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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

Zana A. Lak [1,2], Hans Sandén [1], Mathias Mayer [1,3], Douglas L. Godbold [1] and Boris Rewald [1,*]

1 Department Forest and Soil Sciences, Institute of Forest Ecology, University of Natural Resources and Life Sciences Vienna, 1190 Vienna, Austria; zana.lak@boku.ac.at (Z.A.L.); hans.sanden@boku.ac.at (H.S.); mathias.mayer@boku.ac.at (M.M.); douglas.godbold@boku.ac.at (D.L.G.)

2 College of Agriculture, Forestry Department, Salahaddin University Erbil, Erbil 44001, Kurdistan Region, Iraq

3 Forest Soils and Biogeochemistry, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), 8903 Birmensdorf, Switzerland

* Correspondence: boris.rewald@boku.ac.at; Tel.: +43-1-47654-91219

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Abstract: Belowground competition is an important structuring force in terrestrial plant communities. Uncertainties remain about the plasticity of functional root traits under competition, especially comparing interspecific vs. intraspecific situations. This study addresses the plasticity of fine root traits of competing *Acer pseudoplatanus* L. and *Fagus sylvatica* L. seedlings in nutrient-rich soil patches. Seedlings' roots were grown in a competition chamber experiment in which root growth (biomass), morphological and architectural fine roots traits, and potential activities of four extracellular enzymes were analyzed. Competition chambers with one, two conspecific, or two allospecific roots were established, and fertilized to create a nutrient 'hotspot'. Interspecific competition significantly reduced fine root growth in *Fagus* only, while intraspecific competition had no significant effect on the fine root biomass of either species. Competition reduced root nitrogen concentration and specific root respiration of both species. Potential extracellular enzymatic activities of β-glucosidase (BG) and *N*-acetyl-glucosaminidase (NAG) were lower in ectomycorrhizal *Fagus* roots competing with *Acer*. *Acer* fine roots had greater diameter and tip densities under intraspecific competition. *Fagus* root traits were generally more plastic than those of *Acer*, but no differences in trait plasticity were found between competitive situations. Compared to *Acer*, *Fagus* roots possessed a greater plasticity of all studied traits but coarse root biomass. However, this high plasticity did not result in directed trait value changes under interspecific competition, but *Fagus* roots grew less and realized lower N concentrations in comparison to competing *Acer* roots. The plasticity of root traits of both species was thus found to be highly species- but not competitor-specific. By showing that both con- and allospecific roots had similar effects on target root growth and most trait values, our data sheds light on the paradigm that the intensity of intraspecific competition is greater than those of interspecific competition belowground.

Keywords: *Acer pseudoplatanus*; competition below ground; extracellular enzymes; *Fagus sylvatica*; intraspecific and interspecific competition; toot economic spectrum; toot respiration; tree root traits

1. Introduction

There is strong evidence that belowground competition is an important structuring force in terrestrial plant communities including forests [1–4]—and often at least equally intense as aboveground competition [5–7]. Resource competition below ground is either based on exploitation (i.e., reduction of water and nutrient availability) or interference (i.e., the release of allelopathic compounds inhibiting growth or uptake), governed via fine roots of neighboring trees and their symbionts. In addition, intransitive mechanisms (i.e., competition mediated by factors other than resources, e.g., soil biota) are increasingly considered [8–11]. Competition occurs if negative effects on performance or fitness of a target plant (or components of it) result from the presence of another plant [12]. The magnitude of competitive effects (i.e., competition intensity) is a function of the plant's competitive ability (see [13] for a discussion on competitive response and effect components). Intraspecific competition is often assumed to be more intense due to a nearly complete niche overlap [14]. However, over-proportional reductions of fine root biomass in mixed stands and even over-proliferation of fine roots in soil volumes shared by allospecific roots have been reported [15–17]—pointing towards either greater or reduced root competition intensities under interspecific competition.

Evidence for global patterns of functional variation in plants, such as wood and leaf economic spectra [18,19], indicate that functional traits can enhance our mechanistic understanding of species' trade-offs (i.e., resource acquisition vs. conservation), community composition (e.g., influenced by competitive abilities) and ecosystem function [20,21]. As effects of shoot traits on individual plant strategies are increasingly understood, mechanisms behind species coexistence are progressively unraveled. For example, three key functional traits—wood density, specific leaf area and maximum height—have been shown to consistently influence competitive interactions in trees [22], but are also altered by co-occurring species (e.g., leaf trait variation in beech, [23]). In contrast, variation among functional traits of (tree) fine root systems remains poorly quantified [21,24–26]. Although it becomes increasingly clear that soil resource availabilities are among the key drivers of root trait variation [27,28], few studies have addressed the effects of direct competitive interactions on the plasticity of root functional traits, particularly physiological root traits (e.g., [11,29,30]). This is unfortunate, as trait dissimilarities—as well as increasingly plastic traits and intraspecific trait variations per se—are widely considered to reduce competition intensities and thus increase the probability of species' coexistence [31–34].

As it remains unclear which root traits are most strongly associated with resource limitation [24]—and perhaps also methodological difficulties to quantify resource limitations in (temperate) soils—previous research in forests has largely focused on biomass allocation patterns under competition. Either reduced, increased or unaffected fine root biomasses, depending on tree species' competitive abilities, have been reported [2,17,35]. It remains controversial whether a high degree of trait organization, i.e., one 'root economic spectrum', exists among (tree) fine roots [28,36,37]. However, the suggested traits (e.g., specific root length (SRL), nitrogen (N) content, specific uptake and respiration rates, and lifespan) are strongly related to resource acquisition or conservation [26]—and thus also central to access mechanisms of exploitative competition. In particular, the fast proliferation of active root surface area into heterogeneously distributed, resource-rich patches might be an important strategy to pre-empt resources ([8,38,39], but see [40]). Once space has been occupied, traits related to resource mobilization (such as extracellular enzymes exuded by roots or their fungal symbionts [41]) have to be considered. In sum, more detailed information on the plasticity of root functional traits under competition will facilitate our understanding of plant communities and ecosystem properties.

Mixed stands are increasingly perceived as a way to (partially) mitigate economic and ecological consequences of climate change [42,43]. Thus, the identification of factors which influence their regeneration is key [44–46]. In this context, European beech (*Fagus sylvatica*), the dominant tree species of a potential natural vegetation in large parts of the sub-mountainous altitude range of Central Europe [47], is favored. Sycamore maple (*Acer pseudoplatanus*) is a common competitor of beech—largely sharing its ecological spectrum, but possessing faster growth on resource-rich sites [47].

Successful regeneration of *A. pseudoplatanus* in beech-dominated stands of Central Europe has been attributed to the species' intermediate-to-high shade tolerance coupled with fast height growth if more light becomes available [44,45,48]. *Fagus* and *Acer* are, moreover, associated with different mycorrhizal symbionts, which might influence their nutrient acquisition strategies [49,50]. While ectomycorrhizal symbionts of *Fagus* are able to release enzymes to mobilize nutrients [51], arbuscular symbionts of *Acer* are suggested to (largely) lack these enzymatic abilities [52]. Admixing of beech with maple trees has been recently suggested to foster complementarity effects and to reduce competition within European mixed stands [23]. However, knowledge on the belowground interactions of *F. sylvatica* and *Acer* sp. is rare [30,53,54]—although Simon, et al. [55] underlined the high significance of competitive interactions of *Fagus sylvatica* with other vegetation components on its performance under progressing climate change.

This study aims to identify which root traits of *Acer pseudoplatanus* and *Fagus sylvatica* seedlings adapt under intra- and interspecific competition for a nutrient-rich soil patch. For this purpose, seedlings of both species were grown in a microcosm experiment. Microcosms were connected by competition chambers—wherein inserted roots were set foraging for nutrients. Root traits in intra- and interspecific neighborhoods were compared to roots growing without a competitor (i.e., solation). It is hypothesized that resource competition for a nutrient-rich spot (1) affects growth (biomass) and N concentration of *Acer* and *Fagus* fine roots negatively but to species-specific extents if growing with a con- or allospecific root, i.e., indicating different competition intensities. Furthermore, it is hypothesized that intra- and interspecific competition result in (2) differentiated, directed responses of specific root traits and that fine root biomass is not the sole trait affected, and (3) root trait plasticity is highly species-specific.

2. Material and Methods

2.1. Experiment Set-Up

The experiment was conducted in a ventilated polytunnel greenhouse under slightly increased temperature conditions (approximately 5 °C above ambient daily average) and near ambient lighting conditions (approximately 80%–90% of ambient photosynthetically active radiation, midday; data not shown), in Tulln, Austria (48°19′05.0″ N 16°03′58.2″ E). The experiment took place between mid-April and early September 2017. Two common broad-leaved European tree species of economic importance were studied: *Acer pseudoplatanus* L. (A, Sycamore maple) and *Fagus sylvatica* L. (F, European beech). *Acer pseudoplatanus* is strictly associated with arbuscular mycorrhizal (AM) fungi, while *Fagus sylvatica* is associated with ectomycorrhizal (ECM) fungi [56]. Two-year-old bare-rooted seedlings of similar height (0.9–1.1 m), stem diameter and crown characteristics were obtained in early April 2017 (before budburst) from a local nursery (Natlacen GmbH, Pilgersdorf, Austria); seed certification numbers A/31807-01/2013 (*Acer*) and D092021322711 (*Fagus*). Two-year-old seedlings were chosen because the early developmental stage is crucial for seedling establishment, in particular under competition for limited resources [57], and practical aspects; two-year-old seedlings featured sufficiently long roots while being not too big for the deployed microcosm systems [58].

A microcosm system with two attached 'competition chambers' (CC), allowing for root competitive interactions between seedlings at distinct locations, was developed (Figure 1). The CC are analogous to previously utilized *in situ* competition chambers described in Rewald and Leuschner [17]—allowing us to study intra- and inter-specific root competition effects in a highly controlled manner by minimizing parallel 'non-target' competition (e.g., the co-occurrence of intra- and inter-specific competition in studies using a 'shared'-pot design). In brief, the system comprises two large microcosms (7 L soil) interconnected by two small competition chambers (CC; 1 L soil). Initially, all compartments were filled with a nutrient-poor, sand–silty clay substrate (Supplementary Table S1). 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 intra- and interspecific

CC; thus, intra- and interspecific CCs hold two fine root axes, inserted from opposite sides (Figure 1). In the isolation treatment (i.e., no competition), one opening (i.e., towards the competitor's microcosm) was sealed and only one fine root segment (of the target species) was inserted. See Supplementary Materials S1 for details.

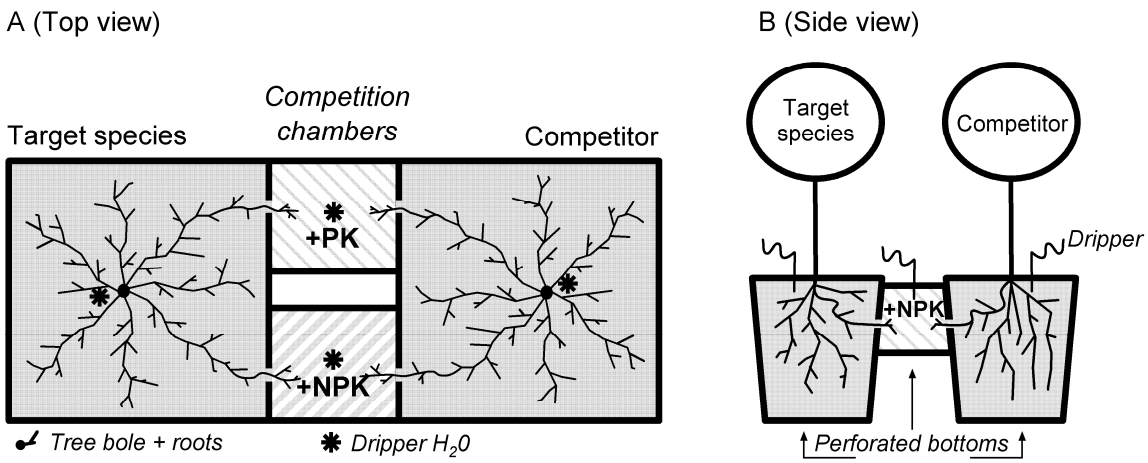

Figure 1. Microcosm system to study competition for localized nutrient-rich soil patches (i.e., competition chambers (CC) with NPK fertilization); (**A**) top view, (**B**) side view. Five-cm long sections of target (focal) species and competitor species' fine roots were inserted from opposite sides into the CC (in intra- and interspecific competition treatments); in the isolation (no competition) treatment, the apertures between the competitor and both CC were closed from one side (not displayed). Positions of tree boles (in microcosms with nutrient-poor substrate) and drippers are displayed. Nutrient-rich fertilizer (+NPK) was applied to the CC only; CC fertilized with a nutrient solution lacking nitrogen (+PK) are not part of this manuscript to keep manageable length and focus. Drawings not at scale.

Thus, three different competition treatments (Table 1) were established per species: (1) isolation (ISO; 'no competition'; only a fine root segment of the target species was inserted into each CC); (2) intraspecific competition (INTRA; roots of two seedlings of the same species, either *Acer* or *Fagus*, were inserted thru opposite sides of the CC); (3) interspecific competition (INTER; roots of one *Fagus* and *Acer* seedling each were inserted from opposite sides of the CC).

Table 1. Experimental set-up with three competitive situations belowground (i.e., isolation, intra- and interspecific competition) for a nutrient-rich spot by roots of two species, *Fagus sylvatica* (F) and *Acer pseudoplatanus* (A), resulting in six treatments (three per species). A:F and F:A originate essentially from the same competition chambers, but a distinction is made whether *Acer* (A:F) or *Fagus* (F:A) is considered the 'target species' (vs. competitor). Abbreviations of treatments are used throughout the manuscript; the number of realized replicates are given.

Type of Root Competition §	Target Species' Root	Belowground Competitor	Treatment (Abbrev.)	Realized Replication (n)
Isolation (ISO; no competition)	*Acer*	none	A	18
	Fagus	none	F	16
Intraspecific competition (INTRA)	*Acer*	*Acer*	A:A	48
	Fagus	*Fagus*	F:F	52
Interspecific competition (INTER)	*Acer*	*Fagus*	A:F	40 *
	Fagus	*Acer*	F:A	39 *

§ Aboveground competition was kept constant with both con- and allospecific neighbors growing at equal distances; * unequal numbers result from one lost *Fagus* sample during lab processing.

An automated, pressure-compensated drip irrigation system was installed ensuring ample water supply. The amount was increased over the growing season in a stepwise manner according to evapotranspiration. The CCs were manually fertilized once per week with 0.05 L of Hoagland solution (+NPK) to create nutrient-rich 'hotspots'. See Supplementary Material S1 for details.

In total, 131 microcosm systems holding 262 trees were set up; 7 were later excluded (see below), resulting in 124 analyzed microcosms (Table 1). A distance of 10 cm was kept between microcosms. The microcosms were set up randomly and in alternating directions (i.e., target species either placed north or south of competitor), to ensure a homogeneous competition environment above ground. After four months of fertilization (May–August), the CCs were harvested in early September 2017.

2.2. Harvesting of NPK Fertilized Competition Chambers

During harvest, the roots were (i) cut at the competition chambers' (CC) apertures (from the inside towards the microcosm), (ii) the bottom of the CC was opened and the substrate was emptied into a bowl, and (iii) the root origin (i.e., from one of the two microcosms) was marked. Subsequently, the apertures (towards the microcosms) were investigated for additional root in-growth (i.e., beside the initially inserted root axis), and roots were investigated for viability using morphological criteria and color [17]. If additional root in-growth or dead roots were detected, the CC were excluded from further analyses (7 CC in total), see realized replicate numbers (Table 1). While induced root death might be a competition mechanism [29], it cannot be ruled out that dead roots resulted from damage that occurred during installation. The roots were carefully rinsed under tap water and untangled into separate root branches—according to the microcosm of origin—keeping the roots moist at all times. No spatial segregation of roots in the CC was noticed during harvest. Subsequently, coarse root segments (diameter (d) > 2mm) were manually dissected from fine roots (d ≤ 2 mm) using a caliper and stored separately.

2.3. Specific Fine Root Respiration

Within 10 min after harvesting, surface-moist fine root sub-samples were entered into 55 mL plastic tubes with lids and placed in a climate cabinet (20 °C) for temperature acclimation (~10–15 min). Subsequently, the roots were blotted surface dry and the CO_2 efflux was determined at 20 °C with an infra-red gas-analyzer (IRGA; EGM-5, PP-Systems International, Inc. Amesbury, MA, USA) as ΔCO_2 (ppm)—recording ppm values every 30 sec for 4–5 minutes (+60 s 'deadband' to stabilize CO_2 readings before measurements). Slopes of ΔCO_2 were calculated by linear regressions (as no curve flattening, requiring polynomial approaches, was observed); specific (fine) root respiration rates (RR_S; nmol CO_2 g^{-1} s^{-1}) were calculated, taking headspace, air pressure, temperature, and root dry mass (see below) into account.

2.4. Potential Enzymatic Activity

For measuring the potential enzymatic activity (PEA) of root tips, 4–5 root tips (~2 mm in length) were randomly sampled per root branch within 10 minutes after harvesting. Root tips were placed in reaction tubes filled with deionized water, and stored for 14–20 h at 4–5 °C. Two of the sampled tips were used the next morning to determine PEAs (nmol cm^2 h^{-1}) at the rhizoplane, using high-throughput photometric and fluorometric microplate assays [59,60]. Four enzymes were measured: acid phosphatase (PEA_{AP}, releasing inorganic phosphate from organic matter), β-glucosidase (PEA_{BG}, hydrolyzing cellobiose into glucose), leucine-amino-peptidase (PEA_{LAP}, breaking down polypeptides), and *N*-acetyl-glucosaminidase (PEA_{NAG}, breaking down chitin). Methylumbelliferone-complexed substrates were used for AP (4-methylumbelliferyl phosphate), BG (4-methulumbelliferyl β-D-glucopyranoside) and NAG (4-methylumbelliferyl *N*-acetyl-β-glucosaminide); 7-amino-4-methylcoumarin (AMC) was used for LAP (L-leucine-7-amido-4-methylcoumarin hydrochloride). All root tips were subsequently imaged and analyzed for surface area (see below).

2.5. Root Morphology, Biomass and Root Competition Intensity

Fine (d ≤ 2 mm) and coarse (d > 2mm) root samples (including samples for respiration measurements) were stored in tap water at 4–5 °C until further processing. Fine root samples were individually imaged with a flatbed scanner (Expression 10000XL with transparency unit, Epson, Japan; 600 dpi, grey-scale). Images were analyzed with the software WinRhizo 2012b Pro (Regent, Quebec, Canada) for morphological root traits including length (cm), surface area (cm^2), volume (cm^3), and average diameter (RD; mm). In addition, root tip density (RTD; n cm^{-1}) was calculated by dividing recorded tip numbers by length.

Root samples were dried to constant mass (65 °C) and weighed (±0.1 mg); fine root (FRB) and coarse root (CRB)) values were recorded (g DM, dry matter). Specific root area (SRA; cm^2 g^{-1}) and tissue density (TD; g cm^{-3}) were calculated for dried fine root samples. In addition, total FRB (i.e., sum of target and competitor fine root biomass) was calculated per CC with intra- and interspecific competition.

To measure the strength of competition, three relative competition intensity (RCI) indices were calculated for each species using the fine root biomass (FRB). $\overline{RCI}_{Intra\ vs\ Iso}$ standardizes fine root biomass (FRB) in intraspecific mixtures (INTRA; i.e., monoculture) with FRB in isolation (ISO, i.e., 'alone', Equation (1); this study), $\overline{RCI}_{Inter\ vs\ Iso}$ standardizes FRB in interspecific mixtures (INTER) with FRB in isolation (Equation (2); sensu [61]), and $\overline{RCI}_{Inter\ vs\ Intra}$ standardizes FRB in interspecific mixtures with FRB in intraspecific mixtures (Equation (3); sensu [62]). Species-specific means ($\overline{FRB}$) under isolation (Equations (1) and (2)) or under intraspecific competition (Equation (3)) were used to calculate mean RCI values by comparison with individual FRB values for n competition chambers (n = 39–52).

$$\overline{RCI}_{Intra\ vs\ Iso} = \frac{1}{n}\sum_{i=1}^{n}\left(\overline{FRB}_{ISO} - FRB_{INTRA}\right) \,/\, \overline{FRB}_{ISO} \quad (1)$$

$$\overline{RCI}_{Inter\ vs\ Iso} = \frac{1}{n}\sum_{i=1}^{n}\left(\overline{FRB}_{ISO} - FRB_{INTER}\right) \,/\, \overline{FRB}_{ISO} \quad (2)$$

$$\overline{RCI}_{Inter\ vs\ Intra} = \frac{1}{n}\sum_{i=1}^{n}\left(\overline{FRB}_{INTRA} - FRB_{INTER}\right) \,/\, \overline{FRB}_{INTRA} \quad (3)$$

2.6. Root and Soil Chemical Analysis

For determination of root carbon (C) and nitrogen (N) concentrations, dried (70 °C, until constant mass) fine roots were ground to powder (Pulverisette 5; Fritsch, Idar-Oberstein, Germany). Fine roots were analyzed by pooling and homogenizing three random samples each per treatment (n = 5). 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. C:N ratios were calculated. See Supplementary Material S2 for details on soil chemical analysis.

2.7. Root Trait Plasticity

The plasticity of root traits under the different competitive situations was calculated as relative distance plasticity index (RDPI) with strong statistical power to test for differences in plasticity of traits within and between species (see [63] for details). Root phenotypic plasticity was determined for the following (normalized) traits: FRB, CRB ('biomass traits'); RD, TD, SRA and RTD ('morphological traits'); and RR_S, PEA_{AP}, PEA_{BG}, PEA_{LAP}, PEA_{NAG} ('physiological traits'). RDPI(X) values were calculated for each specific trait (X) as relative phenotypic distances between individuals ($d_{ij}\rightarrow i'j'$) of the same species under different competition treatments, divided by the sum ($x_{i'j'}$ + x_{ij}). An RDPI ranging from 0 (no plasticity) to 1 (maximal plasticity) was obtained for each species as:

$$RDPI = \Sigma(d_{ij}\rightarrow i'j'/(x_{i'j'} + x_{ij}))/n, \quad (4)$$

where n is the total number of distances. Three 'types' of RDPI were calculated: (1) $RDPI_{Total,}$ considering relative phenotypic distances between all three competition treatments (i.e., ISO | INTRA | INTER), (2) $RDPI_{INTRA\ vs\ ISO}$, considering relative phenotypic distances between traits under intraspecific competition and isolation only, and (3) $RDPI_{INTER\ vs\ ISO}$ considering relative phenotypic distances between traits under interspecific competition and isolation only. RDPI values were calculated by means of the statistical software R, v. 3.5.3, [64] and the R package 'Plasticity' [65]. Subsequently, $RDPI_{Total}$ values of root traits were compared between species. $RDPI_{INTRA\ vs\ ISO}$ and $RDPI_{INTER\ vs\ ISO}$ values of specific traits were contrasted to determine if the plasticity of all or some traits differs between intra- and interspecific competition.

2.8. Statistical Analysis

Statistical analysis was performed using the PC software SPSS v. 24.0 (SPSS, IL, USA) and Microsoft Excel 2013. The data were transformed to obtain a normal distribution when needed (Kolmogorov–Smirnov test) and controlled for homogeneity of variances (Leven's test). Overall differences in root traits between species and competitive situations were tested with a general linear model (GLM) with species and competition as fixed variables (see Supplementary Material S4 for GLM statistics). In addition, all traits were tested for differences between treatments with a *t*-test; RCI was tested for differences between species within competitive environments with a *t*-test; $RDPI_{Total}$ values among species and/or competition treatments were analyzed by GLMs followed by two-sample *t*-tests. Please note, the alpha-value of *t*-tests were not Bonferroni-corrected, following the arguments of Moran [66]. All data represent mean ± standard error (SE). Statistical relationships were considered significant at $p < 0.05$.

3. Results

3.1. Root Biomass and Competition Intensity

The fine (FRB; Figure 2a; Supplementary Table S2), coarse (CRB; Supplementary Figure S1, Table S3), and total root biomass (Supplementary Table S4) of *Acer* were larger than those of *Fagus*. In *Acer,* FRB did not vary significantly under intra- and interspecific competition compared to isolation (i.e., no competition). In contrast, significantly less fine and coarse root biomass were found in *Fagus* under interspecific competition (F:A) compared to isolation. The relative distance plasticity indices ($RDPI_{Total}$; Figure 3a) of FRB and CRB possessed in both species the greatest values across all measured traits (beside $RDPI_{Total}(PEA_{NAG})$ of *Fagus*, see below; Figure 3a).

$RDPI_{Total}$(FRB) of *Fagus* was significantly greater than that of *Acer*, while $RDPI_{Total}$(CRB) values did not differ between species. The RCI values of *Acer* were generally lower than RCI values of *Fagus*; $RCI_{INTER\ vs\ ISO}$ of *Fagus* under interspecific competition tended ($p = 0.06$) to be greater than $RCI_{INTER\ vs\ ISO}$ of *Acer* (Figure 2b).

3.2. Fine Root Respiration

The specific fine root respiration (RR_S) rates were significantly lower in both species grown under intra- and interspecific competition in comparison with isolation (Figure 4). In most cases, RR_S did not differ significantly between species within the same competition treatment; however, the RR_S of *Acer* under intraspecific competition (A:A) was significantly lower than the RR_S of *Fagus* under intraspecific competition (F:F). Within species, the *t*-test indicated no significant differences in RR_S between intra- and interspecific competition, however, a significant interaction in the GLM between species and competition type may hint at *Acer* fine roots respiring more under interspecific competition and *Fagus* under intraspecific competition (Supplementary Table S5).

$RDPI_{Total}$ (RR_S) values of *Acer* and *Fagus*' RR_S differed significantly between species (Figure 3a).

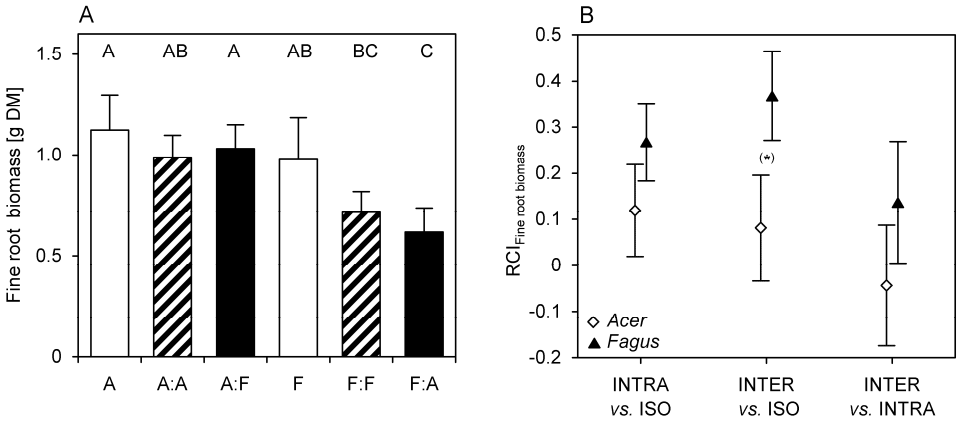

Figure 2. (**A**) Biomass (g DM, dry matter) of fine roots of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) grown under three different competition treatments each into nutrient-rich soil patches. A, *Acer* root grown in isolation (no competition); A:A, *Acer* root grown in competition with another *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 + standard error (SE), n = 39–52; see Supplementary Table S2 for GLM statistics on log-transformed FRB data). (**B**) Relative competition intensity (RCI) based on fine root biomass (FRB) of *Acer* (open rectangles) and *Fagus* (filled triangles) under 1) intraspecific competition (INTRA) relative to isolation (ISO), 2) interspecific competition (INTER) relative to ISO, and 3) INTER relative to INTRA. Tendencies between species are indicated by (*) (*t*-test, $p = 0.06$; mean ± SE, n = 39–52).

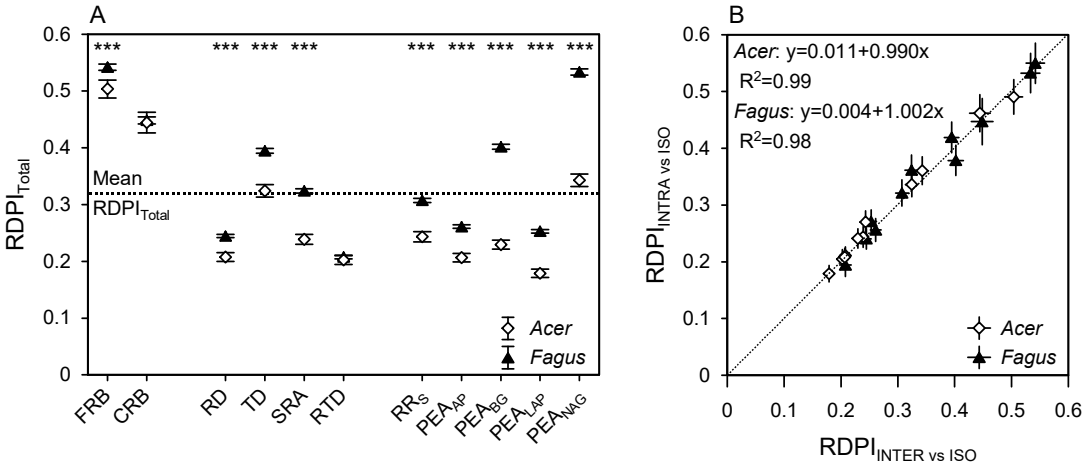

Figure 3. **(A)** Relative distance plasticity indices ($RDPI_{Total}$) for the traits fine root biomass (FRB), coarse root biomass (CRB), root diameter (RD), tissue density (TD), specific root area (SRA), root tip density (RTD), specific root respiration (RR_S) and the potential enzymatic activities (PEA) of acid phosphatase (PEA_{AP}), β-glucosidase (PEA_{BG}), leucine-amino-peptidase (PEA_{LAP}), and N-acetyl-glucosaminidase (PEA_{NAG}) of *Acer pseudoplatanus* (open rectangles) and *Fagus sylvatica* (filled triangles) across isolation, intra- and interspecific competition. The mean $RDPI_{Total}$ value (across traits and species) is shown as the dotted line. Significant differences between species are marked (*t*-test, $p < 0.001$ ***; mean ± three standard errors (3SE)). **(B)** Relative distance plasticity indices under intraspecific competition ($RDPI_{INTRA\ vs\ ISO}$) vs. RDPI under interspecific competition ($RDPI_{INTER\ vs\ ISO}$; mean ± 3SE); linear regressions (formulae and R^2) of trait means are given for *Acer* and *Fagus*; the 1:1 line is drawn for comparison (dotted line).

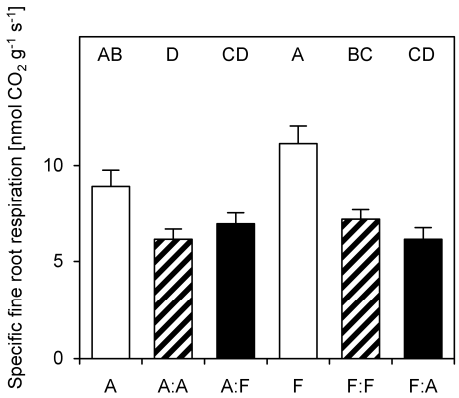

Figure 4. Specific fine root respiration (RR_S; 20 °C) of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) under three different competition treatments in nutrient-rich soil patches. A, *Acer* root grown in isolation (no competition); A:A, *Acer* root grown in competition with another *Acer* root; 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* (mean + SE, n = 15–49; see Supplementary Table S5 for GLM statistics).

3.3. Potential Extracellular Enzymatic Activities

Fagus root tips possessed generally higher potential enzymatic activities (PEA) at the surface than *Acer* root tips (Figure 5). Specifically, the PEA of acid phosphatase (PEA_{AP}) possessed no differences to either intra- or interspecific competition compared to isolation (Figure 5a). Similarly, the PEA of leucine-amino-peptidase (PEA_{LAP}) was unaffected by the competition treatments in both species (Figure 5c). Finally, the PEAs of both β-glucosidase (PEA_{BG}) and *N*-acetyl-glucosaminidase (PEA_{NAG}) were unaffected by competition in *Acer* while being significantly reduced in *Fagus* under interspecific competition (F:A) compared to isolation (Figure 5b,d).

$RDPI_{Total}$ values of PEA_{AP} and PEA_{LAP} ranged in both species from 0.18–0.26; the plasticity of PEA_{BG} had a similar extent compared to PEA_{AP} and PEA_{LAP} in *Acer* but was greater in *Fagus* (Figure 3a). PEA_{NAG} possessed $RDPI_{Total}$ values of 0.34 ± 0.00 and 0.53 ± 0.01 in *Acer* and *Fagus*, respectively.

3.4. Fine Root Morphology

The average fine root diameter (RD) differed significantly between *Acer* grown under intra- (A:A) compared to interspecific (A:F) competition, with lower average diameters under interspecific competition (Figure 6a). The tissue density (TD) and specific root area (SRA) of *Acer* fine roots were generally lower or greater, respectively, compared to *Fagus*; competition had no significant influence on either trait (Figure 6b,c). GLM evidenced no overall competition effect on root diameter (RD), tissue density (TD) and specific root area (SRA; Supplementary Tables S6–S8). The root tip density was generally greater under intraspecific competition as compared to isolation and intraspecific competition (Supplementary Table S9); this effect was largest in *Acer* (Figure 6d). *Fagus* had both higher TD and RTD than *Acer* (Supplementary Tables S8 and S9).

The RD and RTD of both species possessed relatively low plasticity indices under competition, with $RDPI_{Total}$ values of 0.20–0.24; however, $RDPI_{Total}$ (RD) differed significantly between species, with greater RDPI values in *Fagus* (Figure 3a). The $RDPI_{Total}$ (TD) differed significantly between *Acer* and *Fagus*. Similar, $RDPI_{Total}$ (SRA) values differed significantly between species, with a significantly greater plasticity of SRA in *Fagus* compared to *Acer*.

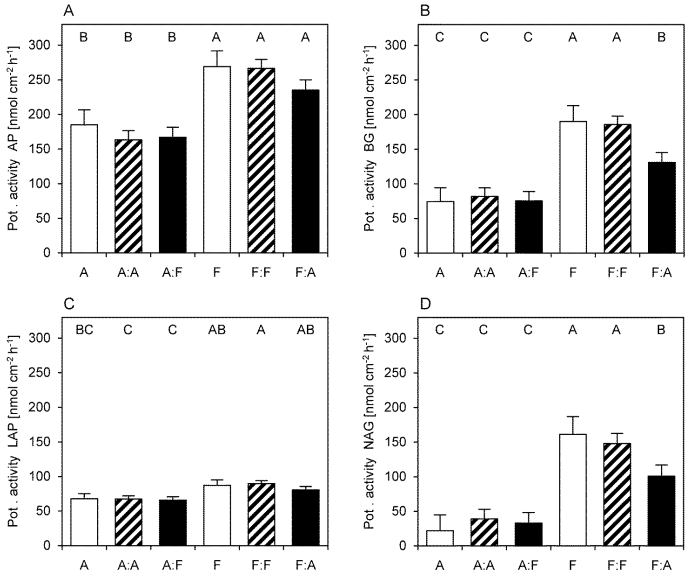

Figure 5. Potential enzymatic activities (PEA; nmol cm^{-2} h^{-1}) of **(A)** acid phosphatase (AP), **(B)** β-glucosidase (BG), **(C)** leucine-amino-peptidase (LAP), and **(D)** *N*-acetyl-glucosaminidase (NAG) on the rhizoplane of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) root tips growing under three different competition treatments each in 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 = 14–50).

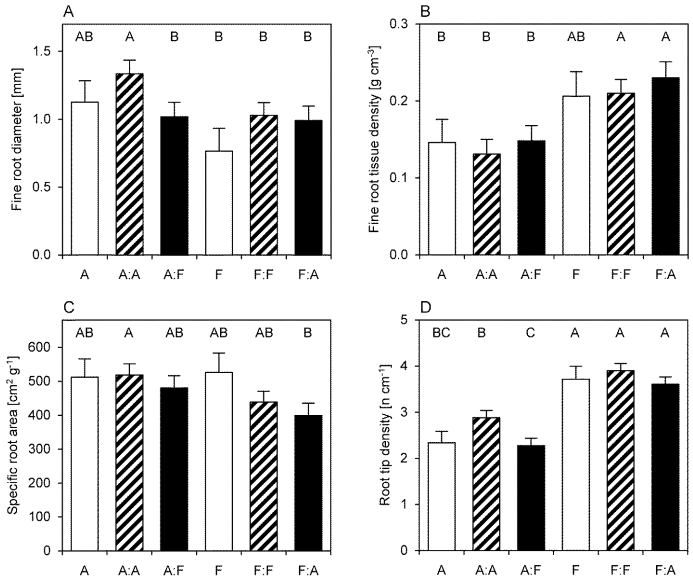

Figure 6. Morphological traits of fine roots. **(A)** Average fine root diameter (RD), **(B)** fine root tissue density (TD), **(C)** specific root area (SRA), and **(D)** root tip density (RTD) of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) grown under three different competition treatments 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 = 16–52; see Supplementary Tables S6–S9 for GLM statistics, partially on log-transformed data).

3.5. Fine Root N Concentrations

No significant differences in total nitrogen (N) concentrations were found between *Acer* and *Fagus* fine roots grown under similar competitive treatments. However, N concentrations were significantly reduced by approximately 7%–33% under competition in both species (Figure 7, Supplementary Table S10). In *Fagus*, the N under interspecific competition (F:A) was significantly lower compared to both intraspecific competition (F:F) and isolation (F), while in *Acer*, the N concentration under interspecific competition was significantly lower compared to isolation only. The low N content under interspecific competition is reflected by a greater C:N ratio under interspecific competition compared to the other two treatments; see Supporting Material S2 for fine root total carbon concentration and C:N ratios (Supplementary Figure S2, Table S11).

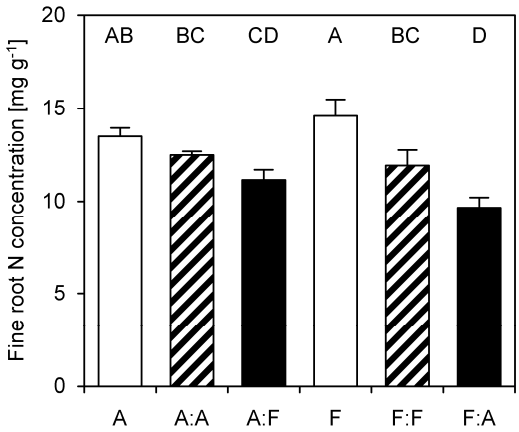

Figure 7. Fine root nitrogen (N) concentration of *Acer pseudoplatanus* (A) and *Fagus sylvatica* (F) grown under three different competition treatments into nutrient-rich soil patches. A, *Acer* root grown in isolation (no competition); A:A, *Acer* root grown in competition with another *Acer* root; 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 S10 for GLM statistics).

3.6. Plasticity Index

Greater relative distance plasticity index (RDPI) values indicate a greater plasticity of a specific trait under different environmental conditions, i.e., here understood as the different competition treatments. *Fagus* roots possessed greater $RDPI_{Total}$ values for the traits FRB, RD, TD, SRA, RR_S and all four PEAs than *Acer* (Figure 3a). For both species, the $RDPI_{Total}$ values for the traits FRB and CRB were above the mean of all traits' $RDPI_{Total}$ across species (0.32 ± 0.02; dotted line in Figure 6b); $RDPI_{Total}$ values of TD, PEA_{BG} and PEA_{NAG} were 30%–50% greater than the mean $RDPI_{Total}$ in *Fagus* only. In both species, the traits RD, RTD, PEA_{AP} and PEA_{LAP} possessed $RDPI_{Total}$ values well below the mean.

The $RDPI_{INTRA\ vs\ ISO}$ and $RDPI_{INTER\ vs\ ISO}$ values per species and trait were comparable to the respective $RDPI_{Total}$ values, but slightly lower (Figure 3). The RDPI values under intra- and interspecific competition, each relative to the trait values under isolation (Control), were highly related and thus aligned closely to a hypothesized 1:1 line (Figure 3b).

4. Discussion

4.1. Influence of Competition for a Nutrient-Rich Soil Spot on Fine Root Foraging Behaviour, Root Nitrogen Status and Root Trait Characteristics

The root biomass production rates, root N concentrations and C:N ratios measured in the competition chambers (CC) were comparable to values from nutrient-rich top soil layers in mature stands dominated by either species [67], suggesting that realistic experimental conditions were

established. No signs of fine root over-proliferation were found [38] but individual root biomasses were in general lower in CCs with a competing root compared to isolation (Figure 2a). The stronger decrease in fine root biomass in *Fagus* as compared to *Acer* seedlings under competition and the relative competition intensity (RCI) indices illustrate that *Fagus* seedlings' roots are affected to a greater extent by roots sharing the same soil volume than *Acers'* (Figure 2b). In accordance, significant effects of neighboring plants on *Fagus* root systems (i.e., reduced total fine root biomass/root length density or shifted rooting depths) were previously reported for e.g., mature mixed stands of *Fagus* and *Picea abies* [35,68,69], although with contrasting results in regard to the shift of *Fagus*' fine root production to deeper or more shallow soil horizons, respectively. In contrast, Leuschner, Hertel, Coners and Büttner [2] reported increasing *Fagus* fine root biomass (i.e., over-proliferation) when competing with *Quercus petraea* roots for N-rich top soil layers, concluding that this competitive replacement of *Quercus* fine roots by faster growing *Fagus* roots indicates asymmetric interspecific root competition in favor of *Fagus* in the studied stand. Although data on *Acer* sp. fine root biomass in monocultures is absent, to the best of our knowledge, Meinen, et al. [70] showed that the (relative) fine root biomass of mature *Acer* sp. increased with increasing tree species diversity level *in situ*. Based on findings on mature trees, we had thus hypothesized that competition intensities between seedling roots also differ largely between competitive situations. The supposed difference between intra- and interspecific situations is usually related to two contrasting ideas—one being that conspecific roots compete for more similar resources and root growth is thus inhibited to a larger extent compared to interspecific situations, where facilitative aspects may dominate [17,71], and another being that plants may recognize their 'kin' and compete less with conspecifics vs. 'strangers', i.e., allospecific roots [32,72]. However, our data does not support either hypotheses for both species as fine root biomasses and competition intensities under intra- and interspecific competition did not differ significantly, indicating similar effects of neighboring roots independent of their identity. However, as the fine root biomass of *Fagus* declined significantly compared to isolation while *Acers*' did not, the available data underscore that root foraging behaviors under interspecific competition are in favor of *Acer* seedlings. Together with previous results, using similar experimental set-ups in situ [17,71,73], our results add to the conclusion that root foraging behaviors (of temperate trees) in shared soil patches are highly species-specific and modulated by the respective environmental conditions [30,71,74]. We thus suggest that our current understanding of root competitive interactions does not yet allow for drawing general predictions on the intensity of root interactions under intra- vs. interspecific competition between species.

While a reduced root resource uptake capacity, as related to reduced fine root biomasses, length or surface, might be 'counterbalanced' in theory by increasing specific uptake rates, the fine root N concentrations of both *Fagus* and *Acer* seedlings decreased under interspecific competition while being significantly lower under intraspecific competition in *Fagus* (Figure 7). Similarly, Simon, et al. [75] reported that under interspecific competition, the inorganic N uptake rates (per root dry weight) decreased by up to 80% in *Fagus* but increased by 30%–50% in *Acer* seedlings, resulting in significantly lower N concentrations in the roots of *Fagus* compared to intraspecific situations. The results of Simon and colleagues are also consistent with earlier reports stating 30%–60% lower/decreased inorganic N accumulation efficiencies/uptake rates in *Fagus* seedlings under competition with *Rubus fruticosus* [74,76]. While we cannot exclude modified N translocation rates (to the shoot or other parts of the root system), our results on root N concentrations are in line with previous reports indicating lower N uptake capacities of *Fagus* roots in comparison to *Acer* seedlings' roots.

Specific root respiration (RR_S) rates depend on three major energy-requiring processes, namely ion uptake and mobilization, growth and defense, and maintenance of living cells, and root respiration represents a major sink of assimilated C [77]. As both species possess relatively similar growth rates at sapling stage [44,48], and RR_S is generally considered to be related to growth rates [77], the similarity of RR_S of *Fagus* and *Acer* within the same competitive situation might come as a limited surprise. However, our results are in contrast to findings on *Pisum sativum* and root tips of *Larix gmelinii*, where (nocturnal) root respiration increased significantly under non-self/interspecific competition for unknown reasons

(see [11,78] and the discussion within). Furthermore, our experiment did not find significant differences in RR_S between intra- and interspecific competition (Figure 4), while Zwetsloot, Goebel, Paya, Grams and Bauerle [69] recently reported that oxygen consumption rates of *Fagus*, and partially *Picea abies*, fine roots (of mature trees during spring) were significantly lower under interspecific competition compared to 'single species' conditions. However, it remains open if Zwetsloot and colleagues measured 'single species' RR_S on roots competing with roots of conspecifics, the same individual, or isolated roots. As respiration is highly related to root N concentrations—as a proxy for the amount of protein—it may serve as a predictor of root tissue activity [26]. Thus, lower root N concentrations and the sum of reduced, RR_S-effective 'activities' such as growth and exudation, may underlie the reduced RR_S rates under competition found in our study. Further studies are needed to untangle the contrastingly reported, potentially direct (e.g., interference competition) or indirect (e.g., resource competition) effects of roots sharing the same soil volume on specific root respiration rates.

In addition to root biomass and root respiration, root exudates (directly or via C transfer to exuding mycorrhiza) can be a substantial sink for assimilated carbon and have a major influence on plant mineralization and nutrient uptake capacity and efficiency. The C investment in symbiotic microorganisms, in our case ectomycorrhiza for *Fagus* and arbuscular mycorrhiza for *Acer*, is reflected in the potential enzymatic activity (PEA) on the root rhizoplane—as among the four analyzed PEAs, roots can only produce phosphatase (AP, Figure 5a). In contrast, NAG in particular has been found to be strongly related to soil fungal biomass [see [79] and references within). The generally higher PEAs in *Fagus* are likely based on the higher enzyme exudation rates of ECM compared to AM fungi [80]; NAG activity has been found to be strongly correlated to Basidiomycota and Ascomycota (both ectomycorrhizal phyla). Changes in PEAs of BG, LAP and NAG are thus likely (co-)related to changes in mycorrhizal colonization rates or identity of the symbionts; both parameters were previously reported to differ between monocultures and mixtures [11,81], but lay beyond the focus of this study. A lower colonization rate of *Fagus* under interspecific competition could thus be another factor underlying the lower extracellular enzymatic activity (reduced PEA_{NAG} and PEA_{BG}) of *Fagus* fine roots and their symbionts under interspecific competition. In contrast, an increase in the enzymatic activity, especially of NAG, would have indicated a change in nutrient foraging strategy from roots to hyphae under competition—however, our study does not provide evidence for such potentially adaptive changes in neither of the two species.

In sum, we could not generally confirm our first hypothesis, namely that that sharing a nutrient-rich spot with another root strongly affects root biomass and N status of fine roots negatively; *Acers'* root growth and N content were, especially under intraspecific competition, affected only to a minor extent. However, our data does provide support for the hypotheses that root competition intensities differ for the two studied tree species but do not confirm that competitive interactions generally differ under intra- and interspecific situations. This strongly contrasts common findings above ground, namely that 'competition within species [is] stronger than between species' [22]—the divergence is likely related to the multitude of resources below- compared to aboveground.

4.2. Species-Specific Plasticity of Functional Root Traits (under Intra- and Interspecific Competition)

In our third hypothesis, we speculated that different competitive neighborhoods trigger distinct, species-specific responses among root traits—potentially increasing the differences between specific root traits. Our results evidence that root traits of *Fagus* seedlings were in general significantly more plastic than *Acer* root traits (Figure 3). This fits with previous studies, which frequently describe the root system (biomass) of *Fagus sylvatica* as being very dynamic and adaptable to competitive situations compared to other Central European tree species (e.g., [17,68,69]). Specifically, we found a high degree of plasticity in biomass-related traits (i.e., FRB, CRB) of both studied species while the morphological root traits studied in this work (i.e., RD, TD, SRA), especially of *Fagus*, possessed limited plasticities. While the high plasticity of fine root biomass fits previous findings on tree roots competing with other tree roots [17], it contrasts the findings of e.g., *Fagus* with herbs where FRB was not responsive [82].

The low plasticity of morphological traits was surprising as it has been frequently hypothesized that e.g., the production of thinner roots with a greater SRL or SRA in response to a specific neighbor could improve nutrient and water uptake under competition [83,84]. Indeed, increased SRL were previously reported for competing, mature *Fagus* trees [68], and *Fagus* seedlings competing with herbs [82]. Furthermore, consistent responses to N enrichment resulting in greater fine root diameters of temperate trees has been reported recently [85]. However, similar to our findings (Figure 6a,c), Lei et al. [86] reported no significant differences in *Fagus* fine-root diameter and SRL between different species richness levels. In *Acer*, on the other hand, fine root diameter (RD) and root tip density (RTD) were significantly greater under intra- compared to interspecific competition (Figure 6a,d). As we did not perform a root-order based analysis, we can only speculate that the increased mean fine RD in *Acer* is related to the increase in RTD, as 'swollen' root tips often feature a slightly larger diameter than the next higher fine root orders. As the N content of *Acer* fine roots was sustained under intraspecific competition, this might point to a benefit of a greater density of 'physiological active' root tips for N uptake [87]; however, studying the underlying (e.g., anatomical) traits of the RD change in greater detail would be necessary to draw general conclusions [85]. Among the physiological root traits studied, *Fagus* showed a high plasticity in PEA_{NAG}. As PEA_{NAG} is related to the presence of fungal symbionts, and ectomycorrhiza are only present in *Fagus*, the high plasticity probably largely reflects different ectomycorrhizal colonization or activity as discussed above. In contrast, $RDPI_{Total}$ values do indicate a generally low plasticity of RR_S and other PEAs. No consistent patterns regarding the influence of competition for local nutrient patches on morphological or physiological root traits have thus emerged in our study.

In summary, our study possesses limited evidence for greater root trait dissimilarities under interspecific competition. This is further supported by comparing the plasticities between isolation and intra- ($RDPI_{INTRA\ vs\ ISO}$), and isolation and interspecific ($RDPI_{INTER\ vs\ ISO}$) competition. They follow a near 1:1 pattern in our study—i.e., indicating very similar trait plasticities irrespective of the competing species (Figure 3b). Thus, most observed changes in root trait values under competition compared to isolation might be rather a 'passive' reaction to resource availability (or a modified stoichiometry) and not directed or even adaptive (to specific competitors)—in the sense of increasing the competitive ability of a specific root. However, as information on root trait values is still scarce in general and not related to different competitive situations but environmental gradients at best [27,28], it seems too early to draw general conclusions on root reactions norms under resource competition. However, due to the marked differences in root traits under isolation compared to 'competition', we suggest that information on the competitive neighborhood (in a shared soil space) is key ancillary data needed to better interpret functional root traits deposited within databases [88,89].

5. Conclusions

In accordance with previous results [30,53,75], our study underscores the inability of roots of *Fagus sylvatica* seedlings to successfully compete with *Acer pseudoplatanus* roots for nutrient-rich soil patches under ambient light conditions. This inability of *Fagus* is embodied by the significantly reduced root biomass placement under interspecific competition, partially in combination with reduced extracellular enzymatic activities, which resulted in low root N concentrations. Our findings can be generally attributed to the different growth patterns of *Fagus sylvatica* and *Acer pseudoplatanus*, at least at the seedling stage. In studies investigating growth performance, *Acer sp.* had a competitive advantage over *Fagus* with increasing light availability and under non-limiting water supply (e.g., [54,90]).

The observed foraging behavior of *Fagus sylvatica* seedlings under competition seems unfavorable to exploit a specific, nutrient-rich soil patch. However, we speculate that a (potentially resource availability- or kin recognition-induced) feedback mechanism may limit root growth (and related C costs, i.e., respiration, exudation and mycorrhizal symbionts; as evident from our data) into 'pre-occupied' soil areas. Limiting C allocation to specific, 'non-efficient' roots (in regard to resource uptake) may result in an overall greater C use efficiency in *Fagus* seedlings. Diverging C allocation

and turnover patterns in specific parts of the root system have been previously shown e.g., in *Pinus sylvestris* [91]. This may foster the ability of highly shade-tolerant *Fagus* seedlings to withstand the limited availability of photosynthetic assimilates—as is prevailing under the light conditions of dense forest understories. Indeed, the importance of C assimilate availability in determining the N uptake capacities of *Acer* and *Fagus* was demonstrated by Simon, Li and Rennenberg [53]—i.e., a reduced light availability severely hampering the ammonium and glutamine uptake of *Acer* but not *Fagus* under interspecific competition. The strongly reduced fine root biomass of *Fagus* might thus be interpreted as a 'self-thinning mechanism', reducing the competitive interactions belowground by curtailing the overlap of root/mycorrhizal zones (and leading to increased root zone 'stratification')—as repeatedly shown for mature *Fagus sylvatica* trees in mixtures (see also theoretical consideration in [38]). While in large parts of Central Europe, the competitive advantage of *Fagus sylvatica* aboveground is clearly related to its ability to tolerate shade in the juvenile state and pre-empt light as mature trees, evidence is thus increasing that *Fagus* 'strength' belowground may not be its competitive effect on neighboring roots per se but its highly plastic and C-efficient root system. To improve our understanding of competitive mechanisms belowground, further studies are needed considering C and nutrient metabolism of roots and their symbionts in relation to whole plant C economics.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/5/528/s1, Table S1: Soil properties in the microcosms and competition chamber. Tables S2–S11: Statistical output of GLMs. Figure S1: Coarse root biomass of *Acer* and *Fagus*. Figure S2: Fine root carbon-to-nitrogen ratio of *Acer* and *Fagus*. Supplementary Material S1: Detailed description of the experimental set-up, irrigation and fertilization, Supplementary Material S2: Methods used to determine soil chemical properties. Supplementary Material S3: Fine root carbon concentration and C:N ratio. Supplementary Material S4: Statistics.

Author Contributions: B.R. and H.S. conceived the experiment. Z.A.L., B.R. and H.S. conducted the experiment and curated and analyzed the data. M.M. calculated the RDPI. Z.A.L. and B.R. wrote the first draft of the paper, Z.A.L., B.R., H.S., D.L.G. and M.M. jointly revised and edited the paper. All authors have read and agreed to the published version of the manuscript.

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