA and FBA) were transferred to glass containers for subsequent exposition of the organisms. *Ceriodaphnia reticulata* neonates less than 48 h old were isolated from the initial culture and placed individually in 100 mL glasses with 25 mL of the corresponding medium (Control, AA, FAA, BA and FBA). Each treatment consisted of 17 (±5) replicates.

Every other day, the media in the glass containers was renewed from the trays to maintain fresh and oxygenated conditions.

Each treatment consisted of 17 (±5) individuals (replicates); each one placed in a separate glass container. The assays lasted 20 days, corresponding to lifespan of *C. reticulata*. Throughout the experiment, the media was renewed and the organisms were fed with *T. obliquus* concentrate (1.4 cells/mL) every other day.

Analyzed variables

Survival and fecundity were recorded every 48 h. Survival was measured by recording the number of organism deaths in each glass container. An organism was considered dead if it exhibited no activity when stimulated. Fecundity was determined based on the total number of neonates and broods from each female.

Data analysis

Survival was analyzed using the Kruskal-Wallis (KW) test with data from days 1 to 20, since normality and homoscedasticity assumptions were not met. Fecundity (total neonates and total broods per female) was analyzed using ANOVA after applying a log10 transformation to meet assumptions. Statistical significance was stepped at $P<0.05$. Pairwise comparisons of estimated marginal means (EMMs) were performed to explore differences between treatments.

All statistical analyses were performed in R (version 2024.09.0+375) (R Core Team 2024). The following packages were used: readxl for reading Excel files, tidyverse for data manipulation and visualization, writexl for writing Excel files, rstatix and lme4 for statistical tests, MASS for model fitting, emmeans for estimated marginal means, PMCMRplus for post tests and ggplot2 and patchwork for graphical representation.

Results

Water quality parameters

Temperature, pH and dissolved oxygen remained relatively constant. However, conductivity increased in both ash-treated media (AA and BA) (Table 1), with the highest values observed in the medium with ashes from grassland A. Both ash media showed an increase in SRP and A_{440}. Conversely, both showed a decrease in NO_3^-+NO_2^-. Additionally, phenolic compounds increased in the medium from grassland B (Supplementary Material-Table S1). For all these variables, except conductivity, the medium with ashes from grassland B showed the greatest differences compared to the control (Table 1).

Survival

Ceriodaphnia reticulata exposed to ashes registered a significant reduction in survival compared to the control (KW: X^2=14.951; df=4; P=0.005), particularly when exposed to both the total ash fraction (AA and BA) and the finest particles and dissolved materials from grassland A (FAA) ($P<0.005$). By contrast, exposure to the finest particles and dissolved materials from grassland B (FBA) did not produce any significant effect when compared to the control ($P>0.05$). The greatest differences regarding the control were observed for AA and FAA treatments (Figure 2).

Table 1. Descriptive statistics for the studied media (control, grassland A ashes [AA] and grassland B ashes [BA]). Mean and standard deviation (SD) of data grouping days 1, 7 and 15 are shown.

Tabla 1. Estadísticas descriptivas para los medios estudiados (control, cenizas de pastizal A [AA] y cenizas de pastizal B [BA]). Se muestra la media y desviación estándar (DE) de los datos agrupando los días 1, 7 y 15.

	Control		AA		BA	
Variable	Mean	SD	Mean	SD	Mean	SD
Temperature (°C)	22	0	22	0	22	1
Conductivity (μS/cm)	1679	12	2130	163	1867	146
Dissolved oxygen (%)	98	15	93	21	94	13
pH	8.0	0.5	7.7	0.5	7.6	0.5
Phenolic compounds (mg/L)	0.59	0.07	0.62	0.05	1.17	0.13
NH_3+NH_4^+ (μg N/L)	194	168	100	61	179	153
SRP (μg/L)	244	23	4602	2253	7478	2566
NO_3^-+NO_2^- (μg N/L)	682	370	348	158	116	142
A_{440} (m^{-1})	0.4	0.1	1.1	0.1	3.6	0.5

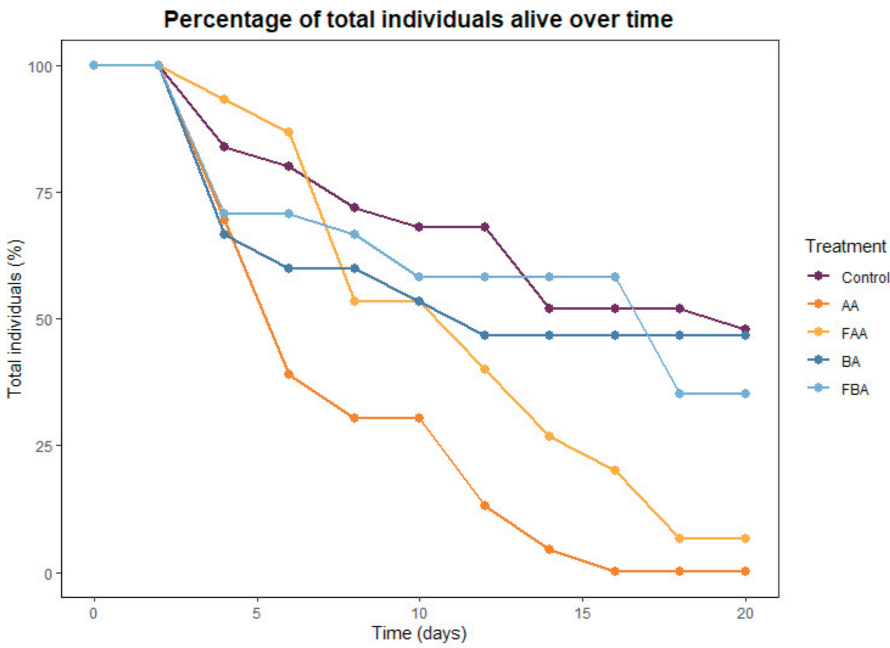

Figure 2. Survival of *Ceriodaphnia reticulata* under the different treatments (control, grassland A ashes [AA], filtered grassland A ashes [FAA], grassland B ashes [BA] and filtered grassland B ashes [FBA]), measured as the percentage of total individuals alive over time.

Figura 2. Supervivencia de *Ceriodaphnia reticulata* bajo los diferentes tratamientos (control, cenizas de pastizal A [AA], cenizas de pastizal A filtradas [FAA], cenizas de pastizal B [BA] y cenizas de pastizal B filtradas [FBA]), medida como el porcentaje total de individuos vivos a través del tiempo.

Fecundity

The total number of births (F=26.46; df=4; P<0.001) and broods (F=28.82; df=4; P<0.001) exhibited a significant decrease in both treatments with grassland A ashes (AA and FAA, P<0.05) (Figure 3) compared to the control. In treatments with grassland B ashes, the total number of births significantly increased (P<0.05), while the total number of broods did not show any significant difference compared to the control (P>0.05) (Figure 3).

Discussion

We evaluated the effects of ashes generated from the burning of two grasslands on zooplankton organisms through chronic assays. Ashes from both grasslands had negative effects on cladoceran survival. However, the filtered ashes from grassland B did not produce significant changes, suggesting that only their largest particles were deleterious. Additionally, ashes from grassland A negatively impacted population

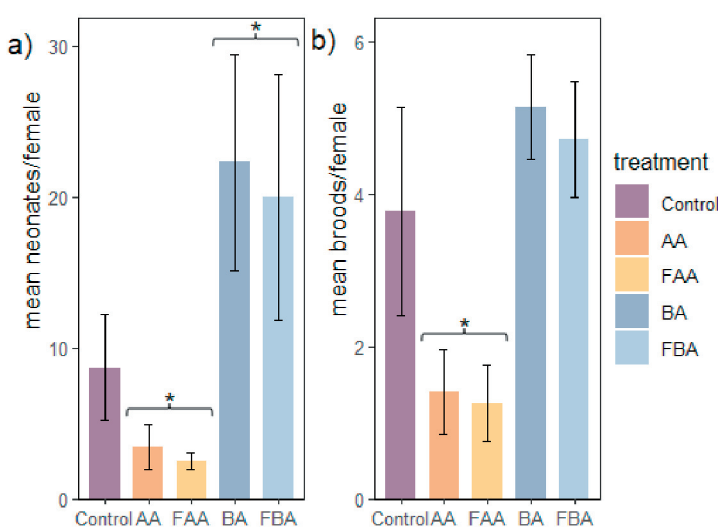

Figure 3. Fecundity of *Ceriodaphnia reticulata* under the different treatments (control, grassland A ashes [AA], filtered grassland A ashes [FAA], grassland B ashes [BA] and filtered grassland B ashes [FBA]), measured as: a) mean of neonates per female, and b) mean of broods per female. (*) indicates significant differences compared to the control (P<0.05).

Figura 3. Fecundidad de *Ceriodaphnia reticulata* bajo los diferentes tratamientos (control, cenizas de pastizal A [AA], cenizas de pastizal A filtradas [FAA], cenizas de pastizal B [BA] y cenizas de pastizal B filtradas [FBA]) medida como: a) media de neonatos por hembra, y b) media de camadas por hembra. (*) indica diferencias significativas respecto al control (P<0.05).

metrics by reducing the total number of broods and neonates, whereas ashes from grassland B stimulated reproduction, leading to a significant increase in neonates.

The observed differences can primarily be attributed to the distinct vegetation species involved in each fire. The solubilization of materials contained in plant ashes can vary widely depending on species' capacity to absorb and accumulate elements from the soil and surrounding environment (Peralta-Videa et al. 2009; Brito et al. 2017). Moreover, the selected areas for this research were influenced by nearby roads, which can introduce anthropogenic sources of contaminants —such as heavy metals— due to traffic emissions. These emissions include pollutant particles generated by vehicle exhaust, tire wear, weathered street surface and gasoline combustion, among others (Sezgin 2003; Ahmed 2006; Wei 2009), which can be absorbed and accumulated by the vegetation (Vymazal 2015), ultimately becoming part of the ash residues upon combustion (Abraham 2017).

Although heavy metal concentrations were not evaluated in this study, it is known that vegetal ashes can contain amounts of these elements sufficient to induce toxicity in aquatic environments (Ignatavičius et al. 2006; Pereira and Úbeda 2010). Additionally, the mineralization of particulate organic matter during fires can enhance heavy metals solubilization (Abraham et. Al 2017). The higher A_{440} and concentration of phenolic compounds in the medium with ashes from grassland B suggest a greater availability of dissolved organic matter (DOM), compared with the medium with ashes from grassland A. The higher DOM concentration might have decreased the toxicity of certain metals since natural DOM is characterized by functional groups able to bind heavy metals (Kungolos 2006). This could explain the greater negative effects on *C. reticulata* observed for ashes from grassland A compared to ashes from grassland B.

The higher concentration of soluble reactive phosphorus (SRP) in addition with higher DOM values in grassland B medium may explain the observed stimulation in reproduction when organisms were exposed to these ashes. Previous research has demonstrated a direct relationship between SRP and DOM with increased zooplankton biomass (Gyllström et al. 2005; Jeppesen et al. 2010; Kissman et al. 2017).

However, while phosphorus solubilization was pronounced, inorganic nitrogen solubilization was comparatively low for both types of ashes. This finding is consistent with previous studies that reported high phosphorus concentrations but low nitrogen concentrations in leachates from different ash types (Harper et al. 2019). This distinction is important because dissolved inorganic nitrogen can have toxic effects on microcrustaceans due to the generation of non-ionized ammonium and nitrite (Yu et al. 2022). In this regard, these effects can be dismissed according to the low values of total ammonia and nitrate plus nitrite observed in this study.

In environmental risk studies, *Ceriodaphnia* sp. is considered relatively equivalent to *Daphnia* (Versteeg et al. 1997) because both belong to the same functional group. Daphnids are filter feeders, and it has been reported that feeding appendages in species like *D. magna* efficiently filter particles ranging from 0.1-35 µm in size (Gophen and Geller 1984; Renzi and Blaskovic 2019). These organisms do not discriminate between particulates; they can ingest both algae and inorganic particulates such as ash. Particles larger than the average mesh size are retained very efficiently and transported to the intestine, where they can aggregate and cause blockages (Zhu et al. 2010). The inability to expel non-nutritive particulates can cause damage due to the additional energetic cost, disrupting the feeding process (Porter and McDonough 1984). This mechanism may explain the observed reduction in survival of *C. reticulata* exposed to total ash particles from both grassland A and B ashes. It also applies to the finest ash particles from grassland A. By contrast, the finest ash particles from grassland B, when isolated, did not cause any significant changes, which might be attributed to differences between vegetal species and/or fire characteristics. The type and size of the finest particulates from grassland B did not appear to negatively affect the feeding efficiency of the organism. Future research that includes particle size measurements is necessary to explore this aspect further.

Additionally, previous studies showed that particles of different nature can adhere to the shell surface of cladocerans (Artells et al. 2013). The small spines and rough texture on the exoskeleton of *C. reticulata* may serve as effective anchors for capturing and retaining particles from the surrounding medium. This accumulation of large ash particles can increase body weight, making swimming and maintaining position in the water column more challenging. This redirection of energy from vital processes to sustain life may reduce the energy available for reproduction, affecting the organism´s balance. According to ecophysiological theories, this could represent an adaptive strategy (Forbes and Callow 1996).

Furthermore, the conductivity of the medium with ash from grassland A was observed to be higher than that with ash from grassland B. Increased conductivity/salinity can result in osmotic stress, leading to higher mortality and reduced reproductive and growth rates (Gutierrez et al. 2024; Jeppesen et al. 2015). This observation is supported by fecundity assays, which showed a decrease in the total number of neonates and broods per female when *C. reticulata* was exposed to grassland A ashes.

To address these issues comprehensively, further research should focus on analyzing ashes produced by burning various plant species typical of wetlands, evaluating a broader range of ash concentrations, including size measurement, and assessing their effects on aquatic organisms from different areas of the system. Investigating the chemical composition of these ashes is crucial, as it could reveal the nutrient contributions they make to water bodies via wind, rain, or runoff. Additionally, understanding how fire may affect the release of toxic substances such as heavy metals from plant materials in areas with different land uses - especially by comparing ashes from plants in agricultural and livestock areas to those from less anthropogenically affected areas - could provide valuable insights.

Conclusions

The ashes produced by grassland burns can negatively impact cladoceran population parameters, although the extent of these effects likely depends on factors such as the type of vegetation burned and the fire's severity. Notably, different impacts were observed when comparing the effects of total ash particles to those of fine particles and dissolved matter. Survival rates were mainly affected by total ash particles, whereas reproduction was influenced by both total ash particles and the combination of fine particles and dissolved materials. Furthermore, while ashes from grassland A impaired reproduction, an opposite effect was observed with ashes from grassland B, highlighting the complexity of the mechanisms involved.

Understanding the factors that determine the severity of burn effects and their relationship with the generated ash could offer valuable insights into their impact on aquatic communities. New findings on these aspects are crucial for enhancing and refining integrated fire management practices, especially in light of the increasing prevalence of wildfires driven by climate change.

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