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RESEARCH ARTICLE

Root growth and belowground interactions in spring wheat / faba bean intercrops

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Abstract
Background and aims Intercrops offer multiple advantages over sole crops. The aim of our study was to characterize root growth and interactions in spring wheat/faba bean intercrops to better understand belowground interactions that govern resource capture.
Materials and methods A field experiment was conducted with one faba bean cultivar and two spring wheat cultivars sown at three sowing densities, defining three intercropping designs. Destructive root coring was conducted (0–100 cm) in the intercrops and sole crops at two development stages. FTIR spectroscopy was used to discriminate the species' root masses. The plant-plant interaction index was calculated to represent the belowground interactions.
Results A negative impact of intercropping on total root mass was observed in the treatment with high sowing density in both stages. For the fully and partial replacement design treatments, plant-plant facilitation was more pronounced than competition in all layers. Competition dominated root growth in the treatment with high sowing density in both stages. Lower sowing densities encouraged deep root growth of wheat (both cultivars) in intercropping. The early root growth in depth and in density of one spring wheat cultivar impacted negatively faba bean root growth. Intercropping resulted in a grain yield

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advantage in both fully and only one partial replacement design treatment.

Conclusion In the intercrops, total root mass and plant-plant interactions were affected more by sowing density than by the spring wheat cultivar. Understanding the effect of sowing density on root growth in intercropping can help to support the design of sustainable intercropping systems.

Keywords FTIR spectroscopy · Intercropping · Crop mixture · Land equivalent ratio · Root carbon and nitrogen

Introduction

Crop mixtures or intercrop or intercropping is the practice of cultivating two or more crops with different rooting abilities, canopy structure, height, and nutrient requirements simultaneously (Hauggaard-Nielsen et al. 2008; Lithourgidis et al. 2011). To study interactions in intercrops, different experimental designs can be applied. A common one is the replacement (substitutive) design, in which the densities of the partners relative to the respective densities of the sole crops add up to 100% (Snaydon 1991). In the additive design, the intercrop is formed by adding the plants of both species in the same densities as in their sole crops; as a result, the total density of the intercrop is higher than the density of sole crops (Snaydon 1991).

The mixture mechanisms that affect intercrop performance are (resource use) complementarity (e.g. through different rooting habits/structures), competition (for light, soil water, and nutrients), and facilitation (e.g. of phosphorus and micronutrient acquisition via root-root interactions) (Vandermeer 1989; Brooker et al. 2015; Stomph et al. 2020; Zhang et al. 2021). So, the behavior and performance of intercrops is governed by complex interactions. According to Justes et al. (2021), Competition occurs when one species has a greater ability to use limiting resources (e.g., nutrients, water, space, light) than others. Complementarity occurs when intercropped plants have different requirements for abiotic resources in space, time, or form. Cooperation (or facilitation) is observed when the modification of the environment by one specie is beneficial to the other(s). Compensation occurs when the failure of one specie is compensated by the other(s) because they differ in their sensitivity to abiotic or biotic stress (Justes et al. 2021; Döring and Elsalahy 2022). The review on interspecific root-root interactions in competition-based and facilitation-based intercropping systems by Yu et al. (2022) describes in detail the mechanisms that drive interspecific below-ground competition (e.g. driven by resource depletion) and facilitation (e.g. due to nutrient or water enrichment or enrichment of beneficial microbiome) in intercropping. Due to the mentioned interactions, intercrops offer the possibility of increasing the productivity of a defined piece of land (Lithourgidis et al. 2011), limiting the use of synthetic fertilizers (Jensen et al. 2020), suppressing weeds (Den Hollander et al. 2007), as well as increasing biodiversity and maintaining and regenerating ecosystem services (Kremen and Miles 2012). Intercrops also minimize risks related to volatile market prices, drought, and/or floods (Brooker et al. 2015; Bedoussac et al. 2015). Further ecosystem services offered by intercrops include below-ground biomass advantage which is directly linked to better nitrogen (N) mineralization and carbon (C) sequestration (Cong et al. 2015) and soil stability which decreases soil erosion (Obalum and Obi 2010, Sharma et al. 2017).

To optimize the intercrop cultivation (e.g. choice of partners, sowing density) and to enhance ecosystem services (e.g. root-based C input for enhanced C sequestration), a better understanding of the underlying mechanisms responsible for belowground growth and interactions in species mixtures and of other ecosystem services is needed (Li et al. 2006; Tosti and Thorup-Kristensen 2010; Bargaz et al. 2015; Brooker et al. 2015; Shao et al. 2019). As root studies are generally laborious, particularly in (in-row) species mixtures, little is known about the effect of intercrop management practices on belowground growth especially under field conditions and in temperate climatic zones. Several methods for root species identification in mixtures have been applied. Methods based on DNA, ^{13}C, or root morphology are time-consuming and need extensive training (Rewald et al. 2012). The monolith excavation method combined with visual distinction (Li et al. 2011; Yu et al. 2022) is rather simple and cheap but less accurate. Infrared spectroscopy has been proven to be a fast tool to discriminate roots of different species such as corn-soybean (White et al. 2011), pea-oat (Naumann et al. 2010), pea-oat and maize-barnyard grass (Legner et al. 2018), faba bean-wheat (Streit et al. 2019), and blue lupin-winter rye (Kemper et al. 2022). Fourier transform infrared (FTIR) spectroscopy can be applied to separate roots of species in mixtures and can also give an estimation of the species specific proportions within a root sample (Meinen and Rauber 2015; Streit et al. 2019; Kemper et al. 2022). In these studies, mean root mass LER (over differential depths) ranged from 0.52 to 1.50 depending on the experimental year and the species (Streit et al. 2019; Kemper et al. 2022).

One important aspect in studying intercrop performance and the linkage of root traits in species mixtures is to understand the effect of management practices such as sowing density and cultivar (*cv.*) selection as a way to improve intercrop design and cultivation (Demie et al. 2022; Yu et al. 2022). The sowing density is important because it dictates the number of intraspecific and interspecific neighbors (Homulle et al. 2022). Sowing density affects aboveground productivity mainly through intra- and interspecific competition for resources capture (Yu et al. 2016). Belowground, studies on the impact of sowing density on root growth are still scarce, especially when sowing densities of both species are varied. To the best of our knowledge, only Wang et al. (2018) evaluated the effect of increasing total sowing density in a maize/spring wheat strip intercropping system on root growth. They found that with increasing sowing density of maize in species mixtures, root growth of the intercropped maize was increased significantly in comparison to the maize sole crop.

Shao et al. (2019) found that genotypes with less variation in root size, as well as medium root size, medium to broad root system, and more inter-row root distribution, help to reduce root-to-root competition and tend to have higher yield at high planting densities in a strip intercropping system. Hence, the genotype plays an essential role in determining the root traits and eventually the complementarity and/or competition between intercropped species.

Currently, knowledge of the root systems contribution to intercrop yield advantage and the related effects of cultivar choice and sowing density is scarce. Specific belowground processes between the species should be considered to improve interspecific facilitation in future species mixture designs (Yu et al. 2022). The aim of this study was therefore to investigate the effect of faba bean and spring wheat intercropping on root and shoot growth as a first step to understand root interactions in intercrops and to study the effects of different sowing densities and cultivars on belowground growth and interactions.

Materials and methods

Site description, field design, and crop management

The research facility Campus Klein-Altendorf (CKA) of the University of Bonn, Germany, is located in Rheinbach near Bonn (50° 37’ 31’’ N, 6° 59’ 21’’ E). The soil at the experimental station was classified as Haplic Luvisol, derived from loess and characterised by a silty-loamy texture with clay accumulation in the subsoil between about 45 and 95 cm soil depth (Barej et al. 2014). The climate at the experimental station can be described as moderately humid with maritime influences. The mean annual air temperature and precipitation are 10.3 °C and 669 mm (1991 to 2020), respectively. In 2021, an in-row mixture trial of spring wheat (*Triticum aestivum* L.) and faba bean (*Vicia faba* L.) with two spring wheat and one faba bean *cv*. and three total sowing densities (*TSD*) representing three types of intercropping designs was established. Each cultivar was also sown as a sole crop. In a subset of these plots, the presented root observations were conducted (Table 1). The sowing densities of sole crops considered in this study are higher than the usually applied densities in Germany, but as the emergence rate is not well known we kept them to better reflect the interactions in intercrops. The sowing densities in grain/m2 and in % are given in Table 2.

The experiment presented in this study of a large in-row mixture experiment. Due to a sowing error, the intended field design could not be fully implemented and there were therefore less than four field replicates available for the current study (Table S1). Therefore, root sampling was repeated four times in the selected plots (one plot for each treatment). The plot size was $15 m^2$ (1.5×10 m) with a row distance of 21 cm and 6 rows per plot.

The preceding crop in 2020 was spring barley. On 30/03/2021, the soil was harrowed to 10 cm soil depth. Soil mineral N was 98 kg ha^{-1} (16 kg ha^{-1} from 0 to 30, 27 kg ha^{-1} from 30 to 60 cm and 55 kg ha^{-1} from 60 to 90 cm) on 17/02/2021. Spring wheat

Table 1 Treatments with spring wheat (*cv.* SU Ahab, *cv.* Anabel) and faba bean (*cv.* Fanfare) and the respective sowing densities at Campus Klein-Altendorf in 2021. The total sowing density (*TSD*) is the sum of both sowing densities

Abbreviation	Description	Sowing density (%)		TSD (%)	Design of the cropping system
		spring wheat	faba bean		
SW_SUAh_100	Sole crop spring wheat SU Ahab	100		100	Sole crop
SW_Ana_100	Sole crop spring wheat Anabel	100		100	Sole crop
FB_100	Sole crop faba bean Fanfare		100	100	Sole crop
FB_33_SW_Ana_33	Intercrop Fanfare x Anabel	33	33	66	Partial replacement
FB_33_SW_SUAh_33	Intercrop Fanfare x SU Ahab	33	33	66	Partial replacement
FB_50_SW_Ana_50	Intercrop Fanfare x Anabel	50	50	100	Full replacement
FB_50_SW_SUAh_50	Intercrop Fanfare x SU Ahab	50	50	100	Full replacement
FB_100_SW_SUAh_100	Intercrop Fanfare x SU Ahab	100	100	200	Additive

Table 2 Sowing density considered for each treatment and the corresponding number of sown grains per m^2

Sowing density (rate) in %	Spring wheat (grains per m^2)	Faba bean (grains per m^2)
33	160	18
50	240	27
100	480	54

cultivars SU Ahab and Anabel and faba bean cultivar Fanfare were sown on 30–31/03/2021. The cultivars are described in Paul et al. (2024). Spring wheat emerged mid-April (BBCH 11/12 on 19/04/2021) and faba bean emerged about one week later. Hand harvest took place on 13/08/2021 (BBCH 99) and machine harvest on 25 August 2021, when both crops were fully ripened. No fertilizers or pesticides were applied.

Root sampling

Root samples were taken with a soil auger with an inner diameter of 9 cm down to 100 cm soil depth in the selected plots on 09/06/2021 and on 05–06/07/2021. The root sampling in the intercrop treatments covered always one faba bean and one wheat plant and the core was placed not exactly above a row but next to the row (from the row to 1.5 cm from the middle of the row) (see Fig. S1). On 09/06/2021, the BBCH stages of wheat and faba bean were 39 (end of shooting) and 63 (full flowering), respectively. On 05–06/07/2021, the BBCH stages of wheat and faba bean were 69 (end of flowering) and 71 (approx. 10% of the pods have a species or variety-specific size achieved), respectively. Samples were taken in eight plots (three sole crops and five intercrops) replicated four times per plot (Table 1). Soil cores were split into ten centimetre sections and stored separately in plastic bags and dried under a plastic crop tunnel before sample preparation and evaluation performed at the University of Göttingen, Germany.

Quantification of root biomass, root carbon and nitrogen contents

The root samples were washed in a root washing machine (custom made, mesh size 1 mm) and cleaned of soil residues and non-root particular organic matter manually. The root samples were frozen in a tea bag between different cleaning, scanning, and drying steps. Roots were scanned with a flat-bed scanner (Expression 12000XL, Epson, Suwa, Japan) and analysed with WinRhizo 2016a software (Régent Instruments Inc., Quebec, QC; Canada) to estimate the root length density (RLD, cm cm^{-3} soil). After scanning, all roots were oven-dried at 40 °C for 48 h and weighted. The samples were ground with an ultra-centrifugal mill (Retsch, ZM 200, Haan, Germany) and stored in glass vials for the next analysis (see Discrimination between species).

Due to low absolute weights in deeper soil layers, the root mass samples of the subsoil layers were pooled for weighing and for the C and N content determination (after the FTIR analyses) resulting in samples soil depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–60 cm, and 60–100 cm. Root C and

N were measured according to ISO 13,878 and ISO 10,694 standards with an elemental analyzer VarioMAX cube (Elementar Analysensysteme GmbH, Langenselbold, Germany).

Discrimination between species

Fourier Transform Infrared Spectroscopy (FTIR)

The roots of the sole crops of the two spring wheat cultivars (SU Ahab and Anabel) and one faba bean cultivar (Fanfare) were used to evaluate the species' root proportion in the intercrop samples. Absorption spectra of the ground root samples of the sole crops, as well as of the intercrops, were measured by the FTIR-ATR spectrometer (Alpha-P with a diamond crystal attenuated total reflection (ATR) device, Bruker Optics, Ettlingen, Germany) with a resolution of 4 cm^{-1} and 32 scans in the spectral range of 4000–400 cm^{-1}. Each sample was measured 3 to 5 times. The evaluation of the FTIR-ATR spectra was conducted with the Opus software Quant 2 (version 7.2, Bruker Optics, Ettlingen, Germany). The FTIR spectra of the sole crop sample species were used for a cluster analysis (Opus software, version 7.2, Bruker Optics) to allow for species discrimination. For the cluster analyses, the spectra were pre-processed by second derivative and vector normalization, the frequency range was reduced and the Euclidian's distance and Ward's algorithm was applied (Fig. S2, S3 and S4). The interspecific heterogeneity for both species was higher than the intraspecific heterogeneity permitting a separation of the two species. Both spring wheat cultivars separately but also combined were clearly separable from faba bean via cluster analysis (Fig. S5). Since the average FTIR spectra of both spring wheat cultivars were very similar, both spring wheat cultivars were combined for the second sampling date analyses (Fig. S5 and S6).

Model establishment

For the quantification of the root proportion of each species in the intercrops root samples, the FTIR spectra of the single species samples were used to generate a model. For establishing a two-species model, a calibration set of 35 "artificial mixtures" was generated in 3% steps from 0 to 100% for spring wheat and faba bean, respectively. These mixtures covered the complete calibration range. 20 additional "artificial mixtures" with known species composition were generated to be used for external calibration of the model. With the FTIR spectra of these calibration mixtures, a model was calculated on the basis of multivariate calibrations with the method of partial least square (PLS) regression using the software Quant 2 (Opus, version 7.2, Bruker Optics, Ettlingen, Germany). The absorption of infrared radiation is correlated to the concentration of compounds in a multi-compound system. The established model was evaluated by an internal validation (cross validation) and was subsequently optimized by the Quant 2 software. This optimization process detected the best data preparation and the best frequency range to explain the actual mixtures of the calibration samples. Six to eight of the proposed optimized models were verified by an external calibration (20 additional "artificial mixtures"). Both internal validation and external calibration were compared with the calculated statistical parameters of each calibration. For the first sampling date for each wheat cultivar, a separate model was generated. The statistical parameters of the model (calibration/internal validation and external calibration) are shown in Tables S2 (first sampling date, 09/06/2021) and S3 (second sampling date, 05-06/07/2021). With the chosen model, the FTIR spectra of the mixed species samples were evaluated with the associated model. The output of this evaluation was the percent share of each species within the mixed species root mass samples which were used for further calculations. Values outside the calibration range (below 0% or above 100%) were corrected to 0% and 100%.

Data analysis and statistics

Root parameters and indexes

Root length density (RLD, in cm cm^{-3}) per layer was calculated using the following equation:

$$\mathrm{RLD} = \frac{\mathrm{Rootlengthperlayer}}{\mathrm{Soilvolumeofthelayer}} \tag{1}$$

The soil volume of each layer is equal to 636 cm^3 (core diameter: 9 cm, sample height: 10 cm).

Root mass (t ha^{-1}) was calculated according to the Eq. (2):

$$\text{Rootmass} = \frac{rootmassforthecorrespondinglayer}{surfaceareaofcylinder} \quad (2)$$

The surface area of cylinder (core auger) is equal to 63.6 cm^2.

Specific root length (SRL; m g^{-1}) was calculated as follows:

$$\text{SRL} = \frac{Rootlengthperlayer}{Rootmassforthecorrespondinglayer} \quad (3)$$

The FTIR method used in this study to separate between the intercropped species allows only to determine the root mass of the two species, separately. Thus, the RLD and SRL in this study refer to the whole intercropping system rather than to the specific crop species.

Various terminologies for characterizing the yield advantages in intercrops exist in the literature, namely, 'overyielding' (Li et al. 2013; Streit et al. 2019; Nelson et al. 2021; Yang et al. 2022) or 'Relative Yield Total' (Willey and Osiru 1972), which is identical to 'Land Equivalent Ratio' (LER) defined by De Wit and Van den Bergh (1965). In the context of our study, we use also the term root mass advantage to characterize the positive effect of intercrops on root biomass.

So, the LER for the faba bean and spring wheat mixtures was calculated for aboveground biomass (LER_{AGB}) at the two growing stages and at harvest as well as for belowground biomass (LER_{Root}) according to Eqs. 4–6. The LER was only calculated for the treatments with fully replacement design. The LER for bean and wheat in intercrops is the sum of the partial LER for bean ($pLER_{Bean}$) and wheat ($pLER_{Wheat}$):

$$\text{LER} = pLER_{Bean} + pLER_{Wheat} \quad (4)$$

$$\text{pLER}_{\text{Bean}} = \frac{\text{Biomassbeanintercrop}}{\text{Biomassbeaninsolecropping}} \quad (5)$$

$$\text{pLER}_{\text{Wheat}} = \frac{\text{Biomasswheatinintercrop}}{\text{Biomasswheatinsolecropping}} \quad (6)$$

The expected values of grain yield, root mass, RLD and SRL were estimated based on the Eq. (7):

$$\text{Y}_{\text{expected}} = p^{*}M \quad (7)$$

Where p is the sowing density of the species in the intercrop divided by the sowing density in the sole crop and M is either the grain yield, root mass, SRL or the RLD of the sole crop.

We applied an adapted version of the 4 C approach of Justes et al. (2021) to find out when and where facilitation or competition dominates. Here, instead of using the pLER as presented in Justes et al. (2021), the calculation is being adapted by dividing the root biomass by the ratio of plant density DR (Eqs. 8–10). The novel index is called plant-plant interaction index (PPII), where:

$$\text{PPII} = \text{PPII}_{Wheat} + \text{PPII}_{Bean} \quad (8)$$

with

$$\begin{aligned} \text{PPII}_{\text{Bean}} &= \frac{\text{Rootmassofbeaninintercrops}}{\text{Rootmassofbeaninsolecrops}} \div DR_{Bean} \\ \text{PPII}_{\text{Wheat}} &= \frac{\text{Rootmassofwheatinintercrops}}{\text{Rootmassofwheatinsolecrops}} \div DR_{Wheat} \end{aligned} \quad (9)$$

and ratio of plant density DR (with density in plants per m²);

$$\begin{aligned} \text{DR}_{\text{Wheat}} &= \frac{\text{Densityofwheatinintercrops}}{\text{Densityofwheatinsolecrops}} \\ \text{DR}_{\text{Bean}} &= \frac{\text{Densityofbeaninintercrops}}{\text{Densityofbeaninsolecrops}} \end{aligned} \quad (10)$$

If $\text{PPII}=1$, neutral effect. If $\text{PPII}<1$, net competition. If $\text{PPII}>1$, net facilitation.

This approach has the advantage of giving the information on the net effect of plant-plant interactions, expressed by plant density.

Statistical analyses

The statistical analyses were performed using the programme R version 4.2.1 (23/06/2022) (R Core Team 2018).

Shoot biomass, root mass and RLD were analysed by a one-factorial analysis of variance (Anova) (factor treatment), as well as two-factorial analysis of variance (factors cultivar and sowing density) for all treatments. Mean values of treatments were compared with a Tukey post-hoc test at a significance level of $\alpha=0.05$. Outliers were detected for each of the response variables (root mass, RLD, FTIR predictions) using the package rstatix in the programme R. Values above- $Q3+1.5$ x IQR or below $Q1–1.5$ x IQR were considered as outliers and were deleted. Q1 and Q3 are the first and third quartile, respectively. IQR is the interquartile range (IQR = Q3 - Q1). A one-sample t-test against 1 was used to test the significance of LER_{root} and one sample t-test against 0.5 was used to test the significance of $pLER_{Wheat}$ and $pLER_{Bean}$. For the calculation of PPII, infinite values induced by 0 when dividing root masses were deleted and not considered in the calculation of the means. Also, we considered the mean across replicates.

Shoot sampling, soil water, and nutrient derivation

Shoot biomass, plant height, number of plants per m^2 and volumetric soil water content at 0, 30, 45, 60 and 90 cm soil depth were measured in the days preceding the two dates when the root sampling took place. Shoot samples for estimation of shoot dry weight were collected destructively with one sample per plot on 06.06 and 06–08/07/2021. Hand harvest of 2 row meters took place on 13/08/2021 in which 1 m from both the 3rd and 4th rows (2m in total per plot) were harvested and ensuring that cuts were made a minimum of 1 m from the plot boundary to reduce boundary effects. Wheat and faba bean were separated manually in case of intercrop treatments. The fresh biomass samples were weighed and (in case of large samples only aliquots) then oven-dried (105° C) until constant weight was reached and weighed again to estimate shoot, straw or grain dry matter. Due to lack of replicates regarding shoot biomass and yield at harvest, the aboveground dataset is only presented as supplementary (Table S4).

The soil water content was measured at soil depth of 0, 30, 45, 60, and 90 cm with a mobile FDR probe (ThetaProbe ML3, ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany) on 07/06/2021 and 05/07/2021. Soil samples from 0 to 30, 30–60, and 60–90 cm soil depth were collected to estimate soil mineral nitrogen (Nmin) before sowing (17/02/2021, pooled samples over field) and one day after harvest (26/08/2021, pooled samples per plot) using a Pürckhauer auger. Nitrate-N and ammonium-N were determined photometrically using a continuous flow analyser (Seal QuAAtro 39, Norderstedt, Germany) after K_2SO4 extraction of the soil sample.

General characteristics of the growth period

The growing season in 2021 can be characterized as chilly in April and May with a normal rainfall pattern, however, a storm with a heavy rainfall occurred on 14–15/07/2021 with about 120 mm of rainfall. In the growth period from 30/03/2021 to 25/08/2021, total rainfall was 395 mm and the mean air temperature was 14 °C (Fig. S7).

Results

Aboveground overyielding in intercrops

Total dry matter grain yield in intercrops varied from 4.5 t ha-1 to 5.6 t ha-1 (Table S4). In intercrops with *cv.* SU Ahab, the grain yield attained values were higher for the treatments of the partial replacement design and fully replacement design but lower that for the additive design (Table 3). For intercrops with the *cv.* Anabel the lower sowing density of the partial replacement treatment (*TSD* = 66%) resulted in grain yield value lower than the expected one. However, for that same cultivar, a value of grain yield attained higher than the expected one was found under fully replacement design (FB_50_SW_Ana_50, *TSD* = 100%).

In intercrops, LER could only be calculated for the fully replacement design treatments (FB_50_SW_Ana_50 and FB_50_SW_SUAh_50), the shoot LER values ranged from 1.03 to 1.42 (Table S5) with a mean across both varieties of 1.28 ± 0.20 at the first sampling date and 1.10 ± 0.10 at the second sampling date. At harvest, the wheat contributed less (lower $pLER_{Bean}$) than the faba bean to the positive grain yield overyielding (1.27 ± 0.28, mean across both cultivars). The comparison between both wheat varieties revealed that the grain yield LER of the intercrops with *cv.* SU Ahab was higher than in intercrops with *cv.* Anabel. The *cv.* SU Ahab seems to be more advantageous for mixtures (higher LER for grains and higher absolute grain yield in mixture) than the cultivar Anabel (Table S5).

Root growth in intercrops

Characterisation of root mass

The cumulated root mass over the soil profile (all soil depths measured) increased from the first to second date by 19% (mean of the two cultivars) for the sole crop wheat and 34% for the sole crop faba bean

Table 3 Attained and expected values (n = 1) of grain yield at harvest (13/08/2021). Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

	SW_ SUAh_100	SW_Ana_100	FB _100	FB_33_ SW_ Ana_33	FB_33_SUAh_33	FB_50_ Ana_50	FB_50_ SUAh_50	FB_100_ SUAh_100
SW GY	4	5.2		2.3	3	2.5	2.7	2.2
expected SW GY				1.716	1.32	2.6	2	4
FB GY			3.4	2.9	2.6	2	2.7	3.1
expected FB GY				1.122	1.122	1.7	1.7	3.4
Total GY	4	5.2	3.4	5.2	5.6	4.5	5.4	5.4
expected Total GY				6.916	2.442	4.3	3.7	7.4

(Table S6). For the intercrops, the greatest increase between the two sampling dates were estimated in treatments FB_50_SW_Ana_50 (46%) and FB_100_SW_SUAh_100 (41%) and the lowest were estimated for the treatments FB_50_SW_SUAh_50 (21%) and FB_33_SW_Ana_33 (20%). On sampling date one (09/06/2021), the significantly highest mean values of total root mass (0–1 m) were observed in the intercrop with wheat cv. SU Ahab with TSD = 66% (FB_33_SW_SUAh_33) and 100% (FB_50_SW_SUAh_50) *TSD* with 2.11 t ha-1 and 2.03 t ha-1, respectively (Table S6).

At the first sampling date (Fig. 1), the lowest root mass values in the topsoil (0–30 cm) were determined for the wheat sole crops. The highest sowing density (*TSD* = 200%) showed lower total root mass as compared to the two other sowing densities in intercropping. For the upper subsoil (30–60 cm), the sole wheat root mass was significantly higher than all intercrop treatments. The intercropping of faba bean with the wheat *cv*. Anabel at the lowest sowing density achieved the lowest root mass value, while the faba bean sole crop achieved the second lowest total root mass at this soil depth. For the deeper subsoil layers (60–100 cm), the faba bean sole crop presented the lowest value. At the first sampling date, spring wheat *cv*. Anabel developed more roots in deeper soil layers as a sole crop and in intercropping in comparison to *cv*. SU Ahab (Fig. 1).

At the second sampling date, no significant differences between the treatments with regard to topsoil root mass were observed (Fig. 2). The intercrops with low sowing density (FB_33_SW_SUAh_33 and FB_33_SW_Ana_33) achieved the significantly lowest values of root mass cultivars in the upper subsoil (30–60 cm). In the deeper soil layer (60–100 cm), faba bean reached the lowest root mass. Results of a two-way Anova (α = 0.05) indicated that the cultivar choice had no significant effect on root mass but sowing density had. Also, no significant interactions between the sowing density and cultivar for root mass were found (Table S7).

Proportion of faba bean and spring wheat root in intercrops

The results of discrimination between species using the FTIR showed that wheat root mass dominated in the subsoil (20/30–100 cm, Fig. 3). In general, there were no significant differences in faba bean root mass proportions between the different treatments. Only in the first sampling date significant differences in 0–10 cm (the very high sowing density led to low faba bean root proportions) and in 60–100 cm depth (the intercrop treatments with wheat *cv.* Anabel had low faba bean root proportions) were observed. The quick and deep rooting ability of the *cv.* Anabel in comparison to *cv.* SU Ahab is illustrated by the greater proportion of faba in intercrops with *cv.* SU Ahab in the deeper soil depths (60–100 cm) at both sampling dates (although the differences were only significant at the first sampling date).

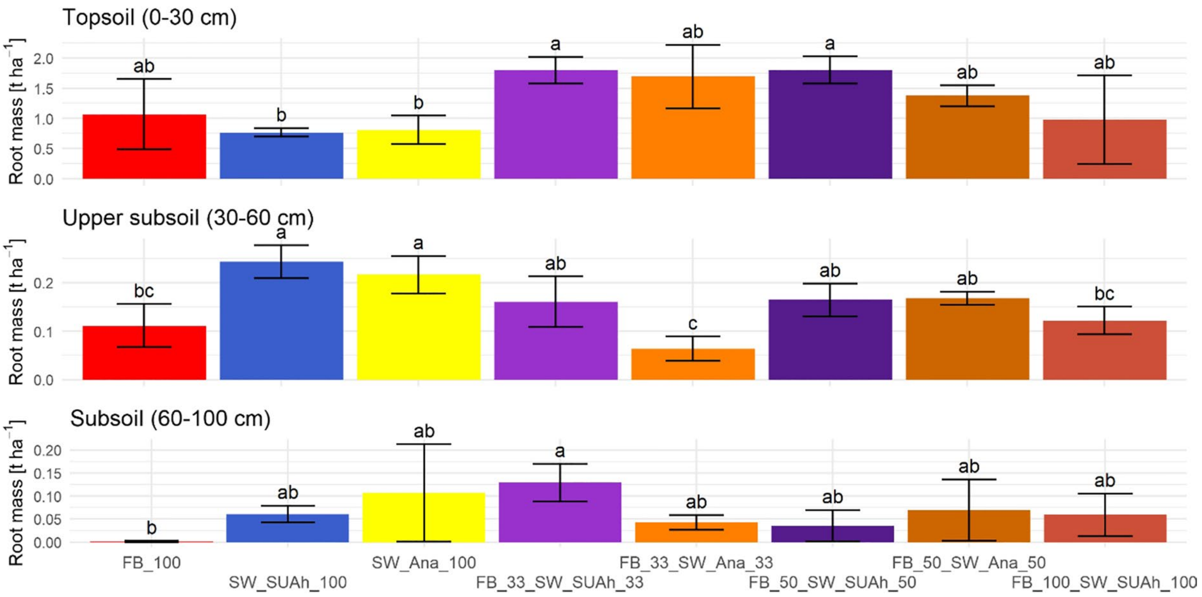

Fig. 1 Mean ($n=4$) total root mass (sum of both crops) in t ha^{-1} at the first sampling date (09/06/2021) for three soil layers. Different letters indicate significant differences (Anova and Tukey post-hoc test, $\alpha=0.05$). Error bars refer to the standard deviation. Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

Root mass advantage in intercropping

At the first sampling date (09/06/2021) in the topsoil and upper subsoil layers (0–40 cm) for intercrops with wheat *cv.* Anabel and 0–30 cm for intercrops with wheat *cv.* SU Ahab), a positive root mass LER was observed (Table 4). A the second sampling date (05/07/2021), the root mass LER was above one for the layers 0–20 cm for the intercrop with *cv.* SU Ahab and above one from the layers 0–60 cm for the intercrops with *cv.* Anabel (Table 4).

Effect of sowing density on root mass of intercrops

The analysis based on the comparison between the attained and the expected values of root mass revealed that, on both sampling dates, under high sowing density (*TSD* = 200%, additive design) the expected values of root mass in 0–1 m soil depth were higher than the attained values (Fig. 4). In contrast, for the lower sowing densities (*TSD* = 66%, partial replacement design and *TSD* = 100%, full replacement design), the attained values were higher than the expected one.

Root length density

On both sampling dates and in all soil layers, the RLD of the tap rooted sole faba bean was lowest (Figs. 5 and 6). In the upper subsoil (30–60 cm), mostly significant differences were found between RLD of faba bean and spring wheat in sole cropping. For the mixed cropping treatments, the RLD in the upper subsoil was higher for the fully replacement treatments (*TSD* = 100%) as compared to the partial replacement ones (*TSD* = 66%) and vice versa in the deeper subsoil 0–100 cm). Thus, lower sowing densities encouraged deep rooting in mixtures.

No significant differences in RLD were observed for the wheat *cv.* SU Ahab for all sowing densities on either sampling date in any soil layer. For the wheat *cv.* Anabel, RLD in the upper subsoil was significantly higher in the 50%-50% treatment as compared to the 33–33% treatment (both dates).

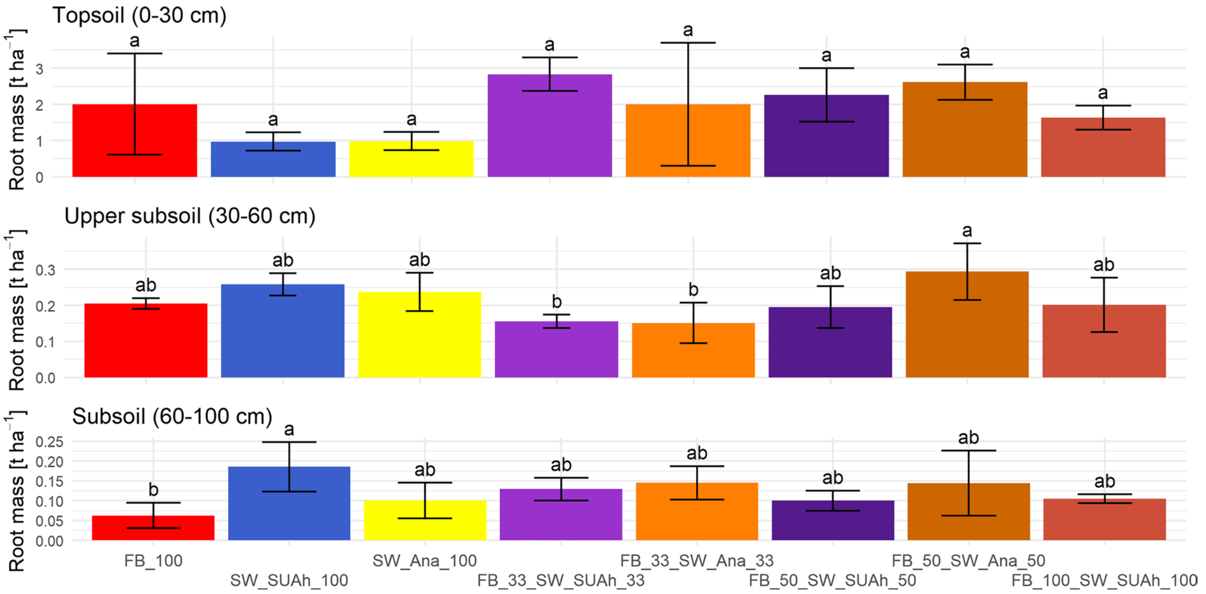

Fig. 2 Total root mass (sum of both crops) in t ha^{-1} of the second sampling date (05–06/07/2021) for three soil layers. Different letters indicate significant differences (Anova and Tukey post-hoc test, α = 0.05). Error bars refer to the standard deviation. Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

For deep subsoil (60–100 cm) and for all treatments, the RLD decreased with soil depth. However, the mean RLD for the subsoil (60–100 cm) was found to be highest in the 33%-33% mixture with the wheat *cv.* SU Ahab. Additionally, in both treatments with *TSD* 66%, the mean RLD from 60 to 100 cm was higher in comparison to the mean RLD of 30–60 cm. Both the intercrops and the spring wheat sole crops attained slightly higher cumulative RLD values than the faba bean, with a mean value over all intercrops and sole crop spring wheat treatments of around 18 cm cm^{-3} compared to 5 cm cm^{-3} for the faba bean (0–1 m soil depth) (Table S8).

Specific root length

On both sampling dates, the mean SRL (all depths) was lower in faba bean compared to spring wheat (Table S9). An enhanced SRL (more fine roots in 0–100 cm) in intercrops as compared with the expected SRL from sole crops was observed. A trend for decreasing mean SLR values with increasing *TSD* in the mixtures was observed.

Belowground interactions in intercrops

Generally, the mean PPII decreased from the topsoil to the subsoil. The analysis of PPII showed that under fully replacement design (*TSD* = 100%) and partial replacement design (*TSD* = 66%), the facilitation were the most dominant interaction. In contrast, the competition between the species was more pronounced in the additive design (Fig. 7), in both growing stages.

Root carbon content

The root C content, calculated as C concentrations (mean: 45%) multiplied by root dry matter, did not change significantly across the treatments for both sampling dates. However there was a trend of higher root C contents in the intercrop treatments compared with the sole crops, with the exception of the treatment with *TSD* = 200% (Fig. S8). For the intercrop

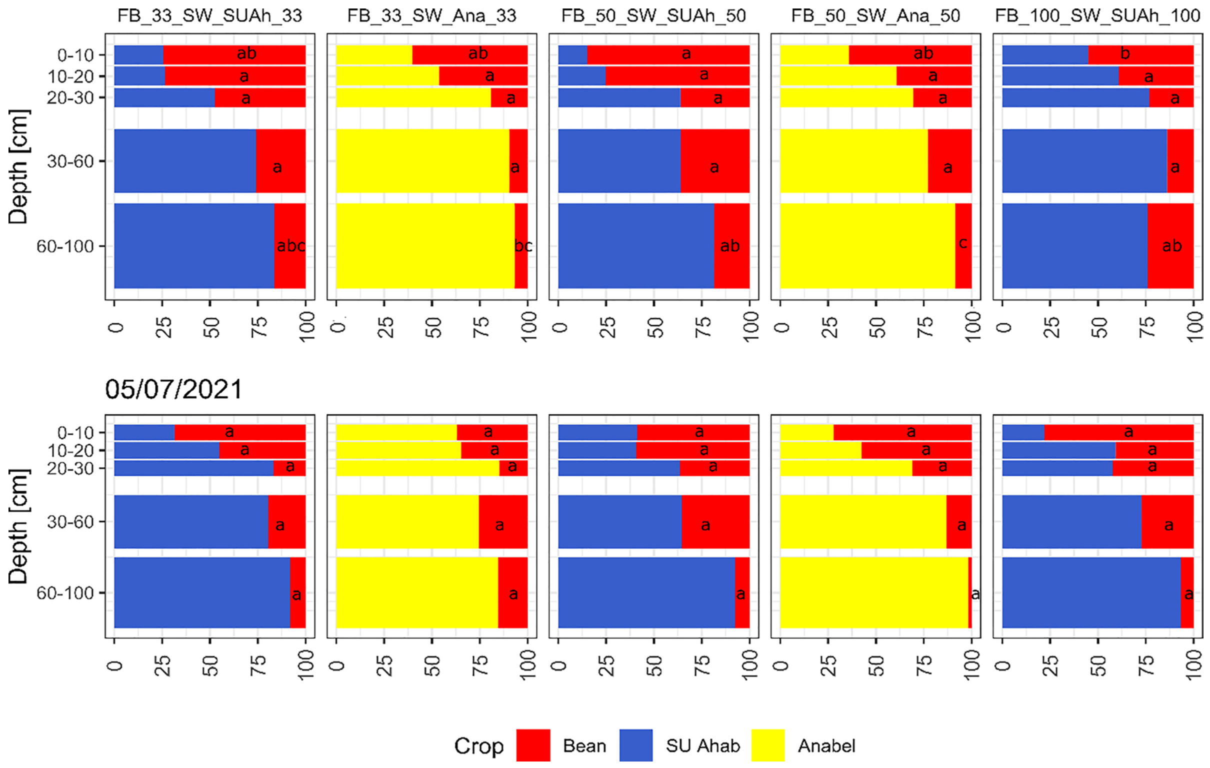

Fig. 3 Mean values ($n=4$) of species proportion of root mass (%) of spring wheat and faba bean in five intercrops. Different letters indicate significant differences (Anova and Tukey post-hoc test, $\alpha=0.05$) between proportion of root mass of faba bean within each soil layer (0–10 cm, 10–20 cm, 20–30 cm, 30–60 cm, 60–90 cm) in 09/06/2021 (top panel) and 05/07/2021 (bottom panel). Treatment abbreviations: FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%)

treatments with wheat *cv.* SU Ahab, there was a decrease of root C content with increasing *TSD*. The opposite trend was observed for the wheat *cv.* Anabel.

Root nitrogen content

The mean root N content were 2.3% (sole faba bean), 0.7% (sole wheat), and 1.2% (intercrop). As expected, the lowest values of root N content were estimated in sole spring wheat treatments (Fig. S9). Root N content in several intercrop treatments was comparable to the sole crop faba bean treatment. On the second sampling date, no significant differences were observed between the intercropping treatments and sole faba bean. However, in faba bean, the root N content was also found to be higher in the deeper soil layers (20–60 cm).

Soil mineral N

Before the establishment of the crops, the initial Nmin was 16 kg ha^{-1} in the topsoil (0–30 cm), 27 kg ha^{-1} in the upper subsoil (30–60 cm) and 55 kg ha^{-1} in the deeper soil (60–90 cm). After harvest, lower Nmin values over the whole soil layers were found in the spring wheat sole crop treatments. The topsoil Nmin values were lower in sole cropping (wheat and bean) as compared to the intercropping treatments (Fig. S10). The highest topsoil value (25 kg ha^{-1}) was determined in the treatment FB_100_SW_SUAh_100. In the upper subsoil 30–60 cm, the lowest value of 7.7 kg ha^{-1} was measured in the intercropping treatment with highest total grain yield and with lowest sowing density (FB_33_SW_Ana_33) followed by both spring wheat sole treatments. Again,

Table 4 Mean values ± standard deviation of root partial land equivalent ratio of bean (pLER$_{Bean}$, $n=4$), wheat (pLER$_{Wheat}$, $n=4$) and root land equivalent ratio (LER, $n=4$) based on root mass of the intercrops with wheat for two sampling dates for the replacement treatment with *cv.* SU Ahab FB_50_SW_Ana_50 and with *cv.* Anabel FB_50_SW_SUAh_50. For the first sampling date (60–100 cm), no values were provided for the treatment FB_50_SW_SUAh_50 due to absence or low root mass in all replicates. No standard deviation was provided for the treatment FB_50_SW_Ana_50 due low number of replicates ($n=1$). * refers to significant differences for pLER from 0.5, for LER from 1 ($p \leq 0.05$, t-test)

			FB_50_SW_Ana_50		FB_50_SW_SUAh_50		
Date	Depth	pLER $_{Bean}$	pLER $_{Wheat}$	**LER$_{root}$**	pLER $_{Bean}$	pLER $_{Wheat}$	**LER$_{root}$**
09/06/2021	0–10	0.62 ± 0.09	0.92 ± 0.097	1.54 ± 0.63	1.16 ± 0.30	0.35 ± 0.32	1.51 ± 0.31
	10–20	0.37 ± 0.18	0.99 ± 0.18	1.35 ± 0.66	1.32 ± 0.36	0.68 ± 0.39	2.01 ± 0.54
	20–30	0.58 ± 0.20	0.86 ± 0.20	1.44 ± 0.23	0.35 ± 0.24	0.64 ± 0.50	0.99 ± 0.52
	30–60	0.35 ± 0.11	0.60 ± 0.20	0.95 ± 0.15	0.57 ± 0.42	0.43 ± 0.17	1.00 ± 0.33
	60–100	0.43	0.13 ± 0.11	0.56			
05/07/2021	0–10	3.05 ± 4.20	0.83 ± 0.22	3.88 ± 4.37	1.46 ± 1.33	1.15 ± 0.50	2.61 ± 0.94*
	10–20	0.76 ± 0.70	0.65 ± 0.29	1.41 ± 0.85	0.64 ± 0.43	0.71 ± 0.44	1.35 ± 0.43
	20–30	0.26 ± 0.18	0.90 ± 0.54	1.15 ± 0.39	0.30 ± 0.15	0.64 ± 0.27	0.94 ± 0.19
	30–60	0.20 ± 0.12*	1.12 ± 0.59	1.32 ± 0.68	0.37 ± 0.29	0.46 ± 0.08	0.84 ± 0.29
	60–100	0.03 ± 0.02*	0.96 ± 0.43	0.99 ± 0.45	0.13 ± 0.09*	0.53 ± 0.26	0.67 ± 0.30

the highest value in 30–60 cm soil depth of 18 kg ha^{-1} was measured in the treatment with the highest sowing density FB_100_SW_SUAh_100. In the deeper subsoil (60–90 cm), soil Nmin was lowest in the intercrop treatments FB_100_SW_SUAh_100 and FB_50_SW_SUAh_50.

Higher topsoil N but low subsoil N were observed in the intercrop treatments with wheat cultivar SU Ahab (slower root growth) as compared to the intercrop treatments with *cv.* Anabel (fast early root growth). Especially in the upper soil layers there was a trend for a higher N depletion (lower Nmin values) in the low sowing density as compared to the high density intercrop treatments.

Soil volumetric water content

In general, the soil volumetric water content around the flowering of spring wheat in July (second sampling date) was higher than at the early sampling date in June (first sampling date). Soil volumetric water content for the spring wheat cultivar Anabel, which indicates the potential to root quickly and deeply, was lower in the sole crop treatment and in mixtures compared to the *cv.* SU Ahab at the second sampling date, particularly at deeper soil depths (Fig. S11). However, in the treatment with the *cv.* SU Ahab as a sole crop and as intercrop (*TSD* = 100%) the lowest soil water content values were measured at 30–60 cm soil depth. In general, soil water depletion was lower for the low density (FB_33_SW_SUAh_33) as compared to the very high density intercrop treatment (FB_100_SW_SUAh_100) (second sampling date, 30–90 cm).

Discussion

Root mass, root length density and belowground interactions

Although calculating root biomass in t ha^{-1} based on soil auger data is a common practice (Chirinda et al. 2012; Streit et al. 2019), we want to emphasize that this approach involves certain uncertainties since the root samples can only represent the root mass in a given soil volume.

Root system extension of wheat often exceeds the one of legumes like faba bean (Gregory et al. 1995; Turpin et al. 2002), though under field conditions, factors such as phenology, sampling technique and sampling depth may influence root growth. The faba bean root mass at flowering (2.3 t ha-1) observed in our study is higher than the values reported in the studies from Rengasamy and Reid (1993), who reported average root mass over years and treatments of approximately 1.4 t ha^{-1} for a sampling depth of 70 cm. These values are also higher than the values reported by Streit et al. (2019) who found values of

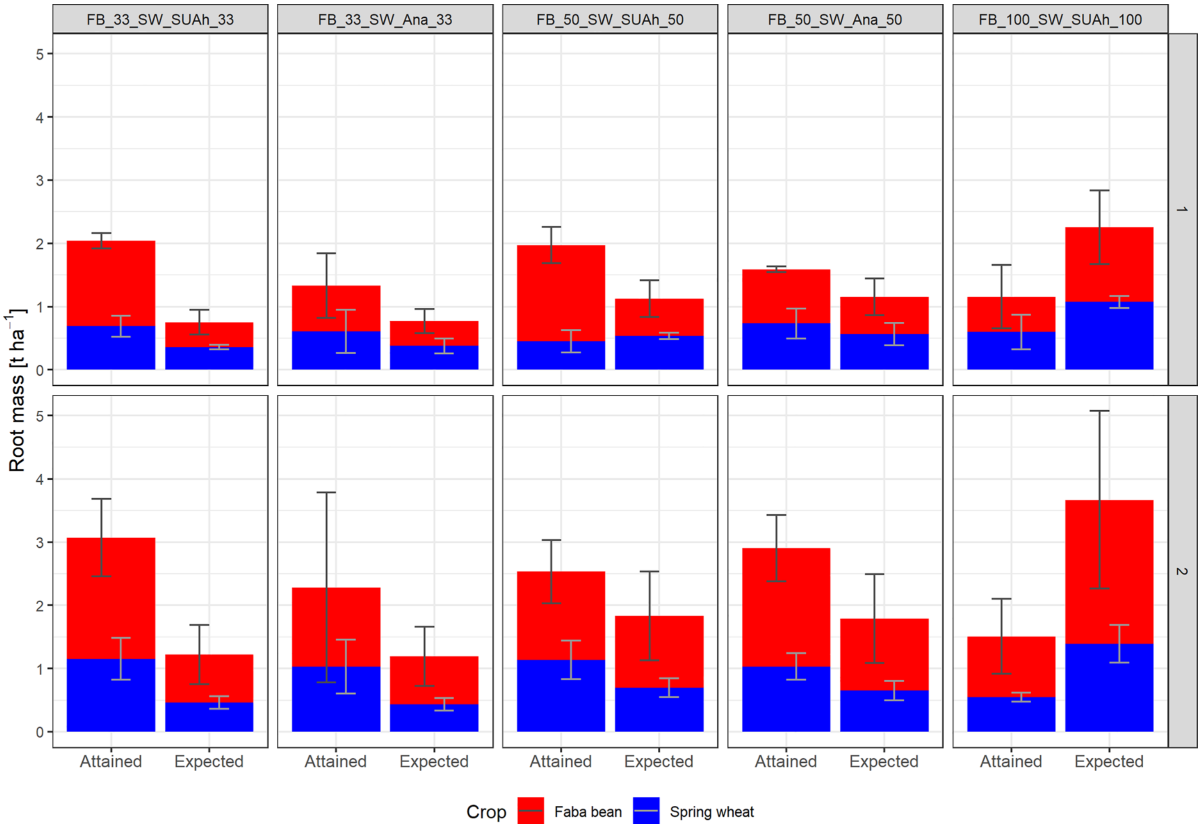

Fig. 4 Expected vs. attained values of mean root mass (t ha^{-1}, $n=4$) over 0–1 m soil depth in intercrops on 09/06/2021 (top panels) and on 5/07/2021 (bottom panels). The error bars refer to the standard deviation. Treatment abbreviations: **FB_33_SW_SUAh_33** = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

around 0.7 t ha^{-1} for a sampling depth up to 60 cm. This difference can be attributed to the higher sowing density considered in our study for the sole cropping treatments and also the sampling technique as we always considered a faba bean in the soil core which overrepresented the faba bean compared to the study of Streit et al. (2019), for instance. Literature revealed high variability for spring wheat root masses ranging from 0.8 t ha^{-1} to 1.4 t ha^{-1} at flowering (Wechsung et al. 1995; Gan et al. 2009). In our study, a spring wheat root mass of 1.4 t ha^{-1} was reached at flowering over the soil depth of 0 to 1 m. This rather high value can be partly attributed to the enhanced sowing density considered for the sole crops compared to the optimal sowing density recommended for spring wheat.

Cereals are generally considered as strong competitors compared to legumes, mainly due to a larger root system and deeper root distribution (Gregory et al. 1995; Hauggaard-Nielsen et al. 2001; Corre-Hellou and Crozat 2005; Bedoussac et al. 2015). Many studies reported that intercrops produce significantly higher root masses as compared to their sole cropping equivalents (Ma and Chen 2016). Root mass advantage was observed in faba bean-maize (Xia et al. 2013) and faba bean-winter wheat intercrops (Streit et al. 2019). In our study, the mean topsoil root LER was above one indicating a root mass advantage in intercropping versus sole cropping. In the upper subsoil, it depended on the spring wheat cultivar, but LER$_{root}$ was always below one in the deeper subsoil (60–100 cm).

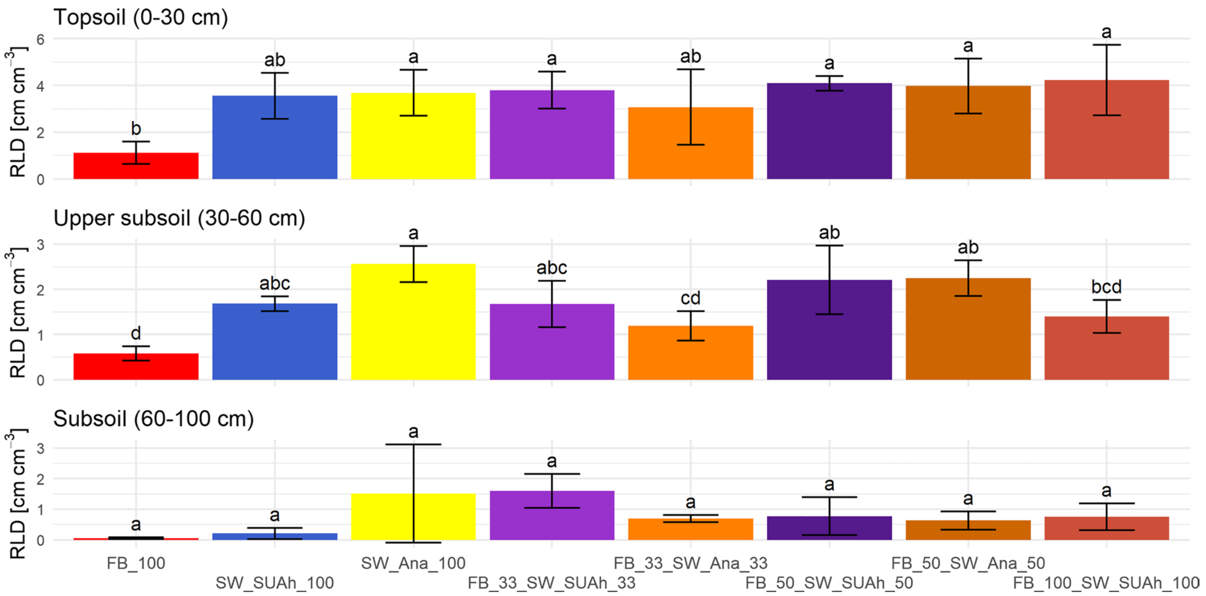

Fig. 5 Mean values ± standard error ($n = 4$) of root length density (RLD, not crop-specific) in cm cm^{-3}, for sole faba bean and sole spring wheat, as well as for the mixtures treatments for cumulated three soil layers in 09/06/2021. Different letters indicate significant differences at each soil depth (Anova and Tukey post-hoc test, $\alpha = 0.05$). Error bars refer to the standard deviation. Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

A combination of tap rooted and fibrous rooted crops is widely recognized as being one of the mechanisms of overyielding in intercrops due to belowground complementarity which may increase water and nutrient acquisition by niche differentiation and due to resource partitioning (Yu et al. 2022). In line with this finding, the attained values of root mass in the intercrop treatments for both wheat and faba bean (0–1 m soil depth) were mostly higher than the expected values (Fig. 4). This applied for both the low density (*TSD* = 66%) and the nearly optimal sowing density (*TSD* = 100%), but not for the very high sowing density (*TSD* = 200%).

It is assumed that belowground biomass advantage during vegetative stages fosters higher resource availability, as well as shoot and grain overyielding. This was especially reported under stress conditions (Fargione and Tilmann, 2005; Hector et al. 2002). The enhanced root growth and development partially compensated competition for light (Amossé et al. 2013), carbon dioxide (Shili-Touzi et al. 2010) and other resources (Wang et al. 2018). The results of aboveground overyielding and interactions in intercrops as described by the plant-plant interaction index (PPII) showed a positive correlation between facilitation, enhanced root growth, facilitation process and overyielding especially for intercrops with the spring *cv.* SU Ahab. However, due to lack of real field replicates, a clear relationship between belowground root interactions and aboveground overyielding could not be statistically tested. Also, the favorable growing conditions characterizing our experimental site and year combination (fertile soil, favorable soil moisture due to plenty of rain) could be a reason behind these observations. Similar studies in contrasting environments should be performed to better assess the relationship between belowground root advantage and aboveground overyielding.

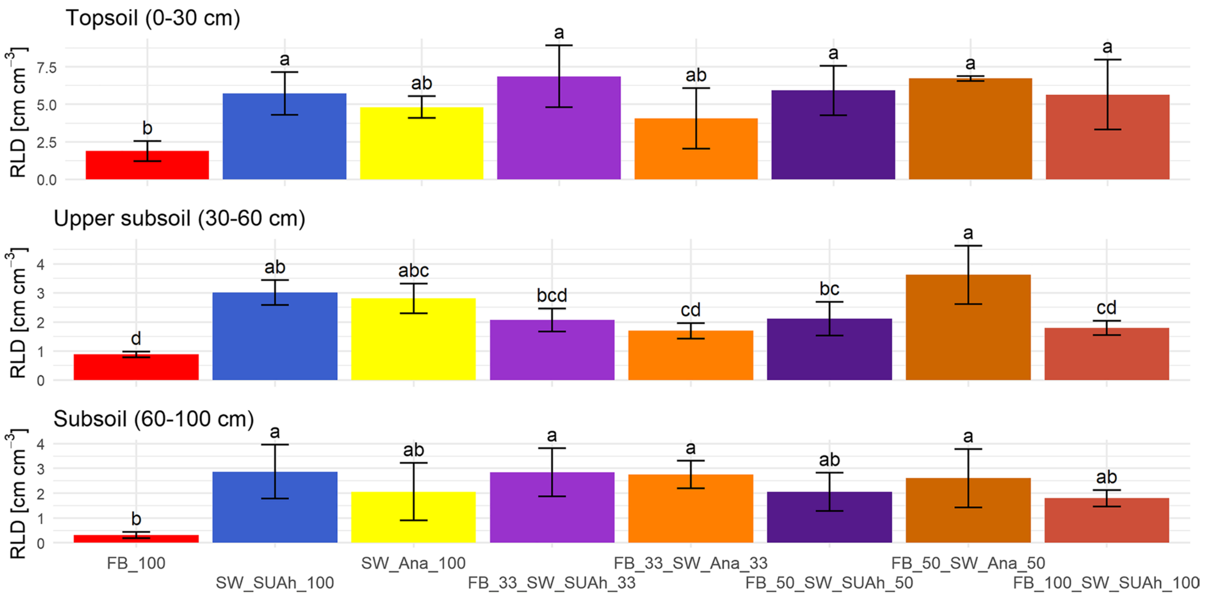

Fig. 6 Mean values ± standard error ($n = 4$) of root length density (not crop-specific) in cm cm^{-3} (RLD), for sole faba bean and sole spring wheat, as well as for the mixtures treatments for cumulated three soil layers in 05/07/2021. Different letters indicate significant differences (Anova and Tukey post-hoc test, $\alpha = 0.05$). Error bars refer to the standard deviation. Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

Sowing density effect on root growth advantage and facilitation and competition

The spatial arrangement in intercropping is an important factor for the above- and belowground growth (Wang et al. 2018; Homulle et al. 2022). In our study, the spatial arrangement was represented by the sowing density that characterized the designs considered in the study, as well as by the completely mixed design or adjacent row design which permitted a high interaction between the species (Homulle et al. 2022; Li et al. 2006). The high sowing density in the additive design resulted in low root biomass over the whole soil profile (Table S6). and enhanced plant-plant competition between faba bean and spring wheat in both growing stages.

In a sole cropped spring wheat experiment, Hecht et al. (2016) found that RLD increased with increasing sowing density in the topsoil (0–10 cm), partly due to greater production of fine roots. The authors argued that light competition forced plants to grow more shoot mass at the cost of investment into roots, in our study an increased sowing density fostered RLD only at the first sampling date and only in 0–10 cm soil depth. However, for the second date there was a decrease of total RLD with increasing *TSD*. Bulson et al. (1997) reported a significant decrease in resource complementarity with increasing wheat and faba bean sowing density. The presented low attained root mass compared to the expected values in the high sowing density treatment (additive design, *TSD* = 200%) indicates high competition under the high sowing density of the additive design.

Cultivar effect on belowground growth and interactions in intercrops

Although statistically there was no significant effect of the cultivar on the root mass, we observed a difference in rooting ability between both spring wheat cultivars (Figs. 1, 2, 5 and 6). The ability of *cv.* Anabel to root quickly and deeply around faba bean flowering as

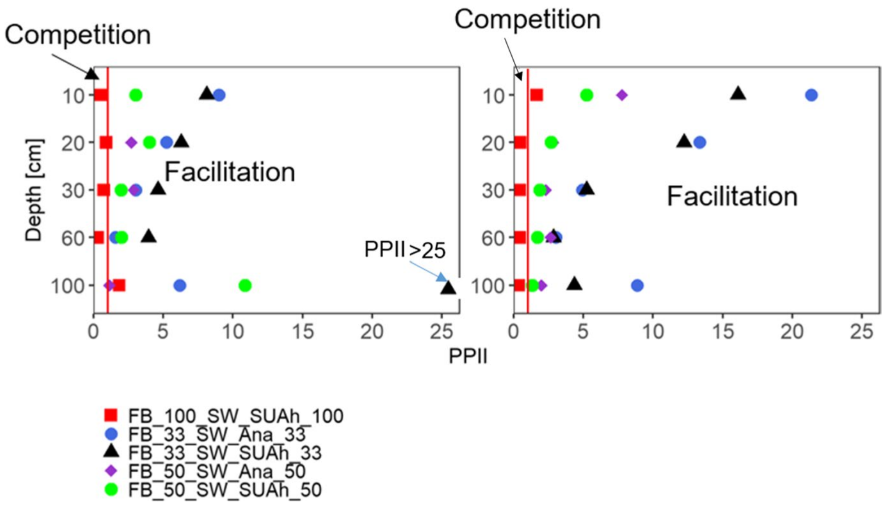

Fig. 7 The mean PPII is shown for each soil depth. The area where PPII > 1 indicates facilitation between the two species. The area where PPII < 1 indicates competition between the two species. The red line shows PPII = 1, indicating a neutral effect. The mean PPII was calculated as the mean of PPII across treatment´s replicates ($n=4$) for the sampling dates in 09/06/2021 (left panel) and 05/07/2021 (right panel). X axis was cut in the value 25, data points > 25 are shown directly after the value 25. Treatment abbreviations: FB_100 = Sole crop faba bean Fanfare, SW_SUAh_100 = Sole crop spring wheat SU Ahab, SW_Ana_100 = Sole crop spring wheat Anabel, FB_33_SW_SUAh_33 = Intercrop Fanfare (SD = 33%) x SU Ahab (SD = 33%), FB_33_SW_Ana_33 = Intercrop Fanfare (SD = 33%) x Anabel (SD = 33%), FB_50_SW_SUAh_50 = Intercrop Fanfare (SD = 50%) x SU Ahab (SD = 50%), FB_50_SW_Ana_50 = Intercrop Fanfare (SD = 50%) x Anabel (SD = 50%), FB_100_SW_SUAh_100 = Intercrop Fanfare (SD = 100%) x SU Ahab (SD = 100%))

compared to *cv.* SU Ahab resulted in lower root mass proportions of faba bean intercropped with *cv.* Anabel compared to intercropped with *cv.* SU Ahab. Moreover, comparing the root growth patterns in intercrops and sole crops in two different growth stages (flowering of wheat and flowering of bean), permitted to better understand the cultivar effect of root growth dynamics in intercrops. Other studies only considered studying root growth around flowering (Streit et al. 2019), where it is assumed that the species reach their maximum root mass (Chirinda et al. 2012). In our study, we found that the early dominance of one spring wheat cultivar (*cv.* Anabel) impacted negatively faba bean root growth in intercrops.

Soil mineral N, soil water, and root carbon and nitrogen in sole crops and intercrops

Soil mineral N below the faba bean at harvest time are usually higher than below cereals (Neugschwandtner et al. 2015), this was not confirmed in our study. For the upper soil layer (0–30), the Nmin in sole crop treatments was higher below faba bean than below spring wheat (both cultivars). However, in the subsoil layers (60–100), Nmin below faba bean sole crops was higher than the one below spring wheat sole crops (both cultivars). This could be attributed to the low RLD of faba bean in deeper layers which decreased the N uptake (Kage 1997). In intercrops, the mineral N content in the topsoil after harvest was greater than in both sole crops, indicating a difference in N uptake rate between intercrops and sole crops. In a long-term experiment, an increase of topsoil organic N content by 11% was observed in intercropping as compared to sole cropping, indicating that increased biological N fixation contributed to increased soil N content (Cong et al. 2015). Moreover, it is widely recognized that N uptake is mainly performed by the fine roots (McCormack et al. 2017). This was also indicated by our study where for the low density treatments with high SRL (higher fine roots compared to the high *TSD* treatment), the N uptake was greater than in the high density treatments.

Plant diversity also affects soil organic C stocks in deeper soil which is more stable and difficult to access for microbes (Chen et al. 2020). Hence, root-based C inputs in deeper soil layers is the major source of soil organic carbon (Yu et al. 2022). We observed no significant effect of intercropping on root C in the deep soil layer (30–100 cm, date 05/07/2021, Fig. S12). In the deeper soil layers (30–100 cm), total C in roots in the mixtures was on average 22% greater than the average root C in sole faba bean and 18% lower than average root C in sole spring wheat (mean of both cultivars), providing a possible mechanism for the divergence in soil C sequestration between sole crops and intercropping systems. Similar trends were observed by Cong et al. (2015).

Characterization of soil water depletion at different soil layers below the root zone is important in evaluating water use pattern and its linkage to the RLD (Moroke et al. 2005). Our results didn´t confirm the positive correlation between RLD and soil water depletion already reported in other studies (Moroke et al. 2005; Zhang et al. 2020).This can be explained by the non-significant differences between the intercrop treatments in term of RLD, found in our study (Figs. 5 and 6).

Implication of the results to better understand intercrops and their belowground interactions

In our study, LER in the fully replacement design revealed that intercropping was favorable to increase the aboveground biomass and yield. The overyielding in terms of yield and aboveground biomass found in this study was already reported in many other contexts. Many studies argue the importance of studying roots in intercrops to better understand the belowground mechanisms that increase their productivity and allow a better resource capture (Ma et al. 2019; Homulle et al. 2022). We demonstrated that high sowing densities of the additive design led to decreased root mass, RLD and SRL and also to competition between the intercropped species which resulted in lower grain yield value compared to the expected one. The early root dominance of spring wheat cultivar was not beneficial for the grain yield. When resources such as soil water become scarce, this may lead to a decreased resource capture. We found that lower sowing densities (i) led to a lower depletion of soil water in the deeper soil layers, (ii) fostered deeper rooting, (iii) led to a depletion of more N in the upper soil layers, and (iv) fostered higher SRL and thus potentially enhanced root N uptake as compared to high density intercrops. Comparing intercropping with sole cropping but also different sowing densities within the intercropping system revealed that there were different depth-dependent processes occurring belowground that affected not only root biomass but also soil mineral N and soil water content and thus their plant availability. Thus, an improved understanding of the effects of the species (or cultivar) combination and the crop management on root growth are essential for better understanding interactions and productivity in intercrops.

Conclusion

In our study, belowground root growth and interactions varied with the different intercropping designs and spring wheat cultivars considered in the study. On both sampling stages, the belowground intercrop advantage decreased under high sowing density due to plant-plant competition. Intercropping of faba bean with a spring wheat cultivar characterized by a rather small root system during faba bean flowering fostered a higher belowground intercrop advantage, as facilitation dominated the plant-plant interactions in intercrops under lower and optimal sowing densities in both growing stages. Further research should focus on finding the optimal sowing density that can enhance aboveground root advantage and improve the facilitation process permitting optimal resource capture and depletion. The effect of spring wheat cultivar choice, although insignificant in this study, seems to have an effect on the total root mass and belowground interactions in intercrops, a generalization of the results should be further researched in the frame of breeding experiments.

Also, we suggest to conduct a similar study under limited growth conditions and with several sampling dates to better assess the relationship between above- and belowground overyielding and support the generalization of the obtained results. Moreover, there is a need to explore the effects of mixtures on soil C and N sequestration to mitigate climate change.

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Author contributions S.S and T.D. conceived the idea, planned the research and designed the experiments. S.S., D.D and M.P conducted the experiment in the field, S.H. and S.S collected the root samples. N.L. processed the root samples and performed the FTIR analysis. S.H. analyzed the data and wrote the article. O.W., T.G., N.L., F.E, E.J., R.K., M.P., and S.S. contributed to data interpretation, writing and editing of the article. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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

References

Amossé C, Jeuffroy M-H, David C (2013) Relay intercropping of legume cover crops in organic winter wheat: effects on performance and resource availability. Field Crops Res 145:78–87

Barej J, Pätzold S, Perkons U, Amelung W (2014) Phosphorus fractions in bulk subsoil and its biopore systems. Eur J Soil Sci 65:553–561

Bargaz A, Isaac ME, Jensen ES, Carlsson G (2015) Intercropping of faba bean with wheat under low water availability promotes faba bean nodulation and root growth in deeper soil layers. Procedia Environ Sci 29:111–112. https://doi.org/10.1016/j.proenv.2015.07.188

Bedoussac L, Journet E-P, Hauggaard-Nielsen H et al (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agron Sustain Dev 35:911–935. https://doi.org/10.1007/s13593-014-0277-7

Brooker RW, Bennett AE, Cong W et al (2015) Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytol 206:107–117. https://doi.org/10.1111/nph.13132

Bulson H, Snaydon R, Stopes C (1997) Effects of plant density on intercropped wheat and field beans in an organic farming system. J Agric Sci 128:59–71

Chen X, Chen HY, Chen C et al (2020) Effects of plant diversity on soil carbon in diverse ecosystems: a global meta-analysis. Biol Rev 95:167–183

Chirinda N, Olesen JE, Porter JR (2012) Root carbon input in organic and inorganic fertilizer-based systems. Plant Soil 359:321–333

Cong W-F, Hoffland E, Li L et al (2015) Intercropping enhances soil carbon and nitrogen. Glob Change Biol 21:1715–1726. https://doi.org/10.1111/gcb.12738

Corre-Hellou G, Crozat Y (2005) Assessment of root system dynamics of species grown in mixtures under field conditions using herbicide injection and 15 N natural abundance methods: a case study with pea, barley and mustard. Plant Soil 276:177–192

De Wit C, Van den Bergh J (1965) Competition between herbage plants. J Agric Sci 13:212–221

Demie DT, Döring TF, Finckh MR, van der Werf W, Enjalbert J, Seidel SJ (2022) Mixture× genotype effects in cereal/legume intercropping. Front Plant Sci 13:846720

Den Hollander NG, Bastiaans L, Kropff MJ (2007) Clover as a cover crop for weed suppression in an intercropping design: II. Competitive ability of several clover species. Eur J Agron 26:104–112

Döring TF, Elsalahy H (2022) Quantifying compensation in crop mixtures and monocultures. Eur J Agron 132:126408. https://doi.org/10.1016/j.eja.2021.126408

Fargione JE, Tilman D (2005) Diversity decreases invasion via both sampling and complementarity effects. Ecol Lett 8(6):604–611

Gan YT, Campbell CA, Janzen HH et al (2009) Root mass for oilseed and pulse crops: growth and distribution in the soil profile. Can J Plant Sci 89:883–893

Gregory PJ, Palta JA, Batts GR (1995) Root systems and root: mass ratio-carbon allocation under current and projected atmospheric conditions in arable crops. Plant Soil 187:221–228

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

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES (2008) Grain legume–cereal intercropping: the practical application of diversity, competition and facilitation in arable and organic cropping systems. Renew Agric Food Syst 23:3–12

Hecht VL, Temperton VM, Nagel KA et al (2016) Sowing density: a neglected factor fundamentally affecting Root distribution and biomass allocation of field grown spring barley (Hordeum Vulgare L). Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.00944

Hector A, Bazeley-White E, Loreau M, Otway S, Schmid B (2002) Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. Ecol Lett 5(4):502–511

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

Jensen ES, Carlsson G, Hauggaard-Nielsen H (2020) Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: a global-scale analysis. Agron Sustain Dev 40:1–9

Justes E, Bedoussac L, Dordas C et al (2021) The 4 C approach as a way to understand species interactions determining intercropping productivity. Front Agric Sci Eng 8:3

Kage H (1997) Is low rooting density of faba beans a cause of high residual nitrate content of soil at harvest? Plant Soil 190:47–60

Kemper R, Döring TF, Legner N et al (2022) Root traits in cover crop mixtures of blue lupin and winter rye. Plant Soil 475:309–328. https://doi.org/10.1007/s11104-022-05366-9

Kremen C, Miles A (2012) Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecol Soc 17(4)

Legner N, Meinen C, Rauber R (2018) Root differentiation of agricultural plant cultivars and proveniences using FTIR spectroscopy. Front Plant Sci 9:748

Li L, Sun J, Zhang F et al (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290

Li L, Sun J, Zhang F (2011) Intercropping with wheat leads to greater root weight density and larger below-ground space of irrigated maize at late growth stages. Soil Sci Plant Nutr 57:61–67. https://doi.org/10.1080/00380768.2010.548307

Li L, Zhang L, Zhang F (2013) Crop mixtures and the mechanisms of overyielding. Encycl Biodivers 2:382–395

Lithourgidis AS, Dordas CA, Damalas CA, Vlachostergios DN (2011) Annual intercrops: an alternative pathway for sustainable agriculture. Aust J Crop Sci 5(4):396–410

Ma Z, Chen HY (2016) Effects of species diversity on fine root productivity in diverse ecosystems: a global meta-analysis. Glob Ecol Biogeogr 25:1387–1396

Ma L, Li Y, Wu P et al (2019) Effects of varied water regimes on root development and its relations with soil water under wheat/maize intercropping system. Plant Soil 439:113–130

McCormack ML, Guo D, Iversen CM et al (2017) Building a better foundation: improving root-trait measurements to understand and model plant and ecosystem processes. New Phytol 215:27–37. https://doi.org/10.1111/nph.14459

Meinen C, Rauber R (2015) Root discrimination of closely related crop and weed species using FT MIR-ATR spectroscopy. Front Plant Sci 6:765

Moroke T, Schwartz R, Brown K, Juo A (2005) Soil water depletion and root distribution of three dryland crops. Soil Sci Soc Am J 69:197–205

Naumann A, Heine G, Rauber R (2010) Efficient discrimination of oat and pea roots by cluster analysis of fourier transform infrared (FTIR) spectra. Field Crops Res 119:78–84

Nelson WCD, Siebrecht-Schöll DJ, Hoffmann MP et al (2021) What determines a productive winter bean-wheat genotype combination for intercropping in central Germany? Eur J Agron 128:126294

Neugschwandtner R, Ziegler K, Kriegner S et al (2015) Nitrogen yield and nitrogen fixation of winter faba beans. Acta Agric Scand Sect B—Soil Plant Sci 65:658–666

Obalum SE, Obi ME (2010) Physical properties of a sandy loam Ultisol as affected by tillage-mulch management practices and cropping systems. Soil Tillage Res 108(1-2):30–36

Paul MR, Demie DT, Seidel SJ, Döring TF (2024) Evaluation of multiple spring wheat cultivars in diverse intercropping systems. Eur J Agron 152:127024

R Core Team (2018) R: eine sprache und umgebung für statistisches rechnen. R stiftung für statistisches rechnen, Wien. https://www.R-project.org

Rengasamy JI, Reid JB (1993) Root system modification of faba beans (Vicia faba L.), and its effects on crop performance. 1. Responses of root and shoot growth to subsoiling, irrigation and sowing date. Field Crops Res 33(3):175–196

Rewald B, Meinen C, Trockenbrodt M et al (2012) Root taxa identification in plant mixtures–current techniques and future challenges. Plant Soil 359:165–182

Shao H, Shi D, Shi W, Ban X, Chen Y, Ren W, Chen F, Mi G (2019) Genotypic difference in the plasticity of root system architecture of field-grown maize in response to plant density. Plant Soil 439:201–217

Sharma N, Singh RJ, Mandal D et al (2017) Increasing farmer's income and reducing soil erosion using intercropping in rainfed maize-wheat rotation of Himalaya, India. Agric Ecosyst Environ 247:43–53

Shili-Touzi I, De Tourdonnet S, Launay M, Doré T (2010) Does intercropping winter wheat (Triticum aestivum) with red fescue (Festuca rubra) as a cover crop improve agronomic and environmental performance? A modeling approach. Field Crops Res 116:218–229

Snaydon RW (1991) Replacement or additive designs for competition studies? J Appl Ecol 930–946

Stomph T, Dordas C, Baranger A, de Rijk J, Dong B, Evers J, Gu C, Li L, Simon J, Jensen ES, Wang Q, Wang Y, Wang Z, Xu H, Zhang C, Zhang L, Zang WP, Bedoussac L, van der Werf W (2020) Designing intercrops for high yield, yield stability and efficient use of resources: are there principles? Adv Agron 1–50

Streit J, Meinen C, Nelson WCD et al (2019) Above- and belowground biomass in a mixed cropping system with eight novel winter faba bean genotypes and winter wheat using FTIR spectroscopy for root species discrimination. Plant Soil 436:141–158. https://doi.org/10.1007/s11104-018-03904-y

Tosti G, Thorup-Kristensen K (2010) Using coloured roots to study root interaction and competition in intercropped legumes and non-legumes. J Plant Ecol 3:191–199. https://doi.org/10.1093/jpe/rtq014

Turpin JE, Herridge DF, Robertson MJ (2002) Nitrogen fixation and soil nitrate interactions in field-grown chickpea (Cicer arietinum) and fababean (Vicia faba). Aust J Agric Res 53:599–608

Vandermeer JH (1989) The ecology of intercropping. Cambridge University Press, Cambridge

Wang Y, Qin Y, Chai Q et al (2018) Interspecies interactions in relation to root distribution across the rooting profile in wheat-maize intercropping under different plant densities. Front Plant Sci 9:483

Wechsung G, Wechsung F, Wall GW et al (1995) Biomass and growth rate of a spring wheat root system grown in free-air CO2 enrichment (FACE) and ample soil moisture. J Biogeogr 22:623–634

White KE, Reeves JB III, Coale FJ (2011) Mid-infrared diffuse reflectance spectroscopy for the rapid analysis of plant root composition. Geoderma 167:197–203

Willey R, Osiru D (1972) Studies on mixtures of maize and beans (Phaseolus vulgaris) with particular reference to plant population. J Agric Sci 79:517–529

Xia H-Y, Zhao J-H, Sun J-H 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

Yang H, Xu H, Zhang W et al (2022) Overyielding is accounted for partly by plasticity and dissimilarity of crop root traits in maize/legume intercropping systems. Funct Ecol 36:2163–2175

Yu Y, Stomph T-J, Makowski D et al (2016) A meta-analysis of relative crop yields in cereal/legume mixtures suggests options for management. Field Crops Res 198:269–279. https://doi.org/10.1016/j.fcr.2016.08.001

Yu R-P, Yang H, Xing Y et al (2022) Belowground processes and sustainability in agroecosystems with intercropping. Plant Soil 476:263–288

Zhang X, Whalley P, Ashton R et al (2020) A comparison between water uptake and root length density in winter wheat: effects of root density and rhizosphere properties. Plant Soil 451:345–356

Zhang W, Gao S, Li Z et al (2021) Shifts from complementarity to selection effects maintain high productivity in maize/legume intercropping systems. J Appl Ecol 58:2603–2613. https://doi.org/10.1111/1365-2664.13989