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Microplastic inclusion in birch tree roots

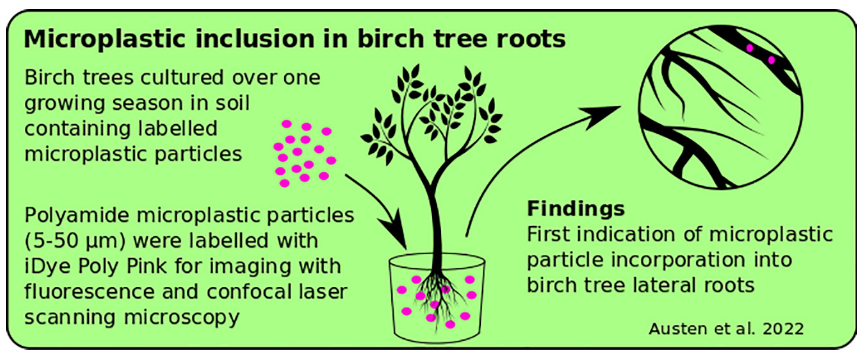
Kat Austen [a,b,*], Joana MacLean [c], Daniel Balanzategui [d,e], Franz Hölker [a]

[a] Leibniz Institute for Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany
[b] Studio Austen, Lehderstrasse 74, 13086 Berlin, Germany
[c] GFZ German Research Centre for Geosciences, Section Geomicrobiology, 14473 Potsdam, Germany
[d] Natural Sciences Unit, German Archaeological Institute, Podbielskiallee 69-71, 14195 Berlin, Germany
[e] Section 4.3 - Climate Dynamics and Landscape Evolution, German Centre for Geosciences, Wissenschaftpark "Albert Einstein", Telegrafenberg, 14473 Potsdam, Germany

HIGHLIGHTS

- Microplastic beads have been detected included in birch tree roots.
- Experiments introduced microplastic beads of 5–50 μm in size to the soil around saplings' roots.
- After 5 months' growth, microplastic beads were observed within the tree roots using fluorescence and confocal laser scanning microscopy.

GRAPHICAL ABSTRACT



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ABSTRACT

In this pilot study, microplastic beads (5–50 μm) were tagged with fluorescent dye and introduced to the soil of potted *Betula pendula* Roth. (silver birch) saplings during the growing season. After five months, root samples were examined using fluorescence- and confocal laser scanning microscopy. This paper presents the first documented indication of the incorporation of microplastic into root tissues of woody plants and discusses the phytoremediation potential of birch in soil with microplastic contamination.

1. Introduction

Microplastics are ubiquitous, anthropogenic contaminants of emerging concern. Due to plastics' inherent durability they are expected to accumulate and persist in the environment. Microplastics originate from both the breakdown of larger plastic fragments and from the release of primary materials from, for example, cleaning products. Microplastics are thereafter easily transported in water, air and soil (Petersen and Hubbart,

* Corresponding author at: Leibniz Institute for Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany.
E-mail address: iam@katausten.com (K. Austen).

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2021). With 4900 Mt. of plastics, 60% of all plastics ever produced since the 1950's, entering aquatic and terrestrial ecosystems (Geyer et al., 2017), it is not surprising that microplastics have reached the bottom of the Mariana Trench (Peng et al., 2018) and the top of Mount Everest (Napper et al., 2020). For the organisms, including humans, co-existing alongside these foreign objects in the so-called plastisphere (Zettler et al., 2013), the impact of microplastic on physiological, morphological, behavioral, and biochemical functions is only beginning to be explored (Petersen and Hubbart, 2021).

Early research on the influence and legacy of microplastics in natural environments has focussed mainly on the distribution of microplastics in aquatic systems and their interaction with aquatic flora and fauna (Qi et al., 2020). While less obvious than in oceans and waterways, microplastics accumulate in soils at levels 4–23 times higher than in marine ecosystems (Horton et al., 2017) through both direct introduction and atmospheric deposition (Qi et al., 2020). On land, microplastic concentration has been observed to be highest in soils near roads and in agricultural environments. Estimations of microplastic concentrations vary, with industrial topsoils reported to contain up to 7% plastic particles by weight, though they are often much lower (Büks et al., 2020; de Souza Machado et al., 2018). In more remote sites, the quantity and range of microplastic in urban forests remains poorly understood (Rillig et al., 2019) although microplastics have been detected in woodlands (Choi et al., 2020) and urban wetlands (Helcoski et al., 2020).

In terrestrial environments, microplastics alter the physicochemical properties of the soil and have the potential to affect plant growth (Khalid et al., 2020) and microbial communities (Qi et al., 2020). However, relatively little is known about how microplastic interacts with higher order terrestrial plants. Recent studies have shown microplastic uptake in the roots of agricultural species such as wheat (Li et al., 2019, 2020b) but until now little attention has been paid to whether longer-lived woody plants are capable of incorporating, and perhaps storing, this foreign material within their tissue.

2. Methods

Two potted juvenile birch trees were grown in a mix of soil and microplastic labelled with fluorescent dye. Polyamide (PA) powder was chosen as the source of microplastic for the experiment as it has been found, along with polypropylene, to dominate in polluted soil environments (Xu et al., 2020). Since particle size is observed to affect incorporation of microplastics into wheat roots (Li et al., 2020a), we used a PA powder with a particle size ranging between five and 50 μm (Nylon 6, AM306010; Goodfellow GmbH, Bad Nauheim, Germany), and labelled it using with iDye Poly Pink 456 dye (Jacquard), which has been shown to have fluorescent stability across a range of plastics (Karakolis et al., 2019). Under fluorescence, the dye exhibits excitation at 531/40 nm and emission at 593/40 nm (Karakolis et al., 2019). According to the method of Karakolis et al. (2019), a dye solution at a concentration of 0.1 gml^{-1} was prepared and used to label 50 g of microplastic material. The dyed microplastics were stored in deionized water at a concentration of 1 gml^{-1}.

In June 2020, 3 g of microplastic was mixed into the soil (15 l, dry weight 7 kg) of two, one-year-old birch saplings. The concentration of microplastic to soil (0.043% by dry weight) falls within the range of environmentally observed microplastic concentrations. Over the subsequent growing season, each tree received adequate and uniform sunlight, nutrients and water for a five-month period, each growing by 30 cm from starting heights of 120 and 122 cm. The trees were located in a sheltered, outdoor area in Berlin during the entire exposure period. Because 2020 exhibited a comparatively hot and dry summer (Deutscher Wetterdienst, n. d.), the trees were kept in partial shade. In addition, they were irrigated every day with about 0.5 ml each. Only organic quality, sandy compost mixture, typical for the Berlin-Brandenburg area, was used to exclude any prior contamination with microplastic. No further fertilization was applied. Tree health was maintained throughout the experiment, observed through assessment of parasites, leaf health and bud health. At the end of this period, the trees were removed from their pots and the number of coarse lateral roots was counted for each tree. For each tree, of 15 coarse lateral roots, four were harvested for image analysis with the remaining roots left intact to maximise the trees' chances of survival for further study.

For fluorescence microscopy, 8 cm sub-sections of the lateral roots were embedded in 5% agarose and longitudinal cross sections cut to a thickness of 20 μm and 40 μm using an advanced core microtome (Liang et al., 2013). 64 slices were analysed in total. Imaging was performed using a light microscope (Leica DMS 2000) attached to a digital camera (Leica DFC420) with a light-emitting diode (LED) providing the fluorescence source. Using green light at 531/40 nm for excitation of the dyed PA particles it was possible to visually identify the microplastics within the root tissue. Confocal Laser Scanning Microscopy has been previously used to identify microplastic in plant tissue (Li et al., 2020b). For CLSM analysis (Liang et al., 2013) (543 nm, Olympus FluoView FV300), transverse root sections 0.8 mm in diameter were cut at thicknesses of 20 μm and 40 μm using an advanced core microtome (Gärtner and Nievergelt, 2010, p.). Prior to the preparation of thin sections, root samples were rinsed using running distilled water for several minutes to dislodge foreign particles (incl. microplastic) from the root surface. Samples were air-dried, mounted in paraffin or agarose, sectioned without fluids to avoid the redistribution of microplastics, and transferred to glass slides. Different layers throughout the slices were checked and the layer with the best resolution for the microplastic particles was used for observations and measurements, which were repeated three times. Regardless of the microscopy technique used, the presence of microplastic was visually confirmed in multiple longitudinal and transverse root sections. Images were corrected for brightness and contrast.

3. Results and discussion

Here, we report findings from a study on the potential for uptake and incorporation of primary microplastic beads into the root tissue of the pioneer species, *Betula pendula* Roth., commonly known as silver birch. Birch trees have been used to remediate contaminated land (Jonczak et al., 2020) by sequestering and storing industrial pollutants in their tissues, which subsequently allows the colonisation of microbial communities that breakdown polyaromatic hydrocarbons (Sipilä et al., 2008) and heavy metals (Rosselli et al., 2003). Common across temperate Eurasian and North American landscapes, birch trees rapidly colonize and stabilise disturbed land allowing for reforestation (Jonczak et al., 2020). Because the species' dense root system lies near the soil surface (Mauer and Palátová, 2012) where the concentration of microplastic pollution has been shown to be highest (Liu et al., 2018), the motivation for this study was, can birch trees offer phytoremediation of plastic-pervaded soils by the direct uptake of microplastic particles?

In this study we show that individual microplastic particles with diameters between 5 and 10 μm can enter the root system of a birch tree from the surrounding soil (Figs.1 and 2). By combining fluorescence and confocal laser scanning microscopy (CLSM), we could locate and visualize labelled microplastic particles embedded among root cell structures. Besides the natural autofluorescence of the tree root, evidence of fluorescing microplastic was found in the root exodermis, cortex and the vascular tissue of a lateral root (Fig. 2), as well as the outer epidermal layer and among root hairs. Together these results give a strong indication for the uptake and incorporation of soil-derived microplastic into juvenile birch roots, and suggest that further investigations addressing the rate of uptake of microplastic and its implications for long term tree health are necessary to determine if birch trees are suitable microplastic remediators.

A total of 64 root sections was examined, in 6 of which it was possible to detect between one and four microplastic particles, with a median of one. The percentage range of tree roots sections in which microplastic particles were observed over the two experimental trees was 5–17%. It was not possible to determine the entry point or mechanism responsible for moving the microplastic across the soil-root interface and transporting it to the inner section of the root. However, studies have already reported the direct uptake of sub- to micrometer sized plastic into the root tissue of annual plants

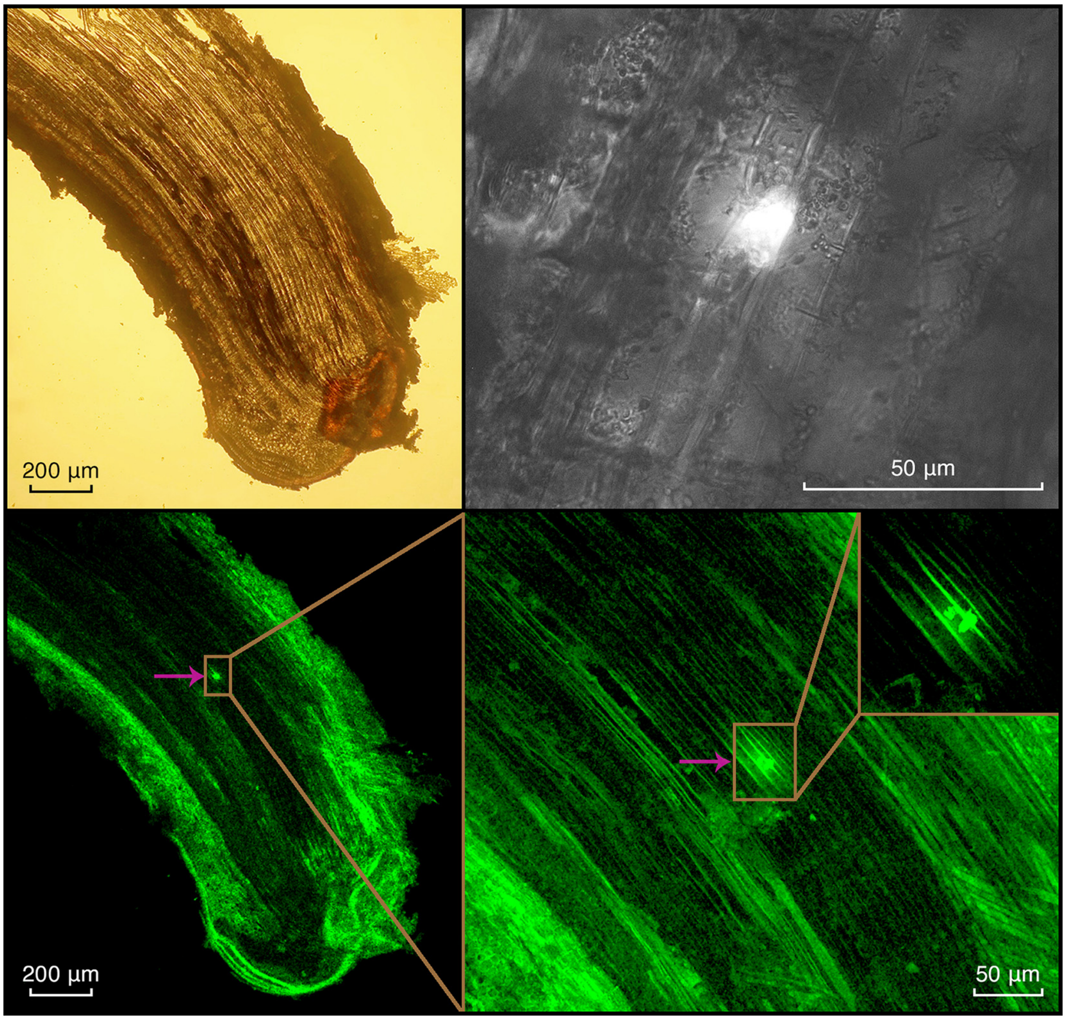

Fig. 1. Longitudinal cross-section showing microplastic particles inside a birch lateral root in a one-year-old birch tree after being exposed to contaminated soil for 5 months. Clockwise from top left shows cross sections using light microscopy, fluorescence microscopy, and confocal laser scanning microscopy. Arrows point to fluorescing microplastic particles. Inset in bottom right panel shows the area indicated by the arrows in the bottom left panel after enlargement. All images have been enhanced using a contrast and brightness correction. Scales are shown in the bottom right or left corner of the respective image.

(Li et al., 2019, 2020a). In the case of wheat and lettuce, microplastic uptake of particles below 10 μm occurred at the site of lateral root emergence through the crack-entry mode followed by the migration between intercellular spaces depending on internal gradients in hydrostatic pressure (Li et al., 2020a). Although the particle size of the PA powder used in this study ranged between 5 and 50 μm, our observations of microplastic particles with diameters less than 10 μm within the tree roots suggests that size may be a limiting factor in microplastic uptake. Because we have also found microplastic in the inner root, our results suggest that micrometer sized microplastic are capable of reaching the vascular tissue of the inner root, but for now we speculate that crack-entry and an apoplastic pathway, as observed in wheat and lettuce (Li et al., 2020a), are responsible for the uptake and transport of these foreign particles between various cell tissues.

4. Conclusions

The results of this pilot study demonstrate that the uptake and storage of microplastics in birch trees warrants further study. For instance, indications from wheat and lettuce studies exposed to smaller particle sizes of 0.2 μm–2 μm suggest that rates of microplastic inclusion in the submicrometre size range in birch tree roots may be significantly higher (Li et al., 2020a). The inclusion of microplastic beads into the root tissue gives a positive indication that other micro-and nano-sized particles arising from the breakdown of plastics in the environment could be incorporated into tree roots. Depending upon further study into the rate of uptake and its effect on short- and long-term tree health, and given the remediation potential of birch for other soil-based pollutants, we posit the potential of birch for long-term soil remediation solutions. Our findings lay the foundation for investigation of the role of birch trees in phytoremediation not only for sites with chemical contamination, but now also for those with substantial microplastic pollution.

Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper.

Credit authorship contribution statement

Kat Austen

Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Roles/Writing - original draft; Writing - review & editing.

Joana MacLean

Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Roles/Writing - original draft; Writing - review & editing.

Daniel Balanzategui

Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Roles/Writing - original draft; Writing - review & editing.

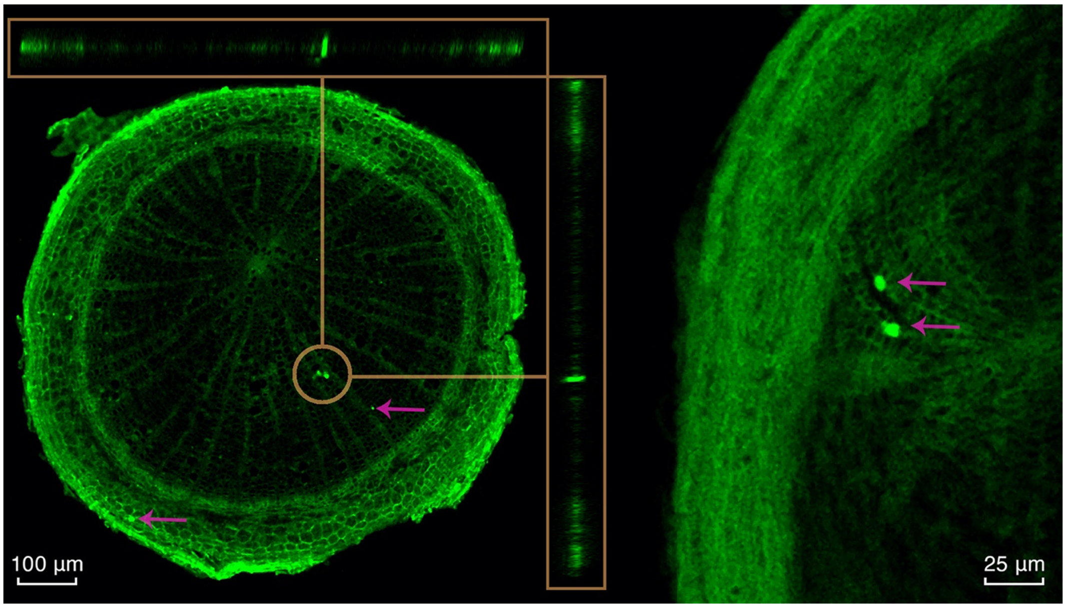

Fig. 2. Confocal laser scanning micrographs of two birch root cross sections showing the incorporation of microplastic particles in a one-year-old tree after being exposed to contaminated soil for 5 months. The location of microplastic particles are indicted by arrows and highlighted inside the circle. In the left image, the upper and right panels show the orthogonal view of the z-stacks, showing the presence of the fluorescing microplastic particle within the sample slice. The location of microplastic particles is indicated by arrows, circle, and guidelines from the circle to each z-stack. All images have been enhanced using a contrast and brightness correction. Scale is shown in the bottom right or left corner of the respsective image.

Franz Hölker
Methodology; Validation; Visualization; Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Büks, F., Loes van Schaik, N., Kaupenjohann, M., 2020. What do we know about how the terrestrial multicellular soil fauna reacts to microplastic? Soil 6, 245–267. https://doi.org/10.5194/soil-6-245-2020.
Choi, Y.R., Kim, Y.-N., Yoon, J.-H., Dickinson, N., Kim, K.-H., 2020. Plastic contamination of forest, urban, and agricultural soils: a case study of Yeoju City in the Republic of Korea. J. Soils Sediments https://doi.org/10.1007/s11368-020-02759-0.
Gärtner, H., Nievergelt, D., 2010. The core-microtome: a new tool for surface preparation on cores and time series analysis of varying cell parameters. Dendrochronologia 28, 85–92. https://doi.org/10.1016/j.dendro.2009.09.002.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782. https://doi.org/10.1126/sciadv.1700782.
Helcoski, R., Yonkos, L.T., Sanchez, A., Baldwin, A.H., 2020. Wetland soil microplastics are negatively related to vegetation cover and stem density. Environ. Pollut. 256, 113391. https://doi.org/10.1016/j.envpol.2019.113391.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190.
Jonczak, J., Jankiewicz, U., Kondras, M., Kruczkowska, B., Oktaba, L., Oktaba, J., Olejniczak, I., Pawłowicz, E., Polláková, N., Raab, T., Regulska, E., Słowińska, S., Sut-Lohmann, M., 2020. The influence of birch trees (Betula spp.) on soil environment – a review. For. Ecol. Manag. 477, 118486. https://doi.org/10.1016/j.foreco.2020.118486.
Karakolis, E.G., Nguyen, B., You, J.B., Rochman, C.M., Sinton, D., 2019. Fluorescent dyes for visualizing microplastic particles and fibers in laboratory-based studies. Environ. Sci. Technol. Lett. 6, 334–340. https://doi.org/10.1021/acs.estlett.9b00241.
Khalid, N., Aqeel, M., Noman, A., 2020. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. Environ. Pollut. 267, 115653. https://doi.org/10.1016/j.envpol.2020.115653.
Li, L., Zhou, Q., Yin, N., Tu, C., Luo, Y., 2019. Uptake and accumulation of microplastics in an edible plant. Chin. Sci. Bull. 64, 928–934. https://doi.org/10.1360/N972018-00845.
Li, L., Luo, Y., Li, R., Zhou, Q., Peijnenburg, W.J.G.M., Yin, N., Yang, J., Tu, C., Zhang, Y., 2020a. Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. Nat. Sustain. 3, 929–937. https://doi.org/10.1038/s41893-020-0567-9.
Li, L., Luo, Y., Peijnenburg, W.J.G.M., Li, R., Yang, J., Zhou, Q., 2020b. Confocal measurement of microplastics uptake by plants. MethodsX 7, 100750. https://doi.org/10.1016/j.mex.2019.11.023.
Liang, W., Heinrich, I., Helle, G., Liñán, I.D., Heinken, T., 2013. Applying CLSM to increment core surfaces for histometric analyses: a novel advance in quantitative wood anatomy. Dendrochronologia 31, 140–145. https://doi.org/10.1016/j.dendro.2012.09.002.
Liu, M., Lu, S., Song, Y., Lei, L., Hu, J., Lv, W., Zhou, W., Cao, C., Shi, H., Yang, X., He, D., 2018. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai,China. Environ. Pollut. 242, 855–862. https://doi.org/10.1016/j.envpol.2018.07.051.
Mauer, O., Palátová, E., 2012. The role of root system in silver birch (Betula pendula Roth) dieback in the air-polluted area of Krusne; hory Mts. J. For. Sci. 49, 191–199. https://doi.org/10.17221/4693-JFS.
Napper, I.E., Davies, B.F.R., Clifford, H., Elvin, S., Koldewey, H.J., Mayewski, P.A., Miner, K.R., Potocki, M., Elmore, A.C., Gajurel, A.P., Thompson, R.C., 2020. Reaching new heights in plastic pollution—preliminary findings of microplastics on Mount Everest. One Earth 3, 621–630. https://doi.org/10.1016/j.oneear.2020.10.020.
Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Du, M., Li, J., Guo, Z., Bai, S., 2018. Microplastics contaminate the deepest part of the world's ocean. Geochem. Perspect. Lett. 1–5. https://doi.org/10.7185/geochemlet.1829.
Petersen, F., Hubbart, J.A., 2021. The occurrence and transport of microplastics: the state of the science. Sci. Total Environ. 758, 143936. https://doi.org/10.1016/j.scitotenv.2020.143936.
Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film residues in the soil environment: a critical review. Sci. Total Environ. 703, 134722. https://doi.org/10.1016/j.scitotenv.2019.134722.
Rillig, M.C., Lehmann, A., Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytol. 223, 1066–1070. https://doi.org/10.1111/nph.15794.

Rosselli, W., Keller, C., Boschi, K., 2003. Phytoextraction capacity of trees growing on a metal contaminated soil. Plant Soil 256, 265–272. https://doi.org/10.1023/A:1026100707797.

Sipilä, T.P., Keskinen, A.-K., Åkerman, M.-L., Fortelius, C., Haahtela, K., Yrjälä, K., 2008. High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of I.E.3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. ISME J. 2, 968–981. https://doi.org/10.1038/ismej.2008.50.

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Change Biol. 24, 1405–1416. https://doi.org/10.1111/gcb.14020.

Deutscher Wetterdienst n.d. Climatological Maps of Germany. URL https://www.dwd.de/EN/ourservices/klimakartendeutschland/klimakartendeutschland.html?nn = 519080 (accessed 11.19.21).

Xu, C., Zhang, B., Gu, C., Shen, C., Yin, S., Aamir, M., Li, F., 2020. Are we underestimating the sources of microplastic pollution in terrestrial environment? J. Hazard. Mater. 400, 123228. https://doi.org/10.1016/j.jhazmat.2020.123228.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the "Plastisphere": microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/es401288x.