Supporting information

Impacts of microplastics on the soil biophysical environment

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Supporting information S1 A: Particle size distribution

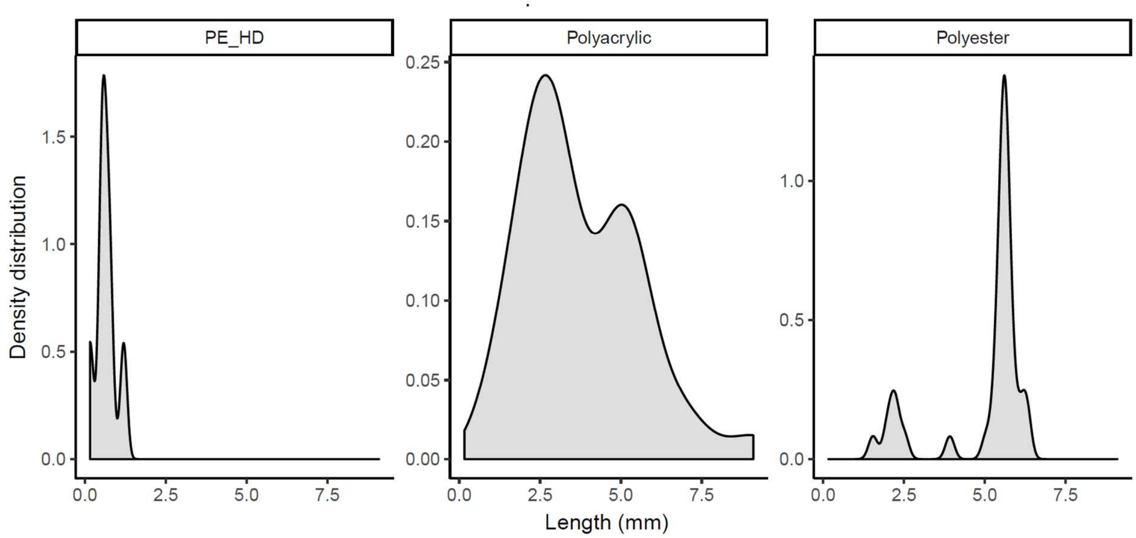

Figure S1 A: Microplastic size distributions for the largest dimension of the self-prepared particles. A subsample of polyethylene high-density (PE_HD) fragments (N= 7), and polyacrylic and polyester fibers (N= 47) were measure under a graduated microscope.

Supporting information S1 B: Information on Experimental set up

Our test soil was a loamy sand, collected at the experimental facilities of the Freie Universität Berlin (52°27'58" N, 13°18'10" E; Berlin, Germany) in October 2016 and stored in the greenhouse. This soil is loamy sandy mineral soil (Albic Luvisol) with nitrogen content of ~ 0.12 %, carbon content of ~ 1.87 %, and consequentially C : N ratio of ~ 15.58. The pH ($CaCl_2$) is ~ 7.1, and phosphorus (CAL; available P) ~ 69 mg kg^{-1} (according to Verbruggen et al[1]).

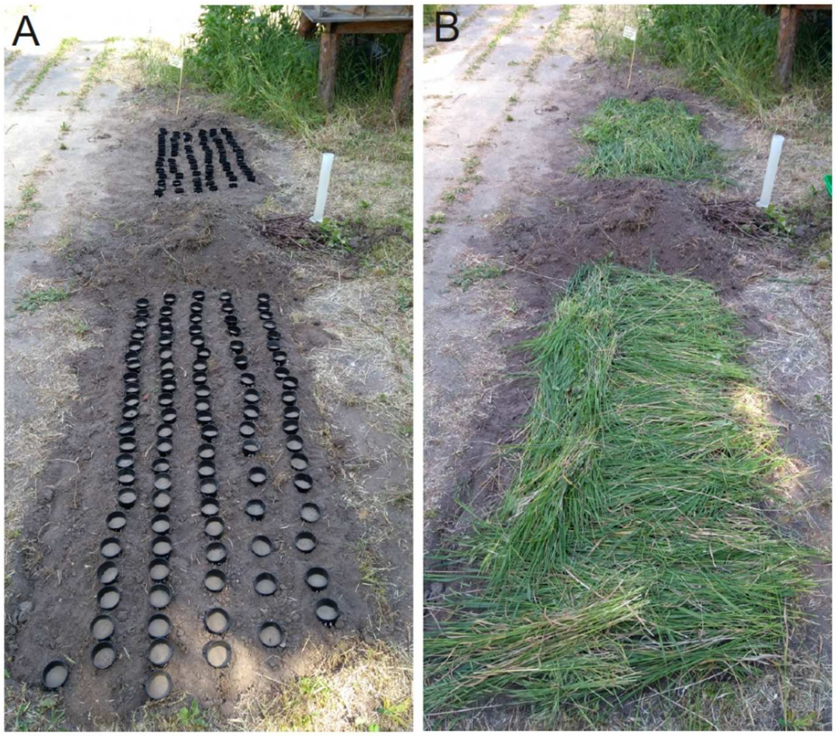

Figure S1 B: Partially buried experimental pots (A) were covered with local grass (B) for 5 weeks during the boreal summer 2017 to undergo natural temperature and humidity diurnal cycles.

Measurement of hydraulic conductivity

Hydraulic conductivity (K) was measured using the flow induction with constant head method, where $K = \frac{\text{Quantity of water (mL)}*\text{Length of soil pot (mm)}}{\text{Area of pot surface (mm}^2)*\text{Time (s)}*\text{Height of head (mm)}}$. Water holding capacity was measured by adding 1 mL min-1 of distilled water to the soil surface until saturation and measuring the difference between dry and wet saturated weight.

Table S1 B: Pipeline of tests and linear models used for statistical inference (further information starts in line 811 of R script)

Phase	Test	Formula	Data set
Exploratory	kruskal.test with Tukey posthoc if significant	Endpoint[a] ~ Treatment[b]	Whole measured data
	kruskal.test with Tukey posthoc if significant	Endpoint [a]~ Type[c]	Whole measured data

	kruskal.test with Tukey posthoc if significant	Endpoint[a] ~ Material[d]	Whole measured data
Testing effects of particle properties	lm, gls, glm whenever appropriate	Endpoint[a] ~ Type[c]	Whole measured data
	lm, gls, glm whenever appropriate	Endpoint [a]~ Material[d]	Whole measured data
Testing effects of microplastics considering concentration per particle type	gls	Endpoint[a] ~ Concentration[e]	Data for soils exposed to polyacrylic fibers
	gls	Endpoint[a] ~ Concentration[e]	Data for soils exposed to polyamide beads
	gls	Endpoint[a] ~ Concentration[e]	Data for soils exposed to polyester fibers
	gls	Endpoint[a] ~ Concentration[e]	Data for soils exposed to polyethylene fragments
Testing effects of microplastics considering semi-quantitative and qualitative metrics of microplastic exposure	gls or glm whenever appropriate	Endpoint[a] ~ Concentration[e] + Volume[f] * Area[g] * Number[h]	Whole measured data
	gls or glm whenever appropriate	Endpoint[a] ~ Concentration[e]	Whole measured data
	gls or glm whenever appropriate	Endpoint[a] ~ Volume[f] * Area[g] * Number[h]	Whole measured data
	AICc test to chose the model	Compare the upper three models and make inference based on the smaller AICc values	Whole measured data

Endpoint[a]: Any measured value of a given soil biophysical parameter, e.g. bulk density, water stable aggregates, hydraulic conductivity, microbial activity, etc.

Treatment[b]: The combination of plastic added and its nominal concentration, e.g. polyester fibers at 0.4 %.

Type[c]: Whether microplastics added were linear (e.g. fibers) or non-linear (beads and fragments).

Material[d]: The polymer added (including effects of its particle size distribution), e.g. polyamide beads.

Concentration[e]: The nominal level of soil exposure, e.g. 2.0 %.

Volume[f]: The average volume of microplastics per Kg of soil for each treatment.

Area[g]: The average surface area of microplastics per Kg of soil for each treatment.

Number[h]: The average number of microplastic particles per Kg of soil for each treatment.

Supporting information S1 C: The formation of soil clumps

The formation of soil clumps (concretions) occurs in soils under spatially confined conditions, such as the experimental pots used in the present study. This phenomenon is commonly seen in soil science as an artefact of experimental conditions, and therefore often assumed to not convey much information. However, this process was significantly affected by microplastic presence in the current study. In order to quantify the formation of these soil clumps after the 5-weeks period, the soil was removed from the experimental pots and gently sieved at 630 µm (only soil smaller than 630 µm was present at the start of the experiment). The soil fraction remaining on the sieve after 20 s was assumed to proportionally reflect concretion formation during the period. The polyester fibers significantly increased concretion formation with, while polyethylene fragments decreased it ($p < 0.05$)**.** No significant effects were observed for the polyacrylic fibers or polyamide beads ($p > 0.05$). However, polyacrylic fibers elicited similar trend as the linear polyester.

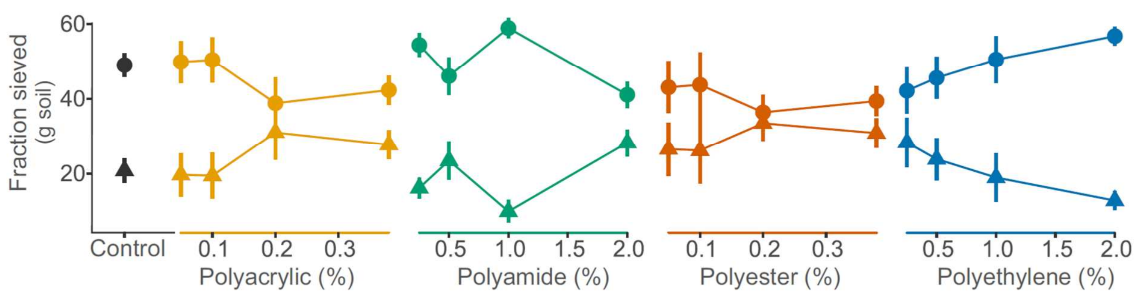

Figure S1 C: The formation of clumps (soil concretions) after 5 weeks of soil exposure to control or various concentrations of polyacrylic, polyamide, polyester, and polyethylene microplastics. The filled circles represent fraction smaller than 630 µm, filled triangles represent clumps larger than 630 µm, while the bars represent the standard error of the mean (Controls N=10, microplastic treatments N=5).

Supporting information S1 D: The accuracy of visual inspection of polyamide beads

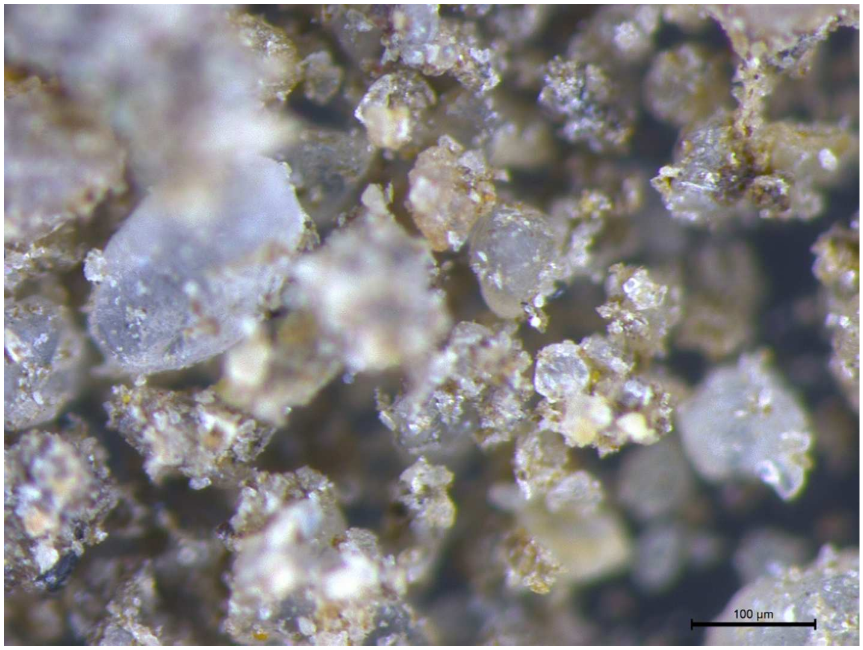

Figure S1 D: The size range of polyamide beads within 15- 20 µm might account for its challenging visual identification under stereomicroscopy. Soil in this image contained 2 % of polyamide beads in mass (~ 10^9 to 10^{10} polyamide particles kg dry soil^{-1}). Mass estimates of microplastic levels in environmental soil samples relying on visual cues might suffer from considerable underestimation.

Supporting information S1 E: Qualitative metrics of plastic exposure yield little information for assessing the potential impacts of microplastics on the parameters considered here

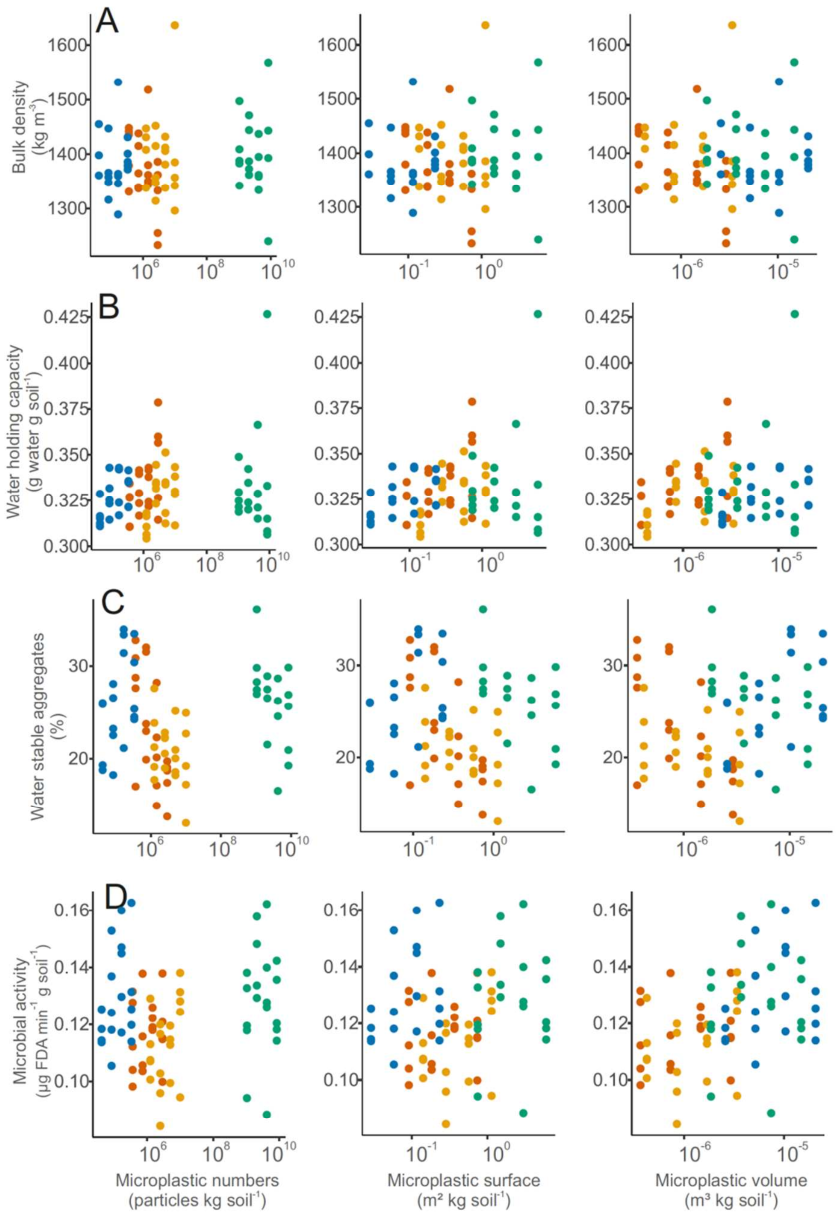

Figure S1 E: Effects of microplastic particles on the soil biophysical environment are not well characterized if idiosyncrasies of particle type and concentration are ignored. Soil bulk density (A), water holding capacity (B), water stable aggregates (C), and microbial activity (D) are presented as function of commony used qualitative metrics when reporting microplastic pollution. In all panels dark gray, yellow, green, red, and blue colors represent respectively control, polyacrylic, polyamide, polyester and polyethylene treatments (i.e. linear microplastics are in warm colors- yellow and red, volumetric microplastics are in cold colors- blue and green).

Supporting information S1 F: No detectable significant effects on hydraulic conductivity

We did not detect significant patterns of microplastic impacts on the hydraulic conductivity using the experimental and statistical approach adopted here (Fig. S1 F). Impacts on the hydraulic conductivity could be expected since the microplastic particles displayed generally particle size distribution, hydrophobicity, and structural properties distinct from those of natural soil minerals. Perhaps the soil (sand loamy) chosen here already displayed hydraulic conductivity (*K*) high enough to be relatively unaffected by the microplastic concentrations and *K* measurement adopted.

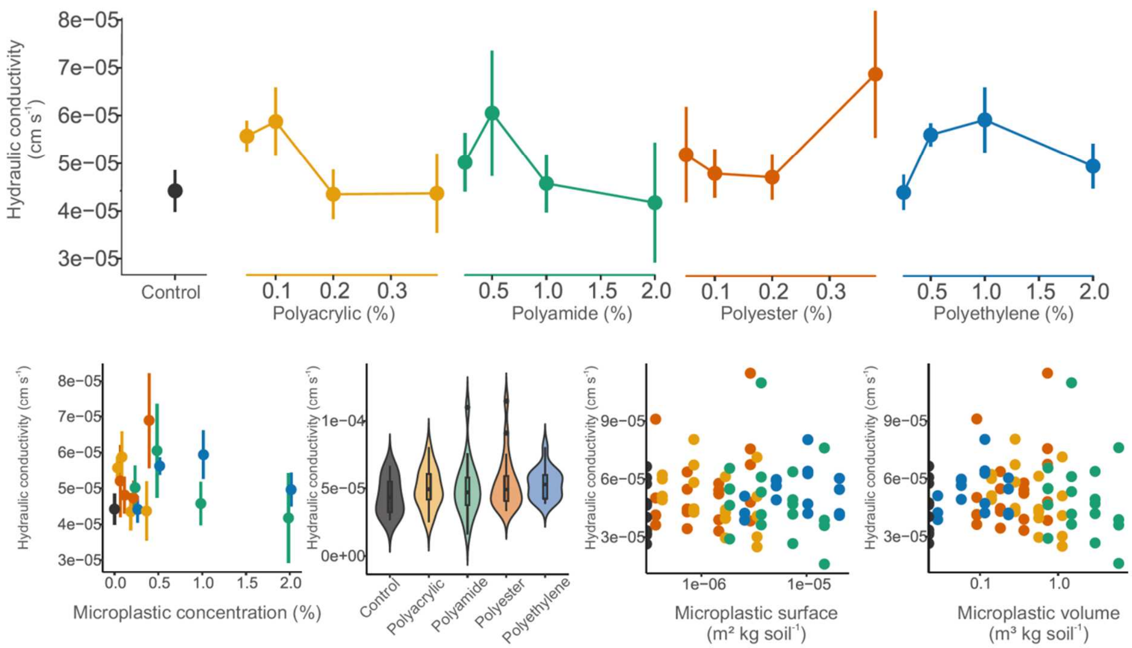

Figure S1 F: Effects of microplastic particles on the soil hydraulic conductivity. The upper panels show hydraulic conductivity per microplastic type highlighting in the x and y axis the range of respectively mean contaminant and response variable. The lower panels present hydraulic conductivity with common exposure metrics in respectively microplastic research. In all panels dark gray, yellow, green, red, and blue colors represent respectively control, polyacrylic, polyamide, polyester and polyethylene treatments (i.e. linear microplastics are in warm colors- yellow and red, volumetric microplastics are in cold colors- blue and green).

Supporting information S1 G: Effects of microplastics on the soil structure

Several changes on the soil structure were observed as a function of microplastic shape. Linear polyacrylic and polyester significantly increased the soil fraction below 630 µm compared to linear or volumetric microplastics ($F= 7.35$, $p < 0.01$). In fact, polyester fibers increased the fraction of that passed the 630 µm regardless of concentration teste ($F= 5.25$, $p < 0.01$). In general, a significant effect of microplastic concentration was observed (F= 6.98, $p < 0.01$), with the other microplastic types generally associated with weaker effects.

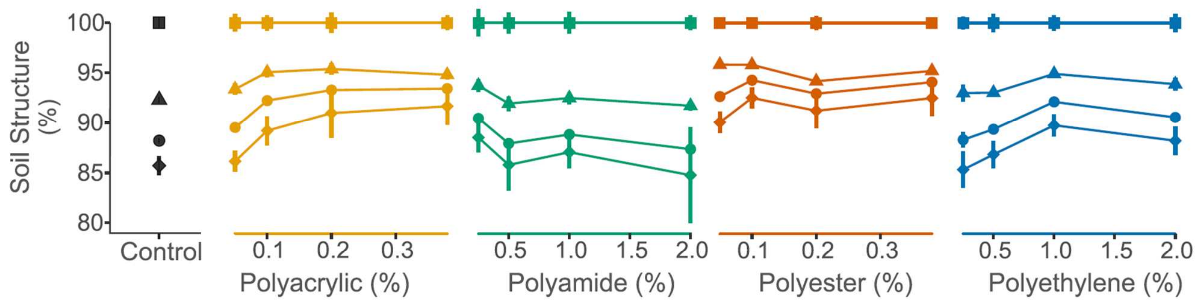

Figure S1 G: Effects of microplastic particles on dry-sieved soil aggregates. The x axis is microplastic treatment and the y axis the percentage of mass of soil in a given size fraction. Rhombuses represent mean values for the fraction smaller than 630 µm, circles denote mean values for cumulative fraction smaller than 1 mm, triangles indicate mean values for cumulative fraction smaller than 2 mm, and squares stand for mean values of the cumulative fraction smaller than 4 mm. Bars indicate the respective standard errors of the mean (N= 5 per microplastic treatments, 10 for controls). Dark gray, yellow, green, red, and blue colors represent respectively control, polyacrylic, polyamide, polyester and polyethylene treatments (i.e. linear microplastics are in warm colors- yellow and red, volumetric microplastics are in cold colors- blue and green).

References

1. Verbruggen, E.; Jansa, J.; Hammer, E. C.; Rillig, M. C.; Vries, F., Do arbuscular mycorrhizal fungi stabilize litter-derived carbon in soil? *Journal of Ecology* **2016,** *104*, (1), 261-269.