

[Supplementary Information]

## **Post COVID-19 pandemic: Biofragmentation and soil ecotoxicological effects of microplastics derived from face masks**

Jin Il Kwak and Youn-Joo An\*

*Department of Environmental Health Science, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Korea*

**\* Corresponding author**

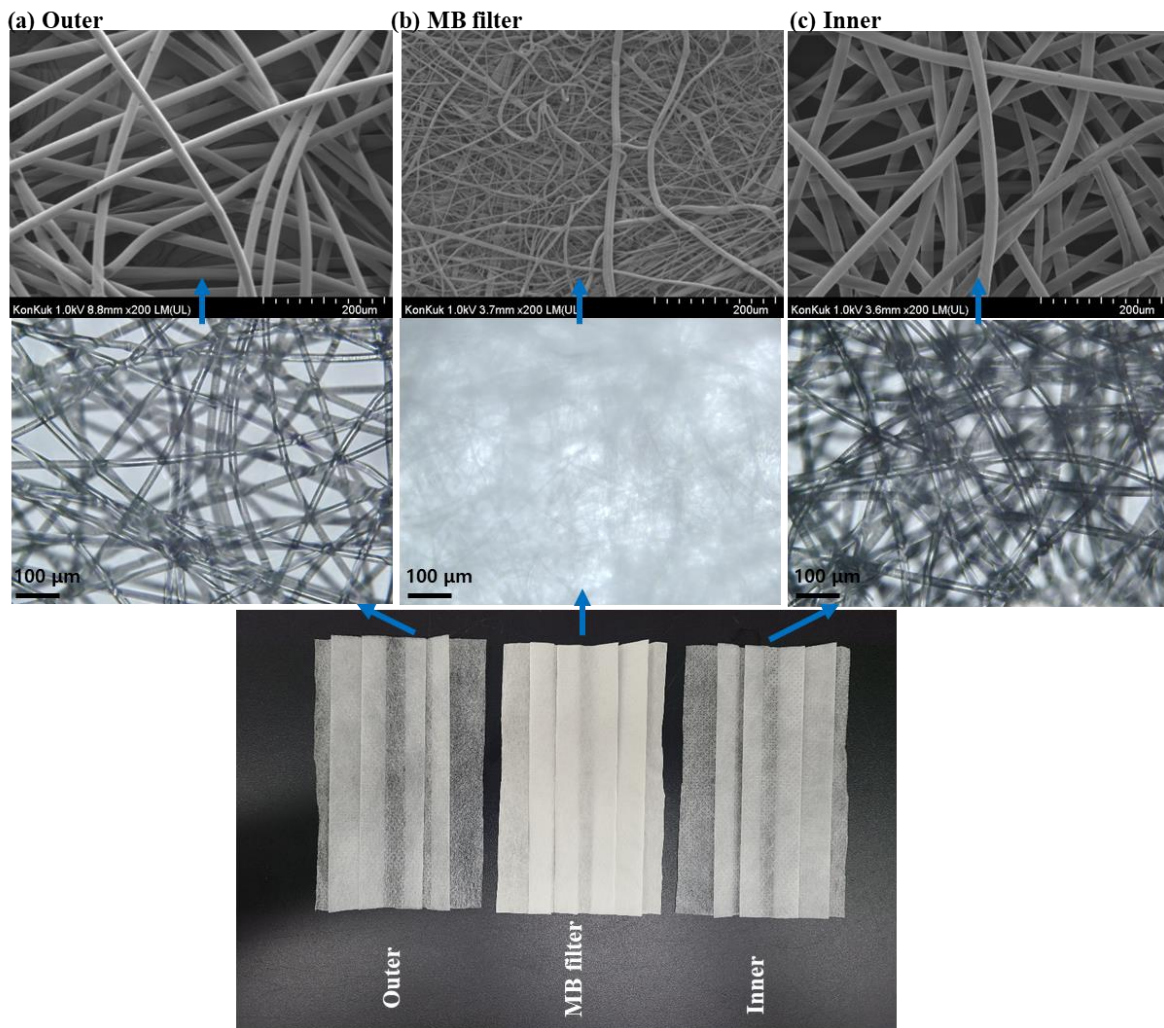
Youn-Joo An

Telephone: +82-2-2049-6090 | Fax: +82-2-2201-6295 | E-mail: [anjoo@konkuk.ac.kr](mailto:anjoo@konkuk.ac.kr)

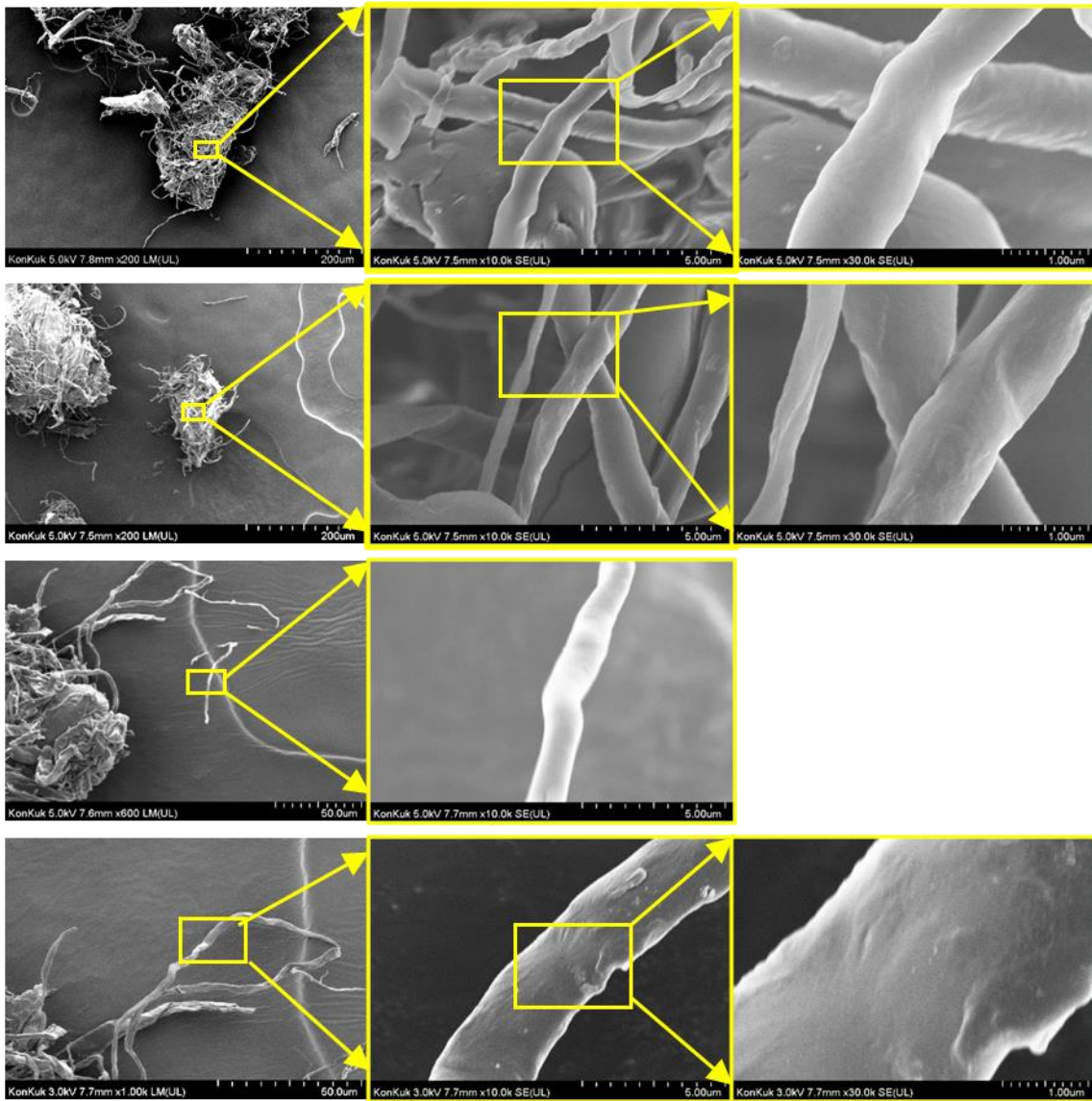
**Table S1.** Score table for earthworm seminal vesicle tissue adapted from Kwak and An (Kwak and An, 2021). Average scores for control and exposed groups were 2.1 and 0.8, respectively. The asterisk indicates significant decreases in the exposed groups ( $p < 0.05$ ).

Score	Seminal vesicle tissue structure disruption		Number of samples	
			Control	Exposed
3	None	Normal development and arrangement of germ cells	4	0
2	Mild	Slightly disordered distribution of germ cells	2	3
1	Moderate	Significantly disordered distribution of germ cells, moderate decrease of mature sperm bundles (decreased by 30% compared with control)	3	2
0	Severe	Severe decrease of mature sperm bundles (decreased by ~50% compared with control)	0	5
Ave.±Std.			2.1±0.9 *	0.8±0.9
Total replicates			9	10

**Fig. S1.** FE-SEM and stereomicroscopic images of the (a) outer layers, (b) MB filter, and (c) inner layers of a pristine mask. Masks were made of polypropylene microfibers.



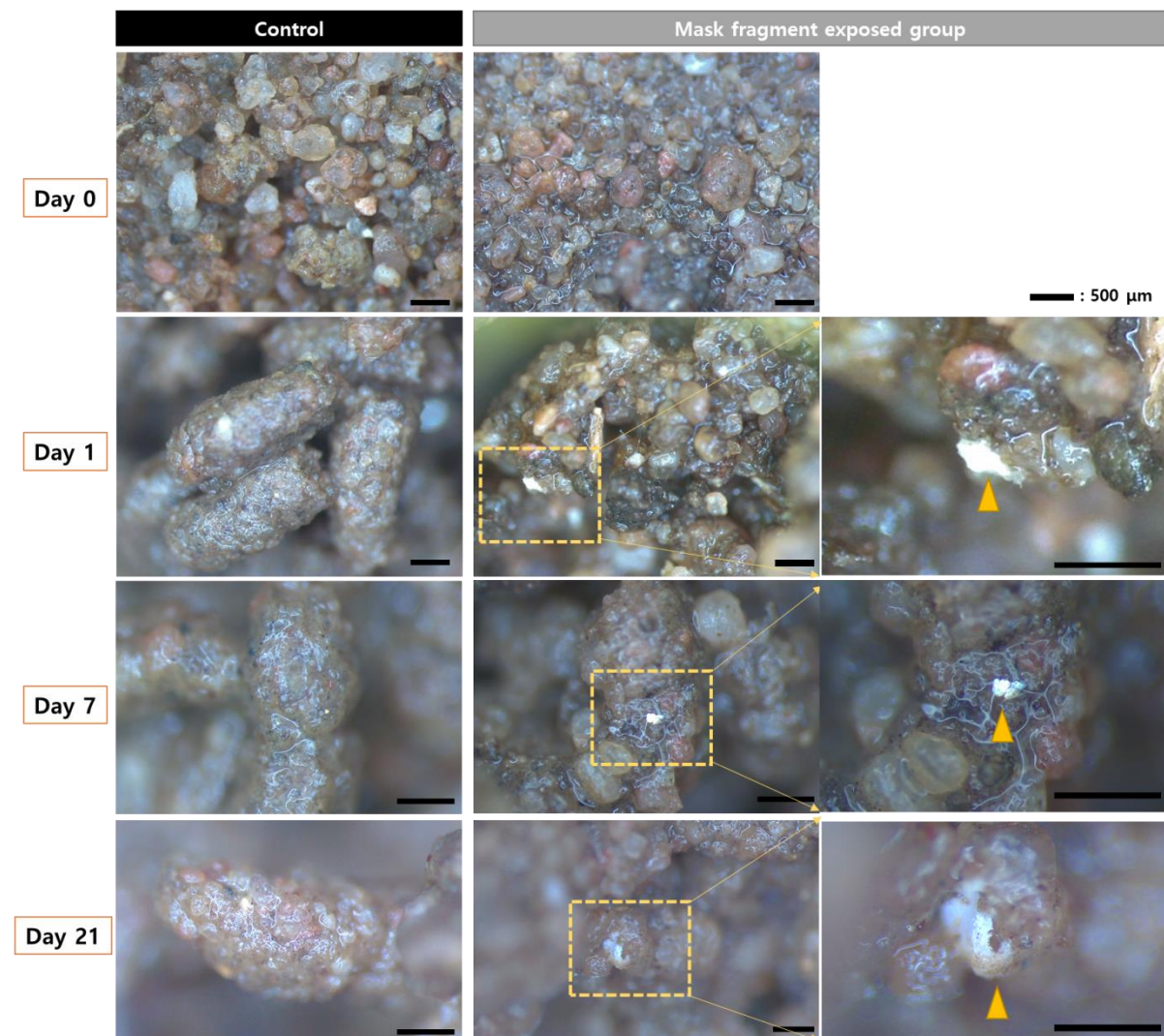
**Fig. S2.** FE-SEM images of pristine MB filter fibers and fragments (size: < 300  $\mu\text{m}$ ).



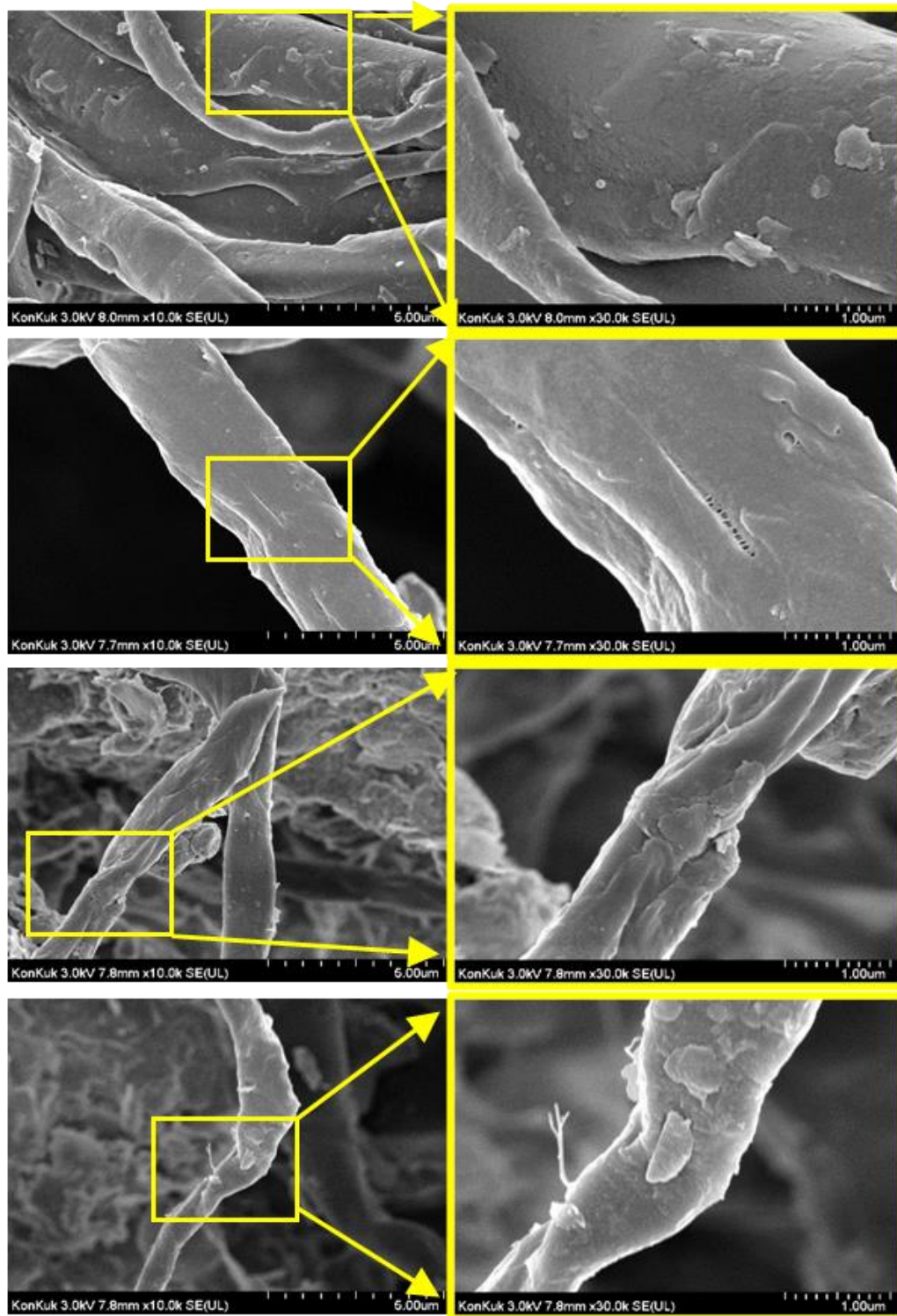
**Fig. S3.** Ingested MB filter fibers and fragments by *F. candida*. Control (untreated), leachate control, and MB filter exposed groups were investigated. Yellow arrows indicate ingested fluorescent mask fragments in (a) juvenile and (b) adult springtail 7 days after exposure.



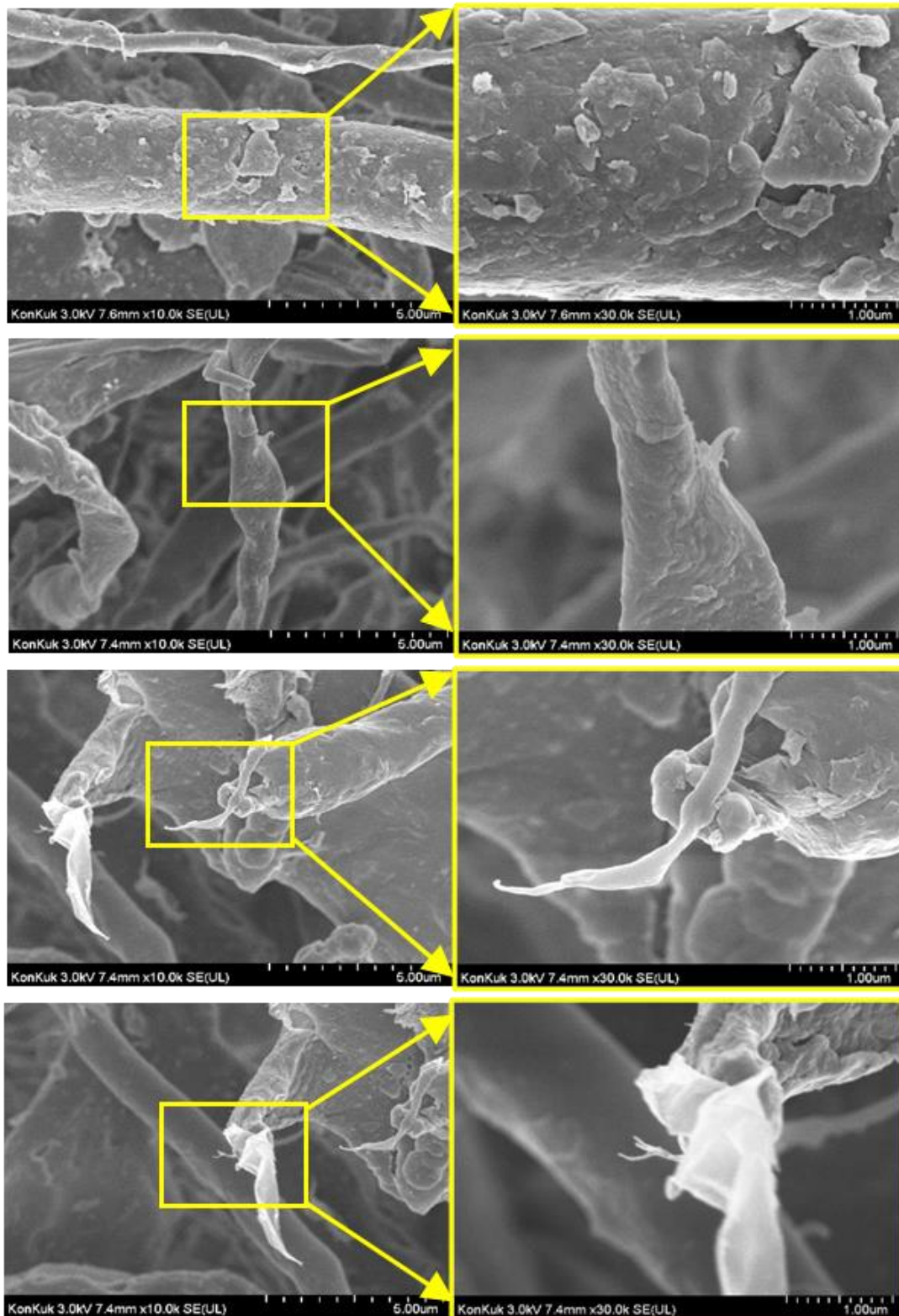
**Fig. S4.** Excreted earthworm casts containing MB filter fibers and fragments.



**Fig. S5.** FE-SEM images of collected MB filter fibers and fragments from earthworm cast 21 days after exposure. These images show the surfaces of four different microfibers, which are rougher than the surfaces of the microfibers of pristine MB filter fragments as shown in Fig. S2.

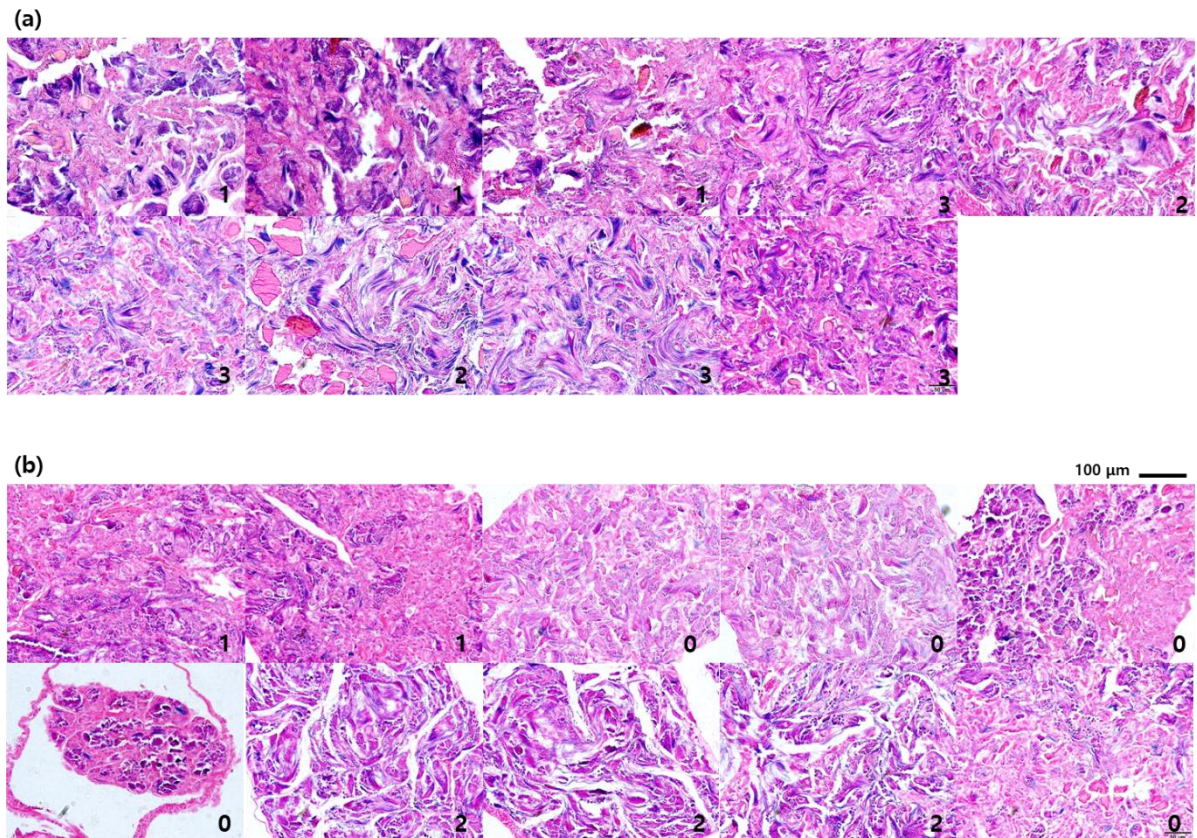


**Fig. S6.** FE-SEM images of collected MB filter fibers and fragments from springtail test soil 28 days after exposure. These images show the surfaces of four different microfibers, which are rougher than the surfaces of the microfibers of the pristine MB filter fragments as shown in Fig. S2.

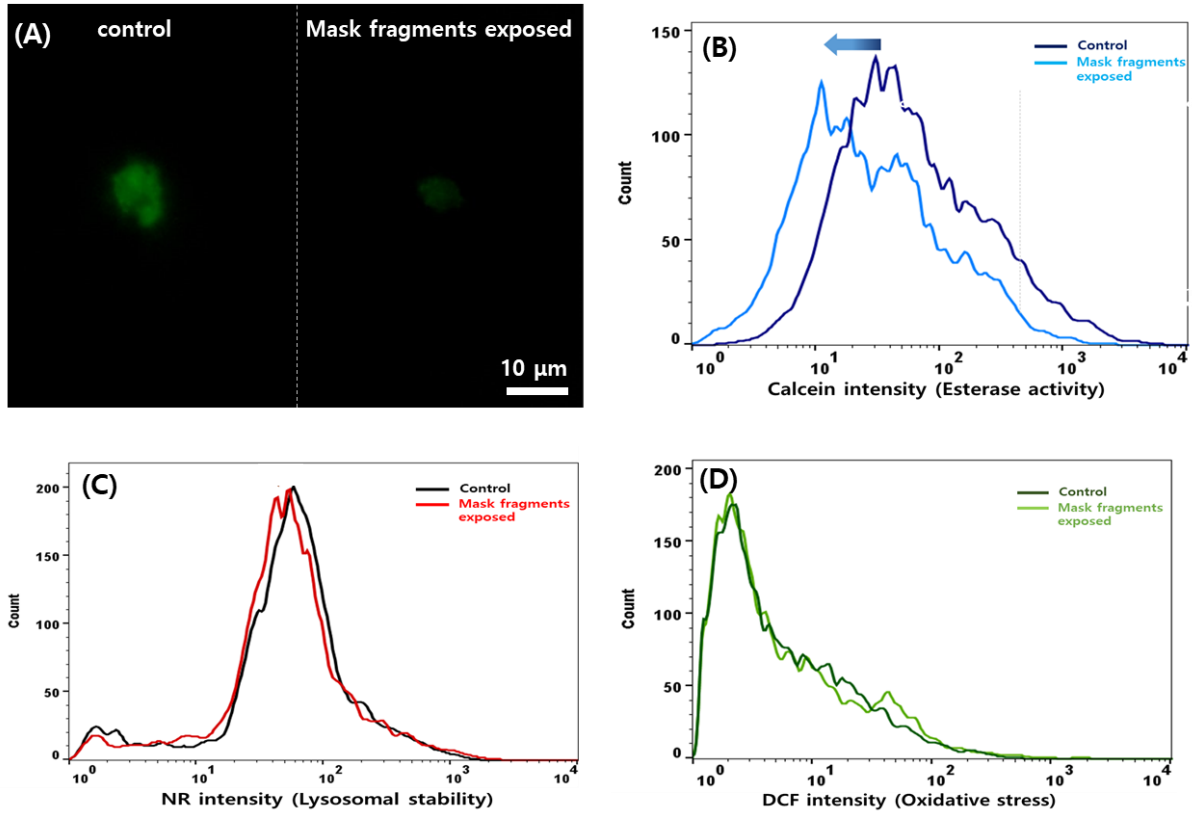




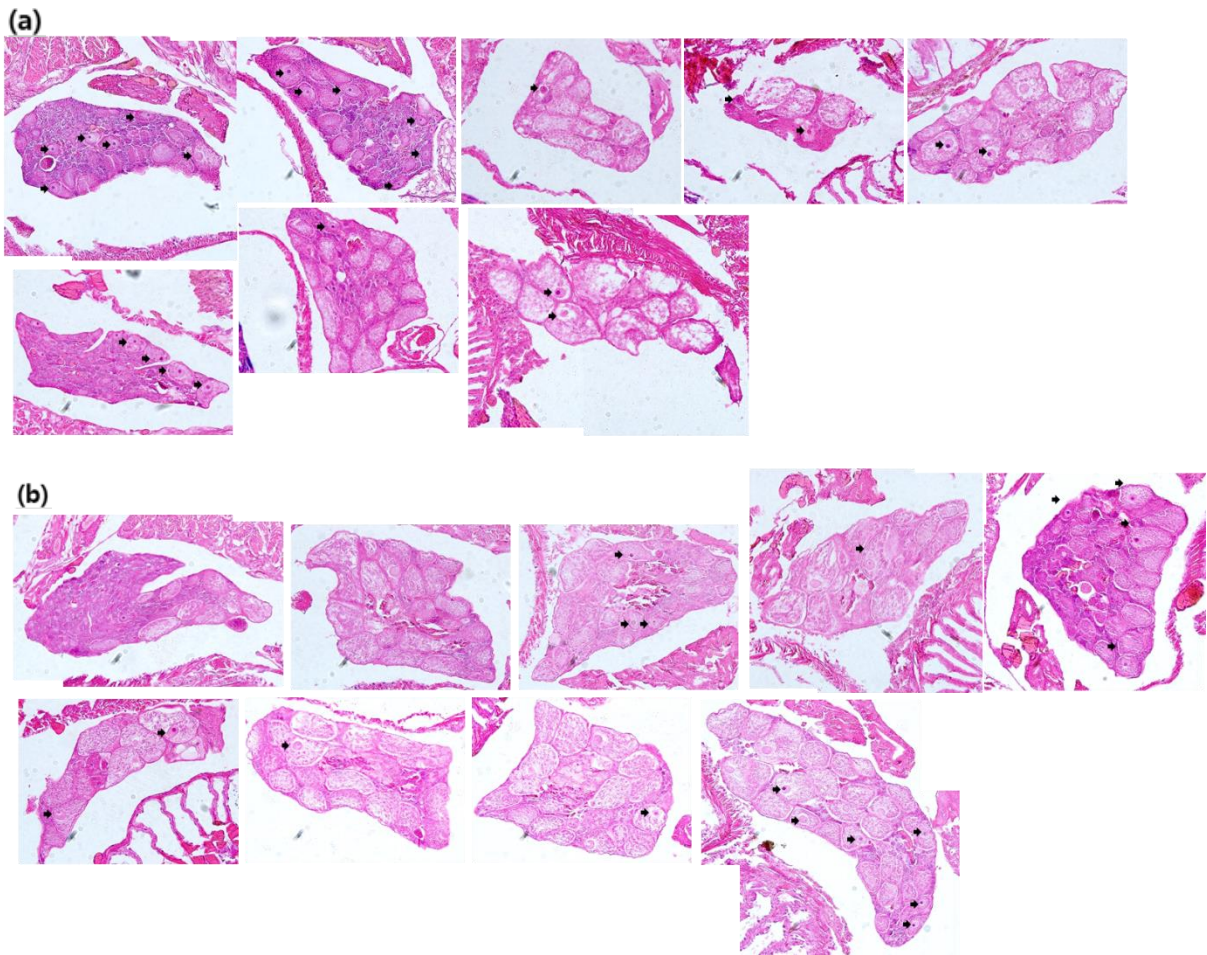
**Fig. S7.** Earthworm seminal vesicles tissues after H&E staining. (a) and (b) indicate control and exposed groups, respectively. In the exposure groups, less mature sperms and spermids (i.e., nuclei stained blue) were observed. Each score was evaluated based on Table S1. The arabic numerals on the bottom of each image indicate seminal vesicle score. In total, nine seminal vesicles from five earthworms and ten seminal vesicles from another five earthworms were investigated in control and exposure groups, respectively.



**Fig. S8.** *In vivo* cytotoxicity of mask MB filter fibers and fragments in earthworm coelomocytes 21 days after exposure. (a) Green, fluorescent images of coelomocytes indicating intracellular esterase activity. (b–d) Representative flow cytometric histograms for esterase activity, lysosomal stability, and oxidative stress, respectively.



**Fig. S9.** Earthworm ovarian tissues after H&E staining. (a) and (b) indicate control and exposed groups, respectively. Black arrows indicate oocytes. In total, eight ovaries from four earthworms and nine ovaries from another five earthworms were investigated in control and exposure groups, respectively.



## Supplementary References

Kwak, J.I., An, Y.-J., 2021. Microplastic digestion generates fragmented nanoplastics in soils and damages earthworm spermatogenesis and coelomocyte viability. *J. Hazard. Mater.* 402, 124034.