**Cigarette butts have adverse effects on initial growth of perennial ryegrass (Gramineae: *Lolium perenne* L.) and white clover (Leguminosae: *Trifolium repens* L.)**

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**Abstract**

Cigarette filters (butts) are currently the most abundant form of anthropogenic litter on the planet, yet we know very little about their environmental impacts on terrestrial ecosystems, including plant germination and primary production. When discarded, filters contain a myriad of chemicals resulting from smoking tobacco and some still contain unsmoked remnants. A greenhouse experiment was used to assess the impacts of discarded filters of regular or menthol cigarette, either from unsmoked, smoked, or smoked cigarettes with remnant tobacco, on the growth and development of *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover). After 21 days, shoot length and germination success were significantly reduced by exposure to any type of cigarette filter for the grass and clover. Although total grass biomass was not measurably affected, the root biomass and root:shoot ratio were less in the clover when exposed to filters from smoked regular cigarettes and those with remnant tobacco. Cigarette filters caused an increase in chlorophyll-a in clover shoots and an increase in chlorophyll-b in grass shoots. Accordingly, whilst the chlorophyll a:b ratio was increased in the clover exposed to cigarette filters, it was decreased in grass. This study indicates the potential for littered cigarette filters to reduce growth and alter short-term primary productivity of terrestrial plants.

**Capsule**

Discarded cigarette filters can reduce the germination success and initial growth of perennial ryegrass and white clover.

**Highlights**

* Cigarette filters can reduce germination success and shoot length of some plants.
* Chlorophyll-a concentrations increased in clover exposed to cigarette filters.
* Root biomass and root-to-shoot ratios of white clover were reduced by cigarette filters.
* Both regular and menthol cigarettes filters can reduce plant growth and germination success.

**1. Introduction**

Since the 1990's cigarette filters (or butts) have maintained their place as the most abundant litter item worldwide, blighting beaches, streets, sidewalks, waterways, and public spaces (Ocean Conservancy 2019). Globally, the number of smokers is steadily increasing with global population growth (Ng et al. 2014) corresponding to an increase in the number of cigarettes being smoked. Each year, ~4.5 trillion cigarette filters are discarded into the environment (Araújo and Costa 2019) due to 76% - 84% of smokers littering their cigarette filters rather than disposing of them into a bin (Patel et al. 2013; Wilson et al. 2014).

Despite their prevalence as litter, there is still a dearth of information about the environmental impacts of cigarette filters. Most filters are composed of up to 12000 cellulose acetate fibres (Novotny et al. 2009), which persist in the environment for an unknown length of time. Furthermore, when discarded, cigarette filters typically contain left-over, unsmoked tobacco (Slaughter et al. 2011). As such, they present a combination of bio-based plastic litter, tobacco, and a myriad of toxicants including nicotine, formaldehyde, heavy metals and a variety of polycyclic aromatic hydrocarbons (PAHs) retained in the filter after smoking (Hoffman and Hoffman 1997; Novotny et al. 2009; Rodgman and Perfetti 2013). Some cigarettes also contain adjuncts such as cloves or other spices but, most commonly 5-methyl-2-(propan-2-yl)cyclohexan-1-ol is added as flavouring and is available in menthol cigarettes. In aquatic habitats, there is evidence that these compounds can leach into waterways (e.g. Green et al. 2014) where they can cause harm to freshwater fauna, including *Ceriodaphnia dubia* (a water flea) (Micevska et al. 2006) and *Pimephales promelas* (fathead minnow)- (Slaughter et al. 2011), and marine organisms including bacteria (Micevska et al. 2006), *Hediste diversicolor* (polychaete); (Wright et al. 2015), several gastropods; (Booth et al. 2015) and *Atherinops affinis* (marine topsmelt) (Slaughter et al. 2011). Interestingly, Slaughter et al. (2011) found that even unsmoked cigarette filters could be toxic to the marine and freshwater fish mentioned above. When in aquatic ecosystems, toxicants can become accessible to organisms depending on their leaching potential. For example, nicotine can be highly soluble in water (16 g L-1; American Chemical Society 2018) especially with alkaline conditions and can leach from cigarette filters over time (Selmar et al. 2018).

In urbanised terrestrial habitats, such as cities, parks and public green spaces, cigarette filters are commonly littered (Schultz et al. 2013), yet very few studies have quantified the amount of cigarette filter litter in these habitats or the consequent environmental effects. Of these studies, Green et al. (2014) found an average density of cigarette filters of 2.7 m−2 (SD = 0.6 m−2) with a maximum of 49 filters m−2 in Berlin, Germany. Schultz et al. (2013) found that the rate of littering cigarette filters in the USA depended on the individual’s age, distance to disposal sites and the amount of litter present. Studies on the impact of cigarette filter litter on ecosystem functioning in terrestrial habitats are particularly limited, but urban birds (such as house sparrows *Passer domesticus* and house finches *Carpodacus mexicanus*) have been observed incorporating smoked cigarette filters into the lining of nests, potentially reducing nest parasites suggesting increased fitness of the offspring (Suárez-Rodríguez et al. 2013). Follow up studies on *C. mexicanus*, however, indicates that the benefits may be short-lived due to toxic damage to the offspring (Suárez-Rodríguez and Garcia 2014). In soil ecosystems, the only experiment conducted, to date, found no effects on the growth, feeding and life-cycle of the woodland snail, *Anguispira alternata*, in response to cigarette filter leachate (up to 4 filters L-1), although initial avoidance behaviour was noted (Gill et al. 2018).

Nicotine has been detected in several plant products, including; food crops, teas and spices, and research shows that plants can take up nicotine either from tobacco smoke or from soil littered with commercial tobacco (Selmar et al. 2015). Even just one littered cigarette filter can contaminate plants within 1m-2 with nicotine (Selmar et al. 2018). Despite their prevalence as waste in terrestrial habitats, the effects of littered cigarette filters on plant germination, growth and chlorophyll content remains largely unknown. A recent short term experimental (48 hours) exposure to leachate from smoked cigarette filters revealed cytotoxic, genotoxic and mutagenic effects on onion plants, *Allium cepa* (Montalvão et al. 2018) thus indicating the potential for wider effects on plants. Furthermore, there is some evidence that plants can absorb components of cigarette smoke from the air in their tissues (Selmar et al. 2015). Cigarette smoke also contains pollutants including heavy metals (e.g. Chiba and Masironi 1992) and polycyclic aromatic hydrocarbons (PAHs) (Ding et al. 2005), and cigarette smoke can also decrease the shoot and root length, and alter the chlorophyll content of common wheat, *Triticum vulgare* (Tileklioğlu et al. 1996) and to decrease the number of leaves grown on chickpea, *Cicer arietinum* (Mondal et al. 2014).

Many temperate grassland ecosystems contain members of the gramineae and leguminosae such as the monocotylodon *Lolium perenne* (perennial ryegrass) and dicotylodon *Trifolium repens* (white clover). They are important forage crops in temperate regions and can also be found in many urban green spaces such as parks and are used in landscaping as part of private lawns and around public buildings. Besides being an economically important forage crop as high quality livestock feed (Brock and Hay 2001), *T. repens* also provides resources for pollinating insects (e.g. Rodet et al. 1998). *Lolium perenne* and *T. repens* are often also grown in combination because of the added value from the atmospheric nitrogen fixation by *T. repens*. It is currently unknown how these plant species respond to littered cigarette filters.

To study potential effects of cigarette filters on the development of two important terrestrial plants, a greenhouse experiment was set up, simulating littered cigarette filters from regular and flavoured tobacco (either as filters from unsmoked, smoked, or smoked cigarettes with remnant tobacco). The following hypotheses were tested: (i) the germination success, growth and chlorophyll content - as a proxy for primary production - of *L. perenne* and *T. repens* is negatively affected by cigarette filters either smoked or unsmoked. (ii) Plants respond in a similar fashion to regular and menthol-flavoured cigarettes, either smoked or unsmoked.

**2. Materials and Methods**

*2.1. Preliminary sampling of cigarette filters as litter and rationale for the experiment.*

In June 2018 the density of littered cigarette filters in urban green spaces in Cambridge, UK was assessed using surveys in three green spaces (Parker's Piece, Jesus Green and Christ Park) in Cambridge, United Kingdom (population: 1.29\*105 in the 2018 census). At each park, a randomly placed 50 x 50 cm quadrat was used to estimate, by counting, the density of cigarette filters, repeated 15 times at each location. The results from this initial survey (section 3.2) provided the rationale for the density of cigarette filters and the length of remnant tobacco used in the experiment

*2.2. Experimental design and setup*

A controlled mesocosm experiment was set up in a north-east facing glass greenhouse at Anglia Ruskin University, Cambridge, UK to test the responses of *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) to filters from regular or flavoured cigarettes. The plants were simultaneously (separately) grown in the greenhouse, but were considered separately, thus the design included the factors “Cigarette” with two levels: *Regular* or *Menthol* and “State” with three levels: *Unsmoked*, *Smoked* (with no remnant tobacco) or *Smoked with Remnant Tobacco* [*SwRT*]. A random pack of regular and menthol cigarettes was purchased and a random subsample was smoked using a manual aspirator in a dedicated, ventilated area. All remnant tobacco from filters from the *Smoked* and *Unsmoked* treatments was removed using a pair of scissors and forceps to ensure that only the filter with its paper wrapper were included. The treatment *Smoked with Remnant Tobacco* contained filters containing ~1 cm of remnant tobacco after smoking. Both experiments (*L. perenne* and *T. repens*) also included a single *Control* treatment, which received a piece of untreated, wooden doweling the same diameter and similar length as the cigarette filters. This takes into account a reduction in available surface area caused by the presence of any of the cigarette filters. In keeping with *a priori* field observations, the Cigarette mesocosms received 1 cigarette filter, equalling observed littering of 61 filters m-2 (corresponding to almost half of the upper quartile found in the preliminary survey). The experiment resulted in two separate, asymmetric designs with n = 5 replicates for each control and treatment making N = 35 replicates (control \* 5 replicates plus 2 cigarettes \* 3 states \* 5 replicates) each for *L. perenne* and *T. repens* (see section 2.4 and Figure S1 for more detail).

Mesocosms were constructed using plant pots with dimensions 11 cm height x 14 cm diameter (~ 1800 cm3). A mixture of air-dried (48 h at room temperature), sandy clay loam top soil (Westland, UK) was homogenised by hand in bulk before being added to the pots to reach a volume of ~1200 cm-3, yielding a mean dry bulk density of ρd = 1.03 ± 0.01 g cm-3. Mesocosms for *L. perenne* received 200 seeds = ~12.1\*103 seeds m-2 equivalent and those for *T. repens* received 150 seeds = ~6.1\*103 seeds m-2 equivalent and were distributed equally over the surface area. All mesocosms containing *L. perenne* or *T. repens* were simultaneously placed in a greenhouse and organised in a completely randomised fashion. Moisture content of each mesocosm was kept at 60% water holding capacity throughout the experimental period via gravimetrically monitoring water loss and adding distilled water when necessary. The experiment ran for 21 days from the 10th of July 2018.

*2.3. Measurement of biological responses: germination success, plant growth, biomass and chlorophyll content.*

The number of germinated plants and the length (mm) of shoots was measured throughout the 21 days of the experiment. Shoot length was estimated using a ruler and measuring the length of plants at 5 random spots in each mesocosm and averaged. On the final day, all mesocosms were destructively sampled, but prior to any other manipulation, the shoots of *L. perenne* or *T. repens* were harvested by hand using a pair of fine secateurs, removing all plant material from the surface. Total fresh above ground plant biomass was recorded and a randomly taken subsample of ~2 g was put aside to determine moisture content gravimetrically after oven-drying at 50°C for 12 h. Another randomly taken subsample was removed for the determination of chlorophyll content. For this, ~ 0.2 g fresh material was added to separate 15 mL, capped centrifuge tubes and stored at -20°C in the dark until processing (~48 h). Chlorophyll was extracted using 90% acetone for 24 h in the dark at -20°C. Chlorophyll-a and –b were estimated via spectrophotometrically measuring the absorbance at 647 nm and 664 nm (Jeffrey and Humphreys 1975) and corrected for dry biomass.

Plant roots were carefully removed from the soil matrix by hand and washed by hand to remove any adhering soil particles. Biomass of fresh roots was determined gravimetrically. Roots were then oven-dried at 50°C to determine dry biomass and to determine dry weight root:shoot ratios.

*2.4. Statistical data analysis*

Using R v 3.5.2 (R core team, 2018), all response variables were checked for normality via q-q plots and Shapiro-Wilkinson tests, homoscedasticity was checked via residual plots and the *car* (v3.0-2) package (Fox and Weisberg, 2018) was used for Levene’s tests using to ascertain assumptions for ANOVA. Some results did not to conform to these assumptions and were transformed (detailed in the statistical results associated with the data). The experimental design had a single control group *Control* (n = 5) for the two orthogonal factors “Cigarette” and “State” and therefore was asymmetric. All results were thus analysed by using the mean squares from two independent ANOVAs (see Figure S2, Tables S1a and S1b for detailed example of calculations). Briefly, the procedure involved partitioning of the variance by calculating: (1) a one-way ANOVA with all treatments as separate levels (All levels: a = 7, n = 5, N = 35); and (2) a full-factorial, two-way ANOVA of “Cigarette” by “State” without the level *Control* (a = 2, b = 3, n = 5, N = 30). The residuals of the 1st ANOVA were used to estimate any differences between the levels within the 2nd ANOVA, allowing the variation associated with *Control* and that of the other treatments to be distinguished (“*Control* vs. Others”), which is contrasted with one degree of freedom (Underwood 1997). When a significant effect (at α = 0.05) in the “*Control* vs. Others” contrast was found, a Dunnett’s test was used to contrast the *Control* versus each level of the significant term using the *multcomp* (v1.4-6) package. Pairwise comparisons for the factors in the 2nd ANOVA were computed using Tukey HSD tests when the main terms were significant (at α = 0.05). Using non-linear least squares (*nls*), plant growth data was analysed assuming a Gompertz model (e.g. Paine et al. 2012):

With the length (mm) of clover or grass for each treatment over time (*t* in days since sowing) and the three unknown parameters *M0*, *r* and *K* being *M0* = length at *t* = 0 in mm, *r* = the growth rate (day-1), and *K* = the asymptote (mm) respectively, using starting values based on observations of the plotted data.

**3. Results**

*3.1. Preliminary sampling of cigarette filters as litter and rationale for the experiment.*

The density of cigarette filters ranged from 0 to 128 m‑2 (median = 16, n = 45). It was observed that 65% of the discarded, smoked cigarette filters still contained remnant tobacco. Therefore randomly selected cigarette filters that contained remnant tobacco were measured in the green spaces in Cambridge to obtain the average length of tobacco from the cigarette remaining, which was on average 9.9 ± 0.8 mm (mean ± SEM, N = 50).

*3.2. Germination success and above ground growth of* L. perenne.

After 21 days, on average 55 ± 23% (mean ± SEM, N= 35) of *L. perenne* seeds had germinated, which ranged from 49 ± 6 (*Regular SwRT*) to 60 ± 5% (*Control*) treatment (Figure 1). There was a significant decrease (~10%) in germination success of *L. perenne* when exposed to any type of cigarette filter (*Control* vs Others: F1,28 = 7.56, P = 0.001, Table S2).

The shoots of *L. perenne* after 21 days were significantly longer in the *Control* compared to all of the other treatments (*Control* vs Others: F1,28 = 70.3, P < 0.001, Table S2), with an average length ranging between 159 ± 3 (*Menthol SwRT*) and 187 ± 5 mm (*Control*) at the end of the experiment (Figure 2). Shoot length was ~13% less when exposed to any of the cigarette filters compared to the *Control*. This is reflected in the estimated coefficients (Table S3a) of the Gompertz growth model, with the estimated *r* (day‑1) and *K* (mm) being ~6% and ~ 17% respectively greater in the *Control* treatments compared to the others, but there was no statistically significant difference between the treatments (Table S3a).

*3.2.2. Above- and belowground biomass, and chlorophyll content of* L. perenne.

At the end of the experiment the biomass of *L. perenne* was not measurably affected by cigarette filters. The yield of dry shoot biomass was on average 31 ± 1 g m-2 (Figure S3a) and the dry root biomass was on average 14 ± 0 g m-2 (Figure S3b). The total above- and belowground dry plant biomass was on average 41 ± 1 g m-2 (Figure S3c). The dry biomass root:shoot ratio for *L. perenne* was on average 0.46 ± 0.02 g g-1 (Figure S3d) with no significant difference (Table S2) between the treatments.

Shoot chlorophyll-a and –b contents were also determined after 21 days of growth. The average chlorophyll-a content was 496 ± 22 µg g-1 dry biomass (Figure 3a), with *L. perenne* not significantly affected by any of the treatments (Table S2). Chlorophyll-b, however, ranged between 344 ± 27 (*Regular Unsmoked*) and 454 ± 67 µg g-1 dry biomass (*Control*) (Figure 3b), was significantly different between the *Control* and the other treatments (*Control* vs. Other: F1,28 = 9.89, P = 0.004). Chlorophyll-b in shoots of the *Control* was ~18% greater than the other treatments. The chlorophyll a:b ratio within *L. perenne* shoots ranged between 1.23 ± 0.02 (*Control*) and 1.34 ± 0.04 (*Menthol Smoked*) (Figure 3c) and was also significantly different between the *Control* and treatments (*Control* vs Others: F = 50.6, P < 0.001, Table S2), with all smoked or remnant cigarette treatments having ~7% higher chlorophyll a:b ratios than that found in the controls.

*3.2.3. Germination success and above ground growth of* T. repens.

Of the *T. repens* seeds sown, overall 56 ± 3% (mean ± SEM, N= 35) germinated, ranging from 42 ± 4 (*Menthol Smoked*) to 73 ± 5% (*Control*) (Figure 4) with the *Control* treatments having significantly better germination success compared to when cigarette filters were present (*Control* vs Others: F1,28 = 23.65, P < 0.001). Approximately 27% more *T. repens* seeds germinated in the *Control* compared to the others. The type of cigarette filter added to the soil was also significantly different (State: F2,28 = 4.46, P = 0.021), especially between filters from *Smoked* or *Unsmoked* cigarettes (P = 0.018, Table S2), with the *Unsmoked* having a ~29% better germination success for *T. repens*.

The shoots in the *Control* were significantly longer than those in all of the other treatments (*Control* vs Others: F1,28 = 69.1, P < 0.001, Table S2), with an average length ranging between 46 ± 5 (*Menthol SwRT*) and 67 ± 4 mm (*Control*) at the end of the experiment (Figure 5). *T. repens* shoots were ~28% longer in the *Control* compared to the other treatments. This is reflected in the estimated coefficients (Table S3b) of the Gompertz growth model, with the estimated *r* (day‑1) and *K* (mm) being ~1% and ~26% respectively greater in the *Control* treatments compared to the others, but there were no significant differences between the coefficients (Table S3b).

*3.2.4. Above- and belowground biomass, and chlorophyll content of* T. repens.

There was no significant difference (Table S2) between the treatments for dry shoot biomass of *T. repens* (Figure 6a) which was on average 18 ± 1 g m-2. The dry biomass of roots was, however, significantly different (Table S2) between the *Control* and the cigarette filter treatments (*Control* vs Others: F1,28 = 10.85, P = 0.003), ranging between 2 ± 1 and 6 ± 1 g m-2 (Figure 6b), with the *Control* having ~57% more dry root biomass than the other treatments. The total above- and belowground dry above- and belowground biomass did not significantly differ (Table S2) and was on average 21 ± 1 g m-2 (Figure 6c). The root:shoot ratio of dry *T. repens* biomass was significantly different between the *Control* and the cigarette filter treatments (*Control* vs Others: F1,28 = 24.57, P < 0.001), ranging between 0.12 ± 0.03 (*Regular SwRT*) and 0.36 ± 0.14 g g-1 (*Control*) (Figure 6d).

The chlorophyll-a content of *T. repens* shoots ranged between 419.45 ± 72.54 (*Control*) and 578.67 ± 32.07 (*Remnant menthol*) µg g-1 dry biomass (Figure 7a), and there was a significant difference between the plants grown in the *Control* compared to the treatments (*Control* vs Others: F1,28 = 4.64, P = 0.040, Table S2). Chlorophyll-b content, on average 352.65 ± 22.96 µg g-1 dry biomass (Figure 7b), however, was not significantly different between any of the treatments (Table S2). The ratio of chlorophyll-a and –b ranged between 1.41 ± 0.01 (*Smoked Menthol*) and 1.86 ± 0.00 (*Control*) (Figure 7c) and was significantly different between the *Control* and treatments (*Control* vs Others: F = 56.6, P < 0.001, *Treatment*: F = 3.2, P = 0.016, Table S2), with all remnant or smoked cigarette treatments having ~20% lower chlorophyll a:b ratios than that found in the controls.

**4. Discussion**

The germination success and initial growth (shoot length during 21 days) of two ecologically and agronomically important plant species was reduced by exposure to smoked and unsmoked cigarette filters. In particular, the germination of *Trifolium repens* was more strongly reduced by smoked cigarette filters and those containing remnant tobacco than by unsmoked filters. This is concerning, because, early detrimental effects from stress on plant development can be sustained over longer terms. An early stage reduction in plant height can reduce the amount of light that is intercepted by the leaves and, therefore, can lead to economic loss by reducing the final biomass and yield of crops (Mathan et al. 2016). *T. repens* also experienced a significant decrease in root biomass in response to the presence of cigarette filters. A reduction in root biomass could jeopardise the ability of the plant to gain water and nutrients from the soil. Root reduction has occurred in response to other environmental stressors such as drought (Zhou et al., 2018). White clover is ecologically important for pollinators (e.g. Rodet et al. 1998) and nitrogen fixation (e.g. Griffith et al 2000), and economically important as it provides biomass for agricultural animal feed (Abberton and Marshall, 2010). Further research is needed to ascertain whether *L. perenne* and *T. repens* could recover from the impacts of cigarette butts over the longer term and whether different species of plant may respond and recover at different rates to this stressor.

Plants can take up pollutants actively such as heavy metals (Tangihu et al. 2011), but also passively such as alkaloids (Yahyazadeh et al 2017), from the soil substrate and they can accumulate these pollutants into their biomass. Lambrechts et al. (2014) found that *T. repens* and *L. perenne* responded with reduced rooting depth and altered root morphology when exposed to Cd or Zn, whereas the aboveground biomass appeared to be unaffected. They found that the two species also responded differently in translocating the pollutants to different parts of the plant, with *L. prenne* accumulating heavy metals in the root biomass whilst *T. repens* was unable to restrict heavy metal translocation. Similarly, Binet et al (2000) found a significant reduction in root and shoot biomass of *L. perenne* when exposed to soil contaminated with PAH.

In the current study, chlorophyll a content increased in *T. repens* and chlorophyll b content decreased in *L. perenne* in response to exposure to cigarette filters. Photosynthetic pigments, such as chlorophylls, play vital roles in harvesting light and changes in their contents are commonly used as biomarkers to indicate stress in plants (Pavlović et al. 2014). For example, chlorophyll a content sometimes increases in plants in response to drought and could be a defensive response to reduce the harmful effects of such environmental stressors (Farooq et al., 2009). On the other hand, chlorophyll a and b content has been found to decrease in other plants such as cotton, *Gossypium hirsutum*, in response to drought (Mssacci et al., 2008) and in grass, *Sorghum* spp. (Sayyad-Amin et al., 2016) and rosy periwinkle, *Catharanthus roseus* (Jaleel et al., 2009) in response to increased salinity. Similarly, in the current study chlorophyll a/b ratios were also altered by cigarette butts in both plants. These ratios are strongly linked to photosynthetic activity and changes can indicate stress in plants (Iori et al., 2017; Zengin and Munzuroglu, 2005).

The effects on shoot length, root biomass and chlorophyll content did not differ depending on whether the cigarette filters were unsmoked, smoked or contained remnant tobacco. This suggests that the bio-based plastic filter itself, rather than the tobacco-related contaminants in the filter after smoking, may contribute to the observed responses. Nevertheless, the majority of cigarette filters, and those used in the current study, are manufactured of cellulose acetate fibres (Register, 2000). Cellulose acetate is a bio-based plastic which has been found to have detrimental effects on plants (including crimson clover, *Trifolium incarnatum*; Maramorosch, 1951, barley, *Hordeum vulgare*; Chada, 1962, cucumber, *Cucumis sativus*; Krizek and Mirecki, 2004, broad bean, *Vicia faba* and alfalfa, *Medicago sativa*; Kieckhefer and Medler, 1960). For example, *C. sativus* grown on cellulose acetate sheets were 25% shorter and had ~50% less shoot and root biomass than those grown on sheets constructed of polytetrafluoroethylene (Krizek and Mirecki, 2004). Furthermore, cellulose acetate cages resulted in mortality of *V. faba* and *M. sativa* after just a few weeks (Kieckhefer and Medler, 1960). These effects were attributed to the presence of the plasticiser, diethyl phthalate in cellulose acetate. Diethyl phthalate can be found in cellulose acetate cigarette filters that are either unsmoked, smoked or smoked with remnant tobacco (Shevchenko, 2012). In isolation, it can be toxic to plants (Cheng, 2012), but also animals (Liu et al., 2009). Diethyl phthalate is slightly water soluble (1 g L-1 at 25℃; Yalkowsky and Dannenfelser, 1992), therefore leaching of this plasticiser could account for the effects on *T. repens* and *L. perenne* observed in the current study. This is, however, only one of many chemicals and toxins present in cigarette filters which may account for the reduction in germination and growth, additional research testing these in isolation would allow a more mechanistic understanding of these effects. It is possible that other potentially toxic substances associated with smoked filters had not leached from the filters into the soil during the 21 days of this study, therefore the immediate (short-term) responses observed may not be attributable to tobacco-related contaminants. Longer-term exposure, promoting further leaching of contaminants, may have a stronger effect on soil flora and fauna.

Very few peer-reviewed studies have assessed the decomposition of cigarette filters under natural conditions, however Bonanomi et al. (2015) found no evidence of decomposition of cellulose acetate filters in soils after 2 years, regardless of environmental conditions (e.g. soil type, moisture, temperature). More recently, Joly and Coulis (2018) compared the decomposition of cellulose acetate filters with recently developed cellulose filters and found that, if discarded onto soil, cellulose acetate cigarette filters will take approximately 14 years to decompose (compared with 7 years in a compost heap), whereas cellulose filters will take 13 years to decompose, six times longer than in a compost heap. It is clear, therefore, that neither cellulose acetate nor cellulose cigarette filters degrade quickly enough to avoid potential ecological damage.

There is now evidence that every aspect of smoking cigarettes, including the smoke (Tileklioğlu et al. 1996), the tobacco (Mondal et al. 2014), the leachate alone (Montalvão et al. 2018) and the discarded filter with associated leachate (current study), may affect the health and productivity of plants. It has been observed by Patel et al. (2013) and Wilson et al. (2014) that the majority of consumers of cigarettes in cities litter their used cigarette filters rather than dispose of them in bins. Interviews conducted by Rath et al. (2012) revealed that this may be due to smokers not viewing cigarette filters as litter. There is an urgent need, therefore, to raise awareness about the fact that, even if biodegradable, cigarette filters can persist in the environment for years, and possibly decades, and that during this time they may cause harmful ecological effects by decreasing the growth and biomass of economically important primary producers with potential cascading effects on ecosystems.

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**Author contributions**

DSG and BB designed the experiment and wrote the initial manuscript, TS and JDSC carried out laboratory work and contributed to the final manuscript.

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**Figures**

Figure1.TIF

**Figure 1.** The germination success of *L. perenne* after 21 days of exposure to either no cigarette filters (Control), or with either regular (without diagonal lines) or menthol (with diagonal lines) filters that had either not been smoked (Unsmoked) or had been smoked (Smoked) or smoked and still have remnant tobacco remaining (SwRT).

Figure2.TIF

**Figure 2.** The shoot growth over 21 days of *L. perenne* exposure to either no cigarette filters (Control, white circles), or with either regular (light grey) or menthol (dark grey) filters that had either not been smoked (Unsmoked, squares) or had been smoked (Smoked, triangles) or smoked and still have remnant tobacco remaining (SwRT, upside down triangles).

Figure4.tif

**Figure 3.** Chlorophyll a (a) and b (b) concentrations (µg-1 g-1 dry shoot biomass) and chlorophyll a to b ratios (c) of *L. perenne* after 21 days of exposure to either no cigarette filters (Control), or with either regular (without diagonal lines) or menthol (with diagonal lines) filters that had either not been smoked (Unsmoked) or had been smoked (Smoked) or smoked and still have remnant tobacco remaining (SwRT).

Figure5.TIF

**Figure 4.** The germination success of *T. repens* after 21 days of exposure to either no cigarette filters (Control), or with either regular (without diagonal lines) or menthol (with diagonal lines) filters that had either not been smoked (Unsmoked) or had been smoked (Smoked) or smoked and still have remnant tobacco remaining (SwRT).

Figure6.TIF

**Figure 5.** The shoot growth over 21 days of *T. repens* exposure to either no cigarette filters (Control, white circles), or with either regular (light grey) or menthol (dark grey) filters that had either not been smoked (Unsmoked, squares) or had been smoked (Smoked, triangles) or smoked and still have remnant tobacco remaining (SwRT, upside down triangles).

Figure7.tif

**Figure 6.** The dry shoot (a), root (b) and combined (shoot plus root) (c) biomass and the dry root to shoot ratio (d) for *T. repens* after 21 days of exposure to either no cigarette filters (Control), or with either regular (without diagonal lines) or menthol (with diagonal lines) filters that had either not been smoked (Unsmoked) or had been smoked (Smoked) or smoked and still have remnant tobacco remaining (SwRT).

Figure8.tif

**Figure 7.** Chlorophyll a (a) and b (b) concentrations (µg-1 g-1 dry shoot biomass) and chlorophyll a to b ratios (c) of *T. repens* after 21 days of exposure to either no cigarette filters (Control), or with either regular (without diagonal lines) or menthol (with diagonal lines) filters that had either not been smoked (Unsmoked) or had been smoked (Smoked) or smoked and still have remnant tobacco remaining (SwRT).