Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplemental Information

Uptake and physiological impacts of nanoplastics in trees with divergent water use strategies

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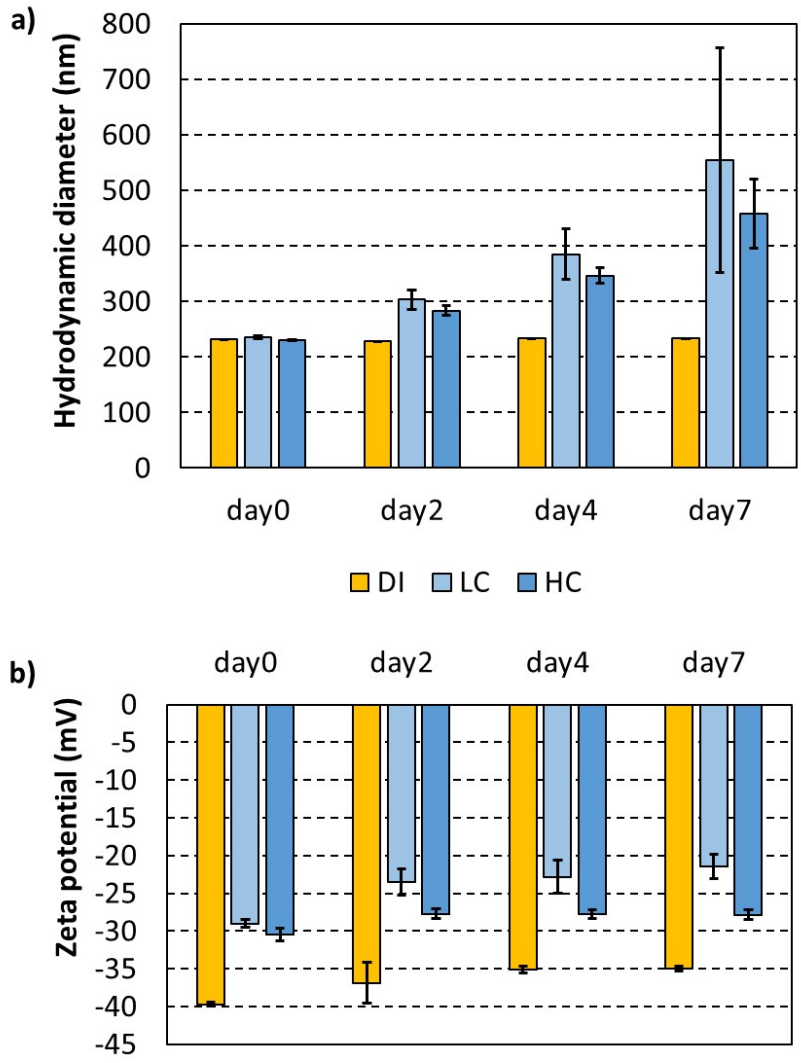

Figure S1. Evolution of the a) hydrodynamic diameter (nm) and b) zeta potential (mV) of the model Palladium doped polystyrene nanoplastics (PS-Pd-NP) in deionized water (DI) at high particle concentration (30 mg/L) and growth media at low (LC) and high (HC) concentrations (10 and 30 mg/L, respectively) over one week. These time points correspond to the time at which solutions were renewed during the exposure experiments. Error bars represent standard deviation.

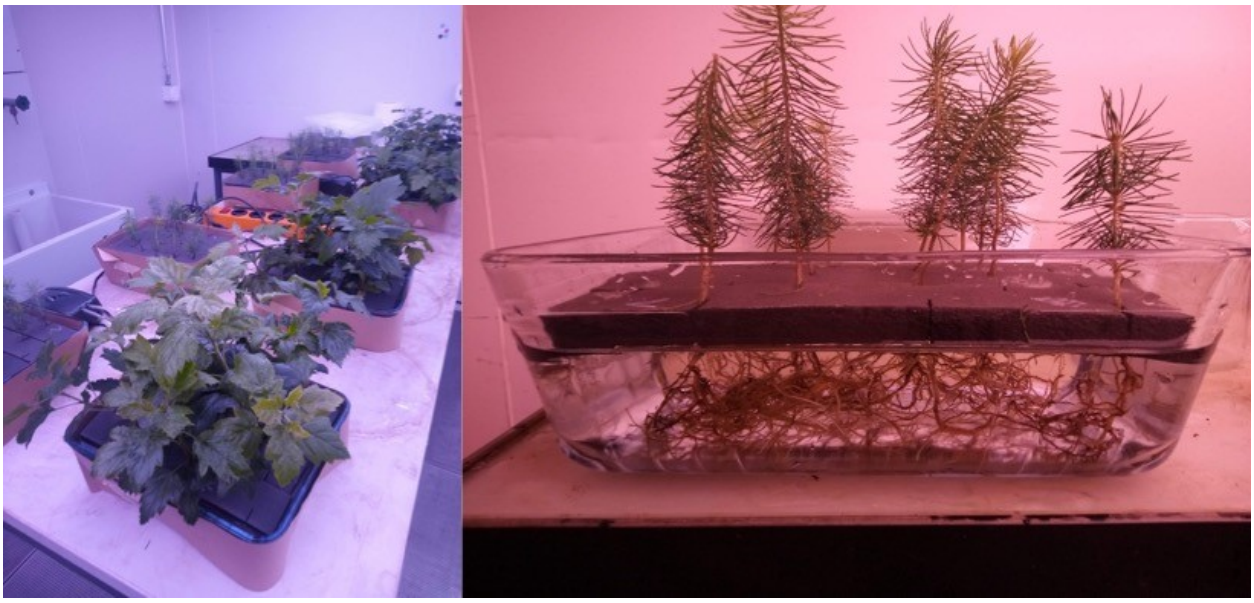

Figure S2: Photos of experiment set-up where 12 trees of each species were grown in a hydroponic system. The root crown of each tree was inserted into the floating mat through a vertical slot. In this way, only the roots were submerged in the liquid solution, whereas the aboveground tissues remained physically isolated from the nutrient solution and potential nanoplastics contamination.

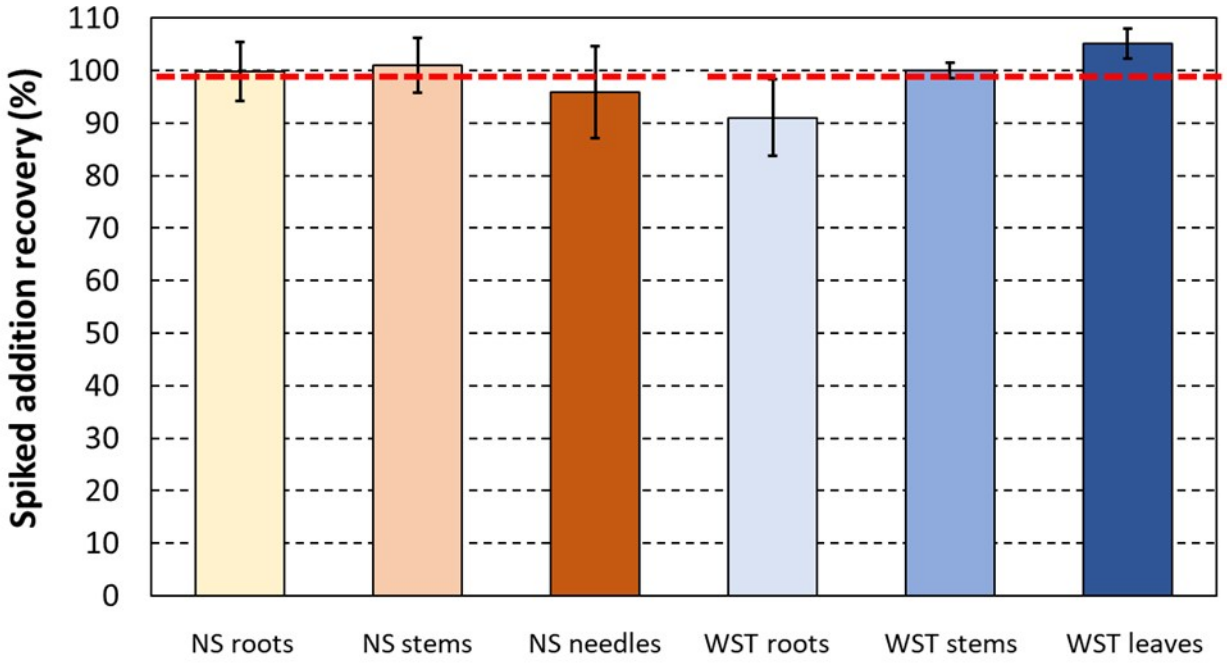

Figure S3. Average nanoplastics spiked addition recovery (%) of PS-Pd-NP dispersions (1 µg/L, 2.5 µg/L, and 5 µg/L) on the different tissues of Norway spruce (NS) and wild service tree (WST) (n=3). Results from all concentrations for each tree organ are graphed together, and error bars represent standard deviations. The red line indicates the average recoveries across all tree organs.

Table S1: p-values of the ANOVA test comparing comparing the NPs uptake between the three tissues groups within one species. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

	Low Concentration (LC)								
	***S. torminalis* (2 weeks)**			***P. abies* (2 weeks)**			***P. abies* (4 weeks)**		
	Est.	**p-value**	**Sig.**	**Est.**	**p-value**	**Sig.**	**Est.**	**p-value**	**Sig.**
Roots vs Leaves	585.64	<0.001	***	1577.11	<0.001	***	2419.83	<0.001	***
Roots vs Stems	572.26	<0.001	***	1549.42	<0.001	***	2420.24	<0.001	***
Stems vs Leaves	13.37	0.580		27.69	0.014	*	0.40	1.000	
	High concentration (HC)								
	***S. torminalis* (2 weeks)**			***P. abies* (2 weeks)**			***P. abies* (4 weeks)**		
	Est.	**p-value**	**Sig.**	**Est.**	**p-value**	**Sig.**	**Est.**	**p-value**	**Sig.**
Roots vs Leaves	1179.55	<0.001	***	2503.56	<0.001	***	2101.55	<0.001	***
Roots vs Stems	1131.63	<0.001	***	2477.30	<0.001	***	2077.28	<0.001	***
Stems vs Leaves	47.91	0.026	*	26.26	0.509		24.27	0.594	

Table S2: P-values of the linear model comparing the NPs uptake at 2 weeks in *Picea abies* vs. *S. torminalis*. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

	***S. torminalis* vs. *P. abies* (2 weeks)**			
Tissue	**Concentration**	**Effect**	**p-value**	**Significance**
Leaves	Low	2.11	0.999	
	High	1.70	0.999	
Stems	Low	-12.20	0.995	
	High	23.36	0.840	
Roots	Low	-989.35	<0.001	***
	High	-1322.30	<0.001	***

Table S3: P-values of the linear model comparing the effects of NPs concentration on uptake. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

Low vs. High Concentration			
***S. torminalis* (2 weeks)**			
Tissues	**Estimate**	**p-value**	**Significance**
Leaves	4.61	0.985	
Stems	39.15	0.261	
Roots	598.52	0.114	
***P. abies* (2 weeks)**			
Tissues	**Estimate**	**p-value**	**Significance**
Leaves	5.02	0.977	
Stems	3.58	0.999	
Roots	931.47	<0.001	**
***P. abies* (4 weeks)**			
Tissues	**Estimate**	**p-value**	**Significance**
Leaves	18.26	0.016	*
Stems	42.94	0.165	
Roots	-300.02	0.853	

Table S4: Interaction plots for the effect of concentration and time on predicted NPs uptake for leaves, stems and roots. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

	2 weeks vs. 4 weeks (*P. abies*)			
Tissue	**Concentration**	**Estimate**	**p-value**	**Significance**
Needles	Low	4.85	0.848	
	High	18.09	0.020	*
Stems	Low	-23.24	0.432	
	High	16.11	0.717	
Roots	Low	847.57	<0.001	***
	High	-383.91	0.228	

Table S5: Estimates and p-values of the linear model for the effects of concentration, time, and species on NPs uptake in leaves, stems and roots. Interactions between concentration-time and concentration-species were included. Significance levels: *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

	Individual and Interaction Effects			
Tissue	**Interaction**	**Estimate**	**p-value**	**Significance**
Leaves	Intercept	3.36	0.361	
	High vs. Low	5.02	0.335	
	4-weeks vs. 2-weeks	4.85	0.352	
	ST vs. PA	2.11	0.684	
	high & 4-weeks vs. low & 2-weeks	13.24	0.045	*
	high & ST vs. low & PA	-0.41	0.956	
Stems	Intercept	31.06	0.009	**
	High vs. Low	3.59	0.827	
	4-weeks vs. 2-weeks	-23.25	0.160	
	ST vs. PA	-12.21	0.458	
	high & 4-weeks vs. low & 2-weeks	39.35	0.094	
	high & ST vs. low & PA	35.57	0.129	
Roots	Intercept	1580.47	<0.001	***
	High vs. Low	931.47	<0.001	***
	4-weeks vs. 2-weeks	847.57	<0.001	***
	ST vs. PA	-989.35	<0.001	***
	high & 4-weeks vs. low & 2-weeks	-1231.49	<0.001	***
	high & ST vs. low & PA	-332.94	0.275	

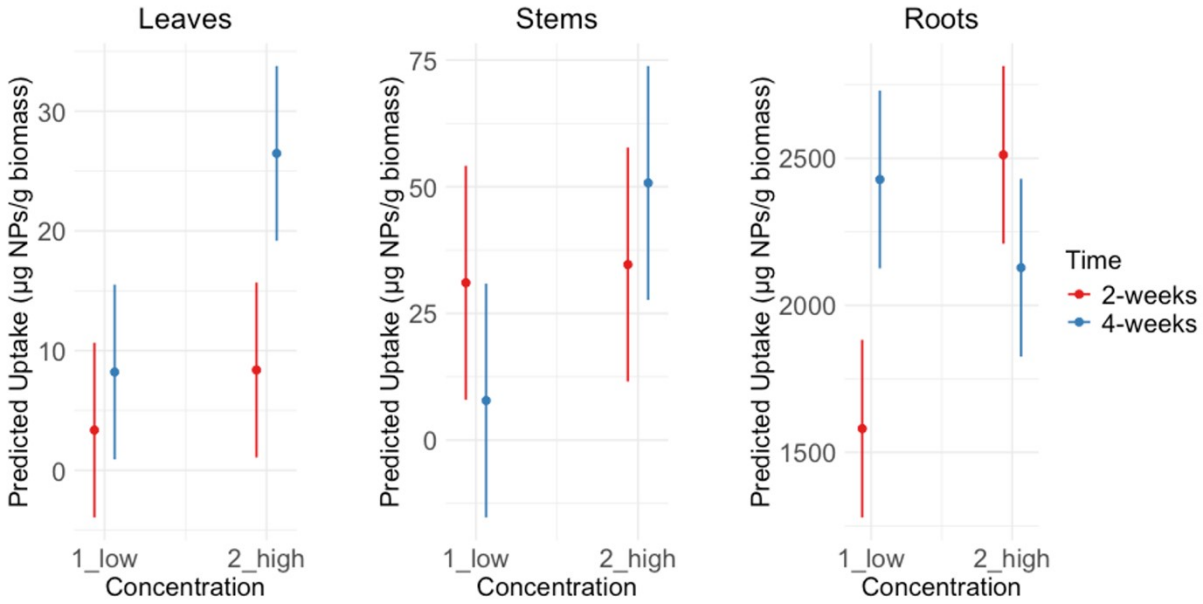

Figure S4: Interaction plots for the effect of concentration and time on NPs uptake for leaves, stems and roots.

Table S6: Dry weight (g) of wild service tree and Norway spruce at the end of the experiments. Data shown are mean values ± SD.

	Wild service tree 2 weeks		
	Control	**LC**	**HC**
Leaves	1 ± 0.39	1.19 ± 0.26	1.08 ± 0.36
Stems	0.42 ± 0.22	0.38 ± 0.16	0.37 ± 0.19
Roots	0.31 ± 0.1	0.31 ± 0.08	0.31 ± 0.17
	Norway spruce 2 weeks		
	Control	**LC**	**HC**
Needles	0.33 ± 0.07	0.4 ± 0.13	0.37 ± 0.09
Stems	0.13 ± 0.04	0.17 ± 0.05	0.13 ± 0.06
Roots	0.17 ± 0.06	0.22 ± 0.07	0.2 ± 0.08
	Norway spruce 4 weeks		
	Control	**LC**	**HC**
Needles	0.18 ± 0.06	0.2 ± 0.07	0.18 ± 0.06
Stems	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.03
Roots	0.13 ± 0.05	0.13 ± 0.04	0.11 ± 0.06

Table S7: Water content (%) in the three different tissues for each species and each exposure time calculated as 100% - 100 (Dry weight / Fresh weight) Data shown are mean values ± SD.

	Wild service tree 2 weeks		
	Control	**LC**	**HC**
Leaves	22.71 ± 14.25	13.58 ± 7.17	14.99 ± 12.76
Stems	49.59 ± 9.07	47.36 ± 12.71	51.56 ± 9.73
Roots	77.12 ± 3.04	76.55 ± 3.54	77.23 ± 2.93
	Norway spruce 2 weeks		
	Control	**LC**	**HC**
Needles	67.04 ± 3.46	67.27 ± 2.74	66.95 ± 1.8
Stems	66.56 ± 4.22	64.41 ± 2.82	68.26 ± 2.84
Roots	78.73 ± 2.19	79.39 ± 2.9	78.86 ± 2.07
	Norway spruce 4 weeks		
	Control	**LC**	**HC**
Needles	64.35 ± 13.77	62.74 ± 18.79	59.94 ± 15.57
Stems	64.2 ± 10.08	59.1 ± 9.3	60.1 ± 20.3
Roots	83.57 ± 2.71	81.25 ± 3.36	84.97 ± 3.84

Table S8: Dry weight shoot-to-root ratios for wild service tree and Norway spruce at the end of the experiments. Data shown are mean values ± SD. F-ratios (the ratio of the between group variance to the within group variance) and p-values were assessed by one-way ANOVA.

Species, duration	**Treatment**			**F-ratio**	**p-value**
	C	**LC**	**HC**		
Wild service tree (2 weeks)	4.76 ± 1.58	5.41 ± 1.69	5.35 ± 1.90	0.808	0.452
Norway spruce (2 weeks)	2.17 ± 0.48	2.09 ± 0.51	2.02 ± 0.47	0.264	0.769
Norway spruce (4 weeks)	2.13 ± 0.81	2.21 ± 0.82	2.55 ± 1.13	0.889	0.418

There were no significant differences in shoot-to-root ratios between the controls and the two treatments for both species. For wild service tree there was a slight, though not significant, tendency for a higher shoot-root ratio in both LC and HC exposure concentrations after two weeks whereas after the same incubation time such trends were not visible in Norway spruce. However, some tendency for increased shoot-root ratios in HC were visible for Norway spruce after four weeks. Two- and four-weeks incubation may not be sufficient to induce clear changes in growth patterns, even in seedlings with their relatively high growth rates. The slight tendency we observed for increased shoot-root ratios in wild service tree after two and in Norway spruce after four weeks might indicate relatively reduced C allocation to the roots, which is known to occur when plants are exposed to stressors (Joseph et al., 2020).

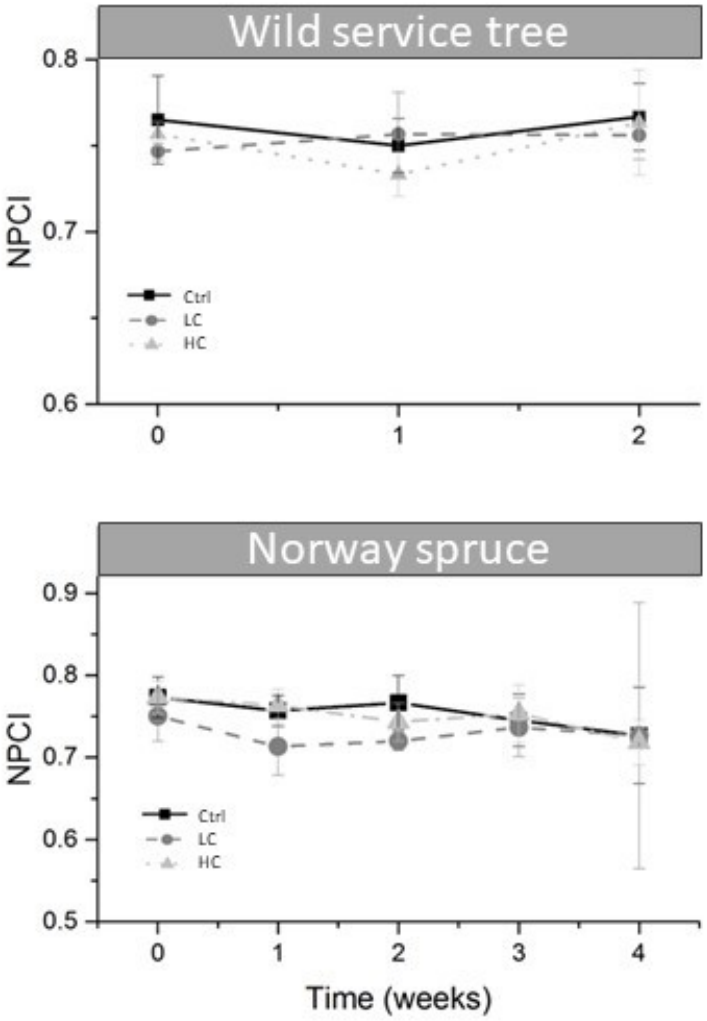

Figure S5: Normalized Pigment Chlorophyll Index (NPCI) of wild service tree and Norway spruce after different exposure times to low (LC) and high concentrations (HC) of plastic nanoparticles. Ctrl: untreated controls. Data shown are mean values (N= 3-9) ± SD.

References

1. Joseph, J.; Gao, D.; Backes, B.; Bloch, C.; Brunner, I.; Gleixner, G.; Haeni, M.; Hartmann, H.; Hoch, G.; Hug, C.; Kahmen, A.; Lehmann, M.M.; Li, M-H.; Luster, J.; Peter, M.; Poll, C.; Rigling, A.; Rissanen, K.A.; Ruehr, N.K.; Saurer, M.; Schaub, M.; Schönbeck, L.; Stern, B.; Thomas, F.M.; Werner, R.A.; Werner, W.; Wohlgemuth, T.; Hagedorn, F.; Gessler, A., Rhizosphere activity in an old-growth forest reacts rapidly to changes in soil moisture and shapes whole-tree carbon allocation. *Proceedings of the National Academy of Sciences* **2020**, 117: 24885-24892.