RESEARCH ARTICLE

A microplastic used as infill material in artificial sport turfs reduces plant growth

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Societal Impact Statement

Plastic pollution is of increasing societal concern, particularly with regard to aquatic environments, where it has received widespread news media attention. However, plastic and plastic byproducts also pollute terrestrial ecosystems, and the potential impacts on organisms are currently poorly understood. Here, we show that a microplastic type used in artificial sport turfs may have negative effects on plant growth. Given the scale of plastic pollution, urgent research is needed to determine the impact of microplastics on terrestrial organisms and their communities, as well as an exploration of alternative, biodegradable materials and measures to reduce the spread of microplastics in nature.

Summary

- The Anthropocene is, among other factors, characterized by the accumulation of plastic in the environment. While studies on the consequences of plastic pollution for animals, particularly in aquatic environments, have increased in recent years, much less is known about potential effects of plastic pollution on plants in terrestrial environments.

- Ethylene propylene diene monomer (EPDM) is a microplastic used in artificial sport turfs. Here, we tested in two separate experiments the effects of different concentrations of EPDM on the performance of *Plantago lanceolata* and on competition between seven grassland-plant species.

- At very low concentrations of the EPDM granules, growth of *P. lanceolata* was slightly improved, but at concentrations of 5% and higher there were strong negative effects on survival and growth. These negative effects were found under low and high nutrient conditions, and for all tested species. The EPDM granules also negatively affected the root weight ratio, which indicates that the root system was more strongly affected than the shoot. Due to the strong negative effects on plant growth, the granules also reduced the competitive interactions between plants.

- Our study shows that it is not only animals in aquatic environments that may be affected by plastic pollution, and that this may also be the case for wild plants in terrestrial ecosystems.

1 | INTRODUCTION

It is now widely accepted that humans have changed the global environment to such an extent that it has been proposed to call the current epoch 'the Anthropocene' (Lewis & Maslin, 2015). This period is characterized by a multitude of environmental changes, and one of them is the accumulation of novel human-made materials, particularly plastics, in the environment (Waters et al., 2016). Plastics are a diverse group of polymers, mostly produced from oil, natural gas or coal. The production of plastics started in the 1930s, and since then has increased dramatically (Jambeck et al., 2015). It has been estimated that between 1950 and 2015 at least 8,300,000,000 tons of plastic - 29 times the weight of all adult humans on Earth (Walpole et al., 2012) - has been produced, and that over half of this has by now been discarded as waste (Geyer, Jambeck, & Law, 2017). Given the high durability of plastic, most of this plastic waste is still present in the environment, and some is even found in remote places such as the Antarctic (Barnes, Galgani, Thompson, & Barlaz, 2009) and in the Mariana Trench (Jamieson et al., 2019).

There is an increasing awareness that plastic waste ends up in aquatic ecosystems, where it can have disastrous effects on wildlife (Vegter et al., 2014). Consequently, research on plastic pollution in aquatic ecosystems has increased in recent years (Eerkes-Medrano, Thompson, & Aldridge, 2015; Jambeck et al., 2015). Much less attention has been paid to the potential effects of plastic pollution in terrestrial environments (Rillig, 2012; Rochman, 2018; Souza Machado, Lau, et al., 2018; Windsor et al., 2019). This is surprising, because the release of plastic in terrestrial ecosystems is likely to be higher than in the oceans. For example, for the European Union, Horton, Walton, Spurgeon, Lahive, and Svendsen (2017) calculated that 4–23 times more plastic is released and retained in terrestrial environments than in the oceans. So, there is a clear need to test the ecological consequences of plastic pollution in terrestrial ecosystems (Rillig, Lehmann, Souza Machado, & Yang, 2019; de Souza Machado, Kloas, Zarfl, Hempel, & Rillig, 2018).

Plastic waste comes in different sizes, and the ecological consequences are likely to depend on the size of the parts or particles and the chemical make-up (de Souza Machado et al., 2019; Windsor et al., 2019). Large organisms, such as sea turtles and whales, may ingest entire plastic bags, and many organisms, also small ones, may ingest small plastic particles (Derraik, 2002). If these particles are smaller than 5 mm, they are usually referred to as microplastics (Barnes et al., 2009; Verschoor, 2015). Plastic particles smaller than 0.1 µm (i.e., nanoplastics) can even pass through cell membranes (Souza Machado, Lau, et al., 2018). Microplastics are either manufactured as such, for example, for use as scrubbing material in cosmetics and toothpaste, or they arise secondarily through disintegration and degradation of large plastic debris (e.g., due to abrasion

or weathering). Microplastics are currently found in most aquatic environments (Dris et al., 2015), but have also been reported in flood plain soils (Scheurer & Bigalke, 2018) and other terrestrial environments (Fuller & Gautam, 2016; Zhang & Liu, 2018). While it has been shown that microplastics can be ingested by soil animals, and can be transported by them vertically and horizontally in the soil (Maass, Daphi, Lehmann, & Rillig, 2017; Rillig, Ziersch, & Hempel, 2017), little is known about potential effects of microplastics on growth of the plants that have their roots in contaminated soil (Qi et al., 2018; Rillig et al., 2019; de Souza Machado et al., 2019).

As there are many different types of plastic, each with different properties, one should test the effects of each type separately. A plastic type that might be relevant for plant growth in terrestrial environments is the synthetic rubber ethylene propylene diene monomer (EPDM). This plastic is present in many materials that are used outdoors, such as roof coatings and car parts (https://www.timcorubber.com/rubber-materials/epdm/), and it is thus likely that through abrasion of these materials, plants can be exposed to EPDM particles. Moreover, in recent decades, many grass sport turfs have, due to their high maintenance costs, been replaced with cheaper artificial sport turfs. To increase the elasticity of the sport turfs, the spaces between the artificial grass blades are filled with small granules. Initially, these granules were made of recycled car tires and contained styrene butadiene rubber, but, because of health concerns, currently most infill particles are made of EPDM (KIMO, 2017). These infill granules easily stick to sports gear and are displaced by heavy rains, and thus spread into the vegetation surrounding the artificial sport turfs (see e.g., Figure 1). For example, in Denmark, an estimated 1.5–2.5 tonnes of infill granules are released each year into the environment by each artificial football field (Lassen et al., 2015).

The potential effects of microplastics on plant growth can be manifold (de Souza Machado et al., 2019). First, microplastics may change the structure and water retention of the soil. This could, depending on the size of the particles and the concentration, increase soil aeration or provide a barrier for root growth. Second, the presence of additives in microplastics, such as plasticizers and flame retardants, may have toxic effects on plants (Andrady, 2017). Rubber granules may also contain other toxic substances, such as heavy metals (Bocca, Forte, Petrucci, Costantini, & Izzo, 2009). Third, the microplastics might bind nutrients, and thereby decrease soil fertility (Vijaya & Reddy, 2008). Furthermore, even if the growth of a plant species is not directly impacted by microplastics, it might be affected indirectly if, for example, soil organisms or competitor plant species are affected (de Souza Machado et al., 2019).

Here, we tested in two separate experiments the effects of different concentrations of a microplastic type on plant performance and its effect on competition between plants. In the first experiment,

FIGURE 1 A photograph illustrating that infill granules (black particles in the image) used in artificial sport turfs can easily spread from the artificial turf into the surroundings, resulting in a relatively high concentration of the granules in the surrounding soils. Dark patches of plastic granules can be seen not only on the artificial sport turf and the tarred path, but also in the patch of grass at the bottom of the photo. © Mark van Kleunen

we grew the common perennial herb *Plantago lanceolata* at different concentrations of a plastic (EPDM) and a natural cork infill material used in artificial sport turfs. To test whether the effect of plastic pollution depends on nutrient availability, we combined this factorially with a fertilizer-addition treatment. In the second experiment, we grew seven grassland species, all common in Central Europe, without competition and in all possible pairwise combinations in the presence and absence of the EPDM granules. We asked the following specific questions: (a) Do the EPDM granules affect plant growth, and how does this depend on the concentration? (b) Does the effect of the EPDM granules depend on the nutrient availability in the soil substrate? (c) Do the granules affect plant competition?

2 | MATERIALS AND METHODS

2.1 | Experiment testing the effects of different concentrations of EPDM granules

To test whether and at what concentrations EPDM microplastic in the substrate affects plant growth, we grew single plants of *Plantago lanceolata* L. (Plantaginaceae) at different concentrations of EPDM

granules. *Plantago lanceolata* is a perennial forb native to Europe, western Asia and northern Africa (Sagar & Harper, 1964), and widely naturalized in other parts of the world (e.g., Alexander, van Kleunen, Ghezzi, & Edwards, 2012). It grows in various grassland habitats and is one of the most common species in Central Europe (e.g., http://www.floraweb.de/webkarten/karte.html?taxnr=4320). It can also frequently be found next to sport fields with artificial turfs, where it is exposed to the granules used as infill material (M. van Kleunen, personal observation).

We bought seeds of *P. lanceolata* from Rieger-Hofmann GmbH. The seeds were soaked in water for 5 days and then, on 11 May 2018, they were sown in a tray (l × w × h: 13.4 cm × 12.2 cm × 4.9 cm) filled with commercial seedling soil (Einheitserde P, Patzer Sinntal-Jossa). The tray was placed in a greenhouse compartment with a day/night temperature of 23/18°C, without additional lighting. On 22 May 2018, single seedlings were transplanted into pots (l × w × h: 7 cm × 7 cm × 6.5 cm). The base substrate used was a 1:1 mixture of quartz sand and vermiculite, which can be easily washed off the roots at harvest. In this base substrate, we had mixed varying amounts of either of two granule materials used as infill material in artificial sport turfs. As plastic-pollution treatment, we used dark green infill granules made of the synthetic rubber EPDM with a size range of 0.5–2.5 mm and a density of 1.6 g/cm^3 (Resedagrün RAL 6011, GranuElastic-Höfer & Stankowska GbR). As control, we used infill granules made of natural cork without artificial additives, and with a size range of 0.5–2.0 mm and a density of 0.225 g/cm^3 (Friedbert Bleile H&D; https://cork-shop.com/). For each of the two granule materials, we created a series of 10 volumetric concentrations: 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64% v/v. We used a geometric increase of the granules' concentration to have a finer resolution at low concentrations. For each granule type and concentration, we had 14 pots. To test whether the effects of the granule materials and the concentrations depended on nutrient availability, seven of these 14 pots were assigned to a low-fertilizer treatment and the other half to a high-fertilizer treatment (totalling 280 pots). In the low- and high-fertilizer treatments, each pot received weekly 50 ml of a 0.5‰ and a 1‰ m/v, respectively, solution of Universol$^®$ Blue (Everris International B.V.), which contains as main macronutrients 18% N (10% N-NO$_3$, 7.5% N-NH$_4$, 0.5% urea), 11% phosphate (P$_2$O$_5$), and 18%, potassium (K$_2$O). The 280 pots were randomly assigned to positions in a greenhouse compartment of the Botanical Garden of the University of Konstanz (Germany). The plants were watered every second or third day until saturation. The temperature was set to vary between 20°C during the day and 15°C during the night. No artificial lighting was used.

One day after transplanting the seedlings (23 May 2018), we measured the length of the longest cotyledon, and counted the number of leaves, as initial size measurements for each plant. That day, we also replaced four seedlings that had died. Seven weeks after the start of the experiment (10 July 2018), we scored survival of the plants. Then we first harvested the aboveground biomass, and after that we washed the roots free from substrate. To determine the length and the average diameter of the roots, a random subsample of

the roots of each plant was digitized with a flatbed scanner modified for root scanning (Epson Expression 10000 XL; Regent Instruments) and WinRhizo software (2017; Regent Instruments Inc.). For each plant, we separately dried the aboveground biomass, the root sample that had been scanned and the remaining roots in a drying oven at 70°C for at least 3 days prior to weighing. We used these data to calculate the total root length per plant, the specific root length (root length divided by the root biomass), the total biomass and the root weight ratio (root biomass divided by total biomass).

The continuous variables, total biomass, root weight ratio and specific root length, were analysed in linear models (LMs) with granule material and fertilizer treatments as main factors, and the granules' concentration as a continuous main effect, using the *lm* function in R (version 3.5.1; R Core Team, 2018). As the increments in concentration of granules were geometric, we took the natural logarithm of the granules' concentration to get a more regular distribution, and we first added 0.1 to each value to avoid undefined values when the concentration was zero. Because graphical inspection of the plots indicated non-linear relationships between some response variables and ln(granules' concentration +0.1), we also included a quadratic component of ln(granules' concentration +0.1), using the *poly* function in R. To test whether the linear or nonlinear effect of the granules' concentration was affected by granule material and fertilizer level, we also included all two- and three-way interactions. If the quadratic term of ln(granules' concentration +0.1) was neither significant as the main factor nor in any interaction, we removed it from the final model. To account for differences in the initial size of plants, we included the length of the longest cotyledon and the number of true leaves (0 or 1) as centered covariates. To improve normality and homoskedasticity of the residuals, specific root length was square-root transformed prior to analysis.

Survival of plants was analysed in a similar way, but with a binomial generalized linear model (GLM) instead of LM. However, because all plants in the treatments with cork granules survived, we restricted this analysis to the treatments with plastic granules, and consequently the term granule material and its interactions were not included in the model. In addition, we used the *predict* function in R to estimate the EPDM granules' concentrations at which 10%, 20% and 50% of the plants died, corresponding to the lethal concentrations LC10, LC20 and LC50, respectively. The associated data files and R syntax are included as supporting information (Dataset S1, Code S1).

2.2 | Experiment testing the effects of EPDM granules on competition

To test whether EPDM granules affected plant competition, we chose seven common European grassland species. Three of those species are perennial grasses (*Alopecurus pratensis* L., *Festuca guestfalica* Boenn. ex Rchb., *Lolium perenne* L.), three of them are perennial forbs (*Galium album* Mill., *Leucanthemum ircutianum* [Turcz.] Turcz. ex DC. [a taxon of the *Leucanthemum vulgare* agg.], *Prunella vulgaris* L.), and one of them is a biennial forb (*Daucus carota* L.). Seeds of *D.*

carota were collected in the wild in the vicinity of Halle (Germany) in 2017, and the seeds of the other six species were bought from Rieger-Hofmann GmbH. As *F. guestfalica* was known to need more time to germinate than the other species, it was sown 10 days earlier (13 July 2018) than the other six species (23 July 2018). The seeds were sown separately for each species into trays (l × w × h: 13.4 cm × 12.2 cm × 4.9 cm) filled with commercial seedling soil (Einheitserde P, Patzer Sinntal-Jossa). The trays were placed in a growth chamber with a daily light period of 16 hr, a temperature of 23°C during the day and 18°C during the night, and 90% humidity.

On 7 August 2018, the seedlings were transplanted into pots (l × w × h: 9 cm × 9 cm × 8 cm) filled with a base substrate consisting of a 1:1 mixture of sand and vermiculite. As plastic-pollution treatment, we mixed into the substrate of half of the pots EPDM infill granules of the same type as the one used in the experiment on testing the effects of different concentrations of EPDM granules. As we were interested in how infill granules could affect growth and competition between plants when the granules spread into the vegetation compared to when not, we used pots with substrate that did not contain any granules as a control treatment. We chose a concentration of 5% (corresponding to c. 22,000 particles per kg of substrate), which is not an unrealistic concentration in close vicinity to artificial sport turfs (Figure 1). This concentration is still lower than the >40,000 microplastic particles/kg found in some arable soils in southwestern China (Zhang & Liu, 2018). The temperature of the greenhouse was set to vary between 25°C during the day and 18°C during the night. No artificial lighting was used.

We grew each of the seven species as target plants without competition, and in competition with a plant of the same species (intraspecific competition) or a plant of one of the other six species (interspecific competition). So, each species was grown in competition with itself and with each of the other species according to a complete diallel design, and also without competition (Figure S1). Each cell of the diallel-by-plastic-pollution treatment combination was replicated three times, resulting in a total of 7 target species × 8 competition treatments (7 competitors + 1 without competitor) × 2 plastic-pollution treatments × 3 replicates = 336 pots, which were randomly allocated to positions in the greenhouse. Five plants that had died during the first 2 weeks were replaced. The plants were watered every day until saturation, and each pot received 50 ml of a 1‰ Universol® Blue solution once a week to avoid nutrient limitation.

One week after planting the seedlings (on 14 and 15 August 2018), we counted the number of leaves, and measured the length of the longest leaf, as initial size measures. Two months after the start of the experiment (8 October 2018), the plants were harvested. We harvested both the target and the competitor plant of each pot, but in the analyses we only used the target-plant data to make sure that the data are non-independent. For all plants, we harvested the aboveground biomass. For the competition-free plants, we also harvested the roots by gently washing them free from substrate. We also tried to harvest the roots of the plants grown in the competition. However, the roots of plants in pots with the grasses *A. pratensis* or *L. perenne* were too intertwined,

and we therefore could not harvest the roots of the plants in those pots. To determine the length and the average diameter of the roots, a random subsample of the roots of each plant in the competition-free treatment was digitized with a flatbed scanner modified for root scanning (Epson Expression 10000 XL; Regent Instruments) and WinRhizo software (2017; Regent Instruments Inc.). For each plant, we separately dried the aboveground biomass, the root sample that had been scanned and the remaining roots in a drying oven at 70°C for at least 3 days. We used these data to calculate the total root length per plant and the specific root length (only for plants in the competition-free treatment), and the total biomass and the root weight ratio (for the subset of plants for which we could harvest the roots; that is, excluding the competition pots with *A. pratensis* or *L. perenne*).

The continuous variables measured in plants with and without competition, aboveground biomass, total biomass and root weight ratio, were analysed in linear mixed models (LMMs) with plastic and competition treatments as fixed factors, using the *lme* function of the 'nlme' package (Pinheiro, Bates, DebRov, & Sarkar, 2018) in R (version 3.5.1; R Core Team, 2018). As the competition treatment had three levels (no, intra- and interspecific competition), we split it into two factors, competitor presence (presence vs. absence of competition) and competition type (intra- vs. interspecific competition). We also included the two-way interactions of competitor presence and competition type with the plastic treatment. To account for differences in the initial size of the plants, we included the length of

the longest leaf and the number of leaves as centered covariates. Furthermore, to account for non-independence of plants of the same target species and for plants with the same competitor species, we included target species and competitor species as random factors. As transformations did not improve homoskedasticity of the residuals, we also included variance structures to model different variances per species and per competition-treatment level using the *varIdent* function.

The survival of plants was analysed in a similar way, but with a binomial generalized linear mixed model (GLMM) instead of an LMM. However, because the complete model did not converge, probably due to extreme low mortality of plants in the treatment without plastic, we simplified the model by removing the competition factors. Specific root length, which was only measured in plants in the competition-free treatment, was, like the other continuous variables, also analysed with an LMM, but without the factors competitor presence and competition type. To improve normality and homoskedasticity of the residuals, specific root length was square-root transformed prior to analysis, and we included variance structures to model different variances per species and per plastic treatment. In all (G)LMMs, to assess the significance of the fixed model terms, we used log-likelihood ratio tests comparing the models with and without the terms of interest (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). The associated data files and R syntax are included as supporting information (Dataset S2, Code S2).
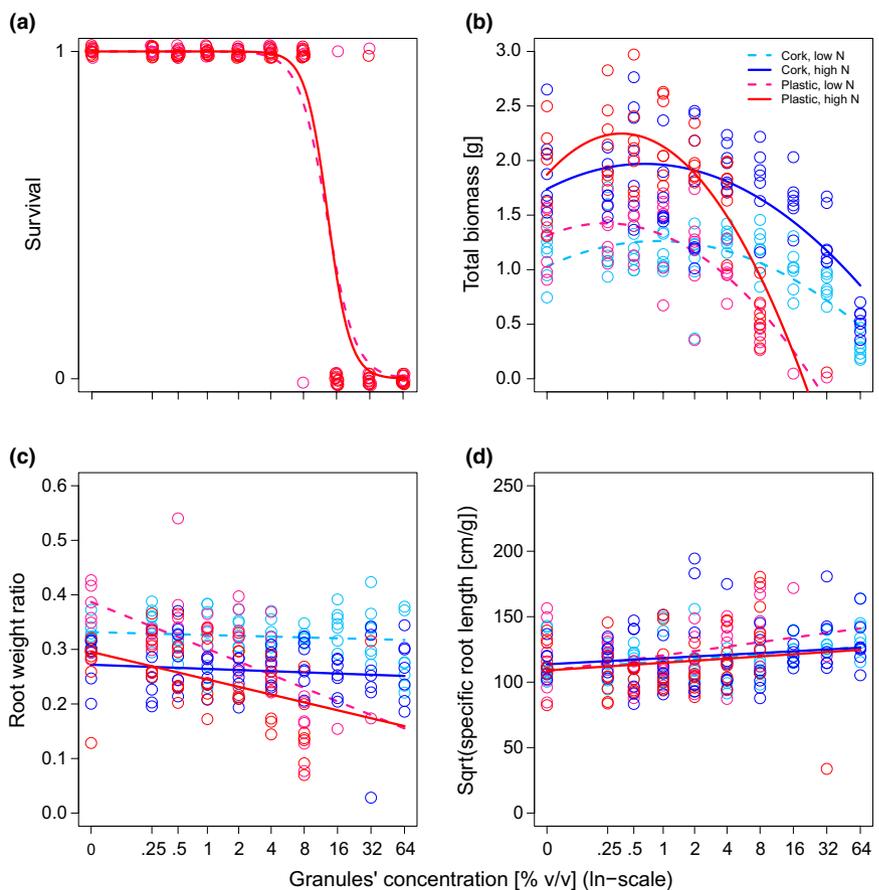

FIGURE 2 Relationships of (a) survival; (b) total biomass; (c) root weight ratio; and (d) specific root length of plants of *Plantago lanceolata* grown at different concentrations of the plastic ethylene propylene diene monomer (EPDM) and cork granules, and under low and high nutrient (N) conditions. Each circle represents a single data point (plant), and the fitted lines are from (generalized) linear models. Because in the presence of cork granules all plants survived, only survival in the presence of plastic granules is shown

3 | RESULTS

3.1 | Experiment testing the effects of different concentrations of EPDM granules

In the treatment with cork granules, all *P. lanceolata* plants survived. However, among the plants in the treatment with plastic granules, survival was reduced in all concentrations above 8% (granules-concentration effect: χ^2 = 128.43, df = 1, p < .001), irrespective of the fertilizer treatment (Figure 2a; Table S1). The estimated LC10, LC20 and LC50 values were 6.8%, 8.7% and 13.1%, respectively, in the low nutrient treatment, and 8.0%, 9.6% and 13.3%, respectively, in the high nutrient treatment.

The total biomass of the surviving *P. lanceolata* plants was strongly affected by the natural logarithm of the granules' concentration in a non-monotonic way (Figure 2b; Table 1). At low non-zero concentrations, plants tended to produce slightly more biomass than in the absence of granules, but biomass rapidly decreased at concentrations above c. 2% (Figure 2b). While the overall effect of EPDM granules was marginally significantly negative (p = .080, Table 1), the negative effect of the granules' concentration was much stronger for the EPDM than for the cork granules (significant GM × GC and GM × GC^2 interactions in Table 1; Figure 2b). Fertilizers increased biomass production and steepened the negative effect of the granules' concentrations (significant F × GC interaction in Table 1),

particularly so for the EPDM granules (significant F × GM × GC^2 interaction in Table 1; Figure 2b).

The root weight ratio and specific root length of *P. lanceolata* decreased and increased, respectively, with the natural logarithm of the granules' concentration in a more or less linear way (Figure 2c,d; Table 1). The specific root length was not significantly affected by the granule material and the fertilizer (Figure 2d, Table 1). However, the root weight ratio was on average lower in the presence of the EPDM granules than in the presence of the cork granules, and this was particularly the case at higher concentrations (significant GM main effect and GM × GC interaction in Table 1; Figure 2c). The fertilizer overall decreased the root weight ratio, irrespective of the material and concentration of the granules (Figure 2c; Table 1).

3.2 | Experiment testing the effects of EPDM granules on competition

In the competition experiment, almost all 168 target plants in the treatment without granules survived, with the exception of one plant of *Festuca guestfalicia*. However, in the treatment with the tested granules, six plants of *Leucanthemum ircutianum* and eight plants of *Prunella vulgaris* died. The GLMM including the factors competition and granule treatment did not converge, but when we excluded the factor competition from the model, it converged and the effect of the tested granules was significant (χ^2 = 13.99, df = 1, p < .001).

TABLE 1 Results of linear models testing the significance of the effects of initial plant size measures, fertilizer treatment, granule material, the concentration of granules and their interactions on total biomass, root weight ratio and specific root length of plants of *Plantago lanceolata*

Model terms	df	Total biomass			Root weight ratio			Specific root length		
		SS	F	p	SS	F	p	SS	F	p
Initial cotyledon length	1	4.49	13.20	**<.001**	0.0006	0.12	.726	1,051.5	2.26	.134
Initial no. leaves	1	0.19	0.56	.455	0.0025	0.54	.463	12.6	0.03	.869
Fertilizer (F)	1	22.88	159.19	**<.001**	0.2126	61.86	**<.001**	121.9	0.27	.602
Granule material (GM)	1	0.44	3.10	.080	0.0512	14.89	**<.001**	51.3	0.12	.735
Granules' concentration (GC)	1	17.30	102.46	**<.001**	0.0773	22.51	**<.001**	5,693.8	12.76	**<.001**
GC^2	1	6.02	41.98	**<.001**	—	—	—	—	—	—
F × GM	1	0.06	0.60	.440	0.0017	0.56	.457	1,007.9	2.26	.134
F × GC	1	0.81	7.78	**.006**	0.0023	0.77	.382	309.6	0.69	.406
F × GC^2	1	0.30	2.88	.091	—	—	—	—	—	—
GM × GC	1	6.94	62.37	**<.001**	0.1027	33.98	**<.001**	312.4	0.70	.403
GM × GC^2	1	1.99	19.34	**<.001**	—	—	—	—	—	—
F × GM × GC	1	0.26	2.51	.114	0.0096	3.20	.075	136.0	0.30	.582
F × GM × GC^2	1	0.53	5.29	**.022**	—	—	—	—	—	—
Residual	226[a]	22.71			0.6886			102,833.0		

Note: p < .05 are marked in bold.
[a]The residual df for root weight ratio and specific root length was 230 instead of 226.

Among the surviving target plants, the presence of the tested granules in the substrate strongly reduced the aboveground biomass (Table 2, Figure 3). This was true for all seven study species (Figure S2). The presence of a competitor also reduced the aboveground biomass of the target plants, and this reduction was smaller in the presence of the tested granules than in its absence (significant G × CP interaction in Table 2, Figure 3). Whether the competitor was of the same species or a different species did not significantly matter for biomass of the target plants. For the subset of species for which we could also harvest root biomass in the presence of competition, the results for total biomass were very similar to those for the aboveground biomass (Table S2, Figure S3).

In the absence of competition, the presence of the tested granules in the substrate reduced the root weight ratio by on average

TABLE 2 Results of a linear mixed model testing the significance of the effects of initial plant size measures, granule treatment, competitor presence and competitor type and their interactions on the aboveground biomass of plants of the seven grassland species in the competition experiment

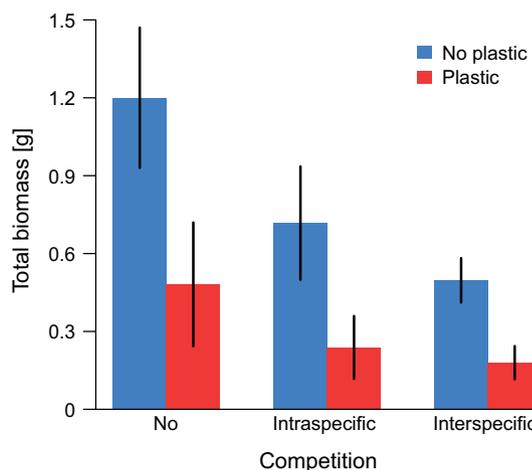| Fixed model terms	df	χ^2	p
Initial no. leaves	1	12.70	**<.001**
Initial length of longest leaf	1	0.98	.323
Granule treatment (G)	1	130.54	**<.001**
Competitor presence (CP)	1	8.33	**.004**
Competition type (CT)	1	0.052	.819
G × CP	1	7.39	**.007**
G × CT	1	1.09	.297
Random model terms			**SD**
Target species			0.1891
Competitor species			0.0303
Residual			0.2163

Note: p < .05 are marked in bold.

FIGURE 3 Mean (±*SE*) of the aboveground biomass averaged across the seven grassland species grown in the presence and absence of plastic (EPDM) granules without competition, with intraspecific competition and with interspecific competition

32.7% (χ^2 = 9.94, df = 1, p = .002), and increased the specific root length of the plants by on average 13.2% (χ^2 = 5.12, df = 1, p = .024). For the subset of species for which we could also harvest belowground biomass in the presence of competition, the negative effect of the tested granules on root weight ratio remained significant, and was not significantly affected by the competition treatment (Table S2, Figure S3).

4 | DISCUSSION

Here we show in two experiments that the addition of EPDM microplastic to the substrate can negatively affect the growth and survival of common grassland species, at least if applied at concentrations of 5% or higher. These negative effects were found both under low and high nutrient conditions, and for all tested species. Due to the strong negative effects on plant growth, the plastic granules reduced the competitive interactions. Previous studies have shown already that plastic particles might negatively affect soil animals that have ingested the particles (Huerta Lwanga et al., 2017) or are otherwise exposed to them (Zhu et al., 2018). Our study shows that the continued accumulation of plastic particles not only threatens animals in aquatic ecosystems, but that in terrestrial ecosystems, it may also threaten the plants that encounter these particles with their roots.

Few other studies have tested the effects of microplastics on plant growth, and the ones that do focus on agricultural crops. Plastic pollution is a relevant topic in agroecosystems, because microplastics can be present as contamination in sewage sludge and compost applied to agricultural land, and may additionally result from plastic mulching (Nizzetto, Langaas, & Futter, 2016; Rillig, Ingraffia, & Souza Machado, 2017; Steinmetz et al., 2016). Atuanya, Aborisade, and Nwogu (2012) found that a 2.50% w/w treatment of polyethylene granules reduced the height of maize plants by 22%. Similarly, Qi et al. (2018) found that biomass of wheat was reduced by a 1% w/w treatment of different plastics. They also found that the magnitude of the reduction was stronger when microplastics were used instead of macroplastics, and that the effect was stronger for biodegradable plastics than for polyethylene. Recently, de Souza Machado et al. (2019) found that some microplastics increased root biomass and morphology of spring onions (*Alium fistulosum*). They proposed that the effects of microplastics on the roots could be caused by changes in soil structure and composition, which have consequences for pore connectivity and water evaporation, as well as by changes in microbial activity (de Souza Machado et al., 2019). However, more research is needed to better understand the mechanisms by which different microplastics can affect plant growth.

Interestingly, the effect of the EPDM granules on biomass production of *P. lanceolata* was non-monotonically dependent on the granules' concentration. Recently, Souza Machado, Lau, et al. (2018) found also non-monotonic effects of microplastics on several proxies of the soil biophysical environment. In that study, however, effects of low concentrations were stronger than effects of higher concentrations. In our study, at low concentrations, the effect of the

EPDM granules on biomass production was actually slightly positive. The same, however, was also the case when we used cork instead of EPDM granules. Possibly, the addition of EPDM and cork granules, which were overall larger than most sand and vermiculite particles, improved the drainage or aeration of the soil. At higher concentrations, however, the effect of the EPDM granules became strongly negative and at concentrations above 8% v/v almost all plants died. At high concentrations of the cork granulate, the plants also produced less biomass than at low concentrations, but these effects were far less negative than those of the EPDM granules, and none of the plants with cork granules died. As the size of the cork and EPDM granules were very similar, our results strongly indicate that the detrimental effect is due to the material of the granules and not due to the presence of granules per se.

The concentrations at which we found negative effects of the EPDM granules on plant growth were relatively high, and such high concentrations are still rare in nature. Nevertheless, high concentrations of granules can be found in vegetation close to artificial sport turfs, where the granules are used as infill material (Figure 1). Moreover, of the few studies that have quantified concentrations of microplastics in soils, some found very high concentrations. For example, Fuller and Gautam (2016) reported that top soils in industrial areas around Sydney (Australia) contained up to 6.9% w/w of microplastics. We used volumetric concentrations, but as the plastic granules had a 2.0 times lower density than the sand-vermiculite mixture, the 5% v/v concentration used in the competition experiment corresponds to a 2.5% w/w concentration. Therefore, although we used relatively high concentrations, these concentrations are not unrealistic.

One potential reason for the negative effect of the EPDM granules on plant growth could be that they reduce the nutrients available to the plants. On the one hand, the granules may dilute the other substrate and thereby dilute the nutrients. However, because the sand-vermiculite mixture that we used is very nutrient poor, and all pots received the same amount of fertilizer solution, this is unlikely to be an explanation. On the other hand, the EPDM granules might bind the soil nutrients and thereby make them unavailable to the plants. If that is the case, we would have expected that in the experiment with *P. lanceolata* the negative effect of the EPDM particles would have been less severe in the high fertilizer treatment. As clear signs of nutrient limitation were not observed, we think that the EPDM granules do not decrease the nutrient availability. Moreover, while nutrient limitation resulted in an increase in the root weight ratio of *P. lanceolata*, which is in line with optimal allocation theory (Bloom, Chapin, & Mooney, 1985), the EPDM granules resulted in a decrease of the root weight ratio. Another possible explanation for the negative effects of the EPDM granules is that they have hydrophobic surfaces. Even though we regularly watered the plants to saturation, the substrate might have dried out more quickly when there were large amounts of EPDM particles in it, causing drought stress to the plants. Finally, although EPDM is supposedly less toxic than other plastics (KIMO, 2017), it could be that some of the additives (e.g., process, light and thermal stabilizers, and

colorants) used in the production of the granules leached into the soil and are toxic to the plants. Clearly more research is needed to identify the exact causes of the negative effects of the tested microplastic on plant growth.

The negative effect of the EPDM granules was stronger for the roots than for the aboveground parts of the plants, as shown by the reduced root weight ratio of plants in the plastic treatments of both experiments. This is likely a result of the roots being in direct contact with the granules, whereas the aboveground parts are not. The inhibitive effect of the EPDM granules on root growth was also reflected in a significant reduction of the total root length in both experiments (Figures S2 and S4, Table S3). The effects of the EPDM granules on root thickness, however, were much smaller. In the *P. lanceolata* experiment, the specific root length was not significantly affected by the EPDM granules, and although the average root diameter increased, this appears to be mainly due to an outlying data point at high EPDM concentrations (Figure S4, Table S3). In the competition experiment, the specific root length was slightly decreased by the granule treatment, and the average root diameter was slightly increased (Figure S5). Overall, this suggests that the EPDM treatment resulted in slightly thicker roots on average. Possibly, this reflects that particularly the development of fine roots was negatively affected by the granules. As the main functions of the fine roots are the uptake of water and nutrients, their impairment can explain the overall reduced growth of plants in plastic treatments.

Competition, as expected, resulted in a reduced growth of the plants. The effect of competition, however, was weaker in the presence than in the absence of the tested granules. Probably, this reflects that the granules reduced the growth of both plants in a pot, and that due to their small sizes they interacted less intensively. Nevertheless, if the two plants are differently affected by the granulate, the competitive balance between them might change. To address this, we calculated the general and specific species combining abilities, according to Wüest and Niklaus (2018), for the treatments with and without plastic granules. The grasses *L. perenne* and *A. pratensis* had the highest general combining abilities (i.e., averaged aboveground biomass in presence of competition), both in the presence and absence of the granules. However, while the forb *P. vulgaris* had the worst general combining ability in the absence of the granules, it had the third-best combining ability in the presence of the granules. Consequently, the rank correlation between the general combining abilities of a species in the absence and presence of the EPDM granules was statistically not significant (Figure S6; Spearman rho = 0.536, *p* = .236). Although non-significance might partly reflect low statistical power (*n* = 7), it also indicates that the species differed in the degree to which they were affected by the granules across the different competitor combinations.

Similarly, the specific combining ability of each pair of species (i.e., the deviation of the total aboveground biomass of each pair from expectation based on the sum of GCAs of the two species) showed no consistent pattern between the treatments with and without granules (Figure S7, Mantel *r* = .170, *p* = .243,

permutations = 999). In the absence of the EPDM granules, the highest and the lowest specific combination abilities were found for the species pairs *D. carota – P. vulgaris* and *G. album – P. vulgaris*, respectively, whereas in the presence of the granules, the highest and lowest values were found for *A. pratensis – L. ircutianum* and *L. perenne – L. perenne*, respectively (Figure S7). Together these results suggest that the tested infill granules may change the competitive interactions between species, and thus could change the composition of plant communities.

The impacts of plastic pollution in marine and freshwater ecosystems are now widely recognized (e.g., Cole, Lindeque, Halsband, & Galloway, 2011). However, little is known about potential impacts in terrestrial ecosystems, particularly with regard to effects on plants (Rillig et al., 2019). We show here that, while low concentrations do not necessarily slow down growth, volumetric concentrations of 5% or higher may have strong detrimental effects on the survival and growth of wild plant species, in the case of the EPDM infill granules that were used in this study. Nevertheless, given the paucity of data, it is still too early to draw general conclusions about the effects of microplastics on plant growth. Clearly more studies on the effects of different plastic types and concentrations are needed, as well as long-term studies in natural communities that also include other soil organisms that might be affected by plastic pollution.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTION

MvK designed the experiments, AB, LG and ZZ did the experiments, MvK and ZZ did the analyses, and MvK led the manuscript writing with inputs from all other co-authors.

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REFERENCES

Alexander, J. M., van Kleunen, M., Ghezzi, R., & Edwards, P. J. (2012). Different genetic clines in response to temperature across the native and introduced ranges of a global plant invader. *Journal of Ecology*, *100*(3), 771–781. https://doi.org/10.1111/j.1365-2745.2011.01951.x

Andrady, A. L. (2017). The plastic in microplastics: A review. *Marine Pollution Bulletin*, *119*(1), 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082

Atuanya, E. I., Aborisade, W. T., & Nwogu, N. A. (2012). Impact of plastic enriched composting on soil structure, fertility and growth of maize plants. *European*. *Journal of Applied Sciences*, *4*, 105. 109.10.5829/idosi.ejas.2012.4.3.270

Barnes, D. K. A., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *364*(1526), 1985–1998. https://doi.org/10.1098/rstb.2008.0205

Bloom, A. J., Chapin, F. S., & Mooney, H. A. (1985). Resource limitation in plants - an economic analogy. *Annual Review of Ecology and Systematics*, *16*, 363–392. https://doi.org/10.1146/annurev.es.16.110185.002051

Bocca, B., Forte, G., Petrucci, F., Costantini, S., & Izzo, P. (2009). Metals contained and leached from rubber granulates used in synthetic turf areas. *Science of the Total Environment*, *407*(7), 2183–2190. https://doi.org/10.1016/j.scitotenv.2008.12.026

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, *62*(12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Core Team, R. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

de Souza Machado, A. A., Kloas, W., Zarfl, C., Hempel, S., & Rillig, M. C. (2018). Microplastics as an emerging threat to terrestrial ecosystems. *Global Change Biology*, *24*(4), 1405–1416. https://doi.org/10.1111/gcb.14020

de Souza Machado, A. A., Lau, C. W., Kloas, W., Bergmann, J., Bacheher, J. B., Faltin, E., … Rillig, M. C. (2019). Microplastics can change soil properties and affect plant performance. *Environmental Science & Technology*, *53*(10), 6044–6052. https://doi.org/10.1021/acs.est.9b01339

de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M. C. (2018). Impacts of microplastics on the soil biophysical environment. *Environmental Science & Technology*, *52*(17), 9656–9665. https://doi.org/10.1021/acs.est.8b02212

Derraik, J. G. B. (2002). The pollution of the marine environment by plastic debris: A review. *Marine Pollution Bulletin*, *44*(9), 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., & Laforsch, C. (2015). Beyond the ocean: Contamination of freshwater ecosystems with (micro-)plastic particles. *Environmental Chemistry*, *12*(5), 539–550. https://doi.org/10.1071/EN14172

Eerkes-Medrano, D., Thompson, R. C., & Aldridge, D. C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Research*, *75*, 63–82. https://doi.org/10.1016/j.watres.2015.02.012

Fuller, S., & Gautam, A. (2016). A procedure for measuring microplastics using pressurized fluid extraction. *Environmental Science & Technology*, *50*(11), 5774–5780. https://doi.org/10.1021/acs.est.6b00816

Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, *3*(7), 5. https://doi.org/10.1126/sciadv.1700782

Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, *586*, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salanki, T., van der Ploeg, M., … Geissen, V. (2017). Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. *Environmental Pollution*, *220*, 523–531. https://doi.org/10.1016/j.envpol.2016.09.096

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., … Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.1260352

Jamieson, A. J., Brooks, L. S. R., Reid, W. D. K., Piertney, S. B., Narayanaswamy, B. E., & Linley, T. D. (2019). Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. *Royal Society Open Science*, *6*(2), 180667. https://doi.org/10.1098/rsos.180667

KIMO. (2017). Microplastic pollution from artificial grass – A field guide. Retrieved from http://www.kimointernational.org/de/feature/microplastic-pollution-from-artificial-grass-a-field-guide/

Lassen, C., Hansen, S. F., Magnusson, K., Norén, F., Bloch Hartmann, N. I., Rehne Jensen, P., ... Brinch, A. (2015). *Microplastics. Occurrence, effects and sources of releases to the environment in Denmark.* Copenhagen, Denmark: Danish Environmental Protection Agency.

Lewis, S. L., & Maslin, M. A. (2015). Defining the Anthropocene. *Nature*, *519*(7542), 171–180. https://doi.org/10.1038/nature14258

Maass, S., Daphi, D., Lehmann, A., & Rillig, M. C. (2017). Transport of microplastics by two collembolan species. *Environmental Pollution*, *225*, 456–459. https://doi.org/10.1016/j.envpol.2017.03.009

Nizzetto, L., Langaas, S., & Futter, M. (2016). Do microplastics spill on to farm soils? *Nature*, *537*, 488.

Pinheiro, J., Bates, D., DebRov, S., Sarkar, D., & R Core Team. (2018). nlme: Linear and nonlinear mixed effects models. R package version 3.1-137. Retrieved from https://CRAN.R-project.org/package=nlme

Qi, Y. L., Yang, X. M., Pelaez, A. M., Lwanga, E. H., Beriot, N., Gertsen, H., ... Geissen, V. (2018). Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. *Science of the Total Environment*, *645*, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229

Rillig, M. C. (2012). Microplastic in terrestrial ecosystems and the soil? *Environmental Science & Technology*, *46*, 6453–6454. https://doi.org/10.1021/es302011r

Rillig, M. C., Ingraffia, R., & de Souza Machado, A. A. (2017). Microplastic incorporation into soil in agroecosystems. *Frontiers in Plant Science*, *8*, 1805. https://doi.org/10.3389/fpls.2017.01805

Rillig, M. C., Lehmann, A., de Souza Machado, A. A., & Yang, G. (2019). Microplastic effects on plants. *New Phytologist*, *223*(3), 1066–1070. https://doi.org/10.1111/nph.15794

Rillig, M. C., Ziersch, L., & Hempel, S. (2017). Microplastic transport in soil by earthworms. *Scientific Reports*, *7*, 6. https://doi.org/10.1038/s41598-017-01594-7

Rochman, C. M. (2018). Microplastics research – From sink to source. *Science*, *360*, 28–29. https://doi.org/10.1126/science.aar7734

Sagar, G. R., & Harper, J. L. (1964). *Plantago major* L., *P. media* L. and *P. lanceolata* L. *Journal of Ecology*, *52*(1), 189–221. https://doi.org/10.2307/2257792

Scheurer, M., & Bigalke, M. (2018). Microplastics in Swiss floodplain soils. *Environmental Science & Technology*, *52*(6), 3591–3598. https://doi.org/10.1021/acs.est.7b06003

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Troeger, J., ... Schaumann, G. E. (2016). Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Science of the Total Environment*, *550*, 690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Vegter, A. C., Barletta, M., Beck, C., Borrero, J., Burton, H., Campbell, M. L., ... Hamann, M. (2014). Global research priorities to mitigate plastic pollution impacts on marine wildlife. *Endangered Species Research*, *25*(3), 225–247. https://doi.org/10.3354/esr00623

Verschoor, A. J. (2015). *Towards a definition of microplastics. Considerations for the specification of physico-chemical properties.* RIVM Letter report 2015–2016. Bilthoven, The Netherlands: National Institute for Public Health.

Vijaya, C., & Reddy, R. M. (2008). Impact of soil composting using municipal solid waste on biodegradation of plastics. *Indian Journal of Biotechnology*, *7*(2), 235–239.

Walpole, S. C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., & Roberts, I. (2012). The weight of nations: An estimation of adult human biomass. *BMC Public Health*, *12*(1), 439. https://doi.org/10.1186/1471-2458-12-439

Waters, C. N., Zalasiewicz, J., Summerhayes, C., Barnosky, A. D., Poirier, C., Ga uszka, A., ... Wolfe, A. P. (2016). The Anthropocene is functionally and stratigraphically distinct from the Holocene. *Science*, *351*(6269), aad2622. https://doi.org/10.1126/science.aad2622

Windsor, F. M., Durance, I., Horton, A. A., Thompson, R. C., Tyler, C. R., & Ormerod, S. J. (2019). A catchment-scale perspective of plastic pollution. *Global Change Biology*, *25*(4), 1207–1221. https://doi.org/10.1111/gcb.14572

Wüest, S. E., & Niklaus, P. A. (2018). A plant biodiversity effect resolved to a single chromosomal region. *Nature Ecology & Evolution*, *2*(12), 1933–1939. https://doi.org/10.1038/s41559-018-0708-y

Zhang, G. S., & Liu, Y. F. (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. *Science of the Total Environment*, *642*, 12–20. https://doi.org/10.1016/j.scitotenv.2018.06.004

Zhu, D., Chen, Q. L., An, X. L., Yang, X. R., Christie, P., Ke, X., ... Zhu, Y. G. (2018). Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. *Soil Biology & Biochemistry*, *116*, 302–310. https://doi.org/10.1016/j.soilbio.2017.10.027

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R.* New York, NY: Springer.

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