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Second Cycle Degree (MSc) in

Sustainable Agriculture

Root growth in biopores of spring wheat, faba bean and intercropping system

Supervisor
Prof. Nicola Dal Ferro

Co-supervisor
Prof. Thomas Döring

Submitted by
Zia Ullah
Student no.
2004956

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Declaration

I affirm that I have independently prepared this thesis. Any parts of the text taken verbatim or analogously from published or unpublished sources are duly acknowledged. The thesis has not been submitted in the same or similar form, or in extracts, within the context of any other examination.

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Zia Ullah

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CKA	Campus Klein-Altendorf
ha	Hectare
RLD	Root length density
%	Percent
BPs	Biopores
N	Nitrogen
SW	Spring wheat
FB	Faba bean
Mix	Mixture
RCBD	Randomized complete block design
m	Meter
LER	Land equivalent ratio
pLER	Partial land equivalent ratio

Abstract

Enhancing root systems, especially through utilization of biopores, can improve crop access to essential resources as climate change effects intensify. In this context, intercropping, also known as mixed cropping emerges as a promising technique to facilitate root growth into deeper soil layers, optimizing water and nutrient extraction and providing a resilient and sustainable solution to agricultural challenges. While crop mixtures have the potential to increase yield compared to sole crops, little is known about their impact on root growth in biopores. Therefore, a study was conducted with the aim of quantifying the root length density and biopore usage of spring wheat and faba bean in pure stands and mixtures. The trial was established at the Campus Klein-Altendorf (CKA), the experimental research farm of the University of Bonn in Germany. Crops were analyzed for root mass, root length density (RLD), biopore usage by roots, and yield parameters. Roots were sampled by soil monolith and profile wall methods up to 70 and 100 cm, respectively. The mean RLD in mixture was highest in top soil (10-20 cm) while for lower depth levels of 40-70 cm, spring wheat exhibited significantly higher root growth than faba bean. Meanwhile, in biopores, faba bean root share was higher than spring wheat and mixture. Mixture showed lowest root share in biopores at 40-50 cm but this share was higher than spring wheat at 60-70 cm, but differences were not statistically significant. The results indicated that the faba bean uses biopores more excessively for root growth than spring wheat. Values of Land Equivalent Ratio (LER) index >1 confirmed the productivity advantage of mixture over sole crops. Overall, the results indicated better yet insignificant root growth for mixtures in biopore and higher crop yield in mixtures.

1. Introduction

Climate change poses significant challenges to agriculture, particularly in terms of water and nutrient availability for crops. As the effects of climate change intensify, it is increasingly important to develop strategies that address these limitations and enhance the root systems of plants. By improving the ability of roots to penetrate into deeper layers, especially through the utilization of biopores, we can provide crops with greater access to essential resources. In this context, intercropping emerges as a promising technique with the potential to facilitate root growth into deeper soil layers, thereby enabling the extraction of water and nutrients necessary for optimal crop development. By exploring the benefits of intercropping/mix cropping and its impact on productivity, we can pave the way for more resilient agricultural practices in the face of climate change.

In order to address the challenge of limited access to water and nutrients in the subsoil, intercropping presents itself as a potential solution. Specifically, intercropping cereals and legume plants, such as spring wheat and faba bean, can offer a promising approach. By sowing these two crops in both sole (single crop) and mix (intercropped) conditions, we can investigate the root growth in biopores and the effect of intercropping. When cereals and legume plants are intercropped, they create a mutually beneficial relationship. The legume plants, known for their nitrogen-fixing abilities, can enrich the soil by converting atmospheric nitrogen into a form usable by plants.

By studying the growth of spring wheat and faba bean in both sole and mix conditions, we can gain insights into the advantages of mix cropping. This approach aims to enhance the root systems of both crops, enabling them to exploit the resource depots in the subsoil more efficiently. By accessing deeper layers, the intercropped plants can tap into additional water and nutrients, potentially leading to improved yields and overall crop performance.

1.1 Biopores and Their Function

Round-shaped biopores (BPs) are formed by crop roots (Han et al., 2015) and soil-dwelling organisms, as well as soil cracks in the structured soil (Stirzaker et al., 1996; Jakobsen and Dexter 1988; Kautz et al., 2014). Typically, the term “biopores” is used to describe the pores larger than 2 mm, but some authors have also included smaller pores with diameter less than 2 mm (e.g. Volkmar, 1996). They may be stable for decades in the subsoil below the plough layer (Hagedorn and Bundt, 2002) and play a key role in mobilizing active nutrients (Kautz et al., 2013a).

The soil contains natural biopores that serve a variety of functions (Kautz, 2015). Biopores facilitate the movement of water and solutes (Edwards et al., 1990; Naveed et al., 2016) as well as air (Dziejowski et al., 1997) through the soil. Oxygen is transported from the soil surface to the deeper soil layers through the soil matrix mainly by the diffusion of gases (Craul, 1992). The concentration of oxygen in the soil air generally decreases with depth due to the roughness and length of the diffusion pathway (Craul, 1992; Lal and Shukla, 2004). While, vertical continuous and large sized-biopores offer straight path for diffusion and convection, ensuring comparatively stable oxygen concentration in these biopores throughout the soil profile (Hillel 1998; Glinski and Lipiec, 1990).

In compacted soil conditions, biopores can serve as a path of least resistance for roots to penetrate the subsoil (Athmann et al., 2013; Colombi et al., 2017; White and Kirkegaard, 2010). The biopore sheath is usually rich in nutrients as a result of root exudation and the effect of root decay (Pierret et al., 1999). Biopores are therefore beneficial for roots to absorb water and nutrients from the subsoil (Han et al., 2017; Kautz, 2015; Mckenzie et al., 2009).

Similarly, crops depend on an extensive root system to acquire nutrients from the subsoil (Lammerts van Bueren et al., 2002). Studies have shown that fodder crops with deep taproots can enhance biopore formation and increase biopore density (BPD) (BPD: number of biopores BP per unit area) in subsoil (McCallum et al., 2004; Kautz et al., 2014), that might enhance plant potential to acquire subsoil resources (Kautz, 2014). The recent studies reported on increased rooting density (Perkons et al., 2014) of winter barley and improved water uptake by spring wheat as a function of increased number of biopores (Gaiser et al., 2012).

1.2 Root Growth in Biopores

Plant roots preferentially elongate through the round-shaped biopores (Arora et al., 2011; Atkinson et al., 2020; Colombi et al., 2017) that can offer access to subsoil water (McKenzie et al., 2009), and nutrients (Kuzyakov and Blagodatskaya, 2015), which is advantageous especially in droughts (Gaiser et al., 2012).

The initial observations that roots preferentially propagate through biopores have been confirmed by multiple studies (Kopke, 1981, and Nakamoto, 1997). For this preference several reasons have been identified. For example, the root system is usually hindered by the soil compaction (Bengough et al., 2011; Correa et al., 2019), while, the biopores offer favorable pathways for plant roots to bypass compacted soil and penetrate deeper soil layers (Atkinson et al., 2020; Colombi e al., 2017). This is of particular importance because soil compaction has been identified as a major limitation to soil exploration by roots (Hoad et al., 2001; Lynch and Wojciechowski, 2015), also slows down root growth and elongation when roots growing through the bulk soil (Bengough, 2012).

1.3 Cereal Legume Mixed Cropping

The ecological performance of cropping systems has been enhanced by a variety of practices, such as intercropping and crop rotation (Wezel et al., 2014). Growing two or more crops simultaneously on the same piece of land is known as intercropping (Willey, 1990; Lithourgidis et al., 2011), and has the potential to improve sustainable agriculture (Maitra et al., 2021). It has three types: relay, strip and mixed intercropping (Li et al., 2013b). Intercropping systems commonly involves growing of cereals and legumes in mixed combinations (Connolly et al., 2001).

In the widespread practice of cereal/legume mixed cropping in natural ecosystem, legume is one of the key species in promoting ecosystem efficiency (Altieri, 1999; Anil et al., 1998; Malézieux et al., 2009; Vandermeer, 1995; Vandermeer et al., 1998). Their ability to fix nitrogen (N) is a key factor in justifying its use in organic farming, which makes them valuable in intercropping system (Bedoussac et al., 2015). Intercropped legumes have proved to be capable of providing a wide range of additional services and of producing substantially higher yields than a sole crop (Willey, 1979).

1.4 Problem statement

The limited understanding of the mixture effect on root growth development in cereal and legume (specifically wheat-faba bean) mixtures poses a problem. While these mixtures have the potential to increase yield compared to sole crops, little is known about their impact on root growth in biopores. Biopores play a crucial role in nutrient uptake, but their response to mixtures remains unclear. Investigating the mixture effect on root growth in biopores is essential for optimizing agricultural practices and improving crop performance. This study aims to uncover the role of biopores in yield improvement in spring wheat-faba bean mixture by evaluating root growth in biopores and back soil. Addressing this knowledge gap is vital for enhancing crop productivity and resource utilization.

1.5 Objectives:

a. To quantify and compare the root length density of spring wheat and faba bean in pure stands and in mixture.
b. Quantifying biopore usage by spring wheat and faba bean in pure stand and in mixtures

2. Material and Methods

2.1 Experimental Site

The field experiment was conducted at Campus Klein-Altendorf (CKA), the experimental research farm of the University of Bonn in Germany in spring season of 2022. It is a conventionally managed research station located at Rheinbach (50° 37′ 9″ N, 6° 59′ 29″ E), North Rhine-Westphalia, at altitude of 186 m above sea level, approximately 40 km south of Cologne. The soil type is a fertile Haplic Luvisol derived from loess with a loamy silt texture (IUSS Working Group WRB 2006). Mean annual temperature is 9.5 °C with a mean annual precipitation of 606 mm (https://www.aussenlabore.uni-bonn.de). The weather conditions during the experimental year (2022) are shown in Figure 1.

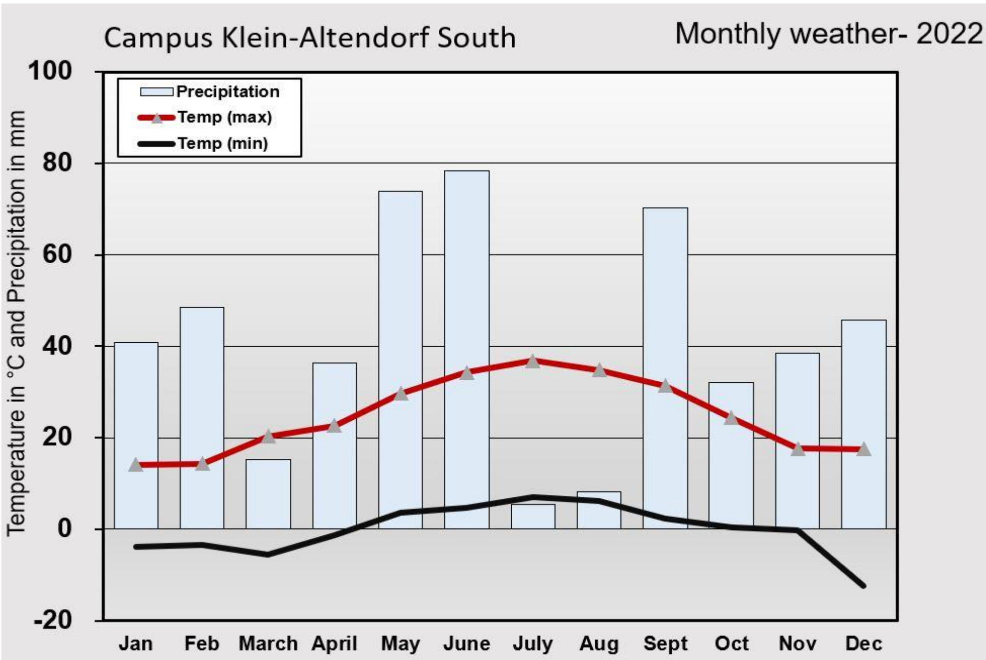


Figure 1: Overview of weather data (precipitation, Maximum and Minimum temperature during experimental year (2022). Source (weather station CKA)

2.2 Treatments

In the year 2022, an individual experimental field served as the platform for cultivating two distinct crop species: spring wheat and faba bean. The sowing was done in April, with careful consideration given to the sowing densities for each crop. Spring wheat (SW) was sown at a density of 400 seeds/m², faba bean (FB) at 40 seeds/m², and a combination of the two, known as the mixture, at a density of 200 seeds/m² for (SW) and 20 seeds/m² for (FB). Manual weeding was carried out twice a month, effectively managing the unwanted plant species that could potentially hinder the growth and yield of the crops and their roots could mix with the roots of the crops during root sampling.

There were four blocks labeled as A, B, C, and D with three treatments (spring wheat, faba bean and their mixture), resulting in a total of twelve plots. The trial covered an area of 180 m^2. A randomized complete block design (RCBD) was adopted for this trial. The seeding rate of both sole crops and combination in the mixture is written below:

1. **Winter Wheat**
   SW 400 seeds/m^2
2. **Faba Bean**
   FB 40 seeds/m^2
3. **Winter Wheat/Faba Bean mixture**
   (SW 200 seeds + FB 20 seeds) / m^2

2.3 Field Experiment Layout

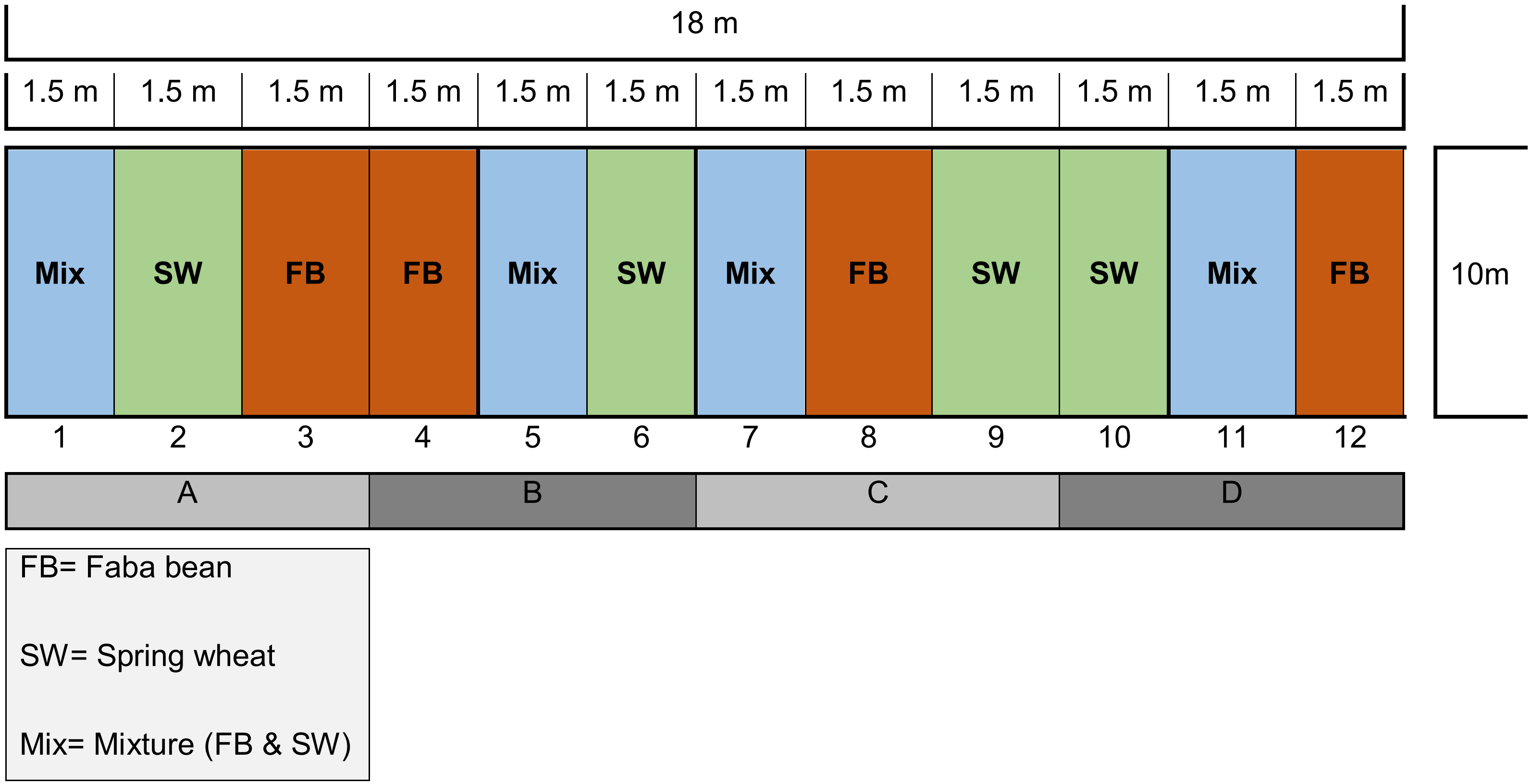


Figure 2: Layout of field experiment at Campus Klein-Altendorf, consisting of four blocks (A, B, C and D), including three treatments of spring wheat (SW), faba bean (FB) and mixture (Mix), resulting in a total of twelve plots.

2.4 Equipment and Software

Tools/Equipment/Software	Method/Purpose
Metallic frame (100 cm x 100 cm) Crop sprayer Birchmeier REC 15 (battery-operated, with round jet nozzle, maximum 3 bar) Spade Tap water (for sprayer)	Profile wall
Buckets Metallic sieves Tap water Tweezers	Root washing
Aryclic glass platter Scanner Epson Perfection V700 Photo Software Epson Scan 3.9.2.1. DE Tap Water Tweezers	Root scanning
RootPainter program	Root image analysis
Vacuum cleaner Palette-knifes	Biopores opening and roots collection

2.5 Root sampling

The following methodology was used to analyze biopores in soil samples. A trench measuring 1.5 meters long and 1 meter deep was initially created across the experimental field, running perpendicular to the rows of plants, using an excavator (Figure 3). Firstly, a plane of 10x40 cm was smoothed in 10 cm depth, and two monoliths measuring 20 cm long, 10 cm wide, and 10 cm high were selected. The plane of 10x40 cm (Figure 4) was then smoothed in 40 cm depth, and a vacuum cleaner was used to uncover biopores. Two areas of 20x10 cm (monolith) were marked, and biopores were categorized into two classes, Small (3-5 mm) and Large (>5 mm). All biopores were marked on transparent plastic using different colored markers for the two categories and were numbered. The biopores in the soil were labeled with needles, and named after the pore number (and plot, depth, replicate). The bulk soil was collected in a bucket and stored in a plastic bag for later root washing. Biopores were then opened with a micro-spoon/micro-spatula, and all roots were removed with tweezers, stored in a small glass bottle labeled with the individual biopore number. This process was repeated for each layer and plot.

Figure 3: Trench across the experimental field measuring 1.5 m wide and 1 m deep. Source (by Zia Ullah and Arslan) (1st June 2022) (Location: Campus Klein-Altendorf)

Figure 4: (a) The smoothened surface measuring 10 x 40 cm at a depth of 40 cm was covered with transparent plastic and labeled for the small and large biopores (2 replications of 10 x 20 cm). (b) Biopores in the soil were labeled with red (>5 mm) and blue (3-5 mm) needles representing the size of biopore. Source (by Zia Ullah and Arslan) (17th June 2022) (Location: Campus Klein-Altendorf)

2.6 Root length density

2.6.1 Profile wall method

The root length density (RLD) was measured using the profile wall method (Böhm 1979) during the anthesis stage. This was done by taking photos of vertical profile with a camera, which were later analyzed using RootPainter software. Therefore, the vertical profile wall was leveled using a spade, perpendicular to the plant rows. Then, a fork was used to scratch the fresh soil and 0.5 cm of soil was then removed from the vertical layer by washing it out with a crop sprayer filled with tap water. A 100 x 100 cm frame (Figure 5) was vertically positioned in the center of a plot, parallel to the horizontal soil surface. The frame was subdivided into six separate frames for six different images, vertically into three (0-35 cm, 35-65 cm, 65-100 cm), and horizontally into two (replicas of 50 cm each).

Figure 5: Measuring RLD (cm/cm^2) by using 100 x 100 cm frame (partitioned into two vertical (50 cm each) and three horizontal portions (35 cm, 30 cm and 35 cm) with profile wall method. Source (by Zia Ullah and Arslan) (Date: 2nd June 2022) (Location: Campus Klein-Altendorf)

2.6.2 Soil Monolith method

Soil monoliths measuring 20 cm in length, 10 cm in width, and 10 cm in depth were collected using spades, scrapers, and hammers (Figure 6). These monoliths were carefully collected in buckets and subsequently transferred into plastic bags at specific depths (10-20 cm, 40-50 cm, 50-60 cm and 60-70 cm). At each depth, two replicates, denoted as 'a' and 'b' in Figure 6, were collected during the soil monolith sampling process. Following collection, all samples were promptly stored in a cold room to maintain soil integrity and preserve their representativeness of the prevailing soil conditions at the time of sampling, facilitating subsequent detailed analysis.

Figure 6: Monolith of size 20 cm (length), 10 cm (width) and 10 cm (depth) were taken at a depth of 10-20 cm, 40-50 cm, 50-60 cm and 60-70 cm. Two replicates (‘a’ and ‘b’) from each plot were taken, resulting in 8 samples per plot (2 replicates and 4 depths). Source (by Zia Ullah and Arslan) (Date: 7th June 2022)

2.7 Washing and sorting of roots

To separate roots from the soil, soil monoliths were soaked in a bucket with tap water and washed by hand using multiple metallic sieves with a minimum 0.50 mm and maximum 4 mm mesh size until all the soil particles washed away and only the roots and some leftover particles from previous crops left in the sieves. Afterwards, roots were sorted with tweezers, taking out the dead roots and non-root particles like compost, and straw leftovers from previous crops. After that, cleaned roots were stored in the freezer until scanning.

2.8 Roots Scanning

To analyze root length and calculate root length density, the roots extracted from the monoliths and biopores were scanned using an Epson Perfection V700 Photo scanner and the compatible software Epson Scan version 3.9.2.1. DE. The root samples from each biopore and monolith were carefully arranged on an acrylic glass platter filled with tap water to ensure they did not overlap, and then scanned using the scanner (figure 7). Subsequently, the scanned images were analyzed using RootPainter software, which facilitated accurate quantification of root length and density.

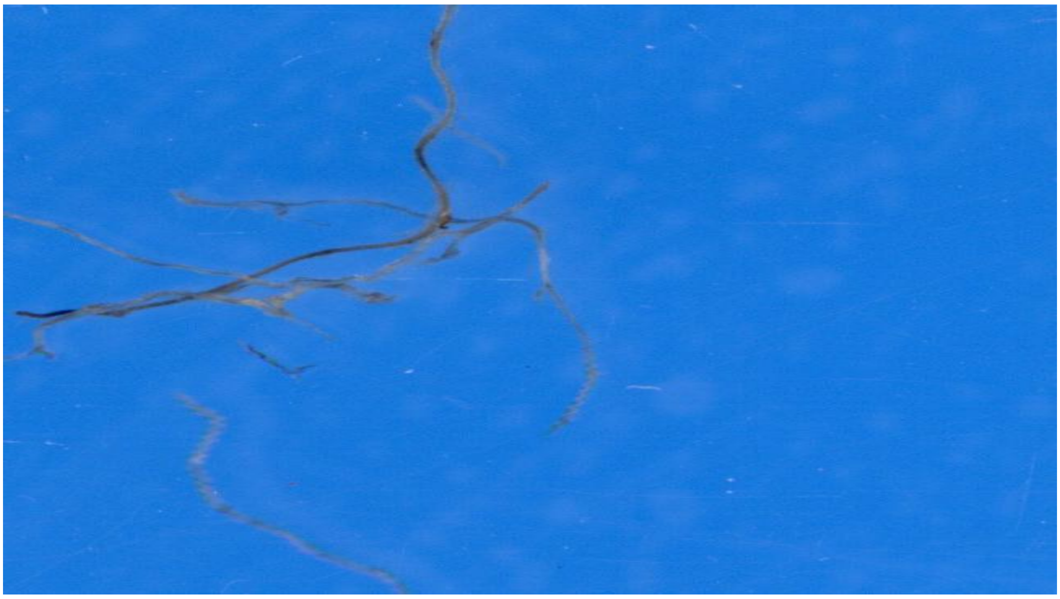
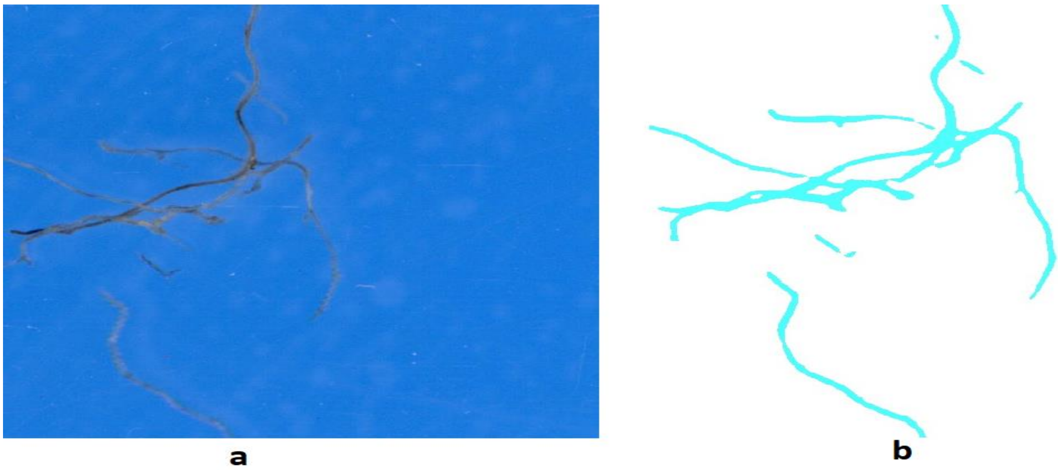


Figure 7: Photo of scanned roots of spring wheat and faba bean

2.9 Root image analysis

Scanned roots were analyzed for root length by using RootPainter software (Smith et al., 2022). Figure 8 shows first the scanned image of roots and then it was annotated by RootPainter program.



a b

Figure 8: **(a)** represents the scanned image of roots, while **(b)** represents the corrective annotation of root image by RootPainter program.

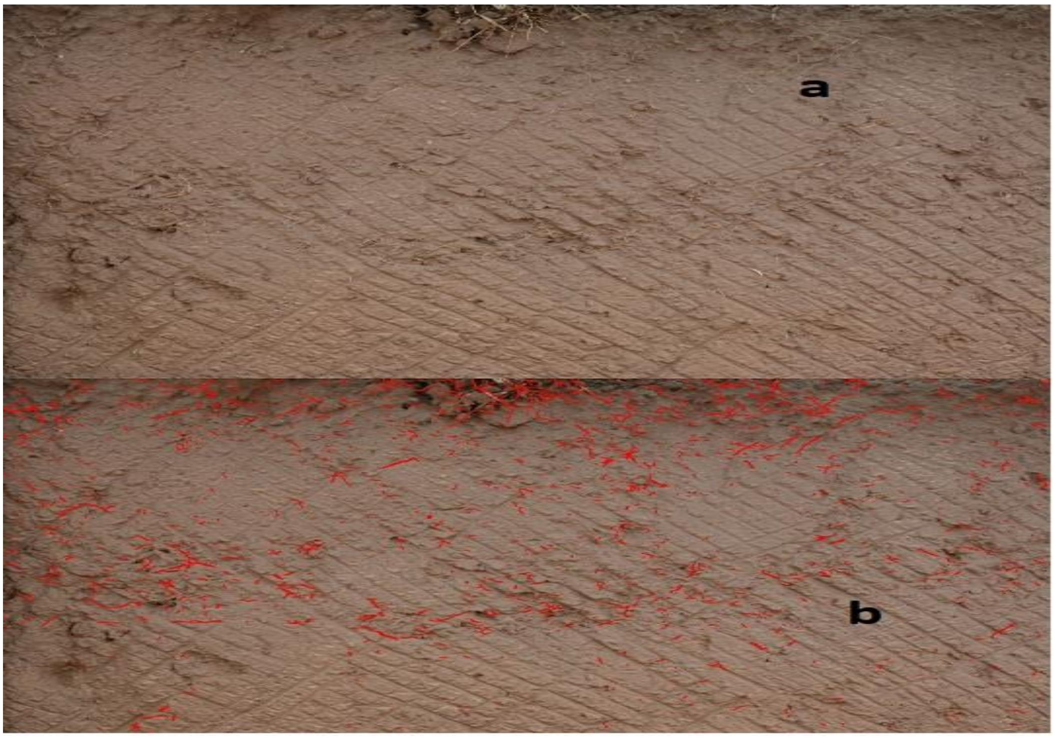


Figure 9: (a) Profile wall Image **(b)** Annotated profile wall image showing roots in red.

2.10 Land Equivalent Ratio (LER)

Shoot performance and grain yield of mixture crops were analysed using the land equivalent ratio (LER) concept (Mead and Willey 1980). LER is the relative land area under sole cropping that is required to produce the same yields as under intercropping. The land use efficiency of an intercrop will be superior to that of corresponding sole crops if LER is> 1. LER is the sum of the partial land equivalent ratios pLER (Eq. 1). Y*i* is shoot dry mass or grain yield in the intercrop of species *i* and M*i* is shoot dry mass or grain yield of the sole crop.

$$\mathrm{LER} = \sum pLERi = \sum \mathrm{Y}i/Mi \qquad (1)$$

2.11 Data analysis and statistics

Data were analyzed with the program R version 4.2.1 with R studio version 1.1.463. Shoot biomass, root biomass, mean of RLD, and root weight in combined soil layers were analyzed by a one-factorial analysis of variance (Anova). After Anova, mean values of treatments were compared with a Tukey test at a significance level of $\alpha = 0.05$.

3. Results

3.1 Root Weight in Biopores

To investigate the root growth in terms of root weight in biopores among distinct crop treatments (spring wheat, faba bean and mixture), across various soil depths (40-50 cm, 50-60 cm and 60-70 cm), was studied (Fig. 10). At the first two depth levels, ranging from 40–50 cm and 50–60 cm, no statistically significant differences in root weight were observed among the three treatments. This suggests that, within the of soil depths (40-60 cm), the treatments exhibited the similar patterns of root growth. While, at the depth of 60–70 cm, a significant difference in root weight were observed within the treatments. Spring wheat demonstrated higher root weight compared to other treatments, while, faba bean exhibited the lowest root weight. The mixture treatment showed intermediate root weight and was not significantly different from sole treatments.

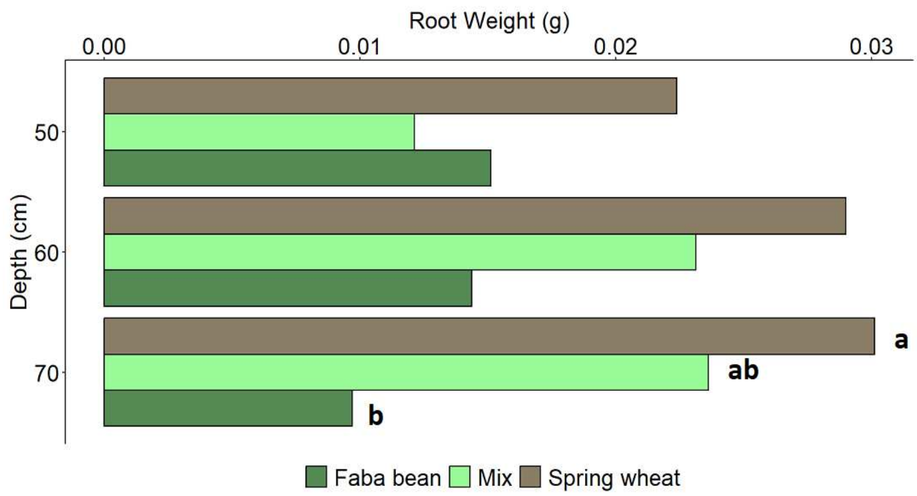


Figure 10: Mean root weight within biopores of three treatments (spring wheat, faba bean, and mixture) at three different depths (40–50 cm, 50–60 cm, and 60–70 cm). The different letters represent the significant differences between the treatments within one layer. Mean values of treatments were compared with a Tukey test at a significance level of $\alpha = 0.05$.

3.2 Root Length Density (RLD)

3.2.1 Monolith

The study was conducted to investigate the impact of different crop treatments- spring wheat, faba bean and the mixture (combination of wheat and faba bean) - on root length density (RLD) across various soil depths (10-20 cm, 40-50 cm, 50-60 cm and 60-70 cm). The root length density (RLD) of crop treatments at different soil depth is depicted in figure 11. At shallow depth, (10–20 cm), no significant differences were observed among the treatments, indicating similar root growth. However, at deeper depths, significant variations were observed. At 40–50 cm, the root length density of spring wheat and mixture was significantly higher compared to faba bean. In the deeper soil layers (50–60 cm and 60–70 cm), the RLD was significantly higher for spring wheat compared to faba bean. However, the mixture showed an intermediate RLD and was not significantly different from sole treatments.

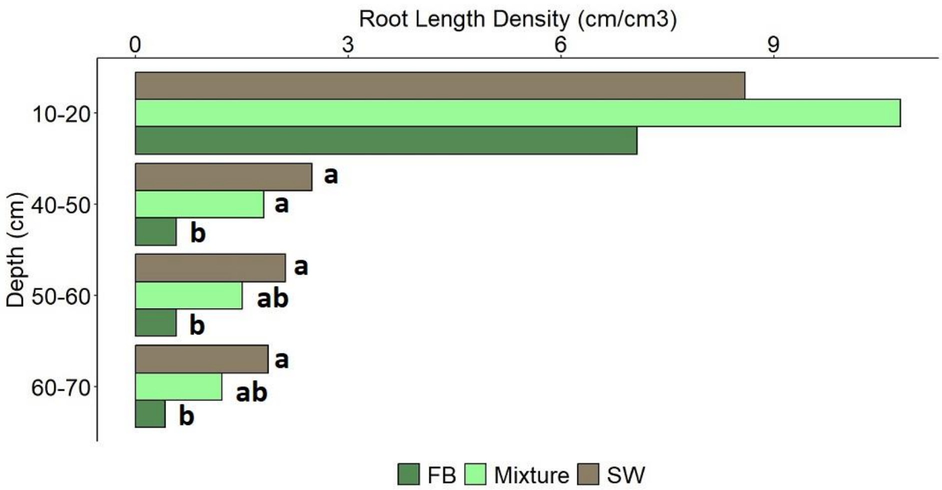


Figure 11: Root length density (RLD) in cm/cm^3 determined with the monolith method for faba bean (FB), Mixture and spring wheat (SW) in different soil depth layers. The different letters represent the significant differences between the treatments within one layer. Mean values of treatments were compared with a Tukey test at a significance level of $\alpha = 0.05$.

3.2.2 Biopores

The root length density (RLD) of spring wheat, faba bean and mixture is depicted in biopores at different soil depths, ranging from 40 – 50 cm to 60 – 70 cm (Fig. 12). At the depth 40 – 50 cm and intermediate depth 50 – 60 cm, no significant differences in RLD were observed among the treatments, indicating that, within these depths, all three treatments exhibited similar root length densities. In contrast, at the deeper soil layer of 60 – 70 cm, a distinct pattern became evident. Spring wheat exhibited the highest RLD at this depth, suggesting its adaptability to explore deeper soil layers and access potentially richer nutrient reserves, however, faba bean showed the lowest RLD among the treatments. The mixture treatment, falling in between, demonstrated an intermediate RLD, signifying a balanced exploration of deeper soil layers.

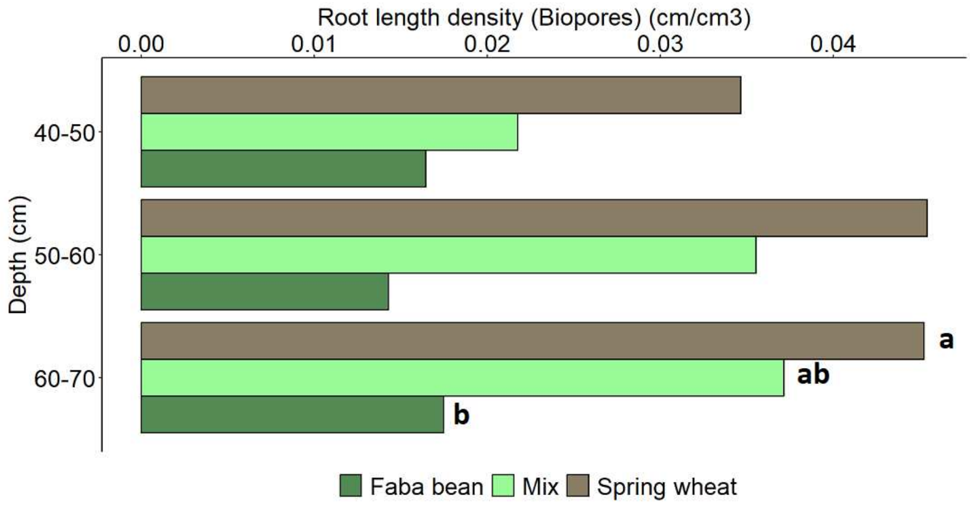


Figure 12: Root length density (RLD) of biopores for spring wheat (SW), faba bean (FB), and mixture. The different letters represent the significant differences between the treatments within one layer. Mean values of treatments were compared with a Tukey test at a significance level of $\alpha = 0.05$.

3.2.3 Profile wall

The root length density was measured in blocks of 5-5 cm until the depth of 100 cm but was grouped into three main depth levels (0-30 cm, 30-60 cm, and 60-100 cm) to analyze the difference in treatments (Figure 13). At the shallow depth of 0-30 cm, spring wheat, mixture, and faba bean exhibited distinct significance differences. Spring wheat and mixture were significantly higher than faba bean. As the deeper soil layer at 30-60 cm was investigated, spring wheat was still significantly higher than faba bean, while the mixture exhibited an intermediate level of root length density and was not significantly different from sole faba bean and spring wheat. In the deepest soil layer, ranging from 60–100 cm no statistical difference were observed among the treatments. This suggests that, at this depth, the treatment displayed a relatively consistent pattern in terms of root length density.

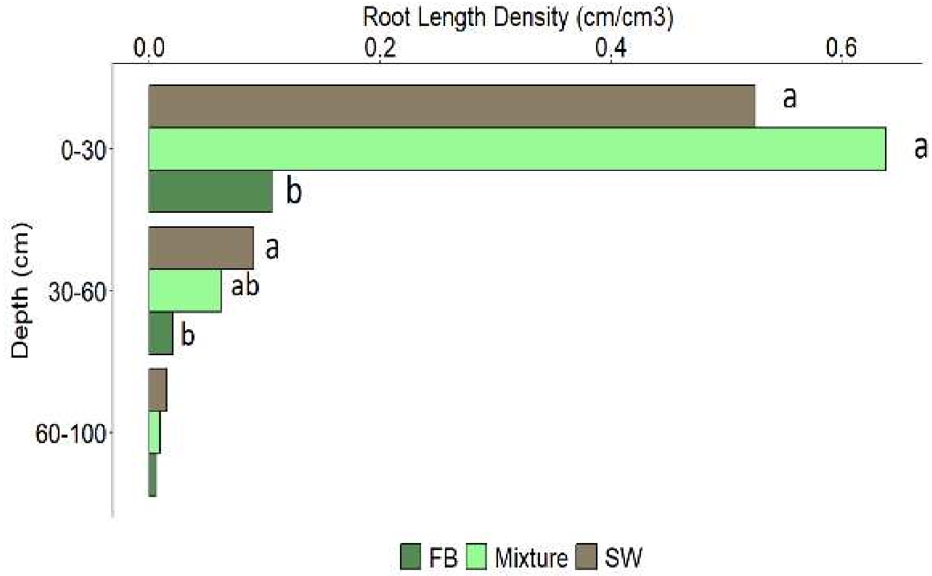


Figure 13: Root length density (RLD) of profile wall (PW) for three treatments (spring wheat (SW), faba bean (FB), and Mixture) at various depths ranging from 0–100 cm. Mean values of treatments were compared with a Tukey test at a significance level of $\alpha = 0.05$.

3.3 Share of roots in biopores

The share of roots in the biopores of treatments were evaluated at different depths ranging from 40-70 cm (Fig. 7). The figure illustrates that the treatment 'faba bean' exhibited a higher proportion of roots compared to other treatments, and this share increased with depth. Specifically, at depths of 40-50cm, 50-60 cm, and 60-70 cm, the share of root was 5.4%, 8%, and 8.3%, respectively. Subsequently, followed by the mixture treatment, with the exception of the 40-50 cm depth (2.3%), showed better performance (4.5% at depth 50-60 cm and 5.6% at 60-70 cm). However, the share of roots for spring wheat was the lowest among the treatments, with proportions of 2.7% at 40-50 cm, 4.1% at 50-60 cm, and 4.3% at 60-70 cm depths. All the treatments exhibited a similar trend, with an increasing share of roots in biopores as depth increased. While, the faba bean dominated in the deeper layers compared to spring wheat.

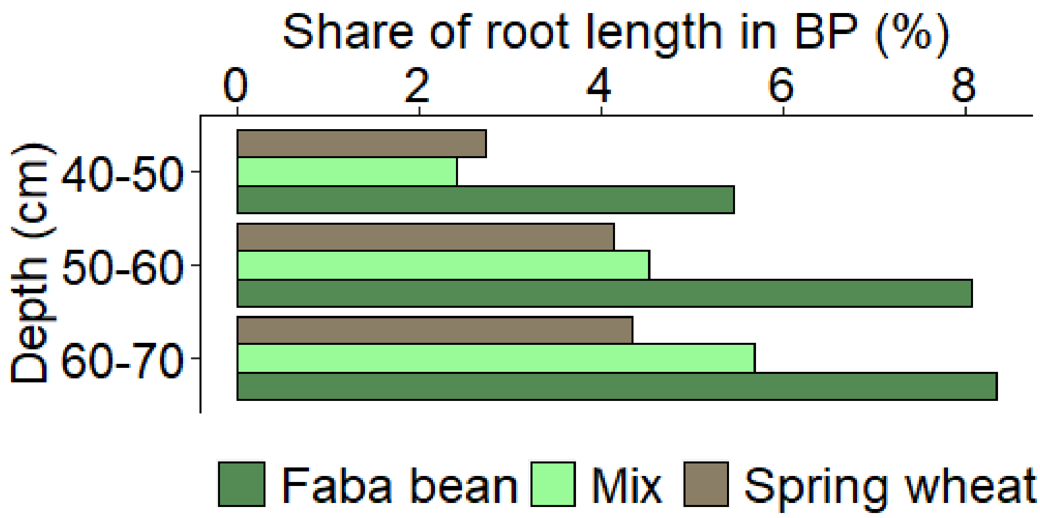


Figure 14: Share of root length in biopores.

3.4 Yield parameters

3.4.1 Land Equivalent Ratio (LER)

The LER diagram, in figure 15 (a, b, and c), shows that all values were falling below the diagonal dotted line. Hence, spring wheat demonstrated a competitive advantage over faba bean in terms of grain yield. In figure 15 (a), for the mixture treatment, three out of four values for the partial land equivalent (pLER) of faba bean were below 0.5, however, one value exceeded 0.5. Conversely, for spring wheat, all values for the pLER consistently above 0.5. This implies that spring wheat in the mixed cropping system contributed to a more efficient use of land, making it the dominant crop in terms of grain yield within the mixture. Similarly, all the shoot values (dry matter at booting and dry matter at maturity) for the mixture treatment, were below the diagonal dotted line (figure 15 (b) and (c). In the context of pLER for faba bean, all values for dry matter both at booting and maturity stages were found to be below 0.5. However, the values for spring wheat, in both booting and maturity stages, consistently exceeded 0.5. During both the booting and maturity stages, it was observed that spring wheat had a suppressive effect on faba bean in terms dry matter production. For the mixture treatment, the mean LER values for grain yield, dry matter at booting, and dry matter at maturity were 1.31, 1.12, and 1.17, respectively.

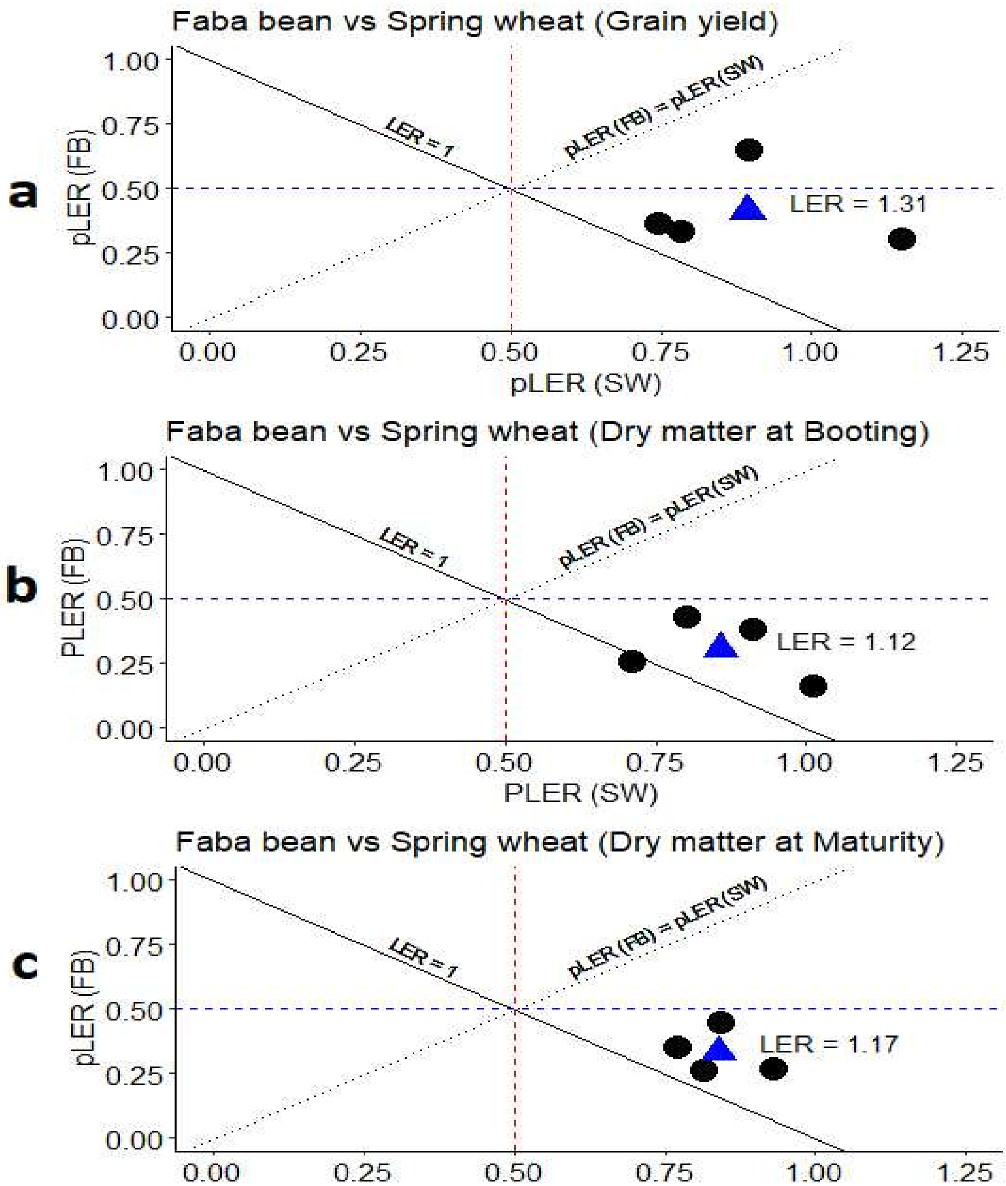


Figure 15: Land Equivalent Ratio (LER) of spring wheat and faba bean. The X-axis represents the Partial land equivalent ratio (pLER SW) of spring wheat, while the Y-axis represents (pLER FB) of faba bean. The solid line corresponds to a land equivalent ratio of 1 (LER = pLER SW + pLER FB). The dashed horizontal and vertical lines represent the expected pLER for the mixture partners. (a) Represents the LER of grain yield (b) indicates the LER of dry mass at booting stage (c) Shows the LER of dry mass at maturity.

3.4.2 Grain yield

Among the treatments examined (16a), the average grain yield for faba bean was 2.69 tons per hectare, while, spring wheat performed higher with in average production of 4.17 tons per hectare. This yield can be considered as a typical result when faba bean and spring wheat are cultivated as a sole crop. However, the mixture treatment, which involves the 50:50-mixture of both faba bean and spring wheat, showed a significant increase in grain yield, averiging 4.71 tons per hectare. Statistically, the mixture and spring wheat were sigificantly higher than faba bean.

3.4.3 Dry matter

The dry matter for all the treatments was evaluated at two different atges: booting and maturity. Figure 16 (b) represents the average dry matter of the treatments at booting while, figure 16 (c) displays the values at maturity, respectively. The treatment showed varying average dry matter values at the booting stage. The mixture treatment presented the highest average dry matter at 3.08 ton per hectare, followed by spring wheat with an average of 2.72 ton per hectare. However, the faba bean showed the lowest average dry matter of 2.44 ton/ha. This pattern persisted in the dry matter evaluation at the maturity stage, with the mixture treatment having the highest average dry matter at 9.56 ton per hectare, followed by spring wheat having the average value of 9.01 ton per hectare. In contrast, faba bean had the lowest average dry matter of 6.21 ton per hectare. The averagedry matter values for faba bean and spring wheat treatments can be attributed to its sole or mono cropping, While the mixture treatment involves the 50:50-mixture of both faba bean and spring wheat. There was no statistical diffrence among the treatments at booting stage, while at maturity, the mixture and spring wheat were significantly higher than faba bean.

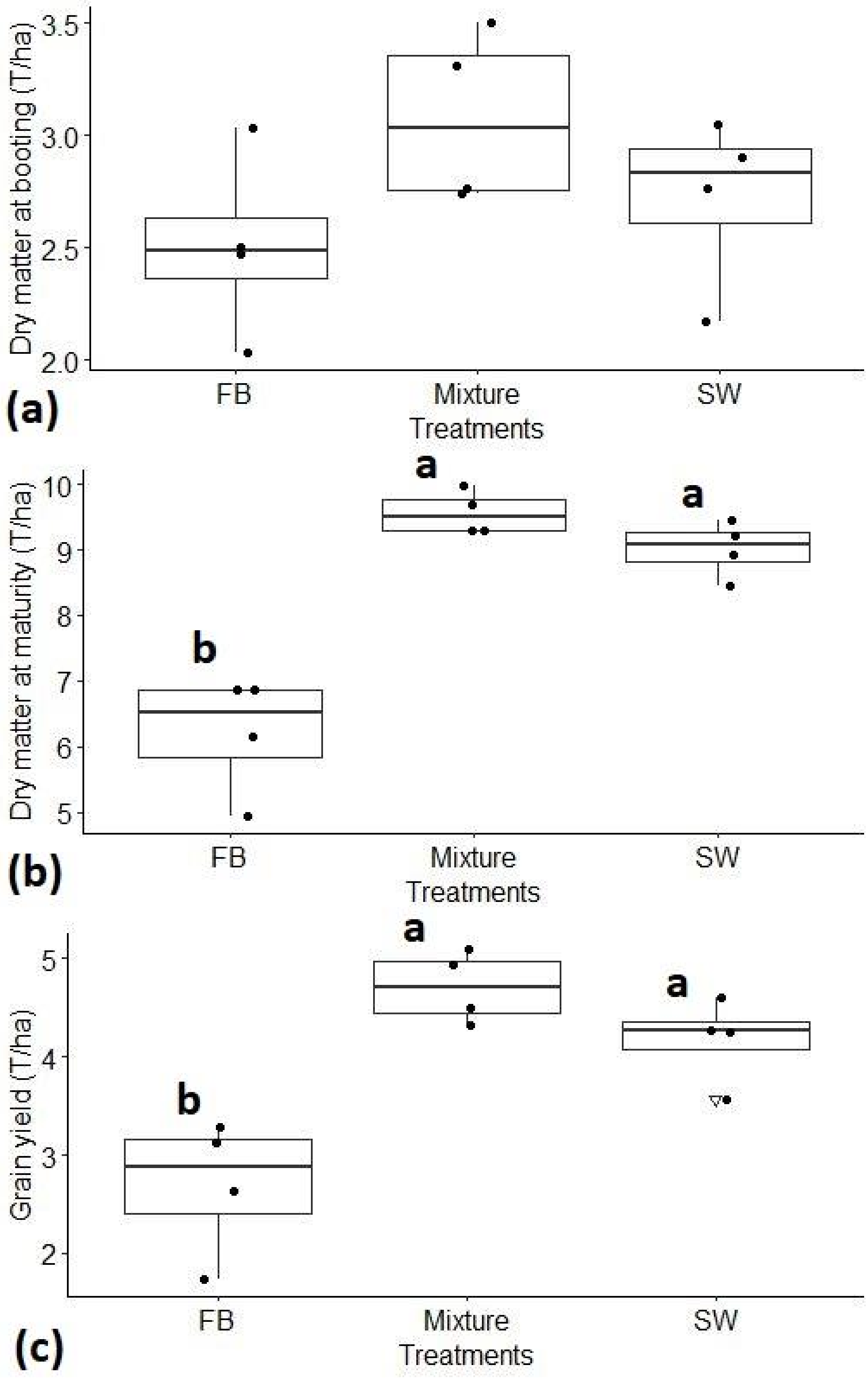


Figure 15: **(a)** Shoot dry matter at booting stage. **(b)** Shoot dry matter at maturity stage. **(c)** Grain yield of three treatments: faba bean, mixture, and spring wheat.

4. Discussion

4.1 Root growth in biopores (root weight, root length density and root share)

The root system expansion of wheat typically surpasses that of legumes such as faba beans (Gregory et al., 1995; Turpin et al., 2002). In our study, the root weight within biopores showed no statistically significant difference between the sole spring wheat, faba bean, and mixture at the first two layers (40-50 cm and 50-60 cm). However, the trend indicates that root weight of spring wheat was higher for depth level of 40-50 cm, followed by the faba bean and then mixture. Conversely, root weight in mixture considerably increased and has become higher than faba bean at 50-60 cm. The same trend followed at 60-70 cm with the difference of root weight becoming statistically higher in spring wheat than mixtures. The general trend indicates that mixture is resulting in more root weight in deeper soil layers. The root mass of sole spring wheat and faba bean in back soil has been measured by researchers at different depths. (Rengasamy and Reid, 1993) reported an average root mass for faba bean approximately 1.4 T/ha for a sampling depth of 70 cm. While, (Streit et al., 2019) found values of approximately 0.7 T/ha for a sampling depth up to 60 cm. Similarly, the literature has shown a wide range of spring wheat root masses with values varying from 0.8 T/ha to 1.4 T/ha (Wechsung et al., 1995; Gan et al., 2009). The difference in the values could be attributed to the soil depth and sampling techniques. Campbell et al., (1977) noted that the decrease in root mass of wheat occurred in the 0 to 75 cm depth, but remained constant below 75 cm. Conversely, in our study, the root mass for spring wheat in the biopores increased from 40 cm to 70 cm.

Cereals are typically considered as strong competitors when compared to legumes. This is primarily attributed to their larger root system and deeper root distribution, as indicated by several studies (Gregory et al., 1995; Hauggaard-Nielsen et al., 2001; Corre-Hellou and Crozat, 2005; Bedoussac et al., 2015). Numerous research studies have also noted that intercropping systems yield substantially higher root masses in comparison to their sole cropping counterparts (Ma and Chen, 2016). However, in our study, the mixture treatment displayed intermediate root weight, which was greater than that of faba bean but lower than that of spring wheat.

Roots have a substantial rate of respiration and need a considerable supply of oxygen, which they can obtain through the air-filled biopores (Lynch et al., 2012). Biopores are supposed to have a special relevance for root growth (Ehlers et al., 1983) and serve as hot spots for nutrient acquisition of crop roots (Kautz et al., 2013). In our study, the root length density (RLD) of sole crops (spring wheat and faba bean) and the mixture in biopores showed no statistically significant difference at a depth of 50 and 60 cm. However, the RLD of spring wheat was dominant, and the RLD of the mixture drastically increased at a depth of 60 cm. At a depth of 70 cm, the RLD of spring wheat was significantly higher than that of faba bean, while the mixture treatment displayed intermediate RLD, which was not significantly different from the sole crop treatments. The RLD of the mixture was also observed to be similar to the RLD of the mixture at a depth of 60 cm. So, The RLD of spring wheat was dominant throughout the layers. Perkons et al., (2013) reported the same results. According to Perkons et al., (2013), the root length density (RLD) of spring wheat in biopores as determined with monolith method was significantly higher in soil depth from 45 to 85 cm. While comparing the RLD of the tap root system (Mallow) and the fibrous root system of spring wheat, (Perkons et al., 2014) reported that spring wheat had a high RLD in the topsoil and less RLD in the subsoil. In contrast, mallow had a significantly lower RLD in the topsoil but a significantly higher RLD in soil depths below 45 cm. Similarly, Athmann et al., (2019) studied the exploration of biopores by four different crops (faba bean, wheat, barley, and oilseed rape), and they found that the RLD for oilseed rape was significantly higher compared to the other three crop species.

In our study, the proportion of roots of faba bean was observed to be higher compared to spring wheat and the mixture. This proportion increased with depth from 5.4% at 40 cm to 8.3% at 70 cm. The share of roots for faba bean at a depth of 60 cm was 8%, and this was not significantly different from the share at 70 cm. This is in contrast to the values reported in the study by Athman et al., (2019), where the share of roots in the biopore was reported as 1% for faba bean at a depth of 0-40 cm. Subsequently, the mixture treatment exhibited a consistent increase, starting at 2.3% at the depth (40 cm) and reaching 5.6% at the deeper layer (70 cm). The difference in share for mixture between each layer was relatively substantial and tended to increase with depth. However, the share of roots for spring wheat was the lowest, with proportions of 2.7% at 40-50 cm, 4.1% at 50-60 cm, and 4.3% at 60-70 cm in biopores compared to the other treatments. Our results differ from those reported by White and Kirkegaard, (2010). In their research on root growth of winter wheat, they illustrated that at depths above 60 cm, 30-40% of roots were located in biopores or soil cracks. Below 60 cm depth, a significantly higher proportion, ranging from 85% to 100%, of roots utilized these pathways for growth.

4.2 Root growth in bulk soil (Monoliths and Profile wall)

Root Length Density (RLD) is a quantitative measure used in the field of plant biology and soil science to describe the spatial distribution and abundance of plant roots within a specific volume of soil. RLD is typically expressed as the total length of plant roots per unit volume of soil. The crop growth and ultimate yield of an intercropping system are closely linked to root expansion, influencing the absorption and efficient utilization of water and nutrients. The distribution and elongation can be expressed as a root length density (RLD) (Adiku et al., 2001). In the literature, root-length densities (RLDs) of cereals have been reported to be in the range of 0.2–2.75 cm/cm^3 in 40–60 cm soil depth (Mun˜oz-Romero et al., 2010; Liu et al., 2011). In our study, the root length density (RLD) of spring wheat fell within this range. Additionally, there was no significant difference in RLD among sole crops (spring wheat and faba bean) and the mixture at a depth of 10-20 cm, with root length densities for spring wheat, faba bean, and the mixture being 8.5, 7, and 10.7 cm/cm^3, respectively. Our results were in line with those of (Li et al., 2005), who reported that the RLD of sole-cropped wheat and faba bean was lower at 0–30 cm depth but not below 30 cm. Additionally, they also observed a higher proportion of faba bean roots in the upper soil layers when intercropped. This might be the reason that, in our study, the root length density (RLD) of the mixture exceeds that of the sole crops at shallow depth.

In our study, within the depth range of 40-70 cm, the root length density (RLD) of spring wheat was significantly higher, ranging from 1.8 to 8.5 cm/cm3, compared to faba bean and the mixture. Despite a decrease in density with depth, spring wheat remained dominant compared to the other treatments.

At depth 40-50 cm, the RLD of the mixture was significantly higher than faba bean. However, at deeper depths (50-70 cm), the mixture showed intermediate RLD and was not significantly different from spring wheat and faba bean.

Additionally, the RLD of sole cropped faba bean decreased with depth. The results were aligning with Manschadi et al., (1998), who illustrated a substantial decrease in the root length density of faba bean when grown as a sole crop, particularly at higher depths. Additionally, (Athmann et al., 2019) reported that the root length density in bulk soil was significantly smaller for faba bean compared to wheat, barley, and oilseed rape.

4.3 Mixture effect on yield parameters

The land equivalent ratio (LER) is a measure of how efficiently multiple crops utilize land compared to a sole crop. An LER value less than 1 indicates that the combined yield of the two crops (spring wheat and faba bean) grown together is less than the sum of their yields when grown separately, while a LER value greater than 1 indicates an increase an overall productivity in the mixed system compared to sole crops. Intercropped legumes have proved to be capable of providing a wide range of additional services and of producing substantially higher yields than a sole crop– expressed as a land equivalent ratio (LER) higher than 1 (Willey, 1979). In our study, for the mixture treatment, in terms of grain yield, three out of four values for the partial Land Equivalent Ratio (pLER) of faba bean were below 50%, while for spring wheat, all values for pLER were above 50%. Similarly, all the shoot values for faba bean were below 50%, and for spring wheat, all values were above 50%. This implies that in the mixed intercropping system, spring wheat had a suppressive effect on faba bean and contributed to a more efficient use of land. On average, intercrops were more efficient in land use than sole crops, with calculated LER greater than 1 (Fig. 15a, 15b, and 15c). The LER value for grain yield, dry matter at booting, and dry matter at maturity were 1.17, 1.12, and 1.31, respectively. Our study confirmed results of other studies, showing the cereal to be dominant in cereal-legume intercrops (Yu et al., 2016; Kemper et al., 2022), Similarly, Ren et al., (2016) reported that legume and cereal intercropping significantly increased cereal crop yield. While, other studies have also argued that intercropping benefited the yields of both crops (Zuo et al., 2004; Laberge et al., 2011). Many intercropping systems have proved to be better than sole crops in terms of yield (Zhang et al., 2007) because intercropping makes better use of one or more agricultural resources (Rodrigo et al., 2001). Such improvements in yield have been attributed almost exclusively to above-ground interactions between intercropped species. However, yield advantages of intercropping systems are due to both above and below-ground interactions between intercropped species (Li et al., 2006).

5. Conclusion

In this study, the root length density of spring wheat and faba bean, as well as biopore usage, were assessed in both pure stands and in a mixture (intercropping) at various soil depths. The study showed that the differences in root length density of the treatments were insignificant at 10-20 cm (top soil), indicating similar root growth. However, at the deeper soil layers (40-70 cm), spring wheat exhibited dominance, and the mixture showed intermediate root growth. The profile wall method revealed that the root length density of the treatments at the 100 cm depth was again similar and did not show any significant difference in root growth.

The results showed that the root length density and root weight of sole crops (spring wheat and faba bean) and the mixture in biopores at 40-60 cm exhibited similar root growth. However, at a depth of 70 cm, spring wheat was dominant, and the mixture showed intermediate root growth. The proportion of roots in biopores for faba bean consistently increased across all soil layers, followed by the mixture treatment, and this share further increased with depth. Meanwhile, the share of roots for spring wheat was the lowest among the treatments. This indicated the higher efficiency of faba bean roots for using biopores in deeper soil layers.

The study also went on investigate grain yield, shoot biomass, and the mixture effect. In terms of grain yield, both the mixture and spring wheat were significantly higher, and shoot biomass at maturity was also significantly higher. The mixture treatment demonstrated a more efficient use of land, evident in higher values for both grain yield and shoot biomass, especially for spring wheat compared to faba bean. This suggests that in the mixed intercropping system, spring wheat exerted a suppressive effect on faba bean, contributing to a more effective land utilization. The LER values for grain yield, dry matter at booting, and dry matter at maturity were 1.17, 1.12, and 1.31, respectively. These Land Equivalent Ratio (LER) values, all greater than 1, support the idea that mixture is more productive than sole spring wheat and faba bean.

In the context of root growth in biopores, exploring the deeper soil layers (>70 cm) is essential, as differences in root growth become apparent at those depths.

Similarly, in mixture treatment, roots needs to be discriminated using latest techniques i.e. Fourier transform infrared (FTIR) spectroscopy.

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