Effects of Microplastic Fibers and Drought on Plant Communities

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ABSTRACT: Microplastics in soils can affect plant performance, as shown in studies using individual plants. However, we currently have no information about potential effects on plant community productivity and structure. In a plant community consisting of seven plant species that co-occur in temperate grassland ecosystems, we thus investigated the effect of microplastics (i.e., microfibers) and drought, a factor with which microfibers might interact, on plant productivity and community structure. Our results showed that at the community level, shoot and root mass decreased with drought but increased with microfibers, an effect likely linked to reduced soil bulk density, improved aeration, and better penetration of roots in the soil. Additionally, we observed that microfibers affected plant community structure. Species such as Calamagrostis, invasive in Europe, and the allelopathic Hieracium, became more dominant with microfibers, while species that potentially have the ability to facilitate the establishment of other plant species (e.g., Holcus), decreased in biomass. As microfibers affect plant species dominance, the examination of cascade effects on ecosystem functions should be a high priority for future research.

INTRODUCTION

Microplastics, a diverse group of polymer-based particles (<5 mm), are becoming recognized as an important new global change factor potentially influencing terrestrial ecosystems. Between 1950 and 2015, global plastic waste is estimated to have been 6300 million tonnes. The abrasion of large plastic objects during manufacture or in the environment, the erosion of tires when driving, or the abrasion of synthetic textiles during washing are just some of the sources of large amounts of microplastics that will end up not only in the oceans but also in terrestrial systems.

Microplastics can enter soils via soil amendments, plastic mulching, irrigation, diffuse urban runoff, flooding, and atmospheric fallout. As consequence, microplastics in soil may appear in the form of fibers, films, granules, presenting a variety of shape, composition, and abundance in concentrations that may reach up to 7% close to industrial areas. Therefore, microplastic effects on terrestrial systems depend on the microplastic type and the plant-soil system involved. For instance, agriculture lands would be highly affected by microplastic films as plastic mulching is intensively used to promote plant productivity, while rivers or roadsides would tend to be more affected by microplastic fragments or microfibers. Likewise, microplastic effects in terrestrial systems may be influenced by the soil type (e.g., sandy vs loamy soil) and by the plant species, as depending on their identity, they respond differently to environmental stressors.

The effects of microplastics on plant performance have been observed in some species, such as crops or plants growing as single individuals or in a population but not in a community context. For example, Triticum aestivum (wheat) exposed to films or Lolium perenne (grass) exposed to fibers had shown reduced biomass while Allium fistulosum (crop) exposed to fibers had shown an opposite effect. Likewise, Plantago lanceolata (forb) and Allium fistulosum had contrasting responses to microplastics in relation to root morphological traits (e.g., root length). This variety of individual plant responses suggests that different plant species in a community could be affected to a different degree by the addition of microplastics in the soil, which could potentially affect plant productivity and community structure. Therefore, in a community, some species may be better able to take advantage of the changes in soil properties due to the presence of microplastics.

Depending on the microplastic effects on different plant species in any given community (e.g., invasive or facilitative species), different ecosystems functions could be affected. Dry grasslands species show different responses to drought through the adjustment of their root morphological traits; therefore, microfibers could affect root traits as well, as a consequence of their effects on soil-water dynamics, which at the end, would modify resource availability affecting plant competition. Previous research provides evidence that grasses and herbs may have contrasting strategies to face drought. For example, Festuca and Holcus respond with...
increased specific root length (i.e., root finesses), likely promoting mycotrophy for resource acquisition, while herbs such as Achillea or Potentilla respond with decreased specific root length but instead increase root biomass, likely as a strategy to promote water acquisition.\textsuperscript{17,20} Thus, we could expect that at the community level, root morphological traits may be affected by drought, an effect likely intensified by microfibers in the soil. Additionally, we could expect a better performance of fast growing species (e.g., most grasses), as they could rapidly access the limited water.\textsuperscript{21} Several models suggest that grasses and herbs could have complementary resource use under stressful conditions,\textsuperscript{22,23} which suggest that microplastics exacerbating the effects of drought may influence species abundance.

Among microplastics, microfibers are considered one of the most predominant microplastic types in the soil.\textsuperscript{7,8} The linear shape, size, and flexibility of such particles could reduce soil bulk density and soil aggregation,\textsuperscript{14} although they can also entangle soil particles and thus contribute to the soil aggregate formation process\textsuperscript{16} so that one of the most critical effects of microplastic fibers is on soil structure and soil-water dynamics. Microplastic fibers can enhance water holding capacity and keep water saturation for longer periods,\textsuperscript{14} but it is possible that they increase soil water evaporation as additional channels for water movement may be created.\textsuperscript{24} Changes in soil-water dynamics could alleviate or exacerbate drought, a phenomenon which is predicted to increase over the next few decades\textsuperscript{25} in many regions worldwide, with the consequences on plant communities structure and ecosystem functionality remaining unknown.

In this study, we established a community of grasses and herbs including plants with invasive, allelopathic, or facilitative characteristics, which naturally co-occur in temperate dry grasslands in northeastern Germany. We assessed the effect of microfibers in the soil and drought on plant productivity and structure. We hypothesized that microfibers and drought would strongly alter not only productivity but also community composition and that microfibers may exacerbate the effects of drought on plant communities.

\textbf{Materials and Methods}

\textbf{Species Selection.} We selected seven plant species, including grasses such as Festuca brevirepila, Holcus lanatus, and Calamagrostis epigejos and herbs such as Achillea millefolium, Hieracium pilosella, Plantago lanceolata, and Potentilla argentea to shape community modules, typical of temperate grasslands ecosystems. Seeds of these plant species were obtained from a commercial supplier in the region (Riegler-Hofmann GmbH, Blaufelden, Germany). We will refer to plant species by their generic names from now on. All are common, frequently co-occurring grassland species in Central Europe that naturally grow in the same patch as seen in field observations in dry grasslands in the Brandenburg region (Germany). As microplastic fibers could alleviate or exacerbate drought, we selected plant species that exhibit contrasting responses to drought conditions. Growing as single species, Festuca and Holcus have decreased root biomass with drought, while Achillea, Plantago, or Hieracium show the opposite trend. Other traits, such as root diameter or root finesses (SRL), also differ among the selected species as drought increases.\textsuperscript{17}

\textbf{Microplastic Fibers.} Polyester fibers (Rope Paraloc Mamutec polyester white, item number, 8442172, Hornbach.de) were manually cut with scissors, and a length of 5.0 mm was established as an upper size threshold in order to generate microplastic fibers. They had a length of 1.28 ± 0.03 mm and a diameter of ∼30 µm. Size distribution is given in Figure S1, and mechanical properties of polyester fibers are listed in Table S1.

\textbf{Soil Preparation.} We collected sandy loam soil (0.07% N, 0.77% C, pH 6.66) from Dedelow, Brandenburg, Germany (53° 37' N, 13° 77' W) where our plant species naturally grow. The soil was dry when collected, and then it was sieved (4 mm mesh size), homogenized, and mixed with microplastic fibers at a concentration of 0.4%. A 12 g portion of microfibers (∼763333 fibers g\textsuperscript{-2} microplastic) was mixed into 3 kg of soil for each pot. Soil preparation was done separately for each experimental unit: microfibers were separated manually and mixed with the soil for 30 min in a large container, before being placed into each individual pot, to help provide an equal distribution of microfibers throughout the soil (optical inspection) and the correct microfiber concentration. This was done in order to reflect the fact that after accumulation at the top of the soil, microplastics can move along soil profile due to soil biota activity, properties of the soil particles, and plant processes such as root growth.\textsuperscript{26,27} Twenty experimental units (pots) were established. Half had soil with microfibers, while the other half had soil without added microfibers. Soil was mixed in all experimental units in order to provide exactly the same disturbance.

\textbf{Experimental design.} In May 2019, we established the experiment in a controlled glasshouse with a daylight period set at 12 h, 50 klx, and a temperature regime at 22/18 °C day/night with a relative humidity of ∼40%. Prior to germination, ∼200 seeds per species were surface-sterilized with 10% sodium hypochlorite for 5 min and 75% ethanol for 2 min and then thoroughly rinsed with sterile water. Then, the seeds were germinated in trays with sterile sand and individual seedlings of similar size were transplanted into pots (16 cm diameter, 16.5 cm height, 3L) 3 days after germination. Twenty-one holes were dug in a grid, keeping a distance of 2.5 cm among them (Figure S2). Seedlings were randomly distributed, and each planting hole received one plant individual, so that a community module shaped by three individuals of each of the seven plant species was established per pot. Pots were well-watered (100 mL twice a week) during the first 3 weeks of growth. Then, half of them were kept at approximately 70% of soil water holding capacity (WHC) by adding 200 mL of water, while the other half were kept at approximately 30% WHC by adding 50 mL of water. By using 30% WHC, the plant species we selected, were under stress conditions due to drought.\textsuperscript{17,18} We determined the respective amount of water for our soil type. Pots were watered by hand twice a week for two months by gently spraying distilled water on the soil surface. We thus had 20 experimental units in a fully crossed orthogonal design that includes two microfiber treatments (one with and the other without added microfibers, also called “present” and “absent”) and two drought treatments (with and without drought, also called “drought” and “well-watered”), with five replicates each (n = 5). All pots were randomly distributed in the chamber, and their positions were shifted twice to homogenize environmental conditions during the experiment. Pots were weighed weekly to verify maintenance of their respective water content. No changes in the watering amount or frequency was needed. Likewise, no water drained out from the pots, so that microfibers added to the soil did not leave the pots. The percolation behavior of the fibers is
unknown, but given their linear shape, it would be expected to be much slower compared to that of beads. At harvest, shoot mass was sorted by plant species. Roots were carefully removed from the soil and gently washed (roots could not be separated by species). Morphological traits were determined for fine roots (i.e., <2 mm in diameter): length, surface area, volume, and root average diameter were measured on a fresh sample of the plant community by scanning the roots using the WinRhizo™ scanner-based system (v.2007; Regent Instruments Inc., Quebec, Canada). Then, roots were dried at 60 °C for 72 h as some trait calculations are based on their dry weight: root tissue density (RTD; mg cm⁻³), specific root length (SRL; cm mg⁻¹), and specific root surface area (SRSA; cm² mg⁻¹). Root and shoot masses were measured after the samples were dried at 60 °C for 72 h. In additional pots with and without added microfibers, we extracted a soil core and measured its volume to obtain soil bulk density (n = 4).

Statistical Analyses. The experiment had a fully crossed orthogonal design where microfibers, drought, and their interaction were considered as fixed factors. Community root traits: root average diameter (RAD), root tissue density (RTD), specific root length (SRL), and specific root surface area (SRSA), along with community shoot and root mass, shoot mass per species, and soil bulk density were analyzed using general linear models. Shoot mass per species accounted for the shoot mass of the neighbors (the other six plant species) as a covariate. Community root:shoot ratio included shoot mass as the sum of all plant species per pot. Soil bulk density was examined using microfibers as factor. A general linear model is a regression model that assumes normality, homogeneity, and independence of the residuals. Model residuals were checked to validate these assumptions. When necessary, we implemented the varIdent() function to account for heterogeneity in the microfiber and drought treatment, respectively (see details in the tables). Principal component analysis (PCA) of the shoot mass per species was performed using the function “prcomp” and “fviz_pca” from the package “factoextra”. Ellipses in the PCA graph grouped the different treatments with a confidence level of 0.95. To analyze changes in plant community composition, in terms of biomass, we log-transformed biomass per species and used the function Manova from the package “MASS”. We tested the null hypothesis by means of the Pillai trace statistic, which accounts for the variance between groups and is robust to violation of multivariate normality and homogeneity of the variance-covariance matrix. Manova is based on the Mahalanobis distance which makes it not only appropriate but even advantageous in terms of statistical power in our case where there are no zeros in species biomass, i.e., no richness effects. Community evenness or equitability describes how

Table 1. Results from Linear Models on Plant Community and Root Morphological Traits Response to Microplastic Fibers (i.e., Microfibers, M), Drought (D), and Their Interaction (M x D)a

|                      | Plant community traits | Root morphological traits |
|----------------------|------------------------|---------------------------|
|                      | df Shoot mass Root mass Root:shoot Evenness | RAD RTD SRL SRSA |
| Microfibers (M)      | 1 4.25 (0.05) 11.20 (0.004) 7.67 (<0.01) 5.44 (0.03) | 1.88 (0.18) 0.43 (0.52) 1.80(0.19) 1.83 (0.19) |
| Drought (D)         | 1 1524.6 (<0.01) 19.52 (<0.01) 1.43 (0.24) 0.32 (0.57) | 12.7(<0.01) 14.04(<0.01) 2.49(0.13) 0.006(0.9) |
| M x D               | 1 0.22 (0.64) 0.20 (0.65) 2.00 (0.17) 1.19 (0.29) | 0.001 (0.96) 1.48 (0.24) 0.03(0.86) 0.36 (0.55) |

aCommunity evenness is based on species biomass. Root morphological traits: root diameter (RAD), root tissue density (RTD), specific root length (SRL), and specific root surface area (SRSA). F and p-values (in parentheses) are shown. All significant values (<0.05) in bold. Heteroscedasticity was corrected for shoot and root mass in the microfiber treatment, evenness in the water treatment, and root:shoot in both.
uniform in biomass each species in the community was. It was calculated using the function “diversity” from the package “vegan”\textsuperscript{33}. The larger the differences in biomass between species the less uniform the community, as some species will dominate.\textsuperscript{34} All statistical analyses were done with R version 3.5.3.\textsuperscript{35} Results shown throughout the text are mean values ±1 standard error (SE).

\section*{RESULTS}

Plant community biomass was independently affected by drought and microfibers (Table 1). Community shoot and root mass decreased from well-watered to drought conditions by ~47\% and ~53\%, respectively, but by contrast, shoot mass increased by ~6\% and root mass by ~90\% when the soil had added microfibers (Figure 1a,b). Community root:shoot ratio increased when microfibers were added to the soil and was not
range of the variation. Data points are shown as circles; 

Figure 4. Microplastic fibers and drought effects on (A) root diameter (RAD), (B) root tissue density (RTD), (C) specific root length (SRL), and (D) specific root surface area (SRSA). The box in each boxplot shows the lower, median, and upper quartile values, and the whiskers show the range of the variation. Data points are shown as circles; n = 5.

significantly affected by drought (Table 1, Figure 1c). Plant community structure, in terms of biomass, was affected by drought and microfibers (Figure 2, Table 1, Figures S2–S4). All plants survived until the end of the experiment. Drought decreased biomass for all plant species, while microfibers had a different effect depending on plant species identity and drought conditions (Figure 3). Microfibers added to the soil increased shoot mass of Calamagrostis by ~66% under well-watered conditions and of Hieracium by ~85% under drought conditions, but microfibers decreased shoot mass of Holcus by ~78% under well-watered conditions and of Festuca by 51% under drought conditions (Figure 3). Community evenness, in terms of biomass, was only affected by microfibers (Table 1), which increased when the microfibers was added to the soil. Under drought conditions (with and without microfibers), evenness was similar to that under well-watered conditions with microfibers (Figure 1d). At the community level, root traits were affected by drought but not microfibers (Table 1, Figure 4). Root diameter increased by ~24% with drought while RTD decreased by ~83% (Figure 4a,b). Although no significant changes were detected for SRL or SRSA (Table 1), both tended to decrease when microfibers were added to the soil (Figure 4c,d). SRL and SRSA decreased by ~88% and 94%, under well-watered conditions, and by 81% and 85%, under drought conditions, respectively. Soil bulk density decreased by ~12% when microfibers were added in the soil (Figure S3).

■ DISCUSSION

Plant Productivity. Drought negatively affected plant biomass (a proxy of productivity), while microfibers in soil had the opposite effect. Although the threshold of drought depends on the species identity and its environmental adaption, the reduced shoot mass due to drought is a well-known phenomenon that can be generalized across plant species, as photosynthesis is one of the key processes to be affected by water deficit. Contrary to the shoot response, the response of root biomass to drought is less predictable as it varies depending on the plant species identity (e.g., plant functional group), but at the level of plant community, we observed that root biomass was reduced by drought while it was increased by microfibers. Although some species tend to invest more biomass into longer-lasting root organs, in order to optimize water uptake over time, our pattern aligns with the idea that biomass of fine roots is often reduced with drought as a consequence of reduced transpiration and respiration rates. Additionally, it is likely that the increase in root biomass in soil with microfibers can be linked to reduced soil bulk density and an increase in soil macroporosity, which ultimately, improved aeration and facilitated a better penetration of roots in the soil matrix. Root mass increases will promote water and nutrient uptake, rhizodeposition, microbial activity, and mycorrhizal associations, which translate to an increase in shoot biomass. In terms of gauging effect sizes, plant communities under drought conditions with microfibers had similar root biomass as those under well-watered conditions without microfibers. Water availability would increase with microfibers in the soil, as fibers would keep water content higher for longer periods, as was observed in soils similar to ours. Soil water holding capacity is strongly related to soil microporosity and thus to soil bulk density. Additionally, as water holding capacity is positively correlated with soil aggregation, and microfibers may contribute to this soil property by helping to entangle soil particles, microplastic fibers would indirectly promote water holding capacity by their positive effects on soil aggregation. All these potential positive effects of microfibers on WHC would suggest an amelioration of drought conditions. However, biomass increase was only seen for root and not for shoot biomass, which suggests that microfibers did not sufficiently ameliorate the negative effects of drought at the level at which we applied drought here. Along with plant biomass, drought also modifies root morphological traits. Our results showed that drought would
but not microfibers affected root morphological traits at the community level. Root diameter increased while root tissue density decreased with drought, which can be linked with the fact that roots with these characteristics may support faster resource acquisition with a lower investment.49,50 We did not find a clear response in SRL or SRSA, traits that are typically highly responsive to drought17,18 likely due to the presence of different species that counteract the effect of drought. However, our data showed that at community level, SRL and SRSA tended to decrease, likely in order to diminish the risk of hydraulic rupture and face drought.51,52

Our results showed that in the short term, microfibers increased plant productivity and modified root morphological traits; however, we do not currently know what the long-term responses will be, as additional factors could come into play. For instance, the increase in root biomass may create channels in the soil profile, which having been influenced by the drought conditions could facilitate downward movement of the microfibers.27,53 Additionally, microplastics, depending of the degradability, hydrophobicity, electric charge, or roughness, can also serve as vectors for pathogens, as their surface may harbor potential pathogenic bacteria.54,55 Thus, when microplastics are added into the soil, pathogens would easily be translated into the soil matrix, as seemingly they do not distinguish between natural (e.g., wood) and artificial surfaces.55 After some time, the accumulation of soil pathogens due to microplastics can suppress plant growth causing negative plant–soil feedback.56 In addition, toxic substances already present or absorbed onto microplastics37,58 would negatively affect not only plant roots (growth, and thus rhizodeposition) but also soil biota composition and microbial activity, which could then secondarily affect plant growth. Likewise, the high carbon content of microplastic9 could lead to a microbial N immobilization, with consequences for plant community productivity and composition.

**Plant Community Structure.** Plant community structure, here, in terms of biomass per species in our community modules, was affected by drought and microfibers in the soil. Drought stress was such that all plant species had reduced biomass irrespective of the presence of microfibers in soil, while the effect of microfibers on plant growth was variable and depended on the water conditions and plant species. Under drought conditions and with microfibers in soil, *Festuca* had decreased growth, while *Hieracium*, a species that through allelopathy can reduce the germination and growth of neighboring species,60 had increased growth. This suggests that under drought conditions, the decrease of soil bulk density due to microfibers led to a better performance of *Hieracium*, which may in turn have had a detrimental allelopathic influence on *Festuca*.

Microfibers in the soil promoted the growth of the highly invasive species *Calamagrostis*, especially under well-watered conditions. *Calamagrostis* is a species that is widespread across the whole of temperate Eurasia. It uses the guerrilla strategy of clonal growth to spread rapidly, reducing community diversity by competitive exclusion of other plant species.61 In fact, *Calamagrostis* has invaded many high-value seminatural grasslands of Central Europe, contributing to biodiversity decline.62 Different efforts have been dedicated to counteract the growth of this species: mowing, grazing, or biological control using hemiparasitic plants.52,63 However, efforts to control this invasive species could be made more difficult if *Calamagrostis* in fact grows better with microfibers in the soil, whose strategy of clonal growth could promote its dominance in the field.

Moreover, microfibers tended to decrease biomass of *Holcus*, albeit slightly. *Holcus* is a native species in Europe with a rapid clonal growth64 that would facilitate the establishment of different plant species through the amelioration of microclimatic conditions65 as it performs well in bare soils and disturbed areas.54 Likewise, species such as *Holcus* could potentially reduce invasiveness of *Calamagrostis* by creating a more competitive environment for that species. The negative effect of microfibers on *Holcus* in our experiment could thus have contributed to the increased success of *Calamagrostis*.

Our results clearly showed contrary responses of plant species growing in a community to microfibers in the soil. Future research on this topic should include different plant species and growth forms (e.g., trees) and explore responses over more than one growing season. Additionally, future research should explore the effects of other microplastics on different plant communities to achieve a greater generalizability of the patterns observed here.

**Potential Consequences for Ecosystem Functioning.** Plant communities often respond more rapidly to human activities or altered environmental constraints in terms of evenness than those of richness.66 Our results show that for our selected species, microfibers added to the soil at 0.4% concentration, without drought, strongly affect community evenness, in terms of biomass, which could impact ecosystem functions. When synergistic interactions are important for the community, reduced evenness may have negative consequences67 but when an ecosystem function is highly dominated by few species,68 an increase in evenness could reduce ecosystem functionality. Microplastics cannot only modify community evenness, in terms of biomass, but also potentially affect community evenness in terms of species abundance or survival. Litter decomposition, which is a key process enabling the recycling of carbon and nutrients, will be altered, as litter production and quality change along with plant relative abundances.69 Soil properties, such as aggregate stability, aeration, and moisture content, which are strongly affected by microplastics,70 would influence the accessibility of soil carbon to active decomposer enzymes71 and thus litter decomposition and soil carbon storage. Additionally, in communities that are dominated by species highly resistant or resilient to disturbance, a change in relative abundance of plants could affect community resistance or resilience.36 Such effects on ecosystem functioning should be a high priority for future research. In addition, it will be important to test effects of not only microplastic fibers, which are highly abundant worldwide, but also other microplastic types that may have potential consequences for ecosystem functioning; for instance: microplastic films in agricultural lands or microplastic fragments (e.g., car tires) in urban ecosystems. Future research should also include different concentrations of microplastics in the soil as they may vary depending on the proximity to the emitting source, the microplastic type, and the ecosystem studied.

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**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c01051.

Microplastic fiber size distribution (Figure S1); photographs of the experimental setup (Figure S2); micro-
plastic fiber effects on bulk density (Figure S3); properties of polyester fibers (Table S1); results from linear models on shoot mass per plant species (Table S2); eigenvalues of the principal component analysis for plant community structure (Table S3); results from multivariate analysis of variance of plant species biomass (Table S4) (PDF).

### ACKNOWLEDGMENTS

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