Dear Editor,

Thank you very much for considering our work for publication in *Journal of Experimental Botany*. We went through the comments provided by the two reviewers, and we addressed them as exhaustively as possible. We hereby submit a revised version of our manuscript, which we believe has been significantly improved thanks to the reviewers’ comments. Two aspects of the manuscript have notably been strengthened:

1. We now decompose the net effect of varietal interactions into complementarity and selection effects, as suggested by reviewer 1. These new results revealed that complementarity effects were the most important contributors to varietal interactions.
2. We better explain why reduced biomass at the seedling stage is not necessarily a “negative effect”. In the absence of other competing species (e.g., weeds) and under limited-resource conditions, intraspecific competition can become a strong determinant of yield per unit area. Seedling growth and vigor being traditionally considered as a competitive trait, higher biomass at the seedling stage can compromise yields due to higher inter-plant competition. This hypothesis is supported by previously published results where seedling biomass measured on the same phenotyping platform was found to negatively correlate with grain yield measured in the field across two species (durum and bread wheat), nearly 1000 varieties, and 42 environments (Colombo *et al.*, 2022).

We provide below a line-by-line response (bold text) to the comments raised by the two reviewers, as well as a revised version of the manuscript with tracked changes. Because reviewer 2 provided his minor feedback in the form of comments in the pdf file, we have responded to these comments with direct changes in the text. We attract your attention to the fact the manuscript has increased in length, going from 4,075 to 5,185 words. Following comment 1) from reviewer 1, the abstract now exceeds the 200-word limit (278 words). For this reason, and because we could not find any mention of this in the [instructions for authors](https://academic.oup.com/jxb/pages/manuscript-preparation), we did not add a “Conclusion” section to the manuscript as asked by reviewer 1. We will of course add this section if you think it is important from an editorial perspective.

We genuinely believe that these changes considerably improved our paper, and we hope that this new version will be suitable for publication in *Journal of Experimental Botany*.

Looking forward to hearing from you.

Sincerely,

Germain Montazeaud, on behalf of all co-authors.

**Reviewer 1**

This is an interesting study on variety mixtures of wheat under resource limited conditions conducted in greenhouse. The manuscript is well structured and the idea is interesting and relevant in addressing underlying mechanisms of variety mixtures to reduce intra-specific competition. While previous studies have widely reported the contributions of aboveground functional traits (i.e., plant height, specific leaf area, leaf C/N ratio) to inter- and intra-specific interactions, little is known about how root interactions might increase productivity, especially among different cultivars or genotypes. This study explores the effects of wheat variety mixtures on reducing intra-specific belowground competition and finds that this reduction is closely related to root area.  
Generally, the study is well presented, and I enjoyed reading it. However, I also have several questions and believe that the manuscript should be revised thoroughly before being published. I have listed my major points in detail below and hope that these may help to clarify a few things in the revision.

**We thank reviewer 1 for acknowledging the scientific value and the novelty of our work.**

Major points  
1) Your abstract is well and clearly written, but you should state more clearly the research gap and the novelty of your work. For research gap, you might state more clearly about why it would be useful to fill it, how you intend to do so and what the novelty of your research is (either the method and/or the results).

**We now clearly state that the novelty of our study is to characterize belowground competition between varieties using high throughput root phenotyping methods while previous studies focused on the aboveground compartment (l. 33-37). We also clarified the originality of our results, showing that seedling biomass reduction most likely reflected a relaxation of belowground competition rather than a negative interaction between varieties, and that early competition between seedlings was mainly captured by a single trait, that is the average root area of the varieties (l. 40-47).**

Lines 39-40: The manuscript mentions that the strength of competitive ability is related to root area, but it does not specifically explain how the root system influences plant competitive ability. The most significant and novelty results are not adequately highlighted in Abstract.

**We have now clarified the link between root area and competition in the abstract (l. 40-47).**

2) The Introduction is well-written and logically clear. However, there is insufficient description (review) of how the root system can reduce competition and lower TOCs (Tragedy of the Commons). The current state of research in this area, as conducted by previous studies, is also not clearly stated.

**We now clearly explain how the root system contributes to TOC citing seminal and more recent papers on this topic (e.g., Gersani *et al.*, 2001, see l. 84-95). In addition, we present the different approaches that have been proposed to lower TOCs via root systems using either (i) direct selection on root traits, e.g., selecting narrower root systems as early proposed by Donald (1968), (ii) group selection and kin selection to favour the evolution of less competitive root systems (cf. all the literature on Darwinian Agriculture (Denison *et al.*, 2003) and evolutionary agroecology (Weiner *et al.*, 2010), (iii) trait diversity in species or varietal mixtures to generate complementarity effects or selection effects, citing both intercropping and grassland studies (l. 96-116). In case we missed some key studies that used root systems to lower TOCs, we will be happy to include any additional suggestions from reviewer 1.**

3) The manuscript does not calculate selection and complementarity effects, although these are discussed in the abstract, hypothesis in Introduction section, and Discussion sections of the paper. I recommend that the authors calculate the diversity effects, including selection and complementarity effects. Even if it is challenging to measure belowground biomass, I believe that aboveground biomass data can be used for these calculations.

**We would like to thank reviewer 1 for this suggestion, which we think has considerably improved the manuscript. We did not calculate these effects in the first version because we thought we could easily tell apart the complementarity and the selection effects from RY values in such simplified communities with only two varieties. It appears that the additive partitioning is indeed much more informative: instead of just a selection effect, as proposed in the first version of the manuscript, we see that complementarity effects are in fact the most important contributors to RYT in this experiment (see Figure 4, new Methods l. 311-320, new results l. 385-394, and new Discussion l. 480-517).**

4) Line 224: I do not fully understand here and the rest of the results analysis sections. Since it was mentioned earlier in the “phenotype” description (Line 186-188) that the roots in mixtures cannot be distinguished, so I am confused how is the root RY calculated here? There seems to be a contradiction between the earlier and later descriptions.

**Indeed, this point was not very clear in the first version of the manuscript. We chose to measure root traits as close as possible to the harvest date in order to have sufficiently developed root systems and to synchronize as much as possible root trait measurements and biomass measurements in order to increase the chance to detect causal relationships between them. Root traits were measured by analysing images of the roots of the seedlings directly taken through the walls of the Rhizotubes®. At this date however, the root systems of adjacent plants were largely overlapping, so the image analysis software was unable to separate individual plant root systems. At harvest in contrast, we extracted the plants from their Rhizotubes®, so we could manually disentangle their roots using aboveground parts that were more clearly separated. Then it was possible to measure individual root biomass for each plant and compute RY. This is now explained l. 225-231 & l. 238-241.**

5) There is no Conclusion section in this manuscript. That is, there is no summary provided after the Discussion section. I suggest adding this section. Please clearly state the novel findings of your work, whether they are methodological or results-based, as requested in the instructions for authors. Additionally, it would be beneficial to include some comments on how these findings will inform future agroecological management strategies.

**The revised version has increased in length, going from 4,075 to 5,185 words. Thus, we rather chose not to include a conclusion section, which is not mandatory according to the** [instructions for authors](https://academic.oup.com/jxb/pages/manuscript-preparation)**. As we discuss the implication of our results for plant breeding and agroecological practices in the last section of the Discussion (“Practical implications for plant breeding”, l. 564-596), we thought that there would be little added-value to write a Conclusion section. However, we are willing to reconsider adding this section if the editor deems it useful.**

**Reviewer 2**

General comments to the authors:

This is overall a very interesting study and the paper is nicely written. Everything is quite clear, I added some minor comments in the text, but here are my main comments:

**Thank you for this nice evaluation. We here only reply to the major comments, and we addressed the minor points by direct edition in the text.**

1. In your data analyses, I wondered why you used this method to calculate one value per treatment/mixture, and why you did not simply calculate RY per treatment/mixture/block and then use block as a random factor in your model.

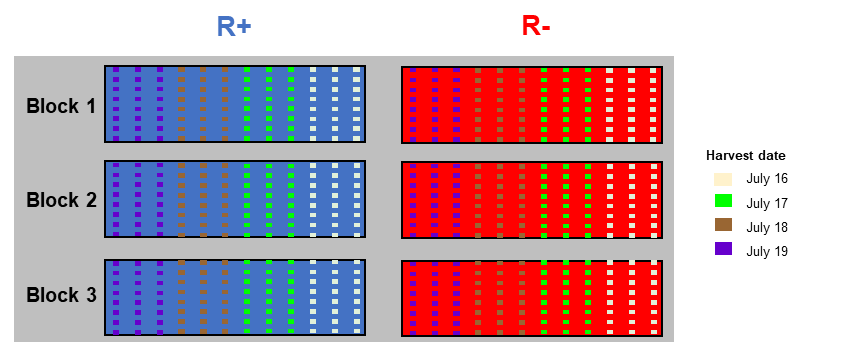
**We agree with you that it would have been more optimal (i.e., higher statistical power) to compute RY within each block\*treatment combination, and then use block in the final model. Unfortunately, given the size of the experiment (e.g., 3240 plants to be phenotyped at harvest), we could not harvest all the Rhizotubes® on the same day, and thus harvested over four days. In order to avoid cofounding effects between harvest date and the other factors such as treatment or spatial blocks, we harvested one quarter of each block\*treatment combination every day (cf Figure 1). As a consequence, within a given block\*treatment combination, the Rhizotubes had four different harvest dates (Figure 1). This means that for most mixed stands Rhizotubes®, the pure stands of two components of the mixture were harvested at two different dates, which were themselves potentially different from the harvest date of the mixed stands Rhizotube®. Then, it was impossible to compare the biomass of the mixed vs pure stands within block\*treatment combinations. We now explain this rationale l. 270-277.**

Figure 1 – Spatial distribution of harvest dates across the experiment

1. The main thing that I think is missing in your story, is why a reduction in biomass at the seedling stage would actually be beneficial for grain yield. When we read it, it sounds like it's "bad" to grow mixtures because they actually have less biomass than expected based on the pure stands. I seem to understand that biomass at the seedling stage is negatively correlated with grain yield? This seems strange to me, but if this is true, it should be explained much earlier.

**Indeed, this point was not clear enough in the first version of the manuscript. The hypothesis is that, under some conditions where intraspecific competition (i.e., competition between plants of the same crop species) is a significant contributor to grain yield per unit area, it is actually better to select and assemble plants that are less competitive, as this reduction in competition will translate into higher final yields. This is not a new idea, since it was early advanced by plant breeders and agronomists during the Green Revolution (Donald, 1968). Early seedling vigour and growth are classically targeted by plant breeders as a favourable trait to compete against weed species (Lemerle et al., 1996; Bertholdsson, 2005; Hendriks et al., 2022), meaning that seedlings with higher biomass are more competitive. However, in the absence of weeds, and when resources are limiting just as in our experiment, it might be the case that too much competition between seedlings actually impairs grain yields. In that case, reduction in biomass at the seedling stage could be a way to increase final yield, as we propose in our study. This negative relationship between seedling biomass measured in controlled conditions and final yields measured in the field has been robustly characterized, using seedling data from the same phenotyping platform as the one we used in our study (4PMi platform): Colombo et al. compared platform traits and traits measured in the field, notably grain yield, using 750 varieties of bread wheat and 200 varieties of durum wheat. They found that seedling biomass measured in the platform was negatively correlated to grain number per square meter and grain yield over 42 contrasted environments (see Figure 2, 3, and 4 in Colombo *et al.*, 2022). We now explain this idea in more details in the manuscript (l. 506-517, l. 590-594).**

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