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Cigarette butt effects on diatom health in a stream ecosystem

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Abstract Cigarette butts are a prevalent form of litter containing numerous toxic chemicals. Because cigarette butts are frequently deposited on the ground and carried into water bodies, greater understanding of the toxic effects of cigarette butts in aquatic ecosystems is needed. We examined the toxicity of cigarette butts to algal growth and diatom health—especially pertinent because of the strong ecological role of these organisms. We modified the agar-based nutrient-diffusing substrate method by using cigarette butt leachate (at 10, 5, 2.5, and 1.25 butts/l concentrations), a whole cigarette butt, and a plain agar control. After incubating for 10 days in a small stream, the biofilms from the diffusing substrates were assessed for algal biomass and diatom health (chloroplast intactness and size of lipid bodies in two abundant species of *Navicula*). There were no significant differences among the cigarette butt treatments for algal biomass or diatom health; hence, evidence of toxic effects was not found. Other studies have demonstrated cigarette butt leachate toxicity to fish and aquatic invertebrates, but these studies were done in closed systems. In contrast, in open stream ecosystems, effluent may be quickly diluted and carried away by water flow, and the complex chemical environment of streams likely includes leachate from a variety of riparian leaves that fell into the stream (i.e., algae are naturally exposed to low concentrations of a wide variety of secondary chemicals). Our results do not preclude the finding of toxicity of cigarette butt effluent to algae, including diatoms, in standard toxicity tests.

Keywords CB · Cigarette butt toxicity · Chemical diffusing substrates · Emerging contaminant · Pollutants

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Introduction

Cigarette butts (CBs) are frequently littered (Bator et al. 2011; Patel et al. 2013; Rath et al. 2012; Slaughter et al. 2011) and comprise one of the most prevalent forms of litter in the environment. CBs are

commonly deposited in terrestrial locations frequented by people, including building entrances (Lee et al. 2013), shopping areas or locations where cigarettes are sold (Marah and Novotny 2011; Roder Green et al. 2014), and along streets (Healton et al. 2011; Moriwaki et al. 2009). Cigarette butts are also commonly found along shorelines (Araújo and Costa 2019), where they are both directly deposited and washed in from terrestrial habitats (Novotny et al. 2009).

Cigarette butts contain a variety of toxic chemicals that can leach into the environment and pose potential harm to ecosystems (Moriwaki et al. 2009). Among the approximately 4,000 chemicals in CBs (Kurmus and Mohajerani 2020; Shevchenko 2012) are toxins such as nicotine and nicotine derivatives (Roder Green et al. 2014) and metals, including heavy metals (Moerman and Potts 2011; Moriwaki et al. 2009; Pelit et al. 2013). As a consequence, CBs can be considered as hazardous waste (Torkashvand et al. 2020). Despite efforts to find commercial uses for recycled CBs (e.g., Kadir and Mohajerani 2011; Kurmus and Mohajerani 2020; Marinello et al. 2020), these CBs continue to be deposited in the environment because many smokers do not consider them as significant litter (Bator et al. 2011; Rath et al. 2012) and likely to do realize their toxic nature.

The toxicity of CBs, often in the form of CB leachate, has been borne out in toxicity studies using a wide variety of organisms (21 studies are graphically summarized in Fig. 1), including both traditional toxicology test organisms, such as cladocerans, fathead minnows, and the bacterium *Aliivibrio fisheri*, and locally relevant species, such as tidepool snails and freshwater mussels. Most toxicity studies have used aquatic organisms, especially those in freshwater. From a food web perspective, several examples of vertebrate predators and both invertebrate and vertebrate omnivores have been tested (Fig. 1). In contrast, autotrophs have been underrepresented in CB toxicity studies, despite the importance of autotrophs in food webs and their potential ability to take up chemicals from CBs (uptake by terrestrial plants: Selmar, 2018).

We were interested in assessing the toxicity of CBs to benthic freshwater algae, focusing on diatoms. Because diatoms and other algae are important base members of the aquatic food webs, toxicology studies conducted on these and other algae could lead to greater understanding of toxicity ramifications within aquatic ecosystems. The endpoint of many toxicity studies is organismal death, but in microbial communities, such as unicellular algal communities, following the fate of individual cells is impractical. As an alternative, changes in biomass, as measured by chlorophyll *a* concentration, are monitored. This sublethal endpoint is consistent with sublethal effects of CBs on other organisms, for example, behaviors to reduce exposure: clamping the shell opening to the substrate by tidepool snails and avoidance by terrestrial snails (Booth et al. 2015; Gill et al. 2018; respectively) and cell nuclear abnormalities in house finches and onions (Suárez-Rodríguez and Marías Garcia 2014; Montalvão et al. 2019b; respectively). Sublethal measures of diatom health have also been developed—including chloroplast condition and changes in lipid bodies. Specifically, stress in diatom cells can produce chloroplast contraction and increases in the size and/or number of lipid bodies (Pandey 2014).

In a departure from previous studies, we tested the potential toxicity of CBs to algae under ambient environmental conditions rather than investigating toxicity effects under laboratory conditions. Using a modification of nutrient-diffusing substrates, we tested whole CBs and CB effluent effects on algal communities in an urban stream, measuring effects on algal biomass (as chlorophyll *a*) and diatom health. We hypothesized that whole CBs would have the greatest toxic effects and that these effects would attenuate with decreasing effluent concentration.

Methods

Smoking cigarettes and preparing agar treatments

Purchased cigarettes (Marlboro 100's, Phillip Morris, Richmond, Virginia) were artificially smoked inside a fume hood. A metal aquarium manifold with adjustable airflow to the five outlets was fitted with plastic tubing that held the filter end of cigarettes in three of the ports (airflow to the other two ports was blocked), which allowed three cigarettes to be smoked at the same time. A large needle-less syringe, connected by tubing to the air pump port of the manifold, was used to pull air through the cigarettes and manifold, which effectively smoked the lit cigarettes. The syringe was temporarily removed and

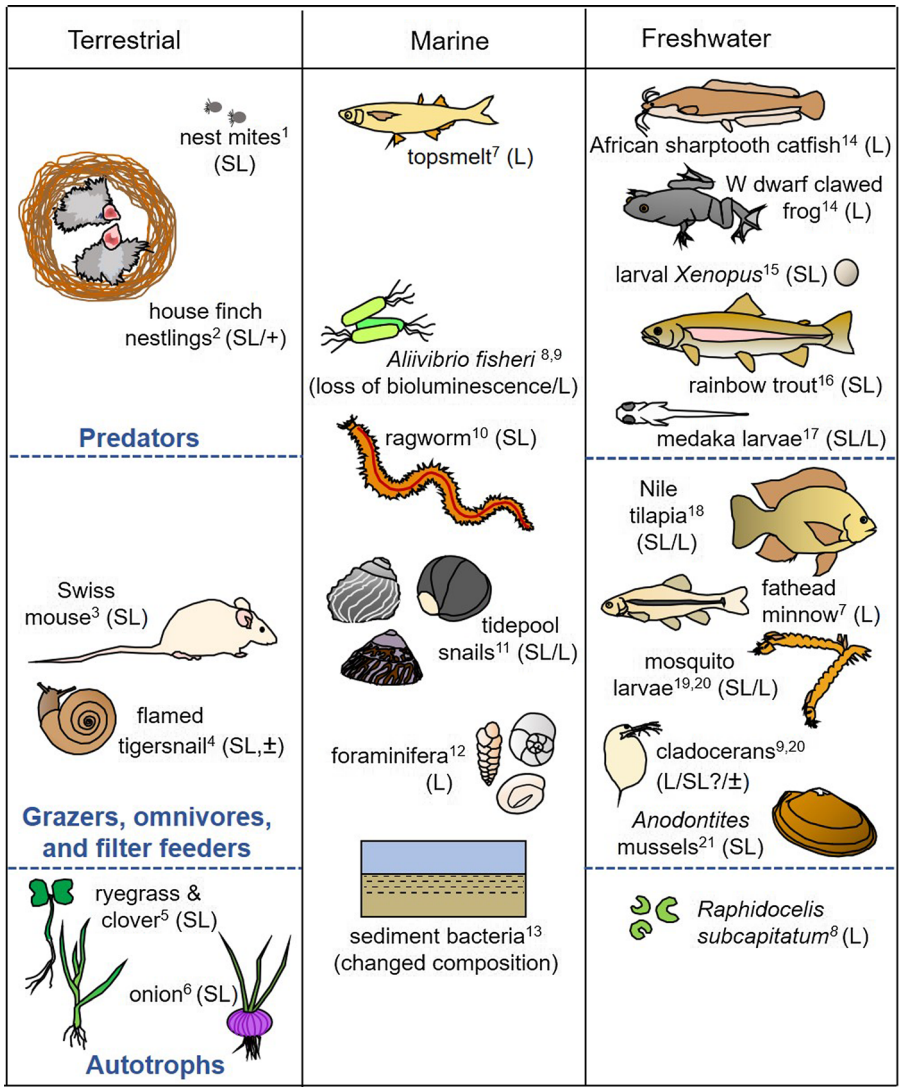

Fig. 1 Summary of CB toxicity studies with respect to each organism's habitat type (terrestrial, marine, or freshwater) and food web position. General results of studies are indicated by: SL = sublethal (including behavior, developmental or cellular damage); L = lethal (although lethality is concentration dependent); ± = neutral effects; and + = positive effects; with results described for microbes. Citations are indicated by superscript numbers as follows: 1 = Suárez-Rodríguez et al. (2013); 2 = Suárez-Rodríguez and Macías Garcia (2014); 3 = Cardoso et al. (2018): 4 = Gill et al. (2018); 5 = Green et al. (2019); 6 = Montalvão et al. (2019b) 7 = Slaughter et al. (2011); 8 = Bonanomi et al. (2020); 9 = Micevska et al. (2006); 10 = Wright et al. (2015); 11 = Booth et al. (2015); 12 = Caridi, (2020); 13 = Quéméneur et al. (2020); 14 = Lawal and Ologundudu (2013): 15 = Parker and Rayburn (2017); 16 = Yabes (2018); 17 = Lee and Lee (2015); 18 = Osuala et al. (2017); 19 = Dieng, (2011); 20 = Register (2000); and 21 = Montalvão, (2019a)

emptied between 'puffs.' The cigarettes were smoked to a pre-marked length of 1.5 cm beyond the filter, which yielded the filter, unsmoked tobacco, and an edge of burned tobacco. When split open, the smoke residue within filters was visually indistinguishable from human-smoked cigarettes of the same brand. Prepared butts were kept dry in an air-tight jar and used for the experiment within a week.

To make cigarette butt effluent, 10 cigarette butts were soaked in 1 L of deionized water for 24 h (Osuala et al. 2017), producing an effluent 10 times the lethal concentration for two tested fishes (Slaughter et al. 2011). This water was used as the full concentration

effluent in making the agar gels. Lower concentrations of effluent were made by diluting the effluent to one-half, one-quarter, and one-eighth concentrations. The resulting concentrations of effluent were 10, 5, 2.5, and 1.25 butts/L. In addition to butt effluents, a control 'effluent' of only deionized water and a whole cigarette butt treatment were used. Three nutrient treatments of potassium phosphate (phosphorus using 13.6 g KPO_3/L), sodium nitrate (nitrogen using 6.8 g $NaNO_3$/L), and a mix of both were used to characterize the nutrient conditions of the stream (Fairchild, 1985).

The nine treatments were used to make agar plates (Table 1; Fig. 2a). To ensure filling of the 25-ml plastic petri dishes (diameter = 6 cm) with agar, 250 mL of each treatment (whole cigarette butt or leachates, nutrients, and a control) were made. Agar was added to treatments at a concentration of 24 g of agar per L of solution, and then, the solutions were individually microwaved until the agar dissolved; at which point solutions were poured into labeled petri dishes. Whole smoked cigarette butts were placed in the thickening agar for the full butt treatment. A total of 72 agar gels were made (= 8 replicates per treatment).

Stream description and field methods.

The experimental site was in Bishop Creek, a second-order urban stream that crosses part of the University of Oklahoma campus, including Brandt Park (where it is impounded to form the Duck Pond; 1.03 hectares) and the South Research Campus. The location used was at the upstream edge of the Jimmie Austin Golf Course. At this location, the stream is shaded by riparian trees, is deeply incised—such that the sandstone bedrock is partly exposed—and has a stream bed that includes the remnants of a previous concrete wall (Fig. 2b). Consequently, the reach has a riffle-pool structure with a streambed of bedrock, natural and man-made rocks, and fines (primarily sand). Most other areas of Bishop Creek have a sandy streambed.

Each of the 8 replicates was positioned on a single 0.3 × 0.3 m concrete paver by gluing empty petri dish bottoms to the paver blocks in a pattern to minimize downstream effects of drifting chemicals among treatments (Fig. 2c). Blocks were labeled, as were the petri dish locations and the 9 treatments were randomly assigned to petri dishes within each block. Before placement in the stream, treatment agars were placed into the petri dish bottoms. Each petri dish was covered with thin cellulose filter paper, attached to the sides of the dish with two rubber bands, so the top was a flat surface. Blocks were placed in shaded pools in the stream and blocks were oriented so that the stream current would flow over blocks in a similar direction (Fig. 2d).

Current velocity (Marsh–McBirney Flo-Mate), water depth of each paver, and pH (Oakton Pocket pH tester) were recorded for each block at the start of the experiment (data in Online resource S2). Water depth over the blocks averaged 17.9 (SE = 3.3) cm and the current velocity averaged 0.05 (SE = 0.02) cm/s. The pH of the water was 8.05 throughout the study area.

The experiment ran for 10 days, starting on September 2, 2019 and ending on September 12, just prior to a large rainstorm, which would have washed out the experiment. Although the filter papers over the agar plates have been used in previous studies (Bergey 2008; Biggs and Kilroy 2000), after five days we noted that some of the filters were beginning to tear at the edges of the petri dishes and, consequently, a layer of fine polyester mesh was added over the paper filters to

Table 1 The nine tested treatments, which include a control, a whole CB, serial dilutions of concentrated CB effluent, and three nutrient treatments (without CB effluent)

Abbreviation	Treatment	Chl *a*	Diatom health
C	Control	x	x
Butt	Whole smoked cigarette butt	x	x
1*x*	Full effluent concentration, 10 butt/L	x	x
1/2*x*	Half effluent concentration, 5 butt/L	x	x
1/4*x*	Fourth effluent concentration, 2.5 butt/L	x	
1/8*x*	Eighth effluent concentration, 1.25 butt/L	x	
N	Nutrient treatment, 6.8 g $NaNO_3$/L	x	
P	Nutrient treatment, 13.6 g KPO_3/L	x	
N + P	Nutrient treatment, 6.8 g $NaNO_3$ + 13.6 g KPO_3/L	x	

Treatments used in analyses are indicated with an 'x'

Fig. 2 Field methods. **a** Two replicates of agar plates with nutrient treatments; from left: control, nitrogen, phosphorus, N + P, full butt and ¼, ½, and full dilutions. **b** Study site on Bishop Creek. **c** Labeled concrete paver with attached petri dish bottoms. **d** One replicate paver in a pool at the start of the experiment (direction of water flow over the paver is indicated by the yellow arrow)

stabilize the filters. One block of replicates was lost because of disturbance, most likely by a beaver, as bite marks were present on some of the agar disks and freshly chewed twigs were nearby.

At harvest, each filter/polyester mesh circle covering the agar disks was halved. One half was placed in formaldehyde and one half wrapped in aluminum foil, iced, and frozen upon return to laboratory.

Chlorophyll *a* concentration and diatom health

We evaluated diatom health characteristics for four treatments (control, whole butt, full CB effluent, and half CB effluent), using the formaldehyde-preserved samples. We assessed two species of diatom genus *Navicula*—namely, *Navicula rostellata* Kütz. 1844 and *Navicula eidrigiana* Carter 1979 (identified using: Spaulding and Edlund 2008). These species were common in the samples and readily distinguished at 400 × magnification by their boat-shape and capitate (*N. rostellata*) or rounded (*N. eidrigiana*) ends. Like most *Navicula*, these species have two plate-like chloroplasts, appressed to girdle and visible as lines along the two sides of the frustule from valve view (Cox 1996). Healthy individuals have small lipid bodies, with 4 in *N. rostellata* and 2 in *N. eidrigiana*.

Samples were cleaned of formaldehyde by adding water, settling, and decanting. From each slide, a target of 50 diatoms of each *Navicula* species was scored according to lipid globule and chloroplast

condition. The actual number counted per species averaged between 46 and 47 Chloroplast health was graded in three categories using percent size reduction: 0–10% (healthy), 35–85% (unhealthy) or 90–100% shrunken (dead), based on illustrated percent loss categories in Pandey (2017) and illustrated in Fig. 3. Lipid bodies were graded as either healthy or abnormal (an increase in size or number) in comparison with their normal condition.

Chlorophyll *a* (Chl *a*) analysis used the ethanol extraction procedure and equation described in Biggs and Kilroy (2000). Frozen half-circle filters (area = 8.48 cm^2) were added to tubes with 5 ml of 95% ethanol, heated and extracted overnight in a refrigerator. Absorbance readings at 663 and 750 nm wavelengths (pre- and post-acidification) produced phaeophytin-corrected chlorophyll *a* concentrations. Most samples were diluted prior to reading absorbances because of high pigment concentration.

Data were analyzed using 2-way ANOVA, with the treatments and the block locations as factors and $\alpha = 0.05$. Two of the tested variables (chloroplast contraction and lipid body characteristics) met the normality and equal variance assumptions without transformation; Chl *a* data required a log transformation to meet the normality assumption. For a significant ANOVA result, treatment or location differences were evaluated using Tukey's multiple comparison test.

Results

At harvest, the treatments and concrete pavers were coated with a brownish biofilm, comprised mostly of diatoms and silt. Although a distinct CB odor was present in all the CB treatments at the start of the experiment, only the butt treatment had a consistently noticeable odor at the end of the experiment. In addition to the loss of one entire block of replicates, a single sample was apparently stepped on during the experiment and destroyed.

Chlorophyll *a* concentrations averaged 54.7 mg/m^2 (SE = 4.8, $N = 62$). Concentrations were not significantly different among treatments ($p = 0.451$; Table 2A) and there were no trends among the CB treatments, although there was a trend toward greater algal biomass in the P nutrient treatment versus the N and N + P treatments (Fig. 4).

Based on counts of approximately 50 cells of each species, most *N. rostellata* and *N. eidrigiana* had healthy appearing chloroplasts, with little or no shrinkage. Empty and near-empty cells were much less common than live diatoms. The percent of healthy chloroplasts (with 10% or less reduction from full size; e.g., Fig. 3A) did not significantly differ among the control and three CB treatments (butt, 1*x* and 1/2*x*; Fig. 5a, b; Table 2).

Lipid bodies were not apparent in healthy *Navicula*. Neither lipid body characteristics nor chloroplast health differed among any of the cigarette butt treatments (the *p* values > 0.10; control, butt, 1*x* and 1/2*x*; Fig. 5c, d; Table 2). Lipid body characteristics of

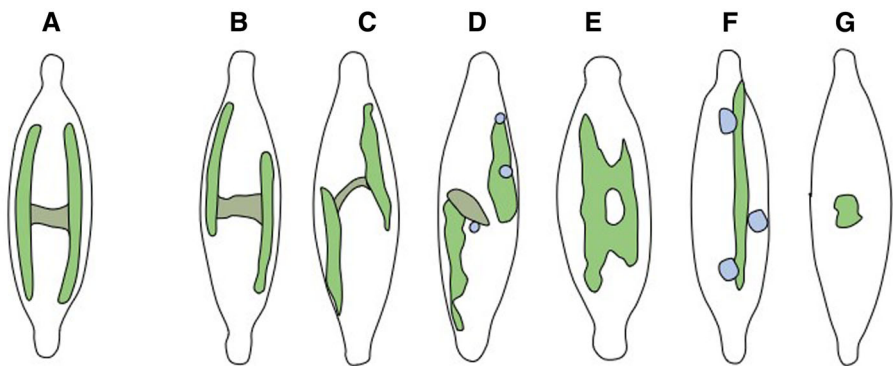

Fig. 3 Valve view indicators of diatom health. **A** healthy chloroplasts (in green) with little for no shrinkage. **B–G** increasing shrinkage of chloroplasts. Lipid bodies are shown as blue circle and are visible in D and are enlarged in F. Illustrations are tracings of photographed *Navicula rostellata*

Table 2 Statistical tables for (A) chlorophyll *a*, (B) diatom chloroplast health, and (C) diatom lipid body condition, summarizing results of 2-way ANOVAs. Statistically significant treatments are bolded

		df	*F*	*p*
(A) Chlorophyll a concentration				
Biofilm	Treatment	8	0.996	0.451
	Block	6	9.014	**< 0.001**
	Residual	47		
(B) Percent healthy chloroplasts				
N. rostellata	Treatment	3	0.341	0.796
	Block	6	0.926	0.501
	Residual	18		
N. eidrigiana	Treatment	3	0.6	0.623
	Block	6	1.67	0.186
	Residual	18		
(C) Percent abnormal lipid globules				
N. rostellata	Treatment	3	0.296	0.828
	Block	6	1.039	0.428
	Residual	18		
N. eidrigiana	Treatment	3	0.865	0.478
	Block	6	4.7	**0.0048**
	Residual	18		

Treatments are listed in Table 1

N. eidrigiana differed among blocks (locations; Table 2).

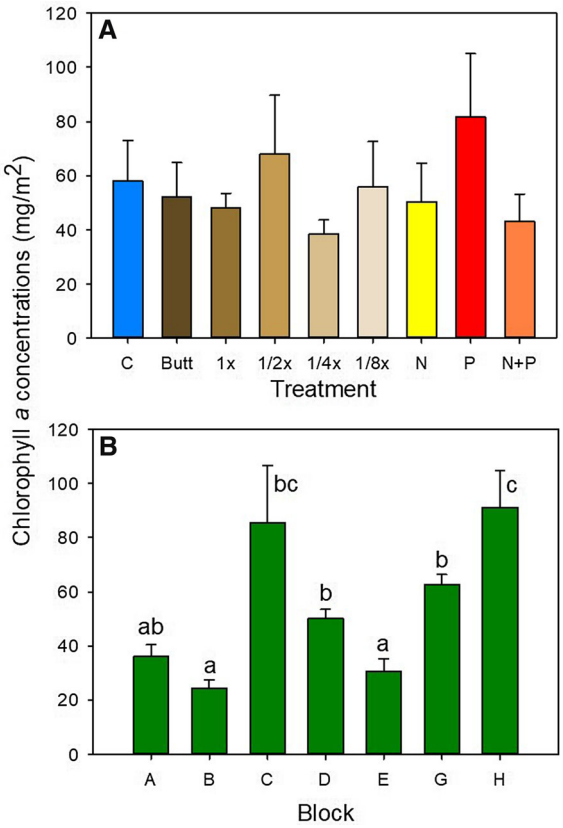

Fig. 4 Mean chlorophyll *a* concentration of the biofilm for (A) each of the 9 treatments, which are detailed in Table 1, and (B) the 7 blocks, each of which is in a different pool in the stream. Significantly different chlorophyll *a* concentrations are indicated by different letters on top of bars. Error bars are + 1 SE

Discussion

Despite testing whole CBs and an effluent concentration 10 times the level toxic to fish (e.g., Slaughter et al. 2011), our experiment did not find evidence of toxicity of CBs to benthic algae under field conditions. This result would at first appear inconsistent with the multitude of studies using other organisms that showed toxicity ranging from acute lethality to sublethal effects, some with possible long-term ramifications. However, our study differs from most previous studies in three respects: (1) the in situ setting in a water body, (2) the complex chemical environment of streams, and (3) the length of the study relative to target species' generation time.

We tested the toxicity of CBs under ambient conditions in a stream—in contrast to most other studies that used controlled laboratory conditions. Two other study systems also used ambient conditions. House finches incorporating CBs as fibrous nestlining material provide a natural experiment to study CBs as deterrents to ectoparasites (Suárez-Rodríguez and Garcia 2017; Suárez-Rodríguez et al. 2013) and the effects on nestlings (Suárez-Rodríguez and Macías Garcia 2014). As in our field-based study, no lethality was demonstrated. The second ambient-conditions study examined the decay of CBs in the laboratory and in the field. Field-based CBs in grasslands developed a more fungal dominated community than did the laboratory samples over a 5-yr decomposition experiment, although the decomposing CBs continued to be toxic to the microalga *Raphidocelis subcapitatum* in laboratory toxicity tests (Bonanomi, 2020).

In both our study and the studies involving CBs as nest material, the open environment meant that

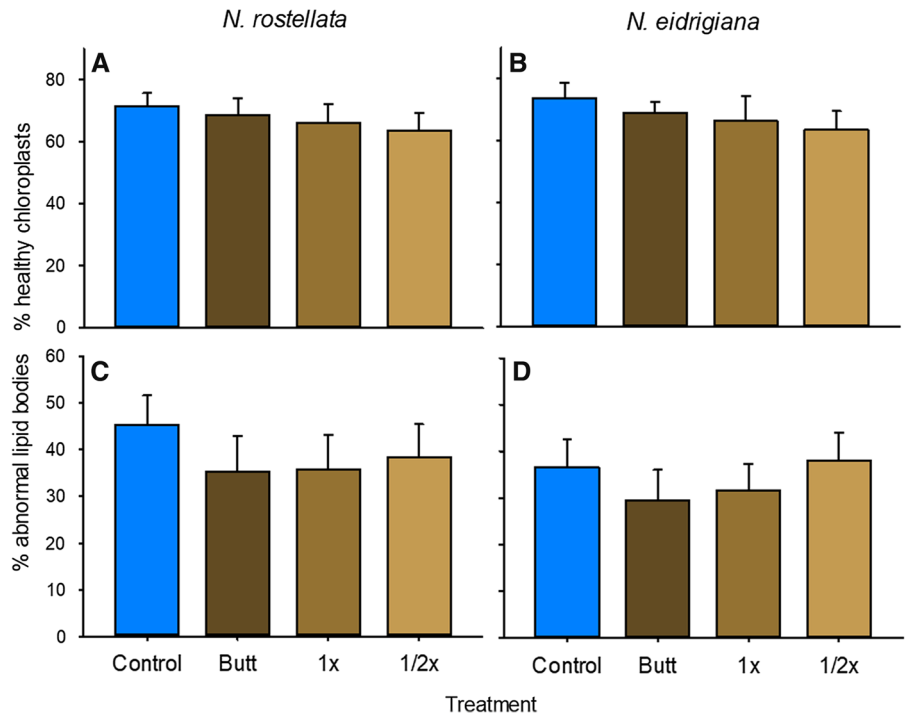

Fig. 5 Mean percent abundance of diatoms showing health metrics for the control and 3 CB treatments. **A**, **B** Percent of healthy chloroplasts and **C**, **D** percent with abnormal (enlarged) lipid bodies for each of the two species of *Navicula*. Error bars are + 1 SE

chemicals diffusing from the CBs (or agar-infused CB leachate) were carried away by water and air currents, respectively. This chemical diffusion results in spatial and temporal gradients of chemicals—likely producing the observed avoidance rather than death of nest ectoparasites (Suárez-Rodríguez et al. 2013) and the observed lack of toxicity in our study of stream algae. Leaching into water has been better documented. Most nicotine is leached in 10 h (Rodar Green et al. 2014). Benzene, toluene, and related chemicals are in low concentration in CBs, but also leach quickly, with one to two -thirds of the chemicals that are leached in one day leaching within the first 15 min (Dobaradaran et al. 2021). Most polycyclic aromatic hydrocarbons likewise leach rapidly, although the heavier compounds leached primarily between days 14 and the end of the study at 21 days (Dobaradaran 2020). Metals vary in leaching rates, with some (e.g., Pb, Ni, Zn) leaching primarily during the first day and others (e.g., Fe, Mn) continuing to leach over a 34-day period (Moerman and Potts 2011). This rapid leaching of CBs is reflected in the frequent use of a 24-h leaching period to produce effluent for toxicity tests (e.g., Micevska et al. 2006; Lee and Lee, 2015; Cardoso, 2018; Micevska et al. 2006; Osuala et al. 2017; Slaughter et al. 2011; Wright et al 2015; this study). Research on the leaching rates of specific chemicals beyond 24-h and leaching from agar diffusing substrates (if this technique is used for additional CB studies) are needed to better understand the effects of CBs in aquatic ecosystems.

We found no indication of toxicity, but other studies also using diffusing substrates have demonstrated impacts on algae from exposure to a variety of chemicals, including nutrients (Bergey 2008; Fairchild and Lowe 1984; Pringle and Bowers 1984), metals (DeNicola et al. 2018; Hirst et al. 2004; Pandey and Bergey 2018; Pandey et al. 2014), an insecticide (Francoeur et al. 1999), and acidification (DeNicola et al. 2018; Hirst et al. 2004).

The rate of diffusion from chemical diffusing substrates decreases with time (Corkum 1996). Although such temporal leaching of chemicals may have contributed to the lack of effects in our study, the whole CB treatment retained a strong fresh CB odor though the end of the experiment, indicating that chemicals were likely still diffusing, but no effect on algal health or biomass was detected in this treatment.

Instead, we found pool-to-pool differences in algal biomass, likely due to the differences in the current, sunlight, sediment accumulation, and other conditions that varied among the block locations in the stream. This small-scale site effect has been observed in other studies (Bergey 2008; Mosisch et al. 2001).

Tobacco contains a wide variety of plant secondary compounds and their derivatives (Rodgman and Perfetti 2013). Indeed, about one half of the 4000 compounds found in cigarette smoke occur in tobacco leaves (Engstrom et al. 2003). These compounds include alkaloids (notably nicotine), polycyclic aromatic hydrocarbons, and phenols (Rodgman and Perfetti 2013). Tobacco leaves are not alone in containing secondary compounds; plants produce a wide range of secondary compounds, which may defend plants against predators and pathogens (Agrawal and Weber 2015; Zaynab et al. 2018), increase tolerance to cold drought or UV and aid in reproduction (including providing the color and aroma of flowers) (Samanta et al. 2011).

The leachate from CBs may not have affected benthic algae because dilute concentrations of plant secondary compounds are normal components of stream waters. Leaf litter is a significant component of stream ecosystems in supporting the decomposer base of stream food webs (Wallace et al. 1997). As the first step in decomposition, leaves falling into streams leach soluble compounds, which can reduce the weight of dry leaves by as much as 30% (Bärlocher 2020). Plant secondary compounds are among these soluble leachates (Ardón and Pringle 2008; Bärlocher 2020). Exposure to leaf litter leachates may be particularly high during seasonal leaf fall in forested streams, as large amounts of leaves enter streams (as indicated by seasonally high dissolved organic compound concentrations: Meyer et al. 1998). Leaf leachate can be toxic to aquatic organisms (e.g., Beleza et al. 2019; Manusadžianas, 2014). Whereas toxicity may be evident in laboratory toxicity tests, by extrapolating this toxicity to small lakes, Alonso et al (2020) concluded that leachates from natural leaf fall would have little or no toxic effects on aquatic invertebrates.

Although lasting only 10 days, our study investigated relatively long-term effects. Diatoms divide every 2–3 days (Rivkin 1986); hence, our experiment encompassed 3–5 generations. Over this interval, the combination of diffusion of leachates from the treatments and multiple generations of diatoms obscured any toxic effects, which differs from the toxic effects found with nutrient-diffusing metal substrates (Pandey and Bergey 2018). Only one other study involved multiple generations—Quéméneur et al. (2020) investigated microbial diversity changes in marine sediment over a 96-h period and documented changes in microbial composition including a decrease in the relative abundance of autotrophic Cyanobacteria. Unlike our experiment, the marine sediment test was a laboratory test done in a closed system—a design that does not include the diffusion of leachates that would occur under natural conditions.

This study found no significant effect of cigarette butt leachate on diatom health within the context of a stream environment; however, it is likely that a closed-system study would find toxicity to diatoms. Our experiment also did not test the uptake of CB leachate chemicals by algae or the possibility of bioaccumulation within algae over time—conditions that could impact stream food webs.

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Author contributions All authors contributed to the experimental design, methodology, data analysis, writing, and/or editing.

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Availability of data and materials Data are contained in the text or in the supplementary files (Online resource S2).

Declarations

Conflicts of interest The authors have no conflicts of interest to declare relevant to the content of this article.

References

Agrawal AA, Weber MG (2015) On the study of plant defence and herbivory using comparative approaches: how important are secondary plant compounds. Ecol Lett 18:985–991. https://doi.org/10.1111/ele.12482

Alonso A, Vázquez de Aldana BR, Castro-Díez P, Medina-Villar S, Pérez-Corona ME (2020) Effects of leaf litter extracts from four tree species on aquatic invertebrates: an ecotoxicological risk assessment approach. Aquat Ecol 54:1155–1168. https://doi.org/10.1007/s10452-020-09800-x

Araújo MCB, Costa MF (2019) A critical review of the issue of cigarette butt pollution in coastal environments. Environ Res 172:137–149. https://doi.org/10.1016/j.envres.2019.02.005

Ardón M, Pringle CM (2008) Do secondary compounds inhibit microbial-and insect-mediated leaf breakdown in a tropical rainforest stream, Costa Rica? Oecologia 155:311–323

Bärlocher F (2020) Leaching. In: Bärlocher F, Gessner M, Graça M (eds) Methods to study litter decomposition, 2nd edn. Springer, Switzerland, pp 37–41

Bator RJ, Bryan AD, Schultz PW (2011) Who gives a hoot? Intercept surveys of litterers and disposers. Environ Behav 43:295–315. https://doi.org/10.1177/0013916509356884

Beleza S, Campos D, Nunes B, Pestana JLT (2019) Ecotoxicological responses of *Chironomus riparius* to *Eucalyptus* and *Alnus* leaf leachates: from biochemical to life-history effects. Ecol Indic 106:105473. https://doi.org/10.1016/j.ecolind.2019.105473

Bergey EA (2008) Does rock chemistry affect periphyton accrual in streams? Hydrobiologia 614:141–150. https://doi.org/10.1007/s10750-008-9450-9

Biggs BJF, Kilroy C (2000) Stream periphyton monitoring manual. NIWA, Christchurch

Bonanomi G et al (2020) The fate of cigarette butts in different environments: decay rate, chemical changes and ecotoxicity revealed by a 5-years decomposition experiment. Environ Pollut 261:114108. https://doi.org/10.1016/j.envpol.2020.114108

Booth DJ, Gribben P, Parkinson K (2015) Impact of cigarette butt leachate on tidepool snails. Mar Pollut Bull 95:362–364. https://doi.org/10.1016/j.marpolbul.2015.04.004

Cardoso LS et al (2018) The exposure to water with cigarette residue changes the anti-predator response in female Swiss albino mice. Environ Sci Pollut Res 25:8592–8607. https://doi.org/10.1007/s11356-017-1150-4

Caridi F et al (2020) Cigarette butts, a threat for marine environments: lessons from benthic foraminifera (Protista). Mar Environ Res 162:105150. https://doi.org/10.1016/j.marenvres.2020.105150

Corkum LD (1996) Patterns of nutrient release from nutrient diffusing substrates in flowing water. Hydrobiologia 333:37–43. https://doi.org/10.1007/BF00020962

Cox EJ (1996) Identification of freshwater diatoms from live material. Chapman & Hall, London

DeNicola DM, Stapleton MG, Onufrak AJ (2018) Examination of effects of acid and metals on benthic algae in streams using chemical diffusing substrata. Ecol Indic 88:17–26. https://doi.org/10.1016/j.ecolind.2017.12.071

Dieng H et al (2011) Discarded cigarette butts attract females and kill the progeny of *Aedes albopictus*. J Am Mosq Control Assoc 27:263–271. https://doi.org/10.2987/11-6124.1

Dobaradaran S, Schmidt TC, Kaziur-Cegla W, Jochmann MA (2021) BTEX compounds leachates from cigarette butts into water environment: a primary study. Environ Pollut 269:116185. https://doi.org/10.1016/j.envpol.2020.116185

Dobaradaran S et al (2020) Polycyclic aromatic hydrocarbons (PAHs) leachates from cigarette butts into water. Environ Pollut 259:113916. https://doi.org/10.1016/j.envpol.2020.113916

Engstrom P, Clapper M, Schnoll R (2003) Physiochemical composition of tobacco smoke. In: Kufe D, Pollock R, Weichselbaum R, Bast RJ, Gansler T, Holland J, Frei EI (eds) Holland-Frei cancer medicine, 6th edn. BC Decker, Hamilton

Fairchild GW, Lowe RL (1984) Artificial substrates which release nutrients: effects on periphyton and invertebrate succession. Hydrobiologia 114:29–37. https://doi.org/10.1007/bf00016599

Francoeur SN, Biggs BJF, Lowe RL (1999) Inhibition of algae and invertebrates by malathion from insecticide-diffusing substrata. J Freshwat Ecol 14:179–186. https://doi.org/10.1080/02705060.1999.9663669

Gill H, Rogers K, Rehman B, Moynihan J, Bergey EA (2018) Cigarette butts may have low toxicity to soil-dwelling invertebrates: evidence from a land snail. Sci Total Environ 628–629:556–561. https://doi.org/10.1016/j.scitotenv.2018.02.080

Green DS, Boots B, Da Silva CJ, Starkey T (2019) Cigarette butts have adverse effects on initial growth of perennial ryegrass (Gramineae: *Lolium perenne* L.) and white clover (Leguminosae: *Trifolium repens* L.). Ecotoxicol Environ Saf 182:109418. https://doi.org/10.1016/j.ecoenv.2019.109418

Healton CG, Cummings KM, Connor RJ, Novotny TE (2011) Butt really? The environmental impact of cigarettes. Tob Control 20:i1

Hirst H, Chaud F, Delabie C, Jüttner I, Ormerod SJ (2004) Assessing the short-term response of stream diatoms to acidity using inter-basin transplantations and chemical diffusing substrates. Freshwat Biol 49:1072–1088. https://doi.org/10.1111/j.1365-2427.2004.01242.x

Kadir AA, Mohajerani A (2011) Recycling cigarette butts in lightweight fired clay bricks. Proc Inst Civ Eng - Constr Mater 164:219–229. https://doi.org/10.1680/coma.900013

Kurmus H, Mohajerani A (2020) The toxicity and valorization options of cigarette butts. Waste Manag 104:104–118. https://doi.org/10.1016/j.wasman.2020.01.011

Lawal M, Ologundudu S (2013) Toxicity of cigarette filter leachates on *Hymenochirus curtipes* and *Clarias gariepinus* in Nigeria. J Environ Ext 11:7–14

Lee JGL, Ranney LM, Goldstein AO (2013) Cigarette butts near building entrances: what is the impact of smoke-free college campus policies? Tob Control 22:107–112. https://doi.org/10.1136/tobaccocontrol-2011-050152

Lee W, Lee CC (2015) Developmental toxicity of cigarette butts—an underdeveloped issue. Ecotox Environ Saf 113:362–368. https://doi.org/10.1016/j.ecoenv.2014.12.018

Manusadžianas L et al (2014) Ecotoxicity effects triggered in aquatic organisms by invasive *Acer negundo* and native *Alnus glutinosa* leaf leachates obtained in the process of aerobic decomposition. Sci Total Environ 496:35–44. https://doi.org/10.1016/j.scitotenv.2014.07.005

Marah M, Novotny TE (2011) Geographic patterns of cigarette butt waste in the urban environment. Tob Control 20:i42–i44. https://doi.org/10.1136/tc.2010.042424

Marinello S, Lolli F, Gamberini R, Rimini B (2020) A second life for cigarette butts? A review of recycling solutions. J Hazard Mater 384:121245

Meyer JL, Wallace JB, Eggert SL (1998) Leaf litter as a source of dissolved organic carbon in streams. Ecosystems 1:240–249. https://doi.org/10.1007/s100219900019

Micevska T, Warne MSJ, Pablo F, Patra R (2006) Variation in, and causes of, toxicity of cigarette butts to a cladoceran and microtox. Arch Environ Contam Toxicol 50:205–212. https://doi.org/10.1007/s00244-004-0132-y

Moerman JW, Potts GE (2011) Analysis of metals leached from smoked cigarette litter. Tob Control 20:i30–i35. https://doi.org/10.1136/tc.2010.040196

Montalvão MF et al (2019a) How leachates from wasted cigarette butts influence aquatic life? A case study on freshwater mussel *Anodontites trapesiali*. Sci Total Environ 689:381–389. https://doi.org/10.1016/j.scitotenv.2019.06.385

Montalvão MF, Sampaio LLG, Gomes HHF, Malafaia G (2019b) An insight into the cytotoxicity, genotoxicity, and mutagenicity of smoked cigarette butt leachate by using *Allium cepa* as test system. Environ Sci Pollut Res 26:2013–2021. https://doi.org/10.1007/s11356-018-3731-2

Moriwaki H, Kitajima S, Katahira K (2009) Waste on the roadside, 'poi-sute' waste: Its distribution and elution potential of pollutants into environment. Waste Manag 29:1192–1197. https://doi.org/10.1016/j.wasman.2008.08.017

Mosisch TD, Bunn SE, Davies PM (2001) The relative importance of shading and nutrients on algal production in subtropical streams. Freshwat Biol 46:1269–1278. https://doi.org/10.1046/j.1365-2427.2001.00747.x

Novotny TE, Lum K, Smith E, Wang V, Barnes R (2009) Cigarettes butts and the case for an environmental policy on hazardous cigarette waste. Int J Env Res Public Health 6:1691–1705

Osuala F, Abiodun O, Igwo-Ezikpe M, Kemabonta K, Otitoloju A (2017) Relative toxicity of cigarette butts leachate and usefulness of antioxidant biomarker activity in Nile Tilapia *Oreochromis niloticus* (Trewavas, 1983). Ethiopian J Environ Stud Manag 10:75–88

Pandey LK, Bergey EA (2018) Metal toxicity and recovery response of riverine periphytic algae. Sci Total Environ 642:1020–1031. https://doi.org/10.1016/j.scitotenv.2018.06.069

Pandey LK et al (2017) The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. Water Res 118:39–58. https://doi.org/10.1016/j.watres.2017.01.062

Pandey LK, Kumar D, Yadav A, Rai J, Gaur JP (2014) Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. Ecol Indic 36:272–279. https://doi.org/10.1016/j.ecolind.2013.08.002

Parker TT, Rayburn J (2017) A comparison of electronic and traditional cigarette butt leachate on the development of *Xenopus laevis* embryos. Toxicol Reports 4:77–82. https://doi.org/10.1016/j.toxrep.2017.01.003

Patel V, Thomson GW, Wilson N (2013) Cigarette butt littering in city streets: a new methodology for studying and results. Tob Control 22:59–62. https://doi.org/10.1136/tobaccocontrol-2012-050529

Pelit FO, Demirdöğen RE, Henden E (2013) Investigation of heavy metal content of Turkish tobacco leaves, cigarette butt, ash, and smoke. Environ Monit Assess 185:9471–9479. https://doi.org/10.1007/s10661-013-3266-4

Pringle CM, Bowers JA (1984) An *in situ* substratum fertilization technique: diatom colonization on nutrient-enriched, sand substrata. Can J Fish Aquat Sci 41:1247–1251. https://doi.org/10.1139/f84-150

Quéméneur M, Chifflet S, Akrout F, Bellaaj-Zouari A, Belhassen M (2020) Impact of cigarette butts on microbial diversity and dissolved trace metals in coastal marine sediment. Estuar Coast Shelf Sci 240:106785. https://doi.org/10.1016/j.ecss.2020.106785

Rath JM, Rubenstein RA, Curry LE, Shank SE, Cartwright JC (2012) Cigarette litter: smokers' attitudes and behaviors. Int J Env Res Public Health 9:2189–2203

Register K (2000) Cigarette butts as litter- toxic as well as ugly? Underwat Nat 25:23–29

Rivkin RB (1986) Radioisotopic method for measuring cell division rates of individual species of diatoms from natural populations. Appl Environ Microbiol 51:769–775

Roder Green AL, Putschew A, Nehls T (2014) Littered cigarette butts as a source of nicotine in urban waters. J Hydrol Part D 519:3466–3474. https://doi.org/10.1016/j.jhydrol.2014.05.046

Rodgman A, Perfetti TA (2013) The chemical components of tobacco and tobacco smoke, 2nd edn. CRC Press, Boca Raton

Samanta A, Das G, Das SK (2011) Roles of flavonoids in plants. Int J Pharm Sci Technol 6:12–35

Selmar D et al (2018) Uptake of nicotine from discarded cigarette butts—a so far unconsidered path of contamination of plant-derived commodities. Environ Pollut 238:972–976. https://doi.org/10.1016/j.envpol.2018.01.113

Shevchenko V (2012) Characterization of chemical compounds in cigarette filters leachates. Masters thesis, San Diego State University

Slaughter E, Gersberg RM, Watanabe K, Rudolph J, Stransky C, Novotny TE (2011) Toxicity of cigarette butts, and their chemical components, to marine and freshwater fish. Tob Control 20:i25–i29. https://doi.org/10.1136/tc.2010.040170

Spaulding S, Edlund M (2008) Navicula. In: Diatoms of North America. https://diatoms.org/genera/navicula. Accessed 10 Jan 2021

Suárez-Rodríguez M, Garcia CM (2017) An experimental demonstration that house finches add cigarette butts in response to ectoparasites. J Avian Biol 48:1316–1321. https://doi.org/10.1111/jav.01324

Suárez-Rodríguez M, López-Rull I, Macías Garcia C (2013) Incorporation of cigarette butts into nests reduces nest ectoparasite load in urban birds: new ingredients for an old recipe? Biol Lett. https://doi.org/10.1098/rsbl.2012.0931

Suárez-Rodríguez M, Macías Garcia C (2014) There is no such a thing as a free cigarette; lining nests with discarded butts brings short-term benefits, but causes toxic damage. J Evol Biol 27:2719–2726. https://doi.org/10.1111/jeb.12531

Torkashvand J, Farzadkia M, Sobhi HR, Esrafili A (2020) Littered cigarette butt as a well-known hazardous waste: a comprehensive systematic review. J Hazard Mater 383:121242. https://doi.org/10.1016/j.jhazmat.2019.121242

Wallace JB, Eggert SL, Meyer JL, Webster JR (1997) Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104. https://doi.org/10.1126/science.277.5322.102

Wright SL, Rowe D, Reid MJ, Thomas KV, Galloway TS (2015) Bioaccumulation and biological effects of cigarette litter in marine worms. Sci Rep 5:14119. https://doi.org/10.1038/srep14119

Yabes LJ (2018) Bioaccumulation of organic compounds from smoked cigarette litter in the freshwater rainbow trout, *Oncorhynchus mykiss*. Masters thesis, San Diego State University

Zaynab M, Fatima M, Abbas S, Sharif Y, Umair M, Zafar MH, Bahadar K (2018) Role of secondary metabolites in plant defense against pathogens. Microb Pathog 124:198–202. https://doi.org/10.1016/j.micpath.2018.08.034