

Can forest trees take up and transport nanoplastics?

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Plastic contamination of ecosystems has increased dramatically over the last decades, raising concerns about the negative impacts of plastic particles on aquatic and terrestrial systems. In recent years, the focus of most research has shifted from large fragments (macroplastic) to micro- (<5 mm) and more recently to nano-plastic (<1000 nm) particles as more evidence has come to light about their ubiquity in water, soils, and living systems, and their effects on ecosystem and human health. In this study, we investigate nanoplastic uptake in the roots of seedlings (1-2 years old) of three different tree species and assess their transport to different tissues. Parts of the main roots of silver birch (*Betula pendula* Roth), sessile oak (*Quercus petraea* Matt. [Liebl.]), and Norway spruce (*Picea abies* [L.] Karst.) were immersed for one or four days in a suspension containing ¹³C-labelled nano-sized polystyrene particles (¹³C-nPS; 99% ¹³C, d = 28 ± 8 (1 σ) nm). Carbon stable isotope analysis showed significant ¹³C enrichment ($P < 0.05$) in the immersed part of the root after one day of treatment in all three species, and after four days in *Q. petraea* alone. Signals of significant ¹³C enrichment were also found in the aboveground tissues of the trees. The stem of *B. pendula* in particular showed a significant ¹³C enrichment after one day of treatment ($P < 0.01$). This indicates that nanoplastic particles can be taken up through tree roots into the tree's central cylinder, where they are subsequently conveyed through the tree by acropetal transport via the xylem.

Keywords: Forest Trees, Nanoplastic, Polystyrene

Introduction

Plastics are synthetic polymers derived mainly from petroleum. Plastic production rates have increased steadily over the past decades, as have the attendant rates of waste production and pollution (Jambeck et al. 2015, Geyer et al. 2017). A lightweight, low-cost product, plastic is also resilient, durable, and easily transported and is therefore ubiquitous in modern life. The longevity of plastic is also the reason for its accumulation in the environment. Plastics have become a source of pollution affecting almost every ecosystem on the planet.

Plastic pollution is currently a key concern

for human society, and its mitigation is a big challenge for future research and policy making (Mitrano & Wohlleben 2020). Plastic litter starts as macroplastics, such as bottles or packaging, which slowly fragment into micro- (<5 mm) and nano-sized particles (<1000 nm – Allen et al. 2019). Such small particles can rapidly disperse across many ecosystems (De Souza Machado et al. 2019). Most studies to date have focused on aquatic systems, such as rivers, lakes, and oceans. However, only about 5% of the annual terrestrial plastic waste ends up in marine ecosystems. The fate of the remaining plastic litter is still largely un-

known due to the fragmentation of plastic into nanoparticles (Jambeck et al. 2015, De Souza Machado et al. 2018).

Research has only recently started to focus on terrestrial ecosystems after decades of scrutinizing the fate and impact of plastics on marine and freshwater ecosystems. Microplastics have been found in floodplain soils (Scheurer & Bigalke 2018), agricultural soils (Rillig et al. 2017), forests (Choi et al. 2020), and glaciers (Ambrosini et al. 2019). The range of ecosystems in which these particles are found indicates that micro- and nanoplastics can be transported by wind (Rezaei et al. 2019), and are therefore likely to also contaminate forest ecosystems. Atmospheric transport seems to be the most important pathway explaining the presence of plastic in remote areas and regions worldwide (Dris et al. 2016, Gasperi et al. 2018, Bergmann et al. 2019, Brahney et al. 2020, 2021, Materić et al. 2021). However, the fate of micro- and nanoplastics in the different ecosystems is almost unknown due to the analytical challenge of their detection in the environment (Wagner & Reemtsma 2019, Lehner et al. 2019, Patil et al. 2022).

A current challenge is to understand the micro- and nano-sized plastic pools and fluxes in terrestrial ecosystems, as well as the impact of plastic particles on plants and ecosystem functioning. Microplastics can affect the biophysical properties of soil, but our understanding of the complex relationships between microplastics, soil

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abiotic properties, microbial communities, and plants is still limited (De Souza Machado et al. 2019, Lozano et al. 2021a, 2021b). Wang et al. (2022) recently found that microplastics affect physical, chemical, and microbiological soil properties and that polymer type, dose, shape, and size can have different impacts in soils. Changes in soil properties may then affect the growth and development of plant roots, with potential consequences for ecosystem functioning.

Microplastics and nanoplastics can be absorbed by plant root hairs (Azeem et al. 2021). Indeed, Bosker et al. (2019) found a germination delay effect following the accumulation of microplastics in the root hairs of cress seedlings. Sun et al. (2020) recently demonstrated nanoplastic uptake in *Arabidopsis thaliana* (L.) Heynh. by root tips and subsequent negative physiological effects. Giorgetti et al. (2020) showed that onion seeds germinating in polystyrene nanoplastic suspensions exhibited decreased root growth and signs of cyto- and genotoxicity. In contrast, experiments with aquatic macrophytes have shown that growth depression only occurs when nanoplastic concentrations in the sediment exceed concentrations unlikely to be found in the environment (Van Weert et al. 2019). Van Weert et al. (2019) used solutions with 0.03, 0.1, 0.3, 1, and 3% nanopolystyrene concentrations, which covers the range of concentrations likely to be found in the environment. An experiment with duckweed by Dovidat et al. (2020) showed that although nanoplastic particles attached to roots, they were not detected within the plant. Li et al. (2020) demonstrated submicrometer plastic uptake in crop plants via a crack-entry pathway through roots. In a recent hydroponic experiment, Liu et al. (2022) found evidence of both nano- and micro-plastics uptake in rice seedlings through the roots and subsequent transport to aerial parts. Apoplastic transport was assumed to be the main pathway for plastic particles reaching aboveground tissues. Nanoplastic absorption by roots from

colloidal solutions and transport in higher plants (*Murraya exotica* L.) has been shown by Zhang et al. (2019). As with this study, the authors found that transport did not occur in the xylem and instead assumed it to be restricted to the apoplast of the lignified epidermis of roots and stems.

At present, there is a limited understanding of the impact of nanoplastics on tree physiology and forest health, and it is still unclear whether trees are able to take up nanoplastic particles via their roots. To assess whether and, if so, to what extent nanoplastics are taken up by trees through their roots, we immersed the roots of seedlings from three different forest tree species in a ^{13}C -labelled nano-sized polystyrene particle suspension (^{13}C -nPS) with a concentration similar to that observed in soils of polluted terrestrial ecosystems (Huerta Lwanga et al. 2016, Windsor et al. 2019). We further investigated whether nanoplastics can be transported to different aboveground tissues.

Materials and methods

Nanoplastic preparation, pre-processing, and characterization

Styrene- $^{13}\text{C}_8$ (Sigma-Aldrich, Buchs, Switzerland, ≥ 99 atom % ^{13}C) was used to synthesize batches of spherical ^{13}C -nPS of 28 ± 8 (1 σ) nm in size following the procedure of Al-Sid-Cheikh et al. (2020). Unreacted monomers were removed by ultrafiltration (exclusion size of membrane: $30,000 \text{ g mol}^{-1}$). The hydrodynamic diameter (z average) of the particles was determined by dynamic light scattering (DLS) on a Zetasizer (Nano-ZS[®], Malvern Instruments, UK). Secondary electron images recorded on a dedicated scanning transmission electron microscope (STEM, HD 2700 Cs[®], Hitachi, Japan) indicated that the particles were mostly spherical and not aggregated (Fig. S1 in Supplementary material).

Plant material and greenhouse setting

In February 2019, 36 seedlings of three different tree species, previously grown

outdoors in a forest nursery, were potted into 12 cm diameter plastic pots with a mixed soil substrate ("Containererde", Ökohum GmbH, Switzerland). One-year-old silver birch (*Betula pendula* Roth), two-year-old sessile oak (*Quercus petraea* Matt. [Liebl.]), and two-year-old Norway spruce (*Picea abies* [L.] Karst.) were used. As each plant was potted, its main root was directed through a central hole at the bottom of the pot so that it was protruding out of the pot. This part of the root was then put into a smaller pot containing the same substrate, beneath the first pot (a sketch of the experiment set-up is shown in Fig. 1a).

This set-up enabled easy access to part of the rooting system for the ^{13}C -labelling procedure (see below). The seedlings were then grown under natural light conditions in a greenhouse. The position of each seedling in the greenhouse was randomly changed once a week, and the pots were watered to field capacity twice a week.

Nanoplastic uptake experiment

At the end of August 2019, the main root of each seedling was carefully removed from the lower pot and rinsed with demineralised water to remove any adhering soil particles. The tips of the main roots (R1) were inserted into 15 ml Falcon[®] tubes (VWR, Dietikon, Switzerland) containing 12 ml of quarter-strength Hoagland nutrient solution (Fig. 1b). On the same day, ^{13}C -nPS were added to the nutrient solutions of six plants of each species to reach a 0.2% mass concentration (according to Huerta Lwanga et al. 2016). The other six individuals of each species were used as controls. The acropetal part of the main root (R2) that was not immersed in the suspension (Fig. 1b) was covered daily with a fresh wet paper towel to prevent desiccation during the experiment.

For each species and treatment, three seedlings were sampled after one day of exposure to the ^{13}C -nPS. The remaining three seedlings were sampled four days after the ^{13}C -nPS was added. For the four-day-long exposure, the amount of suspension in the tubes was monitored daily and nutrient solution was added as needed to account for evaporation and absorption by the roots.

Plant harvest

After one or four days of treatment, the tips and the rest of the main roots that were immersed in the ^{13}C -nPS suspension, plus a section of the root that was moistened by the suspension (~ 1cm, following Gessler et al. 2002), were collected from each plant (R1 – Fig. 1b). The individual root pieces were washed intensively with demineralised water for five minutes, then dried with a paper towel and weighed using a precision laboratory balance (PM 200, Mettler-Toledo, Columbus, OH, USA). The rest of the plant was subdivided into the following sections (see Fig. 1b): the upper

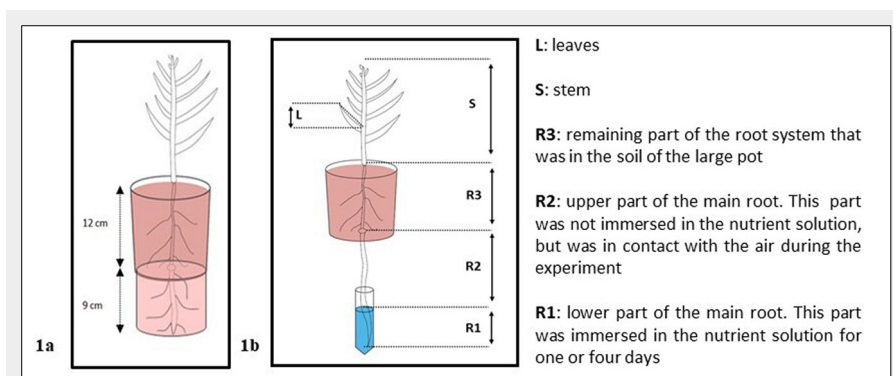


Fig. 1 - Experiment set-up (not to scale). Average stem heights of the different forest tree species were: 18 cm for *Q. petraea*, 20 cm for *P. abies*, and 42 cm for *B. pendula*. (a) Illustration of the potting preparation of a forest seedling; (b) illustration of a seedling during the experimental phase. The five different plant tissues that were analysed are also indicated (R1, R2, R3, S, L).

part of the main root outside of the exposure medium (R2), the remaining part of the root system in the main pot (R3), the stem (S), and the leaves (L). The roots (R3) were washed with tap water to remove adhering soil particles. All sections were weighed to obtain fresh weights, then oven dried at 60 °C for four days to obtain dry weights.

Stable isotope analysis

After drying, each of the five different tissues of the seedlings were homogenized using a ball mill for 1.5 minute at a frequency of 30 cycles per second (MM 400®, Retsch, Haan, Germany). For every tissue, first controls and then treatments were milled to avoid any possible contamination. One milligram of the homogenised material was weighed into a tin capsule then combusted in an elemental analyser (EA-1110®, Carlo Erba, Milan, Italy). The resulting CO₂ was analysed in a coupled isotope-ratio mass-spectrometer (Delta Plus XL®, Thermo, Bremen, Germany). The ratio of ¹³C/¹²C in the sample indicates its relative deviation in per mil from the international standard, V-PDB, which is given as δ¹³C. Laboratory standards and international standards with known δ¹³C values were used for the calibration of the measurements, resulting in a precision of 0.2‰ for δ¹³C.

Data analysis and statistics

For each of the different tree species, we calculated the difference in δ¹³C between the tissues of the plants treated with ¹³C-nPS and the control plants. We refer to this difference in δ¹³C as Δδ¹³C. Positive Δδ¹³C values indicate ¹³C-enrichment in treated plants. We used RStudio Team 2020 to test for the significance of ¹³C enrichment by performing a mixed analysis of variance (ANOVA), an analysis of variance (ANOVA), and a one-sided t-test for each of the five tissues. These tests detect ¹³C-incorporation in or adsorption to a specific tissue in a specific species and at a specific time. Finally, the ¹³C mass balance for each tissue in each plant was calculated to quantify the amount of ¹³C or ¹³C excess (in g ¹³C) contained in each tree’s compartment (Tab. S1 in Supplementary material).

Results and discussion

The addition of ¹³C-labelled nanopolystyrene led to a very high enrichment in the incubated parts of the roots (Fig. 2). It also caused a significant overall δ¹³C increase in all three tree species, indicating that trees were able to take up nanopolystyrene through their roots and incorporate it in their tissues (ANOVA – Tab. 1).

The part of the rooting system immersed in the polystyrene solution (R1) showed positive Δδ¹³C values in all three species after both one- and four-day-long treatments (P < 0.0001), indicating ¹³C enrichment (Fig. 2). The ¹³C enrichment is statistically significant (one-sided t-test: P < 0.05) in all three species following the one-day treatment,

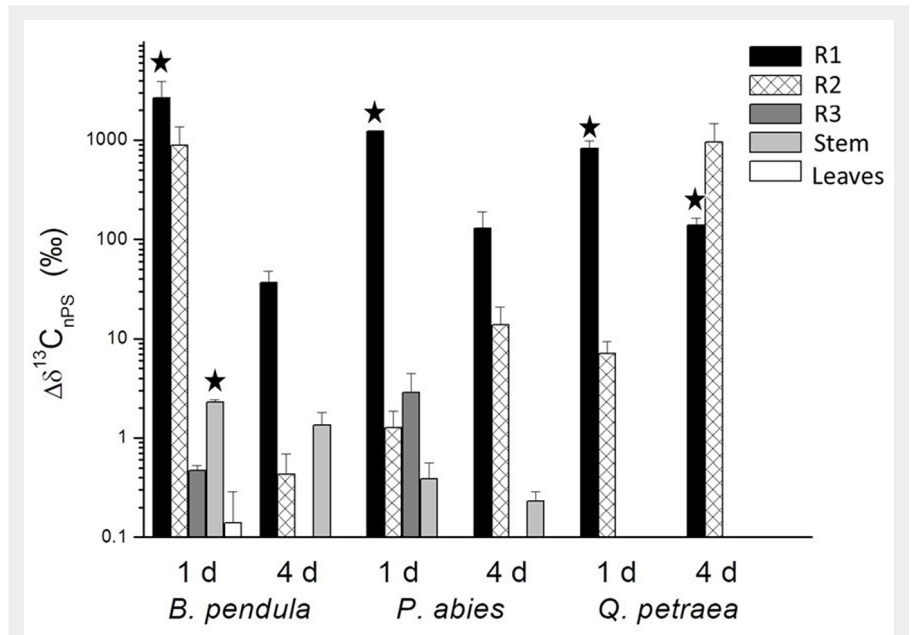


Fig. 2 - Differences in ¹³C between plants with ¹³C-nanopolystyrene (Δδ¹³C) and controls. The Δδ¹³C between the plants treated with ¹³C-labelled nanopolystyrene and the control plants for various tissues of all three tree species at different times of exposure (1d= one-day treatment; 4d= four-day treatment). Means and standard errors of 3 replicates. Black stars indicate statistically significant differences (P < 0.05) between control and treatment, tested by a one-sided t-test. (R1): lower part of the main root, which was immersed in the nutrient solution; (R2): upper part of the main root, which was not immersed in the nutrient solution but was in contact with the air during the experiment; (R3): remaining part of the root system that was in the soil of the large pot.

and in *Quercus petraea* following the four-day treatment (Fig. 2). Studies of both freshwater plants and an ornamental shrub have shown that nanoplastics can attach to the root surfaces (Zhang et al. 2019, Dovidat et al. 2020). If the intensive root washing failed to remove all ¹³C-nPS particles, the enrichment could reflect strong binding to the root surfaces and/or particle uptake via the roots (Sun et al. 2020).

The part of the root not immersed in the polystyrene solution (R2) showed positive Δδ¹³C values in all three species (P < 0.01, Tab. 1) and hence ¹³C enrichment. Seven out of 18 labelled seedlings showed Δδ¹³C values > 5‰, which is well above the natural variability of δ¹³C in unlabelled seedlings, with a standard deviation of 0.8‰.

In the part of the root system that re-

mained in the soil (R3), the labelling did not change δ¹³C values significantly. However, the Δδ¹³C R3 value of one out of 18 treated *P. abies* seedlings (after one day of treatment) exceeded the standard deviation of control trees, indicating a ¹³C enrichment (Fig. 2).

Leaf tissues showed significant differences in Δδ¹³C at the treatment level (P < 0.05 – Tab. 1). A slightly positive Δδ¹³C value was found in *B. pendula* after one day of treatment (0.14 ‰ ± 0.47 – Fig. 2), but this value was not statistically significant.

In stem tissues, Δδ¹³C values depended upon tree species (treat × species: P < 0.001 – Tab. 1). The enrichment of δ¹³C in the stems of *P. abies* and *Q. petraea* remained below detection limit, but the stem tissues of *B. pendula* (Δδ¹³C = 2.28 ‰ ± 0.45)

Tab. 1 - ANOVA testing the effects of exposing three tree species to ¹³C-labelled nanopolystyrene for one and four days on δ¹³C values in various tissues. F-values from ANOVA are shown. (***): p < 0.001; (**): p < 0.01; (*): p < 0.05.

Factor	df	All tissues	Root1 (R1)	Root2 (R2)	Root3 (R3)	Stem	Leaves
Tissue	4	66.8***	-	-	-	-	-
Treat	1	74.8***	178***	8.14**	0.05	2	4.63*
Species	2	4.1*	0.79	0.69	7.8**	6.9**	17.9***
Time	1	8.4**	22.7	0.02	1.3	0.58	0.01
Species × Treat	2	0.6	0.2	0.82	2.15	40.3***	1.41
Tissue × Treat	4	49.2***	-	-	-	-	-

were significantly enriched (one-sided t-test: $P < 0.01$) after one day of treatment (Fig. 2), indicating the presence of nanopolystyrene. Estimating the ^{13}C excess according to Ruehr et al. (2009) revealed that this enrichment of *B. pendula* stems represents 0.19% of the total ^{13}C -labelled polystyrene added to the exposure media (Tab. S1 in Supplementary material). This percentage corresponds to the ratio between the average ^{13}C content in the stem tissue ($4.44 \cdot 10^{-5}$ g) and the average ^{13}C content in the incubation solution (0.0232 g). In details, the incubation solution contained 0.0232 g of ^{13}C (12.6 ml, or approximately 12.6 g) solution with a polystyrene concentration of 0.2%. This results in 0.0252 g of polystyrene per incubation vial, with approx. 93% of the molecules consisting of C and 99% labeled with ^{13}C . Thus, the ^{13}C content in the incubation vial is 0.0232 g ^{13}C . Whereas the average ^{13}C content in the stem tissue ($4.44 \cdot 10^{-5}$ g) is the result of the following multiplication: $2.495 \cdot 10^{-5}$ (^{13}C atom% excess equalling the enrichment of 2.28‰) \cdot 3.56 g stem tissue biomass (g dry weight) \cdot 0.5 (C content in g C per g dry weight).

The enrichment in the stem may be the result of ^{13}C uptake in the central cylinder of the root and subsequent acropetal transport via the xylem. An alternative explanation, as suggested by Zhang et al. (2019), is that ^{13}C -nPS is transported in the apoplast of the lignified root epidermis without crossing the endodermis (and thus without reaching the central cylinder).

The ^{13}C enrichment in the stem of *B. pendula* (Fig. 2) and the overall treatment effect for leaves (Tab. 1) suggests that long-distance transport of nanoplastics from the roots to the shoot occurs in trees. *Betula pendula* is an early successional species with high water use (Leuschner 2002); its transpiration rates are higher than those of the two late successional species studied here (*Q. petraea* and *P. abies*). Xylem transport rates might therefore explain the significant accumulation of labelled ^{13}C in the stems of *B. pendula* but not in the stems of the other two species.

It is important to note that the ^{13}C signal of added nanopolystyrene gets diluted in seedling biomass when transported out of the treated part of the root into the other plant compartments. For example, if one assumes that the majority of the 5% of the label found in the R1 root of *P. abies* after one day of treatment ($\Delta\delta^{13}\text{C} = 1237\% \pm 19$ – Fig. 2) is uniformly transported to R2, R3, and aboveground tissues, this label is diluted by a factor of 0.021 (the biomass of R1 is 2.1% of the biomass of R2 + R3 + S + L; see Tab. S1 in Supplementary material). This would result in an average ^{13}C enrichment of 1.30‰. The ^{13}C enrichment in the stems of *B. pendula* exceeds this value ($\Delta\delta^{13}\text{C} = 2.28\% \pm 0.45$), implying higher uptake of nanoplastics. The ^{13}C enrichment in the root parts R2 and R3 and stems of *P. abies* after one day of treatment is in this

range but is not significant due to the high variability among individuals.

Even if signs of ^{13}C enrichment were found in all the tissues, $\Delta\delta^{13}\text{C}$ values of the aboveground tissues were close to the detection limit, revealing some discrepancies between the two different exposure times. When comparing the two exposure times, more statistically significant values were found after one day of treatment as compared after four days of treatment. We speculate that nanoparticles change root morphology and functioning over time, resulting in reduced uptake and transport to other tissues. Indeed, Zhang et al. (2019) found that polystyrene nanoparticles created a physical barrier in the root micropores and significant injuries at the epidermal root cell level. We therefore assume that longer incubation times did not lead to higher uptake in our experiment. This does not explain the lower enrichment after 4 days of exposure (found in some cases), but this may be due to the fact that ^{13}C enrichment was mostly close to the detection limit.

In conclusion, the use of ^{13}C -nPS in our experiment gave some first evidence of the potential uptake of nanoplastics in trees. The highest ^{13}C enrichments from ^{13}C -nPS were obtained from roots immersed in the exposure media and may be the result of particle adsorption on the root surface. We speculate that ^{13}C -nPS enters into roots via a crack-entry mode, as described by Li et al. (2020). ^{13}C -nPS might be transported to the stem tissue in some species (*B. pendula*) via the transpiration stream in the xylem. Although the ^{13}C enrichment in stems of *B. pendula* was significant and an overall treatment effect on leaves was observed, ^{13}C enrichment remained low, which can most likely be attributed to dilution in the large stem biomass. Future experiments with different exposure times, higher concentrations of ^{13}C -nPS, or the use of more easily detected isotopes (e.g., ^{14}C) would help to identify the magnitude of within-tree transport of nanoplastics. As indicated by this study, the uptake of nanoplastics by trees may affect tree physiological functions and allow nanoplastics to enter the food chain in forest ecosystems, as has been observed in marine environments.

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Authors' contributions

Conceptualization: MEM, PC, AG, IB, FH; Methodology: MEM, PC, AG, IB, FH, RG, MASC; Collection of data: MEM, PC, AG, PB; Data analysis: MEM, AG, MS, FH; Writing-original draft preparation: MEM, PC, AG, IB, FH; Writing-review and editing: MEM, AG, PC, IB, RG, MS, PB, FH, MASC, GO; Funding acquisition: PC, AG; Supervision: PC, AG, IB, FH.

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Supplementary Material

Fig. S1 - Secondary electron (SE) images.

Tab. S1 - Detailed data on dry weight, $\delta^{13}\text{C}$, atom % and the amount of ^{13}C (g) in each tissue of each replicate plant.

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