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# Effect of microfibers combined with UV-B and drought on plant community



Chemosphere

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**SEVIER** 

# HIGHLIGHTS GRAPHICAL ABSTRACT

- Polyester fiber-microplastics increased height and shoot biomass for all plant species, especially invasive species *G. parviflora.*
- Drought negatively affected height and shoot biomass for all plant species.
- At the same conditions, *G. parviflora*  would occupy a unique ecological niche and affect the growth of native species.

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# ABSTRACT

There is an increasing recognition that microplastics contamination in soils has become an important threat for terrestrial ecosystems, and can interact with drought. In addition, due to the increasingly serious environmental pollution and the destruction of the ozone layer, the UV-B radiation to the earth's surface has gradually increased. However, we currently have no information about potential effects of microplastics, UV-B, and drought on plant communities. In order to make up for the vacancy, we conducted an experiment with grassland plant communities. Polyester fiber microplastics (absent, present), UV-B (fully transparent polythene film, attenuating UV-B radiation), and soil water conditions (well-watered, drought) were applied in a fully factorial design. A plant community consisting of four indigenous species and one invasive species, co-occurring in the terrestrial ecosystem of the northern temperate zone was established, and we investigated the effects of microplastics, UV-B, drought and their interactions on plant functional traits and plant community structure. We found that shoot and root biomass decreased with drought but increased with microfibers, and drought significantly decreased specific leaf area at the community level. Physiological and biochemical indexes of individual species and plant community were affected by microfibers, UV-B, drought and their interaction to a varying degree. More importantly, five species were divided into three clusters along PC1 corresponding to individuals from *G. longituba and P. depressa*, *B. bipinnata* and *M. sativa*, plus *G. parviflora,* which indicated that at the same conditions, *G. parviflora* would occupy unique ecological niches that affect the growth of native species. Our research offers insights into the mechanisms of the coexistence of native and invasive plants, as well as the ecological consequences of microplastics and other environment factors on plant communities.

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### **1. Introduction**

Microplastics, a group of different polymer particles with a diameter between 100 nm and 5 mm [\(Hidalgo-Ruz et al., 2012](#page-8-0)), show many shapes, and possess a high degree of physical and chemical diversity ([Helmberger et al., 2020](#page-8-0); [Rillig et al., 2019a](#page-8-0)). These microparticles originate from many sources, including the cosmetics industry ([Boucher](#page-8-0)  [and Friot, 2017](#page-8-0)), tire abrasion [\(Boucher and Friot, 2017](#page-8-0)), flooding, soil amendments, irrigation, plastic mulching, atmospheric fallout [\(Dris](#page-8-0)  [et al., 2016\)](#page-8-0), and the loss of synthetic fibers during washing, as well as the degradation of larger plastic objects (Bläsing and Amelung, 2018; [Rillig and Matthias, 2012](#page-8-0)), which are widespread in all ecosystems on earth [\(Rachman, 2018](#page-8-0)). It is speculated that in next 30 years, microplastic fragments will be close to 12 billion metric tons [\(Geyer et al.,](#page-8-0)  [2017\)](#page-8-0), which may pollute not only oceans but also the terrestrial ecosystems. If the current trend of unreasonable use of plastics without effective management strategies continues, our global environment may eventually be overloaded with plastics. Given that, microplastics, as a prominent emerging pollutants, have been widely regarded as one of the most significant new global change factors potentially affecting ecosystems ([Machado et al., 2018a; Rillig and Matthias, 2012](#page-8-0)), which have attracted worldwide attention in aquatic and terrestrial ecosystems.

Microplastic effects in terrestrial ecosystems have recently come into focus [\(Zhou et al., 2018;](#page-9-0) [Zhang et al., 2018](#page-9-0)), after about a decade of research being limited to aquatic environment ([Koelmans et al., 2017](#page-8-0); [Auta et al., 2017;](#page-8-0) [Nizzetto et al., 2016b;](#page-8-0) [Duis and Coors, 2016](#page-8-0)). Currently, the amount of microplastic pollution in terrestrial ecosystems is far greater than that reported in aquatic systems [\(Zhang and Liu, 2018](#page-9-0); [Machado et al., 2017](#page-8-0); [Horton et al., 2017\)](#page-8-0), and terrestrial ecosystem, particularly soil, has become a major sink for microplastics [\(Mahon](#page-8-0)  [et al., 2017;](#page-8-0) [Nizzetto et al., 2016a](#page-8-0)). Compared to aquatic domains, terrestrial areas, such as soil, are more susceptible to microplastics contamination ([Auta et al., 2017](#page-8-0); [Nizzetto et al., 2016a\)](#page-8-0). Researches on the impact of microplastics contamination on soil physical properties and soil biota has become increasingly apparent ([Ng et al., 2021](#page-8-0); [Machado et al., 2019\)](#page-8-0). Microplastic fibers have a variety of sizes, linear shapes and flexibility, which are identified as one of the most abundant types in the soil and can potentially influence soil hydrodynamics mainly through their connection with soil aggregation (Zhang and Liu, [2018;](#page-9-0) [Dris et al., 2015\)](#page-8-0). Once microplastic fibers accumulate in soil, they can alter soil characteristics ([Machado et al., 2019](#page-8-0)), such as reducing bulk density [\(Machado et al., 2018b](#page-8-0)), decreasing soil water-stable aggregation ([Machado et al., 2019](#page-8-0)), increasing soil water holding capacity ([Machado et al., 2018b](#page-8-0)), and increasing volume of *>*30 μm soil pore structure ([Zhang et al., 2019](#page-9-0)), ultimately affecting the function of soil ecosystem [\(Liu et al., 2017\)](#page-8-0) and biodiversity ([Rillig and Matthias, 2012](#page-8-0)). In addition, microplastics effects on plant performance growing as single individual have been well documented [\(Kleunen et al., 2020; Machado](#page-8-0)  [et al., 2019](#page-8-0); [Boots et al., 2019](#page-8-0)), and microplastics had different effects on different plant species, which might potentially affect plant community ([Rillig et al., 2019b\)](#page-8-0). However, the consequences of microplastics on plant community composed of native species and invasive species remain largely unknown.

Microplastic fibers in the soil may change soil water holding capacity by affecting soil aggregates, thereby altering soil water conditions, and potentially interact with drought ([Lozano et al., 2021\)](#page-8-0). The effects of microplastics and drought on plant community ([Lozano and Rillig,](#page-8-0)  [2020\)](#page-8-0), soil ecosystem functions and multi-functionality ([Lozano et al.,](#page-8-0)  [2020\)](#page-8-0) are starting to become apparent. At the same time, due to the depletion of the ozone layer, the increase in UV-B radiation flux reaching terrestrial ecosystems has become a global environmental problem in the near future ([Rong et al., 2018](#page-8-0); [Sanchez-Lorenzo et al., 2017](#page-9-0); [Zhang](#page-9-0)  [et al., 2017;](#page-9-0) [IPCC, 2013](#page-8-0)), which has important impact on plant growth and development ([Suchar and Robberecht, 2018\)](#page-9-0) as well as soil biological activity ([Díaz-Guerra et al., 2018\)](#page-8-0). It can be seen that with the increasing pollution of microplastics to soil on a global scale,

microplastics, UV-B radiation and drought are common stress factors and may occur at the same time. However, our knowledge about the potential interactive and adaptation mechanisms of plants to the combined stress of microplastics, UV-B radiation and drought are still unknown. Therefore, it is becoming more necessary to better understand the response of plant community functional traits, plant adaptation strategy, and plant community dynamics to microplastics contamination combined with UV-B radiation and drought.

To do that, we established a full factorial experiment with three factors polyester fiber-microplastics (absent, present), UV-B radiation (full spectrum, attenuating UV-B radiation), and soil water conditions (well-watered, drought), and built herbaceous community composed of four indigenous species and one invasive species, which naturally cooccur in temperate grasslands in northeastern China. We hypothesized that (1) microfibers, UV-B radiation and drought would strongly alter plant community functional traits, and microfibers might alleviate the effects of drought and UV-B radiation on plant communities; (2) Different plant species owned different adaptation strategies, and plant adaptation strategies determined the response of plant to microfibers, UV-B radiation, drought and their interactions. With this current research, we aimed to take the first steps to fill the gaps left by previous findings and focused on the previously neglected area of microplastics, UV-B and drought in the soil-plant system.

# **2. Materials and methods**

# *2.1. Plant species selection*

Four indigenous species *Bidens bipinnata*, *Plantago depressa*, *Medicago sativa* and *Glechoma longituba* and one invasive species *Galinsoga parviflora* from temperate grassland ecosystem in northeast China were selected to build plant community modules. Seeds of these plant species were obtained from the wild in Liaoning Province.

#### *2.2. Soil and polyester fiber-microplastics preparation*

The soil was collected from Shenyang, China where plant species naturally grow, then sieved with 2 mesh, and homogenized. Polyester fibers (Taili chemical fiber products, Shandong, item number, 5698) were cut with scissors in order to generate microplastic with a length of 3.00 mm and a diameter of 0.030 mm (Fig. S1). The polyester fibermicroplastics were sterilized by microwave, and thoroughly blended with the soil in a large container. A 12 g of polyester fiber-microplastics was sneaked into 3 kg of soil for each pot (21.2 cm diameter, 15.3 cm height, 5500 mL) [\(Lozano and Rillig, 2020](#page-8-0)). A total of 48 experimental pots were set up, half of which were added with polyester fiber-microplastics in the soil, and the other half without polyester fiber-microplastics served as control.

# *2.3. Design of the spectral attenuation experiment*

Two different kinds of spectral irradiance treatments were established through specific plastic filters installed on frames transmitting radiation: (i)*>*280 nm, transmitting about 95% of the entire solar spectrum (0.05-mm thick transparent polythene film, 3904CF; Okura); (ii)*>*315 nm, attenuating UV-B radiation (0.125-mm thick polyester film, Autostat CT5; Thermoplast). The plastic filters were mounted to 1.2 m  $\times$  1.0 m iron frames with an inclination of approximately 20<sup>°</sup>, facing south (Fig. S1C). Additional plastic filters were attached to the east and west sides of frames in order to attenuate solar radiation early and late in the day.

# *2.4. Experimental design*

The experiment was conducted at the Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China (41.90◦N, 123.59◦E), <span id="page-2-0"></span>which was characterized by temperate continental monsoon climate with an average annual temperature of 6.2–9.7 ◦C and a mean annual rainfall of 600–800 mm. The annual frost-free period was 155–180 days. In June 2020, we conducted the experiment in a growth chamber with a day and night temperature of 25/18 ◦C, a relative humidity of 40%, and a daylight period set at 10 h, 50 klx. Before germination, 300 seeds per species were cleansed with 5% potassium permanganate for 25 min for surface sterilization and thoroughly rinsed with sterile water subsequently. For the sake of the consistent seedling emergence time, the sowing time can be determined according to the germination time of each plant species in the pre-experiment. The seeds were germinated in sterile sand trays and individual seedlings with similar size were transplanted into pots (top diameter  $= 21.2$  cm, bottom diameter  $= 17.5$  cm, height = 15.3 cm, 5.5 L) 7 days after germination. Fifteen holes were randomly dug in each pot (Fig. S1B), and three individuals of each of the five plant species were randomly distributed and a plant community was established per pot. The whole experiment was laid out in three replicate randomized blocks of filters, giving 6 filter frames in total (Fig. S1C). Under the centre of each frame, eight pots (four with polyester fibermicroplastics and four without) were randomly put one shelf, giving a total of 48 pots. Half of them were kept at approximately 70% of soil water holding capacity (WHC) by adding 150 mL of water, while the other half were kept at about 30% WHC by adding 65 mL of water. The pots were weighed every two days to verify the maintenance of their respective moisture content. The positions of the pots were rotated randomly once a week to guarantee that all seedlings grew in similar light conditions during the experiment. So three factors, including microfibers, UV-B radiation, and drought, 8 kinds treatments were obtained. At harvest, shoot biomass was classified by plant species, and roots were carefully removed from the soil and gently washed (roots could not be separated by species).

#### *2.5. Determination of plant functional traits*

All plants survived until the end of the experiment, and plant growth, physiological and biochemical indicators were measured and recorded at harvest. Plant height was measured using a steel tape measure. Specific leaf area (SLA) was calculated as leaf area divided by leaf dry mass (after the leaf was dried to a constant weight at 65 ◦C). Leaf area was calculated using Image J [\(Abramoff et al., 2003](#page-8-0)) and leaves were scanned with a CanonScan LiDE 210 (Canon Inc., Tokyo, Japan). Root and shoot biomass were measured after the samples were dried at 65 ◦C for 72 h. The optical leaf clip Dualex Scientific (Force-A, France) was used to measure the contents of flavonoids (flav), anthocyanins (anth), and leaf chlorophyll (chl) between 9 and 11 a.m., during August 2020. The quantum yield of photosystem (PSII) under ambient sunlight (Fv'/Fm') and after dark adaptation (Fv/Fm) and was determined by a portable Mini-PAM fluorometer (WALZ, Germany) between 9 and 11 a.m., during August 2020. Before the measurement of Fv/Fm, a dark adaptation clip was placed on the leaf for 30 min in order to allow dark acclimation.

# *2.6. Statistical analyses*

All statistical data analyses were performed using IBM SPSS Statistics 21 and Rstudio. The experiment adopted a completely crossed orthogonal design where microfibers, UV-B, drought and their interaction were considered as fixed factors. The effects of microfibers, UV-B, drought and their interaction on each functional trait, including plant height, shoot biomass and specific leaf area per species, plant community shoot biomass, root biomass, specific leaf area were analyzed using general linear models. Shoot biomass, plant height and specific leaf area per species accounted for the shoot biomass, plant height and specific leaf area of the neighbors (the other four plant species) as a covariate. The differences of plant height, shoot biomass, and specific leaf area individual species, as well as shoot biomass, root biomass and specific leaf area at community level were analyzed by one-way ANOVA using Tukey

HSD tests to further compare the effect of a specific variable within each group. Standardized plant trait data were subjected to a principal component analysis (PCA) using the package "factoextra" and "Facto-MineR" with the Rstudio in order to analyze the coordinated trait response to microfibers, UV-B, and drought. Ellipses in the PCA graph grouped the different treatments with a confidence level of 0.95.

#### **3. Results**

#### *3.1. Plant growth index at individual and community levels*

The effects of microfibers, UV-B radiation, drought, and their interaction on growth index of per species and plant community existed difference (Table 1, Table S2). Drought decreased height (3.95%– 35.05%) and shoot biomass (4.3%–32.78%) for all plant species, and microfibers increased height (3.71%–57.17%) and shoot biomass (11.67%–72.68%) for all plant species, while UV-B had different effects on plant height and shoot biomass depending on plant species, microfibers and drought [\(Fig. 1](#page-3-0)A, [Fig. 1](#page-3-0)B). At the individual level, plant height and shoot biomass of *B. bipinnata* were affected by microfibers ( $F =$ 15.81,  $P < 0.01$ ;  $F = 34.51$ ,  $P < 0.01$ ), UV-B ( $F = 10.11$ ,  $P < 0.01$ ;  $F =$ 8.47,  $P < 0.01$ ), drought (F = 12.96,  $P < 0.01$ ; F = 20.65,  $P < 0.01$ ), microfibers  $\times$  drought (F = 9.71, *P* < 0.01; F = 37.73, *P* < 0.01) and UV-B  $\times$  drought (F = 5.96, P = 0.02; F = 31.73, P < 0.01) (Table S1). The height and shoot biomass of *G. parviflora* were affected by microfibers (F  $= 7.90, P < 0.01; F = 28.18, P < 0.01$  and microfibers  $\times$  UV-B (F = 4.04,  $P = 0.05$ ;  $F = 7.41$ ,  $P = 0.01$ ) (Table S2). The height of and shoot biomass of *M. Sativa* were affected by UV-B ( $F = 12.03, P < 0.01; F =$ 45.29,  $P < 0.01$ ) (Table S2). And the height and shoot biomass of *P. depressa* were affected by microfibers (F = 22.09, *P <* 0.01; F = 9.35, *P <* 0.01), drought (F = 15.50, *P <* 0.01; F = 9.73, *P <* 0.01), and microfibers  $\times$  drought (F = 5.94, P = 0.02; F = 6.45, P = 0.02) (Table S1). With regard to SLA, microfibers, UV-B, drought and their interaction had no significant effects on SLA of *G. longituba*, *B. bipinnata*, and *G. parviflora*, while, the SLA of *M. Sativa* was affected by microfibers  $(F = 23.89, P < 0.01)$ , UV-B (F = 18.48,  $P < 0.01$ ), and microfibers  $\times$ UV-B  $\times$  drought (F = 10.90, P < 0.01), and the SLA of *P. depressa* was affected by microfibers  $\times$  UV-B (F = 6.38, P = 0.01), and microfibers  $\times$ drought (F = 9.54,  $P < 0.01$ ) ([Fig. 1](#page-3-0)C; Table S2).

At the community level, shoot and root biomass decreased with drought but increased with microfibers ([Fig. 2\)](#page-4-0). Community average shoot and root biomass increased from absent microplastics to microplastics condition by 27.60%–46.39% and 23.97%–49.88% ([Fig. 2](#page-4-0)A, [B](#page-4-0)), respectively. No matter with or without polyester fiber-microplastics, shoot biomass existed highest under well-watered with 15.57 g, 11.85 g, 14.81 g, and 12.71 g, and shoot biomass obviously decreased from well-watered to drought condition (*P <* 0.01) ([Fig. 2](#page-4-0)A). In addition, root biomass existed the highest under well-watered condition with 6.24 g, 5.8 g, 5.20 g, and 4.87 g, and root biomass obviously decreased from well-watered to drought conditions ( $P < 0.01$ ) [\(Fig. 2B](#page-4-0)). The effect of UV-B on plant community shoot and root biomass varied with microfibers and drought ([Fig. 2](#page-4-0)A, [B\)](#page-4-0). Plant community shoot biomass, and

### **Table 1**

Results from general linear models on shoot biomass, root biomass and SLA of plant community response to polyester fiber-microplastics (Microfibers, M), UV-B radiation (UV–B), drought (D) and their interactions (M x D, M x UV-B, D x UV-B, M x D x UV-B). F and p values (in parenthesis) are shown.

	df	Shoot biomass	Root biomass	SLA
Microfibers (M)	1	1488.19(<0.01)	402.74(<0.01)	2.99(0.09)
UV-B radiation (UV-B)	1	22.66(<0.01)	11.81(<0.01)	0.94(0.34)
Drought (D)		796.84(<0.01)	262.35(<0.01)	7.88(0.01)
$M \times UV-B$	1	$27.70(<0.01$ )	6.85(0.01)	1.10(0.30)
$M \times D$	1	1.86(0.180)	1.84(0.18)	0.06(0.81)
$UV-B \times D$	1	79.01(<0.01)	13.33(<0.01)	0.60(0.44)
$M \times I$ IV-B $\times$ D	1	3.64(0.064)	4.68(0.04)	0.16(0.69)

<span id="page-3-0"></span>

Fig. 1. Plant height (A), shoot mass (B) and specific leaf area (SLA) (C) of different plant species growing in a community under drought and well-watered conditions, full spectrum and attenuating UV-B radiation, with or without polyester fiber-microplastics in the soil. Plant species are designated by their species names. FS-W: full spectrum + well-watered; FS-D: full spectrum + drought; AUVB-W: attenuating UV-B radiation + well-watered; AUVB-D: attenuating UV-B radiation + drought; M: with polyester fiber-microplastics; CK: without polyester fiber-microplastics.

<span id="page-4-0"></span>

**Fig. 2.** The effect of polyester fiber-microplastics, UV-B, drought and their interaction on shoot biomass (A), root biomass (B), and SLA (C) of plant community. FS-W: full spectrum + well-watered; FS-D: full spectrum + drought; AUVB-W: attenuating UV-B radiation + well-watered; AUVB-D: attenuating UV-B radiation + drought; M: with polyester fiber-microplastics; CK: without polyester fiber-microplastics.

<span id="page-5-0"></span>root biomass were affected by microfibers ( $F = 1488.19$ ,  $P < 0.01$ ;  $F =$ 402.74,  $P < 0.01$ ), UV-B (F = 22.66,  $P < 0.01$ ; F = 11.81,  $P < 0.01$ ), drought (F = 796.84,  $P < 0.01$ ; F = 262.35,  $P < 0.01$ ), M  $\times$  UV-B (F =  $27.70, P < 0.01; F = 6.85, P = 0.01$ , UV-B  $\times$  D (F = 79.01, P < 0.01; F = 13.33,  $P < 0.01$ ) ([Table 1](#page-2-0)). Microfibers and UV-B had no significant effect on SLA, however, drought significantly decreased SLA by 2.74% o 6.28% ([Fig. 2C](#page-4-0), [Table 1\)](#page-2-0).

# *3.2. Physiological and biochemical indexes at individual and community levels*

At the community level, anthocyanins were affected by microfibers  $(F = 5.19, P = 0.03)$  and drought  $(F = 27.38, P < 0.01)$  (Table 2), and the content of anthocyanins decreased with drought (Fig. S2A). Chlorophylls were affected by microfibers ( $F = 12.27$ ,  $P < 0.01$ ), drought ( $F = 12.27$ 15.94,  $P < 0.01$ ), microfibers  $\times$  UV-B (F = 5.40,  $P = 0.02$ ), and microfibers  $\times$  UV-B  $\times$  drought (F = 4.68, P = 0.03) (Table 2), and chlorophylls increased with drought and microfibers (Fig. S2B). Flavonoids were independently affected by UV-B ( $F = 27.15$ ,  $P < 0.01$ ) (Table 2), and attenuating UV-B radiation could decrease the contents of flavonoids (Fig. S2C). YII (Fv'/Fm') was affected by UV-B (F = 25.99, *P <* 0.01), drought (F = 5.99,  $P = 0.02$ ), UV-B  $\times$  microfibers (F = 9.90,  $P < 0.01$ ), and UV-B  $\times$  drought (F = 5.77, P = 0.02), while Fv/Fm was affected by UV-B (F = 12.61,  $P < 0.01$ ), and drought (F = 7.02,  $P = 0.01$ ) (Table 2). At the individual level, anthocyanins, chlorophylls, flavonoids, YII and Fv/Fm of *G. longituba*, *B. bipinnata*, *G. parviflora*, *M. Sativa*, and *P. depressa* were affected by microfibers, UV-B, drought and their interaction to varying degree (Table S2).

# *3.3. Coordinated trait response to microfibers, UV-B, and drought*

In the plant functional traits principal component analysis (PCA) with all five species combined, 8 leaf traits showed obviously divergent differentiation, and grass individuals were separated into species with low overlap along the first principal component axis (PC1). PC1 explained 30.96%–39.49% of the total variation of the 8 variables among individuals, and the second principal component (PC2) explained 17.47%–24.84% of the total variation of the 8 variables, the cumulative contribution rate accounting for 50.87%–60.15% ([Fig. 3](#page-6-0)). Under different treatments, we observed that the five species were divided into three clusters along PC1 corresponding to individuals from *G. longituba and P. depressa*, *B. bipinnata* and *M. Sativa*, plus *G. parviflora*  ([Fig. 3](#page-6-0)), which demonstrated *that G. longituba and P. depressa* owned the similar adaptive strategy, and *B. bipinnata* and *M. Sativa* owned the similar adaptive strategy.

# **Table 2**

Results from general linear models on physiological and biochemical indexes of plant community response to polyester fiber-microplastics (Microfibers, M), UV-B radiation (UV–B), drought (D) and their interactions (M x D, M x UV-B, D x UV-B, M x D x UV-B). F and p values (in parenthesis) are shown.

	df	Anth	Chl	Flav	YII	Fv/Fm
Microfibers	1	5.19	12.27	0.72	0.04	0.00
(M)		(0.03)	$(0.01)$	(0.40)	(0.85)	(0.96)
UV-B	$\mathbf{1}$	0.54	2.86	27.15	25.99	12.61
radiation		(0.47)	(0.10)	$(0.01)$	$(0.01)$	$(0.01)$
$(UV-B)$						
Drought (D)	1	27.38	15.94	2.79	5.99	7.02
		$(0.01)$	$(0.01)$	(0.10)	(0.02)	(0.01)
$M \times I$ JV-B	1	0.42	5.40	0.00	9.90	0.01
		(0.52)	(0.02)	(0.95)	$(0.01)$	(0.91)
$M \times D$	1	1.01	0.03	3.88	0.83	0.18
		(0.32)	(0.87)	(0.05)	(0.37)	(0.67)
$UV-B \times D$	$\mathbf{1}$	0.56	0.72	0.34	5.77	0.00
		(0.46)	(0.40)	(0.56)	(0.02)	(0.96)
$M \times I$ JV-B $\times$	1	1.89	4.68	0.37	0.14	3.47
D		(0.17)	(0.03)	(0.54)	(0.71)	(0.07)

# **4. Discussion**

# *4.1. Response of plant community functional traits to microfibers, UV-B, and drought*

As our hypothesized, polyester fiber-microplastics, UV-B radiation, drought, and their interaction strongly alter plant community functional traits. In our study, we demonstrated that drought negatively affected plant shoot biomass and root biomass, while microfibers had the opposite effect, which was consistent with previous study indicated that drought reduced above-ground and root biomass, while microfibers increased biomass at the community level [\(Lozano and Rillig, 2020](#page-8-0)). What's more, the findings from [Machado et al. \(2019\)](#page-8-0) also reported that the root biomass significantly increased after adding polyester fibers. Although the threshold of drought depends on the characteristics of the species and its adaptation to the environment, the decline in above-ground biomass caused by drought is a proverbial phenomenon, which is ubiquitous in plant species ([Eziz et al., 2017\)](#page-8-0), as water deficit seriously affects the photosynthesis of plants. Intriguingly, it was well established that plant community shoot biomass and root biomass increased with microfibers under drought conditions, which suggested that microfibers did effectively ameliorate the negative effects of drought under the moisture condition which we applied drought here, and this was well exemplified in studies from [Lozano and Rillig \(2020\)](#page-8-0). It is likely that increase in root biomass in microfibers soil might be related to the positive influence of microfibers on soil aeration, bulk density and water retention ([Machado et al., 2019\)](#page-8-0). Microplastic fibers are generally hydrophobic ([Prorokova et al., 2012](#page-8-0)), a characteristic that is positively related to soil aggregation under drought conditions ([Zheng](#page-9-0)  [et al., 2016](#page-9-0)), which ultimately improved aeration ([Machado et al.,](#page-8-0)  [2019\)](#page-8-0), helped hold water [\(Rillig et al., 2019a](#page-8-0), [b\)](#page-8-0), and stimulated better penetration of the root system into the soil matrix and facilitated the growth of root ([Lozano and Rillig, 2020\)](#page-8-0). However, some studies have shown that microplastics have no effect [\(Judy et al., 2019](#page-8-0)) or passive influence on plants in the terrestrial ecosystem ([Zhou et al., 2021](#page-9-0); [Qi](#page-8-0)  [et al., 2018](#page-8-0), [2020](#page-8-0); [Boots et al., 2019\)](#page-8-0). This might account for at least that different types of microplastics have different effects on plant biomass ([Boots et al., 2019](#page-8-0)). In contrast to the aboveground biomass response of individual species, the response of underground biomass to drought is unpredictable as it varies according to the characteristics of the plant species [\(Bloor et al., 2018\)](#page-8-0). Nevertheless, the increase in root biomass will accelerate the absorption of water and nutrients, increase rhizodeposition, and improve soil microbial activity [\(Bennett et al., 2017](#page-8-0); [Putten and Wim, 2017\)](#page-8-0), which will contribute to the increase of aboveground biomass. Collectively these studies suggested that the impact of drought on plant community might be altered when microplastics and other global change factors come into play.

In addition, microfibers in the soil promoted the high-speed growth of invasive species *G. parviflora*, especially under well-watered conditions [\(Fig. 1](#page-3-0)A, [B](#page-3-0)). UV-B had different effects on plant height and shoot biomass depending on plant species, microfibers and drought ([Fig. 1](#page-3-0)A, [B](#page-3-0)). In our study, under the condition of microfibers and drought, the attenuation of UV-B could increase plant height and aboveground biomass of *G. parviflora* and *P. depressa*, while the rest of the biomass dropped after filtering out UV-B. This suggested that under microplastics and drought conditions, the attenuation of UV-B led to a better performance of *G. parviflora* and *P. depressa*.

As anticipated, plant physiological and biochemical index were affected by microplastic fibers, UV-B, drought, and their interactions (Table 2, Table S2). As far as we know, flavonoids are an important class of secondary metabolites, of which anthocyanins are the most wellknown flavonoids. It is generally believed that anthocyanins are closely related to the plant defense, signal transduction, adaptation, and growth and development ([Chen et al., 2010](#page-8-0); [Shao et al., 2009\)](#page-9-0), and could improve the drought resistance of plants ([Castellarin et al., 2007](#page-8-0)). In our study, anthocyanins were effected by microfibers and drought at

<span id="page-6-0"></span>

*(caption on next page)* 

**Fig. 3.** Principal component biplot of plant functional traits for all five grass species combined. Plant functional traits included are height (H), shoot biomass (SB), specific leaf area (SLA), anthocyanins (anth), Chlorophyll (Chl), flavonoids (flav), YII (Fv'/Fm'), and Fv/Fm. Points are the trait scores of individuals without fertilization (closed circles) and with fertilization (open circles). M–FS–W: with polyester fiber-microplastics + full spectrum + well-watered; AM–FS–W: without polyester fiber-microplastics + full spectrum + well-watered; M–FS–D: with polyester fiber-microplastics + full spectrum + drought; AM–FS–D: without polyester fiber-microplastics + full spectrum + drought; M-AUVB-W: with polyester fiber-microplastics + attenuating UV-B radiation + well-watered; AM-AUVB-W: without polyester fiber-microplastics + attenuating UV-B radiation + well-watered; M-AUVB-D: with polyester fiber-microplastics + attenuating UV-B radiation + drought; AM-AUVB-D: without polyester fiber-microplastics  $+$  attenuating UV-B radiation  $+$  drought.

the community level, and the contents of anthocyanins decreased with drought (Fig. S2A), which was line with the expectation illuminated that the concentration of secondary metabolites increased under moderate drought stress, while the contents of secondary metabolites were inhibited under severe drought stress [\(Yan et al., 2007\)](#page-9-0). This might be due to the fact that plants use sufficient raw materials to synthesize flavonoids under moderate drought conditions. At the same time, the key enzymes for synthesizing flavonoids have high activity, and the content of flavonoids increases. However, under severe drought conditions, plants can only use more material and energy for growth, which limits the supply of raw materials for the synthesis of flavonoids. In addition, the activity of key enzymes for the synthesis of flavonoids is relatively low. In our study, the soil moisture content was about 30%, belonging to the severe drought stress, which inhibited the synthesis of flavonoids and anthocyanidins, supported by the results obtained by [Zahir et al. \(2014\).](#page-9-0)

Chlorophyll serve as an important indicator to measure the photosynthetic capacity of plants [\(Hui et al., 2003](#page-8-0)), and the increase of chlorophyll could improve the photosynthetic efficiency. In our research, the contents of chlorophylls increased with drought and microfibers (Fig. S2B). As indicated by [Liu et al. \(2021\)](#page-8-0) documented that drought was beneficial to the increase of leaf chlorophyll contents and alleviate the damage of photosynthetic structure. In addition, the increase of chlorophyll contents of plants can compensate for the decrease of photosynthetic rate caused by the decrease of  $CO<sub>2</sub>$  concentration. What's more, differential responses of chlorophyll content in plants based on the polymer type have been found in marine primary producers when exposed to microplastics [\(Green et al., 2016,](#page-8-0) [2017\)](#page-8-0). Polystyrene nanoplastics reduced chlorophyll contents of *Scenedesmus obliquus*  ([Besseling et al., 2014\)](#page-8-0), while, polyethylene microbeads have no effect on chlorophyll content of *Lemna minor* (Kalíková [et al., 2017\)](#page-8-0). This suggested that the response of plants to microfibers in terrestrial and aquatic habitats existed difference, which warrants more investigation given the crucial position of primary producers within ecosystems including soils ([He et al., 2018](#page-8-0)).

Flavonoids, as plant protection elements, were independently affected by UV-B radiation [\(Table 2](#page-5-0)), and attenuating UV-B radiation could decrease the contents of flavonoids (Fig. S2C). In other words, with the increase of UV-B radiation, the content of flavonoids in plants increased. The accumulation of flavonoids in plants can provide plants with a barrier against UV-B radiation and reduce the damage caused by UV-B radiation to plants ([Tattini et al., 2014](#page-9-0)). Our results illuminated interaction of microfibers with global change factors such as UV-B radiation and drought has a profound impact on the functional traits of plants in the short term, but its long-term response is still unclear. Additionally, future research should explore the effects of other microplastics on different plant communities to achieve a greater generalizability of the patterns observed here.

#### *4.2. Coordinated leaf trait response to microfibers, UV-B, and drought*

Vegetation is strongly coupled with environmental conditions ([Medinski et al., 2010\)](#page-8-0). As we hypothesized, plant community will be affected by environmental factors, resulting in changes in plant strategies and traits, thereby affecting species diversity and ecosystem functions. Under different treatments, we observed that the five species were divided into three clusters along PC1 corresponding to individuals from *G. longituba and P. depressa*, *B. bipinnata* and *M. Sativa*, plus *G. parviflora* 

([Fig. 3\)](#page-6-0). Our results suggested that *G. longituba and P. depressa* owned the similar adaptive strategies, and *B. bipinnata* and *M. Sativa* owned the similar adaptive strategies. In our study, a significant extent of overlap in plant functional traits between *P. depressa* and *G. longituba and* was obtained, which indicated that *P. depressa* and *G. longituba existed competitive exclusion*. Our results confirmed and extended earlier observations that a high degree of overlap of plant functional traits should confer some resistance of one species to colonization of another species due to niche saturation [\(Funk et al., 2008](#page-8-0)). While, the invasive species of *G. parviflora* owned the unique adaptation strategies, which indicated that at the same conditions, *G. parviflora* would occupy unique ecological niches that affect the growth of native species. Due to the well-documented link between environment factors and plant functional traits, it is likely that environment factors plays a crucial role in determining trait differences between native and invasive species. Our results showed that three global change factors (microplastics, UV-B and drought) influenced plant community, which in turn might affect ecosystem services [\(Manning et al., 2018;](#page-8-0) [Díaz et al., 2018](#page-8-0)) and thus impact various aspects of human well-being. Future research on this topic should include different plant species and growth patterns and explore responses over more than one growing season, and investigate whether invasive and native species have shifted their trait spaces in response to different environment factors to test the applicability of these results under field conditions.

#### **5. Conclusions**

In conclusion, we found that shoot and root biomass decreased with drought but increased with polyester fiber-microplastics, and drought significantly decreased SLA at the community level. Furthermore, drought negatively affected height and shoot biomass for all plant species, and polyester fiber-microplastics increased height and shoot biomass for all plant species, especially invasive species *G. parviflora*, while UV-B had different effects on plant height and shoot biomass depending on plant species. Physiological and biochemical indexes of per species and plant community were affected by polyester fibermicroplastics, UV-B radiation, drought and their interaction to varying degree. More importantly, five species were divided into three clusters along PC1 corresponding to individuals from *G. longituba* and *P. depressa*, *B. bipinnata* and *M. Sativa*, plus *G. parviflora*, which indicated that at the same conditions, *G. parviflora* would occupy a unique ecological niche and affect the growth of native species. Our results showed that in the short term, polyester fiber-microplastics combined with other global change factors such as drought and UV-B had different effects on plant biomass and plant functional traits, which paving the way towards an improved understanding of the effect of microfibers interact with other factors on plant community in the terrestrial ecosystem.

# **Credit author statement**

Jiaojiao Deng: Experimental design, Investigation, Data curation, Writing-original draft preparation. Li Zhou: Data analysis, Data Visualization. Dapao Yu: Data Visualization. Qiangwei Wang: Experimental design, Reviewing, Revising. Wangming Zhou: Reviewing, Revising.

# <span id="page-8-0"></span>**Declaration of competing interest**

The authors declare no conflict of interest.

#### **Declaration of competing interest**

The authors declare no conflict of interest.

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# **Appendix A. Supplementary data**

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