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Abiotic and Biotic Factors Influencing the Effect of Microplastic on Soil Aggregation

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Abstract: Plastic is an anthropogenic, ubiquitous and persistent contaminant accumulating in our environment. The consequences of the presence of plastics for soils, including soil biota and the processes they drive, are largely unknown. This is particularly true for microplastic. There is only little data available on the effect of microplastics on key soil processes, including soil aggregation. Here, we investigated the consequences of polyester microfiber contamination on soil aggregation of a sandy soil under laboratory conditions. We aimed to test if the microfiber effects on soil aggregation were predominantly physical or biological. We found that soil biota addition (compared to sterile soil) had a significant positive effect on both the formation and stabilization of soil aggregates, as expected, while wet-dry cycles solely affected aggregate formation. Polyester microfiber contamination did not affect the formation and stability of aggregates. But in the presence of soil biota, microfibers reduced soil aggregate stability. Our results show that polyester microfibers have the potential to alter soil structure, and that these effects are at least partially mediated by soil biota.

Keywords: soil aggregation; microplastic; polyester; fiber; soil microbes; wet-dry cycle

1. Introduction

We live in a plastic age [1], and an increasing number of ecosystem compartments is shown to contain various amounts of microplastics. Microplastics are defined as pieces of plastic smaller than 1 mm (or <5 mm in some definitions). While most work on microplastic in the environment has taken place in aquatic systems, soils have come into focus more recently [2], and it is now proposed to consider microplastic pollution as a factor of global change, also in terrestrial ecosystems [3].

Data on the presence of microplastic particles in soils are slowly accumulating, with methods for their detection currently under heavy development. The current status is that microplastics are found in soils world-wide, even in rather remote areas not immediately under human influence [4]. Given the assumed persistence of this material in the environment [2], it is important to understand its effects. Initial studies have shown that microplastic can affect soil physical properties [5,6], most notably soil aggregation, and soil biota. The evidence for the interplay of soil biota and microplastic is recently advancing with examples for microplastic ingestion and utilization [7–9], but also its transport and incorporation into the soil matrix [10,11].

When considering the type of microplastic, most pronounced effects on soil parameters were detected for microfibers, which in a field 'garden' experiment (in the absence of plants) led to decreased water-stability of macroaggregates. The mechanisms underpinning this effect are presently unknown, but could involve purely physical effects (perhaps steric hindrance, or unstable entanglement of soil particles) or biological effects, through effects on soil biota contributing to soil aggregation, or a combination of both.

We here present results from a laboratory incubation during which soils were allowed to interact with polyester microfibers. We tested for pure physical effects by using sterile soils, and for biological effects by adding a 'microbial filtrate' to reestablish a living soil component. We also tested if wet-dry cycles would alter the effects of microplastic on soil aggregation, since microfibers and microplastic films are also known to influence water holding capacity and soil evaporation [5,6].

2. Materials and Methods

To test the influence of biotic and abiotic factors on the interplay of microplastic and soil aggregation, we conducted two experiments: in the first experiment, we investigated the impact of wet-dry cycles on a sterile microplastic soil aggregate system, while in the second experiment, we evaluated the influence of soil microbes on the same test system but without wetting-drying cycles. Each experiment comprised a fiber treatment. The two experiments followed a 2 × 2-factorial design with 10 replicates resulting in two-times 40 experimental units. The experiments were designed to have identical controls and were set up on two consecutive days, which allowed pooling of control units for increased statistical power during the analyses (for further information see statistics section).

2.1. Microplastic Fibers

The applied fiber treatment consisted of a polyester fiber and a fiber-less control. We used polyester fibers (Glorex Inspirations, Bastelwatte, 100% polyester, item number: 6252105) which were manually cut to approximately 5.0 mm length to generate microplastic fibers. These were mixed in the test soil at concentration of 1.0 g polyester in 1.0 kg soil (w:w). Fibers were separated manually before mixing in the soil to guarantee an equal distribution throughout the soil sample. For the fiber-less control, we also mixed the soil to provide the same disturbance as was applied to the fiber treatment. We prepared the whole soil before separating into the individual experimental units.

2.2. Soil

We used a local soil which has a sandy silt texture (Albic Luvisol following FAO classification; 74% sand, 18% silt, 8% clay), with 6.9 mg/100 g P (calcium-acetate-lactate), 5.0 mg/100 g K (calcium-acetate-lactate), 0.12% total N and 1.87% total C content and a soil pH of 7.1 [12].

2.3. Experiment 1—Wet-Dry Cycles

We established a 2-dimensional soil system in 6 cm petri dishes (made of Polystyrene) by adding 12 g soil. The soil was previously air-dried, pre-sieved (<2 mm), and stored in glass test tubes in which it was autoclaved subsequently on two following days. The system had a fill height of max. 4 mm.

To produce the microbial wash, we used half of the (non-sterile) soil amount for filling the petri dishes; hence the microbial wash had 50% soil strength. For the preparation, the soil was soaked with twice as much deionized water as needed for watering the soil systems. Subsequently, we let the soil sit for 10 min, thoroughly stirred for 2 min two-times, and then decanted the solution through a 53 µm mesh to exclude nematodes and microarthropods. Finally, the microbial wash was autoclaved, so that we only added the organic matter and nutrients, but no living microbes. This step was necessary to keep the system settings as comparable as possible to those of experiment 2.

The incubation started after activating the system by adding 3.2 mL sterile microbial wash as droplets added at 1 cm height at random positions; this amount of water was chosen to reach 60% of the soil water holding capacity. Prior to the addition, the solution was shaken thoroughly to avoid settling of soil particles at the bottom of the storage bottle. The water was allowed to soak, then systems were sealed and stored in the dark at room temperature (25 °C).

For the application of the wet-dry cycles treatment, petri dishes were slightly opened and placed in a drying oven. Before use, the oven was cleaned with 70% ethanol. Samples were allowed to dry at 25 °C. After 24 h, samples were re-wetted carefully by adding again 3.2 mL sterile, deionized water (to

not add any more organic matter). Wet-dry cycles were applied on three occasions, with one event each week for three weeks.

After 63 days, the experiment was harvested by drying the opened systems at 30 °C for 2 days. Subsequently, samples were stored in their closed petri dishes in zip lock bags filled with silica gel to avoid re-moistening.

2.4. Experiment 2—Soil microbes

We used the same soil system as in experiment 1, but this time we introduced a microbial treatment instead of a wetting-drying event. The microbial treatment consisted of a living and a sterile autoclaved microbial wash, which was prepared as described under experiment 1 with the exception that we split the soil solution into two portions: one was stored at 4 °C until use the next day, while the other portion was autoclaved to produce the sterile microbial wash.

We applied the two different microbial washes (sterile and alive) to their designated systems by adding 3.2 mL of the respective solution. We assumed a movement of microbes via water flow during the soaking and equilibration process. Additionally, we chose a rather flat and 2-dimensional system to further support the spread of the added microbes. The incubation, storage and harvest followed the same protocol as in experiment 1.

2.5. Soil Aggregation Measurements

We evaluated two indices for our soil samples: (1) new formed aggregates >2.0 mm and (2) the percentage of water-stable aggregates. For this, samples were prepared by carefully extracting the soil from the petri dishes and passing them through a 4 mm sieve to break up drying artifacts.

Next, we measured the soil fraction >2.0 mm to determine the amount of aggregates formed *de novo* from a soil sample <2.0 mm after 9 weeks of incubation. We placed the prepared samples on a 2.0 mm sieve. The sieve was moved vertically two times to allow samples to separate while avoiding abrasion. The weight of the fraction remaining on the sieve was used for calculating the new formed aggregates following the equation: new formed aggregates = (aggregates > 2 mm/12.0) × 100.

Next, we combined the >2.0 mm fraction with the remaining soil sample and carefully mixed both before taking a 4.0 g sub-sample for determination of water stability of aggregates. We followed a modified protocol by Kemper and Rosenau [13]. Briefly, the percentage of water stable aggregates was determined by placing samples (4.0 g consisting of aggregates <4.0 mm) on small sieves with a mesh size of 0.25 mm. We used capillary re-wetting with deionized water and inserted samples into a sieving machine (Agrisearch Equipment, Eijkelkamp, Giesbeek, Netherlands). During the sieving process, samples were agitated for 3 min. The agitation and re-wetting causes the treated aggregates to slake. The process of slaking is determined by the volume of entrapped air inside the aggregates and the re-wetting intensity. During the process, samples separate into water-unstable and water-stable (>0.25 mm) fractions; the latter fraction was additionally corrected for any included debris (i.e., coarse matter), to allow calculations of the percentage of water-stable aggregates (%WSA) per sample: %WSA = (water stable fraction-coarse matter)/(4.0 g-coarse matter).

2.6. Statistics

We evaluated the impact of the abiotic and biotic factors on the interplay of microplastic fibers and the two soil aggregation indices by a generalized least square model for each experiment separately in the 'nlme' package [14]. We pooled the control data of both experiments to increase statistical power. For the resulting imbalanced model, we implemented type III sums of squares. Model residuals were checked for heteroscedasticity and normal distribution. We implemented the varIdent() function to account for heterogeneity in the drought and soil microbe treatment, respectively. If necessary, data were square root transformed. All statistics were conducted in R [15], and we generated plots via the graphic package 'ggplot2' [16]. All data used for analyses and plotting are available in the Supplementary Data.

3. Results

3.1. Experiment 1—Wet-Dry Cycles

We found a significant effect of the applied wet-dry cycles on *de novo* formed aggregates (Figure 1a); this effect was only present as a non-significant trend for the aggregate stability (Figure 1b). The addition of polyester microfibers did not influence any of the two tested soil aggregation indices (Table 1).

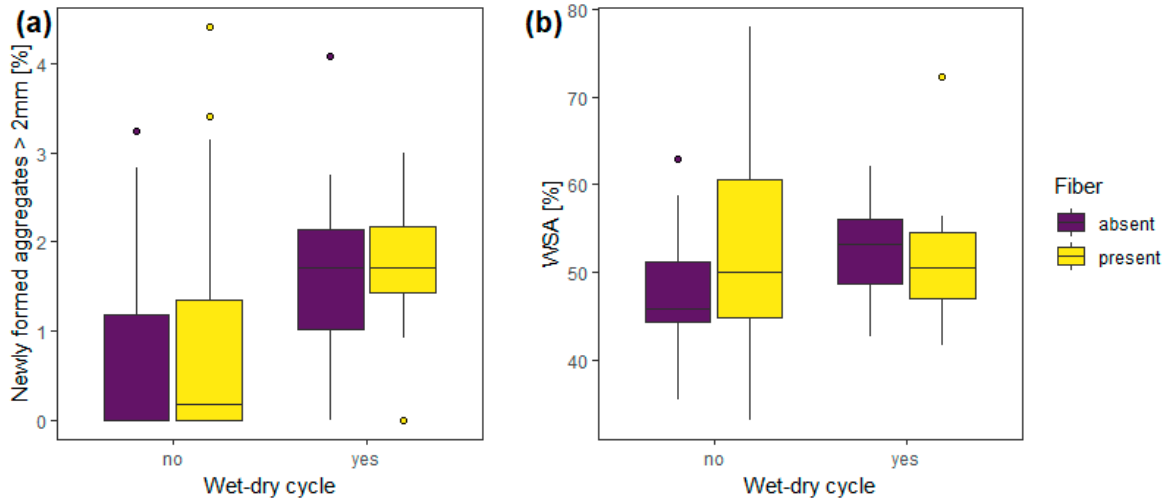

Figure 1. Impact of wet-dry cycles on the effect of polyester microfibers on (**a**) new formed soil aggregates (in %) and (**b**) water-stable aggregates (in %). The boxplots represent 25th and 75th percentile, median and outlying points. Presented data were untransformed. Corresponding model statistics can be found in Table 1.

Table 1. Results of generalized least square model on abiotic and biotic factors influencing the interaction of microplastic fibers and soil aggregation (new formed aggregates and water stable aggregates (WSA)). Significant *p*-values are shown in bold. Data were square root transformed with the exception of WSA in experiment 2.

		Newly Formed Aggregates			WSA		
		df	**F**	**P**	**df**	**F**	**P**
Experiment 1	wet-dry cycle	1, 56	9.97	0.01	1, 56	2.56	0.12
	fibers	1, 56	0.63	0.43	1, 56	2.03	0.16
	wet-dry cycle: fibers	1, 56	0.19	0.67	1, 56	1.13	0.29
Experiment 2	Soil microbes	1, 56	96.68	<0.0001	1, 56	8.18	**0.01**
	fibers	1, 56	0.63	0.43	1, 56	2.26	0.14
	Soil microbes: fibers	1, 56	0.14	0.71	1, 56	6.45	**0.01**

3.2. Experiment 2—Soil Microbes

The soil biota treatment significantly increased the amount of newly formed aggregates but the addition of polyester fibers did not affect this pattern (Figure 2a). For the water-stable aggregates, we could detect an increase in aggregate stability for the fiber-less samples under the influence of soil biota. However, the application of microfibers neutralized this positive contribution of soil biota to soil aggregation (Figure 2b).

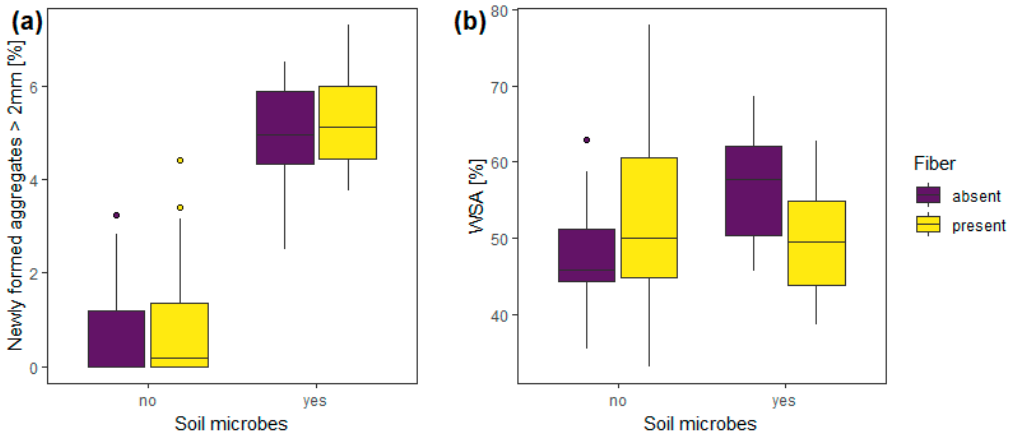

Figure 2. Impact of alive or inactivated soil microbes on the effect of polyester microfibers on (**a**) new formed soil aggregates (in %) and (**b**) water-stable aggregates (in %). The boxplots represent 25th and 75th percentile, median and outlying points. Presented data were untransformed. Corresponding model statistics can be found in Table 1.

4. Discussion

We here carried out a set of laboratory experiments aimed at understanding better how microfibers can influence soil aggregation. For this, we tested the contributions of wet-dry cycles and soil biota to soil aggregate formation and stability.

4.1. Wet-Dry Cycles and Soil Microbial Effects on Soil Aggregation

We found that the applied treatments (soil microbes and wet-dry cycles) increased aggregate formation but had only limited impact on aggregate stability (Figures 1 and 2). In experiment 1, we investigated the physical impact of wetting and drying cycles on soil aggregation of sterilized soil. We found an overall positive effect with a rather small magnitude. This low impact of the wet-dry cycles can be explained by the lack of soil microbes in our experimental system. It is known that soils with high microbial activity more strongly respond to wetting and drying [17] than soils with soil biota excluded e.g., [18]. In our tested meadow soil, we found that the beneficial contribution of the drying process apparently set off the detrimental effect of the wetting phase, thus leading to overall increased aggregate formation and stability [19–21]. The lack of soil biota can further decrease stability of aggregates during wetting and drying cycles [18].

In experiment 2, we evaluated the influence of soil biota on soil aggregation and found an overall positive effect; the presence of soil biota increased the formation and stability of aggregates (Figure 2). This supports the findings of a recent meta-analysis which showed that soil biota, including animals, bacteria and fungi, have an overall positive effect on soil aggregation [22]. Additionally, this agrees with the literature showing for natural and cultivated soils with sandy or clayey soil texture that the overall stability was reduced under soil biota exclusion (e.g., via soil sterilization [18,23] or application of sterilization solutions [24,25]). Soil biota are known to improve soil aggregation via a multitude of biological, biophysical and biochemical mechanisms see [21,26,27].

4.2. Microfiber Effects on Soil Aggregation

We artificially contaminated the test soil with polyester microfibers and found in both experiments that the addition of fibers caused no detectable effect in the new formed aggregate fraction. The fibers were added at a concentration of 0.1% (w:w); as demonstrated by Machado et al. [5] polyester fiber concentrations >0.2% clearly affected stability of aggregates. Hence, the concentration of microfibers is a critical factor which has to be considered in future studies. However, there is no verified estimate reported so far stating microfiber concentrations in natural or urban soils; mainly due to insufficient

analytical tools and protocols [28]. Thus incorporating realistic microfiber concentrations in test soils remains rather arbitrary.

Additionally, we detected a high variability in the water-stable aggregates in the presence of microfibers. This finding is in accordance with Machado et al. [5] who also detected a fiber-induced increase in the variability of water-stable aggregates which was not as pronounced for microplastic beads. The manual manufacturing of fiber fragments could be potentially responsible for this variability. Hence, there is an urgent need for techniques for production of large microfiber fragments of standardized length as is available for small microfiber fragments <100µm [29].

4.3. Interactive Effects of Microplastic and Biotic and Abiotic Factors

We found that for water-stable aggregates, polyester microfibers reduced the effect of the treatments (soil microbes and wet-dry cycle) compared to the control; in fact, it had the opposite direction, as supported by the significant interaction term in experiment 2 (Table 1). For experiment 1, a similar but not significant pattern could be found. This pattern indicates that the microfibers tested here influenced soil aggregation via physical and biological mechanisms.

Microfibers get incorporated into different aggregate size fractions [30]. While they provide no gluing or cementing function and exhibit no pronounced entanglement potential while generally decreasing bulk density [5], it can be expected that microfibers reduce the overall stability of aggregates. On the biological side, soil communities are hypothesized to shift under the influence of microplastic by e.g., its potential utilization as a carbon containing resource [31]. Although plastic can be seen as persistent compound, there is evidence from marine and soil systems that there are microbes utilizing plastic polymers [8,9]. Additionally, by influencing the soil structure and bulk density, microplastic could affect the available pore space in soil and hence microhabitats of soil microbes. Nevertheless, given that we added a microbial consortium in our experiment (likely consisting of bacteria, fungi, protozoa and others) we cannot pinpoint which organisms contributed to the effect we observed here, i.e., reduced soil aggregation in the presence of microfibers.

In summary, our study adds to the increasing database of microplastic effects on soil by showing that microfibers tend to decrease soil aggregate water-stability, and that a biological rather than a physical effect contributes to this outcome. Future studies should now dissect the biological responses in greater detail to come to a more detailed mechanistic understanding of microplastic effects.

Supplementary Materials: Supplementary data (raw data) are available at http://www.mdpi.com/2571-8789/3/1/21/s1, for this study.

Author Contributions: A.L. and M.C.R. designed the research; K.F. performed the research; A.L. conceived and performed the data analyses; A.L. and M.C.R. wrote the manuscript; all authors contributed to the final version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thompson, R.C.; Swan, S.H.; Moore, C.J.; vom Saal, F.S. Our plastic age. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* **2009**, *364*, 1973–1976. [CrossRef] [PubMed]
2. Rillig, M.C. Microplastic in terrestrial ecosystems and the soil? *Environ. Sci. Technol.* **2012**, *46*, 6453–6454. [CrossRef] [PubMed]
3. Machado, A.A.D.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M.C. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Chang. Biol.* **2018**, *24*, 1405–1416. [CrossRef] [PubMed]
4. Scheurer, M.; Bigalke, M. Microplastics in Swiss floodplain soils. *Environ. Sci. Technol.* **2018**, *52*, 3591–3598. [CrossRef] [PubMed]
5. Machado, A.A.D.; Lau, C.W.; Till, J.; Kloas, W.; Lehmann, A.; Becker, R.; Rillig, M.C. Impacts of microplastics on the soil biophysical environment. *Environ. Sci. Technol.* **2018**, *52*, 9656–9665. [CrossRef] [PubMed]

6. Wan, Y.; Wu, C.; Xue, Q.; Hui, X. Effects of plastic contamination on water evaporation and desiccation cracking in soil. *Sci. Total Environ.* **2019**, *654*, 576–582. [CrossRef]
7. Lwanga, E.H.; Gertsen, H.; Gooren, H.; Peters, P.; Salanki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A.A.; Geissen, V. Microplastics in the terrestrial ecosystem: Implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). *Environ. Sci. Technol.* **2016**, *50*, 2685–2691. [CrossRef]
8. Lwanga, E.H.; Thapa, B.; Yang, X.M.; Gertsen, H.; Salanki, T.; Geissen, V.; Garbeva, P. Decay of low-density polyethylene by bacteria extracted from earthworm's guts: A potential for soil restoration. *Sci. Total Environ.* **2018**, *624*, 753–757. [CrossRef]
9. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the "plastisphere": Microbial communities on plastic marine debris. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146. [CrossRef]
10. Lwanga, E.H.; Gertsen, H.; Gooren, H.; Peters, P.; Salanki, T.; van der Ploeg, M.; Besseling, E.; Koelmans, A.A.; Geissen, V. Incorporation of microplastics from litter into burrows of *Lumbricus terrestris*. *Environ. Pollut.* **2017**, *220*, 523–531. [CrossRef]
11. Maass, S.; Daphi, D.; Lehmann, A.; Rillig, M.C. Transport of microplastics by two collembolan species. *Environ. Pollut.* **2017**, *225*, 456–459. [CrossRef] [PubMed]
12. Rillig, M.C.; Mardatin, N.F.; Leifheit, E.F.; Antunes, P.M. Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates. *Soil Biol. Biochem.* **2010**, *42*, 1189–1191. [CrossRef]
13. Kemper, W.D.; Rosenau, R.C. Aggregate stability and size distribution. In *Methods of Soil Analysis. Part I—Physical and Mineralogical Methods*, 2nd ed.; Lute, A., Ed.; SSSA: Madison, MI, USA, 1986; pp. 425–443.
14. Pinheiro, J.; Bates, D.; DebRoy, S.; Sarkar, D.; Team, R.C. *Nlme: Linear and Nonlinear Mixed Effects Models*, R package version 3.1-137; 2018.
15. R Development Core Team. *R: A Language and Environment for Statistical Computing*, version 3.4.1; 2014.
16. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2009.
17. Cosentino, D.; Chenu, C.; Le Bissonnais, Y. Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. *Soil Biol. Biochem.* **2006**, *38*, 2053–2062. [CrossRef]
18. Tisdall, J.; Cockroft, B.; Uren, N. The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. *Aust. J. Soil Res.* **1978**, *16*, 9–17. [CrossRef]
19. Denef, K.; Six, J.; Bossuyt, H.; Frey, S.D.; Elliott, E.T.; Merckx, R.; Paustian, K. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. *Soil Biol. Biochem.* **2001**, *33*, 1599–1611. [CrossRef]
20. Dorioz, J.M.; Robert, M.; Chenu, C. The role of roots, fungi and bacteria on clay particle organization—An experimental approach. *Geoderma* **1993**, *56*, 179–194. [CrossRef]
21. Six, J.; Bossuyt, H.; Degryze, S.; Denef, K. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Till Res* **2004**, *79*, 7–31. [CrossRef]
22. Lehmann, A.; Zheng, W.S.; Rillig, M.C. Soil biota contributions to soil aggregation. *Nat. Ecol. Evol.* **2017**, *1*, 1828–1835.
23. Sutton, J.C.; Sheppard, B.R. Aggregation of sand-dune soil by endomycorrhizal fungi. *Can. J. Bot./Rev. Can. Bot.* **1976**, *54*, 326–333. [CrossRef]
24. Molope, M.B.; Grieve, I.C.; Page, E.R. Contributions by fungi and bacteria to aggregate stability of cultivated soils. *J. Soil Sci.* **1987**, *38*, 71–77. [CrossRef]
25. Roldan, A.; Garciaorenes, F.; Lax, A. An incubation experiment to determine factors involving aggregation changes in an arid soil receiving urban refuse. *Soil Biol. Biochem.* **1994**, *26*, 1699–1707. [CrossRef]
26. Bronick, C.J.; Lal, R. Soil structure and management: a review. *Geoderma* **2005**, *124*, 3–22. [CrossRef]
27. Lehmann, A.; Leifheit, E.F.; Rillig, M.C. Mycorrhizas and soil aggregation. In *Mycorrhizal Mediation of Soil-Fertility, Structure, and Carbon Storage*, 1st ed.; Johnson, N., Gehring, C., Jansa, J., Eds.; Elsevier: Amsterdam, The Netherlands, 2016; pp. 241–262.
28. Blasing, M.; Amelung, W. Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.* **2018**, *612*, 422–435. [CrossRef]
29. Cole, M. A novel method for preparing microplastic fibers. *Sci. Rep.* **2016**, *6*, 34519. [CrossRef] [PubMed]

30. Zhang, G.S.; Liu, Y.F. The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci. Total Environ.* **2018**, *642*, 12–20. [CrossRef]
31. Rillig, M.; de Souza Machado, A.; Lehmann, A.; Klümper, U. Evolutionary implications of microplastics for soil biota. *Environ. Chem.* **2018**. [CrossRef]