**Mixing cultivars reduces early root competition in wheat under water and nutrient limitation**

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Cultivar mixtures, agroecology, relative yield, stress-gradient hypothesis, complementarity, high-throughput root phenotyping, root area, competitive hierarchy

# Abstract

Cultivar mixtures, defined as the concurrent cultivation of several varieties of the same crop in the same field, are receiving growing interest, as increasing genetic diversity in the field could improve agricultural sustainability. Ecological theory predicts that positive interactions between cultivars should led to a more efficient use of resources, especially when multiple resources become scarcer (the “stress-gradient hypothesis”). In line with this prediction, cultivar mixtures yield slightly higher than their pure stand components on average. However, mixing effects are also very variable, and we still poorly understand how cultivar interactions are affected by resource limitation. In this study, we tested whether diversity in root traits could promote positive interactions between cultivars, and whether this pattern would be exacerbated under limiting resource conditions. We grew 36 durum wheat (*Triticum turgidum* ssp. *durum*) genotypes in pure stands and in 54 binary mixtures in a high-throughput root phenotyping platform under two resource conditions: a control (C), and a water- and nutrient-limited treatment (S). We then compared the biomass of three-week-old seedlings in mixed vs pure stands and tested the relationship between the relative biomass of the mixtures and their trait composition, assessed by the average trait value of the mixtures and the absolute trait difference between varieties in the mixture. We found that mixed stands produced less biomass than predicted from their pure stands, particularly in the S treatment. A single trait, the average 2D projected area of the root system, explained up to 50% of the relative biomass production of the mixtures in the S treatment. Our results indicate that this biomass reduction most likely resulted from relaxed competition in mixed relative to pure stands, and that the area of the root system captured the competitive hierarchy between cultivars. Plastic changes in root area also contributed to mitigate competition in mixtures. Overall, our results suggest that root area and plasticity in root area are promising breeding targets to reduce intra-specific competition at the seedling stage and important traits to account for when assembling cultivar mixtures.

# Introduction

Decades of experimental ecology have established a general positive relationship between plant diversity and ecosystem functioning (Tilman et al. 1996, 2001; Hector et al. 1999). Ecosystems with a higher number of species tend to be more productive, more efficient at regulating pathogens, at recycling nutrients, and at buffering abiotic stresses (Hooper et al. 2005; Hector and Bagchi 2007; Tilman et al. 2014). Species diversity is generally thought to improve ecosystem functioning via two main effects: the complementarity effect, and the selection effect (Loreau and Hector 2001). The complementarity effect results from differences in ecological niches between species that have different resource requirements and hence experience less competition, which ultimately translates into a more efficient conversion of resources into ecosystem functions (e.g., Roscher et al. 2008; Mueller et al. 2013). Communities with more species are also more likely to contain species which are the most efficient at performing a given function; such “efficient” species might be even more efficient in a diverse community than in a monospecific community (e.g., highly competitive species often benefit from relaxed competition in a mixture), which corresponds to the selection effect (e.g., Fargione et al. 2007; Li et al. 2018).

Similar ecological effects can be exploited in crops by mixing different species at the same time within the same field (i.e., intercropping, Vandermeer 1992). For example, one of the most ancient intercrop known as the “three-sisters” (maize, bean, squash) combines species with different root foraging strategies that complement each other and achieve greater yield in mixture than grown separately (Zhang et al. 2014), which is commonly referred to as overyielding. Mixing species that get their nitrogen from the soil (e.g., cereals) with species that can fix nitrogen from the atmosphere (e.g., legumes) is another way to achieve complementarity effects and ultimately overyielding (Bedoussac et al. 2015). However, intercropping also raises several challenges because the components of the intercrop can differ in many aspects of their life cycle (germination, growth rate, phenology, plant architecture, etc), which complicates agronomic management (Lemken et al. 2017; Huss et al. 2022). Moreover, they often have different harvest products that need to be separated from each other.

Cultivar mixtures, that is the cultivation of a mixture of genotypes of the same species in the same field, allows to address most of these challenge by mobilizing plant diversity at the intra-specific level (Smithson and Lenné 1996). Historically, cultivar mixtures have been shown to be very efficient at limiting pathogen spread and disease severity compared to monogenotypic stands, especially in grain cereals (Wolfe 1985; Mundt et al. 1995; Zhu et al. 2000; Finckh and Wolfe 2006). This beneficial effect has recently renewed the interest in cultivar mixtures, as they may help reduce pesticide use and thus support the agroecological transition (Barot et al. 2017; Borg et al. 2018; Snyder et al. 2020; Wuest et al. 2021). Extensive research in phytopathology has helped identify the different epidemiological and evolutionary mechanisms that underlie the protective effect of cultivar mixtures against pathogens (Finckh and Mundt 1992; Mundt et al. 1995; Finckh et al. 2000; Vidal et al. 2017). For example, recent results suggest that some cultivars might be able to improve the basal immunity of their neighbors, a phenomenon referred to as neighbor-modulated susceptibility (NMS, Pélissier et al. 2021, 2023).

In addition to their beneficial effects on pathogen control, cultivar mixtures could also help optimize resource use and ultimately increase crop yield (Hajjar et al. 2008; Barot et al. 2017; Snyder et al. 2020). Ecological mechanisms at play in natural ecosystems or in intercropping such as niche complementarity could similarly improve resource use efficiency by reducing competition in intraspecific mixtures. In addition, the niche dimensionality theory in ecology predicts that such positive effects of plant diversity are expected to strengthen with multiple resource limitation, as the stress-gradient hypothesis (SGH, Bertness and Callaway 1994; Maestre et al. 2009). Therefore, cultivar mixtures could help maintain high productivity under low-input farming practices and more variable and extreme climates (Adu-Gyamfi et al. 2015). Yet, how resource limitations affect the relative productivity of mixture remains poorly understood (Reiss and Drinkwater 2018; Alsabbagh et al. 2022).

In natural ecosystems, plant functional traits have been used to better understand and predict the effect of plant diversity on ecosystem processes, notably productivity (McGill et al. 2006; Garnier et al. 2015). These traits are defined as any morphological, physiological or phenological feature which impact fitness, and are measurable at the individual level without reference to the environment or any other level of organization (Violle et al. 2007). They capture how individuals interact with their abiotic and biotic environment, and ultimately how they contribute to processes occurring at higher levels of organization (community, ecosystem). For example, the mean values of leaf functional traits of dominant species can be used to predict primary productivity or litter decomposition rates at the community level (Garnier et al. 2004). Similarly, differences in functional traits between species or genotypes can capture differences in ecological niche (Violle and Jiang 2009) or in competitive ability (Wagg et al. 2017; Cadotte 2017), and in turn inform us on the ecological effects that drive productivity at the community level (e.g., complementarity or selection effects). Yet, even if plant traits are commonly used in agriculture, for example to adapt management practices or to guide plant breeding programs, very few studies have applied trait-based approaches to cultivar mixtures (Montazeaud et al. 2018, 2020; Kong and Zhao 2023).

Root traits play a central role in the functioning of natural ecosystems (Bardgett et al. 2014). Preliminary results suggest that the traits with the strongest effect on mixture performance are not necessarily the ones commonly measured in agricultural applications. In particular, morphological and architectural root traits have been shown to determine multiple facets of mixture performance (Montazeaud et al. 2018, 2020). However, the relationship between root traits and mixture performance remains poorly understood, notably because root traits are difficult to measure and highly plastic in response to both biotic and abiotic factors. A better understanding of this relationship could thus help us identify the mechanisms underlying mixing effects, and guide mixture assembly based on measurable features.

In the present study, we used durum wheat (*Triticum turgidum* ssp*. durum*) as a crop model to investigate the effect of root trait composition on mixture performance. We used a panel of 36 cultivars preliminary shown to exhibit contrasted responses to combined water and nitrogen limitation, and grew them in monogenotypic plots and binary mixtures in a highly controlled high throughput root phenotyping platform under both non-limiting and limiting water and nutrient conditions. Based on ecological theory, we hypothesized that (i) mixtures would produce more biomass than their monogenotypic counterparts (i.e., overyielding), (ii) overyielding would be higher under resource-limited conditions, (iii) resource-use complementarity would be the main effect driving overyielding under resource-limited conditions, (iv) complementarity effects would arise from differences in root traits between cultivars.

# 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; (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 field and plaform data, a subset of 36 genotypes exhibiting contrasted responses to resource availability ranging from very stable to very unstable genotypes on a set of below- and aboveground traits 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). Monocultures (here refereeing to groups of a single genotype, n = 36) and mixtures (here referring to groups of two genotypes, n = 54) were grown in RhizoTubes®, transparent pots designed for high-throughput root phenotyping (Figure 1), with six plants per RhizoTube®. The six plants were of the same genotype in monocultures, whereas two genotypes were grown in alternate positions in the mixtures (Figure 1). The 36 monocultures and the 54 mixtures were grown under a combined water and nutrient stress (S treatment) or under a control treatment without stress (C 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 1).

## Growth conditions

Seeds were sown into Petri dishes on the 22nd of June 2019 and kept at 4°C during two days. Seedlings were then transferred into the RhizoTubes® on the 24th and harvested about three weeks later between the 16th and the 19th of July. Plants were at the tillering stage at harvest. Temperatures were maintained around 20-25°C, relative humidity around 70-80%, and photoperiod was set to 16h from 6am to 10pm, with an average PAR of 330 µmol.m-².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 (see the description of the solution in Supplementary Table 2). 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 S 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).

## Phenotyping

Root traits were measured at the RhizoTube® level based on image analysis, using images taken on the 15th of July (i.e., one day before harvest). DESCRPTION OF IMAGE ANALYSIS PROCESS. Three root traits were computed following image processing: root depth, corresponding to the distance between the top of the RhizoTube® and the deepest root pixel; root length, which was 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.

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 at XX °C during XX days 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), with the Fieldspec 2500© (Analytical Spectral Devices, Inc. (ASD), Boulder, CO, USA) spectrometer. We measured one NIR spectrum per leaf per plant in each RhizoTube® (i.e., six spectrum per RhizoTube®). 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 to nitrogen content using the calibration descibed in Ecarnot et al. DESCRPTION OF LEAF N COMPUTATION FROM NIRS. Because root traits could only be computed at the RhizoTube® level, we pooled aboveground traits either by summing (# leaves, # tillers, shoot biomass, root biomass, total biomass) or averaging (leaf N) the trait values of the six plants to obtain one trait value per RhizoTube®. Root:shoot ratio was also computed at the RhizoTube® level by dividing the total root biomass by the total shoot biomass of the RhizoTube®.

## Statistical analysis

Unless otherwise stated, we performed all statistical analyses with R v. 4.3.2 (R Core Team 2019).

We first tested the effect of the treatment (control *vs* stressed) and the stand type (pure *vs* mixed) on the different traits and biomass components (above and belowground). We used mixed linear models with a given trait or biomass component as the response variable, treatment, stand type and their interaction as fixed effects. To test whether stands differed across treatment and displayed different responses to the treatment depending on their cultivar composition, we included the genotypic identity of the stand (cultivar identity in pure stand, identity of the cultivar pair in mixed stands) as a random intercept and random treatment slope to the model. We finally included two covariates as fixed effects: block, and harvest date or measurement date (except for root traits which were all measured on the same day). We assessed the significance of the fixed effects with standard analyses of variance and *F* statistics computed with Kenward-Roger's approximations for the degrees of freedom (Supplementary Table 3). We fitted the mixed model with the *lmer()* function (package *lme4*), and checked significance with the *anova()* function (package *lmerTest*).

To compare the relative performance of mixed *vs* pure stands, we computed the Relative Yields (RYs, de Wit and van den Bergh 1965) of the cultivars in mixed stands, using each component biomass separately (aboveground, belowground, and total biomass). We first separated our dataset between the pure and mixed stands. Then, we summed the biomass of all plants of the same genotype within each RhizoTube® (i.e., 6 plants in pure stands, 3 plants in mixed stands). We used the pure stands dataset to compute pure stand reference productivity. To do so, we fitted a linear mixed model with biomass as the response variable, measurement date, block, and treatment as fixed effects, and cultivar identity as a random effect (here we included only the random effect of the cultivar on the intercept, as adding the random effect on the 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 cultivar with the estimated fixed effect of the treatment (C or S) to obtain cultivar pure stand reference biomass values adjusted for the effects of block and measurement date within each treatment. For mixed stands, we also fitted a linear mixed model with each cultivar biomass as the response variable, measurement date, block, and treatment as fixed effects, and cultivar 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 cultivars, such that cultivar 1 and cultivar 2 in a mixed stand had pair identities cultivar1-cultivar2, and cultivar2-cultivar1, respectively. In a first step, we summed the BLUP of each cultivar pair (intercept in the C treatment, intercept + slope in the S treatment) with the estimated fixed effect of the treatment (C or S) to obtain the productivity of each cultivar in mixed stands adjusted for the effects of the block and measurement dates within each treatment. We then divided these mixed stand values by the pure stand reference values to obtain RYs for each cultivar within each mixture. Under the null hypothesis that the cultivar produced equal amount of biomass in mixed than in pure stand, RY = 0.5 because there are half the number of plants of a cultivar in mixed stands (n = 3) compared to pure stands (n=6). RY > (<) 0.5 means that the cultivar produced more (less) biomass in mixed than in pure stand. In a second step, we summed the RYs of the two cultivars in the mixed stand to obtain the Relative Yield Total (RYT, de Wit and van den Bergh 1965) of the mixture. RYT = 1 means that the mixture as a whole produced more biomass than expected from the productivity of the components grown in pure stands, whereas RYT > (<) 1 means that the mixture produced more (less) biomass than expected. We tested whether RYTs were significantly different from 1 using a two-sided *t* test. Then, we compared RYTs between the C and S treatment using a linear mixed model with RYT as the response variable, treatment as a fixed effect, and cultivar pair identity as a random effect. We assessed the significance of the fixed effects as detailed above (Supplementary Table 4).

To assess whether trait composition of the mixture explained RYT variability, we used traits measured in pure stands to predict mixed stands RYTs. This approach is based on the hypothesis that pure stand traits are the information available to agronomists and plant breeders when designing cultivar mixtures. First, we computed reference trait values for pure stands that we corrected for design effects and measurement dates. As described above, we fitted a linear mixed model with pure stand trait as the response variable, block, measurement date, and treatment as fixed effects, and cultivar identity as a random effect. Depending on the traits, cultivar identity random effect was specified both on the intercept and the treatment slope or only on the intercept (i.e., when adding the random slope led to singular models due to very low variance on the slope). We then computed pure stand reference trait values as the sum of the BLUP of each cultivar (intercept in the C treatment, intercept + slope in the S treatment when random slope was specified) with the estimated fixed effect of the treatment (C or S). Then, for each mixed stand, we computed both the average and the absolute trait difference between the two cultivars using the pure stand reference trait values. Finally, we fitted a full linear model with RYT as the dependent variable and all trait averages and all trait differences as independent variables (*lm()* function from the *stats* package). We standardized all dependent and independent variables (*μ* = 0, *σ* = 1) and ran a backward model selection (*glmulti()* function from the *glmulti* package). We used the second-order Akaike Information Criterion (AICC, Sugiura 1978) to rank the models and performed model-averaging inference based on the top 10 models using the *coef()* function (*glmulti* package). We report parameter estimates and their 95% unconditional confidence interval computed as ±1.96 unconditional sampling standard deviation, variable importance, and adjusted R-squared averaged over the top 10 models (Burnham and Anderson 2002). Detailed information on the top 10 models are available in Supplementary Table 5.

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 genotype per treatment. We also checked the relationship between RYs and the hierarchical distance in root area between the cultivar and its neighbor in pure stands. Hierarchical distance was the difference between the focal root area and the neighbor root area, divided by the focal root area (i.e., positive values mean that the focal had higher root area that 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 cultivars 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 architecture

The limitation of water and nutrients strongly reduced total plant biomass (1947.35 mg in the S treatment vs 2364.71 in the C treatment, *F*1,87.77 = 144.49, *p* < 0.001,Figure 2f). 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 2X), less leaves (Figure 2a), and less tillers (Figure 2b), they produced higher root biomass (Figure 2X), 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 control to 0.82 in the stressed treatment (+71%, F1,87.78 = 3966.58, *p* < 0.001, Figure 2g). Leaf nitrogen concentration increased in the S treatment (3.32 vs 2.95%, F1,87.19 = 525.03, *p* < 0.001, Figure 1D).

## Comparison of pure vs mixed stands

We did not detect any significant effect of the stand type (pure vs mixed) or its interaction with resource availability (C vs S treatment) on seedling traits or biomass (Supplementary Figure 2 and Supplementary Table 3). However, RYT indices showed that mixtures produced less biomass than expected from the biomass of their components grown in pure stands (average RYT for total biomass = 0.97, *t*107 = -4.10, *p* < 0.001, Figure 3a). This was true for both above- and belowground biomass (average RYT for shoot biomass = 0.97, *t*107 = -3.03, *p* = 0.003, Figure 3b; average RYT for root biomass = 0.97, *t*107 = -4.53, *p* < 0.001, Figure 3c). RYTs were significantly lower in the S than in the C treatment for the three biomass components (shoot biomass: RYT = 0.94 vs 1.00, *F*1,53 = 26.23, *p* < 0.001, Figure 3a; root biomass: RYT = 0.94 vs 0.99, *F*1,53 = 32.56, *p* < 0.001, Figure 3b; total biomass: RYT = 0.94 vs 0.99, *F*1,53 = 34.87, *p* < 0.001, Figure 3c).

## Effect of trait composition on mixture biomass

RYTs were highly variable in both treatments (Figure 3). The trait composition of the mixtures poorly explained RYT variability in total biomass observed in the C treatment (Figure 4a, average adjusted R² over the top ten models = 0.09). In contrast, trait composition explained up to 49% of RYT variation in the S treatment (Figure 4b). Most of this variability was explained by the average root area of the two cultivars grown in mixture (R² = 47% in a model with average root area as the single explanatory variable), which had a negative effect on RYT (Figure 4b, Supplementary Table 5): mixing two genotypes with higher average root area resulted in a decrease in biomass production in mixture compared to pure stands. We obtained similar results when performing the analysis on shoot biomass alone (Supplementary Figure 3a and 3b, Supplementary Table 5). In contrast, traits had higher explanatory power on root biomass RYT in the C treatment (Supplementary Figure 3c, average adjusted R² over the top ten models = 0.33), with strong negative effects of average leaf number and average root area. In the S treatment, however, as for the other biomass components, average root area was the main explanatory trait with a negative effect on RYT (Supplementary Figure 3d).

## Relationship between root area and mixture biomass

To further investigate the potential mechanisms underlying the strong negative relationship between RYT calculated on each biomass component and average root area in the S treatment, we tested whether our results fitted with the hypothesis that root area might be a proxy of competitiveness under resource limited conditions. Under this hypothesis, we expect genotypes with a high root area to experience strong inter-plant competition in pure stands, because both plants have a high root area. Such strong competition might result in an overinvestment in biomass to outgrow the neighbor. Those genotypes, when grown in mixed stands, are more likely to be paired with genotypes that have a lower root area, leading to relaxed competition in mixtures compared to pure stands. Such relaxed competition could in turn lead to a disengagement from the “arms race” between plants, potentially resulting in lower above and belowground biomass production, and thus low RYTs when mixing genotypes with hight root area in pure stands. In line with this hypothesis, there was a strong positive relationship between the total biomass of the pure stands and their root area (Figure 5a). This relationship was stronger in the S treatment (Figure 5a, Pearson’s *R* = 0.87, slope = 0.079 mg.mm-2, *p* < 0.001) than in the C treatment (Pearson’s *R* = 0.74, slope = 0.0446 mg.mm-2, *p* < 0.001). Additionally, consistent with our hypothesis, cultivars with the highest root areas in pure stands and the highest hierarchical distances in root area with their neighbors were the ones with the strongest biomass reduction in mixed stands in the S treatment (Figure 5b, Pearson’s *R* = -0.70, *p* < 0.001; Figure 5c, Pearson’s *R* = -0.51, *p* < 0.001). These relationships were not significant in the C treatment (Figures 5b and 5c). Finally, the strongest biomass reductions occurred in mixtures where the observed root area was lower 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 S (Pearson’s *R* = 0.80, *p* <0.001,slope = 1.93 %-1) than in the C treatment (Pearson’s *R* = 0.54, *p* <0.001,slope = 0.91 %-1). Altogether, these results support the idea that low RYTs in the S 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 cultivars.

## Individual biomass reflects individual competitiveness rather than community performance

Contrary to our expectations, mixing cultivars did not result in increased seedling biomass. In fact, we observed the opposite pattern: mixed stands produced less biomass than expected based on their pure stand components, especially under water and nutrient limitations. At first glance, we might conclude that this result contradicts ecological theory, notably the Stress Gradient Hypothesis that predicts a shift towards more positive interactions as abiotic conditions become less favorable (Bertness and Callaway 1994; Maestre et al. 2009). However, individual cultivar responses to mixture cultivation combined with root trait data together suggest that such reduction in relative biomass in mixtures was the consequence of intense competition in pure stands, rather than evidence for decreased performance in mixed stands. This interpretation is based on the hypothesis that early biomass does not reflect final community performance that would better be approximated by latter established reproductive biomass, but rather individual plant competitiveness. In line with this interpretation, seedling growth rate and early vigor are traditionally targeted by plant breeders as favorable traits for competitiveness against weeds (Lemerle et al. 1996; Bertholdsson 2005; Hendriks et al. 2022). Hence, our results suggest an overall relaxation of competition intensity in mixed relative to pure stands at the seedling stage, especially under limiting water and nutrient resources.

## Root area as the main driver of competitive hierarchy

We identified root area as the main driver of plant biomass and mixing effects in our experiment. The average root area of the two cultivars in pure stands could explain up to 50% of RYT variability under resource-limiting conditions. A higher root area in monogenotypic cultures was associated with a greater biomass reduction in mixture, especially when the mixture partner had a lower root area. These results support the idea of an early-stage belowground competitive hierarchy between cultivars (Kunstler et al. 2012), where higher root area is associated with higher competitive ability. In line with these results, 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).

## No evidence for niche complementarity between seedlings

We found no evidence of complementarity effects, and no effect of functional trait diversity on mixed stand biomass, including under resource limitation. This could be explained by the fact that functional differences between cultivars were not large enough 3-weeks after sowing to generate complementarity effects. A second hypothesis is that we did not measure the “right” traits to detect complementarity effects, notably belowground. Indeed, even if root functional traits are involved in many ecosystem processes (Bardgett et al. 2014), diversity in architectural and morphological root traits have rarely been shown to associate with complementarity effects in ecological studies (Fort et al. 2014; Bakker et al. 2018). In cultivar mixtures, previous studies also failed to find evidence of niche complementarity based on root trait differences (Montazeaud et al. 2018). Overall, this study confirm that root trait differences alone are less likely to generate complementarity effects in cultivar mixtures than they are for example in intercropping systems where differences in root foraging strategies between species can be more significant (Zhang et al. 2014; Homulle et al. 2022; Schmutz and Schöb 2023).

## 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 S 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; Schiffers et al. 2011; Burns and Strauss 2012). In cultivar 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 cultivars (Tschurr et al. 2023; Kong and Zhao 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 cultivars, 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 directly on group performance (i.e., selecting on population yield), or by targeting key traits related to competitive ability. Our study suggests that selecting genotypes with reduced root area in monogenotypic stands and reduced root area plasticity in response to other neighboring genotypes could be a way to reduce competition intensity between seedlings, which could then translate into higher yields. In line with this hypothesis, excessive growth of the root system in the topsoil has been shown to reduce collective yield in oat and barley (Vain et al. 2023). In wheat, modern varieties that have been subject to selection for increased population yield for many generations display reduced root proliferation in presence of a neighbour compared to old landraces (Zhu et al. 2019). Such phenotypes should also be favoured in cultivar mixtures, where the objective would be to minimize the average root area between cultivars. More generally, our results show that maximizing functional diversity within the mixture, as emphasize in most theoretical framework (e.g., Barot et al. 2017; Kopp et al. 2023), may only produce favourable effects for some traits, e.g., but for others, more attention should be paid to the average trait value.

# Figure legends

**Figure 1: Experimental design.** (**a**) Schematic representation of the experiment, each dark square representing a RhizoTube®. (**b**) and (**c**) close-up views on RhizoTubes® showing wheat seedlings and their roots. (**d**) Spatial distribution of the seedlings within the RhizoTubes® in both pure (up) and mixed (bottom) stands. Different colors represent different genotypes.

**Figure 2: Effect of resource limitation on plant growth and architecture.** Comparison of number of leaves (**a**), number of tillers (**b**), leaf nitrogen content (**c**), shoot biomass (**d**), root biomass (**e**), total biomass (**f**), Root:Shoot Ratio (**g**), root length (**h**), and root area (**i**) between the control (C, blue) and the stressed (S, red) treatments. Points and error bars represent the mean ± 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* < 0.01, \*\*\*: *p* < 0.001, complete analysis of variance is reported in Supplementary Table 3).

**Figure 3: Effect of resource limitation on cultivar interactions.** Comparison of Relative Yield Total (RYT) indices on shoot biomass (**a**), root biomass (**b**), and total biomass (**c**) between the control (C, blue) and the stressed (S, red) treatments. Points and error bars represent the mean ± standard deviation. The number of observations in each treatment is reported below each violin plot. Symbols in the title of the plots represent the significance of a two-sided *t*-test testing if the mean RYT is significantly different from 1 (\*\*: *p* < 0.01, \*\*\*: *p* < 0.001).Symbols above the plots represent the significance of the treatment effect on RYTs (\*\*\*: *p* < 0.001, complete analysis of variance is reported in Supplementary Table 4).

**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 C treatment (**a**), and S 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 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 5). 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. Colors 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 () 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 mixture RY computed on total biomass and the average total biomass produced by the two components in pure stands (n = 54 per treatment), **(c)** relationship between RY computed on total biomass and root area measured in pure stands (n = 108 per treatment), **(d)** relationship between RYT computed on total biomass and total biomass measured in pure stands (n = 108 per treatment). Pearson correlation coefficients (*R*) and *p-*values (*p*) refer to simple linear models fitted independently in the C (blue, circle) and S (red, triangles) treatments.

# Legends for Supplementary Materials

**Supplementary Table 1: Cultivar information.**

**Supplementary Table 2: Description of the nutrient solution.**

**Supplementary Table 3: Analysis of Variance (ANOVA) of traits and biomass.** 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 both the intercept and the slope of the treatment effect (C vs S 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 4: Analysis of Variance (ANOVA) of RYTs.** 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 both the intercept and the slope of the treatment effect (C vs S treatment). 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 5: Ten best fitting models between RYT and mixture trait composition.** The top-ten models are ranked according to their AICc. ∆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 C treatment (blue) and the S 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 16thto the 19th of July 2019.

**Supplementary Figure 2: Effect of the stand type on seedling growth and architecture.** Comparison of number of leaves (**a**), number of tillers (**b**), leaf nitrogen content (**c**), shoot biomass (**d**), root biomass (**e**), total biomass (**f**), Root:Shoot Ratio (**g**), root length (**h**), and root area (**i**) between pure and mixed stands. Points and error bars represent the mean ± standard deviation. The number of observations in each stand type are reported below each violin plot. Symbols above the plots represent the significance of the treatment effect (n.s.: not significant, complete analysis of variance is reported in Supplementary Table 3).

**Supplementary Figure 3: Relationships between the trait composition of the mixtures and their above and belowground RYTs.** Standardized effects of traits on mixture RYTs measured on above- (**a** and **b**) and below- (**c** and **d**) ground biomass in the C treatment (**a** and **c**) and S 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 5). 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. Colors 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 () are also reported.