forests

MDPI

Supplementary Material

Plasticity of root traits under competition for a nutrient-rich patch depends on tree species and possesses a large congruency between intra- and interspecific situations

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Supplementary Material S1 – Experimental set-up

The experimental microcosm system comprises of two large microcosms (20 × 20 cm on top, 35 cm deep, 7 l soil) interconnected by two smaller competition chambers (CC; 10 × 10 cm on bottom, 12 cm deep, 1 l soil). Initially, all compartments were filled with nutrient poor substrate (Supplementary Table S1) at a density of ~1.3 g cm^{-3}. The substrate was prepared by mixing 75% (vol./vol.) washed quartz sand (particle size distribution 0.9-1.2 mm) and 25% (vol./vol.) mineral soil (silty clay loam) in a concrete blender; sand was added to improve drainage, reduce nutrient contents and to allow for non-destructive, relatively fast root harvest. The soil was sieved (2 mm) beforehand breaking-up large aggregates and to remove stones and organic fragments. The mixed substrate was homogenized across mixer batches. The bottom of microcosms and CC was perforated (i.e. multiple holes covered by a thin layer of rock wool (microcosms) or 1 mm-sized plastic mesh (CC)), allowing for free drainage. One microcosm each was tightly interconnected to opposite sides of the CC by metal rivets, forming an aperture of 2.3 cm inner diameter (Figure 1).

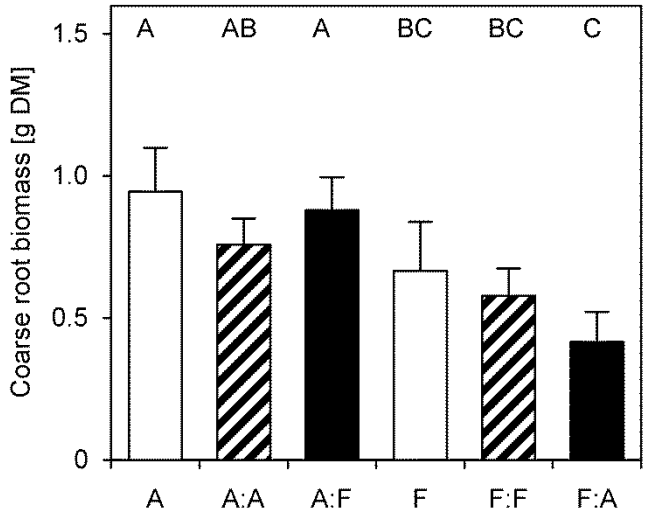
Subsequently, one tree seedling was planted per microcosm by mid-April 2017, and one 5-cm long, 'average'-branched (comprising two root orders), terminal fine root axis per plant was carefully inserted into each CC (Figure 1). Inserted roots were selected from individual seedlings' root systems as available (i.e. of sufficient length) and not recut to size. In the isolation treatment (i.e. control / 'no competition'), the rivets/apertures towards the competitor microcosm were sealed and only one fine root segment was inserted. We assume that self-competition was reduced to a minimum under isolation.

Supplementary Table S1. Soil properties in the microcosms and competition chamber (before NPK fertilization) (mean, n = 3). See Supplementary Information S3 for methodology

Variable	Mean
Total Carbon (mg/g)	5.24
Inorganic carbon (mg/g)	3.20
Total Nitrogen (mg/g)	0.33
Al (mg/g)	4.93
Ca (mg/g)	5.61
Fe (mg/g)	9.07
K (mg/g)	1.20
Mg (mg/g)	1.86
Mn (μg/g)	154.15
Na (mg/g)	0.13
P (μg/g)	175.71
S (μg/g)	120.54
pH (H_2O)	8.5
pH ($CaCl_2$)	7.8

An automated, pressure-compensated drip irrigation system was installed (NMC Junior, Netafim, Israel); one dripper was placed into each microcosm (2 l h^{-1}; ca. 5 cm from the centre/bole) and CC (1.2 l h^{-1}, centred; Figure 1). The microcosms were synchronously and uniformly irrigated with 0.5-2 l of tap water (per tree individual) every 2-3 days ensuring ample water supply. The amount was increased over the growing season in a step-wise manner according to evapotranspiration (determined by weekly measurements of volumetric soil water content using a calibrated soil moisture meter (Field Scout, Spectrum Tech., Plainfield, USA; data not shown)). Similar, the CC were irrigated with tap water (0.1-0.3 l every 2-3 days) according to demand (data not shown). The CC were manually fertilized once per week with 0.05 l of Hoagland solution ('+NPK'; applied ~12 h after an irrigation event; starting point: 2 weeks after tree planting) to create nutrient rich 'hotspots'. As uptake of nutrients from the soil, particularly nitrate, is dependent on water supply [1], water stress and potential confounding effects were avoided. The second CC was fertilized with a modified Hoagland solution without nitrogen ('+PK'; Figure 1); this fertilization treatment is not part of this manuscript in order to keep its length manageable.

1. Fotelli, M.N.; Rennenberg, H.; Geáler, A. Effects of drought on the competitive interference of an early successional species (*rubus fruticosus*) on *fagus sylvatica* l. Seedlings: N-15 uptake and partitioning, responses of amino acids and other n compounds. *Plant Biology* **2002**, *4*, 311-320.



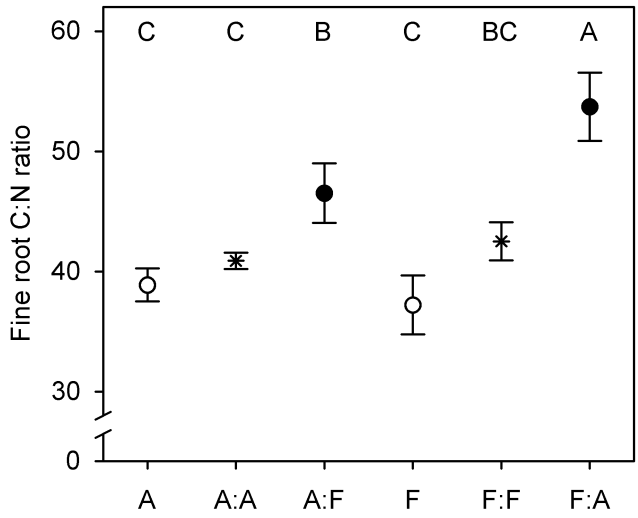
Supplementary Figure S1. Coarse root biomass (g DM, dry matter) of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) grown under three different competition treatments into nutrient-rich soil patches. A, *Acer* root grown in isolation (no competition); A:A, *Acer* root grown in competition with another *Acer* root; A:F, *Acer* grown in competition with *Fagus*; F, *Fagus* grown in isolation; F:F, *Fagus* grown in competition with *Fagus*; F:A, *Fagus* grown in competition with *Acer*. Significant differences between treatments are indicated by different letters (see Supplementary Tables S3 for GLM statistics; mean+SE, n=13-52).

Supplementary Material S2 – Methods used to determine soil chemical properties

For determination of carbon (C) and nitrogen (N) concentrations, dried (70°C, until constant mass) soil substrate were ground to powder with a planetary mill (Pulverisette 5; Fritsch, Idar-Oberstein, Germany. Three soil substrate samples, collected during experimental set-up, were analysed as technical replicates. Total C and N concentrations (mg g^{-1}) were determined by dry combustion using a TruSpec CN analyser (Leco, St. Joseph, USA) according to the Austrian ÖNORM L1080 protocol. Carbon to N (C:N) ratios were calculated. Nutrient contents (Al, Ca, Fe, K, Mg, Mn, Na, P and S) of soil were analysed after acid hydrolysis in nitrohydrochloric acid by ICP-OES (Optima 8300; Perkin Elmer, Waltham, USA); see Austrian ÖNORM protocols L 1085 and L 1202 for technical details. Soil pH was measured in both deionize water and 0.01M $CaCl_2$ suspensions (n = 6); see Austrian ÖNORM protocol EN 15933 for technical details.

Supplementary Material S3 – Fine root carbon concentration and C:N ratio

No significant differences among fine roots' carbon (C) concentrations, ranging between 510±3 mg g^{-1} and 535±3 mg g^{-1} in *Acer* (A:A) and *Fagus* (F) respectively, were found (data not shown). In both *Acer* and *Fagus* the C:N ratios of fine roots were significantly greater under interspecific competition (A:F and F:A, respectively) compared to both intraspecific competition and isolation (Supplementary Figure S2). The greatest C:N ratio was found in *Fagus* fine roots growing under interspecific competition.



Supplementary Figure S2. Fine root carbon-to-nitrogen (C:N) ratio of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) grown under three different competition treatments into nutrient-rich soil patches. A, *Acer* root grown in isolation (no competition); A:A, *Acer* root grown in competition with another *Acer* root; A:F, *Acer* grown in competition with *Fagus*; F, *Fagus* grown in isolation; F:F, *Fagus* grown in competition with *Fagus*; F:A, *Fagus* grown in competition with *Acer*. Significant differences between treatments are indicated by different letters (t test, $p < 0.05$, mean±SE, n=4-5; see Supplementary Table S11 for GLM statistics).

Supplementary Material S4 - Statistics

Supplementary Table S2. Statistical output of GLM on fine root biomass (FRB, g), with species and competition as fixed factors. The data was log-transformed before analysis

Tests of Between-Subjects Effects

Dependent Variable: log (FRB+1)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.279[a]	5	.056	2.075	.070
Intercept	9.769	1	9.769	363.391	.000
comptype	.026	2	.013	.483	.617
species_A	.161	1	.161	6.003	.015
comptype * species_A	.017	2	.008	.312	.733
Error	5.484	204	.027		
Total	17.725	210			
Corrected Total	5.763	209			

a. R Squared = .048 (Adjusted R Squared = .025)

Supplementary Table S3. Statistical output of GLM on coarse root biomass (CRB, g), with species and competition as fixed factors. The data was log-transformed before analysis

Tests of Between-Subjects Effects

Dependent Variable: crblog

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.188[a]	5	.038	2.141	.064
Intercept	5.087	1	5.087	289.350	.000
comptype	.027	2	.014	.775	.463
species_A	.101	1	.101	5.719	.018
comptype * species_A	.030	2	.015	.847	.431
Error	2.602	148	.018		
Total	8.758	154			
Corrected Total	2.790	153			

a. R Squared = .067 (Adjusted R Squared = .036)

Supplementary Table S4. Statistical output of GLM on total root biomass (FRB+CRB, g), with species and competition as fixed factors. The data was log-transformed before analysis

Tests of Between-Subjects Effects

Dependent Variable: FRCRBlog

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.398[a]	5	.080	1.733	.129
Intercept	15.663	1	15.663	341.151	.000
comptype	.043	2	.021	.466	.628
species_A	.259	1	.259	5.648	.018
comptype * species_A	.003	2	.002	.037	.964
Error	9.182	200	.046		
Total	28.709	206			
Corrected Total	9.580	205			

a. R Squared = .042 (Adjusted R Squared = .018)

Supplementary Table S5. Statistical output of GLM on specific fine root respiration (RRs, nmol g^{-1} s^{-1}), with species and competition as fixed factors

Tests of Between-Subjects Effects

Dependent Variable: RRBIO(nmol CO2 g-1 s-1)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	368.496[a]	5	73.699	5.180	.000
Intercept	10259.311	1	10259.311	721.140	.000
comptype	266.301	2	133.150	9.359	.000
species_A	10.475	1	10.475	.736	.392
comptype * species_A	115.080	2	57.540	4.045	.019
Error	2788.397	196	14.227		
Total	14230.364	202			
Corrected Total	3156.893	201			

a. R Squared = .117 (Adjusted R Squared = .094)

Post Hoc Tests

comp type

Multiple Comparisons

Dependent Variable: RRBIO(nmol CO2 g-1 s-1)

Tukey HSD

(I) comp type	(J) comp type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mono	Inter	3.0640*	.76992	.000	1.2457	4.8823
	Mix	3.0398*	.79802	.001	1.1551	4.9244
Inter	Mono	-3.0640*	.76992	.000	-4.8823	-1.2457
	Mix	-.0243	.58347	.999	-1.4022	1.3537
Mix	Mono	-3.0398*	.79802	.001	-4.9244	-1.1551
	Inter	.0243	.58347	.999	-1.3537	1.4022

Based on observed means.
The error term is Mean Square(Error) = 14.227.

*. The mean difference is significant at the .05 level.

Supplementary Table S6. Statistical output of GLM on root diameter (RD, mm), with species and competition as fixed factors. The data was log-transformed before analysis

Tests of Between-Subjects Effects

Dependent Variable: dimlog

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.189[a]	5	.038	2.412	.038
Intercept	14.502	1	14.502	927.410	.000
comp	.081	2	.041	2.605	.076
spce	.087	1	.087	5.541	.020
comp * spce	.031	2	.016	1.004	.368
Error	3.159	202	.016		
Total	21.648	208			
Corrected Total	3.347	207			

a. R Squared = .056 (Adjusted R Squared = .033)

Post Hoc Tests

comp

Multiple Comparisons

Dependent Variable: dimlog

Tukey HSD

(I) comp	(J) comp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mono	Intra	-.0382	.02496	.279	-.0971	.0207
	Inter	-.0021	.02570	.996	-.0628	.0585
Intra	Mono	.0382	.02496	.279	-.0207	.0971
	Inter	.0361	.01906	.144	-.0089	.0811
Inter	Mono	.0021	.02570	.996	-.0585	.0628
	Intra	-.0361	.01906	.144	-.0811	.0089

Based on observed means.
The error term is Mean Square(Error) = .016.

Supplementary Table S7. Statistical output of GLM on specific root area (SRA; cm^2 g^{-1}), with species and competition as fixed factors

Tests of Between-Subjects Effects

Dependent Variable: SRA (cm2 g-1)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	505998.99[a]	5	101199.799	1.999	.080
Intercept	40234707.6	1	40234707.6	794.576	.000
comptype2	160379.167	2	80189.584	1.584	.208
species2	128865.639	1	128865.639	2.545	.112
comptype2 * species2	75447.081	2	37723.541	.745	.476
Error	10431158.4	206	50636.691		
Total	58856405.8	212			
Corrected Total	10937157.4	211			

a. R Squared = .046 (Adjusted R Squared = .023)

Supplementary Table S8. Statistical output of GLM on tissue density (TD; g cm^{-3}), with species and competition as fixed factors. The data was log-transformed before analysis but still not equal variance was achieved

Tests of Between-Subjects Effects

Dependent Variable: TDlog2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.036[a]	5	.007	3.743	.003
Intercept	.792	1	.792	406.962	.000
comptype2	.002	2	.001	.627	.535
species2	.025	1	.025	12.957	.000
comptype2 * species2	.001	2	.000	.230	.795
Error	.391	201	.002		
Total	1.393	207			
Corrected Total	.427	206			

a. R Squared = .085 (Adjusted R Squared = .062)

Supplementary Table S9. Statistical output of GLM on root tip density (RTD; n cm^{-1}) with species and competition as fixed factors. The data was log-transformed before analysis

Tests of Between-Subjects Effects

Dependent Variable: logtippdensity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.450[a]	5	.490	18.383	.000
Intercept	35.449	1	35.449	1329.677	.000
comptype2	.371	2	.186	6.960	.001
species2	1.775	1	1.775	66.596	.000
comptype2 * species2	.007	2	.003	.125	.883
Error	4.825	181	.027		
Total	51.405	187			
Corrected Total	7.276	186			

a. R Squared = .337 (Adjusted R Squared = .318)

Post Hoc Tests

comp type 2

Multiple Comparisons

Dependent Variable: logtippdensity

Tukey HSD

(I) comp type 2	(J) comp type 2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mono	Intra	-.0892*	.03431	.027	-.1703	-.0081
	Inter	.0045	.03508	.991	-.0784	.0874
Intra	Mono	.0892*	.03431	.027	.0081	.1703
	Inter	.0937*	.02622	.001	.0317	.1556
Inter	Mono	-.0045	.03508	.991	-.0874	.0784
	Intra	-.0937*	.02622	.001	-.1556	-.0317

Based on observed means.
The error term is Mean Square(Error) = .027.

Supplementary Table S10. Statistical output of GLM on root nitrogen (N) concentration (mg g^{-1}), with species and competition as fixed factors

Tests of Between-Subjects Effects

Dependent Variable: N.concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	67.357[a]	5	13.471	6.938	.001
Intercept	4137.290	1	4137.290	2130.661	.000
Comp	59.183	2	29.592	15.239	.000
species	.697	1	.697	.359	.555
Comp * species	7.791	2	3.896	2.006	.158
Error	42.719	22	1.942		
Total	4382.535	28			
Corrected Total	110.077	27			

a. R Squared = .612 (Adjusted R Squared = .524)

Post Hoc Tests

Competation

Multiple Comparisons

Dependent Variable: N.concentration

Tukey HSD

(I) Competation	(J) Competation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mono	Intra	1.8352*	.62318	.020	.2697	3.4007
	Inter	3.6397*	.66099	.000	1.9793	5.3002
Intra	Mono	-1.8352*	.62318	.020	-3.4007	-.2697
	Inter	1.8045*	.66099	.032	.1441	3.4650
Inter	Mono	-3.6397*	.66099	.000	-5.3002	-1.9793
	Intra	-1.8045*	.66099	.032	-3.4650	-.1441

Based on observed means.
The error term is Mean Square(Error) = 1.942.

Supplementary Table S11. Statistical output of GLM on C:N ratio, with species and competition as fixed factors

Tests of Between-Subjects Effects

Dependent Variable: CN.ratio

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	782.208[a]	5	156.442	8.829	.000
Intercept	51904.185	1	51904.185	2929.386	.000
Comp	665.491	2	332.746	18.780	.000
species	39.294	1	39.294	2.218	.151
Comp * species	87.608	2	43.804	2.472	.108
Error	389.806	22	17.718		
Total	52477.584	28			
Corrected Total	1172.014	27			

a. R Squared = .667 (Adjusted R Squared = .592)

Post Hoc Tests

Competation

Multiple Comparisons

Dependent Variable: CN.ratio

Tukey HSD

(I) Competation	(J) Competation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mono	Intra	-3.6475	1.88247	.152	-8.3764	1.0813
	Inter	-12.0619*	1.99666	.000	-17.0776	-7.0462
Intra	Mono	3.6475	1.88247	.152	-1.0813	8.3764
	Inter	-8.4144*	1.99666	.001	-13.4301	-3.3986
Inter	Mono	12.0619*	1.99666	.000	7.0462	17.0776
	Intra	8.4144*	1.99666	.001	3.3986	13.4301

Based on observed means.
The error term is Mean Square(Error) = 17.718.