

RESEARCH ARTICLE

Tire abrasion particles negatively affect plant growth even at low concentrations and alter soil biogeochemical cycling

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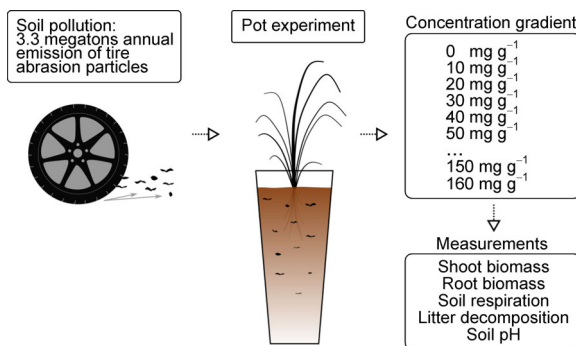
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HIGHLIGHTS

- Tire abrasion particles reduced above-ground and belowground biomass.
- Soil respiration and soil pH increased with increasing amount of added tire particles.
- Litter decomposition is affected by addition of tire particles.
- Effects are apparent already at the lowest added concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Tire particles (TPs) are a major source of microplastic on land, and considering their chemical composition, they represent a potential hazard for the terrestrial environment. We studied the effects of TPs at environmentally relevant concentrations along a wide concentration gradient (0–160 mg g⁻¹) and tested the effects on plant growth, soil pH and the key ecosystem process of litter decomposition and soil respiration. The addition of TPs negatively affected shoot and root growth already at low concentrations. Tea litter decomposition slightly increased with lower additions of TPs but decreased later on. Soil pH increased until a TP concentration of 80 mg g⁻¹ and leveled off afterwards. Soil respiration clearly increased with increasing concentration of added TPs. Plant growth was likely reduced with starting contamination and stopped when contamination reached a certain level in the soil. The presence of TPs altered a number of biogeochemical soil parameters that can have further effects on plant performance. Considering the quantities of yearly produced TPs, their persistence, and toxic potential, we assume that these particles will eventually have a significant impact on terrestrial ecosystems.

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1 Introduction

Microplastic pollution has become a global threat to oceans, rivers and soils (Blettler et al., 2018; Haward, 2018; Rillig and

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Lehmann, 2020). The emerging research on terrestrial ecosystems has been focused on microplastic originating from littering, sewage sludge or mulching. However, a major source of microplastic particles on land are tire particles (TPs) originating from abrasion of tires on roads (Baensch-Baltruschat et al., 2020; Evangelidou et al., 2020). TPs are considered microplastic as they contain a large part of synthetic polymers, have a solid state, are insoluble in water, and have particle size ranges typical of microplastic (1–1000 μm) (Hartmann et al., 2019). Kole et al. (2017) estimate the global annual emission of particles from tires to be more than 3.3 megatons. Although partially retained by separate sewer systems in urban areas, approximately two thirds of these particles end up in soils via runoff or transport by air (Panko et al., 2013). Amounts of TPs in the soil vary a lot: depending on the distance from the road, transport dynamics and the traffic frequency, concentrations can range from 0.1–117 g kg^{-1} (Wik and Dave, 2009; Wagner et al., 2018; Baensch-Baltruschat et al., 2020).

Considering the composition of the tires, they represent a potential hazard for the terrestrial environment. Tires usually contain 40%–50% rubber (natural and synthetic), 30%–35% fillers (typically carbon black, silica and chalk), 15% softeners (e.g., oils), 2%–5% vulcanization agents (S and ZnO) and further additives (Brandsma et al., 2019; Baensch-Baltruschat et al., 2020). Additionally, tires can release other contaminants such as heavy metals and polycyclic aromatic hydrocarbons (PAHs) into the environment (Kreider et al., 2010). Considering these numbers, it is surprising that the effects of TPs in the terrestrial environment have not received more attention so far.

Microplastics often have positive effects on plant performance (de Souza Machado et al., 2018; Lehmann et al., 2020), but also negative effects can occur (Qi et al., 2018; van Kleunen et al., 2020). Tire particles have been tested in past studies for their suitability as potting substrate for plants. The amendment with TPs unambiguously induced morphological and anatomic alterations, as well as growth limitations in the aboveground biomass and altered nutrient concentrations in plant tissue (Schulz, 1987; Bowman et al., 1994). The authors identified the toxicity of zinc (Zn) as the main problem. More recent studies shifted toward a focus on ecotoxicology, mainly in aquatic ecosystems. Here, toxic effects of TP leachates were connected with Zn, other heavy metals and organic components such as phthalates, resin acids or benzothiazoles (Baensch-Baltruschat et al., 2020).

The impact of TPs on soil biota and soil functions relevant to plant performance is largely unknown. Microplastic in general can affect the growth, metabolism, reproduction and mortality of a wide range of the soil fauna, especially at high concentrations and small particle sizes (Büks et al., 2020). A number of soil functions, such as decomposition or soil aggregation, as well as microbial activity can be altered by microplastics (de Souza Machado et al., 2019). One recent study in a soil ecosystem looked at the effect of TPs on earthworms, showing altered gut microbiota of the animals

and impaired survival and reproduction rates (Ding et al., 2020). Not only the particles themselves can be responsible for the observed effects but also the additives contained in the materials (Baensch-Baltruschat et al., 2020). To our knowledge there is no empirical study showing the effect of TPs on plant growth and soil biogeochemical cycling at environmentally relevant concentrations along a wide concentration gradient. Studies used either very high concentrations, such as for potting substrates with up to 66% of the substrate (Bowman et al., 1994), or used rather low concentrations of $\leq 30 \text{ mg g}^{-1}$ (Schulz, 1987; Ding et al., 2020). In this study, we cover a response surface between 0 and 160 mg g^{-1} , with the latter being a realistic maximum concentration for TPs in German soils (Baensch-Baltruschat et al., 2020). We performed this study in the light of increasing global traffic volume and thus increased tire wear emissions and the lack of knowledge on the effect of TPs on the terrestrial environment. Given the significant concentrations of toxic components in the TPs we expect negative effects on plant performance, while at the same time carbon compounds and a reduced bulk density will lead to stimulating effects on microorganisms.

2 Materials and methods

We performed a pot experiment with natural soil and a plant, using a concentration gradient of TP pollution from 0–160 mg g^{-1} soil with 3 replicates per concentration level. We tested the effects of TPs on plant growth, soil pH and the key ecosystem process of litter decomposition and soil respiration.

2.1 Soil and tire particles

For the experiment, a soil-sand mix was prepared. The soil was collected at a field site in Berlin and was classified as Albic Luvisol following FAO classification (loamy sand, 6.9 $\text{mg P } 100 \text{ g}^{-1}$ soil (calcium-acetate-lactate), 0.12% N (total), 1.87% C (total), see Leifheit et al. (2015), 56 $\mu\text{g Zn g}^{-1}$ soil (aqua regia digestion)). The air-dried soil was sieved with a 2 mm sieve and mixed with autoclaved sand (50:50 w/w). We diluted the soil with sand in order to facilitate the harvest of the belowground material. The pH of the soil sand mixture was 5.41 (EN 15933:2012). The experimental system was established in white polypropylene pots (Ray Leach Containers, Stuewe and Sons Inc.) with a cotton wadding at the bottom and initially filled with 70 g autoclaved sand.

We produced tire abrasion particles from a used car tire (Goodyear M+ S 195/60R15 88H) using a portable belt grinding machine (Bosch PBS 75 AE). The particles had an average size of 125 μm within a range of 34–265 μm (determined by photographic analysis, see Fig. S1). Heavy metals in the tire particles were determined with an aqua regia digestion: Cr $175 \pm 7 \text{ mg kg}^{-1}$, Pb $357 \pm 29 \text{ mg kg}^{-1}$, Zn $5089 \pm 40 \text{ mg kg}^{-1}$, Ni $95 \pm 3 \text{ mg kg}^{-1}$ and Cu $453 \pm 10 \text{ mg kg}^{-1}$ (ICP-

OES Perkin Elmer Optima 2100DV). TPs typically have a density of 1.13–1.16 g cm⁻³ (Rhodes et al., 2012).

The concentration of microplastic was increased in steps of 10 mg g⁻¹ soil, starting at 0 mg TPs g⁻¹ soil-sand-mix, up to 160 mg g⁻¹, giving 17 different concentration levels with 3 replicate pots per level, summing up to 51 pots in total (see Table 1 for details on added amounts of TPs and the corresponding added heavy metal concentrations). The TP concentrations were chosen based on literature values, using current environmentally relevant concentrations and prospective concentrations of accumulating TP in soil for Germany (Klöckner et al., 2019; Baensch-Baltruschat et al., 2020).

2.2 Litter and plant

For the analysis of microplastic effects on litter decomposition we produced mesh bags with a size of 3 cm × 2.5 cm containing 300±10 mg of tea leaf litter (Lipton Green Tea, Sencha Exclusive Selection) (see Lehmann et al., 2020).

Seeds of *Allium porrum* (variety “De Carentan,” Floraself) were sterilized with a 0.5% solution of NaClO-bleach for 10 min and 70% ethanol for 40 s. Sterilized seeds were washed with demineralized water and germinated in autoclaved sand. Seedlings were watered with demineralized water.

2.3 Experimental set-up

The experiment was set up in a randomized manner: a labeled pot was picked at random and filled with the soil-microplastic mixture. For each pot, the tire particles were mixed manually with 50 g of soil-sand-mix in a glass beaker by stirring with a metal spatula. The mesh bag was placed in the center of the pot when half of the soil-microplastic-mix was added and finally the rest of the soil-microplastic-mix was filled into the pot.

The growth substrate (the soil-microplastic mix) was watered to 60% water holding capacity (WHC) and this soil moisture was kept constant during the experiment by gravimetric watering with deionized water for all pots.

Seedlings of similar height were selected and placed in the center of the pot. Pots were placed in a randomized manner on two trays and randomized again once per week. Greenhouse conditions were 22/18°C (day/night) with a daylight period of 12 h, illuminance: 50 klx, and a relative humidity ~40%. Plants were grown for 6 weeks and the experiment was harvested destructively.

2.4 Harvest

The harvest was done in a randomized manner. The upper layer of the soil-sand-mixture was discarded from the analysis

Table 1 Amount of tire particles (TP) and the corresponding concentrations of heavy metals that were added to the experimental soil.

TP (mg g ⁻¹)	Corresponding concentrations of heavy metals				
	Zn (mg kg ⁻¹)	Pb (mg kg ⁻¹)	Cr (mg kg ⁻¹)	Ni (mg kg ⁻¹)	Cu (mg kg ⁻¹)
0	0	0	0	0	0
10	50.9	3.6	1.8	1	4.5
20	101.8	7.1	3.5	1.9	9.1
30	152.7	10.7	5.3	2.9	13.6
40	203.6	14.3	7	3.8	18.1
50	254.5	17.9	8.8	4.8	22.7
60	305.3	21.4	10.5	5.7	27.2
70	356.2	25.0	12.3	6.7	31.7
80	407.1	28.6	14	7.6	36.2
90	458.0	32.1	15.8	8.6	40.8
100	508.9	35.7	17.5	9.5	45.3
110	559.8	39.3	19.3	10.5	49.8
120	610.7	42.8	21	11.4	54.4
130	661.6	46.4	22.8	12.4	58.9
140	712.5	50.0	24.5	13.3	63.4
150	763.4	53.6	26.3	14.3	68.0
160	814.2	57.1	28	15.2	72.5

^aThe background concentration of Zn in the experimental soil was 56 mg kg⁻¹.

in order to exclude soil that was influenced by external effects like airborne spores from bacteria or fungi. Below a subsample of soil was taken and stored at 4°C for the subsequent measurement of soil respiration. The above-ground biomass was cut, roots were carefully taken out of the soil, washed and the soil, all plant parts and the mesh bags were dried at 40°C. Plant available zinc was extracted from 5 g of dried soil with 50 mL of diethylenetriaminepentaacetic acid (DTPA) (ICP-OES Perkin Elmer Optima 2100DV).

Roots and shoots were weighed. Dried litter was removed from the mesh bags and the weight loss determined gravimetrically. Soil respiration was measured with the 'MicroResp Soil Respiration SystemTM' using 2 g of soil per measurement and two technical replicates. CO₂ production was measured spectrophotometrically before and after incubation of 6 h at room temperature. After the measurements, samples were dried and soil water content was determined.

2.5 Statistics

We estimated the response surfaces of the measured variables along the gradient of tire particle concentration, fitting a generalized additive model (Hastie and Tibshirani, 1986; Wood, 2017). We consider fitting a generalized additive model to be a reasonable approach, because we do not know the shapes of the curves *a priori*. A spline curve was fitted based on restricted maximum likelihood (Wood, 2011), and the other parameters were set as default. We used the package "mgcv" v1.8-23 (Wood, 2018) in R v4.0.2 (R Core Team, 2020). Model performance was assessed based on adjusted R-squared, and the fitted curve shapes with the 95% Confidence Intervals (CIs). Note that, while we report *p*-value, we do not assess whether a result is statistically significant or not based on *p*-value but focus on the strength of the variable association with variability explained (R^2), following the suggestion by the American Statistical Association (Wasserstein et al., 2019). The R script with the data set is available for reproducibility at 'tire-particles-in-soil' (github repository, full link available below).

3 Results

The addition of tire abrasion particles negatively affected shoot and root growth. Shoot dry weight started to decrease with the lowest TP addition of 10 mg g⁻¹ and further decreased until it leveled off around 60 mg g⁻¹ (adjusted $R^2 = 0.20$, $p = 0.009$; Fig. 1A). Root weight slightly but continuously decreased with increasing TP addition (adjusted $R^2 = 0.07$, $p = 0.09$; Fig. 1B).

The decomposition of the tea litter slightly increased with lower additions of TPs but at TP concentrations larger than 60 mg g⁻¹ decreased slightly with increasing concentration of TPs until an average slightly lower than that of the control was reached (adjusted $R^2 = 0.30$, $p = 0.0005$; Fig. 1C). Soil pH

increased until a TP concentration of 80 mg g⁻¹ and afterwards seemed to level off toward the end (adjusted $R^2 = 0.54$, $p = 6e-09$, Fig. 1D). The concentration of plant available zinc in soil and soil respiration clearly increased with increasing concentration of added TPs (adjusted $R^2 = 0.91$ and 0.63, $p = 2e-16$ and $2e-12$, respectively; Fig. 1E and 1F).

4 Discussion

In this study, we show that there is a direct effect of pure laboratory-produced TPs under controlled laboratory conditions. In previous studies Zn has been shown to be predominantly responsible for detrimental effects on plants, as this metal showed the highest increase in concentration in plant tissue, causing growth reductions, alterations of morphology and anatomy (Schulz, 1987; Bowman et al., 1994). According to the guidelines for safe limits for agricultural soil by the European Commission (EC, 2002) soils should have <300 mg Zn kg⁻¹, <300 mg Pb kg⁻¹, <140 mg Cu kg⁻¹, <75 mg Ni kg⁻¹ and <150 mg Cr kg⁻¹. According to these thresholds, the tire material used for this experiment contaminated the soil with Zn with TP additions of ≥ 50 mg g⁻¹ soil. This is around the concentration level at which plant growth was not further reduced. We assume that the plants reduced their growth with starting contamination and stopped growing when Zn contamination reached a certain level in the soil. This assumption is supported by the fact that concentrations of bioavailable zinc in our experimental soil increased with increasing additions of TPs (Fig. 1E).

In some countries thresholds for safe limits for agricultural soils are much lower (EC, 2002), e.g. Denmark has a limit for good soil quality for Cr at 30 mg kg⁻¹. Accordingly, our experimental soil was slightly contaminated with Cr and could have also impacted plant performance. Root growth slightly decreased with increasing concentration of TPs, indicating increased uptake of contaminants, especially Zn, which induced a growth reduction. Plants generally restrict metals more to root than shoot biomass as a stress-avoidance strategy to circumvent negative impacts of heavy metal pollution of the soil (Audet and Charest, 2008). This could explain the increasing effect of TPs on roots and the leveling off effect on shoots.

The availability of heavy metals depends on the pH of the soil and is higher in the lower pH range (Xian and In Shokohifard, 1989). Our experimental soil was acidic at the start of the experiment (pH 5.3), which probably facilitated their uptake. The TP addition increased the soil pH but saturated around pH 6.5 (see Fig. 1D and next section). The effect of TPs on plants will differ with abiotic conditions such as soil pH, but also moisture, texture and temperature. A change in soil pH will not only affect bioavailability of heavy metals to plants but also soil processes.

The presence of TPs alters a number of biogeochemical soil parameters that can influence the decomposer

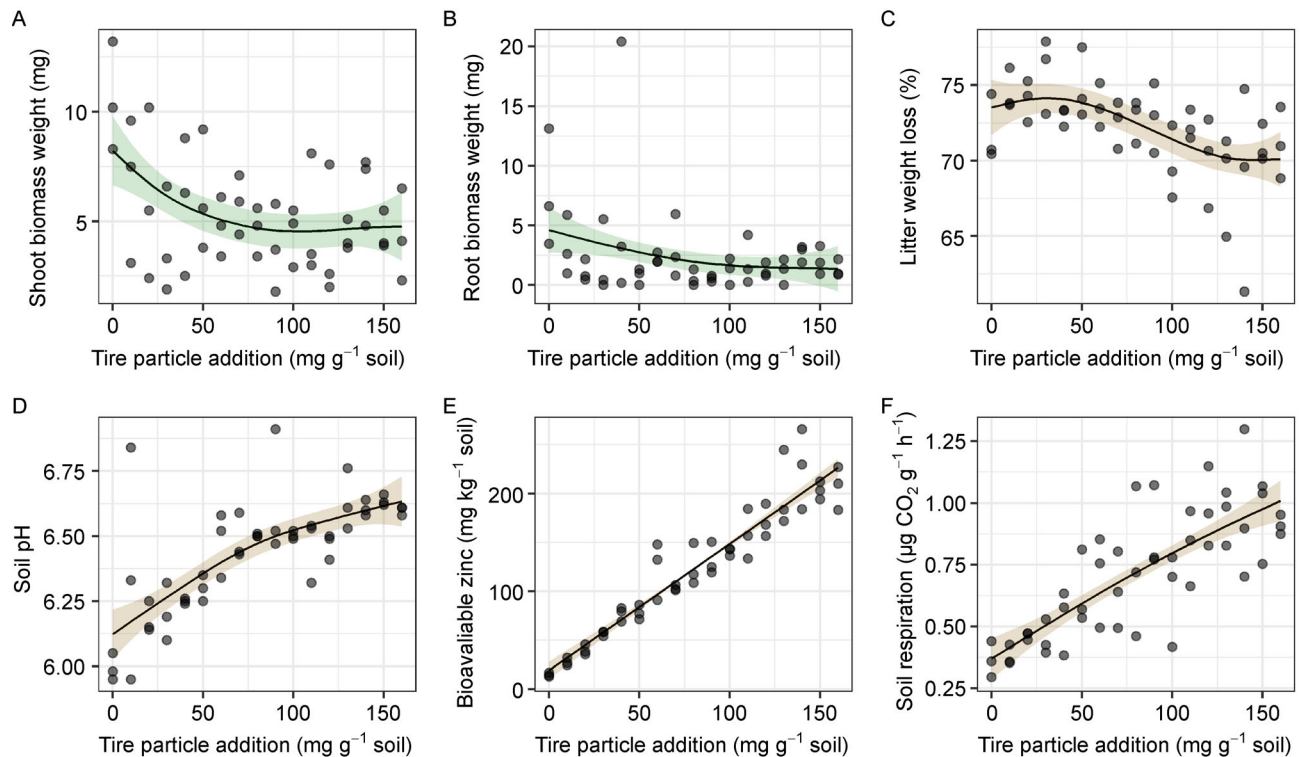


Fig. 1 Individual data points for shoot (A) and root (B) biomass weight (mg), litter weight loss (%) (C), soil pH (D), bioavailable zinc (mg kg⁻¹ soil) (E) and soil respiration (F). Lines indicate smoothed splines of response variables in response to the concentration gradient of tire particles, colored areas represent the 95% CIs.

community: i) TPs contain toxic components that leach from the particles, such as Zn. Zn has been shown to have harmful effects on some soil fungi and bacteria (Babich and Stotzky, 1978). ii) TPs alter bulk density and soil aeration, with consequences for water and oxygen availability. iii) TPs contain carbon, which introduces a change in resource availability. iv) TPs contain CaCO₃ as filler and thus increase the soil pH, which can influence decomposer activity and composition (Smolders and Degryse, 2002; Krishna and Mohan, 2017; Tao et al., 2019; Baensch-Baltruschat et al., 2020). v) Some components of TPs are hydrophobic (e.g., antioxidants and PAHs) (CSTEE, 2003; Wagner et al., 2018), thus potentially changing water relations in the soil. Some of these changes might have a stimulating effect on decomposers at low TP concentrations (such as improved aeration or elevated pH), while others (such as Zn contamination) have increasingly negative impacts on the decomposer community, thus cumulatively overshadowing the previous positive impacts.

At optimal soil moisture conditions, such as in our experiment with constant adjustment to 60% WHC, decreased soil bulk density and enhanced substrate aeration likely improved the conditions for microbial activity. Additionally, TPs represent a carbon source, as carbon black and the synthetic polymers contain a considerable amount of carbon (Kreider et al., 2010). Although TPs and associated particles from other sources (e.g., road abrasion) generally have a low

bioavailability (Wik and Dave, 2009), some of these compounds leach from the TPs (Wagner et al., 2018) and thus are available as a resource to microorganisms and can boost their activity. However, regarding the toxicity of the particles, we assume that the toxic components only selectively affected soil fungal and bacterial species (Babich and Stotzky, 1978). Other species that are less sensitive to these pollution agents might have profited from the carbon addition. Our findings are in line with field observations where spots closer to a road showed increased soil respiration compared to spots more far from the road (Kocher et al., 2001).

5 Conclusions and perspectives

In the environment TPs form heteroaggregates together with road wear particles (e.g., road abrasion particles, de-icing salts), brake ware and particles from atmospheric deposition (Charters et al., 2015). The morphology, chemical composition and potential toxicity of these 'tire- and roadwear particles' (TRWP) will be different from pure TPs (Baensch-Baltruschat et al., 2020), with differing impacts on the environment (Rillig et al., 2019). For example, in the presence of ions from de-icing salts, Zn forms complex ZnCl species that exert greater toxicity than Zn alone (Babich and Stotzky, 1978). However, there is a wide range of observed toxicity of tire containing particles, which can be attributed to differences in particle

composition, applied concentrations, test designs and species sensitivity.

Pristine TPs are prone to degradation, resulting in an alteration of their crystalline structure, chemical composition, shape and surface texture. Degraded TRWP can release sorbed compounds and leachate over time (Councell et al., 2004; Smolders and Degryse, 2002), leading to an accumulation of harmful components in the soil. Considering the quantities of yearly produced TRWP, their persistence, and toxic potential, we assume that these particles will eventually have a significant impact on the terrestrial environment. Effects on plant diversity and soil functions (filtering, medium for plant growth, habitat for soil organisms, carbon reservoir) should be the focus of further studies.

Author contributions

EFL and MCR designed the study. HLK and EFL performed the study. HLK, EF and EFL performed the laboratory work. MR performed the statistical analysis. EFL led the writing of the manuscript and wrote the first draft. All authors contributed to the writing of the manuscript.

Data availability

'Tire-particles-in-soil'. Github repository. Data set and R code available at <https://github.com/Dr-Eva-F-Leifheit/tire-particles-in-soil>.

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Electronic supplementary material

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