



Far-red light perception by the shoot influences root growth and development in cereal-legume crop mixtures

Jin L. Wang · Jochem B. Evers ·
Niels P. R. Anten · Yitong Li · Xiaoyi Yang ·
Jacob C. Douma · Hannah M. Schneider

Received: 18 January 2024 / Accepted: 8 August 2024 / Published online: 30 August 2024
© The Author(s) 2024

Abstract

Background and aims Above- and below-ground signals for and responses to competition in mixed-species stands have mostly been studied separately. This leaves unclear if aboveground signals specific to mixed stands have consequences for how plants respond and compete belowground. This study investigated the effect of an aboveground signal, the ratio of red to far-red light (R/FR), on plant biomass allocation and root architecture and morphology when plants grow with different species.

Methods A greenhouse experiment with a mixture of wheat (*Triticum aestivum*) and faba bean (*Vicia faba*) and their respective mono stands was carried out in deep pots (22.6 L, 72 cm in depth) for 52 days of growth. Light-emitting diode (LED) lights were

used to enrich FR levels (i.e., reduce the R/FR) of the light environment.

Results Exposure of the shoots to FR-enriched light predominantly affected adventitious roots, with species-specific effects. Especially in species mixtures, increased FR caused wheat to produce more adventitious roots per tiller, while adventitious root mass of faba bean decreased. The influence of FR on rooting depth (D75, i.e. depth of 75% of root biomass) was species specific, with higher FR levels causing a reduction in D75 in wheat, but a greater D75 in faba bean.

Conclusions Our findings demonstrate the plasticity of adventitious roots; they are highly responsive to the R/FR level to which the shoot is exposed and to whether neighboring plants are of the same or a different species. This highlights the complexity of plants' responses to environmental cues and how they modify interspecific interactions.

Responsible Editor: Gwendolyn Kristin Kirschner.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-024-06903-4>.

J. L. Wang (✉) · J. B. Evers · N. P. R. Anten · Y. Li ·
X. Yang · J. C. Douma · H. M. Schneider
Centre for Crop Systems Analysis, Wageningen University,
PO Box 430, 6700 AK Wageningen, The Netherlands
e-mail: jin.wang@wur.nl; jin.wang.cn@outlook.com

H. M. Schneider
Leibniz Institute of Plant Genetics and Crop Plant
Research (IPK), Gatersleben, Corrensstr. 3, 06466 Seeland,
OT, Germany

Keywords Crop mixture · Intercropping · R/FR ·
Biomass allocation · Root vertical distribution · Root
morphology

Introduction

In both natural vegetation and crop stands, increased species richness may increase whole-stand performance in terms of growth, reproductive yield or resource-use efficiency (van der Plas 2019). Many

studies have focused on understanding the mechanisms driving these positive effects, particularly in agriculture, as mixed-species cropping is presented as a means towards more sustainable biodiversity-positive production (Kelty 1992; Malezieux et al. 2009; Li et al. 2023).

Mixed-species cropping, also known as intercropping, refers to simultaneously growing more than one crop species on a field. Mixed-species crops typically use resources more efficiently than pure stands (i.e. monocrops) (Li et al. 2023). The mechanisms underlying the effect of species richness on performance include: (i) competition between species is on average less intense than competition between plants of the same species; (ii) facilitation between two species, with at least one of the species positively affecting the other. The former is also called the complementarity effect, which is one of the key factors that enhance the performance of mixed cropping (Li et al. 2020). Reduced interspecific competition, in turn, can be due to: (i) both species being intrinsically different from each other, which reduces the competition due to less overlap in resource types and spatial and temporal niches; and/or (ii) species exhibiting plastic responses to each other's presence or size. Environmental cues drive phenotypic plasticity, which may contribute significantly to the complementarity effect and, consequently, lead to overyielding (Zhu et al. 2015; Zhang et al. 2022).

Combinations of cereal and legume species typically have enhanced belowground resource-use efficiency, which can be partially attributed to differences in root system architectures (Li et al. 2006). Cereals, being monocotyledonous (monocot), have fibrous root systems with a cylindrical root system shape. Legumes, on the other hand, being dicotyledonous (dicot), have a taproot systems and conical root system shape (Herben et al. 2022). The different strategies of crop root foraging create different ecological niches, e.g., topsoil by the relatively shallow, slower-growing root system of legume and deeper exploitation by the deeper and faster-growing cereal root system (Hamblin and Tennant 1987; Ofori and Stern 1987). These differences in vertical root distributions between cereals and legumes have for example been documented in barley/pea (Hauggaard-Nielsen et al. 2001) and maize/faba bean systems (Xia et al. 2013).

While intrinsic differences in resource capture between species have been widely studied (McKane

et al. 2002; Silvertown 2004; Turnbull et al. 2016), the role of plasticity in mixed cropping has received less attention (Zhu et al. 2015). Plant roots are highly plastic (Schneider and Lynch 2020), typically showing strong morphological and physiological responses to changes in e.g., water status (Fromm 2019), nutrient availability (Gruber et al. 2013), and other abiotic stress (Karlova et al. 2021). In mixed stands, plants will typically modify their root system in response to their neighboring species (Homulle et al. 2022; Yu et al. 2022). However, which environmental cues drive these root responses remains unclear.

In pure and mixed plant stands, plants respond to environmental cues from neighboring plants such as light signals, biogenic volatile components, shading, changes in local nutrient concentration and root exudates (Pierik et al. 2013). An important cue for plants to infer upcoming competition is the ratio of red (R) to far-red (FR) light. While plants absorb relatively more photosynthetically active radiation (PAR, radiation with wavelengths between 400–700 nm), FR light (700–780 nm) is reflected and transmitted. When compared to a single plant, those growing in dense crop stands receive lower PAR, with a lower ratio of red (R, 600 to 700 nm, a part of PAR) to FR light. This low R/FR is an early warning for plant light competition, as a reduction of R/FR can occur at an early stage of plant growth through horizontally reflected FR, prior to the onset of actual shading (Casal 2012; Pierik and de Wit 2014). When plants continue to grow, the canopy closes and actual shading happens with red and blue light depletion (Keuskamp et al. 2011). Plants respond to these signals through a suite of responses including an increase in shoot/root ratio, decrease in shoot branching and tillering, stem internode elongation, leaf hyponasty and early flowering, collectively known as the shade-avoidance syndrome (SAS) (Ballaré and Pierik 2017).

The nature and dynamics of the R/FR cues perceived by a plant are largely driven by the aboveground architectural traits and growth dynamics of its neighbors and therefore may be dependent on whether neighbors are con- or hetero-specific (i.e. same species or different species). In addition, in the case of mixed crops, temporal and spatial R/FR distribution also depends on whether species are sown simultaneously or at different times as in relay-cropping. For example, in a maize-soybean relay mixed cropping, the intercropped soybean plants received

lower R/FR and PAR than soybean in sole crops, and this difference became larger at lower row distances between maize and soybean (Yang et al. 2014). A simulation exercise also showed that the difference of canopy structure between the wheat and pea species provided different values of R/FR (Didier et al. 2018). To date, several studies have investigated how above-ground traits in crops respond to the light environment in mixed stands, especially responses in tiller/branch initiation and shoot architecture (Yang et al. 2014; Wang et al. 2017; Li et al. 2021). For example, in maize/wheat mixed cropping, plasticity in wheat tillering mainly attributed to 23% more total light capture (Zhu et al. 2015).

While SAS responses at shoot level to low R/FR have been explored in depth for decades (Ballaré and Pierik 2017), low R/FR effects on whole-plant biomass allocation and root distribution and architecture have been much less studied. Low R/FR could directly influence root development via repressing auxin signaling and transport around the developing lateral root primordium (van Gelderen et al. 2021). Also, the shoot may respond to low R/FR and develop a different canopy structure, therefore resulting in different light capture and assimilation of carbon, which may subsequently influence root growth. However, contradicting results of altered R/FR on biomass allocation to roots in different plant species exist. For example, research on soybean suggests that under low R/FR conditions, there is a reduction in total plant biomass and no effect on biomass allocation to roots (i.e. root/shoot ratio) (Murphy and Dudley 2007; Green-Tracewicz et al. 2011). However, studies on common lamb's-quarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), and tomato (*Solanum lycopersicum* L.) seedlings show that low R/FR conditions have no significant effect on total plant biomass of all plants while lamb's-quarters showed a decreased shoot/root ratio (Ma and Upadhyaya 2016).

The capture and utilization of resources in mixtures such as light aboveground and water and nutrients belowground have been studied independently for the past decades (Keating and Carberry 1993; Morris and Garrity 1993; Yin et al. 2020). While the signaling pathways between shoot to root have been widely studied on individual plants (Shabala et al. 2016; Ko and Helariutta 2017), the potential relevance of such mechanisms for mixed cropping studies

have received less attention. An improved understanding of this may allow better informed selection of key traits for mixed cropping, e.g. the appearance and growth of different root classes and decisions on the mixed cropping design. It is not clear if mixed cropping specific light signals have consequences for how plants respond and compete belowground, when growing next to a different species (Gundel et al. 2014). Thus, it is important to explore: (i) the existence, type and magnitude of root responses to variation in the strength of the aboveground R/FR signals present in mixed stands, and (ii) if this light signal influences root interaction between species. In this paper we address the following question: to what extent do R/FR light signals in mixed-species stands cause responses in growth and development of different root classes and how does this interact with the species of neighboring plants?

To address these questions, we conducted a pot experiment on a typical cereal/legume mixed cropping system consisting of wheat and faba bean, as they have dissimilar root system architectures. As the response to R/FR ratio is species dependent (Demotes-Mainard et al. 2016), we chose these two species, also the same cultivars as they have proven response to low R/FR in field conditions (Dong et al. 2024). They were grown as sole crops or in a mixture and were exposed to different levels of FR enrichment. The study's finding will provide information on the impact of an aboveground light signal (R/FR) on root architecture and morphology when (i) analyzing the functioning and performance of mixed species vegetations, and (ii) designing the density and spatial arrangement of mixed cropping systems involving cereals and legumes, as the variation of arrangement will result in different R/FR environment.

Materials and methods

Experimental design

An experiment was conducted from 25 March to 16 May 2022 in a temperature-controlled greenhouse of the Unifarm facility at Wageningen University & Research in the Netherlands. The climate was set to 22 °C/17 °C day/night temperatures with 70% air humidity. The experiment was laid out in a split-plot design with three replicate blocks. The blocks

were laid in a North–South orientation, and the three blocks were parallel located from East to West. In each block, three levels of FR enrichment (i.e., high, medium and low R/FR) were randomly assigned as the main-plot treatment. In each light treatment plot, three species combinations (wheat/wheat, faba bean/faba bean and wheat/faba bean) were randomly assigned to six pots as the sub-plot factor. Pots were watered daily with drip irrigation system to maintain 80% of water holding capacity based on the estimation of water loss per plant. On 22 April, biological control (*Neoseiulus californicus*, *Stratiolaelaps scimitus*, *Frankliniopsis vespiformis*, *Eretmocerus eremicus* and *Encarsia formosa*) and fungicide (Amistar, 2 mL L⁻¹ diluted, 625 mL m⁻²) was applied as a precautionary measure.

Pot set up and soil conditions

Polyvinyl chloride (PVC) tubes with a diameter of 20 cm and a height of 78 cm (22.6 L in volume) were used for the pots in this experiment. A plastic liner in the pots (100 µm thickness PE transparent tubular film, SPRINTIS Schenk GmbH & Co. KG) with drainage holes was used to facilitate root sampling at harvest. Thermal isolation foil was wrapped around the outside of the pot to minimize soil temperature fluctuations. The growth substrate used in this experiment was a mixture (v/v, 1:1) of cyclone sand (Van Leusden) and agriculture soil (30 cm topsoil, with 4.4 mg N kg⁻¹ and 0.38 mg P kg⁻¹) which was air dried for 48 h and sieved to 2 mm before mixing. The nutrient solution was added during the soil mixing process and was evenly distributed in the pot. The nutrients (mg kg⁻¹ of dry substrate) supplemented included 15.59 NO₃ from KNO₃, 2.23 NH₄ from NH₄NO₃, 16.43 P from KH₂PO₄, 31.03 K from KNO₃ and KH₂PO₄, 21.22 Ca from CaCl₂·2H₂O, 3.22 Mg from MgSO₄·7H₂O, 0.04 B from H₃BO₃, 0.02 Mn from MnSO₄·H₂O, 0.02 Zn from ZnSO₄·7H₂O, 0.005 Cu from 150 CuSO₄·5H₂O, 0.006 Mo from Na₂MoO₄·5H₂O, 0.004 Co from CoCl₂ and 1.48 Fe-DTPA. Pots were filled with this substrate to a depth of 72 cm at a 1.46 g cm⁻³ bulk density. The mixed substrate had a water holding capacity of 14% (v/v). Throughout the experiment pots were watered every day by dripping irrigation system based on the estimation of water loss to ensure moisture levels were approximately 80% water-holding capacity.

Light treatment setup

Shading screens and additional lights (Philips, SON-T) were utilized in the greenhouse to control the light conditions. To avoid direct sun light on the pots and artificial light sources at night from lighting surrounding the greenhouse, the shading screens were closed when radiation levels exceeded (1125 µmol m⁻² s⁻¹) in the daytime, and from 19:00 to 7:30 during the night. The additional lights were consistently operational during daytime (16 h). In total, six light bulbs were used, covering an area of 40 m². Together with natural light, photosynthetic photon flux density (PPFD) was approximately 200–400 µmol m⁻² s⁻¹ during solar noon. To ensure uniform distribution of light across all plants, the pots were rotated by 180 degrees every week.

To investigate the effect of reduced R/FR while maintaining a constant PAR, an FR enrichment source was introduced into the experiment. This methodology was well established in the shade avoidance studies with *Arabidopsis* (Casal 2012). To set up three R/FR levels, supplemental FR light was set at 7.9, 15.8, 23.7 µmol m⁻² s⁻¹ in the high, medium and low R/FR treatments, respectively (note that more FR means a lower R/FR ratio). The FR light was provided by LED light bars (Philips GP LED production 2.1 Dyn DR/W/B/FR 120). The LED light bars were installed at a height of 85 cm from the floor on two sides of each light treatment unit to mimic the early light signals reflecting from neighboring plants (Fig. 1). Each light bar had ten evenly distributed light points. The light bars were turned on three days after transplanting when the shoots emerged from the soil. The distance between the light treatment units was 95 cm east–west and 65 cm north–south. R/FR ratio was measured (SKL 904 SpectroSense2) to the direction of the far-red light with horizontal distance of 15 cm from the light source at pot level (78 cm) in different time of a day for all six pots (Fig. S1).

Seed germination and transplanting

Wheat (*Triticum aestivum* L. cv. Nobless) and Faba bean (*Vicia faba* L. cv. Fanfare) were used for this study. On 22 March, seeds of both species were pregerminated on wet blotting paper in petri dishes at room temperature, then graded by eye for uniformity.

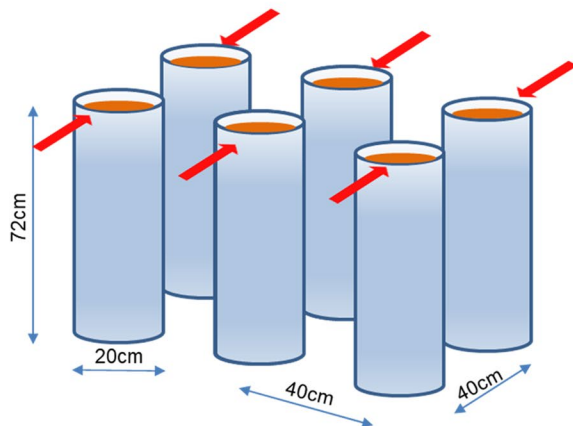


Fig. 1 Left panel: the diagram of pots and light position in a light treatment compartment. Red arrows represent the FR light direction from the LED bars which were positioned



85 cm in height from the floor. Pots were 40 cm in distance to each other. Right panel: photo of the LED light setup. The length and the width of the supporting frame is 140 cm × 60 cm

On 25 March they were transplanted to pots. The planting depth for wheat and faba bean seeds were 2 cm and 4 cm, respectively. There were two seeding positions in pots of pure stand faba bean, six seeding positions in pots of pure stand wheat, and four seeding positions (one for faba bean and three for wheat) as replacement design in pots of wheat/faba bean mixture (Li et al. 2016). To ensure the desired number of plants growing in each pot, two seeds were placed carefully in each seedling position, and covered with growing substrate. Between 29 and 30 March (4 and 5 days after transplanting, DAT), the extra plants were carefully thinned by removing the shoots (thus preventing disrupting the remaining seedling).

Data collection

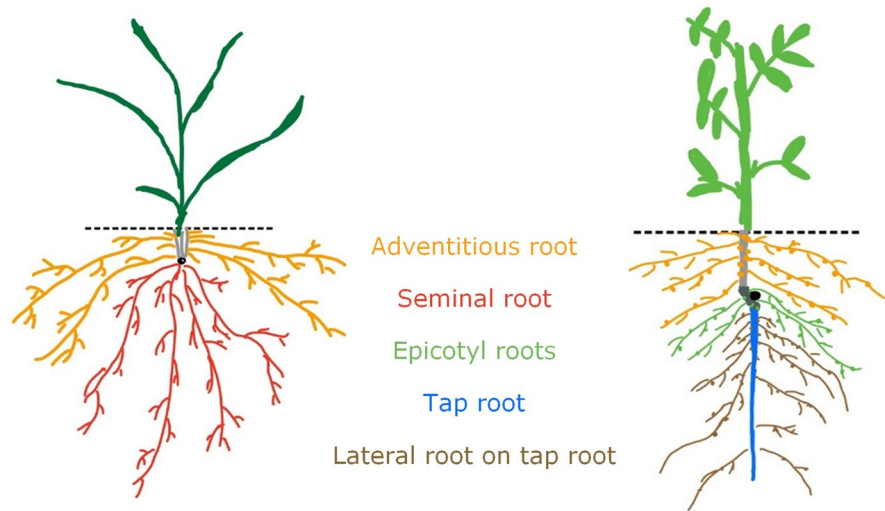
Both species were sampled and measured separately. Wheat from pure stand and mixture was regarded as pure wheat and mixed wheat, respectively. The same definitions were applied to faba bean. Shoots were cut off at the soil surface on the 16 May (52 DAT) and the number of tillers in wheat were counted. Plant height and leaf area of both species, internode length of faba bean (counting from soil surface) was measured. Then the leaves, stems and reproductive organs (flowers and spikes/pods) of both species were dried at 70 °C for 48 h

and weighed separately. Soil with roots was carefully taken out by pulling out the plastic liners from the pots and then vertically divided into six equal bulks. Each soil bulk was a cylinder of 20 cm in diameter and 12 cm in height, representing one of the six soil layers. The top bulk with the soil surface was regarded as first layer and the subsequent layers were counted downwards. For the first layer, soil was gently washed out with running water in a coarse sieve (1.6 mm mesh size), minimizing damage to the root structure.

Then, the faba bean roots were divided into three categories: adventitious roots (with attached lateral roots), epicotyl roots (with attached lateral roots) and lateral roots that initiate from taproot (i.e. LR-tap, only first 3 cm of the taproot was sampled, see Fig. 2). Since plants were experiencing the light treatment continuously from the time of transplanting and lateral root density is not altered with tissue age, we chose the first 3 cm of the taproot as a representative tissue for lateral branching density. The wheat roots were divided into two categories: adventitious roots (with attached lateral roots) and seminal roots (with attached lateral roots) (Fig. 2). The number of roots in different categories were counted before further measurement.

For the remaining soil layers, a 1.6 mm sieve was used to sieve out the roots from the soil, then

Fig. 2 Schematic presentation of root systems of wheat (left) and faba bean (right). The text colors are the same with the root categories. Dashed lines represent the soil surface



roots were cleaned with running water. All roots were stored in 60% ethanol (v/v) at 4 °C before they were scanned. For pots with both species, roots of faba bean and wheat were distinguished based on differences in color and diameter. Faba bean roots were dark colored and thick, while wheat roots were light colored and thin. Root samples in different categories in the first layer and representative samples in the remaining layers were placed in a 20 × 25 cm transparent tray with water and then scanned with the dual scanner (EPSON perfection V700 photo, J221A) and analyzed by RhizoVision (Version 2.0.3) (Seethepalli et al. 2021). After that nodules on the root system were removed and stored separately, dried and weighed. Root samples were oven dried at 70 °C for 72 h and weighed.

Calculation of variables

Root length and root surface area was the output of RhizoVision, from which the root length density (RLD), specific root length (SRL), specific root area (SRA) were estimated based on the dry weight of the sample and presented on a whole-plant level (weighed average throughout the soil depth and root classes) (Table 1). Biomass allocation to organs was quantified as the fraction of biomass invested in leaves (leaf mass fraction, LMF), stem (stem mass fraction, SMF), roots (root mass fraction, including nodule mass, RMF) and reproductive organ biomass (ROMF). The ROMF in wheat was the combination of flower and spike, and in bean was flower and pods (Table 1). D75 is the depth above which 75% of the total root biomass within the pot was located (Schenk

Table 1 Definition of the root variables

Abbreviation	Explanation	Definition	Units
D75	Depth of 75% of root biomass	depth above which 75% of the total root biomass within the pot was located	cm
RLD	Root length density	root length / soil volume	cm cm ⁻³
SRL	Specific root length	root length / root mass*	cm mg ⁻¹
SRA	Specific root area	root surface area / root mass	cm ² mg ⁻¹
LMF	Leaf mass fraction	leaf mass / plant mass	g g ⁻¹
SMF	Stem mass fraction	stem mass / plant mass	g g ⁻¹
RMF	Root mass fraction	root mass / plant mass	g g ⁻¹
ROMF	Reproductive organ mass fraction	wheat: (spikes mass + pod mass) / plant faba bean: mass (flower mass + pod mass) / plant mass	g g ⁻¹

*Mass of the plant materials is the dry weight

& Jackson 2002). The value of D75 was estimated by linear interpolation between the cumulative root weights of samples in six vertical soil bulks (Schenk and Jackson 2002; Trachsel et al. 2013, see Supplementary Material for details).

Statistical analysis

We used R (R Core Team 2022) and lme4 package (Bates et al. 2015) to perform a linear mixed effects analysis of the relationship between tested variables and two factors (mixed/mono stands and FR light intensity). We performed the analysis for each species separately. Multiple measurements (pots) per replicate were averaged before statistical analysis. As fixed effects, light intensity was considered as a continuous variable and cropping system was a categorical variable. The random effect on each light compartment (sub-plot) was nested in the random effect on replicate block effect (main-plot). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Models with the lowest second-order Akaike Information Criterion (AICc) were chosen.

Results

Biomass allocation to roots for wheat and faba bean was most sensitive to FR enrichment

Plants expressed shade-avoidance responses, with decreased wheat tiller number, increased wheat plant height and increased faba bean internode length, while leaf area of both species was not influenced to FR enrichment (Fig S4 and S5). Mixed wheat had more shoot biomass (on average of all light treatment, 0.55 g, 0.46 g, 0.33 g of leaf, stem, and reproductive organs per plant, respectively) than the pure wheat, while allocating more biomass (on average of all light treatment, 0.06 g) to roots than the pure wheat (Fig. S3, a–d). Wheat root biomass was positively correlated with FR enrichment (low R/FR), with a slope of $4.0 \text{ mg per } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.9 \text{ (SE)}$, and root biomass of mixed wheat was 8.0% greater than wheat in pure stand (Fig. S3,d, (root_biomass

mixed wheat—root_biomass pure wheat)/ root_biomass pure wheat). In faba bean, the biomass of leaf, stem, and reproductive organs were significantly reduced (1.45 g, 0.82 g, 0.49 g, respectively) in mixed faba bean compared to pure faba bean while FR enrichment did not influence allocation to those organs (Fig. S3, e–h). However, high FR enrichment (low R/FR) significantly reduced the root biomass of faba bean, and this effect had a strong interaction with the cropping system, with the relationship being more strongly negative in mixture (slope $-67.7 \text{ mg per } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 31.5 \text{ SE}$) than in pure faba bean (Fig. S3, h, slope $-8.0 \text{ mg per } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 22.3 \text{ SE}$).

Total whole-plant biomass and biomass fraction were partially influenced by the FR enrichment

To assess whether the observed changes in absolute organ biomass were influenced by alterations in allocation strategy or plant size, the total biomass and biomass fraction were further investigated. Increasing the intensity of FR enrichment (lowering R/FR) had a positive effect on wheat total biomass both in the pure and mixed stand, with an increase of $19.1 \text{ mg per } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 7.6 \text{ (SE)}$ per unit of FR enrichment (Fig. S2). In addition, wheat whole-plant biomass in the mixture was 31.8% increased compared to pure wheat, from $5.80 \text{ g} \pm 0.15 \text{ (SE)}$ to $4.40 \text{ g} \pm 0.07 \text{ (SE)}$. Faba bean biomass decreased with FR enrichment with a slope of $-48.8 \text{ mg per } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 43.8 \text{ (SE)}$, and the mixture had 31.7% less biomass than pure faba bean per plant (Fig. S2), from $7.64 \text{ g} \pm 0.57 \text{ (SE)}$ to $11.19 \text{ g} \pm 0.19 \text{ (SE)}$.

For wheat, the leaf mass fraction (LMF) declined slightly by increase of FR enrichment (slope = 0.0006), while on average of the light treatments the LMF was 5.6% greater in the mixed than in the pure wheat, from $0.342 \text{ g g}^{-1} \pm 0.003 \text{ (SE)}$ to $0.324 \text{ g g}^{-1} \pm 0.005 \text{ (SE)}$. Stem mass fractions (SMF), reproductive organ mass fraction (ROMF) and root mass fraction (RMF) were not significantly affected by enrichment, while mixed wheat is 3.9% greater than pure wheat in ROMF from $0.211 \text{ g g}^{-1} \pm 0.004 \text{ (SE)}$ to $0.203 \text{ g g}^{-1} \pm 0.003 \text{ (SE)}$, and 17.7% smaller in RMF (Fig. 3), from $0.121 \text{ g g}^{-1} \pm 0.002 \text{ (SE)}$ to $0.147 \text{ g g}^{-1} \pm 0.005 \text{ (SE)}$.

For faba bean, LMF and ROMF were 9.8% and 23.8% lower in the mixed faba bean than the pure faba bean, from $0.327 \text{ g g}^{-1} \pm 0.004 \text{ (SE)}$ to

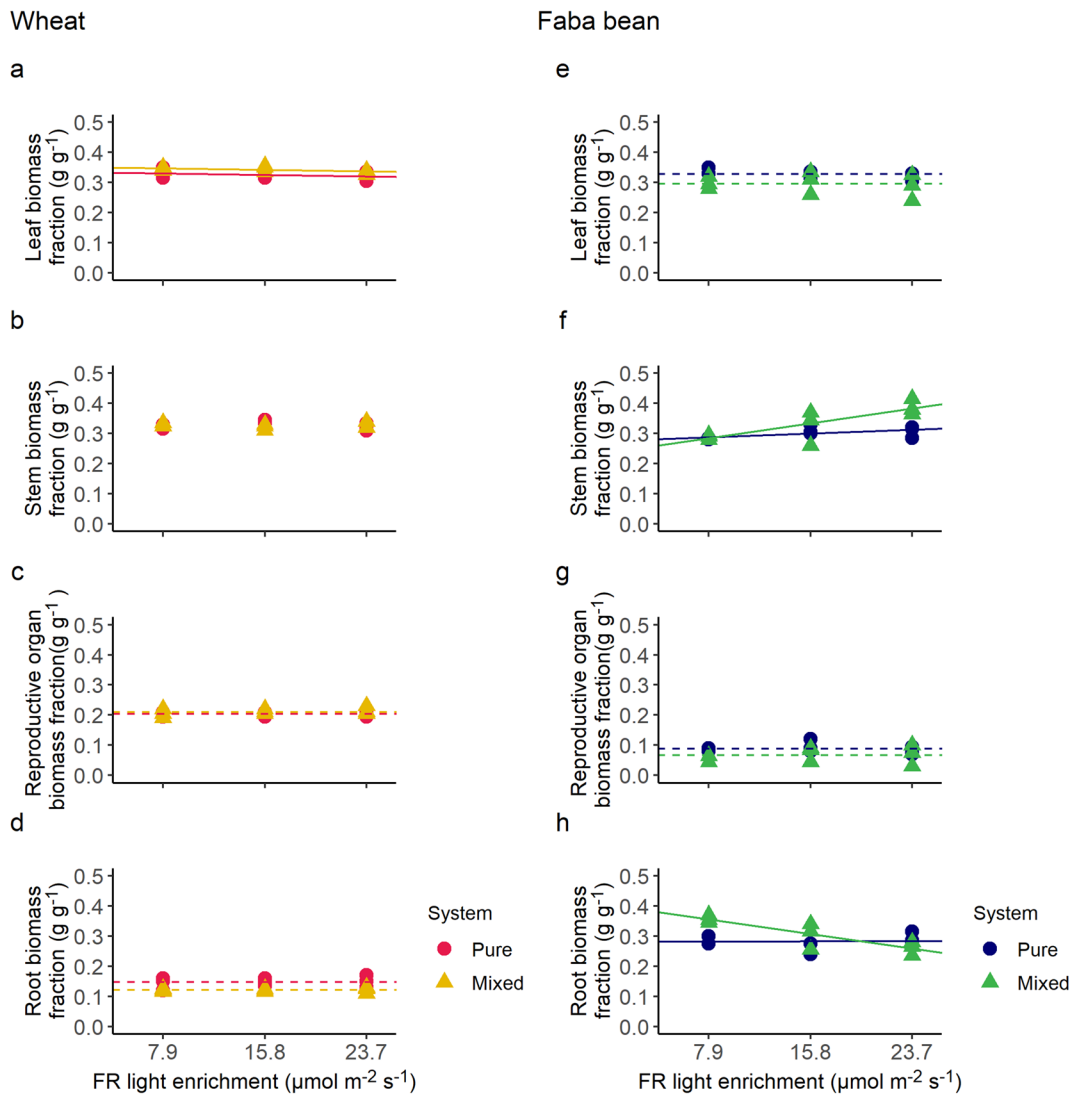


Fig. 3 Dose response curve of the wheat (left panel) and faba bean (right panel) dry biomass fraction of leaf (**a**, **e**), stem (**b**, **f**), reproductive organs (**c**, **g**), and root (**d**, **h**) to different levels of FR enrichment ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Lines show the predicted

biomass fraction in different FR enrichment base on the outcome of best fitted model. Dashed lines indicate FR enrichment effect was not significant

$0.295 \text{ g g}^{-1} \pm 0.010 \text{ (SE)}$ and from $0.088 \text{ g g}^{-1} \pm 0.005 \text{ (SE)}$ to $0.067 \text{ g g}^{-1} \pm 0.008 \text{ (SE)}$, respectively. However, the SMF increased with amount of FR enrichment, and this relationship was steeper in the mixed (slope= 0.0061) than in the pure faba bean (slope= 0.0015) as indicated by the significant FR by species-combination interaction. On the other hand, FR enrichment decreased the RMF of mixed faba bean (slope= -0.0061), while in the pure faba bean the influence was smaller (slope= 0.0001) (Fig. 3).

Faba bean ($0.295 \text{ g g}^{-1} \pm 0.009 \text{ SE}$) had a greater RMF than wheat ($0.134 \text{ g g}^{-1} \pm 0.006 \text{ SE}$).

Adventitious roots were the most responsive root class to FR enrichment

The root morphological traits were also investigated. Roots located in the topsoil layer (0–12 cm of soil depth) were analyzed based on root class (adventitious roots and seminal roots for wheat,

and adventitious roots, epicotyl roots and lateral roots emerging from the tap root (LR-tap) for faba bean; Fig. 2). While the number of wheat adventitious roots was not affected by FR enrichment nor cropping system, interaction between those factors was found on seminal root number (Table 2). The number of adventitious roots per tiller in wheat increased with the intensity of FR enrichment for both pure and mixed wheat (Table 2). The biomass of wheat adventitious roots increased with FR enrichment. Seminal root biomass in the mixed wheat were 31.19% lower compared

to pure wheat, on average of light treatment, from $56.9 \text{ mg} \pm 5.0$ (SE) to $82.7 \text{ mg} \pm 7.1$ (SE) (Table 2). For faba bean, there was no significant difference between the number of adventitious roots, epicotyl and lateral roots emerging from the taproot (LR-tap) under different FR enrichment levels and species combinations (Table 2). There was however a significant FR enrichment by species-combination interaction effect on adventitious root biomass, and adventitious root biomass declined more strongly with FR enrichment in the mixed than in pure faba bean (Table 2).

Table 2 Number and biomass of wheat adventitious roots, seminal roots; faba bean adventitious roots, faba bean epicotyl roots and lateral roots from the taproot. Values are

Mean $\pm$ SE, $n=3$. Asterisks denote significant probability level: *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, — = not significant, n.a. = not applicable

FR enrichment ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	Wheat adventitious root number per plant	Wheat seminal root number per plant	Wheat adventitious root number per tiller	Wheat adventitious root mass per plant (mg)	Wheat seminal root mass per plant (mg)	
Pure wheat						
7.9	19.1 ± 1.1	13.8 ± 0.5	4.9 ± 0.2	137.3 ± 17.7	93.7 ± 10.5	
15.8	20.5 ± 0.4	12.3 ± 0.9	5.8 ± 0.4	201.9 ± 33.9	72.9 ± 4.3	
23.7	20.3 ± 0.6	10.4 ± 1.2	6.2 ± 0.4	210.1 ± 29.8	81.5 ± 19.0	
Mixed wheat						
7.9	21.7 ± 1.2	8.9 ± 0.7	5.2 ± 0.3	197.0 ± 10.0	44.1 ± 2.5	
15.8	21.4 ± 1.6	11.9 ± 2.4	6.1 ± 0.1	143.1 ± 3.3	68.5 ± 7.7	
23.7	20.3 ± 2.0	11.0 ± 0.8	6.3 ± 0.4	223.1 ± 14.8	58.2 ± 8.8	
Interaction	-	*	-	-	-	
System	-	n.a	-	-	*	
Light	-	n.a	*	*	-	
FR enrichment ($\mu\text{mol m}^{-2} \text{ s}^{-1}$)	Faba bean adventitious root number per plant	Faba bean epicotyl root number per plant	Faba bean lateral root from tap root number per plant	Faba bean adventitious root mass per plant (mg)	Faba bean epicotyl root mass per plant (mg)	Faba bean lateral root from tap root mass per plant (mg)
Pure faba bean						
7.9	35.8 ± 2.6	19.7 ± 1.6	25.5 ± 1.8	467.1 ± 87.4	421.1 ± 48.4	407.5 ± 74.8
15.8	31.2 ± 7.2	18.3 ± 1.7	30.7 ± 4.6	256.3 ± 25.7	375.5 ± 35.4	399.0 ± 101.8
23.7	37.5 ± 11.4	18.3 ± 2.3	28.3 ± 1.9	388.2 ± 20.9	326.4 ± 33.8	437.8 ± 145.5
Mixed faba bean						
7.9	26.8 ± 3.1	17.5 ± 3.1	26.7 ± 0.6	665.5 ± 150.4	545.8 ± 246.5	457.8 ± 55.5
15.8	24.7 ± 2.9	20.3 ± 3.1	27.8 ± 2.6	332.5 ± 58.7	438.3 ± 224.0	489.8 ± 57.1
23.7	34.0 ± 8.0	14.5 ± 1.3	29.8 ± 2.6	414.2 ± 38.6	210.0 ± 20.4	331.0 ± 122.1
Interaction	-	-	-	*	-	-
System	-	-	-	n.a	-	-
Light	-	-	-	n.a	-	-

Faba bean in mixed system had higher SRA with FR enrichment

To assess root morphological traits of wheat and faba bean, RLD (cm cm^{-3}), SRL (cm mg^{-1}) and SRA ($\text{cm}^2 \text{mg}^{-1}$) were analyzed. No evidence was found for the relationship between FR enrichment and RLD for either crop (Fig. 4). Wheat roots had a greater RLD in mixed wheat ($11.8 \text{ cm cm}^{-3} \pm 0.6 \text{ SE}$) compared to the pure stand ($9.2 \text{ cm cm}^{-3} \pm 0.3$

SE), while a contrary result was found in faba bean with RLD reduced in the mixed faba bean ($2.3 \text{ cm cm}^{-3} \pm 0.2 \text{ SE}$) compared to the mixture ($3.0 \text{ cm cm}^{-3} \pm 0.1 \text{ SE}$). Our results for wheat SRL and SRA showed a similar pattern, with wheat having greater values in the mixed than in the pure stand, and there being no influence of FR enrichment (Fig. 4). Faba bean SRL and SRA was increased with FR enrichment in the mixed stands but decreased in pure faba bean stands.

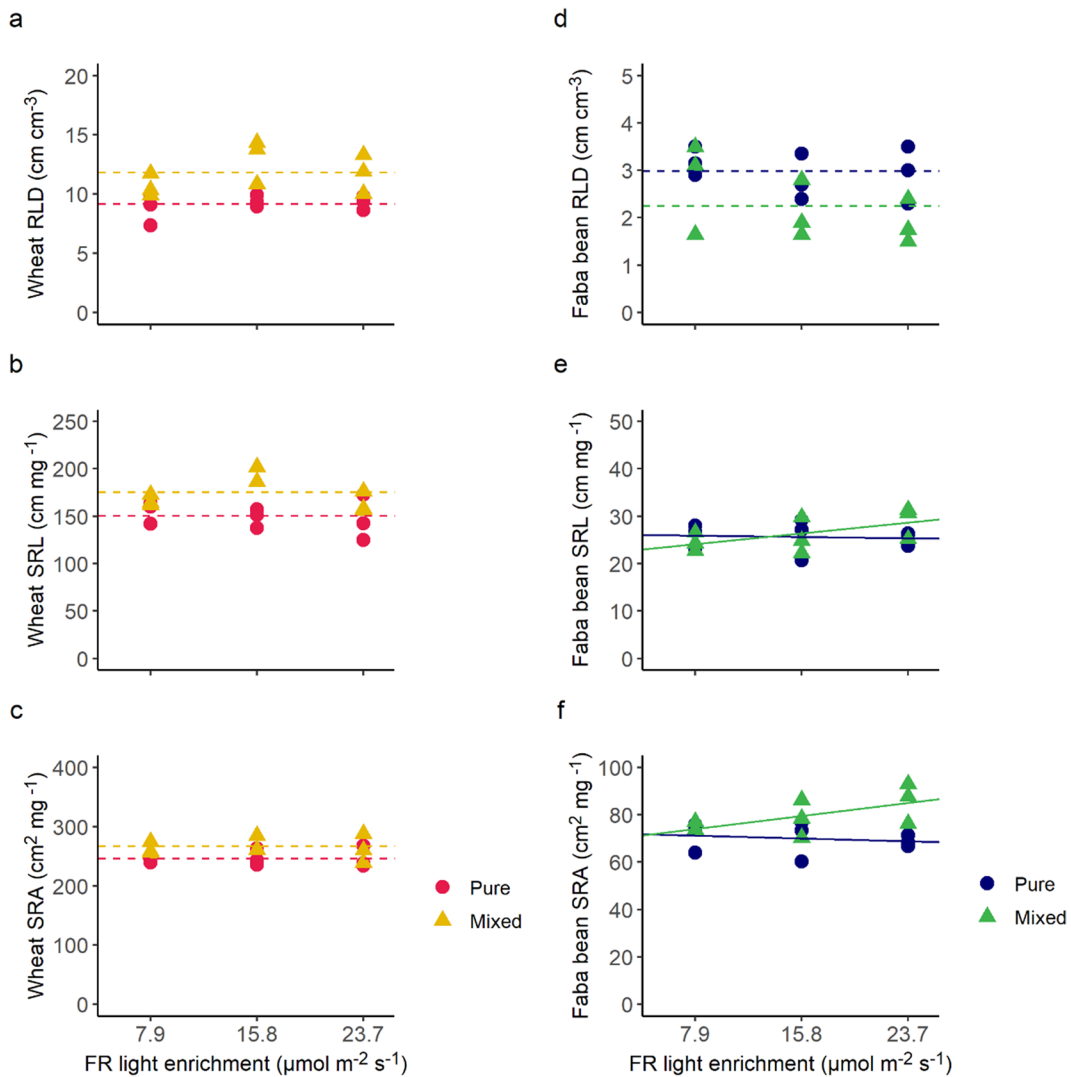


Fig. 4 Root length density (RLD), specific root length (SRL) and specific root area (SRA) of wheat (left panel, a–c) and faba bean (right panel, d–f) under different FR enrichment (μmol

$\text{m}^{-2} \text{s}^{-1}$). Lines show the predicted biomass in different far-red light intensity base on the outcome of best fitted model

Root vertical biomass allocation (D75) showed contrasting responses to the FR enrichment

D75 is a metric indicating vertical root distribution and is defined as the depth at which 75% of the total root biomass is allocated at or above (Fig. 5). For wheat, FR enrichment (low R/FR), restrained the rooting depth while no difference was found between pure and mixed wheat. On the contrary, FR enrichment enhanced the rooting depth of faba bean with it being much (54.7%) lower in mixed faba bean (on average of all light treatments, $32.2 \text{ cm} \pm 1.6 \text{ SE}$) compared to pure faba bean ($17.9 \text{ cm} \pm 1.3 \text{ SE}$, $p < 0.001$).

Discussion

Our results showed a pattern whereby root responses to low R/FR (FR enrichment) of light incident on the shoot differed between faba bean and wheat were context (species $\times$ cropping system) dependent (Fig. 6). The responses of faba bean to low R/FR depended on whether faba bean grew in pure stands or in mixture with wheat. In both species adventitious roots were the most plastic root class, in terms of their response to both low R/FR on the shoot and the presence of hetero-specific neighbors. Faba bean responded to low R/FR light by producing less root

biomass, but this response was much stronger in the mixture than in the pure stand. This could mostly be attributed to similar responses in adventitious root growth. For wheat both low R/FR and growth in mixture resulted in increased root growth and these effects were additive. In addition, wheat produced more adventitious root biomass and a larger number of adventitious roots per tiller when tiller number per plant was constrained by low R/FR. Low R/FR counteracted the niche differentiation in root depth in the wheat/faba bean mixtures, reducing the D75 in wheat and increasing it in faba bean.

Growing with neighbors: resource competition and processing multiple signals

Low R/FR decreased the biomass allocation to faba bean roots, and this effect was stronger in the mixture (Fig. 3). In the mixed faba bean plants, a higher fraction of biomass was allocated to the stem under low R/FR conditions, at the expense of a reduced biomass fraction allocated to the roots (Fig. 3 and S3). This could be attributed to the combined effects of reduced R/FR ratio and potentially other aboveground signals from the neighboring wheat plants, which stimulated a greater proportion of biomass allocation towards the stem. This result is in line with a study with oilseed rape, like faba bean a dicot, where shoot/root ratio was increased with additional FR light (Fortineau

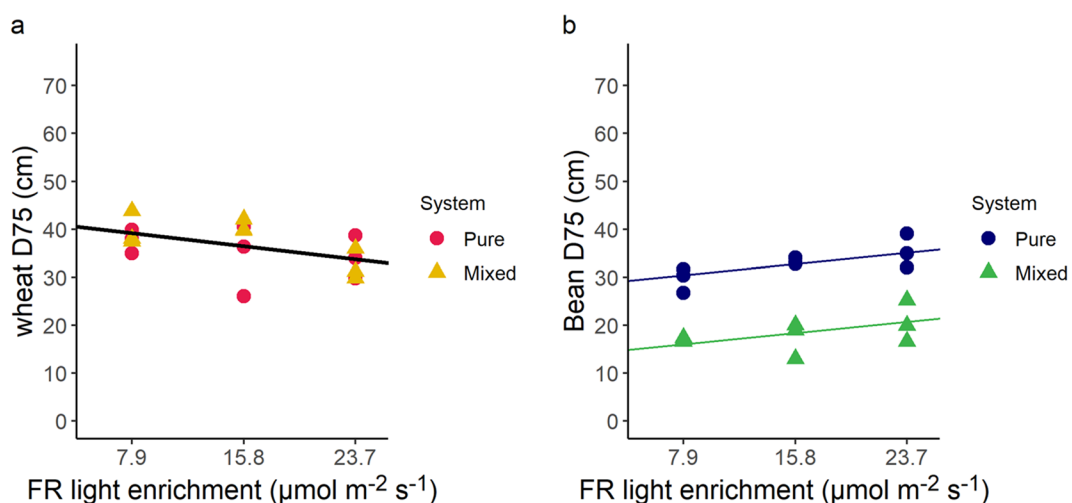


Fig. 5 D75 value of wheat (a) and faba bean (b) under different FR enrichment ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in pure and mixed systems. Lines show the predicted biomass in different FR enrichment base on the outcome of best fitted model

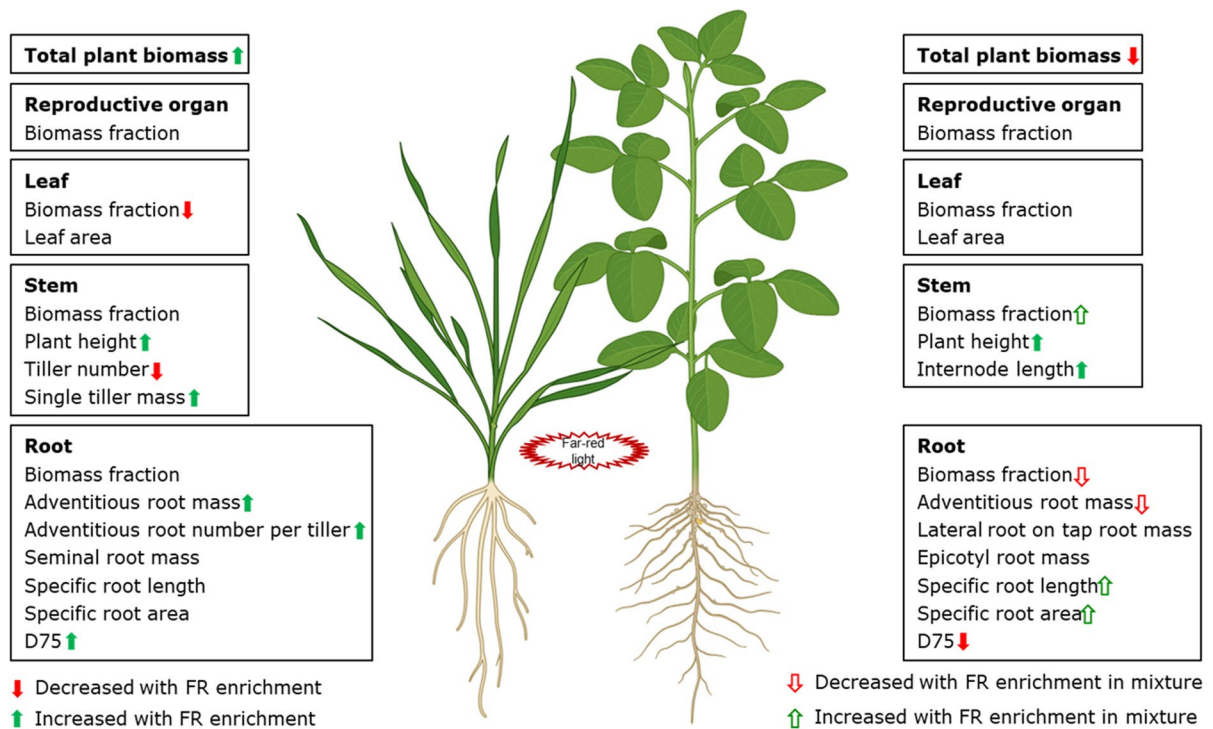


Fig. 6 Model diagram showing the effect of supplemented far-red light in a crop mixture on plant traits of wheat (left) and faba bean (right). Solid arrows indicate an increase (up, green) or decrease (down, red) in a trait value under the far-red light

enrichment. Open arrows indicate the interaction of light treatment and crop system (pure vs mixed) and only the result for the mixed system is presented. Traits without arrows means no response. Created with BioRender.com

et al. 2021). Additionally, the relatively rapid root growth rate of wheat might have resulted in nutrient limitation for faba bean. In species mixtures, wheat is more competitive belowground and typically causes nutrient and water limitation for the less competitive faba bean (Qiao et al. 2015). Specifically, the adventitious root biomass in wheat was enhanced in low R/FR, which strengthened the competitiveness of wheat roots, especially in the topsoil layer.

Supplemental FR can also increase photosynthesis as demonstrated e.g. for soybean and rice (Yang et al. 2020; Huber et al. 2024) which could have affected overall plant size and hence competitiveness. Indeed, in wheat plant biomass was somewhat increased by supplemental FR (Fig. S2). This effect however was small while in faba bean FR enrichment had the opposite effect. Besides the size of root system, root morphological traits also plastically respond to competitiveness of the neighbors (Fig. 4). Wheat roots showed a plastic response to the presence of faba bean while not to low R/FR, by having higher RLD, SRL

and SRA values. This response of mixed wheat was consistent with the previous root studies on wheat/faba bean (Li et al. 2006; Streit et al. 2019). The RLD was lower in the mixed faba bean compared to the pure faba bean, which could be attributed to the suppression from neighboring wheat. While the influence of the low R/FR on pure-faba bean SRL and SRA was negative, it increased these traits in mixed faba bean. Higher SRL and SRA indicate that plants produce more length and surface area for the same amount of carbon invested, which is a known response to interspecific competition (Ravenek et al. 2016). Given the lower biomass allocation to roots in mixed faba bean, plants produced thinner roots to form more surface area for water and nutrient uptake.

For faba bean there was an interactive effect of R/FR reduction and the presence of wheat on SMF, RMF, adventitious root mass per plant, SRL and SRA. This suggests that faba bean is capable of integrating these two signals. This is different from a study on soybean suggesting that plant responses

to the belowground con-species neighbor competition cues occurred independently from responses to the aboveground low R/FR competition cues (Murphy and Dudley 2007). In addition, a common-garden experiment with light competition simulated by a transparent green filter showed that roots of *P. oleracea* would grow towards the green filter which reduced the R/FR, regardless of the location of the nutrient patch (Gottlieb and Gruntman 2022). It is therefore suggested that for faba bean, other than depletion of resources, the presence of neighboring wheat might produce other forms of signals such as root exudates (Wang et al. 2021), volatile organic compounds (Kegge and Pierik 2010) and common mycorrhizal networks (Walder et al. 2012). These additional signals may have contributed to the observed signaling interaction between faba bean and wheat. Furthermore, genetic variation in phenotypic plasticity within one species may also play a role in the response of the root system in crop mixtures (Streit et al. 2019). Therefore, further investigation is needed to explore i) the extent signals other than changes in resources availability play a role in the wheat-faba bean or possibly interspecific interactions, ii) to which extent these are integrated physiologically with the response to low R/FR, and iii) if the root responses in crop mixtures is genotype-specific and interacts with the hetero-specific neighbor.

Adventitious roots are the most sensitive roots to low R/FR and their responses vary between species

Adventitious roots play a major role in the nutrient uptake capacity and stability of plants (Schneider et al. 2020) and in the adaptation of plants to various environments (e.g., flooding and drought) (Steffens and Rasmussen 2016; Gonin et al. 2019). Our results indicate that exposure to low R/FR predominantly affects the adventitious roots (shoot-borne) compared to other root classes, and that these effects differ between species. Several studies on ornamental plants have revealed that supplementing R light with FR light (high R/FR) stimulates adventitious rooting in plants (Christiaens et al. 2016). A study on *Chrysanthemum morifolium* cuttings showed that a low R/FR increased the number, biomass, and length of adventitious roots, and this was most likely associated with either a higher auxin biosynthesis or improved auxin transport (Christiaens et al. 2019). In contrast,

for woody plants, R light (high R/FR) was shown to promote adventitious root initiation by decreasing the accumulation of the phytohormones jasmonate and cytokinins in Norway spruce (*Picea abies* Karst.) seedlings (Alallaq et al. 2020) and by influencing the auxin biosynthesis transport and signaling pathway in *Eucalyptus globulus* (Ruedell et al. 2015). In short, the effect of low R/FR on the development and growth of adventitious roots did not show a clear response across species and cropping systems, and it might be species or growth form (trees vs herbaceous plants) dependent. In our case of wheat, low R/FR increased the biomass of adventitious roots, which is consistent with the ornamental plants (Table 1). However, the fact that in faba bean, low R/FR decreased adventitious root mass (though not number), seems to be in line with the findings for woody species.

For wheat, the adventitious root number per tiller and mass per plant was increased by low R/FR, even though the total number of adventitious roots per plant did not show difference in any FR light intensity in either cropping system (Table 1). Tiller number in wheat decreases under low R/FR (Kasperbauer and Karlen 1986; Evers et al. 2006), which is confirmed in the present study (Fig. S4). The reduction in tiller number could result in less light competition between tillers, leading to greater biomass assimilation per tiller (Fig. S4). Therefore, to support the ensuing increase in water and nutrient demands per tiller, each tiller may have produced more adventitious roots. This plastic response in adventitious roots contributed to the absence of a significant effect of FR enrichment on root biomass fraction (Fig. 3). We showed that low R/FR promotes the initiation of adventitious roots per tiller; however, the whole plant responded to light conditions by forming fewer tillers in wheat.

The timing and type of cues may affect root plasticity. In cereals, seed-borne seminal roots are relevant only for the early seedling stage, and thereafter Adventitious roots (shoot-borne) roots take a major role in root function (Hochholdinger and Zimmermann 2008). Adventitious roots, which are mainly regulated by auxin, represent a major part of the root system (Hochholdinger et al. 2004; Cho et al. 2014) and play a dominant role in root functioning (Hochholdinger and Zimmermann 2008). In dicots, adventitious-root formation follows a similar initiation pattern as lateral-root formation and mainly relies on gene networks regulated by hormones like auxin,

jasmonic acid and strigolactones (Atkinson et al. 2014). High plant density decreased the tiller number and nodal root (shoot-borne) number per barley plant, while nodal roots number per tiller was not affected (Hecht et al. 2019). Nutrient deficiency can also stimulate the growth of maize roots from different classes. For example, a larger number of crown roots (shoot-borne) in maize improves phosphorus acquisition under low phosphorus availability (Sun et al. 2018). Increased seminal root (seed-borne) number in maize also improves nitrogen and phosphorus uptake (Perkins and Lynch 2021). These different root responses to changes in environmental conditions implies that it is essential to understand what and how environmental cues trigger the formation of different root classes in future studies, as it might have as it may have substantial effects on plant growth and stress tolerance.

FR enrichment counteracts interspecific complementary

The low R/FR mainly influenced the adventitious root biomass, which had an indirect effect on the vertical root allocation of wheat and faba bean. Legume crops in general tend to have lower D75 than cereal crops for several reasons: (i) the taproot system of legume leads to a conical shape of the root system, which tends to result in lower D75 values, whereas monocots have cylindrical shapes of root system that tend to make D75 higher; and (ii) legumes tend to place their roots in shallow soil layers to allow nitrogen-fixing rhizobia, whom they host, to efficiently exchange gases with the atmosphere (Fan et al. 2016; Herben et al. 2022). The current study confirms this, with wheat's D75 on average more than 10 cm deeper than that of faba bean (Fig. 5). Wheat D75 did not show active response to the presence of the faba bean neighbor, while for faba bean, a reduced D75 in the mixture suggests that faba bean root development in deeper soil layers might be restrained by the presence of wheat roots and therefore allocate a greater amount of biomass in the topsoil. Together, this provides evidence for the role of plasticity in the size and placement of the root system in response to the presence of another species contributing to improved spatial complementary for soil foraging. However, supplementary FR enrichment mitigated the differences in rooting depth between the two species. Under the low R/FR, wheat tended to have a shallower root system due

to more adventitious root development, while faba bean developed a smaller but deeper root system, as adventitious root growth was constrained (Table 2). The observed interspecific response appears to have resulted in heightened competition among plants, which is undesirable in intercropping systems. Therefore, it is crucial to understand if R/FR signal in intercrops possesses sufficient strength to trigger this root response. Such knowledge would extend our findings from this greenhouse study and contribute to our understanding of how root plasticity in response to above- and belowground cues modify niche differentiation under field conditions.

Limitations, implications for mixed cropping studies and future research

This study investigated if low R/FR would influence the root development during early plant growth, before mutual shading between plants takes place. Therefore, the setup of this experiment, employing the additional FR light to pots with an open canopy, disentangled the effect of low R/FR and low PAR in the dense canopy in the field conditions. Fortineau et al. (2021) discussed the technical limitation of locally applying an FR signal that is sufficiently strong to modify the R/FR, in a heterogeneous and fluctuating outdoor light environment. Compared to the control conditions, the current study also had varied R/FR depending on weather condition, time of the day/year, etc. (Fig. S1). Despite all these limitations, we believe the different dosage of FR light did influence the development of wheat and faba bean, as evidenced by decreasing wheat tiller number, increasing wheat plant height and increasing faba bean internode length (Fig. S4 and S5). These are all typical responses of the shade avoidance syndrome (Demotes-Mainard et al. 2016; Ballaré and Pierik 2017).

Our findings have significant implications for making informed decisions regarding intercropped species choices and optimizing their configuration. The combination of cereal and legume provided spatial niche differentiation due to the intrinsic differences in root system architecture (Herben et al. 2022). If the low R/FR counteracts this differentiation, then it could be considered to use a cultivar with lower plasticity on root growth in response to R/FR. Our results further suggest that it is better to plant faba

bean before wheat, given that wheat does not seem to respond as strongly to faba bean as vice versa. In reality, R/FR in the canopy is more dynamic temporally and spatially, and normally decreases as plants develop (Yang et al. 2014). This could potentially influence the adventitious roots more as they develop later in plant development. Additionally, determining the optimum R/FR patterns through strategic choices in intercrop design and species population densities, can enhance the overall performance of intercropped systems. Planting density plays a major role in the ratio of R/FR in the plant canopy (Ballaré and Pierik 2017). Designing the plant density of both species in the sense of providing the optimal R/FR may benefit not only the light capture (Bongers et al. 2018) and photosynthesis capacity (Yang et al. 2020; Huber et al. 2024) in aboveground, but also uptake of belowground resources. Functional-structural plant modelling (Evers et al. 2019) can be used to explore R/FR in intercrops for optimal complementarity, by exploring the role root plasticity, relative sowing time, land proportion of species, and plant density. Furthermore, understanding the role of light signaling in relay intercropping systems, particularly its effects on adventitious root development and belowground resource uptake, needs further attention. Future research should explore these avenues to unravel the mechanisms behind the observed effects and facilitate the development of sustainable and efficient crop mixtures. Investigating the mechanisms underlying shoot–root signaling, including hormone-mediated processes such as auxin regulation, in response to R/FR perception in the shoot represents an important direction for future research.

Conclusion

This study is, to our knowledge, the first to explore the effect of aboveground light signals (in this case variation in R/FR) in a mixed-species system on the development of roots. In the combination of cereal/legume mixed cropping, crop species have unique strategies to respond to the environment that is altered by neighboring species. This plastic response is crucial to the complementarity effect as it affects the extent to which species can exploit different spatial niches. The current study quantified the extent of the root response to R/FR

signals in mixed-species conditions. Specifically, we found that adventitious roots are the most plastic, responding mainly to either the system or low R/FR (Fig. 2 and 6). In faba bean, changes in biomass allocation to root and root vertical distribution under low R/FR are driven by a decrease in adventitious root growth. Wheat adapts its number of adventitious roots per tiller to the low R/FR and produces more adventitious root biomass. Additional FR counteracts the original spatial niche differentiation, reducing the D75 (i.e. depth of 75% of root biomass) in wheat and increasing it in faba bean (Fig. 6). It is therefore suggested that this effect on the root system should be considered when designing the density and spatial arrangement of the cereal/legume mixed cropping. Further studies could investigate the role of different nutrient regimes on root responses to R/FR signals and examine the long-term effects of mixed cropping and FR enrichment on plant health and productivity, such as yield.

Acknowledgements We would like to acknowledge Wopke van der Werf, Alejandro Morales Sierra, and Zohralyn Homulle for their consultation on statistical analysis. We are grateful to Peter van der Putten for supervising the experiment. We also extend our appreciation to our colleagues for their outstanding assistance in executing this study during the covid-19 pandemic. The comments from three anonymous reviewers are highly appreciated. Financial support by the China Scholarship Council (grant no. 201907720097) is acknowledged.

Author contributions Jin L. Wang, Jochem B. Evers, Niels P. R. Anten and Hannah M. Schneider conceived the ideas and designed methodology; Jin L. Wang, Yitong Li and Xiaoyi Yang collected the data; Jin L. Wang, Yitong Li, Xiaoyi Yang and Jacob C. Douma analyzed the data; Jin L. Wang led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Funding This work was supported by the China Scholarship Council (grant no. 201907720097).

Data availability Upon the acceptance of this manuscript, data that support the findings of this study is intended to be published in the data repository Zenodo. For any inquiries regarding data access or further information, please contact the corresponding author at jin.wang.cn@outlook.com.

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alallaq S, Ranjan A, Brunoni F et al (2020) Red light controls adventitious root regeneration by modulating hormone homeostasis in *Picea abies* seedlings. *Front Plant Sci* 11: <https://doi.org/10.3389/fpls.2020.586140>
- Atkinson JA, Rasmussen A, Traini R et al (2014) Branching out in roots: uncovering form, function, and regulation. *Plant Physiol* 166:538–550. <https://doi.org/10.1104/pp.114.245423>
- Ballaré CL, Pierik R (2017) The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant Cell Environ* 40:2530–2543. <https://doi.org/10.1111/PCE.12914>
- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bongers FJ, Pierik R, Anten NPR, Evers JB (2018) Subtle variation in shade avoidance responses may have profound consequences for plant competitiveness. *Ann Bot* 121:863–873. <https://doi.org/10.1093/aob/mcx151>
- Casal JJ (2012) Shade Avoidance. *The Arabidopsis Book* 10:e0157. <https://doi.org/10.1199/tab.0157>
- Cho S-H, Yoo S-C, Zhang H et al (2014) Rice NARROW LEAF1 regulates leaf and adventitious root development. *Plant Mol Biol Report* 32:270–281. <https://doi.org/10.1007/s11105-013-0675-z>
- Christiaens A, Gobin B, van Huylenbroeck J, van Labeke MC (2019) Adventitious rooting of *Chrysanthemum* is stimulated by a low red:far-red ratio. *J Plant Physiol* 236:117–123. <https://doi.org/10.1016/j.jplph.2019.03.008>
- Christiaens A, Gobin B, Van Labeke MC (2016) Light quality and adventitious rooting: a mini-review. *Acta Hort* 385–394. <https://doi.org/10.17660/ActaHortic.2016.1134.50>
- Demotes-Mainard S, Péron T, Corot A et al (2016) Plant responses to red and far-red lights, applications in horticulture. *Environ Exp Bot* 121:4–21. <https://doi.org/10.1016/j.envexpbot.2015.05.010>
- Didier C, Quentin R, Romain B et al (2018) Influence of neighboring plants on the variation of red to far-red ratio in intercropping system: Simulation of light quality. In: 2018 6th International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications (PMA). IEEE, pp 13–19
- Dong B, Werf W van der, Evers JB, Stomph TJ (2024) Resource capture, plant morphological traits and maize photosynthesis in intercropping. Wageningen University. <https://doi.org/10.18174/653694>
- Evers JB, Vos J, Andrieu B, Struik PC (2006) Cessation of tillering in spring wheat in relation to light interception and red: far-red ratio. *Ann Bot* 97:649–658. <https://doi.org/10.1093/aob/mcl020>
- Evers JB, Van Der Werf W, Stomph TJ et al (2019) Understanding and optimizing species mixtures using functional-structural plant modelling. *J Exp Bot* 70:2381–2388. <https://doi.org/10.1093/jxb/ery288>
- Fan J, McConkey B, Wang H, Janzen H (2016) Root distribution by depth for temperate agricultural crops. *Field Crops Res* 189:68–74. <https://doi.org/10.1016/j.fcr.2016.02.013>
- Fortineau A, Combes D, Richard-molard C et al (2021) Light-cue: An innovative far-red light emitter for locally modifying the spectral cue in outdoor conditions with global consequences on plant architecture. *Plants* 10:2483. <https://doi.org/10.3390/plants10112483>
- Fromm H (2019) Root Plasticity in the Pursuit of Water. *Plants* 8:236. <https://doi.org/10.3390/plants8070236>
- van Gelderen K, Kang C, Li P, Pierik R (2021) Regulation of Lateral Root Development by Shoot-Sensed Far-Red Light via HY5 Is Nitrate-Dependent and Involves the NRT2.1 Nitrate Transporter. *Front Plant Sci* 12: <https://doi.org/10.3389/fpls.2021.660870>
- Gonin M, Bergougnoux V, Nguyen TD et al (2019) What makes adventitious roots? *Plants* 8:240. <https://doi.org/10.3390/plants8070240>
- Gottlieb R, Gruntman M (2022) Can plants integrate information on above-ground competition in their directional responses below ground? *Ann Bot* 130:763–771. <https://doi.org/10.1093/aob/mcac106>
- Green-Tracewicz E, Page ER, Swanton CJ (2011) Shade avoidance in soybean reduces branching and increases plant-to-plant variability in biomass and yield per plant. *Weed Sci* 59:43–49. <https://doi.org/10.1614/WS-D-10-00081.1>
- Gruber BD, Giehl RFH, Friedel S, von Wirén N (2013) Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol* 163:161–179. <https://doi.org/10.1104/pp.113.218453>
- Gundel PE, Pierik R, Mommer L, Ballaré CL (2014) Competing neighbors: light perception and root function. *Oecologia* 1:1–10. <https://doi.org/10.1007/s00442-014-2983-x>
- Hamblin A, Tennant D (1987) Root length density and water uptake in cereals and grain legumes: how well are they correlated. *Aust J Agric Res* 38:513. <https://doi.org/10.1071/AR9870513>
- Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops - A field study employing 32p technique. *Plant Soil* 236:63–74. <https://doi.org/10.1023/A:1011909414400>
- Hecht VL, Temperton VM, Nagel KA et al (2019) Plant density modifies root system architecture in spring barley (*Hordeum vulgare* L.) through a change in nodal root number. *Plant Soil* 439:179–200. <https://doi.org/10.1007/s11104-018-3764-9>
- Herben T, Šásek J, Balšánková T et al (2022) The shape of root systems in a mountain meadow: plastic responses

- or species-specific architectural blueprints? *New Phytol* 235:2223–2236. <https://doi.org/10.1111/nph.18132>
- Hochholdinger F, Zimmermann R (2008) Conserved and diverse mechanisms in root development. *Curr Opin Plant Biol* 11:70–74. <https://doi.org/10.1016/j.pbi.2007.10.002>
- Hochholdinger F, Park WJ, Sauer M, Woll K (2004) From weeds to crops: genetic analysis of root development in cereals. *Trends Plant Sci* 9:42–48. <https://doi.org/10.1016/j.tplants.2003.11.003>
- Homulle Z, George TS, Karley AJ (2022) Root traits with team benefits: understanding belowground interactions in intercropping systems. *Plant Soil* 471:1–26. <https://doi.org/10.1007/s11104-021-05165-8>
- Huber M, de Boer HJ, Romanowski A, et al (2024) Far-red light enrichment affects gene expression and architecture as well as growth and photosynthesis in rice. *Plant Cell Environ* 1:. <https://doi.org/10.1111/pce.14909>
- Karlova R, Boer D, Hayes S, Testerink C (2021) Root plasticity under abiotic stress. *Plant Physiol* 187:1057–1070. <https://doi.org/10.1093/plphys/kiab392>
- Kasperbauer MJ, Karlen DL (1986) Light-mediated bioregulation of tillering and photosynthate partitioning in wheat. *Physiol Plant* 66:159–163. <https://doi.org/10.1111/J.1399-3054.1986.TB01250.X>
- Keating BA, Carberry PS (1993) Resource capture and use in intercropping: solar radiation. *Field Crops Res* 34:273–301. [https://doi.org/10.1016/0378-4290\(93\)90118-7](https://doi.org/10.1016/0378-4290(93)90118-7)
- Kegge W, Pierik R (2010) Biogenic volatile organic compounds and plant competition. *Trends Plant Sci* 15:126–132. <https://doi.org/10.1016/j.tplants.2009.11.007>
- Kelty MJ (1992) Comparative productivity of monocultures and mixed-species stands. Springer, Dordrecht, pp 125–141
- Keuskamp DH, Sasidharan R, Vos I et al (2011) Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in Arabidopsis seedlings. *Plant J* 67:208–217. <https://doi.org/10.1111/j.1365-313X.2011.04597.x>
- Ko D, Helariutta Y (2017) Shoot–root communication in flowering plants. *Curr Biol* 27:R973–R978. <https://doi.org/10.1016/j.cub.2017.06.054>
- Li L, Sun J, Zhang F et al (2006) Root distribution and interactions between intercropped species. *Oecologia* 147:280–290. <https://doi.org/10.1007/s00442-005-0256-4>
- Li C, Dong Y, Li H et al (2016) Shift from complementarity to facilitation on P uptake by intercropped wheat neighboring with faba bean when available soil P is depleted. *Sci Rep* 6:18663. <https://doi.org/10.1038/srep18663>
- Li C, Hoffland E, Kuyper TW et al (2020) Yield gain, complementarity and competitive dominance in intercropping in China: A meta-analysis of drivers of yield gain using additive partitioning. *Eur J Agron* 113:125987. <https://doi.org/10.1016/j.eja.2019.125987>
- Li S, van der Werf W, Zhu J et al (2021) Estimating the contribution of plant traits to light partitioning in simultaneous maize/soybean intercropping. *J Exp Bot* 72:3630–3646. <https://doi.org/10.1093/jxb/erab077>
- Li C, Stomph T-J, Makowski D et al (2023) The productive performance of intercropping. *Proc Natl Acad Sci* 120:e2201886120. <https://doi.org/10.1073/pnas.2201886120>
- Ma L, Upadhyaya MK (2016) Effects of red/far-red light ratio on common lamb's-quarters, redroot pigweed, and tomato plants. *Can J Plant Sci* 97:494–500. <https://doi.org/10.1139/CJPS-2016-0089>
- Malezieux E, Crozat Y, Dupraz C et al (2009) Mixing plant species in cropping systems: concepts, tools and models. A Review. *Agron Sustain Dev* 29:43–62. <https://doi.org/10.1051/agro:2007057>
- McKane RB, Johnson LC, Shaver GR et al (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature* 415:68–71. <https://doi.org/10.1038/415068a>
- Morris RA, Garrity DP (1993) Resource capture and utilization in intercropping: non-nitrogen nutrients. *Field Crops Res* 34:319–334. [https://doi.org/10.1016/0378-4290\(93\)90120-C](https://doi.org/10.1016/0378-4290(93)90120-C)
- Murphy GP, Dudley SA (2007) Above- and below-ground competition cues elicit independent responses. *J Ecol* 95:261–272. <https://doi.org/10.1111/J.1365-2745.2007.01217.X>
- Ofori F, Stern WR (1987) Cereal–Legume Intercropping Systems. *Adv Agron* 41:41–90. [https://doi.org/10.1016/S0065-2113\(08\)60802-0](https://doi.org/10.1016/S0065-2113(08)60802-0)
- Perkins AC, Lynch JP (2021) Increased seminal root number associated with domestication improves nitrogen and phosphorus acquisition in maize seedlings. *Ann Bot* 128:453–468. <https://doi.org/10.1093/AOB/MCAB074>
- Pierik R, de Wit M (2014) Shade avoidance: phytochrome signalling and other aboveground neighbour detection cues. *J Exp Bot* 65:2815–2824. <https://doi.org/10.1093/jxb/ert389>
- Pierik R, Mommer L, Voesenek LA (2013) Molecular mechanisms of plant competition: Neighbour detection and response strategies. *Funct Ecol* 27:841–853. <https://doi.org/10.1111/1365-2435.12010>
- van der Plas F (2019) Biodiversity and ecosystem functioning in naturally assembled communities. *Biol Rev* brv.12499. <https://doi.org/10.1111/brv.12499>
- Qiao X, Bei S, Li C et al (2015) Enhancement of faba bean competitive ability by arbuscular mycorrhizal fungi is highly correlated with dynamic nutrient acquisition by competing wheat. *Sci Rep* 5:8122. <https://doi.org/10.1038/srep08122>
- R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Ravenek JM, Mommer L, Visser EJW et al (2016) Linking root traits and competitive success in grassland species. *Plant Soil* 407:39–53. <https://doi.org/10.1007/s11104-016-2843-z>
- Ruedell CM, de Almeida MR, Fett-Neto AG (2015) Concerted transcription of auxin and carbohydrate homeostasis-related genes underlies improved adventitious rooting of microcuttings derived from far-red treated Eucalyptus globulus Labill mother plants. *Plant Physiol Biochem* 97:11–19. <https://doi.org/10.1016/j.plaphy.2015.09.005>
- Schenk HJ, Jackson RB (2002) The global biogeography of roots. *Ecol Monogr* 72:311–328. [https://doi.org/10.1890/0012-9615\(2002\)072\[0311:TGBORJ\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0311:TGBORJ]2.0.CO;2)
- Schneider HM, Postma JA, Kochs J et al (2020) Spatio-temporal variation in water uptake in seminal and nodal root systems of barley plants grown in soil. *Front Plant Sci* 11:. <https://doi.org/10.3389/fpls.2020.01247>

- Schneider HM, Lynch JP (2020) Should root plasticity be a crop breeding target? *Front Plant Sci* 11:1–16. <https://doi.org/10.3389/fpls.2020.00546>
- Seethepalli A, Dhakal K, Griffiths M, Guo H, Freschet GT, York LM (2021) Rhizovision explorer: opensource software for root image analysis and measurement standardization. *AoB plants* 13(6):plab056. <https://doi.org/10.1093/aobpla/plab056>
- Shabala S, White RG, Djordjevic MA et al (2016) Root-to-shoot signalling: integration of diverse molecules, pathways and functions. *Funct Plant Biol* 43:87. <https://doi.org/10.1071/FP15252>
- Silvertown J (2004) Plant coexistence and the niche. *Trends Ecol Evol* 19:605–611. <https://doi.org/10.1016/j.tree.2004.09.003>
- Steffens B, Rasmussen A (2016) The physiology of adventitious roots. *Plant Physiol* 170:603–617. <https://doi.org/10.1104/pp.15.01360>
- Streit J, Meinen C, Rauber R (2019) Intercropping effects on root distribution of eight novel winter faba bean genotypes mixed with winter wheat. *Field Crops Res* 235:1–10. <https://doi.org/10.1016/j.fcr.2019.02.014>
- Sun B, Gao Y, Lynch JP (2018) Large crown root number improves topsoil foraging and phosphorus acquisition. *Plant Physiol* 177:90–104. <https://doi.org/10.1104/pp.18.00234>
- Trachsel S, Kaeppler SM, Brown KM, Lynch JP (2013) Maize root growth angles become steeper under low N conditions. *Field Crops Res* 140:18–31. <https://doi.org/10.1016/j.fcr.2012.09.010>
- Turnbull LA, Isbell F, Purves DW et al (2016) Understanding the value of plant diversity for ecosystem functioning through niche theory. *Proc R Soc B: Biol Sci* 283:20160536. <https://doi.org/10.1098/rspb.2016.0536>
- Walder F, Niemann H, Natarajan M et al (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. *Plant Physiol* 159:789–797. <https://doi.org/10.1104/pp.112.195727>
- Wang Z, Zhao X, Wu P et al (2017) Border row effects on light interception in wheat/maize strip intercropping systems. *Field Crops Res* 214:1–13. <https://doi.org/10.1016/j.fcr.2017.08.017>
- Wang N, Kong C, Wang P, Meiners SJ (2021) Root exudate signals in plant–plant interactions. *Plant Cell Environ* 44:1044–1058. <https://doi.org/10.1111/pce.13892>
- Xia HY, Zhao JH, Sun JH et al (2013) Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. *Field Crops Res* 150:52–62. <https://doi.org/10.1016/j.fcr.2013.05.027>
- Yang F, Huang S, Gao R et al (2014) Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio. *Field Crops Res* 155:245–253. <https://doi.org/10.1016/j.fcr.2013.08.011>
- Yang F, Liu Q, Cheng Y et al (2020) Low red/far-red ratio as a signal promotes carbon assimilation of soybean seedlings by increasing the photosynthetic capacity. *BMC Plant Biol* 20:148. <https://doi.org/10.1186/s12870-020-02352-0>
- Yin W, Chai Q, Zhao C et al (2020) Water utilization in intercropping: A review. *Agric Water Manag* 241:106335. <https://doi.org/10.1016/j.agwat.2020.106335>
- Yu RP, Yang H, Xing Y et al (2022) Belowground processes and sustainability in agroecosystems with intercropping. *Plant Soil* 476:263–288. <https://doi.org/10.1007/s11104-022-05487-1>
- Zhang Y, Sun Z, Su Z et al (2022) Root plasticity and interspecific complementarity improve yields and water use efficiency of maize/soybean intercropping in a water-limited condition. *Field Crops Res* 282:108523. <https://doi.org/10.1016/j.fcr.2022.108523>
- Zhu J, van der Werf W, Anten NPR et al (2015) The contribution of phenotypic plasticity to complementary light capture in plant mixtures. *New Phytol* 207:1213–1222. <https://doi.org/10.1111/nph.13416>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.