

Mixing varieties mitigates early root competition in wheat under water and nutrient limitation

Running title: mixing varieties mitigates root competition in wheat

Germain Montazeaud^{1,†}, Pierre Roumet¹, Mickaël Lamboeuf², Christian Jeudy², Martin Ecarnot¹, Lise
Malicet-Chebbah¹, Christophe Salon², Hélène Fréville¹

¹AGAP, Université de Montpellier, CIRAD, INRAE, L'institut Agro, Montpellier, France

²Univ. Bourgogne, Agroecol Lab, Univ. Bourgogne Franche Comte, AgroSup Dijon, INRAE, Dijon,
France

[†]corresponding author: germain.montazeaud@inrae.fr

pierre.roumet@inrae.fr

mickael.lamboeuf@inrae.fr

christian.jeudy.2@inrae.fr

martin.ecarnot@inrae.fr

lise.malicet-chebbah@supagro.fr

christophe.salon@inrae.fr

helene.freville@inrae.fr

Date of submission: 14th November 2024

Figures: 5

Tables: 0

Supplementary Figures: 4

Supplementary Table: 6

Word Count (Introduction to beginning of acknowledgements, excluding M&M): 4,075

Highlight

Asymmetric responses to belowground competition between varieties with high vs low root projected area generate positive selection effects in wheat varietal mixtures

Abstract

Competition between plants can lead to a Tragedy of the Commons where over-investments into resource harvesting organs reduce collective performance. Mixing different varieties could resolve such TOCs because different varieties might have different resource requirements (niche complementarity effect) or because the most competitive varieties benefit from being mixed with weaker competitors (selection effect). In the present study, we tested whether such ecological effects could mitigate belowground competition in durum wheat (*Triticum turgidum* ssp. *durum*). We grew 36 varieties in pure stands and in 54 binary mixtures in a high-throughput root phenotyping platform under both controlled conditions (R+) and water and nutrient limitation (R-). Seedlings in mixed stands produced less biomass than predicted from their pure stands in R-. This biomass reduction reflected a relaxation of competition where competitive varieties benefited from having a weaker competitor than themselves (selection effect), and thus disengaged from an arms race for biomass accumulation. The competitive hierarchy between varieties was captured by a single trait, the projected area of the root system which predicted ~50% of the biomass reduction in R-. Our results suggest that root area is a promising breeding targets to reduce intra-specific competition and a key trait to consider for mixture assembly.

Keywords

Agroecology, arms race, competitive hierarchy, high-throughput root phenotyping, niche complementarity, relative yield, root projected area, tragedy of the commons, varietal mixtures, wheat

Abbreviations

RY: Relative Yield; RYT: Relative Yield Total

48 Introduction

49 Competition for resources is a fundamental determinant of plant phenotypes and plant community
50 dynamics (Tilman, 2020). A plant's survival, growth and reproduction can be reduced by highly
51 competitive neighbours that absorb and use resources more quickly and/or more efficiently. To deal
52 with such situations, plants have evolved various mechanisms to detect the presence of neighbours
53 and/or the early exhaustion of resources, and to react by becoming more efficient at capturing
54 resources (Pierik *et al.*, 2013). For example, plants can anticipate light competition by detecting
55 changes in the red:far-red ratios of reflected and transmitted light on the leaves of their neighbours
56 (Ballaré *et al.*, 1990). These changes in light quality in turn trigger a series of phenotypic changes,
57 known as the shade avoidance syndrome, which ultimately lead to more vertical growth in order to
58 out-compete neighbours, e.g., increased plant height or hyponastia of leaves (Ballaré and Pierik,
59 2017). Similarly, competition for belowground resources (water or nutrients) can lead to root
60 proliferation in the presence of a competitor (Robinson *et al.*, 1999; Gersani *et al.*, 2001), which can
61 be triggered either by early detection of resource depletion, or by the detection of chemical markers
62 from the competitors (Schenk, 2006; Pierik *et al.*, 2013).

63 While such response primarily evolved to maximize individual plant fitness under competition, they
64 can lead to a competitive arms race between individuals, and ultimately decrease fitness at the scale of
65 the group, which is known as a Tragedy of the Commons (TOCs, Hardin, 1968). This reduction in
66 collective performance results from two effects: resources allocated to competitive organs are diverted
67 from reproduction, and all individuals suffer from an intense pressure on the resources from their
68 neighbours. Such trajectories have important implications for agriculture, as intense competition
69 between adjacent plants from the same species can reduce yield per unit area (Anten and Vermeulen,
70 2016). This was notoriously identified by agronomists and plant breeders in the context of the Green
71 Revolution: intra-specific competition, especially for light, becomes a strong determinant of yield in a
72 typical high density cereal cropping system with low weed pressure and high fertilizer and pesticide
73 inputs (Donald, 1963, 1968; Jennings and de Jesus, 1968). This observation led to the emergence of a
74 new paradigm for plant breeding based on the idea that selection should target varieties with weak

75 competitive abilities, because such varieties can be grown at high density without wasting resources
76 into competition (Donald, 1968). The major yield gains achieved with the introduction of dwarfing
77 genes contributed to validate this paradigm as these genes mainly reduced above ground plant stature
78 and, *de facto*, the intensity of intra specific competition (Donald, 1968; Jennings and Herrera, 1968).

79 Transitioning towards low-input farming practices notably by reducing the use of fertilizers will
80 reduce the amount of nutrients readily available for plants, and as such, will exacerbate the relative
81 effect of belowground compared to aboveground intra-specific competition. When plants compete for
82 soil resources, game theory models predicts that individuals will invest in root biomass beyond the
83 payoff point where extra soil resource absorption compensate the marginal cost of the extra root
84 biomass, leading to a Tragedy of the Commons (Gersani *et al.*, 2001; Anten and Vermeulen, 2016).

85 Multiple empirical evidences support this prediction: overinvestment in roots in response to
86 competition has been shown to reduce final aboveground biomass in wheat (Zhu *et al.*, 2019) and
87 soybean (Gersani *et al.*, 2001). Topsoil root production also negatively correlates with grain yields in
88 oats and barley (Vain *et al.*, 2023). Then, resolving belowground TOC might be key to maintain high
89 yields under low input farming practices.

90 Belowground TOC could be resolved by conducting direct selection on root architectural and
91 morphological traits in order to select less competitive root systems. However, this option appears
92 particularly challenging given the difficulty to measure root traits at high throughput on many
93 candidates over several generations, and the little information available on their response to selection
94 (Kuijken *et al.*, 2015). Alternatively, one could use the principles that prevent the evolution of TOC in
95 natural ecosystems, notably group selection and kin selection, and apply them in plant breeding to
96 select crops that have less competitive (or more cooperative) root systems, which is the core idea of
97 Darwinian Agriculture and Evolutionary Agroecology (Denison *et al.*, 2003; Weiner *et al.*, 2017).

98 Theoretically, these principles can be applied to avoid TOCs and to select more cooperative crops
99 (Montazeaud *et al.*, 2020; Biernaskie, 2022). However, they are not straightforward to implement in
100 practice, and they rely on high genetic relatedness between individuals, meaning that they produce
101 varieties in which individuals are genetically homogeneous. Both ecological and agronomic research,

in communities, suggest that there are multiple benefits to increase genetic diversity within crop stands. For example, mixing different varieties in the same field can be very efficient at limiting pathogen spreads and disease severity (Wolfe, 1985; Mundt *et al.*, 1995; Zhu *et al.*, 2000; Finckh and Wolfe, 2006), and, on average, varietal mixtures yield 2-5% more than expected on the basis of their pure stand components, which is known as overyielding (Kiær *et al.*, 2009; Borg *et al.*, 2018; Reiss and Drinkwater, 2018; Beillouin *et al.*, 2021).

Reducing intra-specific competition for resources while maintaining genetic diversity in the field could thus be the most optimal way to take advantage of intra-specific plant-plant interactions in agriculture. Two ecological mechanisms are known to reduce competition in diversified plant communities: the niche complementarity effect and the selection effect (Loreau and Hector, 2001). The niche complementarity effect results from differences in ecological niches between species that have different resource requirements and hence experience less competition (MacArthur and Levins, 1967), which ultimately translates into a higher biomass production in mixture (e.g., Roscher *et al.* 2008; Mueller *et al.* 2013). Through the selection effect, species are the most efficient at performing a given function are even more efficient at this function in a mixture than in a pure stand, i.e., they are “selected” by the mixture, which they rapidly dominate (Loreau and Hector, 2001). For example, competitive species can benefit from relaxed competition in mixture because the mixture allow them to escape an arms race with themselves. If the dominant species gains more yield than the sub-dominant loses, it can generate overyielding. Such an effect has for example been shown to contribute to overyielding in tree communities (Schmid and Niklaus, 2017; Williams *et al.*, 2017) and in inter-specific crop mixtures (Li *et al.*, 2018). Varietal mixtures could thus help escaping TOCs by relaxing intra-genotypic competition through either complementarity or selection effects while maintaining intraspecific genetic diversity in the field. However, the current literature provides little insight into the ecological effects underlying varietal mixture performance under contrasted resource levels (Borg *et al.*, 2018).

In the present study, we investigated the potential of varietal mixtures to reduce intra-specific belowground competition at the seedling stage in durum wheat (*Triticum turgidum* ssp. *durum*). We

used a panel of 36 varieties previously shown to exhibit contrasted responses to combined water and nitrogen limitation, and grew them in monogenotypic pots and binary mixtures under highly controlled climate condition in a high throughput root phenotyping platform under both non-limiting and limiting water and nutrient conditions. We hypothesized that (i) root responses to neighbours are already expressed at the seedling stage, (ii) such responses are more important under resource limited conditions, (iii) overinvestment in biomass at the seedling stage reflects a response to neighbour competition (iv) varietal mixtures can mitigate such response either through complementarity or selection effects

Material and Methods

Plant material

This study made use of field data collected on a diversity panel of 250 durum wheat genotypes, which was assembled during the EU Project SolACE (<https://www.solace-eu.net/>) to analyse wheat responses to combined water and nitrogen limitations (Collet, 2022). This panel comprised genotypes from four different collections (Collet, 2022) (i) “CREA”, with Italian cultivars, worldwide cultivars, and breeding lines selected by CREA (Consiglio per la Ricerca in agricoltura e l’analisi dell’Economia Agraria, Italy); (ii) “EPO”, an highly diverse collection of fixed lines derived from an evolutionary pre-breeding population (David et al 2014); (iii) “GPDUR”, with old and modern cultivars from various geographic areas including Western Europe; (iv) “UNIBO”, a diversity panel comprising genotypes from pre-breeding programs, elite lines, and representative genotypes of several worldwide breeding programs since the 1970s. Based on both field and platform data, a subset of 36 genotypes exhibiting contrasted yield responses to resource availability (i.e., ranging from stable to unstable yields) was selected by the European consortium to perform further experiments. Information on the 36 genotypes can be found in Supplementary Table 1.

Experimental design

Based on the 36 genotypes, we designed a set of 54 binary mixtures selected at random, with each genotype observed in three different mixtures (Supplementary Table 2). Pure stands (here referred to as

to groups of a single genotype, $n = 36$) and mixtures (here referring to groups of two genotypes, $n = 54$) were grown in RhizoTubes® (Jeudy *et al.*, 2016), transparent pots designed for high-throughput root phenotyping (Figure 1a & 1b), with six plants per RhizoTube®. The six plants had the same genotype in pure stands, whereas two genotypes were grown in alternate positions in the mixtures (Figure 1c). The 36 monocultures and the 54 mixtures were grown under combined water and nutrient limitation (R- treatment) or under optimal growth conditions (R+ treatment). All monocultures and mixtures were replicated three times within each treatment following a randomized complete block design within treatment, leading to a total of 540 RhizoTubes® (Figure 1c). In addition, six RhizoTubes® per experimental block per treatment (i.e., 36 RhizoTubes® in total) were grown with the commercial variety ANVERGUR and were used as controls to check for environmental heterogeneity in the greenhouse.

Growth conditions

Seeds were first disinfected with a solution of 6 g/L of active chlorine (4 tablets/L of a standard commercial chlorine) in which they were immersed, agitated for 15 min, and finally rinsed 10 times with sterile water on the 22nd of June 2019. They were then soaked overnight, and sown into Petri dishes on the 23rd. They stayed at 4°C during 24h before being transplanted on the into the RhizoTubes® on the 24th. RhizoTubes® have a diameter of 17 cm and a depth of 49.5 cm (Jeudy *et al.*, 2016). They were filled with a 25:75 mixture of sand (Biot B4, Silices et Refractaires de la Méditerranée) and perlite. Temperatures were maintained around 20-25°C, relative humidity around 70-80%, and photoperiod was set to 16h, with an average PAR of 330 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ during the day. Seedlings were daily provided with a liquid nutrient solution that contained water, N, P, K and all micronutrients required for plant growth (Supplementary Table 3). The water content of each RhizoTube® was monitored each day, and the amounts of nutrient solution were adjusted to maintain the RhizoTubes® at 100% of their water storage capacity (Supplementary Figure 1). In the R- treatment, the provision of nutritive solution was stopped on June 28th (four days after seedling transfer), causing the water content to decrease, ultimately reaching 55% of the full storage capacity by the end of the experiment (Supplementary Figure 1). Plants were harvested between the 16th and

the 19th of July, i.e., about three weeks after transplantation, and were at the beginning of the tillering stage.

Phenotyping

Root traits were measured for each RhizoTube® based on image analysis as described in Jeudy *et al.*, (2016). We used images taken on the 15th of July. Because the roots of the different plants were overlapping in most RhizoTube® at the end of the experiment, we were unable to measure root traits for individual plants, and instead computed root traits at the level of the RhizoTube® as a whole. Three root traits could be computed following this aggregation: root depth, corresponding to the distance between the top of the RhizoTube® and the deepest root pixel; root length, the total length of roots detected on the image; and root area, the 2D projected area of the total root system. Root area combines information on both root length and root diameter. We did not consider root depth in our analyses because root tips reached the bottom of the RhizoTubes® in most cases, leading to highly left-skewed trait distribution and very low trait variability.

We also measured a set of aboveground plant traits in order to evaluate the relative importance of belowground relative to aboveground traits in explaining mixture biomass. At harvest, we extracted plants from the RhizoTubes® by carefully separating their rooting systems. For each plant, we counted the number of leaves on the main tiller (hereafter “# leaves”) and the total number of tillers (hereafter “# tillers”). Above and belowground biomass were then separated and dried before weighing to determine shoot biomass, root biomass, root:shoot ratio, and total biomass. Leaf nitrogen content (hereafter “leaf N”) was measured with Near-Infrared Spectrometry (NIRS). We measured one NIR spectrum per leaf per plant in each RhizoTube® (i.e., six spectrum per RhizoTube®) using the Fieldspec 2500© (Analytical Spectral Devices, Inc. (ASD), Boulder, CO, USA) spectrometer. NIRS measurements were done one day before the harvest (i.e., on the 15th for the Rhizotubes harvested on the 16th, on the 16th for the RhizoTubes® harvested on the 17th, etc). NIR spectra were converted into nitrogen content using the calibration described in (Ecartot *et al.*, 2013).

207 Statistical analysis

208 We performed all statistical analyses with R v. 4.3.2 (R Core Team, 2019).

209 We first tested the effect of the treatment (R+ vs R-) on the different traits and biomass components
210 (above and belowground) using only pure stand data summed per Rhizotube® (except leaf N which
211 was averaged per Rhizotube®). We used mixed linear models with a given trait or biomass
212 component as the response variable, treatment as a fixed effect, and genotype identity as a random
213 intercept and random treatment slope. We also included two covariates as fixed effects: block, and
214 harvest date or measurement date (except for root traits which were all measured on the same day).
215 We assessed the significance of the fixed effects with standard analyses of variance and F statistics
216 computed with Kenward-Roger's approximations for the degrees of freedom (Supplementary Table 4).
217 We fitted the mixed model with the *lmer()* function (package *lme4*), and checked significance with the
218 *anova()* function (package *lmerTest*).

219 To compare the relative biomass of mixed vs pure stands, we computed the Relative Yields (RYs, de
220 Wit and van den Bergh 1965) of the varieties in mixed stands for each biomass component
221 (aboveground, belowground, and total biomass):

222 (1)
$$RY_{ijk} = \frac{B_{ijk}}{B_{iik}},$$

223 Where RY_{ijk} is the Relative Yield of the variety i grown in mixture with the variety j in the treatment
224 k , B_{ijk} is the biomass (aboveground, belowground, or total biomass) of the variety i grown in mixture
225 with the variety j in the treatment k and, and B_{iik} is the pure stand reference biomass of the variety i in
226 treatment k . To compute RYs, we first separated our dataset between the pure and mixed stands. Then,
227 we summed the biomass of all plants of the same genotype within each RhizoTube® (i.e., 6 plants in
228 pure stands, 3 plants in mixed stands). We used the pure stands dataset to compute pure stand
229 reference biomass. To do so, we fitted a linear mixed model with biomass as the response variable,
230 measurement date, block, and treatment as fixed effects, and variety identity as a random effect (here
231 we included only the random effect of the variety on the intercept, as adding the random effect on the
232 treatment slope led to singular models due to very low variance on the slope). We then summed the

Best Linear Unbiased Predictor (BLUP) of each variety with the estimated fixed effect of the treatment (R+ or R-) to obtain the pure stand reference biomass values adjusted for the effects of block and measurement date within each treatment (i.e., B_{iik}). For mixed stands, we also fitted a linear mixed model with each variety biomass as the response variable, measurement date, block, and treatment as fixed effects, and variety pair identity as a random effect on the intercept and on the treatment slope. Pair identity was constructed as the concatenation of the identity of the focal and neighbor variety, such that variety 1 and variety 2 in a mixed stand had pair identities variety1-variety2, and variety2-variety1, respectively. We then summed the BLUP of pair identity (intercept in the R+ treatment, intercept + slope in the R- treatment) with the estimated fixed effect of the treatment (R+ or R-) to obtain the biomass of each variety in mixed stands adjusted for the effects of the block and measurement dates within each treatment (i.e., B_{ijk}). We then divided these mixed stand values by the pure stand reference values to obtain RYs for each variety within each mixture following formula (1). Under the null hypothesis that the variety produced equal amount of biomass in mixed than in pure stand, $RY = 0.5$ because there are half the number of plants of a variety in mixed stands ($n = 3$) compared to pure stands ($n=6$). $RY > 0.5$ means that the variety produced more biomass in mixed than in pure stand, and $RY < 0.5$ means that the variety produced less biomass in mixed than in pure stand.

In a second step, we summed the RYs of the two varieties in the mixed stand to obtain the Relative Yield Total (RYT, de Wit and van den Bergh 1965) of the mixture:

$$(2) \quad RYT_{(ij)k} = RY_{ijk} + RY_{jik},$$

Where $RYT_{(ij)k}$ is the Relative Yield Total of the mixture containing varieties i and j in treatment k . $RYT = 1$ means that the mixture as a whole produced similar amount of biomass than expected from the productivity of the varieties grown in pure stands, whereas $RYT > 1$ means that the mixture produced more biomass than expected, and $RYT < 1$ means that the mixture produced less biomass than expected.

258 We compared RYT_s between the R⁺ and R⁻ treatment using a linear mixed model with RYT as the
 259 response variable, treatment as a fixed effect, and varietal mixture identity (i.e., non-oriented
 260 concatenation of the names of the two varieties grown in mixture) as a random effect on the intercept.
 261 We assessed the significance of the fixed effects as detailed above (Supplementary Table 5). Finally,
 262 we checked whether RYT_s significantly differed from 1 within each treatment using two-sided *t*-tests.
 263 To assess whether trait composition (above and belowground traits) of the mixture explained RYT
 264 variability, we used traits measured in pure stands to predict mixed stands RYT_s. This approach is
 265 based on the hypothesis that pure stand traits are the information available to agronomists and plant
 266 breeders when designing varietal mixtures. First, we computed reference trait values for pure stands
 267 that we corrected for design effects and measurement dates. As described above, we fitted a linear
 268 mixed model with pure stand trait as the response variable, block, measurement date, and treatment as
 269 fixed effects, and varietal identity as a random effect. Varietal identity random effect was specified
 270 both on the intercept and the treatment slope, except for # leaves where adding the random slope led
 271 to singular models due to very low variance on the slope. We then computed pure stand reference trait
 272 values as the sum of the BLUP of each variety (intercept in the R⁺ treatment, intercept + slope in the
 273 R⁻ treatment when random slope was specified) with the estimated fixed effect of the treatment (R⁺ or
 274 R⁻). Then, for each mixed stand, we computed both the average and the absolute trait difference
 275 between the two varieties using the pure stand reference trait values. Finally, we fitted a full linear
 276 model with RYT as the dependent variable and all trait averages and all trait differences as
 277 independent variables (*lm()* function from the *stats* package). We standardized all dependent and
 278 independent variables ($\mu = 0$, $\sigma = 1$) and ran a backward model selection (*glmulti()* function from the
 279 *glmulti* package). We used the second-order Akaike Information Criterion (AIC_C, Sugiura 1978) to
 280 rank the models and performed model-averaging inference based on the top 10 models using the
 281 *coef()* function (*glmulti* package). We report parameter estimates and their 95% unconditional
 282 confidence interval computed as ± 1.96 unconditional sampling standard deviation, variable
 283 importance, and adjusted R-squared averaged over the top 10 models (Burnham and Anderson, 2002).
 284 Detailed information on the top 10 models is available in Supplementary Table 6.

Finally, to further investigate the ecological mechanisms behind the significant relationship between RYT and trait composition, specifically root area, we assessed the relationship between pure stand biomass and pure stand root area, and between RYs and pure stand root area using simple linear regressions between these variables within each treatment. We used trait and biomass values adjusted for block effects and measurement date effects as explained above, such that we had one value per variety per treatment. We also checked the relationship between RYs and the hierarchical distance in root area between the variety and its neighbour in pure stands. Hierarchical distance was the difference between the focal root area and the neighbour root area, divided by the focal root area (i.e., positive values mean that the focal had higher root area than its neighbor, and *vice versa*). Finally, we tested whether root area plasticity affected RYT in the two treatments. We computed root area plasticity as the difference between the average root area of the pure stands of the two varieties and the observed root area of their mixture, divided by the average of the pure stands. (i.e., positive values mean that root area increased in the mixed relative to the pure stands, and *vice versa*).

Results

Effect of resource availability on plant growth and traits

The limitation of water and nutrients strongly reduced total plant biomass in pure stands (1947.35 mg in the R⁻ treatment vs 2364.71 in the R⁺ treatment, $F_{1,87.77} = 144.49$, $p < 0.001$, Figure 2a). Such biomass reduction was accompanied by a reallocation of resources from the aboveground to the belowground compartment: while plants produced less above-ground biomass (Figure 2b), less leaves (Figure 2d), and less tillers (Figure 2e), they produced higher root biomass (Figure 2c), longer roots (Figure 2h), and had higher root areas (Figure 2i) in the R⁻ treatment. Consequently, the root:shoot ratio increased from 0.48 in the R⁺ treatment to 0.82 in the R⁻ treatment (+71%, $F_{1,87.78} = 3966.58$, $p < 0.001$, Figure 2g). Leaf nitrogen concentration also increased in the R⁻ treatment (3.32 vs 2.95%, $F_{1,87.19} = 525.03$, $p < 0.001$, Figure 2f).

309 **Relative biomass of mixtures**

310 RYT_s were significantly different between the R⁺ and R⁻ treatments for all biomass components
311 (Figure 3 and Supplementary Table 5). In the R⁺ treatment, the biomass production of the mixtures
312 did not significantly differ from the biomass expected from their pure stand components (average
313 RYT for total biomass = 0.99, $t_{53} = -0.46$, $p = 0.06473$, Figure 3a; average RYT for shoot biomass =
314 1.00, $t_{53} = -0.05$, $p = 0.9632$, Figure 3b; average RYT for root biomass = 0.99, $t_{53} = -0.75$, $p = 0.4568$,
315 Figure 3c). In contrast, in the R⁻ treatment, mixtures produced significantly less biomass than
316 expected from the biomass of their components grown in pure stands (average RYT for total biomass
317 = 0.92, $t_{53} = -6.18$, $p < 0.001$, Figure 3a; average RYT for shoot biomass = 0.94, $t_{53} = -4.29$, $p < 0.001$,
318 Figure 3b; average RYT for root biomass = 0.94, $t_{53} = -6.83$, $p < 0.001$, Figure 3c).

319 **Effect of trait composition on mixture biomass**

320 RYT_s were highly variable in both treatments (Figure 3). The trait composition of the mixtures poorly
321 explained RYT variability in total biomass observed in the R⁺ treatment (Figure 4a, average adjusted
322 R² over the top ten models = 0.10). In contrast, trait composition explained up to 49% of RYT
323 variation in the R⁻ treatment (Figure 4b). Most of this variability was explained by the average root
324 area of the two varieties grown in mixture (R² = 47% in a model with average root area as the single
325 explanatory variable), which had a negative effect on RYT (Figure 4b, Supplementary Table 6):
326 mixing two genotypes with higher average root area resulted in a decrease in biomass production in
327 mixture compared to pure stands. We obtained similar results when performing the analysis on shoot
328 biomass alone (Supplementary Figure 2a and 2b, Supplementary Table 6). Traits had higher
329 explanatory power on root biomass RYT in the R⁺ treatment (Supplementary Figure 2c, average
330 adjusted R² over the top ten models = 0.34), with strong negative effects of average leaf number and
331 average root area. In the R⁻ treatment, however, as for the other biomass components, average root
332 area was the main explanatory trait with a negative effect on RYT (Supplementary Figure 2d). Trait
333 differences between varieties, either above or belowground, and either in the R⁻ or R⁺ treatment, did
334 not explain mixture biomass (Figure 4, Supplementary Figure 2).

335 Relationship between root area and mixture biomass

336 We hypothesized that the negative relationship between RYT and the average root projected area of
337 varieties in the mixtures reflected a relaxation of belowground competition for varieties with high root
338 area in monocultures. Under this hypothesis, varieties with high root area in monocultures are highly
339 competitive varieties that reach such high root areas through root proliferation in response to their
340 neighbour in pure stand, which is himself very competitive. Such strong investment into competition
341 in turn results in an overinvestment in biomass (above and belowground) to outgrow the neighbour.
342 Those varieties, when grown in mixtures, are more likely to be paired with varieties that are less
343 competitive, leading to relaxed competition in mixtures compared to pure stands. Such relaxed
344 competition in turn leads to a disengagement from the “arms race” between plants, potentially
345 resulting in lower root area and lower above and belowground biomass production, and thus low
346 RYTs.

347 In line with this hypothesis, there was a strong positive relationship between the total biomass of the
348 pure stands and their root area (Figure 5a). This relationship was stronger in the R- treatment (Figure
349 5a, Pearson's $R = 0.87$, slope = 0.079 mg.mm^{-2} , $p < 0.001$) than in the R+ treatment (Pearson's $R =$
350 0.74 , slope = $0.0446 \text{ mg.mm}^{-2}$, $p < 0.001$). Additionally, consistent with our hypothesis, varieties with
351 the highest root areas in pure stands and the highest hierarchical distances in root area with their
352 neighbours were the ones with the strongest biomass reduction in mixed stands in the R- treatment
353 (Figure 5b, Pearson's $R = -0.70$, $p < 0.001$; Figure 5c, Pearson's $R = -0.51$, $p < 0.001$). At the opposite,
354 varieties which had lower root areas than their neighbours (i.e., negative hierarchical distance to their
355 neighbour on Figure 5c) produced more biomass in mixture than in pure stands ($\text{RY} > 0.5$). This effect
356 was not symmetrical: for a similar hierarchical distance, varieties which were lower in the hierarchy
357 gained less biomass than the biomass loss observed for varieties placed higher in the hierarchy (e.g.,
358 at hierarchical distance = -0.2 , estimated $\text{RY} = 0.53$ while at hierarchical distance = 0.2 , estimated RY
359 = 0.40 , Figure 5c). These relationships were not significant in the R+ treatment (Figures 5b and 5c).
360 Finally, the strongest biomass reductions occurred in mixtures where the observed root area was lower
361 than the root area predicted from the pure stands, i.e., where phenotypic plasticity led to reduced root

area in mixtures (Figure 5d). Again, the relationship between RYT and root area plasticity was stronger in the R- treatment (Pearson's $R = 0.80$, $p < 0.001$, slope = $1.93 \%^{-1}$) than in the R+ treatment (Pearson's $R = 0.54$, $p < 0.001$, slope = $0.91 \%^{-1}$). We obtained identical results when doing these analyses on shoot and root biomass separately, except that the differences between the R+ and R- treatments were less marked for root biomass (Supplementary Figures 3 & 4). Altogether, these results support the idea that low RYTs in the R- treatment resulted from relaxed belowground competition in mixed relative to pure stands.

Discussion

Shift in root allocation under water and nutrient limitation

Wheat seedling growth was strongly affected by the limitation of water and nutrients in our experiment. Resource limitation triggered an overall reduction in biomass, along with a shift in biomass allocation from the aboveground to the belowground compartment. This result is in line with the optimal allocation theory, which states that plants prioritize allocation to increase their uptake of the most limiting resources (Bloom *et al.*, 1985; Weiner, 2004). In our case, the high allocation to roots suggests that belowground resource limitations were much stronger than aboveground resource limitations, a pattern consistent with what we intended to induce with our experimental protocol. In wheat, plasticity of the root-to-shoot ratio and high reallocation to roots have been shown to be advantageous under drought stress (Bacher *et al.*, 2021, 2022). Allocating carbon to the roots enhances access to water through deeper, longer, and more branched roots, which in turns helps maintain high stomatal conductance and physiological activity. We can thus hypothesize that higher root allocation in response to water and nutrient limitation reflected an adaptive response of the varieties.

Biomass reduction in mixed relative to pure stands reflects a relaxation of competition

On average, mixture biomass was not significantly different from pure stand biomass under optimal growth conditions, indicating no significant interactions between varieties. However, when water and nitrogen were limiting, mixtures produced significantly less biomass than expected from their pure stand components. Individual variety responses to mixture cultivation (i.e., RYs) combined with root trait data (incl. hierarchical distance and plasticity in root area) together suggest that such reduction in biomass was the consequence of intense competition in pure stands: water and nutrient limitation triggered an arms race between plants of highly competitive varieties in pure stands, which manifested through root proliferation and ultimately overinvestment in both below and aboveground biomass. According to previous study on root responses to the presence of competitor plants, such proliferation could have been triggered either by a direct perception of the presence of a competitive neighbour through the detection of chemical cues in the rhizosphere, or by the perception of resource depletion caused by the neighbour (Schenk, 2006; Pierik *et al.*, 2013). Such highly competitive varieties experienced reduced competition in mixed stands and thus disengaged from this competitive arms race and produced less biomass. Conversely, their less competitive neighbour experienced stronger competition in mixed than in pure stands, and hence produced more biomass. The net balance between these two effects was an overall reduction in biomass because the biomass gain from the weaker competitor was lower than the biomass loss from the stronger competitor, which explains the average negative RYT observed in the R- treatment. All together, these results suggest a positive selection effect where the dominant genotype (here most competitive) gain more than what is lost by the sub-dominant genotype. Similar ecological mechanisms have been shown to be responsible for the overyielding in forest trees, with larger tree species able to benefit from reduced light competition from their smaller neighbours (Schmid and Niklaus, 2017; Williams *et al.*, 2017). In our case, a “gain” is seen as a reduction of competition intensity which translates into a reduction of biomass. This is because in a cereal such as wheat, at the seedling stage, biomass typically reflects competitive ability. For example, seedling growth rate and early vigour are typically targeted by plant breeders as

411 favourable traits for competitiveness against weeds (Lemerle *et al.*, 1996; Bertholdsson, 2005;
412 Hendriks *et al.*, 2022).

413 **No evidence for niche complementarity between seedlings**

414 We found no evidence of complementarity effects that would be driven by trait differences between
415 varieties, including under resource limitation. This could be explained by the fact that functional
416 differences between varieties were not large enough 3-weeks after sowing to generate
417 complementarity effects. A second hypothesis is that we did not measure the “right” traits to detect
418 complementarity effects, notably belowground. Our phenotyping method did not allow us **accessing**
419 traits such as root diameter or root tissue density. Other traits could not be computed due to root
420 overlapping between adjacent plants (e.g., root angle) or to the reduced size of the Rhizotube® (e.g.,
421 root depth). However, such traits have rarely been found to associated with complementarity effects in
422 ecological studies (Fort *et al.*, 2014; Bakker *et al.*, 2018). Previous studies in rice varietal mixtures
423 also failed to find evidence of belowground niche complementarity driven by differences in
424 morphological and architectural root traits such as rooting depth, root diameter, or specific root length
425 (Montazeaud *et al.*, 2018). Overall, this study confirm that root trait differences alone are less likely to
426 generate complementarity effects in varietal mixtures than they are for example in intercropping
427 where differences in root foraging strategies between species can be more significant (Zhang *et al.*,
428 2014; Homulle *et al.*, 2022; Schmutz and Schöb, 2023).

429 **Root area as the main driver of competitive hierarchy**

430 We identified root area as the main driver of plant biomass and mixing effects in our experiment. The
431 average root area of the two varieties in pure stands could explain up to 50% of RYT variability under
432 resource-limiting conditions. A higher root area in pure stand was associated with a greater biomass
433 reduction in mixture, especially when the mixture partner had a lower root area. These results support
434 the idea of an early-stage belowground competitive hierarchy between varieties (Kunstler *et al.*,
435 2012), where higher root area is associated with higher competitive ability. In line with these results,
436 root functional traits associated with root foraging and absorption potential have already been shown

to shape early-stage competitive hierarchies between grassland species (Fort *et al.*, 2014; Ravenek *et al.*, 2016; Wagg *et al.*, 2017). The very high explanatory power of root area found in our study might be explained by the fact that this trait integrates several functional dimensions of the root system that are classically captured by distinct traits (e.g., root length density, specific root length, or root diameter).

Root plasticity contributes to mixing effects

We found that root area in mixed stands deviated from root area observed in pure stands, indicating a plastic response of the root system to neighbour genotype identity. On average, root area decreased in mixed relative to pure stands, and the stronger the decrease, the lower the RYT especially in the R-treatment. In accordance with ecological theory, our results thus support the view that phenotypic plasticity contributes to mitigate competition intensity in diverse plant communities (Callaway *et al.*, 2003; Schiffrers *et al.*, 2011; Burns and Strauss, 2012). In varietal mixtures, previous studies have already shown that plasticity in aboveground traits such as plant height, specific leaf area, or canopy cover **contribute to overyielding**, notably by increasing trait divergence between varieties (Kong and Zhao, 2023; Tschurr *et al.*, 2023; Su *et al.*, 2024). We here show that plastic root changes may also contribute to mixture performance, although it may not be by increasing differences between varieties, but rather by changing the average trait value.

Practical implications for plant breeding

Crop performance, including grain yield, is affected by trade-offs between individual competitiveness and group performance. As early pointed out by plant breeders (Donald, 1968), and more recently revisited with the concepts of Darwinian Agriculture, or Evolutionary Agroecology, these trade-offs can hold promising opportunities to increase productivity, notably by reversing past selection for individual competitiveness (Denison *et al.*, 2003; Weiner, 2019). This can be achieved either by selecting on group performance, i.e., selecting on population yield (Montazeaud *et al.*, 2020), or by targeting key traits related to competitive ability. Our study suggests that selecting genotypes with reduced root area in pure stands and reduced root area proliferation in presence of neighbours could

be a way to reduce competition intensity between seedlings, which could then translate into higher yields. This result aligns with classical prediction of game theory models: investment in root biomass beyond the payoff point where soil resource absorption compensate the cost of the root system only happens at cost for neighbouring plants, leading to a Tragedy of the Commons (Hardin, 1968; Gersani *et al.*, 2001; Anten and Vermeulen, 2016). It is also supported by empirical data measured in the same phenotyping platform: Colombo *et al.* (2022) found that early seedling biomass measured in the platform was negatively correlated with yield components measured in the field in multiple wheat panels. Interestingly, modern wheat varieties that have been subject to selection for increased population yield for many generations display reduced root biomass at the seedling stage (Colombo *et al.*, 2022), and reduced root proliferation in presence of a neighbour compared to old landraces (Zhu *et al.*, 2019). In oat and barley, excessive growth of the root system in the topsoil is also associated with yield reductions (Vain *et al.*, 2023).

Beyond direct selection on root traits in pure stand varieties, which could be challenging to implement, mixing varieties could be another solution to resolve belowground TOCs. This solution would allow to benefit from other known positive effects of genetic diversity at the same time (e.g., improved disease control, Wolfe, 1985; Mundt *et al.*, 1995; Zhu *et al.*, 2000; Finckh and Wolfe, 2006)). Our study suggests that highly competitive varieties could benefit from relaxed belowground competition in varietal mixtures, which could translate into overyielding through positive selection effects. Conveniently, the choice of the varieties could be guided by their root projected area measured in monoculture. Of course, the effect of relaxed competition at the seedling stage on final grain yield would still need to be validated in field trials. This means that promoting complementarity effects between varieties is not the only way to reduce competition and may only work for some traits, such as phenological traits (Fletcher *et al.*, 2019; Lowry *et al.*, 2020) or traits related to light interception (Su *et al.*, 2024).

Supplementary Data

Supplementary Table 1: List of the 36 wheat varieties used in the experiment

489 **Supplementary Table 2:** List of the 54 binary mixtures used in the experiment

490 **Supplementary Table 3:** Description of the nutrient solution

491 **Supplementary Table 4:** Analysis of Variance (ANOVA) of biomass components and traits in pure
 492 stands to test the treatment effect

493 **Supplementary Table 5:** Analysis of Variance (ANOVA) to test the effect of the treatment on RYTs

494 **Supplementary Table 6:** Ten best fitting models between RYTs on aboveground, belowground, and
 495 total biomass and mixture trait composition

496 **Supplementary Figure 1:** RhizoTubes® monitoring

497 **Supplementary Figure 2:** Relationships between the trait composition of the mixtures and their
 498 above and belowground RYTs

499 **Supplementary Figure 3:** bivariate plots illustrating the interaction between root area, resource
 500 availability and shoot biomass

501 **Supplementary Figure 4:** bivariate plots illustrating the interaction between root area, resource
 502 availability and root biomass

503 **Acknowledgements**

504 We thank the 4PMI platform (Plant Phenotyping Platform for Plant and Microorganisms Interactions)
 505 - UMR Agroécologie, Dijon, France for carrying out the experiment and the phenotypic analyses used
 506 in this work. 4PMI was supported by the project Phenome-ANR-11-INBS-0012. We thank Florian
 507 Fort for providing helpful comments on the manuscript.

508 **Author contributions**

509 Conception of the research and design of the experiment: C. J., C. S, H. F., P. R.

510 Implementation and monitoring of the experiment: C. J., C. S.

511 Root image analysis: M. L.

512 Near-Infrared Spectrometry: M. E.

513 Harvest and trait measurements: H. F., L. M. C., M. E., P. R.

514 Data analysis: G. M.

515 Redaction of the manuscript: G. M. with inputs from all authors.

516 **Conflict of interest**

517 The authors declare no conflict of interest.

518 **Funding**

519 This study received funding from the European Union's Horizon 2020 Research and Innovation

520 Program under grant agreement N°727247 (SolACE - <https://www.solace-eu.net/>).

521 **Data availability**

522 Data and code used for analysis are available at <https://doi.org/10.5281/zenodo.14163518>.

References

- Anten NPR, Vermeulen PJ.** 2016. Tragedies and crops: understanding natural selection to improve cropping systems. *Trends in Ecology & Evolution* **31**, 429–439.
- Bacher H, Sharaby Y, Walia H, Peleg Z.** 2022. Modifying root-to-shoot ratio improves root water influxes in wheat under drought stress. *Journal of Experimental Botany* **73**, 1643–1654.
- Bacher H, Zhu F, Gao T, *et al.*** 2021. Wild emmer introgression alters root-to-shoot growth dynamics in durum wheat in response to water stress. *Plant Physiology* **187**, 1149–1162.
- Bakker LM, Mommer L, van Ruijven J.** 2018. Can root trait diversity explain complementarity effects in a grassland biodiversity experiment? *Journal of Plant Ecology* **11**, 73–84.
- Ballaré CL, Pierik R.** 2017. The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant, Cell & Environment* **40**, 2530–2543.
- Ballaré CL, Scopel AL, Sánchez RA.** 1990. Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. *Science (New York, N.Y.)* **247**, 329–332.
- Bertholdsson N-O.** 2005. Early vigour and allelopathy – two useful traits for enhanced barley and wheat competitiveness against weeds. *Weed Research* **45**, 94–102.
- Biernaskie JM.** 2022. Kin selection theory and the design of cooperative crops. *Evolutionary Applications* **15**, 1555–1564.
- Bloom AJ, Chapin III FS, Mooney HA.** 1985. Resource limitation in plants-an economic analogy. *Annual review of Ecology and Systematics* **16**, 363–392.
- Borg J, Kiær LP, Lecarpentier C, Goldringer I, Gauffreteau A, Saint-Jean S, Barot S, Enjalbert J.** 2018. Unfolding the potential of wheat cultivar mixtures: A meta-analysis perspective and identification of knowledge gaps. *Field Crops Research* **221**, 298–313.
- Burnham KP, Anderson DR.** 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York: Springer-Verlag.
- Burns JH, Strauss SY.** 2012. Effects of competition on phylogenetic signal and phenotypic plasticity in plant functional traits. *Ecology* **93**, S126–S137.
- Callaway RM, Pennings SC, Richards CL.** 2003. Phenotypic plasticity and interactions among plants. *Ecology* **84**, 1115–1128.
- Collet C.** 2022. A novel phenotyping pipeline for root system architecture: evaluation with diversity panels of bread and durum wheat. UCLouvain.
- Colombo M, Roumet P, Salon C, *et al.*** 2022. Genetic analysis of platform-phenotyped root system architecture of bread and durum wheat in relation to agronomic traits. *Frontiers in Plant Science* **13**.
- Denison RF, Kiers ET, West SA.** 2003. Darwinian Agriculture: when can humans find solutions beyond the reach of natural selection? *The Quarterly Review of Biology* **78**, 145–168.
- Donald CM.** 1963. Competition among crop and pasture plants. In: Norman AG, ed. *Advances in Agronomy*. Academic Press, 1–118.

- Donald CM.** 1968. The breeding of crop ideotypes. *Euphytica* **17**, 385–403.
- Ecarnot M, Compan F, Roumet P.** 2013. Assessing leaf nitrogen content and leaf mass per unit area of wheat in the field throughout plant cycle with a portable spectrometer. *Field Crops Research* **140**, 44–50.
- Finckh MR, Wolfe MS.** 2006. Diversification strategies. In: Cooke BM, Jones DG, Kaye B, eds. *The Epidemiology of Plant Diseases*. Dordrecht: Springer Netherlands, 269–307.
- Fletcher A, Ogden G, Sharma D.** 2019. Mixing it up – wheat cultivar mixtures can increase yield and buffer the risk of flowering too early or too late. *European Journal of Agronomy* **103**, 90–97.
- Fort F, Cruz P, Jouany C.** 2014. Hierarchy of root functional trait values and plasticity drive early-stage competition for water and phosphorus among grasses. *Functional Ecology* **28**, 1030–1040.
- Gersani M, Brown J s, O'Brien EE, Maina GM, Abramsky Z.** 2001. Tragedy of the commons as a result of root competition. *Journal of Ecology* **89**, 660–669.
- Hardin G.** 1968. The tragedy of the commons. *Science* **162**, 1243–1248.
- Hendriks P-W, Gurusinghe S, Ryan PR, Rebetzke GJ, Weston LA.** 2022. Competitiveness of early vigour wheat (*Triticum aestivum* L.) genotypes is established at early growth stages. *Agronomy* **12**, 377.
- Homulle Z, George TS, Karley AJ.** 2022. Root traits with team benefits: understanding belowground interactions in intercropping systems. *Plant and Soil* **471**, 1–26.
- Jennings PR, Herrera RM.** 1968. Studies on competition in rice II. Competition in segregating populations. *Evolution* **22**, 332–336.
- Jennings PR, de Jesus J.** 1968. Studies on competition in rice I. Competition in mixtures of varieties. *Evolution* **22**, 119–124.
- Jeudy C, Adrian M, Baussard C, et al.** 2016. RhizoTubes as a new tool for high throughput imaging of plant root development and architecture: test, comparison with pot grown plants and validation. *Plant Methods* **12**, 31.
- Kong X, Zhao G.** 2023. Increasing yield through wheat cultivar mixture that optimizes functional traits within the canopy. *European Journal of Agronomy* **151**, 126977.
- Kuijken RCP, van Eeuwijk FredA, Marcelis LFM, Bouwmeester HJ.** 2015. Root phenotyping: from component trait in the lab to breeding. *Journal of Experimental Botany* **66**, 5389–5401.
- Kunstler G, Lavergne S, Courbaud B, Thuiller W, Vieilledent G, Zimmermann NE, Kattge J, Coomes DA.** 2012. Competitive interactions between forest trees are driven by species' trait hierarchy, not phylogenetic or functional similarity: implications for forest community assembly. *Ecology Letters* **15**, 831–840.
- Lemerle D, Verbeek B, Cousens R d., Coombes NE.** 1996. The potential for selecting wheat varieties strongly competitive against weeds. *Weed Research* **36**, 505–513.
- Li X-F, Wang C-B, Zhang W-P, Wang L-H, Tian X-L, Yang S-C, Jiang W-L, van Ruijven J, Li L.** 2018. The role of complementarity and selection effects in P acquisition of intercropping systems. *Plant and Soil* **422**, 479–493.

- Loreau M, Hector A.** 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**, 72–76.
- Lowry CJ, Bosworth SC, Goslee SC, Kersbergen RJ, Pollnac FW, Skinner RH, Warren ND, Smith RG.** 2020. Effects of expanding functional trait diversity on productivity and stability in cultivar mixtures of perennial ryegrass. *Agriculture, Ecosystems & Environment* **287**, 106691.
- MacArthur R, Levins R.** 1967. The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist* **101**, 377–385.
- Montazeaud G, Rousset F, Fort F, Violle C, Fréville H, Gandon S.** 2020. Farming plant cooperation in crops. *Proceedings of the Royal Society B: Biological Sciences* **287**, 20191290.
- Montazeaud G, Violle C, Fréville H, Luquet D, Ahmadi N, Courtois B, Bouhaba I, Fort F.** 2018. Crop mixtures: does niche complementarity hold for belowground resources? An experimental test using rice genotypic pairs. *Plant and Soil* **424**, 187–202.
- Mueller KE, Tilman D, Fornara DA, Hobbie SE.** 2013. Root depth distribution and the diversity–productivity relationship in a long-term grassland experiment. *Ecology* **94**, 787–793.
- Mundt CC, Brophy LS, Schmitt MS.** 1995. Disease severity and yield of pure-line wheat cultivars and mixtures in the presence of eyespot, yellow rust, and their combination. *Plant Pathology* **44**, 173–182.
- Pierik R, Mommer L, Voesenek LA.** 2013. Molecular mechanisms of plant competition: neighbour detection and response strategies. *Functional Ecology* **27**, 841–853.
- R Core Team.** 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing.
- Ravenek JM, Mommer L, Visser EJW, van Ruijven J, van der Paauw JW, Smit-Tiekstra A, de Caluwe H, de Kroon H.** 2016. Linking root traits and competitive success in grassland species. *Plant and Soil* **407**, 39–53.
- Robinson D, Hodge A, Griffiths BS, Fitter AH.** 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **266**, 431–435.
- Roscher C, Thein S, Schmid B, Scherer-Lorenzen M.** 2008. Complementary nitrogen use among potentially dominant species in a biodiversity experiment varies between two years. *Journal of Ecology* **96**, 477–488.
- Schenk HJ.** 2006. Root competition: beyond resource depletion. *Journal of Ecology* **94**, 725–739.
- Schiffers K, Tielbörger K, Tietjen B, Jeltsch F.** 2011. Root plasticity buffers competition among plants: theory meets experimental data. *Ecology* **92**, 610–620.
- Schmid B, Niklaus PA.** 2017. Biodiversity: complementary canopies. *Nature Ecology & Evolution* **1**, 0104.
- Schmutz A, Schöb C.** 2023. Crops grown in mixtures show niche partitioning in spatial water uptake. *Journal of Ecology* **111**, 1151–1165.

- Su Y, Yu R-P, Xu H-S, Zhang W-P, Yang H, Surigaogoe S, Callaway RM, Li L.** 2024. Maize cultivar mixtures increase aboveground biomass and grain quality via trait dissimilarity and plasticity. *European Journal of Agronomy* **156**, 127160.
- Sugiura N.** 1978. Further analysts of the data by akaike' s information criterion and the finite corrections. *Communications in Statistics - Theory and Methods* **7**, 13–26.
- Tilman D.** 2020. *Resource Competition and Community Structure. (MPB-17), Volume 17.* Princeton University Press.
- Tschurr F, Oppliger C, Wuest SE, Kirchgessner N, Walter A.** 2023. Mixing things up! Identifying early diversity benefits and facilitating the development of improved variety mixtures with high throughput field phenotyping. *The Plant Phenome Journal* **6**, e20090.
- Vain S, Tamm I, Tamm Ü, Annusver M, Zobel K.** 2023. Negative relationship between topsoil root production and grain yield in oat and barley. *Agriculture, Ecosystems & Environment* **349**, 108467.
- Wagg C, Ebeling A, Roscher C, et al.** 2017. Functional trait dissimilarity drives both species complementarity and competitive disparity. *Functional Ecology* **31**, 2320–2329.
- Weiner J.** 2004. Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics* **6**, 207–215.
- Weiner J.** 2019. Looking in the wrong direction for higher-yielding crop genotypes. *Trends in Plant Science* **24**, 927–933.
- Weiner J, Du Y-L, Zhang C, Qin X-L, Li F-M.** 2017. Evolutionary agroecology: individual fitness and population yield in wheat (*Triticum aestivum*). *Ecology* **98**, 2261–2266.
- Williams LJ, Paquette A, Cavender-Bares J, Messier C, Reich PB.** 2017. Spatial complementarity in tree crowns explains overyielding in species mixtures. *Nature Ecology & Evolution* **1**, 0063.
- de Wit CT, van den Bergh JP.** 1965. Competition between herbage plants. *Journal of Agricultural Science* **13**, 212–221.
- Wolfe MS.** 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* **23**, 251–273.
- Zhang C, Postma JA, York LM, Lynch JP.** 2014. Root foraging elicits niche complementarity-dependent yield advantage in the ancient 'three sisters' (maize/bean/squash) polyculture. *Annals of Botany* **114**, 1719–1733.
- Zhu Y, Chen H, Fan J, et al.** 2000. Genetic diversity and disease control in rice. *Nature* **406**, 718–722.
- Zhu Y-H, Weiner J, Li F-M.** 2019. Root proliferation in response to neighbouring roots in wheat (*Triticum aestivum*). *Basic and Applied Ecology* **39**, 10–14.

Figure legends

Figure 1: Experimental design. (a) and (b) close-up views on RhizoTubes® showing wheat seedlings and their roots. (c) Spatial distribution of the seedlings within the RhizoTubes® in both pure (up) and mixed (bottom) stands. Different colours represent different genotypes. (d) Schematic representation of the experiment, each dark square representing a RhizoTube®. The green squares are the control RhizoTubes® and are all grown with the same wheat variety to check environmental heterogeneity.

Figure 2: Effect of resource limitation on plant growth and traits. Comparison of total biomass (a), shoot biomass (b), root biomass (c), number of leaves (d), number of tillers (e), leaf nitrogen concentration (f), root:shoot ratio (g), root length (h), and root area (i) between the R+ (blue) and the R- (red) treatments. Only pure stand data were used, and trait values were summed per Rhizotube® (except for leaf N for which we averaged trait values per Rhizotube®). Points and error bars represent the mean \pm standard deviation. The number of observations in each treatment is reported below each violin plot. Symbols above the plots represent the significance of the treatment effect (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, complete analysis of variance is reported in Supplementary Table 4).

Figure 3: Effect of resource limitation on interactions between varieties. Comparison of Relative Yield Total (RYT) indices on total biomass (a), shoot biomass (b), and root biomass (c) between the R+ (blue) and the R- (red) treatments. Points and error bars represent the mean \pm standard deviation. The number of observations in each treatment is reported below each violin plot. Symbols above violins represent the significance of a two-sided t -test testing if the mean RYT within treatment is significantly different from 1 (***: $p < 0.001$). ANOVAs testing the significance of treatment effect on RYTs are reported in Supplementary Table 5).

Figure 4: Relationships between the trait composition of the mixtures and their performance. Standardized effects of traits on mixture RYTs measured on total biomass in the R+ treatment (a), and R- treatment (b). Backward model selection was performed on a full model with RYT as the response variable and all trait means and differences as explanatory variables. Based on AIC_C, the top-ten

models were retained to compute model-averaged estimates reported on the left side of the panels with their 95% unconditional confidence intervals (Supplementary Table 6). Empty symbols represent trait differences and filled symbols represent trait means. The relative importance of the variables are reported on the right side of the panels and can be interpreted as the probability that the variable appears in the best model. Hatched bars represent trait differences and filled bars represent trait means. Colours refer to the type of traits, with aboveground traits and belowground traits represented in green and brown, respectively. Adjusted R-squared averaged across the top-ten models ($\overline{R^2_{adj}}$) are also reported.

Figure 5: Interactions between root area, resource availability, and biomass. (a) relationship between the total biomass of the pure stands and their root area ($n = 36$ per treatment), (b) relationship between RY computed on total biomass and root area measured in pure stands ($n = 108$ per treatment), (c) relationship between RY computed on total biomass and the hierarchical distance on root area, i.e., the difference between the root area of the focal and the root area of the neighbour, both measured in pure stands ($n = 108$ per treatment), (d) relationship between RYT computed on total biomass and root area plasticity, i.e., the difference between the expected (based on pure stands) and the observed root area ($n = 54$ per treatment). Pearson correlation coefficients (R) and p -values (p) refer to simple linear models fitted independently in the R+ (blue, circle) and R- (red, triangles) treatments.

Legends for Supplementary Materials

Supplementary Table 1: Variety information.

Supplementary Table 2: List of the 54 binary mixtures.

Supplementary Table 3: Description of the nutrient solution.

Supplementary Table 4: Analysis of Variance (ANOVA) of biomass components and traits in pure stands. Pure stand data was summed per Rhizotube® (except leaf N which was averaged per Rhizotube®). We used Type III analysis of Variance using the Kenward-Roger's method on mixed models where the identity of the variety was used as a random effect on both the intercept and the

slope of the treatment effect (R+ vs R- treatment). For each fixed effects, we report the sum of squares (“Sum Sq”), the mean squares (“Mean Sq”), the numerator degrees of freedom (“NumDF”), the denominator degrees of freedom (“DenDF”), the value of the F statistic (“F value”), and the p -value (“Pr(>F)”).

Supplementary Table 5: Analysis of Variance (ANOVA) of RYT_s. Type III analysis of Variance using the Kenward-Roger’s method on mixed models where the identity of the genotypic pair (concatenation of the identity of the two genotypes in a RhizoTube®) was used as a random effect on the intercept. We report the fixed effect of the treatment with the sum of squares (“Sum Sq”), the mean squares (“Mean Sq”), the numerator degrees of freedom (“NumDF”), the denominator degrees of freedom (“DenDF”), the value of the F statistic (“F value”), and the p -value (“Pr(>F)”).

Supplementary Table 6: Ten best fitting models between RYT on aboveground, belowground, and total biomass and mixture trait composition. The top-ten models are ranked according to their AICc. Δ AICc (“delta_AICc”), model weights (“weight”), and adjusted R-squared (“R2_adj”). The “avg” and “diff” suffixes refer to trait averages and trait differences, respectively.

Supplementary Figure 1: RhizoTubes® monitoring. Measurements of nutrient solution inputs (**a**) and water status (**b**) of the RhizoTubes® in the R+ treatment (blue) and the R- treatment (red) over the course of the experiment. Values are averaged over all RhizoTubes®. Seedlings were transferred in the RhizoTubes on the 24th of June, and plants were harvested from the 16th to the 19th of July 2019.

Supplementary Figure 2: Relationships between the trait composition of the mixtures and their above and belowground RYT_s. Standardized effects of traits on mixture RYT_s measured on above- (**a** and **b**) and below- (**c** and **d**) ground biomass in the R+ treatment (**a** and **c**) and R- treatment (**b** and **d**). Backward model selection was performed on a full model with RYT as the response variable and all trait means and differences as explanatory variables. Based on AICc, the top-ten models were retained to compute model-averaged estimates reported on the left side of the panels with their 95% unconditional confidence intervals (Supplementary Table 6). Empty symbols represent trait differences and filled symbols represent trait means. The relative importance of the variables are reported on the right side of the panels and can be interpreted as the probability that the variable

appears in the best model. Hatched bars represent trait differences and filled bars represent trait means. Colours refer to the type of traits, with aboveground traits and belowground traits represented in green and brown, respectively. Adjusted R-squared averaged across the top-ten models ($\overline{R_{adj}^2}$) are also reported.

Supplementary Figure 3: Interactions between root area, resource availability, and shoot

biomass. **(a)** relationship between the shoot biomass of the pure stands and their root area ($n = 36$ per treatment), **(b)** relationship between RY computed on shoot biomass and root area measured in pure stands ($n = 108$ per treatment), **(c)** relationship between RY computed on shoot biomass and the hierarchical distance on root area, i.e., the difference between the root area of the focal and the root area of the neighbour, both measured in pure stands ($n = 108$ per treatment), **(d)** relationship between RYT computed on shoot biomass and root area plasticity, i.e., the difference between the expected (based on pure stands) and the observed root area ($n = 54$ per treatment). Pearson correlation coefficients (R) and p -values (p) refer to simple linear models fitted independently in the R+ (blue, circle) and R- (red, triangles) treatments.

Supplementary Figure 4: Interactions between root area, resource availability, and root

biomass. **(a)** relationship between the root biomass of the pure stands and their root area ($n = 36$ per treatment), **(b)** relationship between RY computed on root biomass and root area measured in pure stands ($n = 108$ per treatment), **(c)** relationship between RY computed on root biomass and the hierarchical distance on root area, i.e., the difference between the root area of the focal and the root area of the neighbour, both measured in pure stands ($n = 108$ per treatment), **(d)** relationship between RYT computed on root biomass and root area plasticity, i.e. the difference between the expected (based on pure stands) and the observed root area ($n = 54$ per treatment). Pearson correlation coefficients (R) and p -values (p) refer to simple linear models fitted independently in the R+ (blue, circle) and R- (red, triangles) treatments.

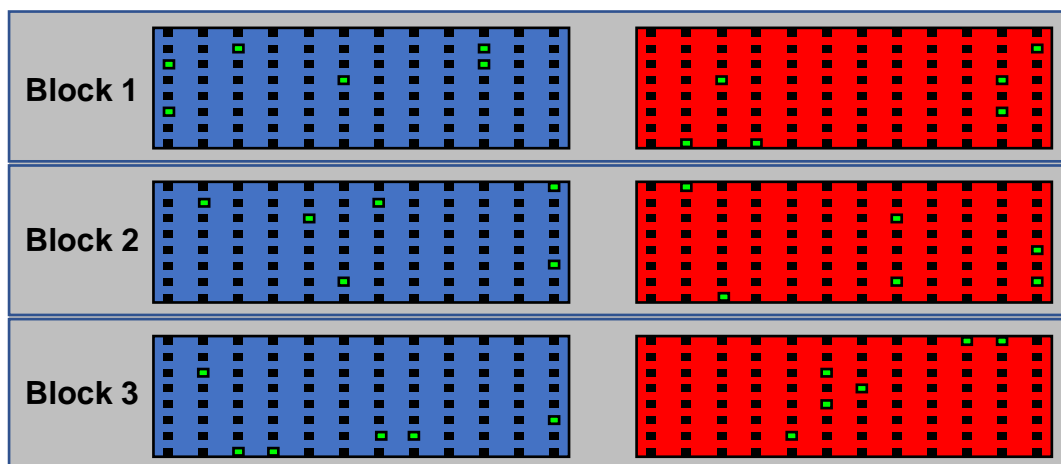
(a)**(b)****(c)****(d)****R+****R-**

Figure 1: Experimental design. (a) and (b) close-up views on RhizoTubes® showing wheat seedlings and their roots. (c) Spatial distribution of the seedlings within the RhizoTubes® in both pure (up) and mixed (bottom) stands. Different colours represent different genotypes. (d) Schematic representation of the experiment, each dark square representing a RhizoTube®. The green squares are the control RhizoTubes® and are all grown with the same wheat variety to check environmental heterogeneity.

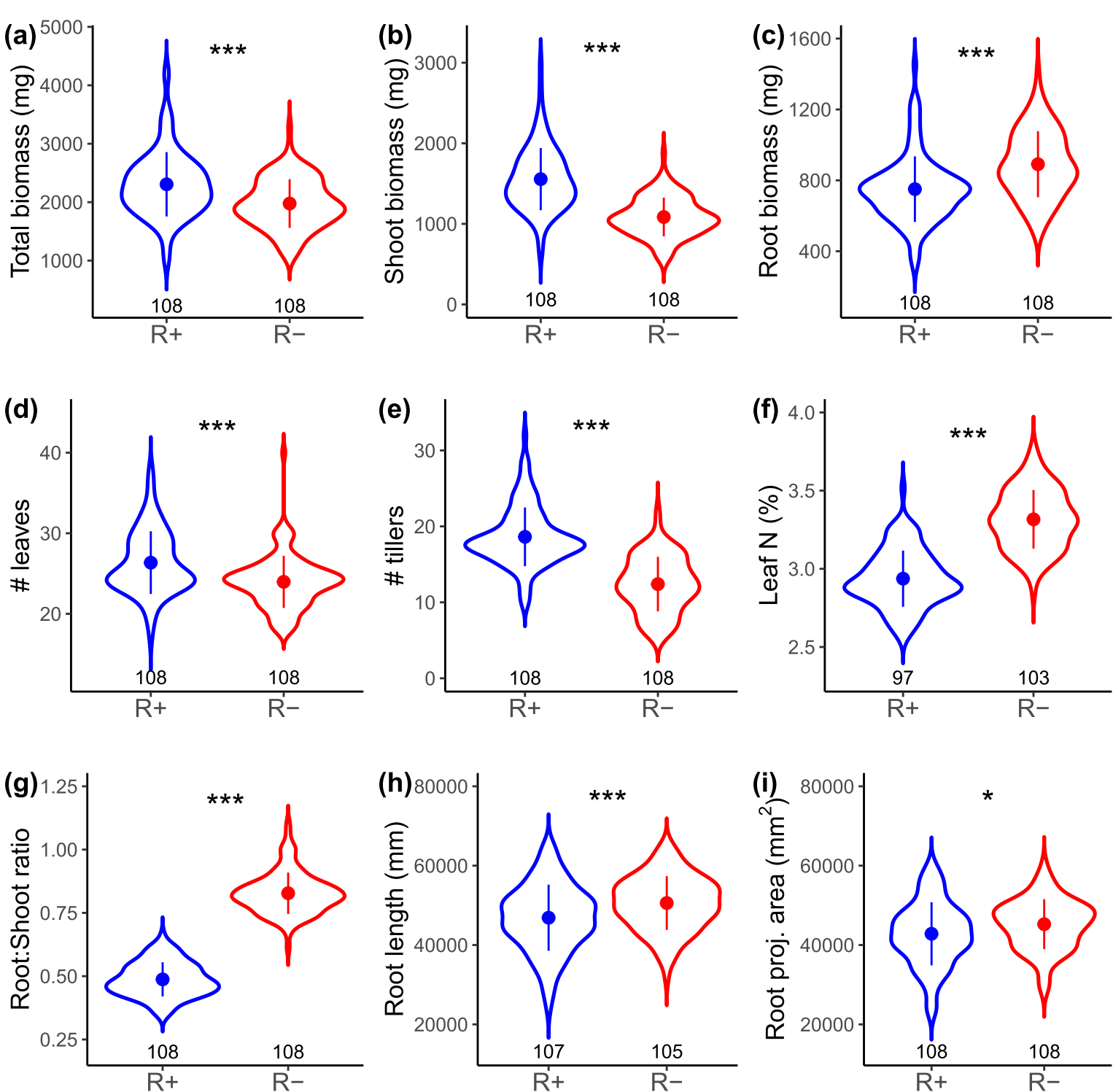


Figure 2: Effect of resource limitation on plant growth and traits. Comparison of total biomass (a), shoot biomass (b), root biomass (c), number of leaves (d), number of tillers (e), leaf nitrogen concentration (f), root:shoot ratio (g), root length (h), and root area (i) between the R+ (blue) and the R- (red) treatments. Only pure stand data were used, and trait values were summed per Rhizotube® (except for leaf N for which we averaged trait values per Rhizotube®). Points and error bars represent the mean \pm standard deviation. The number of observations in each treatment is reported below each violin plot. Symbols above the plots represent the significance of the treatment effect (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, complete analysis of variance is reported in Supplementary Table 4).

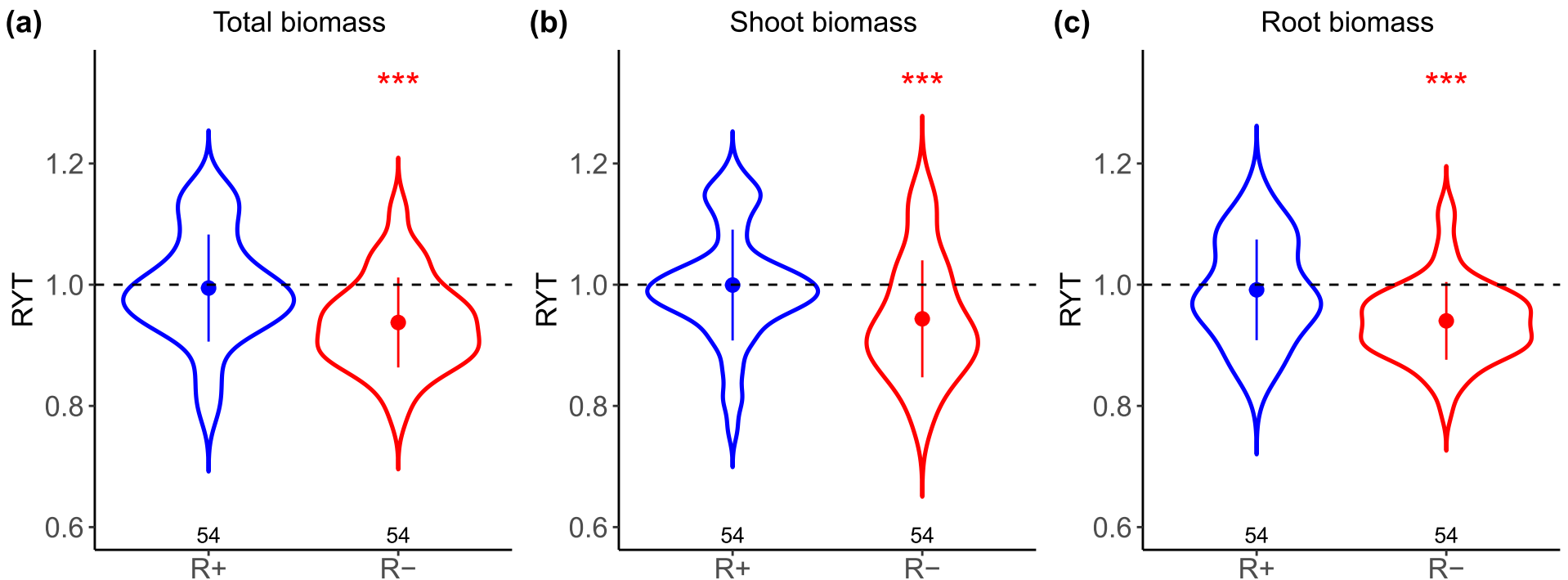


Figure 3: Effect of resource limitation on interactions between varieties. Comparison of Relative Yield Total (RYT) indices on total biomass (a), shoot biomass (b), and root biomass (c) between the R+ (blue) and the R- (red) treatments. Points and error bars represent the mean \pm standard deviation. The number of observations in each treatment is reported below each violin plot. Symbols above violins represent the significance of a two-sided t-test testing if the mean RYT within treatment is significantly different from 1 (***: $p < 0.001$). ANOVAs testing the significance of treatment effect on RYTs are reported in Supplementary Table 5).

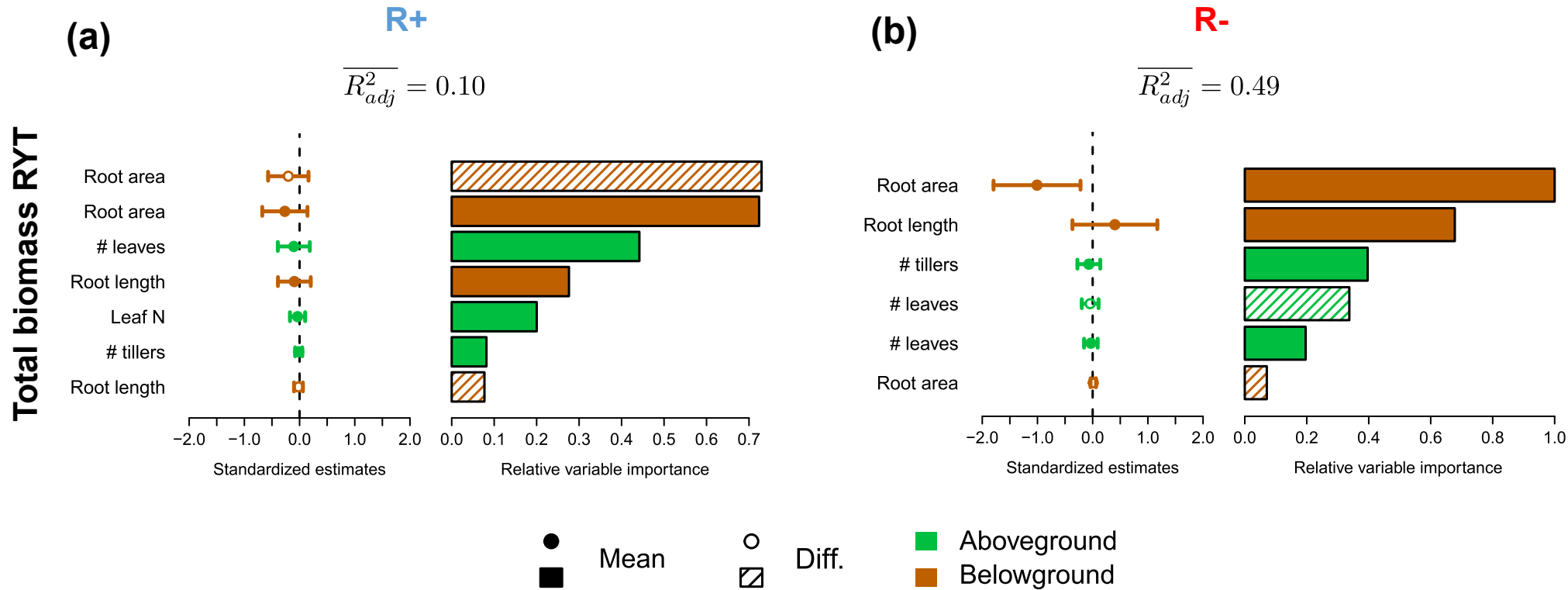


Figure 4: Relationships between the trait composition of the mixtures and their performance. Standardized effects of traits on mixture RYTs measured on total biomass in the R+ treatment (a), and R- treatment (b). Backward model selection was performed on a full model with RYT as the response variable and all trait means and differences as explanatory variables. Based on AIC_C , the top-ten models were retained to compute model-averaged estimates reported on the left side of the panels with their 95% unconditional confidence intervals (Supplementary Table 6). Empty symbols represent trait differences and filled symbols represent trait means. The relative importance of the variables are reported on the right side of the panels and can be interpreted as the probability that the variable appears in the best model. Hatched bars represent trait differences and filled bars represent trait means. Colours refer to the type of traits, with aboveground traits and belowground traits represented in green and brown, respectively. Adjusted R-squared averaged across the top-ten models ($\overline{R^2_{adj}}$) are also reported.

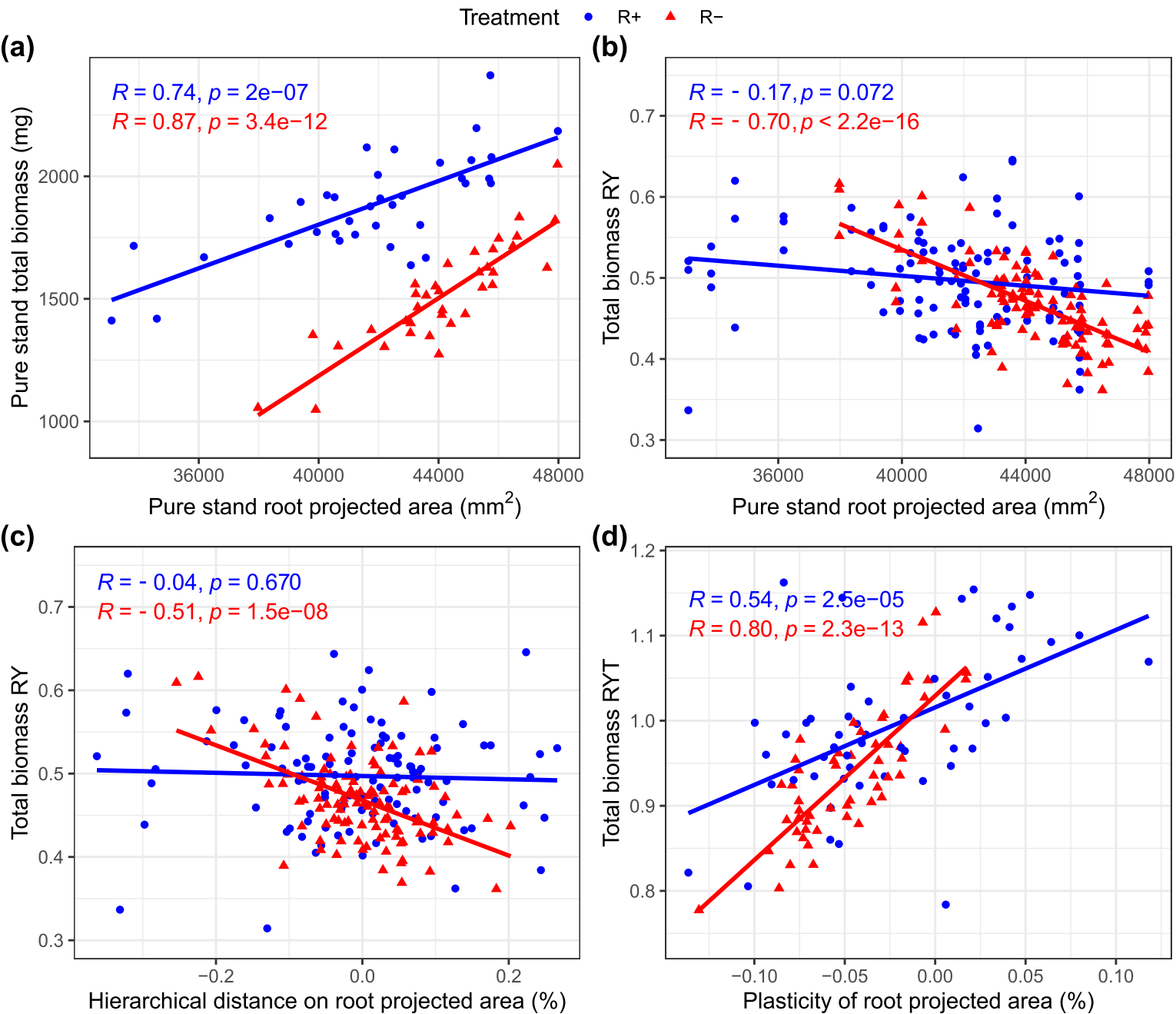


Figure 5: Interactions between root area, resource availability, and biomass. (a) relationship between the total biomass of the pure stands and their root area ($n = 36$ per treatment), (b) relationship between RY computed on total biomass and root area measured in pure stands ($n = 108$ per treatment), (c) relationship between RY computed on total biomass and the hierarchical distance on root area, i.e., the difference between the root area of the focal and the root area of the neighbour, both measured in pure stands ($n = 108$ per treatment), (d) relationship between RYT computed on total biomass and root area plasticity, i.e., the difference between the expected (based on pure stands) and the observed root area ($n = 54$ per treatment). Pearson correlation coefficients (R) and p -values (p) refer to simple linear models fitted independently in the R+ (blue, circle) and R- (red, triangles) treatments.