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Effects of biological and technical subsoil amelioration on root growth and crop performance in cereal crops

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Abstract

In the face of climate change, optimizing root systems to enhance subsoil water and nutrient extraction is increasingly vital for resilient and efficient crop production. Subsoil amelioration approaches such as deep tillage, compost application in the upper subsoil, crop rotations could play an important role to enhance deeper root growth, resource acquisition from subsoil and creation of biopores. Intercropping of cereals and legumes provides a suitable approach to study root growth dynamics in subsoil and utilization of biopores. These approaches advance understanding of how integrated biological and technical subsoil amelioration strategies can optimize root growth, improve subsoil function, and ultimately increase crop productivity. This thesis presents three complementary field studies in Germany focused on subsoil resource exploitation in cereals and legumes. (i) The first study evaluated the combined effects of deep-rooted lucerne as a pre-crop and strip-wise deep tillage with compost on subsequent spring barley and winter wheat. (ii) The second study investigated genotype-dependent responses of spring barley and winter wheat to deep tillage and compost. (iii) The third study quantified root length density and biopore usage in spring wheat and faba bean grown in pure stands and mixtures.

Findings demonstrated that (1) the lucerne together with deep tillage and compost increased subsoil root length density, rooting depth, nutrient uptake, and yield in spring barley under dry conditions, with limited benefits observed for winter wheat when water was abundant.

(2) Deep soil tillage in combination with compost generally promoted root growth and nutrient uptake across genotypes, but yield responses varied, highlighting the importance of root traits in exploiting improved subsoil conditions. Environmental fluctuations across years were one of the key factors in determining the genotype-specific root responses to tillage + compost treatments.

(3) The results of the third study showed that intercropping slightly improved overall root performance in the top soil as well as yield performance, affirming the potential productivity advantages of mixtures. Further, faba bean had a higher share of roots in biopores than wheat, especially in deeper soil layers.

Collectively, these studies show that both biological (intercropping, pre-crops) and technical (deep tillage, compost) strategies can improve root exploration of the subsoil, crop resilience, and productivity especially under drought while revealing limited effects of genotype but more pronounced crop species interactions on the outcomes.

Zusammenfassung

Angesichts des Klimawandels wird die Optimierung von Wurzelsystemen zur Verbesserung der Wasser- und Nährstoffaufnahme aus dem Unterboden für eine widerstandsfähige und effiziente Pflanzenproduktion immer wichtiger. Maßnahmen zur Unterbodenverbesserung wie Bearbeitung im Unterboden, Kompostzugabe im oberen Unterboden und Fruchtfolge könnten eine wichtige Rolle bei der Förderung eines tieferen Wurzelwachstums, der Ressourcengewinnung aus dem Unterboden und der Bildung von Bioporen spielen. Der Mischanbau von Getreide und Hülsenfrüchten bietet darüber hinaus einen geeigneten Ansatz, um die Dynamik des Wurzelwachstums im Unterboden und die Nutzung von Bioporen zu untersuchen. Diese Ansätze fördern das Verständnis dafür, wie integrierte biologische und technische Strategien zur Unterbodenmelioration das Wurzelwachstum optimieren, die Unterbodenfunktion verbessern und letztlich die Ernteerträge steigern können. Dieser Artikel stellt drei sich ergänzende Feldstudien in Deutschland vor, die sich mit der Nutzung von Unterbodenressourcen bei Getreide und Körnerleguminosen befassen. (i) Die erste Studie untersuchte die kombinierten Auswirkungen von tiefwurzelnder Luzerne als Vorfrucht und streifenweiser Bodenbearbeitung mit Kompost auf nachfolgende Sommergerste und Winterweizen. (ii) Die zweite Studie untersuchte die genotypabhängigen Reaktionen von Sommergerste und Winterweizen auf Unterbodenbearbeitung und Kompost. (iii) Die dritte Studie quantifizierte die Wurzellängendichte und die Bioporennutzung bei Sommerweizen und Ackerbohnen, die in Reinkulturen und Mischkulturen angebaut wurden.

Die Ergebnisse zeigten, dass (1), Luzerne in Kombination mit Tiefenbearbeitung und Kompost die Wurzellängendichte im Unterboden, die Wurzeltiefe, die Nährstoffaufnahme und den Ertrag bei Sommergerste unter trockenen Bedingungen erhöhte, während bei Weizen unter wasserreichen Bedingungen nur begrenzte Vorteile beobachtet wurden.

(2) Eine Bodenbearbeitung im Unterboden in Kombination mit Kompost förderte im Allgemeinen das Wurzelwachstum und die Nährstoffaufnahme bei allen Genotypen, aber die Ertragsreaktionen variierten, was die Bedeutung der Wurzelmerkmale für die Nutzung verbesserter Unterbodenbedingungen unterstreicht. Umweltschwankungen über mehrere Jahre hinweg waren einer der Schlüsselfaktoren für die genotypspezifischen Wurzelreaktionen auf Bodenbearbeitung + Kompostbehandlungen.

(3) Die Ergebnisse der dritten Studie zeigten, dass der Gemengeanbau die Wurzelentwicklung im Oberboden sowie den Ertrag leicht verbesserte und damit die potenziellen Produktivitätsvorteile von Mischungen bestätigte. Darüber hinaus wies die Ackerbohne einen höheren Anteil an Wurzeln in Bioporen auf als Weizen, insbesondere in tieferen Bodenschichten.

Insgesamt zeigen diese Studien, dass sowohl biologische (Mischkulturen, Vorfrüchte) als auch technische (tiefe Bodenbearbeitung, Kompost) Strategien die Wurzelausbreitung im Unterboden, die Widerstandsfähigkeit der Pflanzen und die Produktivität verbessern können, insbesondere unter trockenen Bedingungen. Gleichzeitig zeigen sie, dass die Auswirkungen des Genotyps begrenzt sind, während die Wechselwirkungen zwischen den Pflanzenarten einen deutlicheren Einfluss auf die Ergebnisse haben

1 General introduction

Climate change presents new challenges to agriculture (Sillmann et al., 2013), including more frequent and severe droughts in many cropping regions (Olesen et al., 2011) and potential nutrient supply shortages (Cooper et al., 2011). These stresses limit water availability and nutrient uptake, reducing crop growth and yield. With nutrients from topsoil depleted or less accessible from soil degradation and intensive farming, it is crucial to explore alternative sources, particularly subsoil nutrients and water for sustained crop production. In agricultural land, the subsoil can hold up to 50% of total nitrogen stocks (Wiesmeier et al., 2013) and over 25% of phosphorus reserves (Kautz et al., 2013). Additionally, the subsoil can retain moisture during drought conditions (Kirkegaard et al., 2007), though the availability of these resources varies depending on the crop specific factors like rooting depth and architecture to determine the extent to which roots can access subsoil resources. Some non-fertilised experiments showed sustained yields as a result of the uptake of subsoil nutrients (Garz et al., 2000). Encouraging root growth into deeper subsoil layers is one option to achieve more improved nutrient uptake (Lynch & Wojciechowski, 2015). Due to relatively high penetration resistance, root elongation in the subsoil is hampered (Bengough et al., 2011). Soil compaction is detrimental to root growth and development which directly affects nutrient uptake and consequently crop yields (Tracy et al., 2012). It has been observed that deep roots can be formed beyond 1 m soil depth in arable subsoil (Canadell et al., 1996) and can contribute to nutrient uptake (Han et al., 2020).

The cultivation of perennial taprooted fodder crops may promote the formation of large continuous biopores, hence, provide favorable conditions for root growth (Kautz, 2015; Kautz, Perkons, et al., 2013). Growing lucerne (*Medicago sativa* L.) as a taprooted crop not only ameliorates compacted subsoils but also enhances saturated hydraulic conductivity (Rasse et al., 2000), improves infiltration and water availability for subsequent crops (McCallum et al., 2004), and their roots are effective in increasing biopore density in topsoils as well as subsoils (Han et al., 2015; Lucas et al., 2019). By creating large sized biopores in the subsoil, taprooted crops provide a pathway for roots of subsequent crops to explore the deeper soil layers, resulting in higher root growth in subsoil (Han et al. 2015b). On a Haplic Luvisol, biopores were used by barley roots as pathways to quickly penetrate deeper, while at later development stages roots re-entered the less compacted subsoil (Kautz et al., 2013).

Deep tillage refers to processes that are carried out below the conventional cultivation depth and therefore penetrate deeper into the soil (Mollenhauer, 2014). This practice is not used widely, which is due to the high energy demand and costs resulting from the deeper intervention in the soil. Deep tillage has therefore mainly been done to loosen compacted subsoils (Jakobs et al., 2019; F. Schneider et al., 2017). By breaking up compacted soil horizons, deep loosening is intended to allow rainwater to drain more quickly (F. Schneider et al., 2017). An increase in the water retention capacity of the soil for plant-available moisture can be achieved due to the formation of a loosened structure (Mollenhauer, 2014). More porous and loose soil ensures reduced resistance to penetration and results in higher root densities (Jakobs et al., 2017).

Deep tillage across the entire field is often economically impractical. Farmers are also sceptical about this practice due to its labor-intensive nature, time consumption, and potential for causing damage (Frelih-Larsen et al., 2018). A similar study (Bauke et al., 2024) concluded that mechanical subsoil loosening alone was not beneficial, but the combined treatment of subsoil loosening with the addition of organic amendments that maintained the loosened soil structure and provided an additional reservoir of water and nutrients at 50 cm soil depth resulted in better crop performance especially in dry periods. A different study with incorporation of straw in 25 to 34 cm soil depth indicated that loosening the upper subsoil alone had only temporary and limited effects; however, incorporating straw along with deep loosening improved the outcome by supplying additional nutrients and increasing the subsoil's water retention capacity (Getahun et al., 2022). Our first experiment to enhance nutrient uptake, root growth, and crop performance of cereals by promoting the creation of biopores via growing lucerne as a deep-rooting perennial pre-crop and by improving soil structure through deep tillage and compost incorporation. We performed strip-wise deep tillage in rows with 1 meter distance between them with simultaneous incorporation of compost up to 60 cm soil depth as previously tested by Bauke et al. (2024).

While deep tillage and compost incorporation are promising strategies, their interaction with plant genotypes and root traits remains underexplored. Research indicates that genotypic variation in root architecture significantly influences how plants exploit deep soil layers under water- or nutrient-limited conditions (Gao & Lynch, 2016; Uga et al., 2013). Traits such as steeper root angles, deeper rooting depth, and higher specific root length have been associated with improved subsoil foraging, especially under drought or low phosphorus availability (Lynch, 2019; Schneider & Lynch, 2020). Furthermore, the synergy between biological soil amendments (like compost) and genetically determined root traits can affect rhizosphere interactions, nutrient cycling, and microbial activity (Hu et al., 2018; Vries et al., 2018). These insights suggest that optimizing both soil management practices and genotype selection is key to maximizing the effectiveness of technical melioration strategies under climate and resource constraints!

The root characteristics of the genotypes of same species differ depending on the biological, chemical and physical environmental factors (Gregory, 2006), hence it is necessary to investigate the response of different genotypes for deep tillage and compost treatments. It is advantageous for plants to have certain characteristics which can help its roots to penetrate compacted top soil. These include reduction in elongation and enhancement of root diameter. Root character such as hydraulic conductivity, root hair length and density can enhance water uptake (Freschet et al., 2021).

This thesis comprises three interlinked field experiments aimed at improving cereal crop resilience and resource use efficiency by enhancing subsoil root growth and nutrient uptake through biological, technical and genetic factors:

Study 1: Deep tillage and compost combined with precrops

- To evaluate the effects of lucerne, a deep-rooted nitrogen fixing pre-crop, versus ryegrass, a shallow-rooted control pre-crop, on the formation of biopores in the subsoil.
- To assess the impact of these precrops, together with strip-wise deep tillage and compost incorporation, on subsequent cereal root growth (root length density), nutrient uptake, and yield.

- To test hypotheses that lucerne will produce larger diameter roots creating larger biopores, leading to improved subsoil root growth, nutrient capture, and yield compared to ryegrass.
- To determine whether deep tillage combined with compost enhances these effects relative to reference and whether combined application amplifies benefits.

Study 2: Deep tillage and compost with cereal genotype

- To investigate the genotype dependent responses of cereals to deep tillage and compost amendment on root growth, nutrient uptake, and yield.
- To identify genotypes with superior adaptability to subsoil amelioration, informing breeding and management strategies for sustainable cereal production in compacted soils.

Study 3: Spring wheat/faba bean Intercropping effect on root growth in subsoil and biopores

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

Combined, these studies aim to generate comprehensive insights on the biological, technical and genetic factors driving enhanced subsoil resource utilization, contributing to the development of resilient cropping systems with reduced dependence on external inputs.

2 Effects of biological and technical subsoil amelioration on root growth and cereal crop performance

2.1 Introduction

Soil compaction is a widespread limitation to cereal production worldwide, constraining root penetration and reducing access to subsoil water and nutrients (Batey, 2009; Correa et al., 2019). Compacted subsoil layers typically show low porosity and increased mechanical resistance, which inhibit root growth and lead to concentration of roots in shallower soil horizons, ultimately reducing yield potential and resilience, especially under water-limited conditions (Bengough et al., 2011; Pandey & Bennett, 2024). Deep tillage has emerged as a common agronomic practice to physically disrupt compacted layers, increase soil porosity, and improve root exploration at depth (Hamza & Anderson, 2005; Thorup-Kristensen et al., 2020). When combined with organic amendments such as compost, deep tillage can also enhance soil biological activity and nutrient availability, further stimulating root growth. However, these benefits may be transient and depend on crop genotype, soil type, and management history, necessitating further evaluation of genotype-specific responses (Chen et al., 2022). Encouraging the creation of biopores to create pathways for roots of subsequent crops is another strategy to potentially enhance deeper root growth. It is evident that the deep root crops promote the root growth of subsequent cereal crops in subsoil (Behrend et al., 2025). Combining these both approaches could even further create favorable conditions to promote root growth along with subsequent positive effects on yield traits. We hypothesized that:

1) Pre-crop lucerne is expected to produce more roots with diameters greater than 2 mm, resulting in a higher density of large-sized biopores in the subsoil, whereas pre-crop ryegrass, which produces more roots with diameters less than 2 mm, leads to a greater density of small-sized biopores in the subsoil.

2) Lucerne, as a deep-rooted and nitrogen-fixing pre-crop, leads to cereal crops showing (i) enhanced root growth in the subsoil, (ii) higher nutrient uptake, and (iii) higher crop yield

compared to those grown after shallow-rooted ryegrass (*Lolium perenne* L.) as a pre-crop (control).

3) Deep tillage with compost results in (i) enhanced root growth in the subsoil (ii), higher nutrient uptake and (iii) higher crop yield as compared to the control.
4) Combined treatment of deep tillage with compost and lucerne as pre-crop further increases root growth, nutrient uptake, and crop yield, and therefore considerably enhances spring barley (*Hordeum vulgare* L.) and winter wheat (*Triticum aestivum* L.) performance.

2.2 Materials and methods

2.2.1 Experimental site

A field trial was established in 2016 at a commercial farm adjacent to Campus Klein-Altendorf (50°38'06"N, 6°58'36"E), an experimental research farm of the University of Bonn, located in Rheinbach in North Rhine-Westphalia, Germany. The soil type is a Haplic Luvisol derived from loess and characterized by a silty clay loam texture with accumulation of clay between the soil depth of 45 cm and 95 cm. A detailed analysis of chemical properties and soil texture at Klein-Altendorf, near the study site, is provided by Barej et al. (2014). The long-term average annual air temperature (1991-2020) is 10.3°C, and the mean annual precipitation (2011-2021) is 599.1 mm. Total annual precipitation in the experimental years 2017, 2018, 2019, 2020 and 2021 was 599 mm, 422 mm, 616 mm, 492 mm and 790 mm, respectively. Monthly precipitation, maximum and minimum temperature for 2020 and 2021 are shown in Figure 2.1.

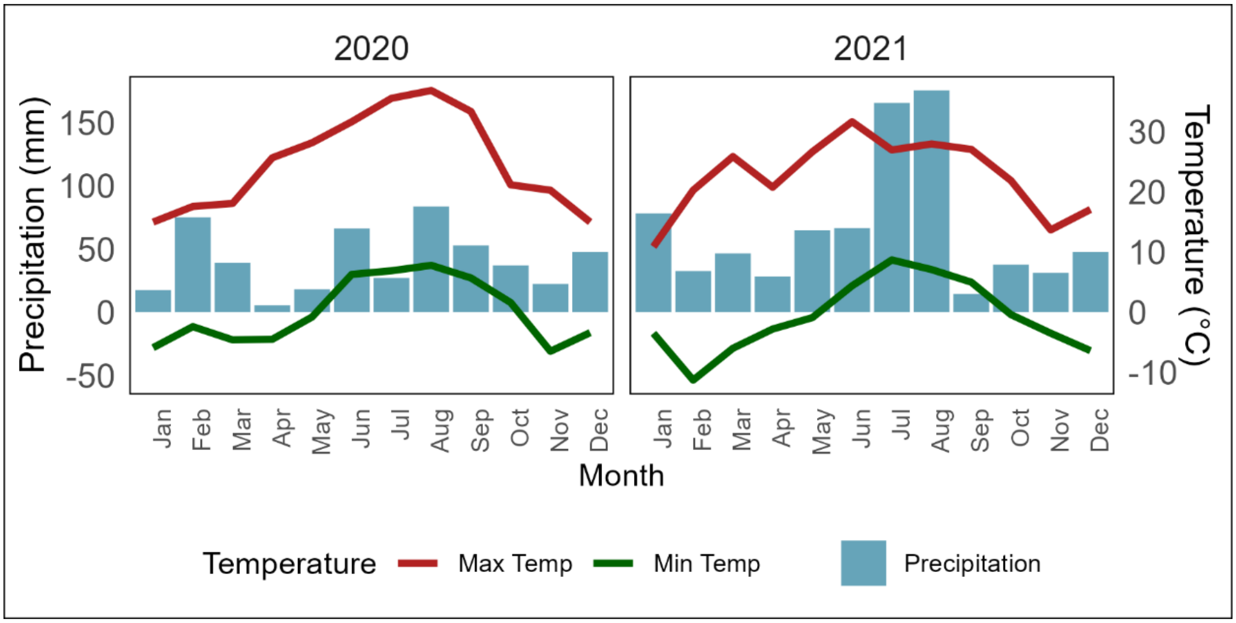

Figure 2.1: Monthly precipitation [mm] and minimum and maximum temperature [°C] of the experimental years (2020 and 2021) at Campus Klein Altendorf.

The climatic water balance at the site was calculated with the Penman-Monteith equation according to FAO (1998) based on data from the weather station at Campus Klein-Altendorf. The climatic water balance at the site was positive in the winter season in all experimental years, but there were considerable differences between the years during the vegetation period with strongly; negative balances from March to August in 2018, 2019 and 2020, but a slightly positive balance in 2021 (Table 2.1).

Table 2.1: Climatic water balance (mm) at Campus Klein Altendorf over the duration of the field experiment.

Season	Climatic water balance (mm)	
	Sep-Feb	Mar-Aug
2017-18	166	-276
2018-19	129	-181
2019-20	195	-286
2020-21	141	24

2.2.2 Treatments and Agronomic Management

Pre-crop treatments included lucerne (L) as deep-rooted crop and ryegrass (G) as control/shallow-rooted crop. Technical amelioration included deep tillage (T), deep tillage with compost amendment (TC) and reference (R) which was without both deep loosening and compost. Deep tillage (T) was done in strips one meter apart from each other to a maximum depth of 60 cm. Biowaste compost (C) from a local compost plant was incorporated at a rate of 3 kg m^{-2} into the subsoil (30-60 cm) during the deep tillage described in (Schmittmann et al., 2021). While the main purpose of incorporating the compost was to stabilize the soil structure after deep tillage, the compost would also supply some additional nutrients to be released upon rhizosphere activity. The resulting soil modifications and the sampling area are shown in Figure 2.2, the nutrient contents of the compost are given in Table 2.2.

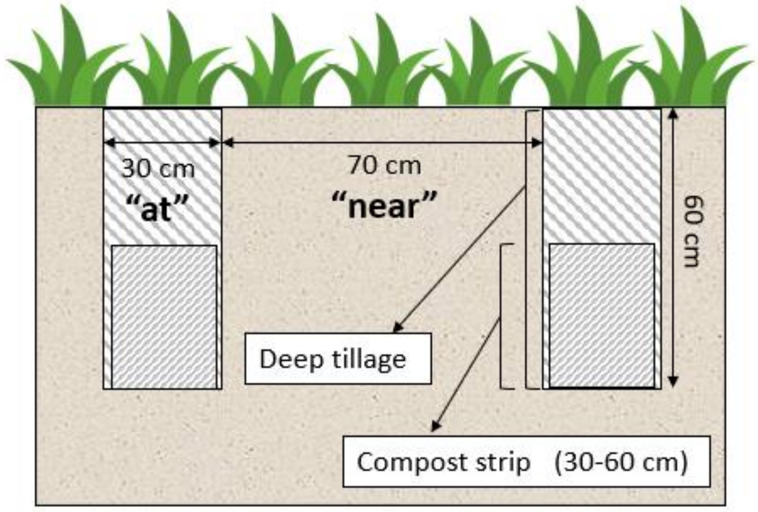

Figure 2.2: Method of technical subsoil amelioration, within this soil profile, the "at" position refers to the 30 cm-wide zone centred over the compost strip and deep tillage, while the "near" position refers to the remaining 70 cm-wide zone between two adjacent strips.

Table 2.2: Nutrient concentrations of compost used for treatments with technical amelioration including compost.

Nitrogen		Phosphate	Potassium oxide	Magnesium oxide	C:N
total [kg t^{-1}]	N_{min} [kg t^{-1}]	[kg t^{-1}]	[kg t^{-1}]	[kg t^{-1}]	
11.7	0.7	5.6	9	4.9	13

The experiment was two-factorial with six treatments conducted in a randomized complete block design (RCBD) with six blocks. Pre-crop treatments were established in 2016, regularly mulched in 2017 and 2018, and tilled in spring 2019. Due to technical reasons, the technical subsoil amelioration could only be carried out after harvest of the first main crop spring wheat (which is not considered in this study) in October 2019. Two subsequent cereal crops were grown in the next two successive years (spring barley in 2020 and winter wheat in 2021). Year-wise information on the treatments, crops and field activities is shown in Figure 2.3. All treatment combinations are listed in Table 2.3.

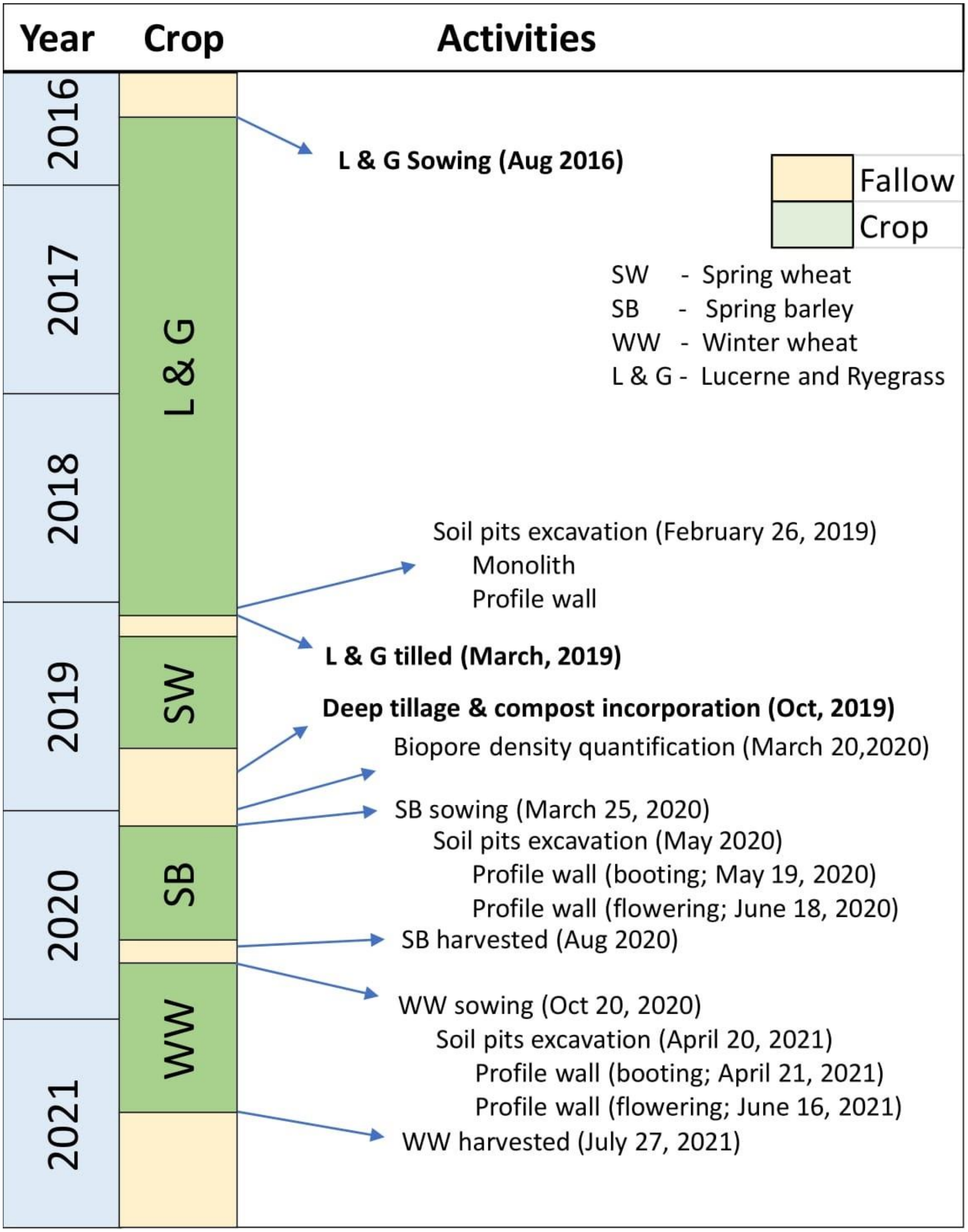

Figure 2.3: Year-wise experiment timeline showing dates of treatment implementation, crop duration and sampling activities.

Table 2.3: Treatment combinations.

		Tillage		
		Reference (R)	Deep tillage (T)	Deep tillage & compost (TC)
Pre-crops	Ryegrass (G)	GR	GT	GTC
	Lucerne (L)	LR	LT	LTC

Spring barley (cv. *RGT Planet*) was sown at a rate of 165.3 kg/ha on March 25, 2020. The fertilizer calcium ammonium nitrate was applied on April 07, 2020 at the rate of 81 kg N/ha. Winter wheat (cv. *Rubisko*) was sown at a rate of 157.5 kg/ha on October 20, 2020, following soil ploughing to a depth of 20 cm. The fertilizer calcium ammonium nitrate was applied on March 6, 2021, at the rate of 56 kg N/ha, and on April 15, 2021, at the rate of 60 kg N/ha. Additional chemical treatments applied to spring barley and winter wheat during the study period are provided in Supplementary Table S5.1.

2.2.3 Belowground data collection

2.2.3.1 Quantification of subsoil biopore density

Biopore density was quantified March 16th to 24th 2020 in four plots that had been cultivated either with perennial ryegrass (treatment GR) or with lucerne (treatment LR) from August2016 to February 2019 followed by spring wheat in 2019 (see Figure 3), areas of 50×50 cm were excavated to a soil depth of 40 cm. Biopores were revealed by carefully flattening the surface with spatulas and removing soil particles with a vacuum cleaner. After applying a metal frame of exactly 0.25 m^2 supplied with a mm scale, the area was photographed using a Nikon D1700. The whole procedure was repeated at 50 and 60 cm soil depth in each plot. Later, biopore densities in the size classes < 1 mm, 1-2 mm, 2-5 mm and > 5 mm were extracted from the images using the deep-learning software Rootpainter V0.2.27 as described in (Smith et al., 2022). As reported by Smith et al. (2022), RootPainter models trained using corrective annotations achieved good accuracy in segmentation of images, including detection of biopores.

2.2.3.2 Quantification of root growth

Soil pits of approximately 2 m depth for lucerne and ryegrass, 1.2 m depth for spring barley and 2 m depth for winter wheat were dug using an excavator in the selected plots approximately 2 m wide and 2 m long. In total, for the two pre-crops (treatments LR and GR), soil pits were established in three field repetitions, resulting in six soil pits in total, while for cereals there were 16 plots in which the soil pits were dug, as four blocks of four treatments (LR, GR, LTC and GTC) were considered.

2.2.3.3 Monolith method

For determining the number of root segments in different diameter classes in the subsoil as a proxy for biopore genesis potential of lucerne and ryegrass, soil monoliths of size 10 cm (depth) x 10 cm (width) x 10 cm (length) were collected at various depths from the established soil pits, first by smoothening with spades, then inserting scrapers with hammers. They were collected into buckets and then stored in plastic bags. After sampling, soil monoliths were stored in a cold room to be washed and scanned later. For a more detailed description of the monolith method, see the study by Kemper et al., (2022, 2025). To separate roots from the soil, monoliths were soaked in a bucket with tap water and washed by hand using multiple metallic sieves with a minimum of 0.50 mm and a maximum of 4 mm mesh size until all the soil particles washed away and only the roots were left in the sieves. Subsequently, roots were manually sorted with tweezers, taking out the dead roots and non-root particles like straw leftovers from previous crops. Afterwards, cleaned roots were stored in the freezer until scanning.

In order to measure root length from monoliths, previously frozen roots were thawed, laid (preferably without overlaps) on to an acrylic glass platter filled with tap water and photo-scanned by Epson V700 at a DPI of 800 in TIFF format and the compatible software Epson Scan version 3.9.2.1. The original scans were converted to JPEG at the same DPI for analysis by WinRhizo program for root length and root diameter. As an indicator for biopore genesis potential of the lucerne and ryegrass, the number of root segments with an assumed length of

10 cm each per m² was calculated from monoliths by dividing total root length per diameter class by monolith height (10 cm) and converting to 1 m².

2.2.3.4 Profile wall

Root length density was quantified by the profile wall method (Böhm, 1979), for the cereals at the developmental stages of booting and flowering. A vertical profile wall was flattened and smoothed with a spade, transversely to the plant rows. Then, a 5 mm thick soil layer was loosened by a metal scraper and later sprayed with water to expose the roots in this 5 mm thick layer. A metal frame was vertically placed in line with the horizontal soil surface and in the middle of the treatment area of a plot, enabling the estimation of root length density.

Pre-crops and spring barley

A 100 cm × 100 cm metal frame with grids of 5 cm × 5 cm was fixed to the profile wall. Root length units (RLU) equivalent to (exposed after spraying process) were visually estimated in each square of the grid in a range of 100 cm width, from surface soil until 100 cm depth. In each grid, root length within biopores was measured separately from root-length in bulk soil, if applicable. RLU were then converted into root length (by multiplying the number of 5 mm root segments by 0.5 cm). Then root length density density (RLD, cm cm^{-3}) was calculated by dividing root length (cm) of each grid by the volume of 12.5 cm³ (0,5 cm × 5 cm × 5cm). Later RLD was grouped to represent three depth levels of 0-30 cm, 30-60 cm, and 60-100 cm. For pre-crops, three additional depth levels (100-130 cm and 130-165 cm and 165-200 cm) were added. For spring barley, at each depth the 5x5 cm estimation squares were grouped into two different positions: The label 'at' refers to the 30 cm amelioration strip, while 'near' refers to the adjacent area of 70 cm between two amelioration strips (Fig. 3).

Winter wheat

The metal frame was separated into 10 grids, vertically into five (0-35 cm, 35-70 cm, 70-100 cm, 100-130 cm, 130-165 cm) and horizontally into two grids (50 cm each). Images were taken from these parts with a camera (Nikon D7100). Later, these images were cut to represent grids of 5 cm x 5 cm area each. These 5 x 5 cm images were analysed for root length with the deep-learning software RootPainter V0.2.27 (Smith et al 2022) and later grouped to represent five depth levels of 0-30 cm, 30-60 cm, 60-100 cm, 100-130 cm and 130-165 cm. Horizontally, as for spring barley the positions ‘at’ and ‘near’ were evaluated separately (Fig. 3). The originally planned assessment of root-length within biopores was not carried out as it turned out to be difficult to train RootPainter to distinguish roots in biopores from roots in the bulk soil.

Root images from profile wall (winter wheat) were analysed with RootPainter software V 0.2.27 (Smith et al., 2022) to measure root length. The volume of soil for each grid for profile wall method was 12.5 cm^3 (5 cm x 5 cm x 0.5 cm). Root length density (RLD, in cm cm^{-3}) was calculated as follows:

$$RLD\ (cm\ cm^{-3})\ = \frac{Root\ length\ (cm)}{Soil\ volume\ (cm^3)} \tag{1}$$

2.2.3.5 *Soil mineral nitrogen*

Soil samples for determining mineral nitrogen (Nmin) content were collected using a Pürckhauer auger at the following depths: 0–30, 30–50, 50–60, 60–70, and 70–100 cm. Sampling was conducted in both 2020 and 2021. In 2020, samples were taken on April 8 and May 19, while in 2021, sampling occurred on March 10 and June 16. Nitrate and ammonium concentrations were analyzed photometrically using a continuous flow analyzer (Seal QuAAtro 39, Norderstedt, Germany) following extraction with potassium sulfate.

2.2.4 Aboveground data collection

2.2.4.1 *Shoot dry matter and grain and straw yield*

Biomass samples were collected at the booting and flowering stages for both spring barley and winter wheat. An area of 0.5 m^2 measured by metal frame was harvested by cutting plants 3 cm above the ground using an electric cutter. The fresh biomass from each plot was weighed to determine total fresh mass. The plant material was then chopped to a uniform size, and a small portion (aliquot) was taken and weighed. This aliquot was dried at 60°C for 24 hours, followed by 105°C for another 24 hours. The dry weight was used to calculate the dry matter percentage and total dry matter (g m^{-2}). Straw and grain yield at maturity were also determined using the same method.

2.2.4.2 Nutrient uptake

Nutrient contents (Carbon, Nitrogen, Phosphorus and Potassium) were analysed from biomass samples at booting and flowering stages for both winter wheat and spring barley. Dried samples were milled with a vibratory disc mill (RS 200 Retsch, Germany). Milled samples were then analysed for nitrogen content with the Dumas method using Elemental analysis (Eurovector EA 3100 dual, Pavia, Italy). Phosphorous content was analysed using a continuous flow analyser, QuAAtro 39 (Seal Analytical, Southampton, England) and for potassium content with atomic absorption spectrometry using PinAAcle 500 (PerkinElmer, Waltham, USA).

2.2.5 Data analysis

Statistical analysis of biopore density, number of root segments, root length density, maximum rooting depth, mineralised nitrogen, shoot dry matter, nutrient uptake and yield was carried out using the statistical programme R (R Core Team, 2022). The R packages *agricolae* and *tidyverse* were used for performing two-factorial analyses of variance (ANOVA) followed by one way ANOVA and Post-Hoc HSD Tukey test. The two-factorial design allowed us to test for interactions between the factors. Because all factor combinations represent realistic management options and interactions were sometimes observed, we chose to present one-way ANOVA results with post-hoc comparisons for every combination, supporting clearer and more useful treatment comparisons for practical application. All the graphs were created with R using ggplot2 and ggpubr package.

2.3 Results

Pre-crop root characteristics and effects on biopore densities in the subsoil

After two and a half years of growth, both lucerne and perennial ryegrass showed a rooting depth of at least 200 cm soil depth. Lucerne had significantly higher RLD only at 130-165 cm depth (Fig. 2.4). Ryegrass had a significantly higher number of fine root segments (< 1 mm diameter), while lucerne had significantly more root segments with a diameter between 1 and 2 mm However, after tillage of the pre-crops there were no clear differences in biopore density regardless of diameter class (Fig. 2.5).

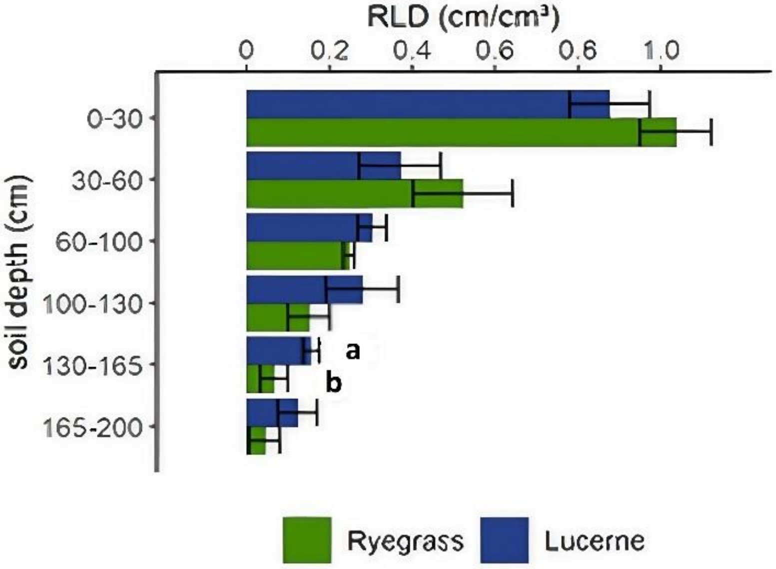

Figure 2.4: Root length density (RLD) for lucerne and perennial ryegrass (spring 2019). The error bars indicate the standard deviation. Significant differences are indicated by different lowercase letters, t-test, $p < 0.05$ (n=3).

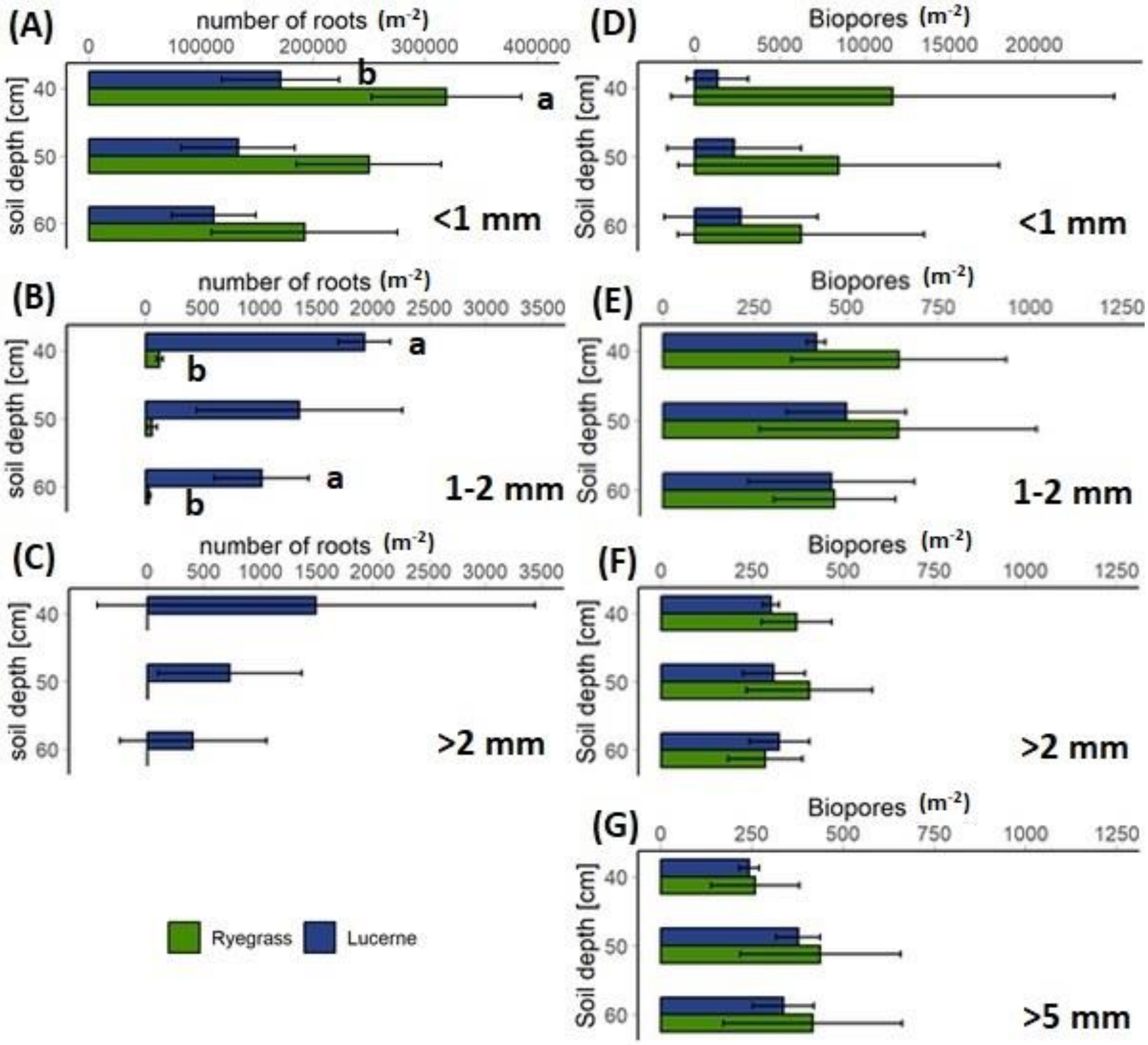

Figure 2.5: Number of root segments of lucerne and perennial ryegrass with 10 cm length (February 2019) and biopore density in the subsoil (spring 2020) in diameter classes < 1mm (A+D), 1-2 mm (B+E) and > 2 mm (C+F), and biopore density in the subsoil in diameter class > 5 mm (G). The error bars indicate the standard deviation. Significant differences among pre-crops within each depth level are indicated by different lowercase letters, one-way ANOVA, $p < 0.05$ (n=4).

2.3.1 Root growth of cereals

Spring barley

At booting stage, RLD determined with the profile wall was significantly higher in the GTC treatment compared to GR at two depth levels of 0-30 cm and 30-60 cm, while no significant differences were found at 60-100 cm (Fig. 2.6 A). At the flowering stage, LTC had significantly higher RLD at 60-100 cm as compared to GTC (Fig. 2.6 B). The percentage of RLD inside large-sized biopores as compared to total RLD showed no statistical differences at any depth level both during booting and flowering – however, overall, much higher shares of roots in biopores were observed during booting (Fig. 2.6 C and D). The highest percentage of root length in biopores was 89% shown after ryegrass without technical amelioration (GR) at 60-100 cm at booting stage. Furthermore, treatments differed in maximum rooting depth: At shooting, spring barley had a mean rooting depth to 80 cm in all treatments, maximum rooting depth increased most in LTC until flowering, while maximum rooting depth did not increase further after booting (Figure 2.6 E and F). Evaluation of root length density was also done for the position factor at both booting and flowering stage. At booting stage, no significant effect of amelioration was noticed at any depth level. However, at flowering stage, it was found that the RLD 'at' the amelioration stripe was higher as compared to the position 'near' the amelioration stripe at the depth levels of 0-30 cm and 30-60 cm, while no significant effect was found at 60-100 cm (Fig. 2.6 G and H). The results of the two-way ANOVA, including p-values for the main effects of pre-crop, deep tillage, and their interactions on RLD and maximum rooting depth, are summarized in supplementary Table S2.2 and S2.4. Significant effects on RLD were observed for deep tillage at depth 0-60 cm for booting stage while interaction of pre-crop and deep tillage was only significant for 0-30 cm for booting stage. Maximum rooting depth showed significant effect of pre-crop treatment and interaction of both factors at flowering stage.

Winter wheat

Root length density showed no significant differences at both booting and flowering stage of winter wheat across all depth levels (Figure 2.7 A+C). For the position factor, at both booting and flowering stage, it was found that the RLD 'at' the amelioration stripe was higher as compared to the position 'near' the amelioration stripe at the depth level of 30-60 cm, while no significant effect was found at other depth levels (Fig. 2.7 B+D). The results of the two-way ANOVA, including p-values for the main effects of pre-crop, deep tillage, and their interactions on RLD and maximum rooting depth, are summarized in supplementary Table S2.3 and S2.6. The only significant interaction of pre-crop and deep tillage for RLD was observed at the depth of 0-30 cm for flowering stage and also lucerne was significantly effective as compared to ryegrass at the same depth. There was no other interaction or main effect found at any other depth. Maximum rooting depth also showed no significant effect of either lucerne and deep tillage + compost and also no interaction both factors.

2.3.2 Soil mineral nitrogen

Mineralized nitrogen was measured at the beginning of the experiment (August 2016) during trial establishment and again in the following year (March 2017). In 2016, no treatment effects were observed; however, in 2017, lucerne showed higher mineralized nitrogen concentrations than ryegrass in the deeper soil layer (50–100 cm). During cereal crops, mineralised nitrogen was found to be significantly affected by treatments only in 2020 at booting and maturity stage, with significantly higher N_{min} contents in either LR or LTC as compared to GR (Fig. 2.8).

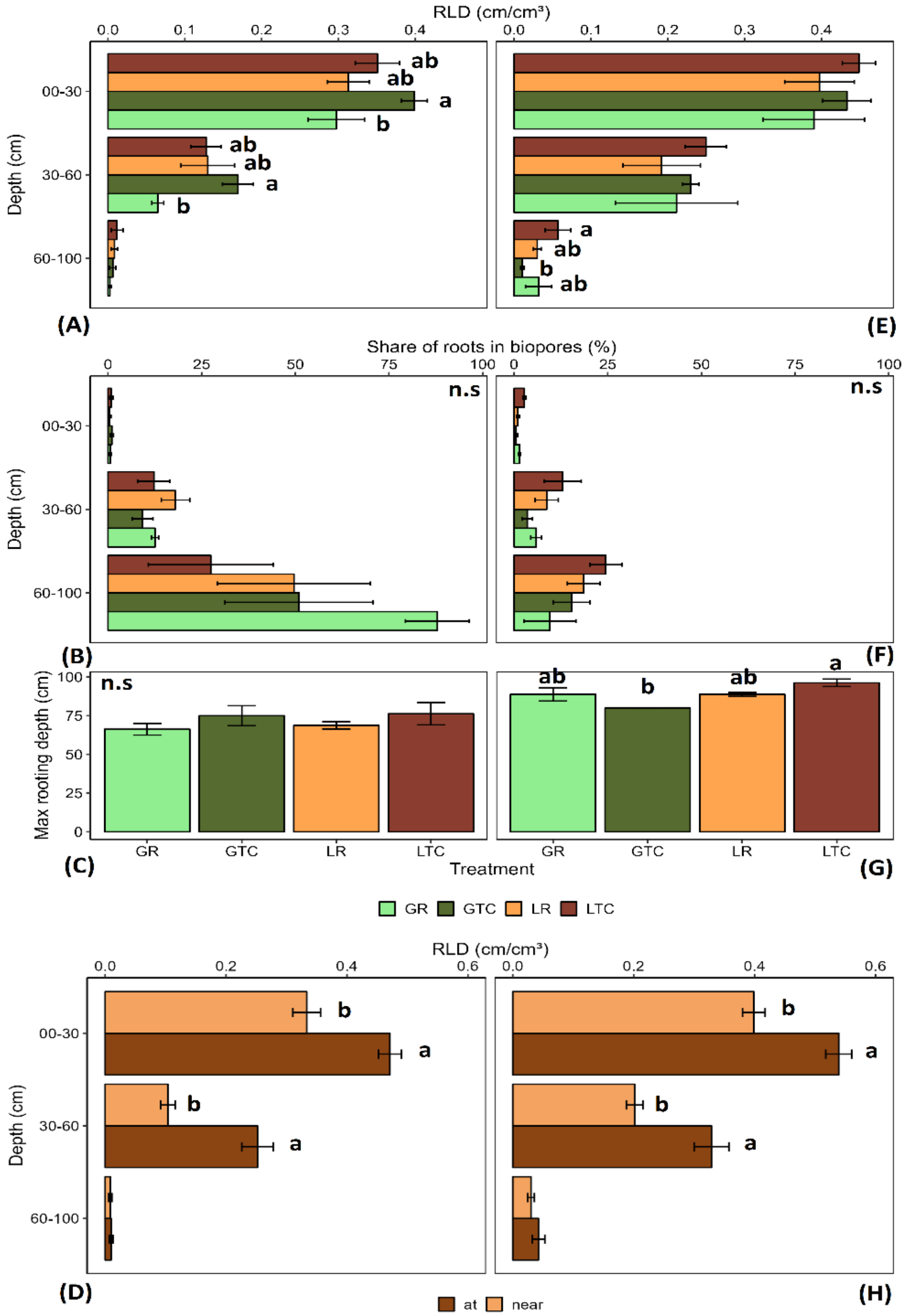

Figure 2.6: Root parameters of spring barley at booting (A-D) and flowering stage (E-H): Root length density (RLD, A+E), Share of roots in biopores (B+F), maximum rooting depth (C+G) and RLD at positions ‘at” and ‘near’ (mean of treatments GTC and LTC, D+H). G and L represent ryegrass and lucerne as pre-crop, respectively, while R and TC represent reference and deep tillage with compost amendments, respectively. Significant differences among treatments within each depth level are indicated by different lowercase letters, HSD Tukey test, $p < 0.05$ (n=4).

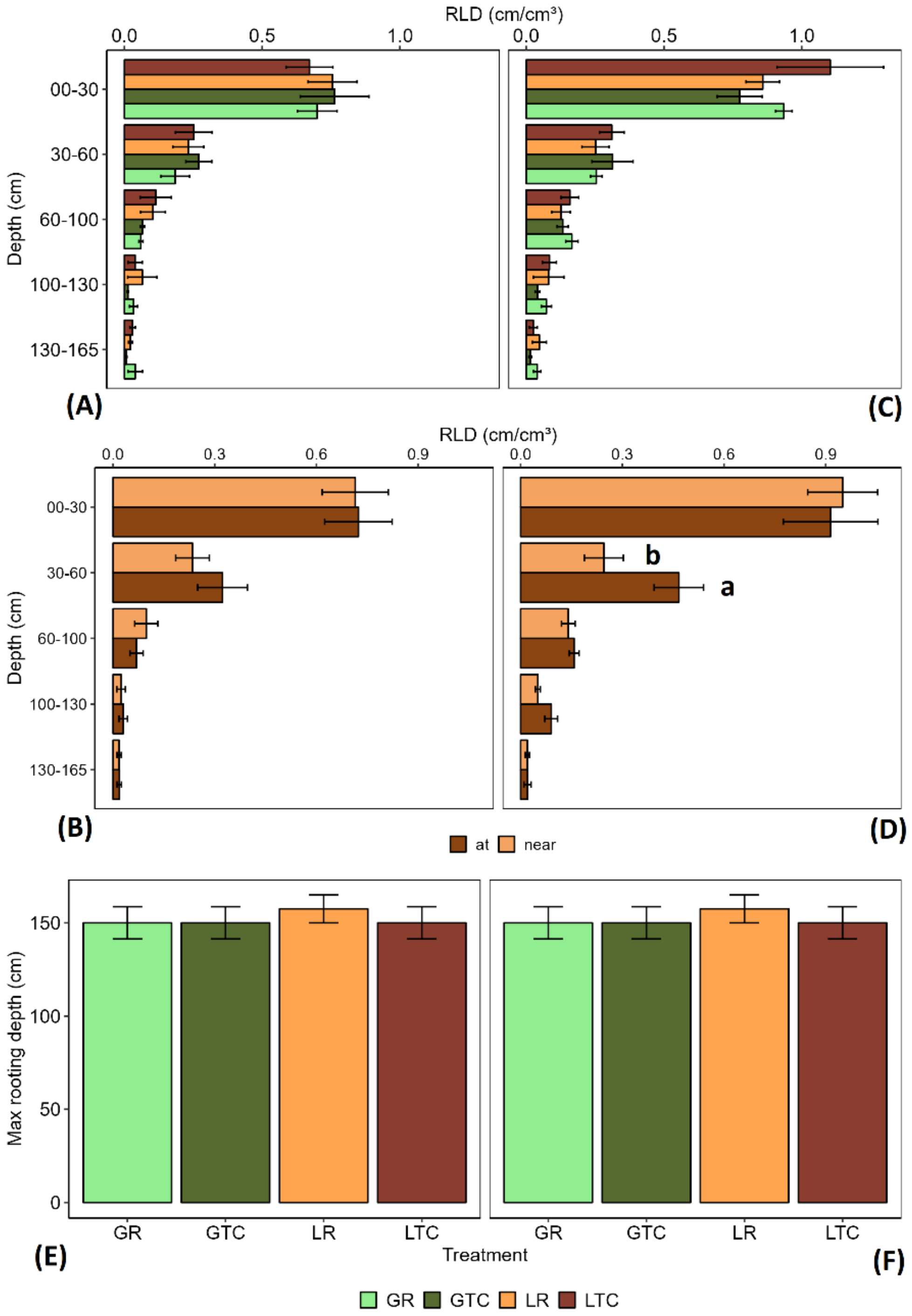

Figure 2.7: Root parameters of winter wheat at booting (A+B) and flowering (C+D): Root-length density (RLD, A+C), RLD at positions ‘at” and ‘near’ (mean of treatments GTC and LTC, B+D) and maximum rooting depth (E+F). G and L represent ryegrass and lucerne as pre-crop, respectively, while R and TC represent reference and deep tillage with compost amendments, respectively. Significant differences among treatments are indicated by different lowercase letters according to HSD Tukey test, $p < 0.05$ (n=4).

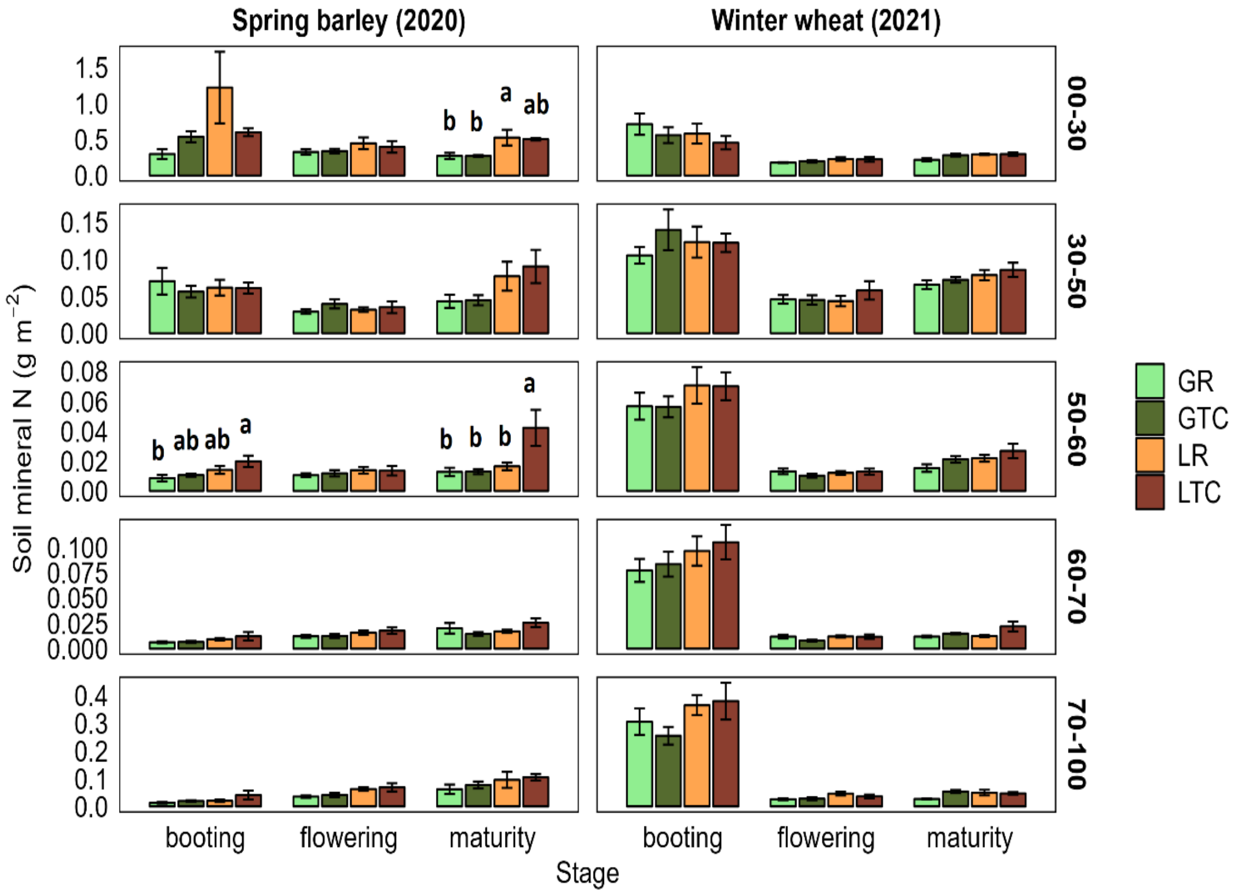

Figure 2.8: Soil mineral nitrogen for 2020 and 2021. G and L represent ryegrass and lucerne as pre-crop, respectively, while R and TC represent reference and deep tillage with compost amendments, respectively. Different lowercase letters indicate significant differences between each treatment within each growth stage according to HSD Tukey test, $p < 0.05$ (n=6).

2.3.3 Aboveground parameters

Nutrient uptake

Lucerne with technical amelioration (LTC) as compared to ryegrass with or without deep tillage (GR, GT) resulted in higher carbon, nitrogen, phosphorus and potassium uptake by spring barley at both booting (Fig. 2.9A) and flowering stages (Fig. 2.9B) except for phosphorus uptake at flowering stage. Technical and biological treatments resulted in no significant effects regarding the uptake of carbon, nitrogen, phosphorus and potassium at both the booting (Fig. 2.10 A) and flowering (Fig. 2.10 B) stage in winter wheat.

2.3.4 Shoot dry matter, grain and straw yield

Spring barley showed highest shoot dry matter both at booting and anthesis stage in response to pre-crop lucerne when combined with deep tillage and compost as compared to the lowest dry matter after pre-crop ryegrass with and without deep tillage (Fig. 2.11 A+B). For winter wheat, no significant treatment effects were found among treatments for shoot dry matter at both booting and flowering stage (Fig. 2.11 E+F). Mean grain yield of spring barley was between 423-516 g m^{-2} across all treatments and showed significant effects among treatments with GR resulting in lower yield as compared to GTC, LT and LTC (Fig. 2.11 C), while there was no significant difference for straw yield (Fig. 2.11 D). Grain and straw yield of winter wheat from 2021 showed no significant differences among the treatments (Fig. 2.11 G+H). Mean grain yield was between 920-1040 g/m^2 across all treatments. The p-values of the two-way ANOVA, for the main effects of pre-crop, deep tillage, and their interactions on dry matter, straw and grain yield for spring barley and winter wheat, are summarized in supplementary Table S2.4 and S2.8. In spring barely, significant effects were observed for deep tillage and pre-crop for dry matter at both booting and flowering and grain yield while no significant interaction was observed among both factors. In winter wheat, there were no main effects or interactions observed for dry matter and grain yield.

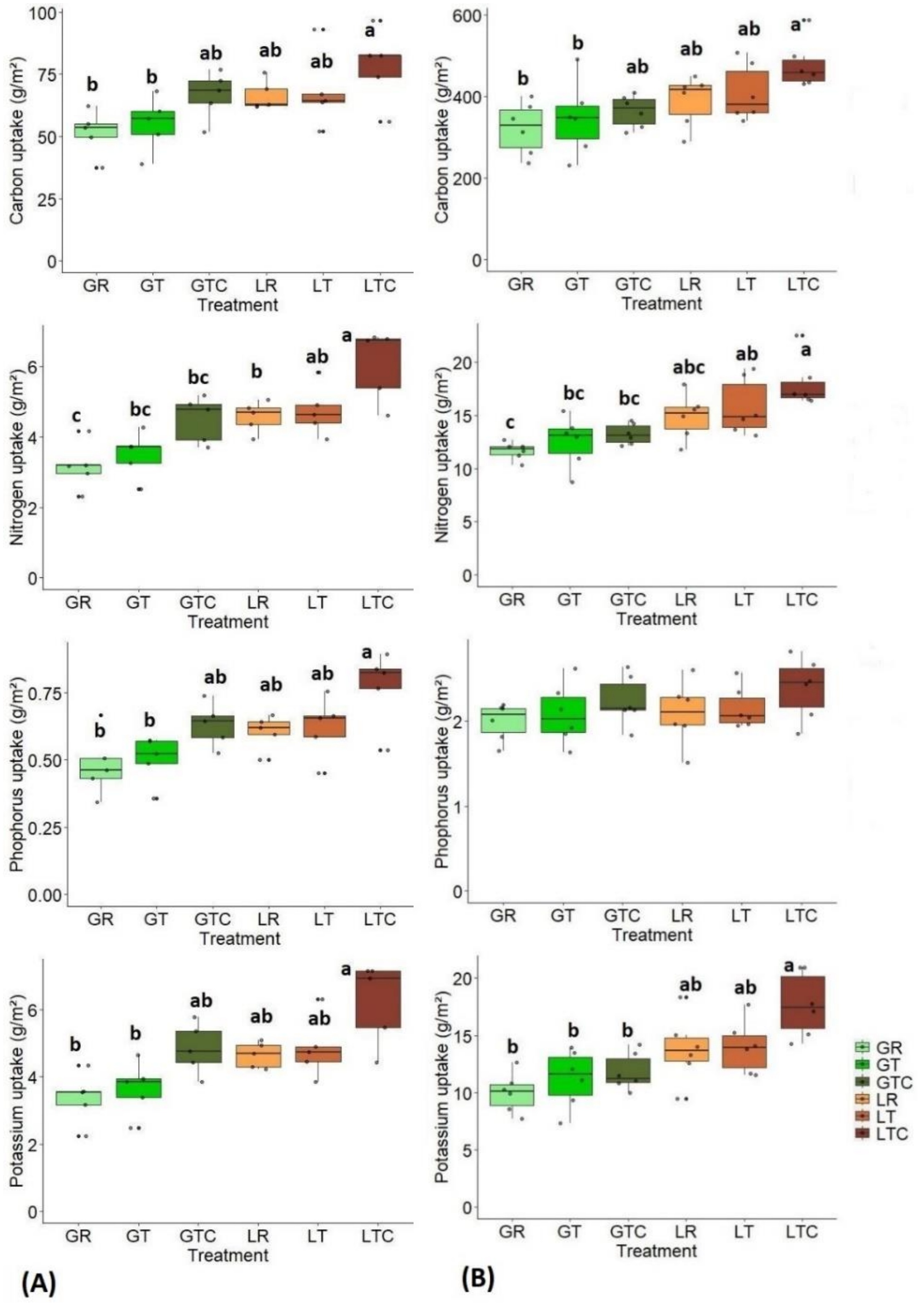

Figure 2.9: Carbon, nitrogen, phosphorus and potassium uptake by spring barley in 2020 at booting stage on left side (A) and flowering stage on right side (B). G and L represent ryegrass and lucerne as pre-crop, respectively, while R, T and TC represent reference, deep tillage and deep tillage with compost amendments, respectively. Different lowercase letters indicate significant differences according to HSD Tukey test, $p < 0.05$ (n=6).

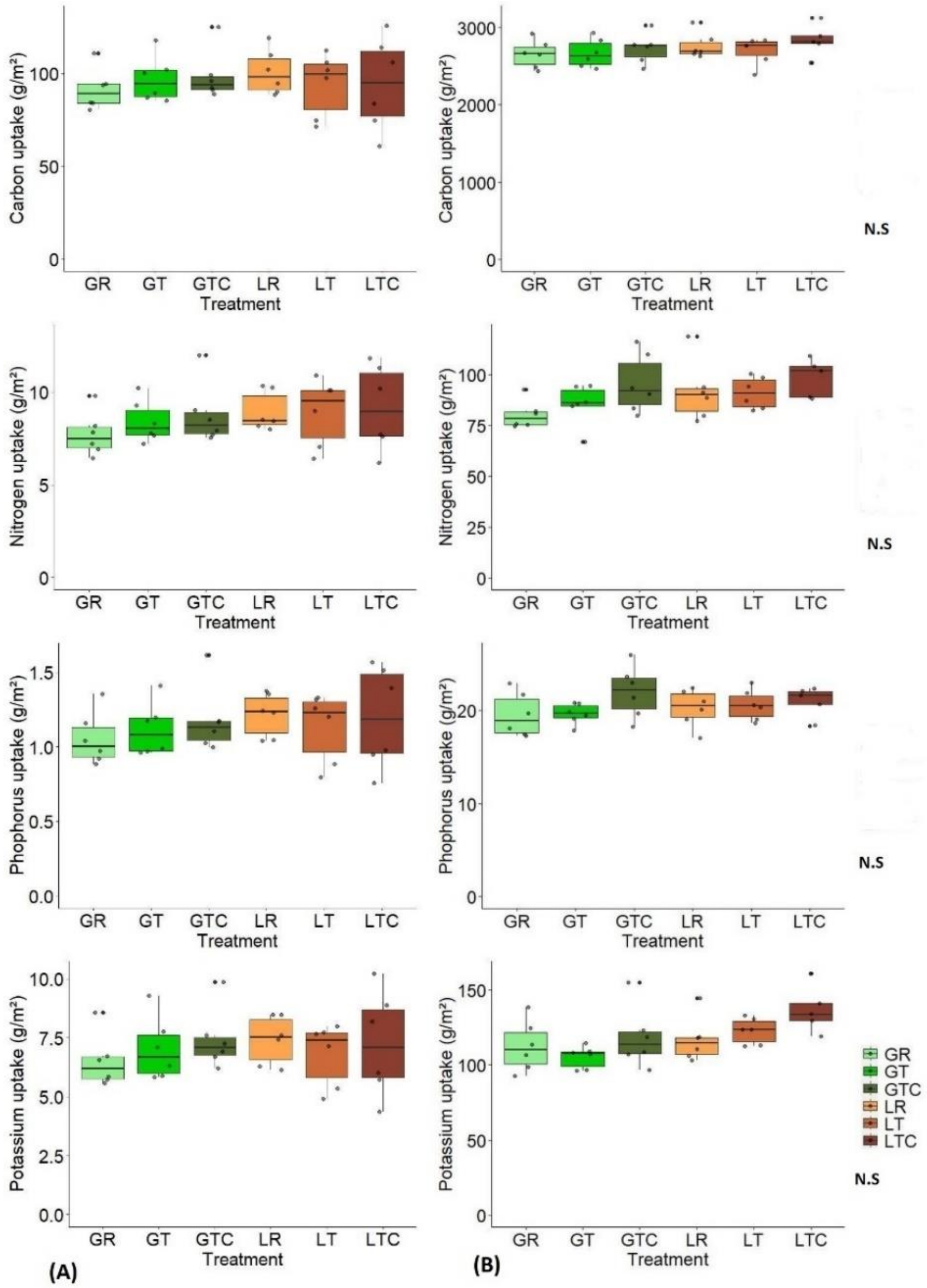

Figure 2.10: Carbon, nitrogen, phosphorus and potassium uptake by winter wheat in 2021. (A) booting stage on left side, (B) flowering stage on right side. G and L represent ryegrass and lucerne as pre-crop, respectively, while R, T and TC represent reference, deep tillage and deep tillage with compost amendments, respectively. No significant differences among treatments at booting and flowering according to HSD Tukey test, $p < 0.05$ (n=6).

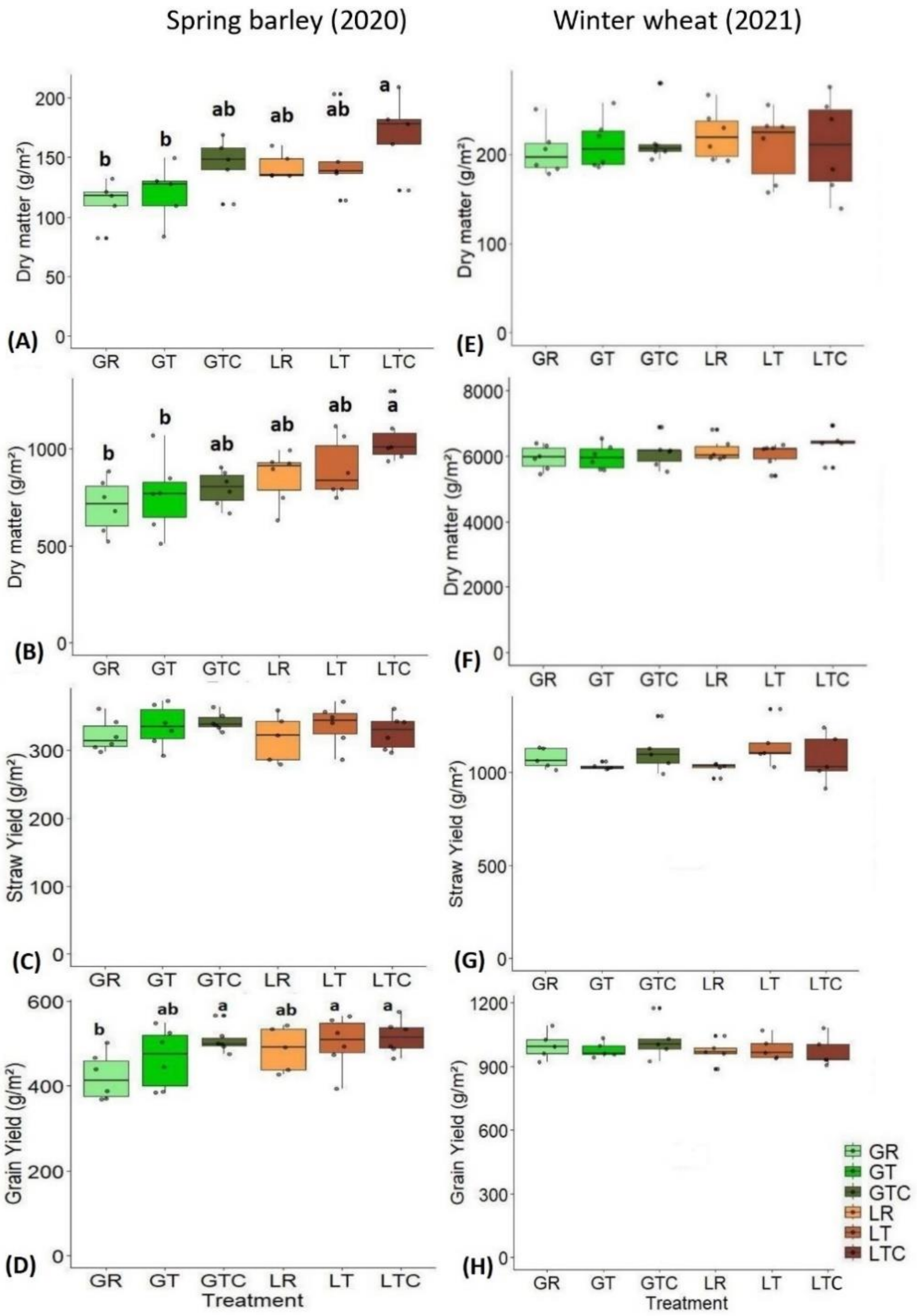

Figure 2.11: Shoot parameters of spring barley (A-D) and winter wheat (E-H): shoot dry matter at booting (A+E), shoot dry matter at flowering (B+F), grain yield (C+G) and straw yield (D+H). G and L represent ryegrass and lucerne as pre-crop, respectively, while R, T and TC represent reference, deep tillage and deep tillage with compost amendments, respectively. Different lowercase letters indicate significant differences according to HSD Tukey test, *p* < 0.05 (n=6).

2.4 Discussion

In this study, we analysed root length density, nutrient uptake, dry matter content and yield of spring barley and winter wheat in response to the two factors: 1. technical subsoil amelioration (reference, deep tillage and deep tillage + compost) and 2. biological subsoil amelioration (pre-crop lucerne as deep-rooted crop and pre-crop ryegrass as control). Year was not considered a factor because of the varying weather conditions of both years, different crops, length of growing seasons and various other factors. As expected, lucerne developed tendentially or significantly higher RLD in the deeper subsoil below 60 cm soil depth (Fig. 4), however, the control pre-crop perennial ryegrass had higher RLD in the topsoil and upper subsoil. Comparing the number of root segments in diameter classes < 1 mm revealed a considerably higher potential of ryegrass to form small-sized biopores, while lucerne had more root segments in diameter classes 1-2 mm and > 2 mm (Fig. 5). However, these differences in biopore genesis potential did not translate into differences in biopore density in the subsoil, which is contrasting our first hypothesis and also earlier studies at the same experimental site where evaluation of biopore density in the subsoil resulted in higher density after taprooted lucerne and chicory as compared to shallow rooted tall fescue (Han et al., 2015; Smith et al., 2022). Han et al. (2022) quantified biopores across various soil types and vegetation histories with multisite data set and found that the effects could persist for decades but they also observed a shift in distribution of pore sizes. Formation of finer pores, blockage of existing pores and reduction in diameter may explain the non-persistence of biopore patterns in our experiment. Notably, we observed very high heterogeneity among biopore sampling areas particularly in the ryegrass plots, indicating that the sampling area of only one area with 0.25 m² per plot was too small for a reliable quantification. In former studies, we included two internal repetitions per plot. Nevertheless, the very high share of roots growing in biopores during booting stage across all treatments indicates preferential root growth of the fibrous-rooted cereal crop in biopores to quicker reach deeper soil layers in this growth phase with little precipitation.

At the booting stage, spring barley resulted in greater RLD in the topsoil and mid-depths (up to 60 cm) under the LTC treatment compared to GR (Fig. 6). By the flowering stage, differences in RLD within the upper soil layers diminished, while LTC maintained higher RLD than GTC in soil layers deeper than 60 cm. The experimental year 2020 was relatively drier than 2021, with not only lower precipitation during the growth season as compared to the long-term annual mean, but also the third year in a row with a negative climatic water balance. Apparently, under these conditions the technical subsoil amelioration (GTC) induced the spring barley grown in this experimental year to root primarily in the ameliorated soil layer of 30-60 cm at booting stage, while root extension to deeper soil layers was not significant as compared to the reference treatment GR. In contrast, technical amelioration combined with the deep-rooting pre-crop lucerne (LTC) supported root growth of spring barley in the 60-100 cm soil layers at flowering stage as compared to GTC. This promotion of root growth into deeper soil layers in LTC resulted in highest shoot biomass and nutrient uptake at both booting and flowering as well as in highest grain yield. GR and GT had lowest shoot dry matter and nutrient uptake during growth and also lowest grain yield, while GTC, LR and LT were intermediate. Thus, both of our hypotheses were confirmed for spring barley that lucerne as a deep-rooted crop (both alone and in combination of deep tillage and compost) enhances root growth, nutrient uptake and crop yield.

In contrast, winter wheat did not respond to the experimental treatments neither belowground nor aboveground in 2021 which was characterized by abundant water supply. These results are in line with our observations from a nearby experimental site at Campus Klein-Altendorf comparing the response of spring and winter sown cereals and oilseed rape to taprooted pre-crops chicory and lucerne and fibrous rooted fescue over a period of 10 experimental years. In that study, spring-sown cereals reacted stronger with increases in grain yield in response to lucerne as compared to fescue than the winter-sown crops (Behrend et al., 2025). Furthermore, in drier years all crops showed pronounced yield increases after lucerne. However, root growth of winter crops responded stronger to pre-crop induced modification of subsoil structure than in the current study Perkons et al. (2014). With visual estimation of RLD at the profile wall, Perkons et al. (2014) were also able to quantify root-length in biopores, which was not possible with our approach of AI based RLD quantification. While visual estimation and AI based quantification of RLD are well correlated and AI based quantification can therefore be considered reliable (Han, Smith, et al., 2021), differentiating roots growing within biopores from roots growing in the surrounding bulk soil requires distinguishing biopore background from bulk soil background. This can easily be done visually, but so far, we did not succeed in training an AI model to recognize these subtle differences. Therefore, future studies aiming at quantification of RLD in biopores should either refer to the more time-consuming visual estimation approach or make sure to train a model that is capable of distinguishing the biopore background beforehand.

The only significant effect observed for winter wheat in our study was higher RLD in the zone of compost incorporation as compared to the adjacent area (Fig. 7), suggesting enhanced soil porosity improved root proliferation pathways within that tillage and compost strip. (Zhao et al., 2019) reported that the compost application at specific depths can enhance root growth in wheat through modifications in soil properties (water stable aggregates and enhanced microporosity). The missing effects observed for winter wheat in response to both biological and technical subsoil amelioration and their combination on other root parameters, shoot growth and nutrient uptake as well as grain yield can presumably be attributed to abundant moisture availability resulting from higher precipitation and a positive climatic water balance in 2021 during critical growth stages, coupled with the extended duration of the winter wheat season resulting in deeper root growth already at the start of the season. These conditions likely prevented root growth limitations, reducing stress levels and limiting the influence of treatment factors on nutrient uptake and crop growth.

With respect to technical subsoil amelioration, a previous study by (Getahun et al., 2022) investigated the effect of loosening and straw incorporation into the upper subsoil, which resulted in higher SOC and soil total nitrogen, but higher crop yields only in the first year spring wheat crop (reported by Getahun et al. (2018)), otherwise only negligible effects on spring cereal crop yields. These treatments were somewhat similar to our technical amelioration, but incorporated the organic amendments at shallower soil depth levels (25-34 cm). Similarly, (McPhee et al., 2023) did not detect effects of application of poultry manure and poppy seed meal into the upper subsoil on vegetable yields. However, other studies using organic amendments with higher nutrient availability such as poultry manure (e.g. Sale et al., 2021) or soils with greater restrictions to crop growth (e.g. Uddin et al., 2022) did report considerable increases in grain yield. Sale et al. (2021) compared surface application and subsoil injection of poultry manure in 30-40 cm deep rip-lines spaced 80 cm apart, and found higher yield increases in the subsoil manuring treatment in the drier experimental year. In our study, mineralized nitrogen showed no pronounced increase as result of treatments in winter wheat while it showed higher mineralized nitrogen at the depth of 50-60 cm in response to LTC as compared to other treatments at booting and maturity stages in spring barley. This combined with higher root density and maximum rooting depth in LTC possibly resulted in higher nitrogen uptake in spring barley but no effect in winter wheat. In winter wheat, no significant differences among treatments were observed for maximum rooting depth, root length density, or mineralised nitrogen.

Previous studies by Jakobs et al. (2019) and Bauke et al. (2024), which investigated technical subsoil amelioration (without pre-crop treatments) on the same crops as in the current study, found stronger effects on root growth and grain yield for spring barley compared to winter wheat. However, during the very dry year of 2018, winter wheat also showed increased root length density (RLD) in the subsoil following amelioration (Jakobs et al., 2019). (Seidel et al., 2022) also reported higher RLD in response to deep tillage and consequently higher yield for spring barley. Additionally, Uhlig et al. (2023) analysed nutrient uptake depth in spring barley using $^{87}Sr/^{86}Sr$ ratio and observed that treatments involving compost incorporation (GTC and LTC) shifted the main nutrient uptake zone deeper, from 10–45 cm to 20–60 cm (GTC) or 10–60 cm (LTC). This suggests that compost applied at 30–60 cm depth likely enhanced nutrient availability, supported by preferential root growth in this meliorated zone as shown in Figures 6 and 7.

The compost thus provided an additional, but spatially restricted reservoir of nutrients to the crops, and the authors concluded that the enhanced root growth into the ameliorated subsoil rather than the additional nutrients from compost was likely the main cause for higher crop nutrient acquisition from the subsoil. Notably, similarly to Bauke et al. (2024), also in the current study measurements of mineral nitrogen do not indicate increased N mineralization and leaching risk after compost incorporation compared to standard management or only mechanical loosening.

Higher total nutrient uptake and grain yields in LTC as compared to GTC suggest further benefits of the nitrogen-fixing and deep-rooting pre-crop lucerne. Increased nitrogen supply ultimately derived from N-fixation is supported by the tendentially to significantly increased soil mineral nitrogen contents (Fig. 8). Increased supply of plant available phosphorous and potassium may have resulted from a nutrient uplift during pre-crop growth from deeper soil layers via P and K mobilization in deeper soil layers and deposition at the soil surface with mulching (Han, Li, et al., 2021). This way, the treatments LR and LTC can enable the utilization of otherwise unexplored nutrients from the atmosphere and deeper subsoil layers for growth of following crops.

Water uptake was not measured in the current experiment, therefore no conclusion can be drawn on how treatments have affected water uptake. However, there is evidence that higher biopore densities in the subsoil resulting from deep-rooting pre-crops can increase water uptake from deeper soil layers from both pot and field experiments as well as modelling studies: (Dresemann et al., 2018; Koch et al., 2021) conducted pot experiments and observed higher wheat shoot biomass especially under limited water supply in the presence of artificial biopores, while (Gaiser et al., 2012) showed higher root growth coinciding with increased water uptake from deeper soil layers of spring wheat after lucerne as compared to other pre-crops in the field. Modelling approaches (Gaiser et al., 2013; Landl et al., 2019; Seidel et al., 2019) also indicate that biopores can improve access to water resources in deeper soil layers. With respect to technical subsoil amelioration, Jakobs et al. (2019) observed lower soil water contents as compared to the control immediately underneath the amelioration furrow when compost was added, indicating enhanced water extraction from this region and therefore also an increased use of subsoil water resources. Similarly, (Sale et al., 2021) identified increased utilization of plant available water in the subsoil as a key benefit of injecting deep-band nutrient-rich organic amendments into the subsoil, with the greatest impact on crop performance in dry years. Furthermore, in Mediterranean-type climates of southern Australia, a yield response of winter crops to deep fertiliser mostly occurred on infertile sandy soils in low rainfall regions (Ma et al., 2009). These results further highlight the potential of subsoil melioration for mitigating adverse effects on crop performance induced by drought periods. Although water uptake potential was not measured in this experiment, it is a vital factor which influences crop growth and response to any soil amelioration. Future research should include measurements of water uptake to better understand its role in crop performance.

As discussed by Bauke et al. (2024), the Haplic Luvisols under study do not contain compacted root restricting layers, which are usually the primary motivation for technical subsoil amelioration (F. Schneider & Don, 2019). The results of our study indicate that under dry conditions and for spring crops, even at fertile sites with an intact soil structure technical subsoil amelioration can improve access to subsoil resources and consequently improve crop growth and grain yield. However, similar effects were achieved with biological subsoil amelioration, which requires much fewer external energy and material resources. Only long-term observations complemented by economic analyses can show whether the additional benefits of combining technical and biological subsoil melioration demonstrated in the current study are persistent and therefore may outweigh the additional financial effort.

3 Root growth and yield effect on different cereal genotypes in response to technical subsoil melioration

3.1 Introduction

Our second experiment aims to enhance root growth and crop performance of cereals by promoting the deeper root growth and improving soil structure through deep tillage and compost incorporation, while investigating the response of different cereal genotypes to this technical melioration.

The supply of nutrients from the subsoil can become increasingly important, especially in dry or depleted topsoils (Kautz et al., 2013). Since roots in the subsoil prefer to grow in macropores, the drilosphere (the area of the soil that is influenced by earthworm activity) has comparatively high levels of organic matter and dissolved substances are also channelled spatially in these areas of the subsoil, macropores can be identified as a hotspot for nutrient mobilisation (Kautz et al., 2013). The subsoil as a nutrient source for plants has hardly been scientifically analysed for a long time. The potential it offers for the nutrient supply of plants is largely unexplored (Kautz et al., 2013).

The root characteristics of the genotypes of same species differ depending on the biological, chemical and physical environmental factors (Gregory, 2006), hence it is necessary to investigate the response of different genotypes for deep tillage and compost treatments. It is advantageous for plants to have certain characteristics which can help its roots to penetrate compacted top soil. These include reduction in elongation and enhancement of root diameter. Root character such as hydraulic conductivity, root hair length and density can enhance water uptake (Freschet et al., 2021).

We hypothesized that the effect of technical melioration (deep tillage and compost) on cereals is genotype dependent.

3.2 Materials and methods

3.2.1 Experimental site

A field trial was established in 2021 at Campus Klein-Altendorf (50°38'06"N, 6°58'36"E), an experimental research farm of the University of Bonn, located in Rheinbach in North Rhine-Westphalia, Germany. The soil type is a Haplic Luvisol derived from loess. Between 1956 and 2014, an average annual temperature at the location was 9.4°C, the average annual precipitation was 603 mm. Total precipitation in the experimental years 2021 and 2022 was 789 mm and 493 mm, respectively. Monthly precipitation, maximum and minimum temperature are shown in figure 3.1.

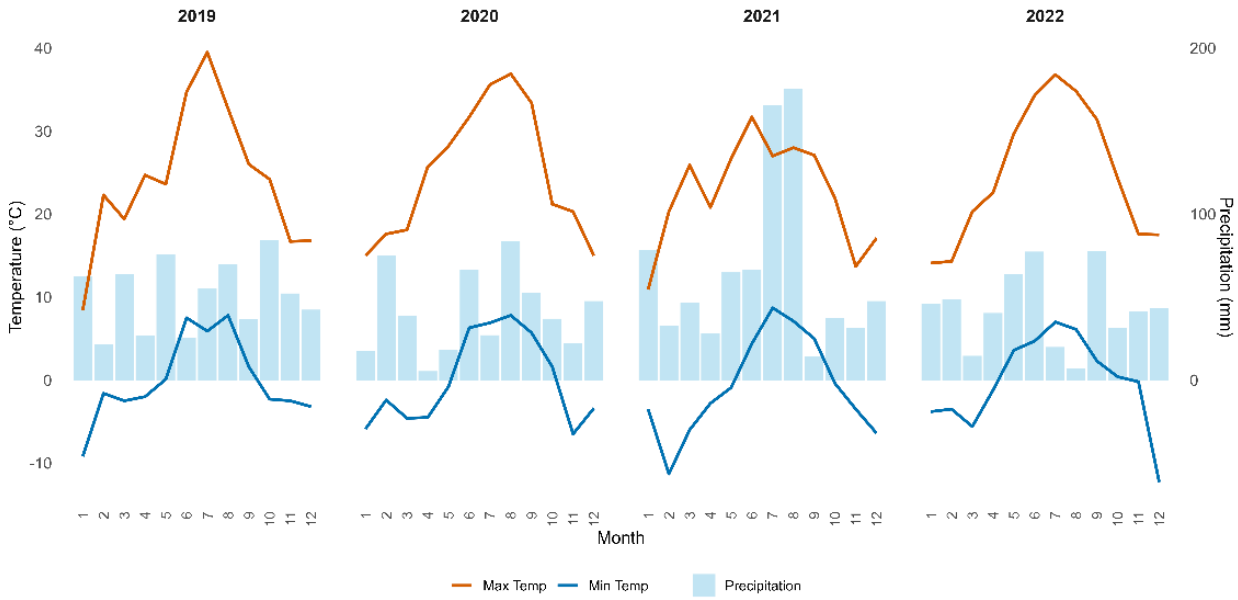

Figure 3.1: Monthly precipitation [mm] and minimum and maximum temperature [°C] of the experimental years (2019 to 2022) at Campus Klein Altendorf.

3.2.2 Treatments and agronomic management

There were two treatment factors involved; tillage and genotypes. Tillage treatments included deep loosening (T), deep loosening with compost amendment (TC) and reference (R) which was without both deep loosening and compost. Deep loosening (T) was done in the middle of each plot with melioration stripes width 30 cm while depth 60 cm. Compost (C) was incorporated into the subsoil during the deep tillage at the 30-60 cm depth (Fig. 2). Two consecutive cereal crops were grown, each with three genotypes: spring barley in 2019 and 2021, followed by winter wheat in 2020 and 2022. Sydney, Eunova and Salome were spring barley genotypes while Milaneco, Trebelir and Capo were winter wheat genotypes. The experiment was two-factorial with nine treatments conducted in a randomized complete block design (RCBD) with three blocks and with a total of 27 experimental units. Winter wheat was sown in October 22, 2021. The nutrient contents of the compost are given in Table 2.2.

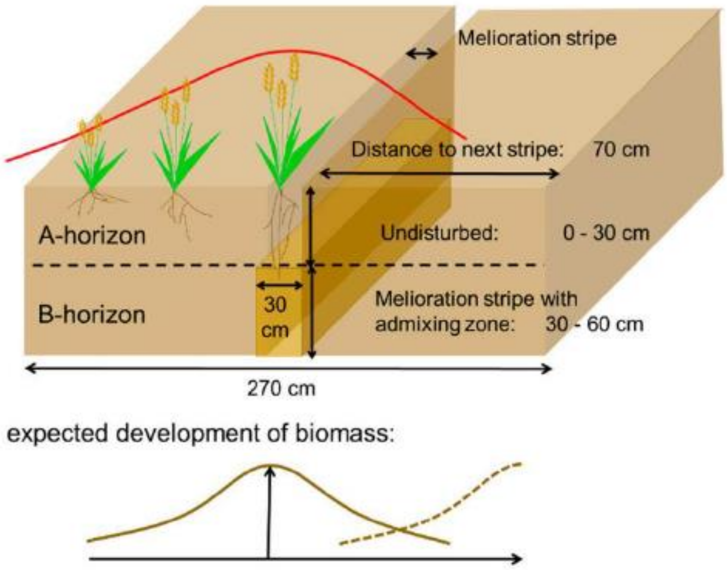

Figure 3.2: Principle of subsoil melioration from the Soil³ project and the expected Gaussian biomass distribution over the compost strip (Schmittmann et al., 2021).

3.2.3 Belowground data collection

Soil core method

Soil core method was used for collection of root samples. Soil core was 80 cm deep and had a diameter of 9.5 cm. It was divided into 8 depth levels, each representing 10 cm depth (0-80 cm). For root analysis, soil core of 80 cm was cut with scrappers to equally 8 depth levels. They were then collected into plastic bags. These samples were taken at two different positions ('in' and ‘at’). One from the plant row (“at”) and second (“in”) from the area between two adjacent plants rows within the compost strip (Fig. 3.3). After sampling, samples were stored in a cold room to be washed and scanned later.

Figure 3.3: Soil core method. Position ‘in’ represents the samples taken from the rows in melioration area (30 cm wide), while position ‘out’ represents the area between two rows.

Washing and sorting of roots

To separate roots from the soil, soil samples 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, depending on treatment, compost particles were left in the sieves. Subsequently, roots were manually sorted with tweezers, taking out the dead roots and non-root particles like compost, and straw leftovers from previous crops. Afterward, cleaned roots were stored in the freezer until scanning.

Root scanning

Washed roots were photo-scanned by Epson V700 at a DPI of 800 in TIFF format and the compatible software Epson Scan version 3.9.2.1. Roots were laid on to an acrylic glass platter filled with tap water and scanned. The scans were converted to JPEG at the same DPI for the further analysis with Rootpainter software V 0.2.27 (Smith et al., 2022).

RootPainter

Root scans from soil core method were analysed with RootPainter software V 0.2.27 (Smith et al., 2022) to measure root length. Root length density (RLD, in cm cm^{-3}) per sample was calculated as follows;

$$RLD\ (cm\ cm^{-3})\ = \frac{Root\ length\ (cm)}{Soil\ volume\ (cm^3)} \tag{1}$$

The volume of soil (depth 10 cm, diameter 9.5 cm) for each depth level was 708.82 cm^3.

3.2.4 Above ground data collection

3.2.4.1 Dry matter and yield

Biomass samples were taken manually at booting and flowering stages for both spring barley and winter wheat. An area of 0.5 m^2 (0.5 m × 1 m) was harvested in each of the 27 plots by cutting the plants approximately 3 cm above the ground using an electric cutter with 8 rows (2 middle rows at melioration stripe and 6 rows near the stripe) up to 1 meter in length. These samples were weighed for each plot to determine the total fresh mass collected. The plants were then chopped to achieve a uniform particle size in preparation for the drying process. For each plot, a small portion of the chopped material, used as an aliquot, was weighed as fresh mass. Drying was carried out first at 60°C for 24 hours and then at 105°C for another 24 hours. The dried material was weighed again to calculate the percentage of dry matter, which was further used to calculate total dry matter (g/m^2). Samples for dry matter and grain yield for both crops at maturity were collected by both manually and machine. For machine harvest, the area was 4 m^2 (1 m wide in the middle × 4 m long).

3.2.4.2 Nutrient uptake

Nutrient contents (C, N, P, and K) were determined from biomass samples collected at the booting and flowering stages of both winter wheat and spring barley. The dried samples were ground using a vibratory disc mill (RS 200, Retsch, Germany). Nitrogen content was measured via the Dumas method using an elemental analyzer (Eurovector EA 3100 dual, Pavia, Italy).

Phosphorus (P) concentrations were quantified with a continuous flow analyzer (QuAAtro 39, Seal Analytical, Southampton, UK), while potassium (K) content was assessed using atomic absorption spectrometry (PinAAcle 500, PerkinElmer, Waltham, USA). Nutrient uptake was calculated by multiplying the nutrient concentration by the corresponding dry matter weight at the booting, flowering, and maturity stages.

3.2.5 Data analysis

Statistical analysis of shoot dry matter, root length density, nutrient uptake, and yield was conducted using the statistical software R (v4.2.1; R Core Team, 2022). The R packages lme4, agricolae, and tidyverse were used to perform two-sided t-tests and linear mixed-effects models, followed by Tukey's HSD test for multiple comparisons where appropriate. All graphs were generated in R using the ggplot2 and ggpubr packages.

3.3 Results

3.3.1 Root length density

3.3.1.1 Spring barley (2019 and 2021)

In spring barley, at soil depths 30-55 cm in Eunova genotype with the deep tillage + compost treatment (TC) showed a higher RLD than the reference at booting stage but in flowering the only positive significant effect was in Sydney at 30-60 cm where TC resulted in higher RLD. Differences between genotypes in RLD were not significant. Cumulated across the depths, RLD was higher in TC than in R in both crops, but not different between genotypes (Table 3.4). In 2021, we found higher RLD in Eunova as at the depth of 40-60 cm in response to TC at flowering, while no other genotype showed any response of TC at any depth (Figure 3.5).

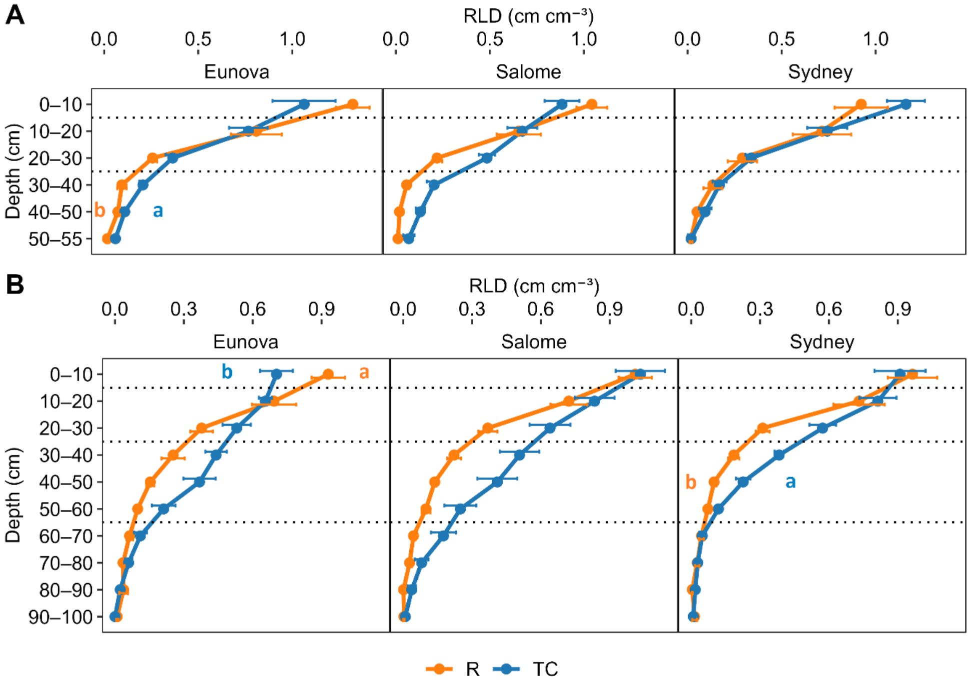

Figure 3.4: Root length density (RLD) for spring barley (2019) at booting and flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

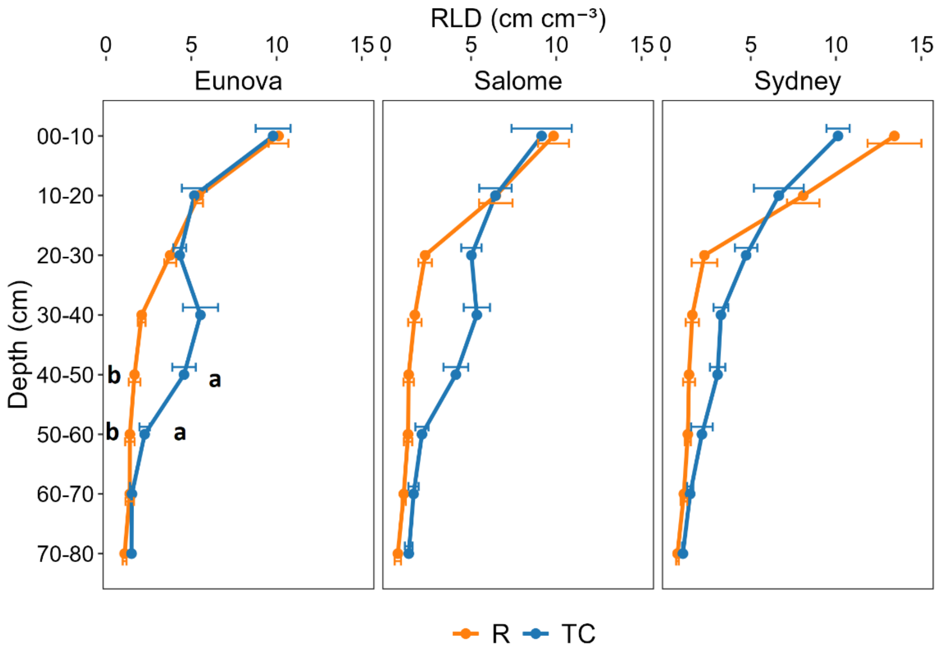

Figure 3.5: Root length density (RLD) for spring barley (2021) at flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

3.3.1.2 Winter wheat (2020 and 2022)

In winter wheat, deep tillage + compost treatment (TC) showed no significant effect on any cultivar across any depth as compared the reference at both booting stage and flowering stage. Differences between genotypes in RLD were not significant (Figure 3.5).

In 2022, at soil depths 40-50 cm the deep tillage + compost treatment (TC) showed a higher RLD than the reference only in cv Trebelir while no significant difference found in other genoypes at any depth level (Fig. 5). In individual genotypes this treatment effect on RLD was not well pronounced. Differences between genotypes in RLD were not significant for all depth levels except 10-20 cm (Table 2). Meanwhile, RLD increased in the subsoil at 30-40 cm in winter wheat as a result of tillage and compost treatment (Fig. 8).

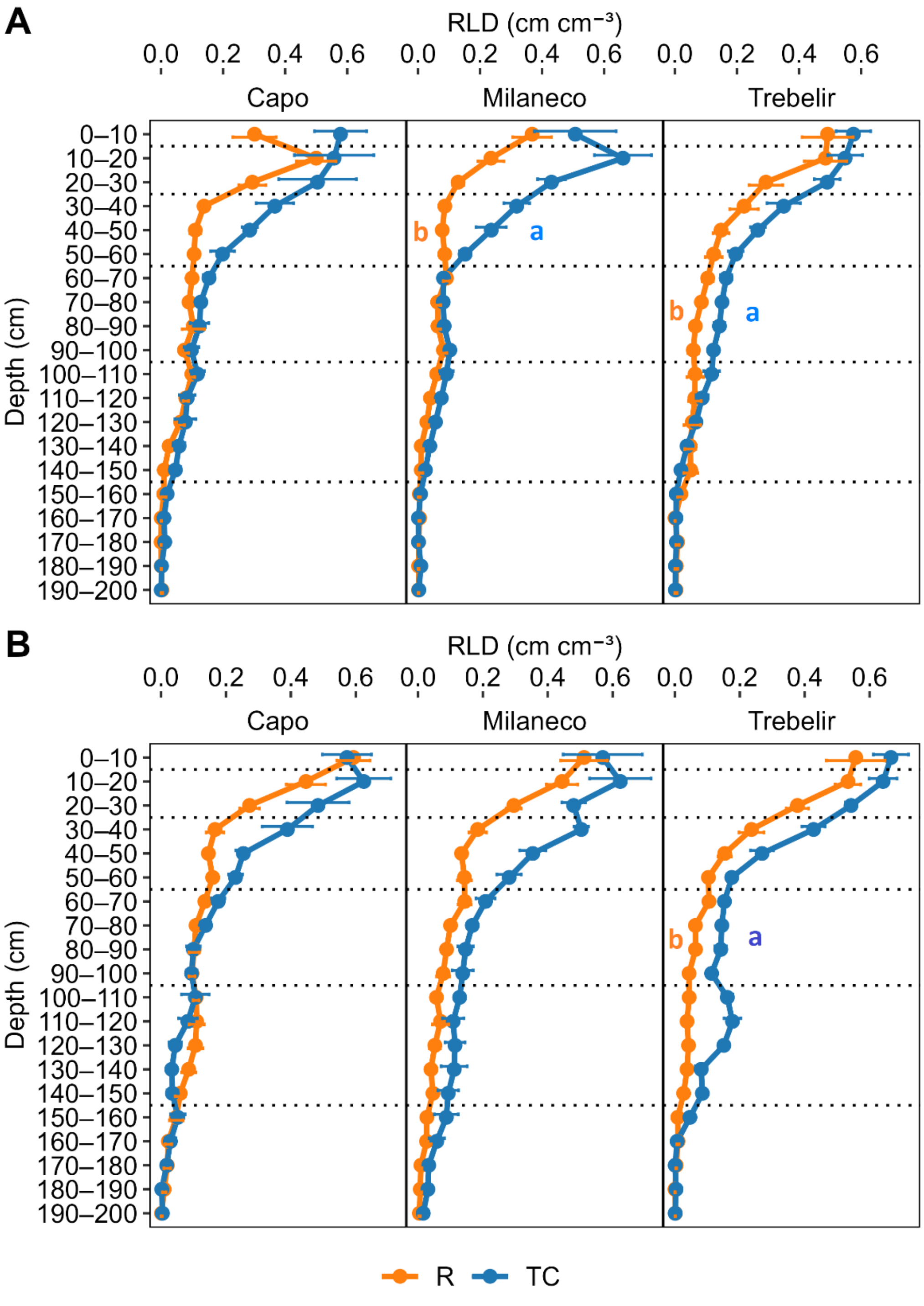

Figure 3.6: Root length density (RLD) for winter wheat (2020) at booting and flowering stage for each depth level. Significance differences are indicated by different lowercase. HSD Tukey test, *p* < 0.05 (n=3).

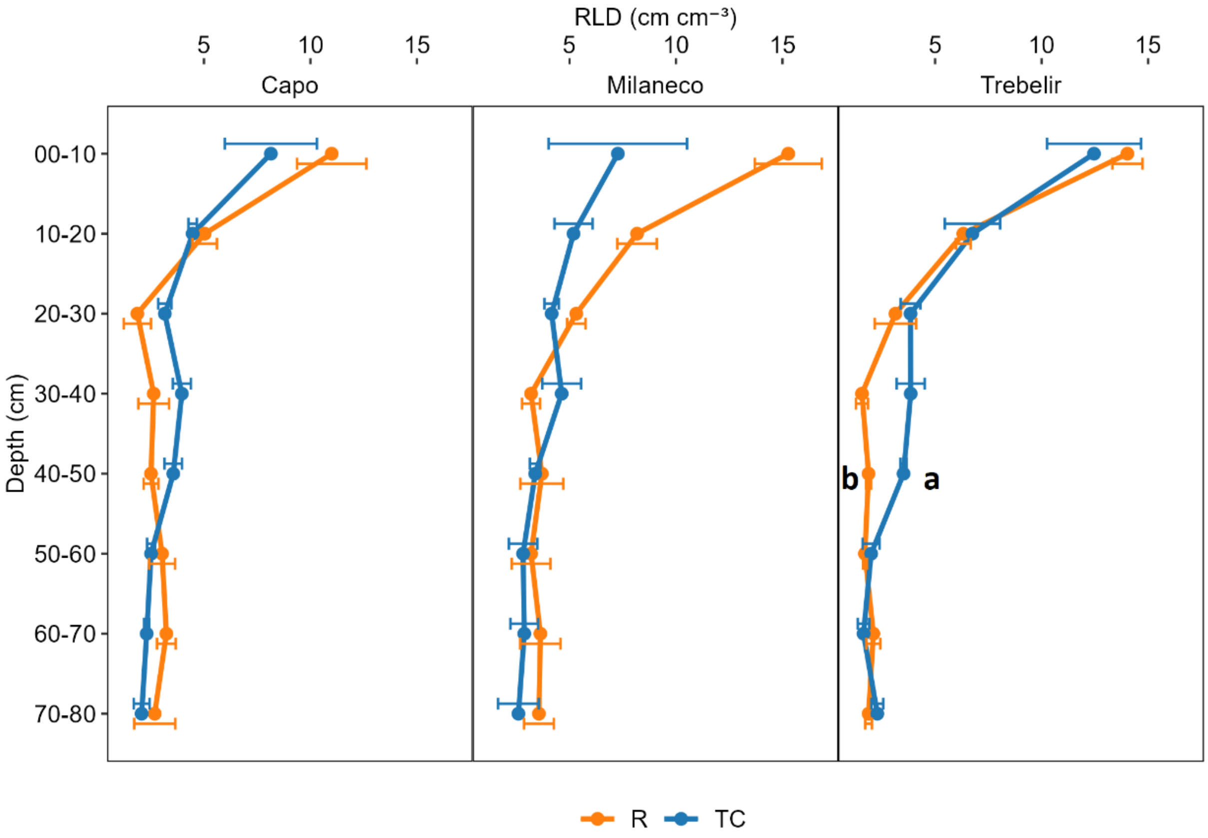

Figure 3.7: Root length density (RLD) for winter wheat (2022) at flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

3.3.2 Dry matter

3.3.2.1 Spring barley (2019 and 2021)

In spring barley, deep tillage + compost treatment (TC) showed a higher Dry matter accumulation than the reference at all stages but it was significantly higher at flowering and maturity stage (Fig. 6). In individual genotypes this treatment effect on dry matter was less evident while tillage treatment specifically TC showed great response. There was no interaction between genotypes and tillage in both 2019 and 2021.

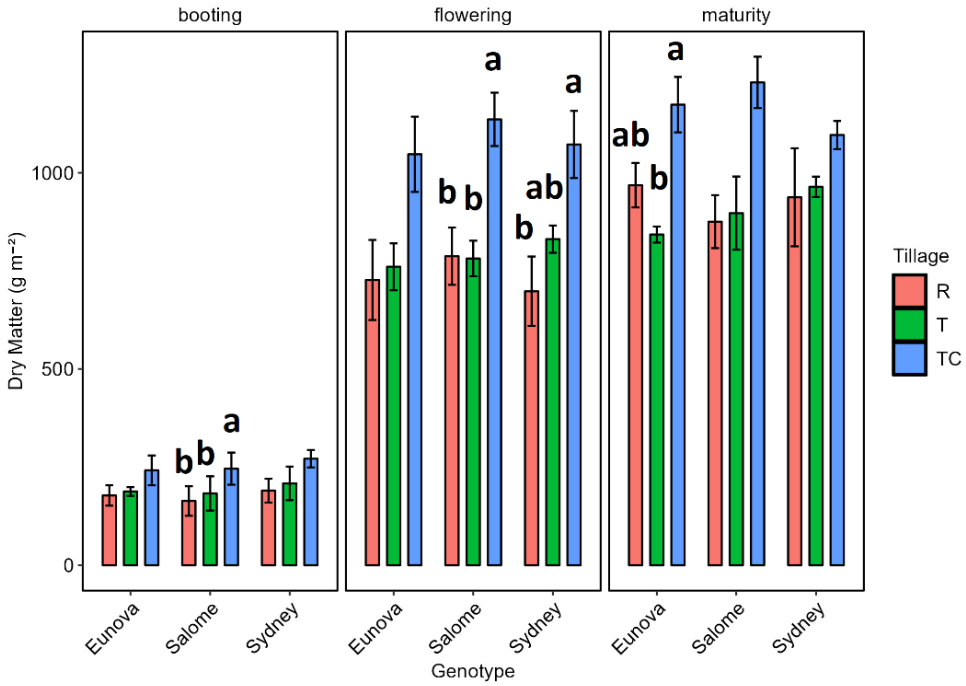

Figure 3.8: Dry matter (g m^{-2}) for spring barley (2019) at booting, flowering and maturity stage. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

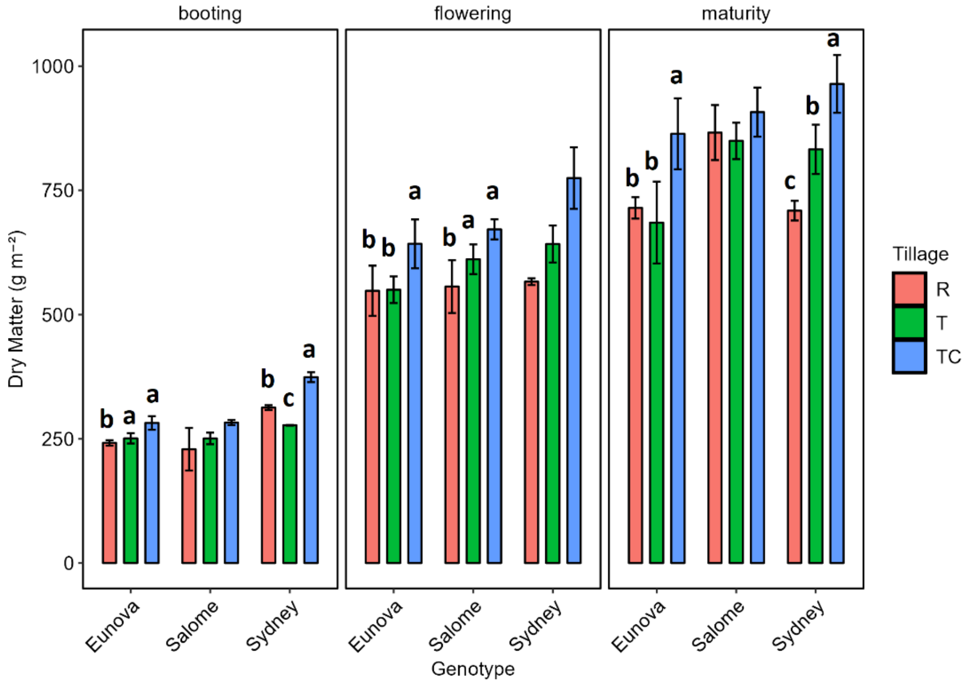

Figure 3.9: Dry matter (g m^{-2}) for spring barley (2021) at booting, flowering and maturity stage. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

3.3.2.2 Winter wheat (2020 and 2022)

In winter wheat, deep tillage + compost treatment (TC) showed a higher Dry matter accumulation than the reference for Capo genotype and only at the maturity stage (Fig. 3.10). In individual genotypes, this treatment effect on dry matter was less evident while tillage treatment specifically TC showed significantly higher dry matter accumulation at maturity stage. There was no interaction between genotypes and tillage in both 2020 and 2022.

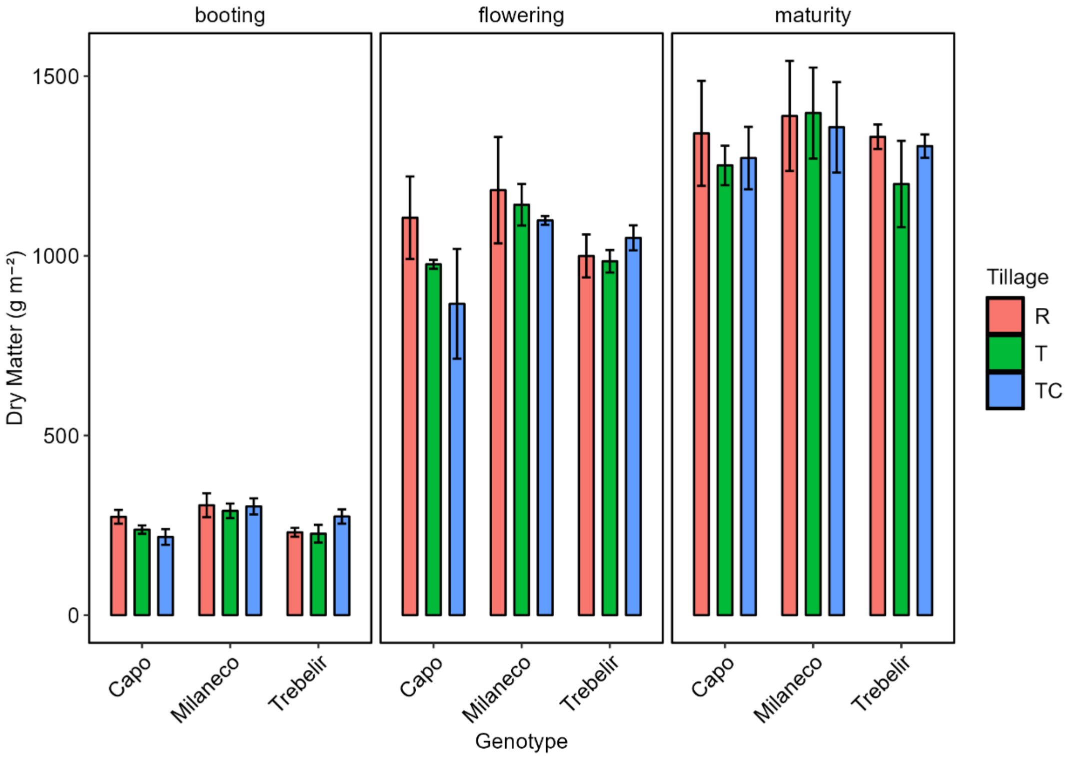

Figure 3.10: Dry matter (g m^{-2}) for winter wheat (2020) at booting and maturity stage. No significant differences were found for tillage treatment at any growth stage and genotype. HSD Tukey test, *p* < 0.05 (n=3).

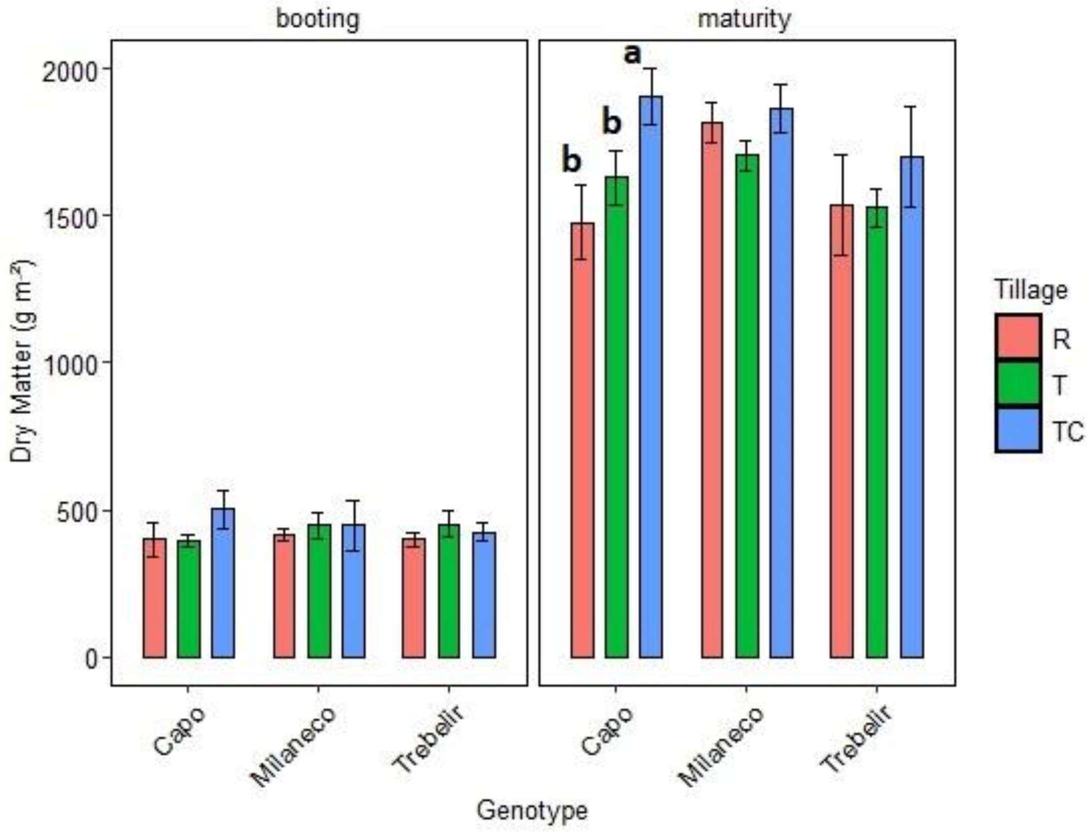

Figure 3.11: Dry matter (g m^{-2}) for winter wheat (2022) at booting and maturity stage. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

3.3.3 Yield parameters

3.3.3.1 Spring barley (2019 and 2021)

In spring barley, the combination of deep tillage and compost (TC) resulted in higher grain yields for the cultivars Eunova and Salome compared to the reference and deep tillage-only (T) treatments in 2019. No significant effects were observed on harvest index or tiller count. However, spike count increased in Sydney in response to the TC treatment (Figure 3.11). Similar results for spike count, harvest index, and tiller count were found in 2021 (Figure 3.12).

Notably, TC increased grain yield in Sydney in 2021, which contrasts with the opposite trend observed in 2019.

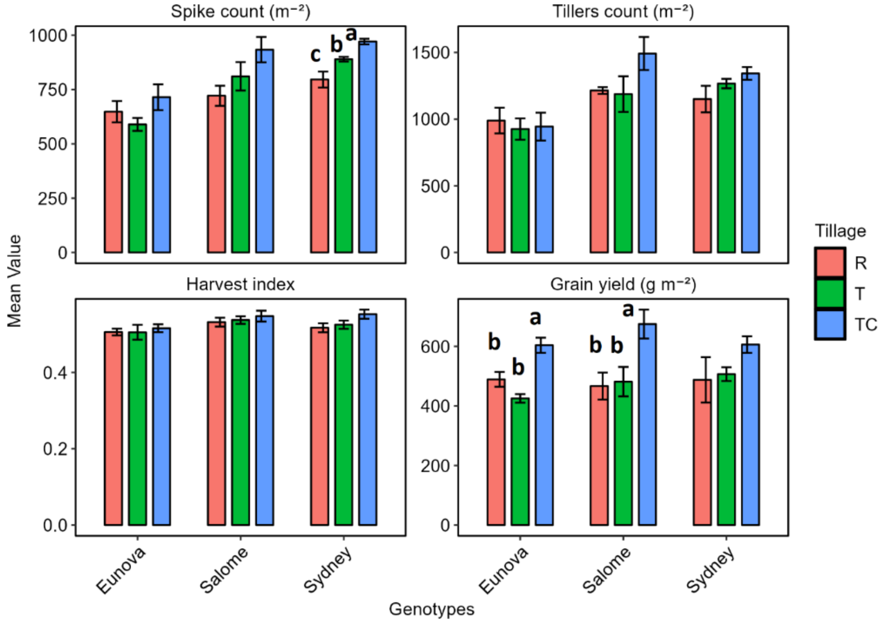

Figure 3.12: Harvest index, spike count, tillers count and grain yield (g m^{-2}) for spring barley (2019). Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

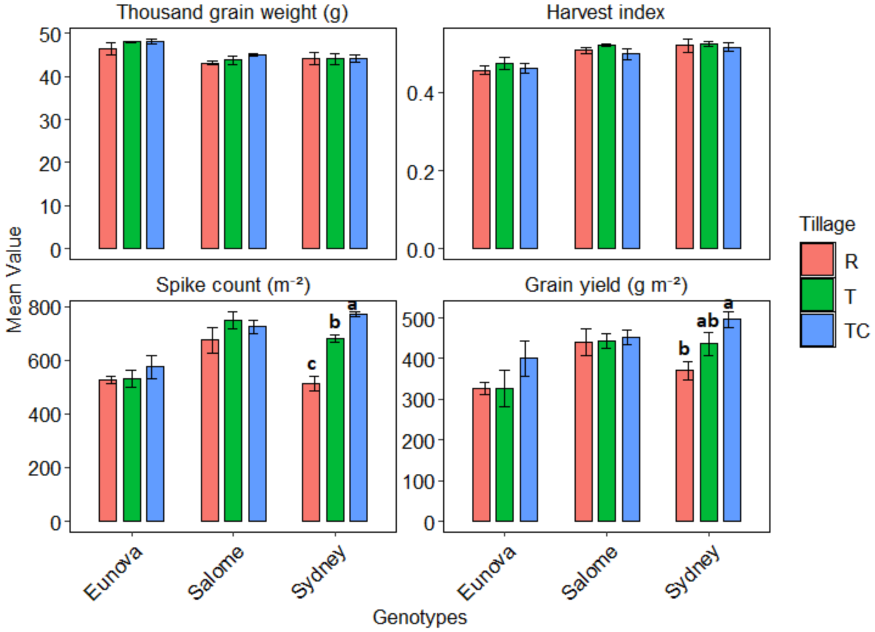

Figure 3.13: Harvest index, thousand grain weight, spike count and grain yield (g m^{-2}) for spring barley (2021). Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

3.3.3.2 Winter wheat (2020 and 2022)

In winter wheat, the combination of deep tillage and compost (TC) did not result in higher grain yields compared to the reference and deep tillage-only (T) treatments in 2020. No significant effects were observed on tillers count and harvest index (Figure 3.14). The response of yield components to the tillage and compost (TC) treatment differed markedly between years. In 2022, the TC treatment increased harvest index in genotype Capo and thousand-grain weight (TGW) in genotype Trebelir; however, grain yield remained unaffected across all genotypes, consistent with observations from 2020 (Figure 3.15). In contrast, tillage and compost treatment significantly influenced spike count in 2022 (P<0.05), whereas no main effect of tillage treatment on spike count was observed in 2020. Genotype effects on grain yield, spike count, and TGW were significant in 2022, with genotype Milaneco producing the highest grain yield; however, these genotypic differences were not statistically significant in 2020 (Figures S3.17 and S3.18). These results indicate substantial year-dependent variation in both treatment efficect and genotype response for yield related traits.

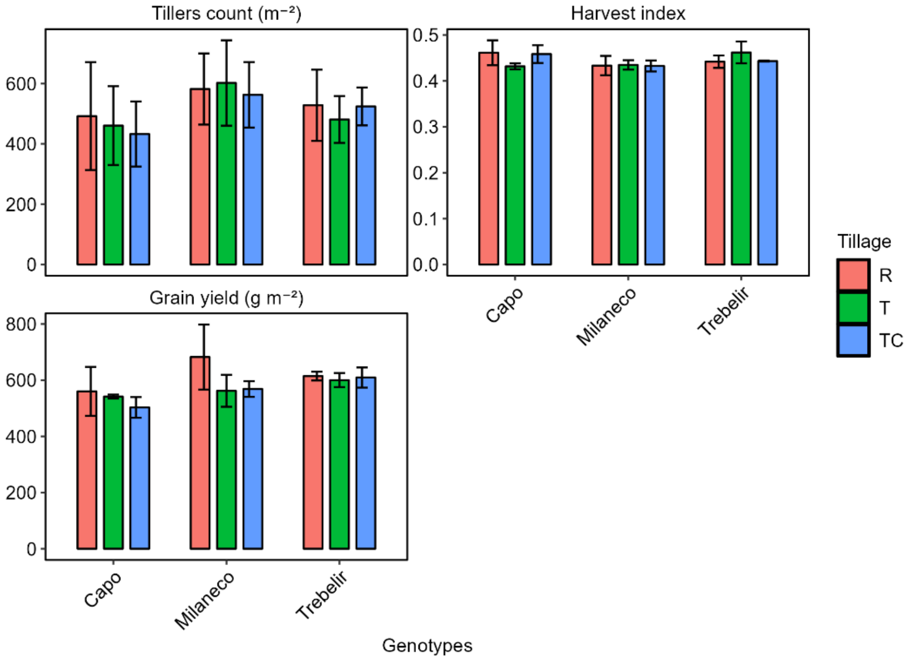

Figure 3.14: Tillers count (m^{-2}), harvest index and grain yield (g m^{-2}) for winter wheat (2020). No significant differences were found for tillage treatment in any genotype. HSD Tukey test, $p < 0.05$ (n=3).

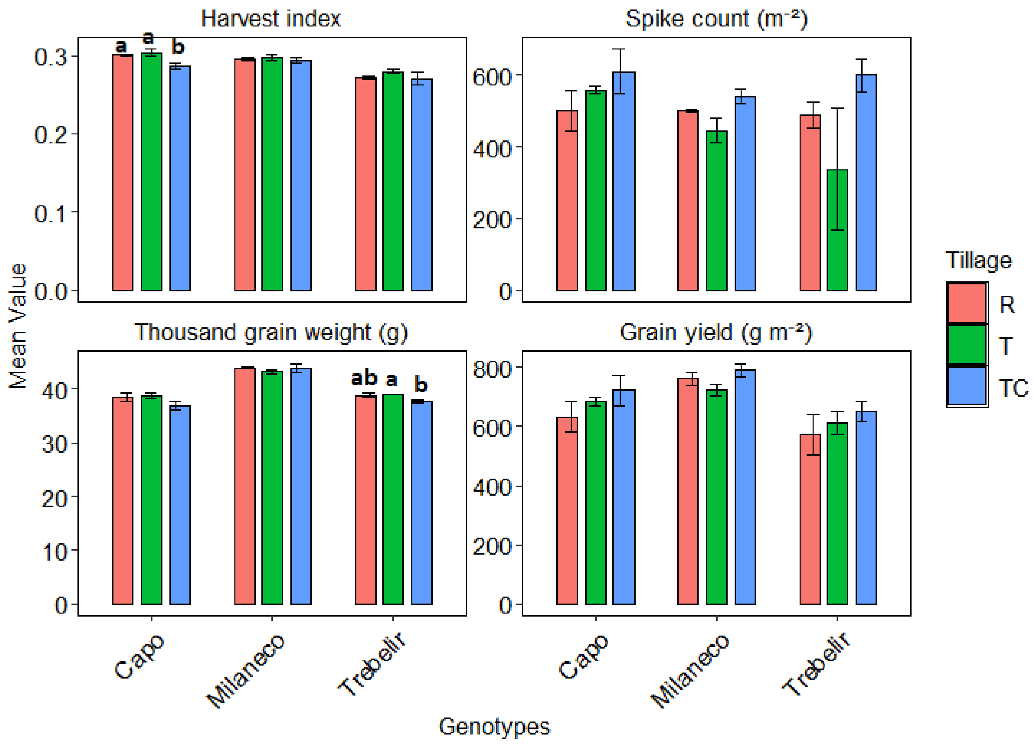

Figure 3.15: Harvest index, spike count, thousand grain weight and grain yield (g m^{-2}) for winter wheat (2022). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

3.4 Discussion

In spring barley, there was no interaction between genotypes and tillage treatments for dry matter and grain yield. For dry matter, results were not consistent at booting and flowering stage but deep tillage + compost produced higher dry matter at maturity stage. At harvest, deep tillage + compost resulted in higher spike count and grain yield in Sydney genotype as compared to R.

Our results confirm that deep tillage can have a lasting positive influence on root development and crop performance, though its effects may vary over time and between crops. In spring barley, we observed a clear positive response in root length density (RLD) and grain yield, particularly in the final year of the study. This suggests that the benefits of soil structural improvement through deep tillage can persist for multiple seasons. These findings are consistent with prior studies indicating that tillage-induced reductions in soil bulk density and improved subsoil porosity can enhance root penetration and water availability in the rooting zone (Hamza & Anderson, 2005;

Chen et al., 2014). However, in winter wheat, these effects were not significant in the last year, indicating a potential decline in the impact of the initial deep tillage treatment or differences in how winter wheat exploits subsoil resources.

Root analysis further revealed that deep loosening promoted root growth into deeper soil layers, with compost addition stimulating root proliferation (Fig. 3.5). The inverse trend of root growth from the topsoil to the subsoil may be attributed to improved nutrient availability due to compost application and reduced soil strength from deep tillage. The enhancement of root length density in deeper horizons aligns with previous work demonstrating that deep loosening facilitates subsoil access by roots (Cai et al., 2014; Himmelbauer et al., 2010). Additionally, compost-derived humic acids are known to stimulate root growth by improving soil chemical conditions and influencing root morphology (Jindo et al., 2012).

Conversely, in winter wheat, deep tillage effects on shoot biomass and grain yield were not significant in the last experimental year. This may indicate a decline in the impact of the initial deep tillage over time or suggest differences in the wheat genotype’s ability to exploit subsoil resources compared to barley.

Variability in genotypic root traits such as root growth angle, diameter, and hydraulic conductivity have been implicated in differential root plasticity and subsoil foraging capacity (Uga et al., 2013; Gao & Lynch, 2016), which could explain the observed genotype-specific responses to amelioration treatments. There is a dramatic variation in genotypes to exploit deep soil layers and deep tillage creates the chances for interactions between genotypes and root depth as some genotypes naturally grow deeper roots and other requirement deep loosening to grow deeper. (Thorup-Kristensen et al., 2020). In this study, specifically for spring barley, the effects of deep tillage combined with compost on root length density (RLD) and yield showed inconsistent responses across genotypes over two years. Specifically, genotypes that responded positively in one year did not necessarily exhibit the same response in the following year, while some genotypes with limited or no response initially demonstrated positive effects later. This variability can be attributed to genotype × environment interactions, where climatic conditions, soil moisture availability, and other environmental factors fluctuate annually, influencing genotypic adaptability and response to soil treatments. Such temporal inconsistency highlights the importance of multi-year trials for accurately assessing genotype-specific responses. Fradgley et al., (2020) documented significant genotype × tillage interactions for root traits; however, same study has also revealed substantial genotype × year interactions, consistent with our observations. Although year was not formally incorporated as a main experimental factor in our investigation, this similar outcome highlights a critical finding: temporal and environmental variability exert substantial influence on genotypic responses to soil management interventions. Our results thus emphasize that genotype × environment × management interactions are complex and multifaceted, with environmental fluctuations across years functioning as a key modulating factor in determining the phenotypic expression of genotype-specific root responses to tillage and soil amendment treatments.

4 Spring wheat/faba bean intercropping effects on roots in subsoil and biopores

4.1 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 (Arslan et al., 2026). In this context, intercropping, i.e., growing two or more crops simultaneously on the same piece of land (Willey, 1990; Lithourgidis et al., 2011), emerges as a promising technique with the potential for efficient nitrogen use and facilitate sustainable intensification of agriculture (Xu et al., 2020). Three types of intercropping have been distinguished: relay, strip and mixed intercropping. Intercropping systems commonly involves growing of cereals and legumes in mixed combinations (Connolly et al., 2001). 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. The ecological performance of cropping systems has been enhanced by a variety of practices, such as intercropping and crop rotation (Wezel et al., 2014).

By sowing these two crops in both sole (single crop) and mixed (intercropped) conditions, we can investigate the benefits and outcomes of this strategy. The legume plants, known for their nitrogen-fixing abilities, can enrich the soil by converting atmospheric nitrogen into a form usable by plants. Intercropping 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. Intercropping a cereal species with high phenotypic plasticity alongside a legume species exhibiting strong physiological plasticity can optimize the yield benefits of intercropping systems by enabling more flexible and complementary resource acquisition (Yang et al., 2022). Plants dynamically adjust root traits such as root length density and lateral root distribution in response to intercropping and resource availability. This phenotypic plasticity in root architecture enhances nutrient uptake and promotes higher crop yields (Liu et al., 2020; Liu et al., 2015).

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). Biopores can serve as a path of least resistance for roots to penetrate the subsoil (Athmann et al., 2013). 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 (Kautz, 2015).

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).

Plant roots preferentially elongate through the round-shaped biopores (Atkinson et al., 2020; Colombi et al., 2017) that can offer access to subsoil water (McKenzie et al., 2009), and nutrients (Kuzyakov & Blagodatskaya, 2015), which is advantageous especially in droughts (Gaiser et al., 2012). The initial observations that root 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 (Lynch & Wojciechowski, 2015), also slows down root growth and elongation when roots growing through the bulk soil (Bengough, 2012).

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; Kokkini et al., 2025). The 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). 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 to maximize subsoil resource use, improve nutrient uptake efficiency, enhance resilience to drought 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 monolith. Addressing this knowledge gap is vital for enhancing crop productivity and resource utilization.

This experiment aimed to;

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

4.2 Material and methods

4.2.1 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 9.4 °C with a mean annual precipitation of 606 mm (493 mm for 2022). Mean monthly precipitation and temperature (maximum and minimum) during the experimental year (2022) are shown in Chapter 3 (Figure 3.1). Field layout of the experiment is shown in Figure S4.1.

4.2.2 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 S4.2). 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 S4.3) 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 remaining 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. The further methodology for root sampling (profile wall and monolith method) and calculation of root length density follows the procedure detailed in Chapter 2 (see Section 2.2.3).

4.2.3 Calucations

Land Equivalent Ratio (LER)

Shoot performance and grain yield of mixture crops were analyzed using the land equivalent ratio (LER) concept (Mead & 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.

$$\mathbf{LER} = \sum \boldsymbol{pLERi} = \sum \mathbf{Y}\boldsymbol{i}/\boldsymbol{Mi} \tag{2}$$

Net Effect Ratio (NER)

The Net Effect Ratio (NER) in intercropping is a measure of the relative yield performance of an intercrop compared to the expected yield based on sole crops weighted by their proportional area in the mixture (Li et al., 2023). It quantifies whether the intercropping system produces more or less yield per unit area than expected if the species grew independently without interaction.

The commonly used formula for NER is:

$$\mathrm{NER} = \frac{Y_1 + Y_2}{P_1 M_1 + P_2 M_2}$$

where:

- Y1 and Y2 are the observed yields of crop species 1 and 2 in intercropping,
- M1 and M2 are the yields of species 1 and 2 in sole cropping,

- P1 and P2 are the proportions (by seed density) of species 1 and 2 in the intercrop, with P1+P2=1.

4.2.4 Statistical data analysis

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

4.3 Results

4.3.1 Root weight in biopores

Root growth was studied 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). At the first two depth levels, i.e., at 40–50 cm and 50–60 cm, no statistically significant differences in root weight were observed among the three treatments (Fig. 4.1). This suggests that, within at soil depths of 40 to 60 cm, the treatments exhibited similar patterns of root growth. In contrast, at the depth of 60–70 cm, significant differences in root weight were observed among the treatments. Spring wheat demonstrated higher root weight compared to the other treatments, while faba bean exhibited the lowest root weight. The mixture treatment showed intermediate root weight and was not significantly different from sole crop treatments.

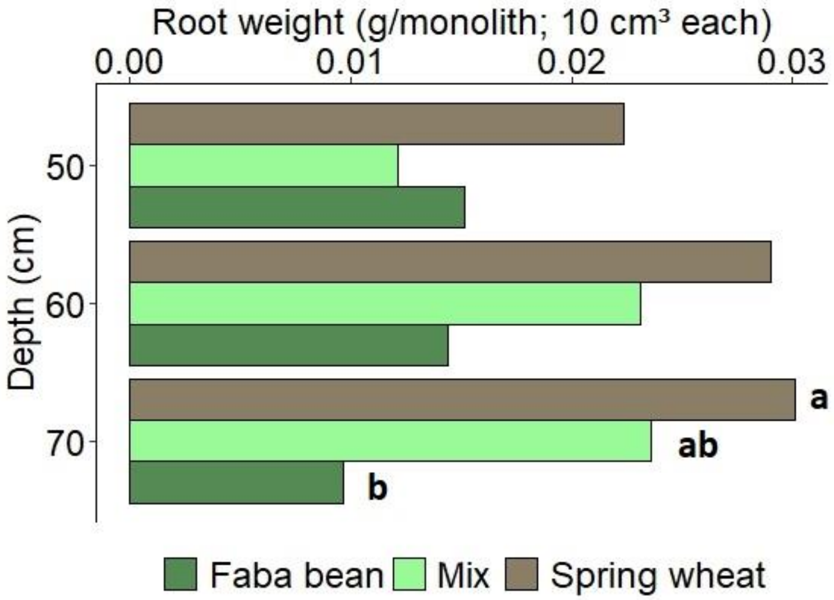

Figure 4.1: Mean root weight (gram per monolith; g/10 cm^3) within biopores of three treatments (spring wheat, faba bean, and mixture) at three different depths (40–50 cm, 50–60 cm, and 60–70 cm). The different lowercase letters represent the significant differences between the treatments within each layer. HSD Tukey test, $p < 0.05$ (n=3).

4.3.2 Root length density (RLD)

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 (Fig. 4.2).

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. 4.3). 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.

The share of roots in the biopores of treatments were evaluated at different depths ranging from 40-70 cm (Fig. 4.4). 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 higher proportions (4.5% at depth 50-60 cm and 5.6% at 60-70 cm). 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. Faba bean dominated in the deeper layers compared to spring wheat.

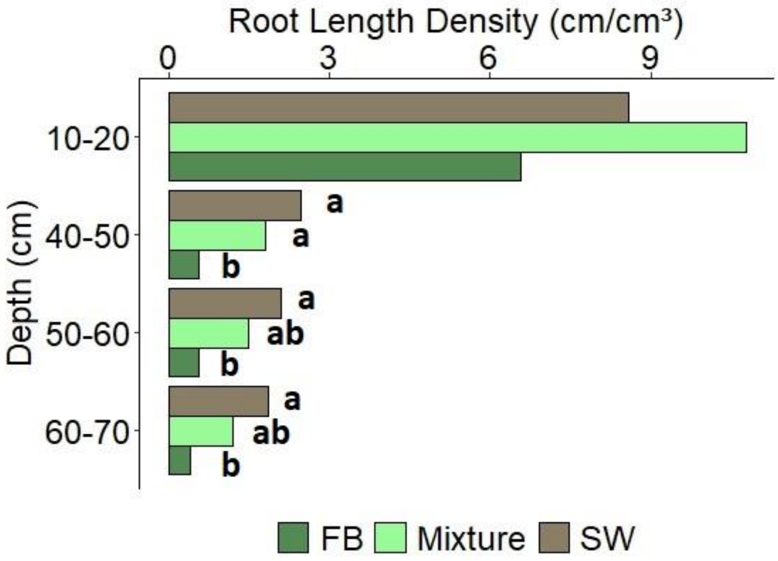

Figure 4.2: Root length density (RLD) in cm/cm^3 determined with the monolith method for faba bean (FB), Mixture and spring wheat (SW) in different soil depth layers. The different letters represent the significant differences between the treatments within one layer. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

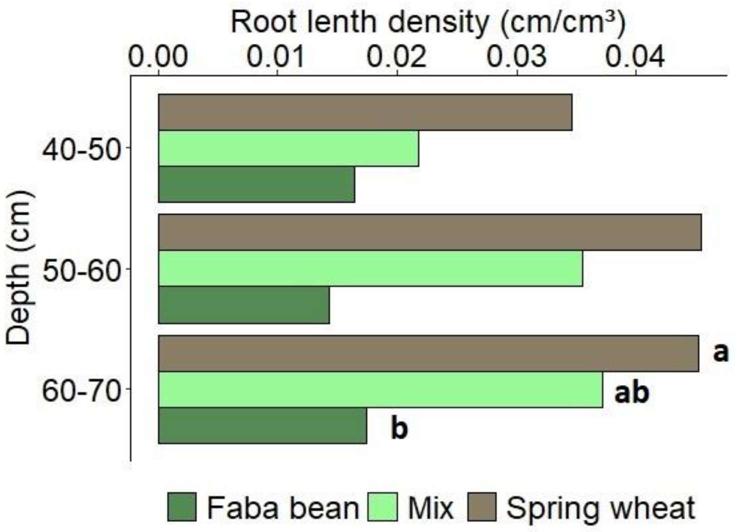

Figure 4.3: Root length density (RLD) of biopores for spring wheat (SW), faba bean (FB), and mixture at different soil depths. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

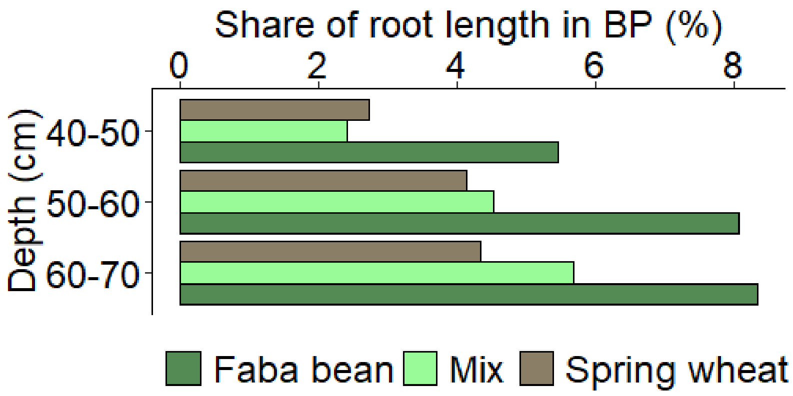

Figure 4.4: Share of root length in biopores (BP). No significance differences were found among crop species in any depth layer. HSD Tukey test, $p < 0.05$ (n=3).

4.3.3 Dry matter and Grain yield

The above-ground dry matter for all the treatments was evaluated at two different stages: booting and maturity (Fig. 5). The treatment showed varying average dry matter values at the booting stage. The mixture treatment presented the highest average dry matter at 3.08 t ha^{-1}, followed by spring wheat with an average of 2.72 t ha^{-1} and the faba bean with the lowest average dry matter of 2.44 t ha^{-1}. 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 t ha^{-1}, followed by spring wheat (average value of 9.01 t ha^{-1}), and faba bean (6.21 t ha^{-1}). However, there were no statistical differences among the treatments at booting stage, while at maturity, the mixture and spring wheat were significantly higher than faba bean. Among the treatments examined, the average grain yield for faba bean was 2.69 t ha^{-1}, while, spring wheat performed higher with in average production of 4.17 t ha^{-1} (Fig. 5). The mixture treatment, which involves the 50:50-mixture of both faba bean and spring wheat, showed a significant increase in grain yield, averaging 4.71 t ha^{-1}. Statistically, the mixture and spring wheat were significantly higher than faba bean.

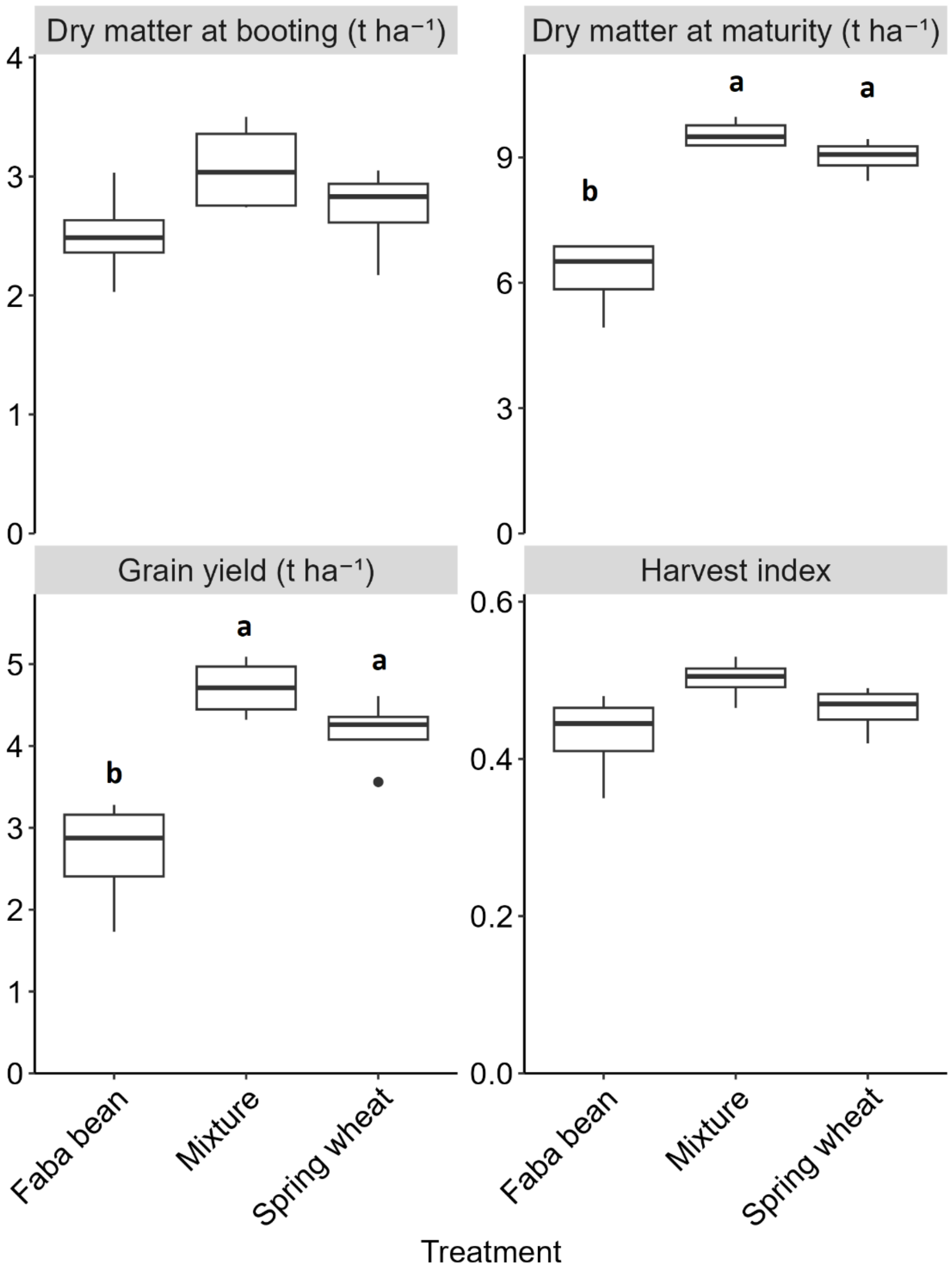

Figure 4.5: Shoot dry matter at booting and maturity stage, harvest index and grain yield of three treatments: faba bean (FB), mixture, and spring wheat (SW). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

4.3.4 Land equivalent ratio on above ground variables (LER)

The LER diagram (Fig. 6), shows that all values fell below the dotted green line. Hence, spring wheat demonstrated a competitive advantage over faba bean in terms of grain yield. For the mixture treatment, three out of four values for the partial land equivalent (pLER) of faba bean were below 0.5, whereas one value exceeded 0.5. Conversely, for spring wheat, all values for the pLER were 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 (Fig. 6). 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, 1.16, and 1.17, respectively. So, in terms of grain yield production, the land use was 31% more efficient in intercropping than in the sole crops.

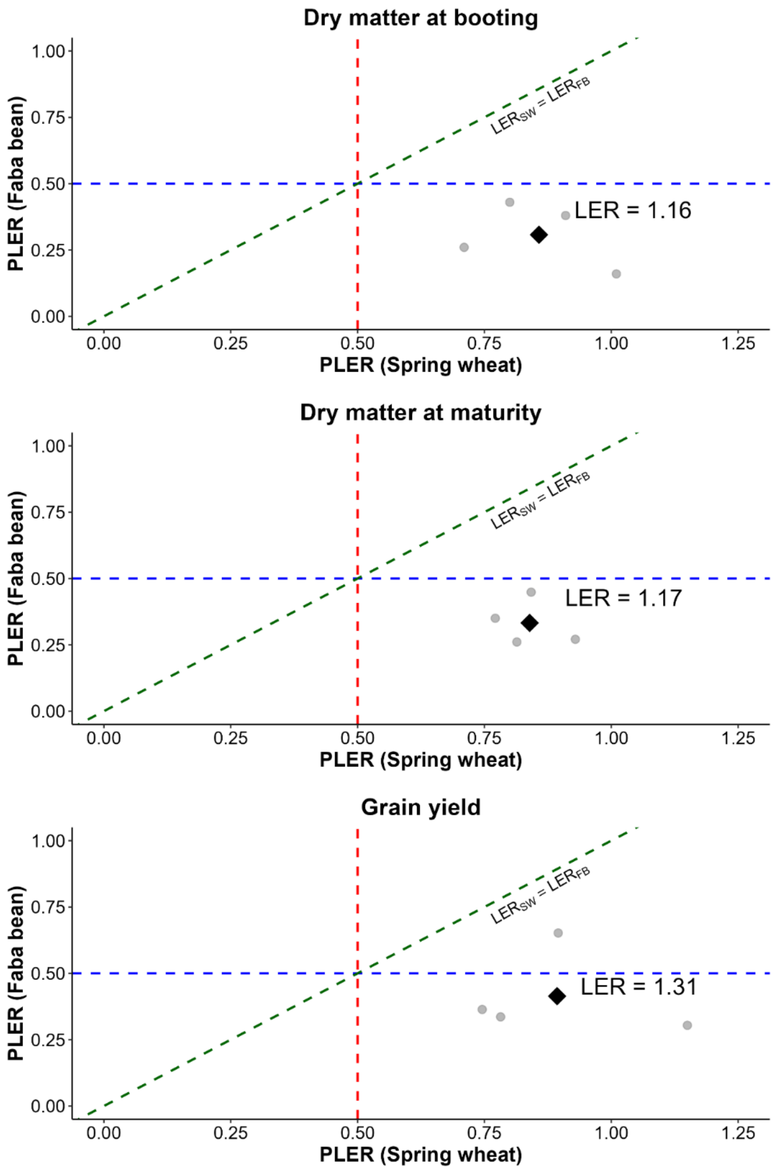

Figure 4.6: Partial Land Equivalent Ratios (pLER) of spring wheat and faba bean and LER for crop mixture. The green dotted line corresponds to a land equivalent ratio of 1 (LER = pLER SW + pLER FB). The dashed horizontal and vertical lines represent the expected pLER for the mixture partners.

4.3.5 Net Effect Ratio (NER)

NER was calculated for root length, above-ground dry matter and grain yield. For root length, NER was only significantly positive in the top soil (10-20 cm). In subsoil, NER was calculated separately for the root length in monolith and biopores. NER within biopores showed increasing trend with soil depth but it was not statistically different from 1 (Figure. 4.7). NER for aboveground dry matter was significantly higher than 1 at only the maturity stage (Figure 4.8). Grain yield also showed significantly positive effect of intercropping as its NER values was 1.39 which depicts the 39% higher grain yield in comparison to the average of the sole crops.

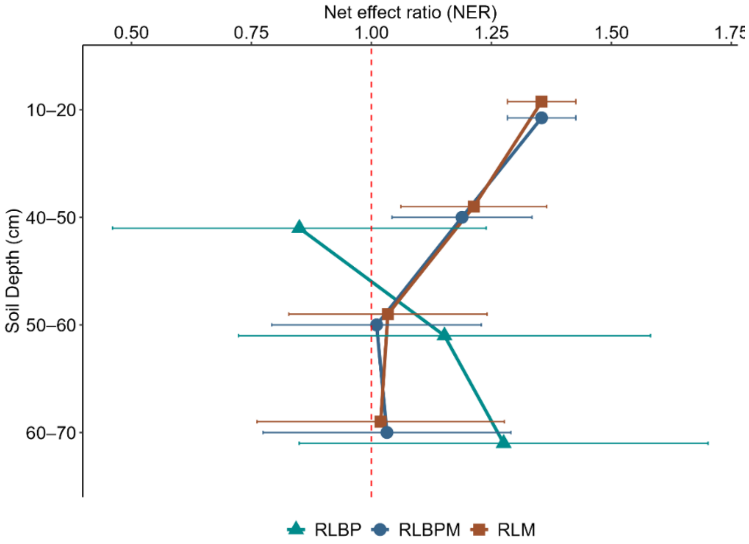

Figure 4.7: Net effect ratio (NER) for root length in biopores (RLBP), monolith (RLM) and total RL (RLBPM) at different depths. Standard error is represented by error bars.

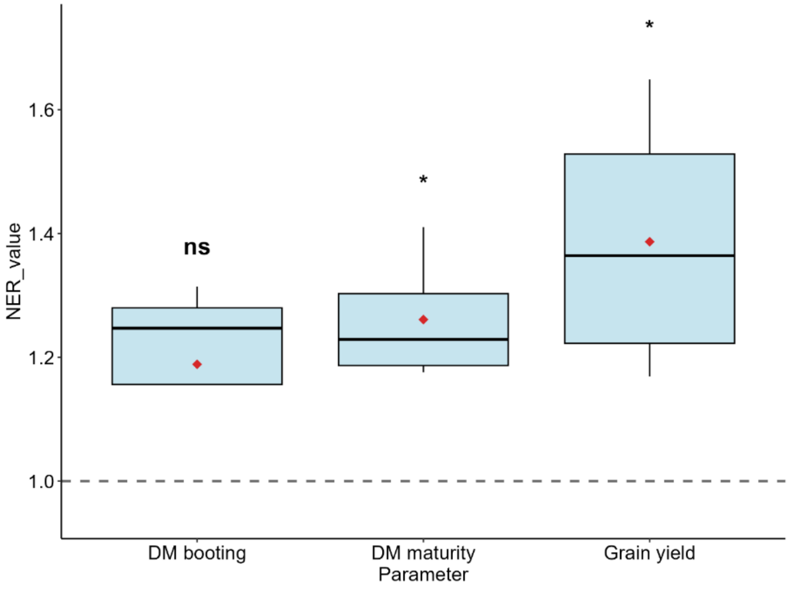

Figure 4.8: Net effect ratio (NER) for dry matter (booting and maturity stage) and grain yield.

4.4 Discussion

Intercropped legumes have proved to be capable of providing a wide range of additional services and of producing substantially higher yields than their respective sole crops – expressed as a land equivalent ratio (LER) higher than 1 (Willey & Osiru, 1972; 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 dominated the mixture, but also contributed more than faba bean to the efficiency of land use. 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), Similarly, Ren et al., (2016) reported that legume and cereal intercropping significantly increased cereal crop yield. 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).

Intercropping spring wheat and faba bean has shown significant advantages in resource use efficiency and productivity, as evidenced by higher Land Equivalent Ratios (LER) and Net Effect Ratios (NER) for both grain yield and dry matter in this study. The observed LER values exceeding unity indicate that the combined cropping system utilized available resources more efficiently than the sole crops grown independently, which aligns with the extensive body of literature documenting the benefits of cereal-legume intercropping systems (Li et al., 2023; Willey & Osiru, 1972). Higher NER values further confirm that the intercrop yields surpassed the expected weighted yields of the monocrops, corroborating findings by Brooker et al. (2024), who emphasized NER as a robust indicator capturing the relative yield advantage contributed by interspecific facilitation and complementarity in intercropping. These enhanced yields and biomass production can be attributed to the complementary temporal and spatial resource use strategies of spring wheat and faba bean. Faba bean, a leguminous crop, enriches soil nitrogen through biological nitrogen fixation, thereby reducing the nitrogen limitation for the cereal component and potentially enhancing photosynthetic efficiency and biomass accumulation (Lo Presti et al., 2024). Such facilitative interactions enhance dry matter accumulation and grain production beyond additive effects. Moreover, deep rooting attributes and differential nutrient uptake patterns improve water and nutrient acquisition, leading to improved total system productivity (Han et al., 2021; Bengough et al., 2011).

However, the root growth measurements in the 10-20 cm and 40-70 cm soil layers, including assessments within biopores in the deeper subsoil, revealed no statistically significant net effect of intercropping on total root length. This indicates that while aboveground yield benefits were observed, root growth dynamics were not markedly altered by intercropping under the conditions studied. Slight increases in root growth within biopores with increasing depth were observed, although these differences were not statistically significant, suggesting that while biopores may offer preferential channels for root penetration in deeper soil horizons, they do not translate into a strong overall root system expansion in the intercrop compared to sole crops. Similar observations have been reported in the literature. For instance, (Hadir et al., 2025) showed that intercrop combinations of spring wheat and faba bean may not always significantly alter root length density or rooting depth; nonetheless, niche differentiation and spatial root distribution optimize water and nutrient uptake efficiency without necessarily increasing total root biomass. The lack of significant difference in root length in this study may also reflect the temporal and spatial complementary use of soil resources, whereby intercrops optimize resource capture through differentiation rather than total root system enlargement (Han et al., 2021). This suggests that different crop species in an intercrop system use soil resources (water, nutrients) at different times and soil depths, reducing direct competition and improving overall resource use efficiency without necessarily expanding the total root system size. (Brooker et al., 2024) also emphasized how differences in root architecture and phenology in intercrops enable spatial-temporal niche differentiation, leading to improved resource capture compared to monocultures. Yu et al. (2016) reviewed root plasticity in intercropping systems and highlighted how complementary root traits reduce belowground competition and improve crop productivity.

Consistent with these physiological interactions, previous field studies have demonstrated increased yield advantages in legume-cereal intercrops. For instance, (Li et al., 2023) reviewed multi-site intercropping trials and reported mean LER values ranging from 1.1 to 1.4, associated with significant yield and resource use improvements. Similarly, (Lo Presti et al., 2024) identified the benefits of optimized sowing ratios in boosting NER and overall system productivity. The findings in this study strongly support the adoption of intercropping spring wheat and faba bean as a sustainable intensification strategy in cereal-based cropping systems, contributing to enhanced food security and sustainable agricultural production.

5 General discussion and conclusions

5.1 Conceptual set-up of studies

Key parameters investigated across three studies in this thesis, specifically focusing on root growth in the subsoil, dry matter accumulation, and grain yield are shown in Figure 5.1. The diagram visually depicts the experimental factors influencing these parameters and the sequence of their assessment over different years and crops. The figure categorizes the experiments into three main groups: Pre-crops combined with deep tillage and compost (Experiment 1), Genotypes with deep tillage and compost (Experiment 2), and Intercropping species (Experiment 3).

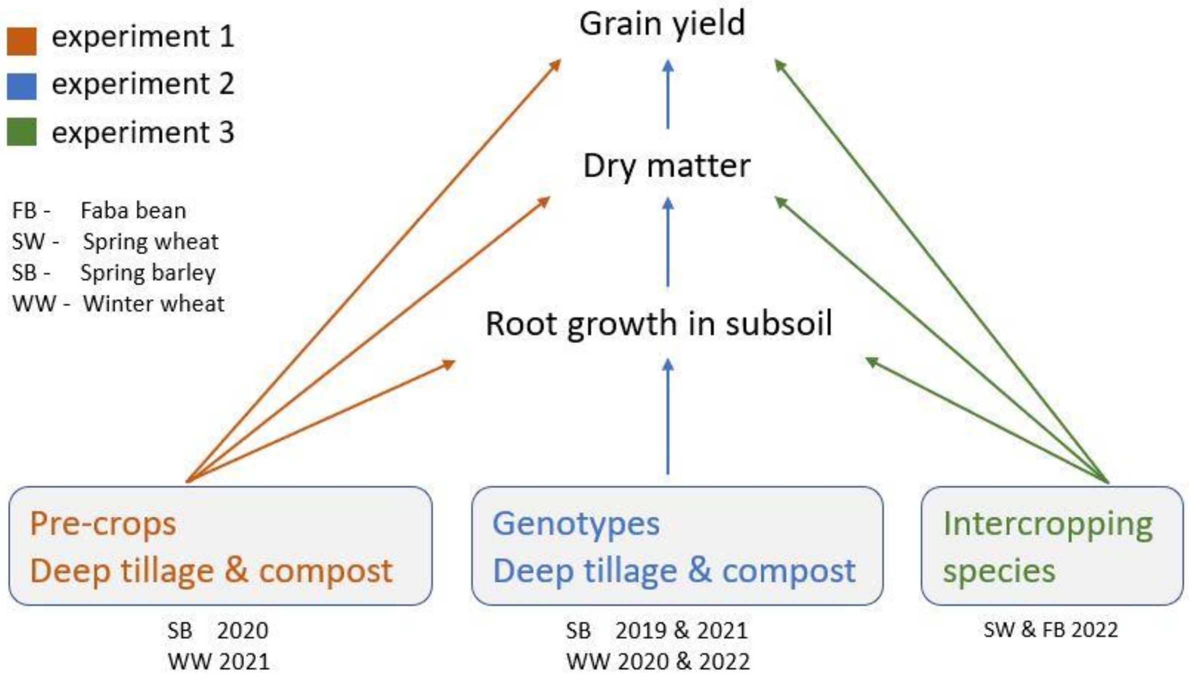

Figure 5.1: Conceptual representation showing key parameters; root growth in subsoil, dry matter, and grain yield measured across three studies and treatments with different crops, genotypes, and management strategies.

5.2 Consistency of results across studies

In both the first and second study, the spring crop had the tendency to show more pronounced effects than the winter crop for grain yield. Compost application has been generally associated with enhanced subsoil water retention and its use, which is critical in dry years and short growing season i.e., spring crops in this case, structurally benefit from subsoil amelioration. Compost incorporation alone can also enhance root growth in the upper subsoil for winter wheat, consistent with findings of improved microporosity and aggregation enhancing root proliferation (Zhao et al., 2019). Biological subsoil improvement via lucerne achieved similar benefits as technical methods but with fewer external inputs. Some long-term studies report stronger subsoil effects and yield benefits for spring crops after lucerne than for winter crops (Behrend et al., 2025). There was also high variability in root data across all experiments due to natural differences in soil and root system distribution.

5.3 Importance ranking of experimental factors

The key experimental factors examined in this thesis include crop species, deep tillage, compost treatment, choice of pre-crop, cereal genotypes, and cropping system (intercropping vs. sole crops). These factors were assessed to understand their relative influence on root length density (RLD) and overall crop performance across different studies. Table 5.1 summarizes which factors were investigated in each chapter of the thesis.

Table 5.1: Summary of experimental factors investigated across thesis chapters, indicating which factors were examined in each study to assess their effects on root length density and crop performance

Factor	**Chapter**
Deep tillage and compost	Chapter 2, 3
Precrops	Chapter 2
Genotypes	Chapter 3
Crop species	Chapter 2, 3, 4

In this thesis, combined technical and biological subsoil amelioration had a clear effect on root growth, crop nutrient uptake and grain yield of spring barley during a drier year (2020). The first experiment demonstrated that lucerne combined with deep tillage and compost significantly increased RLD in the subsoil of spring barley. The second experiment also showed higher RLD in response to deep loosening and compost, with effects more pronounced in spring crops. This may be attributed to the shorter growing season of spring crops, which makes them more responsive to treatments promoting root growth. These results highlight the potential of increasing access to subsoil nutrient and water resources in mitigating impacts of drought. Conversely, winter crops have a longer growing period that naturally allows deeper root development, reducing the relative effect of such management practices. Therefore, in winter crops, rooting time plays a more dominant role in root development than the applied amelioration treatments. Maximum rooting depth was found higher for treatment lucerne with tillage and compost, in comparison to reference treatments in spring barley but not in winter wheat and these results aligns with other root and shoot parameters.

In the third study involving intercropping, root length density (RLD) was primarily evaluated using the net effect ratio due to the inherent differences in rooting systems and architecture between spring wheat and faba bean, which cannot be directly compared. Spring wheat exhibited significantly higher RLD than faba bean. The net effect ratio was not significantly higher than 1 in subsoil, indicating that intercrops did not provide a beneficial effect on root growth either in the soil monolith or within biopores in subsoil. However, there was positive effect in the top soil (10-20 cm). This suggests that intercropping, under the conditions studied, did not enhance root proliferation in subsoil beyond what was observed in sole crops.

In the first study, lucerne as pre-crop developed higher root length density (RLD) in deeper subsoil layers below 60 cm, while perennial ryegrass dominated the topsoil and upper subsoil. Root segment analysis revealed greater formation of small-diameter roots (<1 mm) in ryegrass, suggesting a higher potential for creating fine biopores, whereas lucerne produced thicker root segments (1–2 mm and >2 mm). However, this did not translate into significant differences in subsoil biopore density. Despite this, high proportions of cereal roots grown in biopores during the booting stage indicated preferential root use of existing channels to access deeper soil layers under low precipitation.

In the second study included in this thesis, deep tillage and compost had clear positive effect but it varied across genotypes and was not consistent for all genotypes over four years period. We found that the tillage compost effect on root growth and yield was not genotype dependent, indicating complex interactions beyond genotype alone. It is crucial to consider environmental factors such as soil type, climate, and site-specific conditions, as well as cropping systems, root-soil interactions, and temporal variability. These factors can control genotype performance and may explain inconsistencies observed across years and sites. Including more diverse genotypes and multi-environment trials in future studies will help clarify potential genotype-specific responses and support development of robust, site-adapted management strategies.

5.4 Methodologies of studying crop roots

There were multiple methods used in these studies to study roots, each with its own strengths and limitations (Table 5.2). The profile wall method enables visualization of root distribution along a soil profile and covers greater soil depth but may underestimate root length density by capturing only roots exposed on vertical surfaces. The monolith method quantifies root mass and length by extracting and washing a soil volume, offering precise measurement but it is labor-intensive, sampling limited soil volume and potentially missing spatial variability in heterogeneous sites. Soil coring uses cylindrical samples to analyse roots at multiple depths and locations, facilitating quantitative comparisons, though it may disturb soil structure and miss coarser roots or those in biopores, affecting accuracy.

Table 5.2: Different root sampling/assessment methods in this thesis

Root methods	**Chapter**
Profile wall	Chapter 2,4
Monolith	Chapter 2,4
Soil core	Chapter 3

5.5 Limitations

This section presents the findings and limitations of the intercropping study conducted as part of this thesis, focusing on root growth dynamics in biopores, root measurement methodologies, and challenges of data variability. The study, although conducted over a single year and site with a specific crop combination, provides novel insights into root behaviour in intercrop systems, a relatively unexplored area compared to monoculture or crop sequence systems. Additionally, this section addresses methodological considerations by comparing root measurement techniques used in the study and highlights natural variability in root data that impacts result interpretation and future research design.

The intercropping study reported here was limited to a single year, one site, and a specific crop combination. Despite these constraints, this research is the first to investigate root growth in biopores within intercrop systems. By exploring how roots utilize biopores in the context of intercropping, the study provides pioneering evidence that can inform future experimental designs and field management strategies. Previous research has focused on biopores in monoculture or crop sequence settings, but studies examining their role in intercropping with direct root observations are lacking.

Profile wall and monolith methods have been shown to yield highly divergent root-length density measurements, with profile wall techniques potentially causing considerable underestimation, particularly in crop mixtures and single-year trials, this is most pronounced for absolute root data (Bublitz et al., 2022). In our current study, we also observed important limitations: the monolith method provides only a restricted view of the entire root profile, while the profile wall approach may fail to capture actual roots present in the soil. These inherent biases should be carefully considered when interpreting root data and comparing results across studies.

High variability in root data is another common limitation due to natural differences in soil and root systems. This variability can make it harder to detect treatment effects and interpret results reliably. Acknowledging this limitation highlights the need for careful sampling and replication in future studies to improve data confidence.

5.6 Outlook: Recommendations for future research

The interaction between intercropping and subsoil amelioration needs further study, focusing on different species combinations and proportions of cropping partners. While this study found no significant intercropping effects on root interactions in biopores in the subsoil, likely due to data variability, a positive effect in the topsoil suggests potential benefits worthy of deeper exploration. Additionally, given the substitutive design with limited crop densities used here, future work should assess higher combined crop densities that better represent practical farming systems, with particular emphasis on root growth dynamics. It is important to indicate the key recommendations for future research that build on the findings and limitations of the current studies. We emphasize on exploring subsoil amelioration mechanisms, the interactive effects of intercropping species and densities, and evaluating root growth assessment methods to enhance sustainable agricultural systems.

Further research should address the long-term effects of both technical and biological subsoil melioration on soil properties, crop performance, and farm economic performance under varying site and climatic conditions, to determine site-adapted strategies for adaptation to climate change.

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Supplementary material

Chapter 2

Supplementary Table S2.1. Type, composition, application rate, and timing of chemical treatments applied to spring barley and winter wheat during the 2020–2021 growing seasons.

Chemical treatment	**Spring Barley**	**Winter Wheat**
Fungicide	Adexar (Fluxapyroxad 62.5 g/L + Metconazole 45 g/L); 1.25 L/ha, May 16, 2020 Skyway Pro (Prothioconazole 150 g/L + Bixafen 75 g/L); 1.0 L/ha, June 6, 2020 Osiris (Epoxiconazole 125 g/L + Metconazole 60 g/L); 1.2 L/ha, June 6, 2020	Adexar (Fluxapyroxad 62.5 g/L + Metconazole 45 g/L); 1.25 L/ha, May 20, 2021 Protendo (Prothioconazole 250 g/L); 0.55 L/ha, June 2021 Soliel (Tebuconazole 107 g/L + Bromuconazole 167 g/L); 0.75 L/ha, June 2021
Insecticide	Biscaya (Thiacloprid 240 g/L); 0.3 L/ha, June 6, 2020	Karate Zeon (Lambda-cyhalothrin 100 g/L); 0.075 L/ha, June 2021
Herbicide		Malibu (Flufenacet 60 g/L + Pendimethalin 300 g/L); 4.0 L/ha, November 4, 2020
Growth Regulator		CCC (Chlormequat chloride); 1.0 L/ha, March 25, 2021 Moddus (Trinexapac-ethyl); 0.2 L/ha, March 25, 2021

Supplementary Table S2.2. P-values from two-way ANOVA testing the effects of tillage, pre-crop, and their interaction on root length density of spring barley (2020), analyzed separately for booting and flowering stages and across different soil depth levels.

Stage	Depth (cm)	Tillage	Pre-crop	Tillage × Pre-crop
Booting	00-30	**0.03**	0.58	0.28
	30-60	**0.04**	0.61	**0.04**
	60-100	0.48	0.27	0.97
Flowering	00-30	0.31	0.80	0.93
	30-60	0.46	1.00	0.70
	60-100	0.82	0.09	0.07

Supplementary Table S2.3. P-values from two-way ANOVA testing the effects of tillage, pre-crop, and their interaction on root length density of winter wheat (2021), calculated using root length measured exclusively within biopores. Analyses were performed separately for booting and flowering stages and across different soil depth levels.

Stage	Depth (cm)	Tillage	Pre-crop	Tillage × Pre-crop
Booting	00-30	0.19	0.60	0.89
	30-60	0.88	0.22	0.33
	60-100	0.48	0.51	0.99
Flowering	00-30	0.15	**0.01**	**0.00**
	30-60	0.32	0.07	0.12
	60-100	0.48	0.15	0.08

Supplementary Table S2.4. P-values from two-way ANOVA showing the effects of pre-crop, tillage, and their interaction on dry matter accumulation, carbon (C), nitrogen (N), phosphorus (P), and potassium (K) uptake, and maximum rooting depth of spring barley at booting and flowering stages (2020).

Stage	Parameters	Tillage	Pre-crop	Tillage × Pre-crop
Booting	Dry matter	**0.02**	**<0.01**	0.97
	Carbon uptake	**0.02**	**<0.01**	0.95
	Nitrogen uptake	**<0.01**	**<0.01**	0.87
	Phosphorus uptake	**<0.01**	**<0.01**	0.98
	Potassium uptake	**<0.01**	**<0.01**	0.96
	Maximum rooting depth	0.15	0.73	0.91
Flowering	Dry matter	**0.07**	**<0.01**	0.56
	Carbon uptake	**0.07**	**<0.01**	0.58
	Nitrogen uptake	**0.03**	**<0.01**	0.58
	Phosphorus uptake	0.09	0.28	0.95
	Potassium uptake	**0.02**	**<0.01**	0.30
	Maximum rooting depth	0.81	**0.01**	**0.01**

Supplementary Table S2.5. P-values from two-way ANOVA assessing the effects of tillage, pre-crop, and their interaction on straw and grain yield of spring barley (2020)

Parameters	Tillage	Pre-crop	Tillage × Pre-crop
Straw yield	0.33	0.57	0.71
Grain yield	**0.05**	**0.04**	0.42

Supplementary Table S2.6. P-values from two-way ANOVA testing the effects of tillage, pre-crop, and their interaction on root length density of winter wheat (2021), analysed separately for booting and flowering stages and across different soil depth levels.

Stage	Depth (cm)	Tillage	Pre-crop	Tillage × Pre-crop
Booting	00-30	0.91	0.85	0.45
	30-60	0.38	0.80	0.58
	60-100	0.80	0.15	0.94
	100-130	0.40	0.31	0.92
	130-165	0.42	0.88	0.20
Flowering	00-30	0.70	0.26	0.09
	30-60	0.33	0.96	1.00
	60-100	1.00	0.82	0.26
	100-130	0.64	0.42	0.58
	130-165	0.16	0.56	0.92

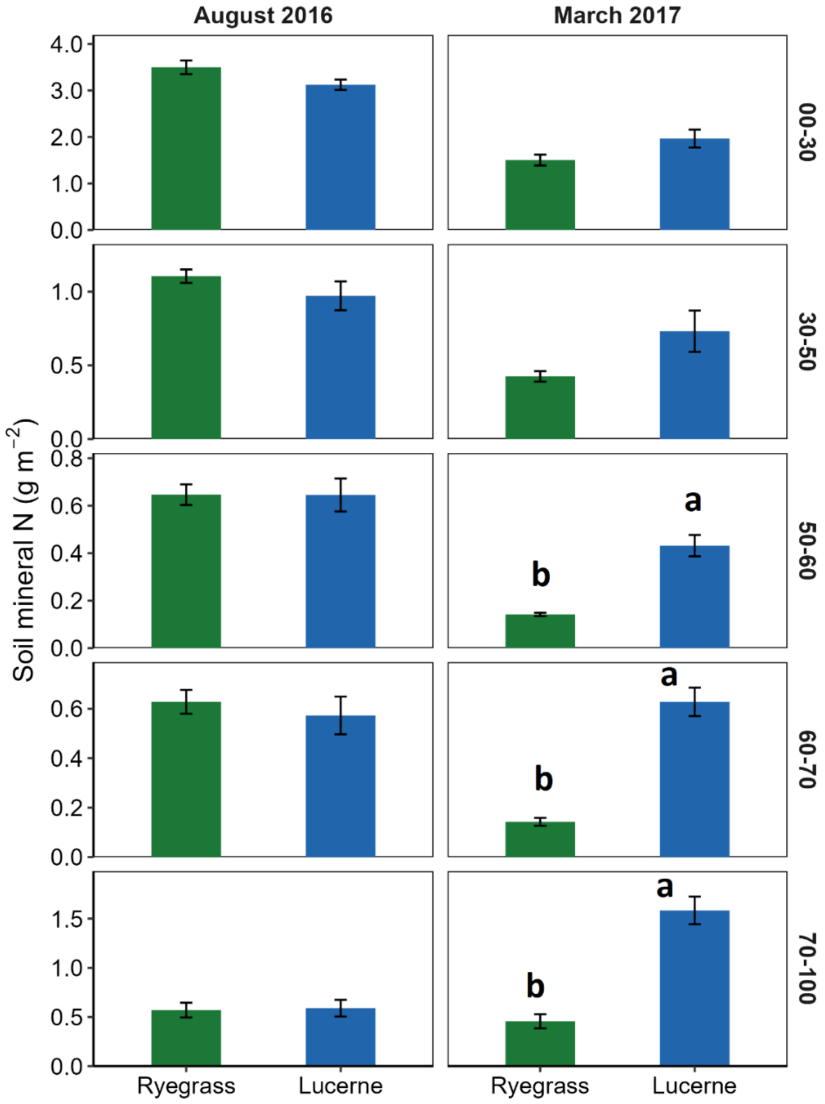
Supplementary Table S2.7. P-values from two-way ANOVA showing the effects of pre-crop, tillage, and their interaction on dry matter accumulation, carbon (C), nitrogen (N), phosphorus (P), and potassium (K) uptake, and maximum rooting depth of winter wheat at booting and flowering stages (2021).

Stage	Parameters	Tillage	Pre-crop	Tillage × Pre-crop
Booting	Dry matter	0.98	0.78	0.52
	Carbon uptake	0.99	0.89	0.38
	Nitrogen uptake	0.54	0.13	0.71
	Phosphorus uptake	0.68	0.32	0.54
	Potassium uptake	0.69	0.71	0.46
	Maxmum rooting depth	0.56	0.56	0.56
Flowering	Dry matter	0.38	0.22	0.82
	Carbon uptake	0.43	0.23	0.85
	Nitrogen uptake	0.06	0.11	0.65
	Phosphorus uptake	0.06	0.64	0.51
	Potassium uptake	0.06	**0.01**	0.42
	Maxmum rooting depth	0.56	0.56	0.56

Supplementary Table S2.8. P-values from two-way ANOVA assessing the effects of tillage, pre-crop, and their interaction on straw and grain yield of winter wheat (2021)

Parameters	Tillage	Pre-crop	Tillage × Pre-crop
Straw yield	0.50	0.82	0.10

Grain yield	0.86	0.33	0.62



Supplementary Figure S2.9. Soil mineral nitrogen (g m^{-2}) for lucerne and ryegrass separately across sampling year and soil depths (cm). Different lowercase letters indicate significant differences according to HSD Tukey test, $p < 0.05$ (n=4).

Chapter 3

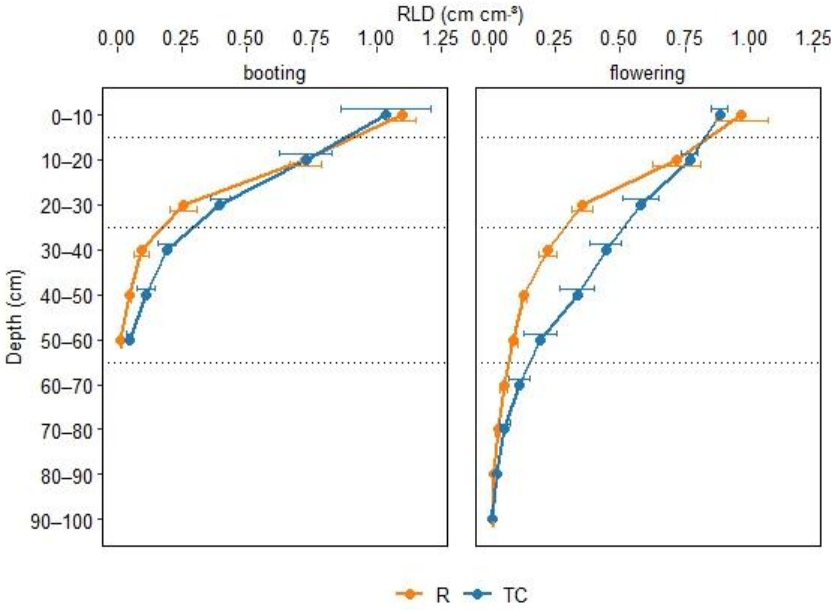


Figure S3.1. Root length density (RLD) for spring barley (2019) at booting and flowering stage for each depth level. No significant differences were found at any depth group. HSD Tukey test, $p < 0.05$ (n=3).

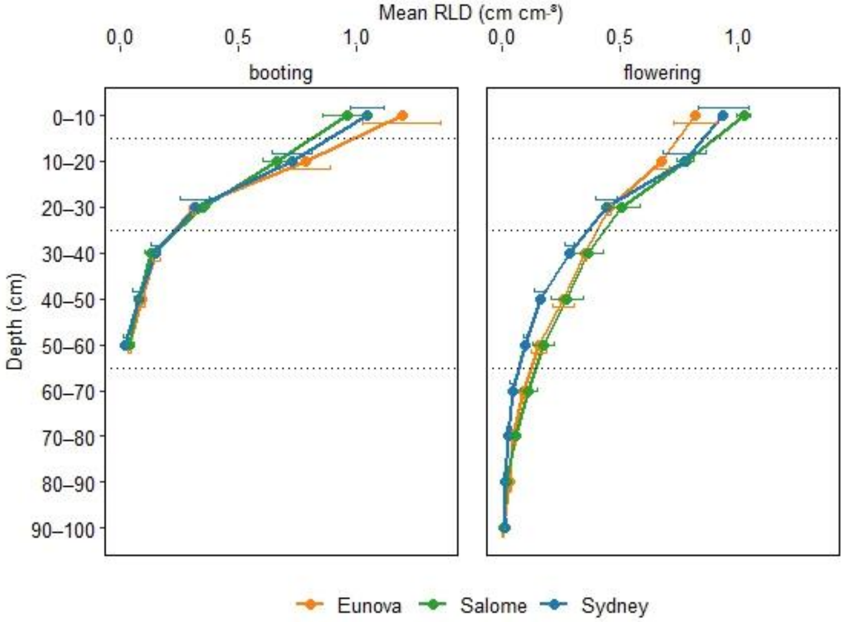


Figure S3.2. Root length density (RLD) for spring barley (2019) at booting and flowering stage for each depth level. No significant differences were found at any depth group. HSD Tukey test, $p < 0.05$ (n=3).

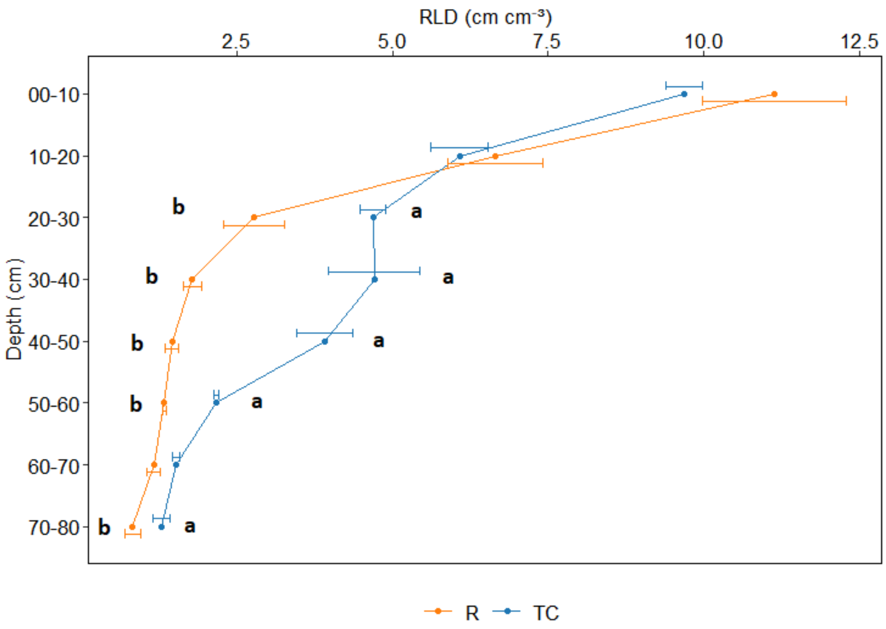


Figure S3.3. Root length density (RLD) for spring barley (2021) at flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

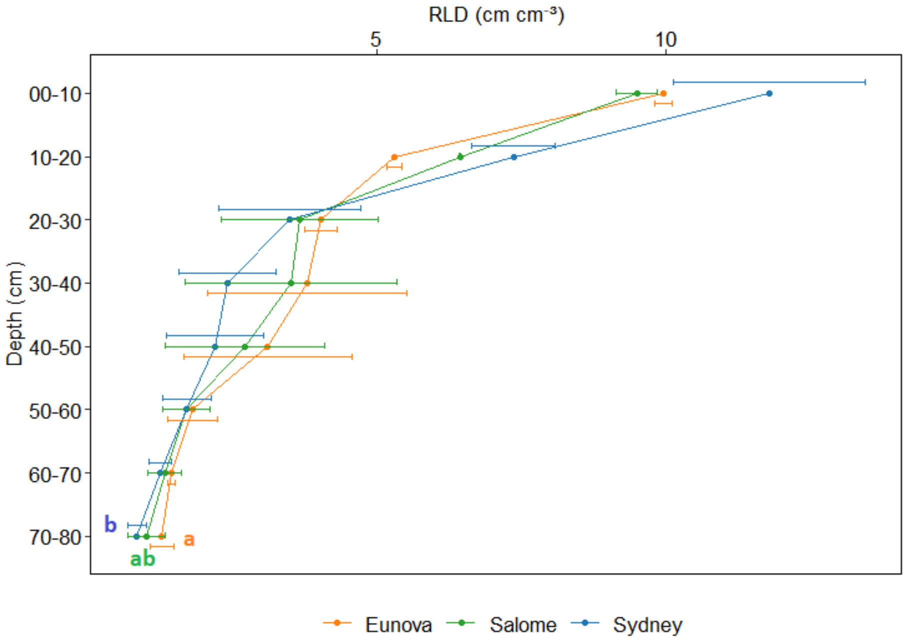


Figure S3.4. Root length density (RLD) for spring barley (2021) at flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

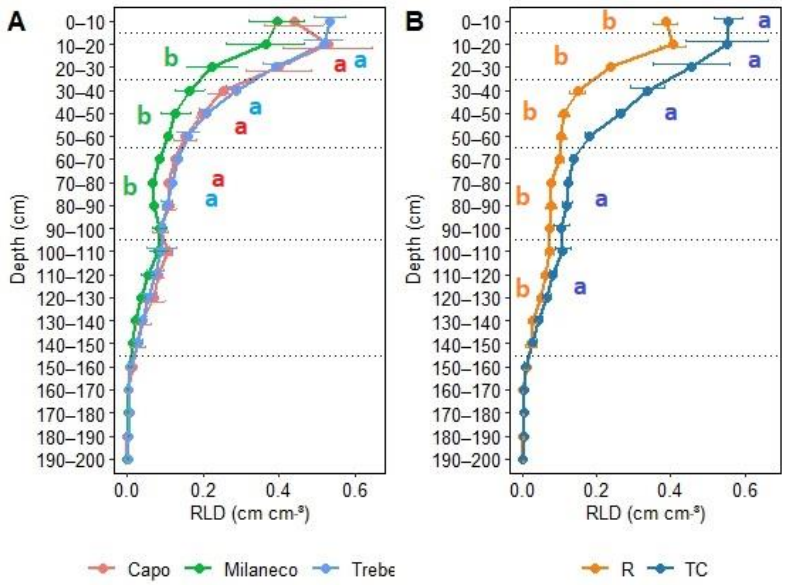


Figure S3.5. Main effects of tillage and genotypes on root length density (RLD) for winter wheat (2020) at booting stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

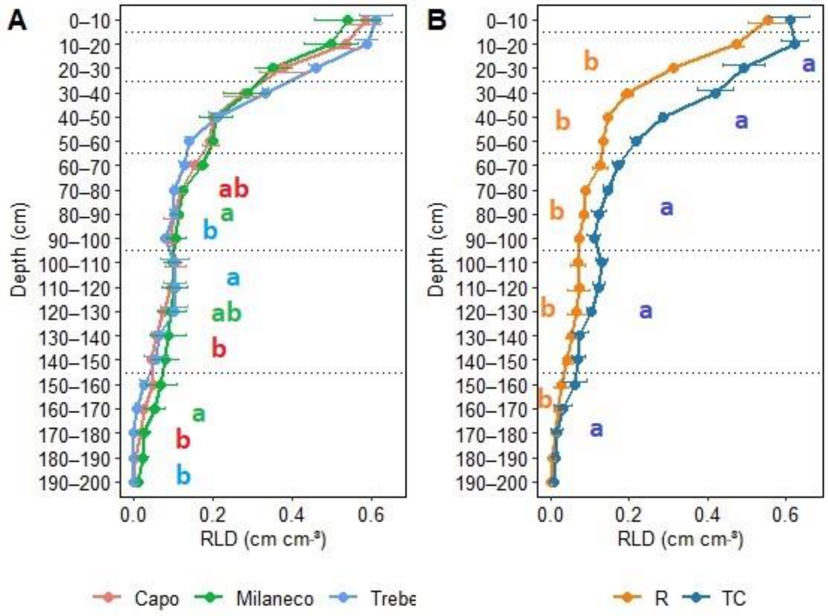


Figure S3.6. Main effects of tillage and genotypes on root length density (RLD) for winter wheat (2020) at flowering stage for each depth level. Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

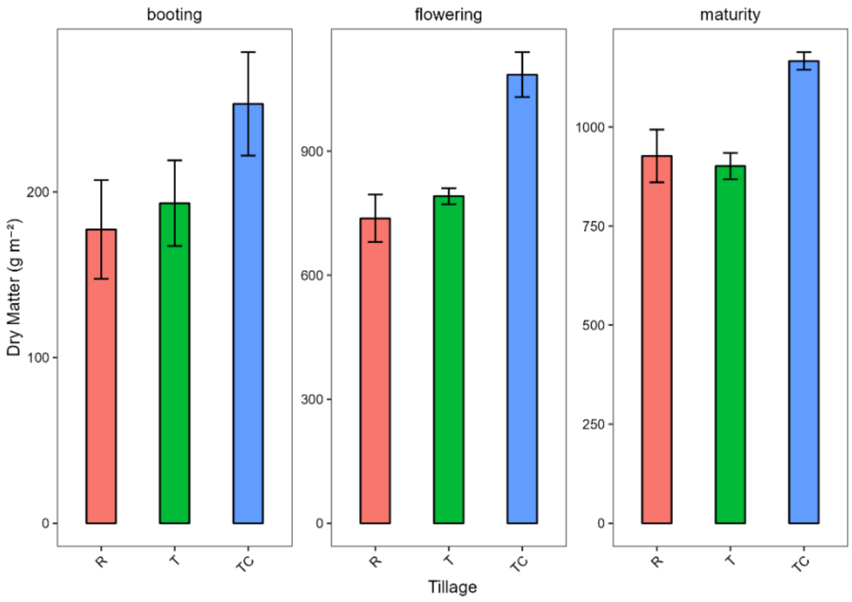


Figure S3.7. Dry matter (g m^{-2}) for spring barley (2019) at booting, flowering and maturity stage for tillage treatment. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

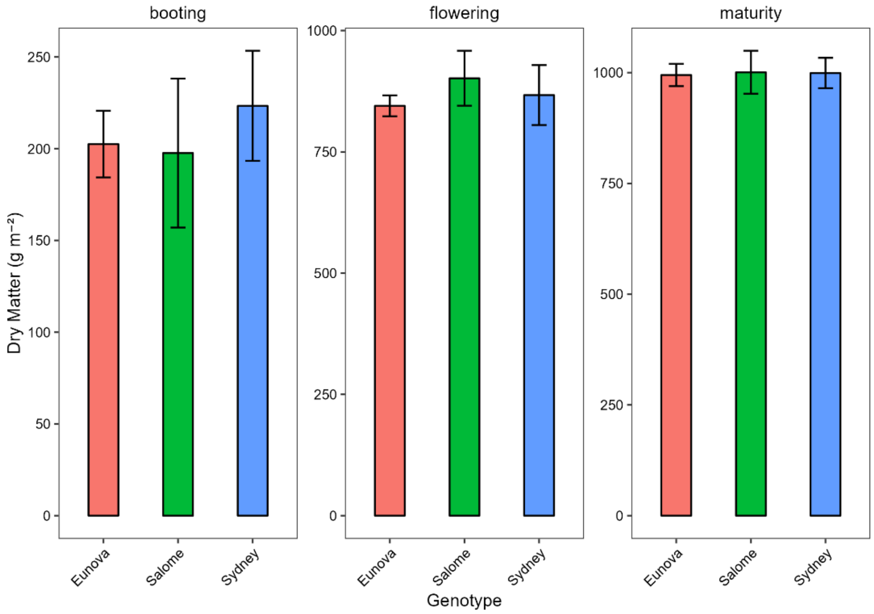


Figure S3.8. Dry matter (g m^{-2}) for spring barley (2019) at booting, flowering and maturity stage for genotype treatment. Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

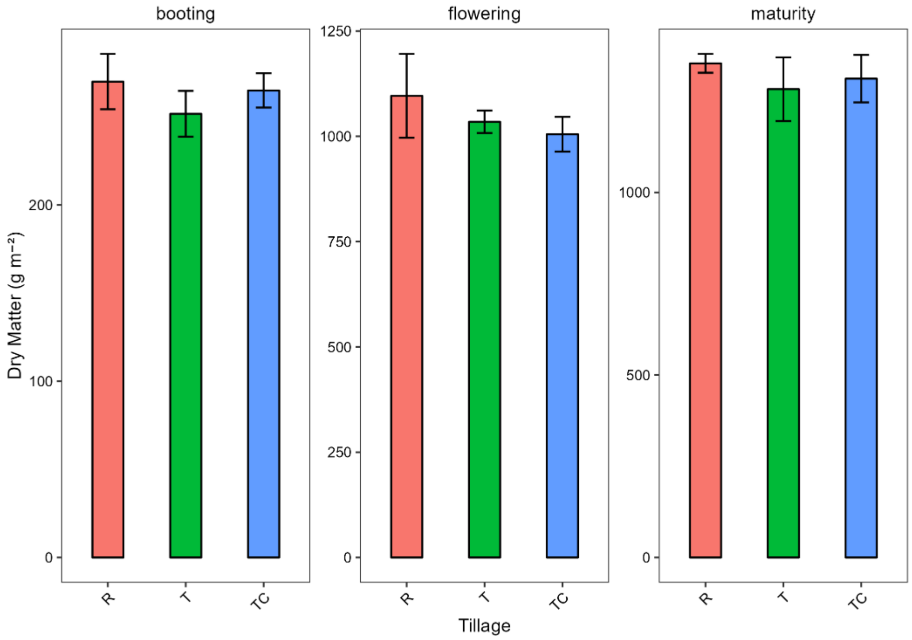


Figure S3.9. Dry matter (g m^{-2}) for winter wheat (2020) at booting, flowering and maturity stage for tillage treatment. No significant differences were found at any growth stage. HSD Tukey test, *p* < 0.05 (n=3).

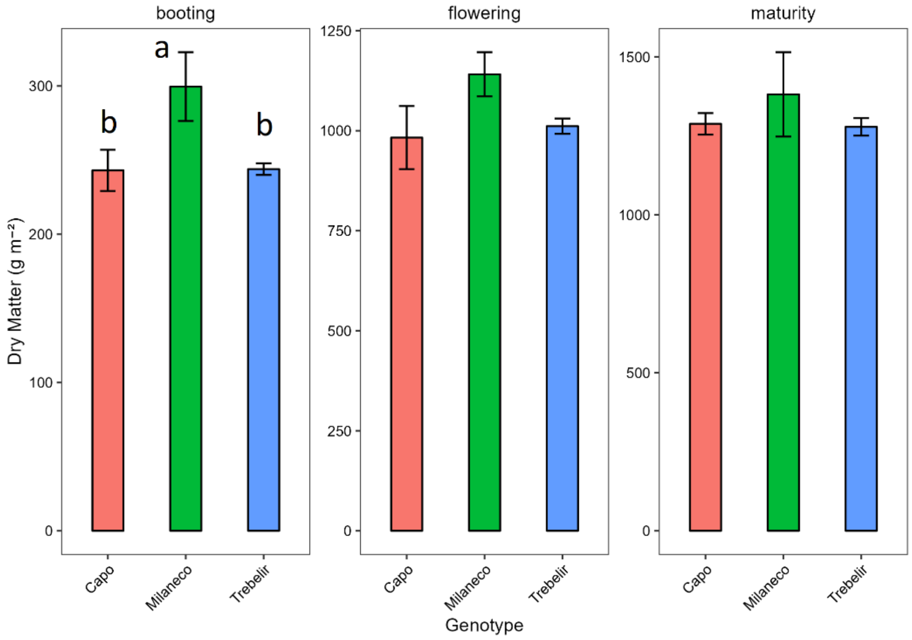


Figure S3.10. Dry matter (g m^{-2}) for winter wheat (2020) at booting, flowering and maturity stage for genotype treatment. Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

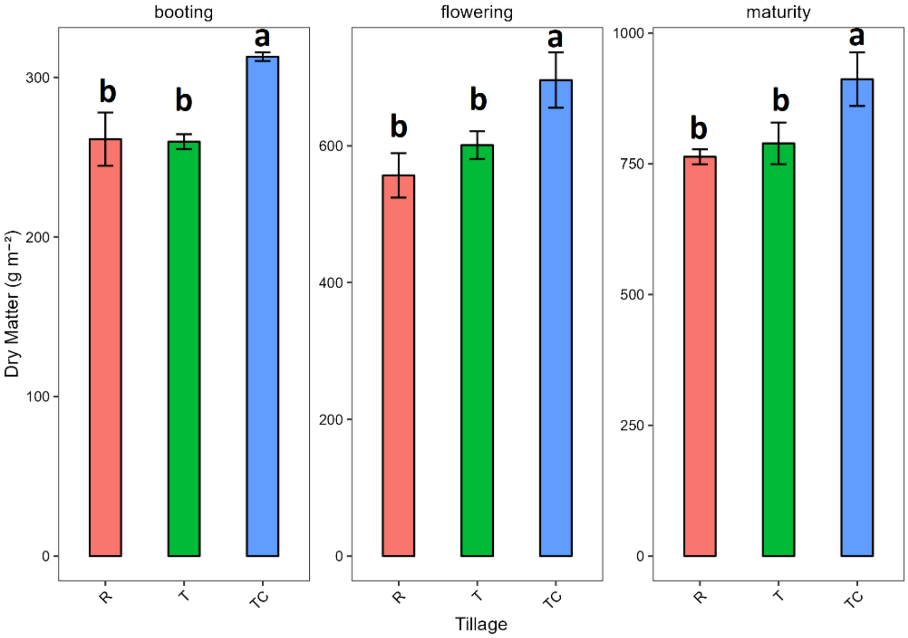


Figure S3.11. Dry matter (g m^{-2}) for spring barley (2021) at booting, flowering and maturity stage for tillage treatment. Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

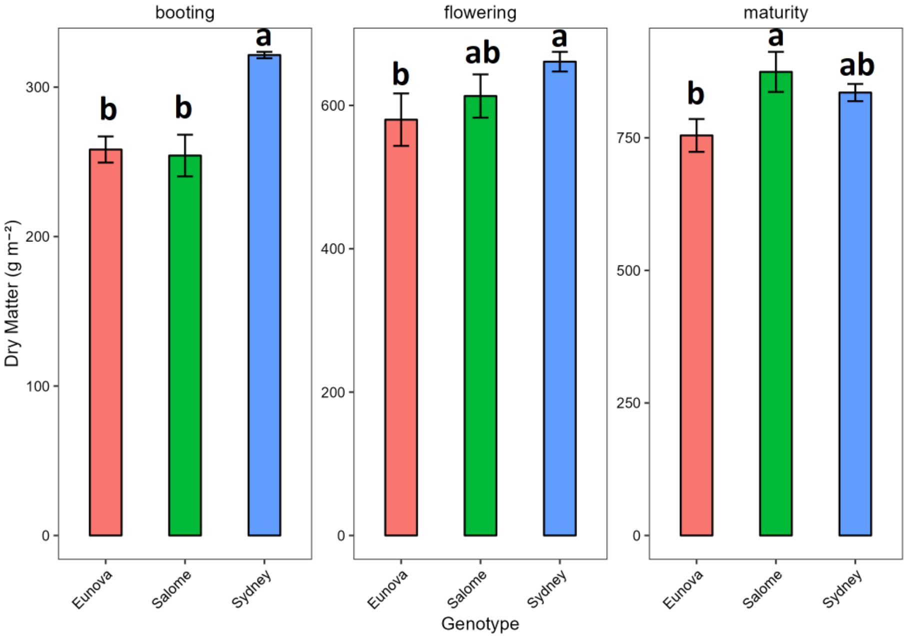


Figure S3.12. Dry matter (g m^{-2}) for spring barley (2021) at booting, flowering and maturity stage for different genotypes. Significance differences are indicated by different lowercase letters. HSD Tukey test, *p* < 0.05 (n=3).

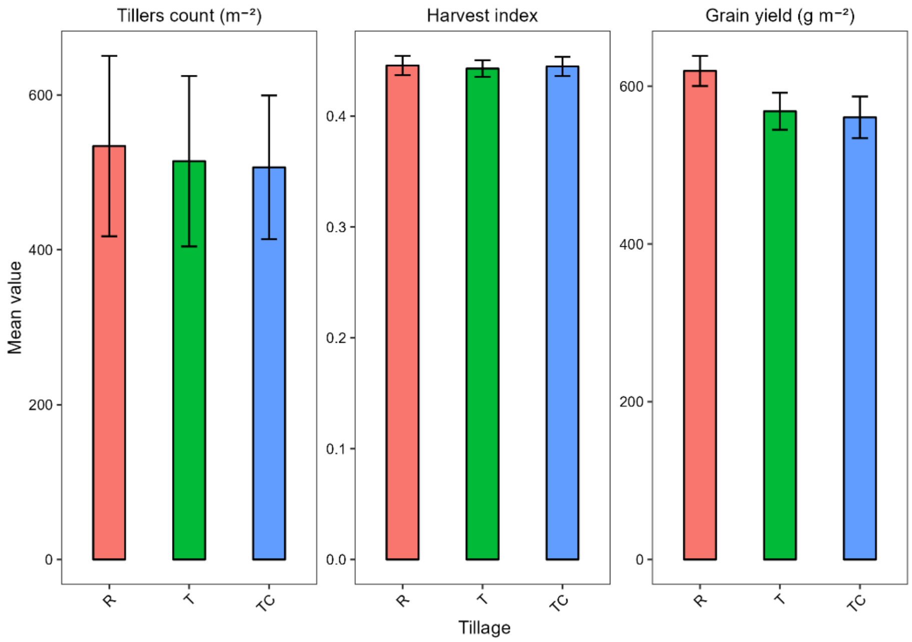


Figure S3.13. Tillers count, harvest index and grain yield (g m^{-2}) for tillage factor in winter wheat (2020). No significant differences were found among tillage treatments. HSD Tukey test, $p < 0.05$ (n=3).

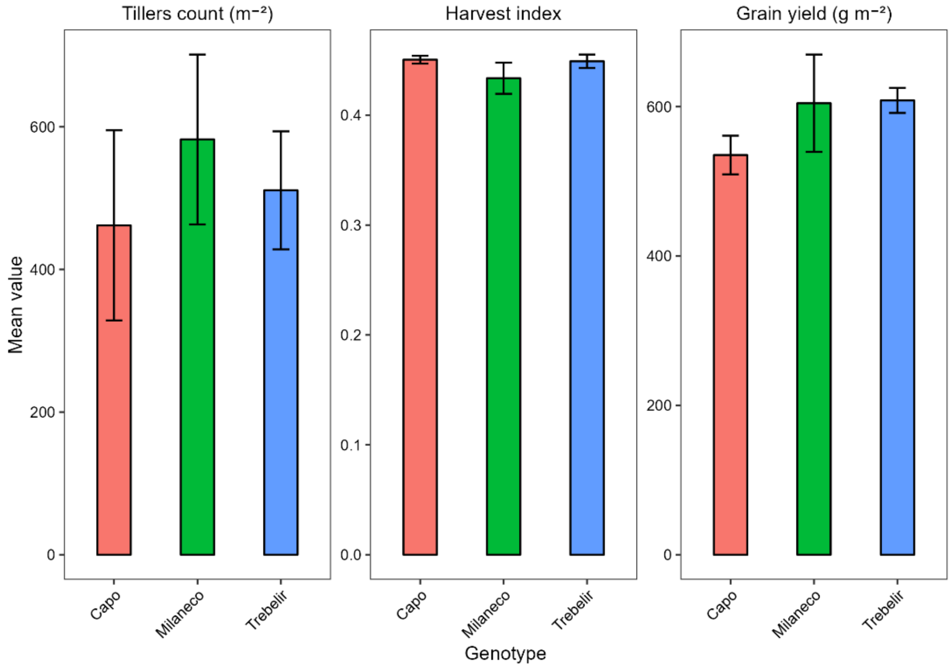


Figure S3.14. Tillers count, harvest index and grain yield (g m^{-2}) for tillage factor in winter wheat (2020). No significant differences were found among genotypes. HSD Tukey test, $p < 0.05$ (n=3).

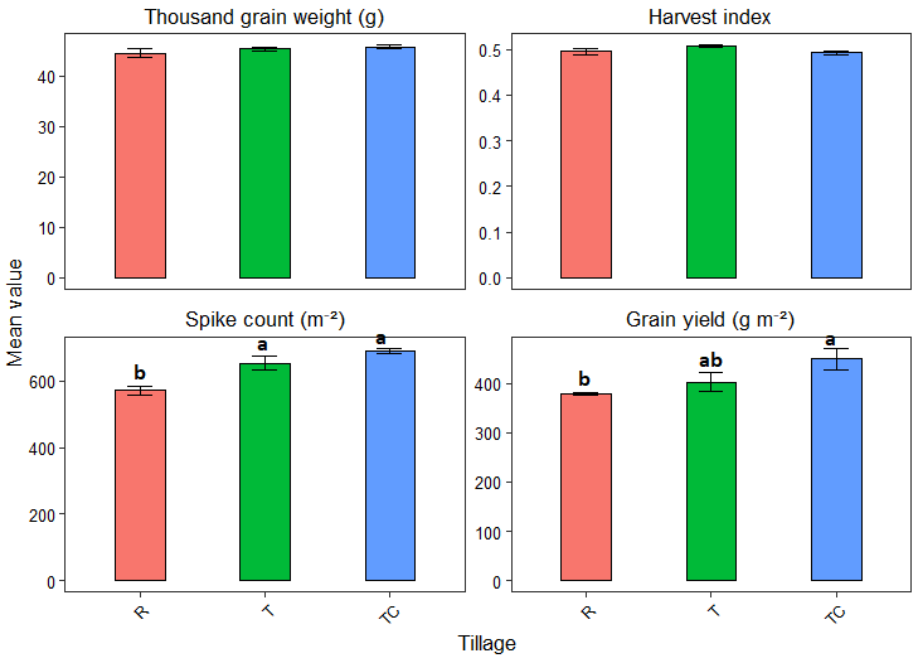


Figure S3.15. Harvest index, spike count and grain yield (g m^{-2}) for tillage factor in spring barley (2021). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

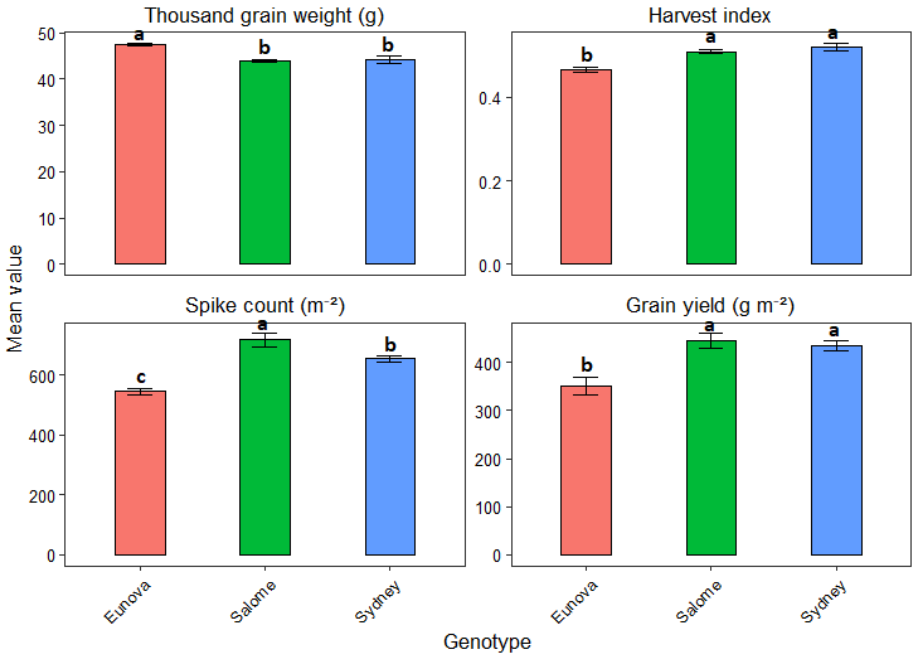


Figure S3.16. Harvest index, spike count and grain yield (g m^{-2}) for genotypes in spring barley (2021). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

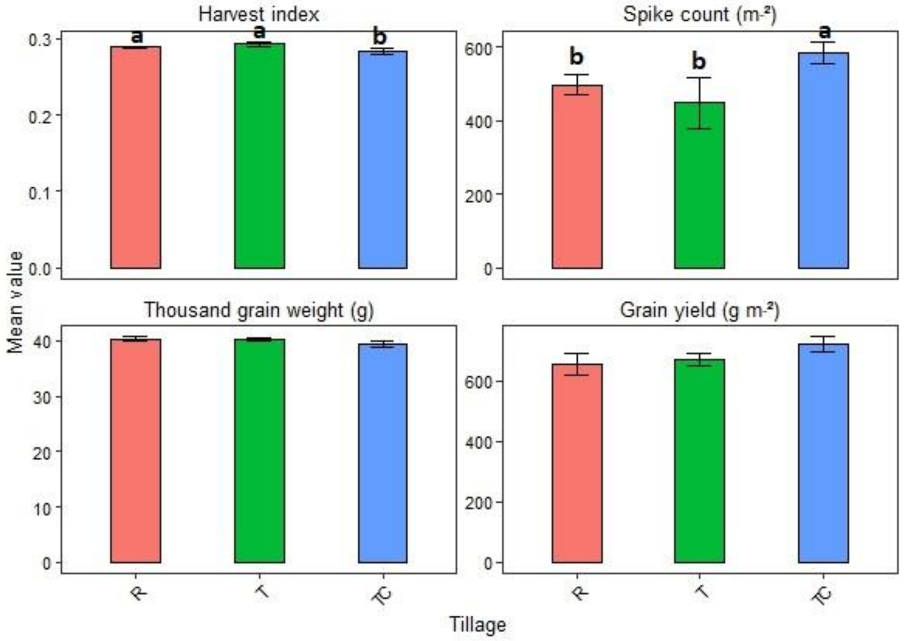


Figure S3.17. Harvest index, spike count and grain yield (g m^{-2}) for tillage factor in winter wheat (2022). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

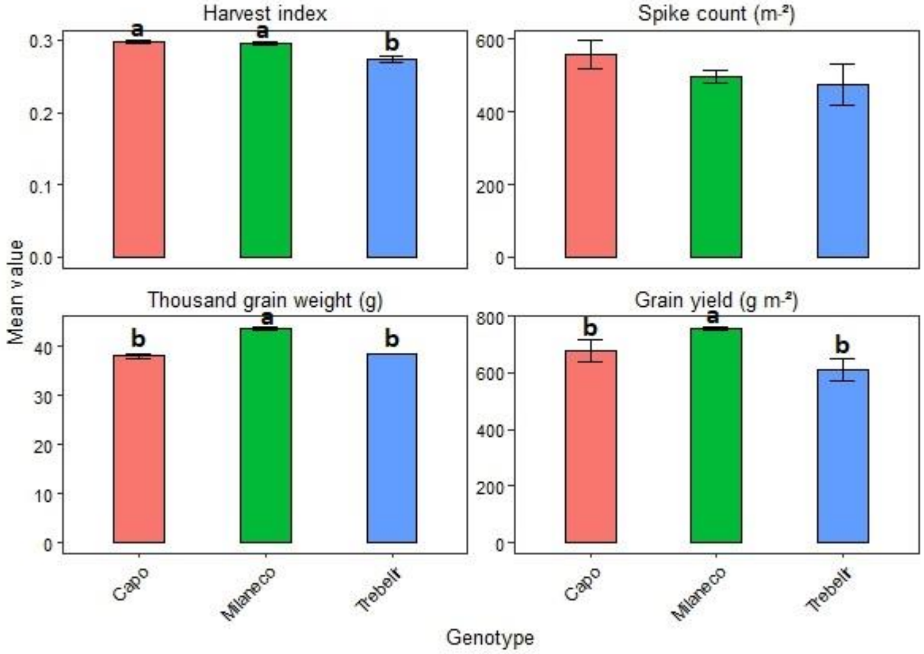


Figure S3.18. Harvest index, spike count and grain yield (g m^{-2}) for genotypes in winter wheat (2022). Significance differences are indicated by different lowercase letters. HSD Tukey test, $p < 0.05$ (n=3).

Table S3.1: Main effects, interaction of genotype and tillage for root length density (RLD) at each depth for spring barley (2021).

	RLD (p-value)							
Factor	**00-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**
Genotype	0.17	0.15	0.62	0.10	0.25	0.94	0.66	0.03 *
Tillage	0.16	0.48	0.00 **	0.00 ***	0.00 ***	0.02 *	0.06	0.00 **
Genotype × Tillage	0.42	0.74	0.15	0.25	0.44	1.00	0.56	0.49

Table S3.2: Main effects, interaction of genotype and tillage for root length density (RLD) at booting at each depth group for winter wheat (2020).

	RLD (p-value)					
Factor	**00-10**	**10-30**	**30-60**	**60-100**	**100-150**	**150-200**
Genotype	0.58	0.03 *	0.04*	0.00 **	0.01 *	0.09
Tillage	0.02 *	0.00 ***	0.00 ***	0.00 ***	0.01 *	0.24
Genotype × Tillage	0.39	0.23	0.46	0.00 **	0.96	0.01 *

Table S3.3: Main effects, interaction of genotype and tillage for root length density (RLD) at flowering at each depth group for winter wheat (2020).

	RLD (p-value)					
Factor	**00-10**	**10-30**	**30-60**	**60-100**	**100-150**	**150-200**
Genotype	0.91	0.45	0.17	0.00 **	0.01 *	0.00 **
Tillage	0.32	0.00 **	0.00 ***	0.00 **	0.00 **	0.01 *
Genotype × Tillage	0.70	0.60	0.46	0.01 *	0.00 **	0.04 *

Table S3.4: Main effects, interaction of genotype and tillage for root length density (RLD) at each depth for winter wheat (2022).

	RLD (p-value)							
Factor	**00-10**	**10-20**	**20-30**	**30-40**	**40-50**	**50-60**	**60-70**	**70-80**
Genotype	0.03 *	0.06	0.01	0.19	0.06	0.16	0.10	0.33
Tillage	0.00 **	0.13	0.56	0.01 **	0.08	0.62	0.14	0.48
Genotype × Tillage	0.04 *	0.12	0.13	0.70	0.14	0.78	0.92	0.57

Table S3.5: Main effects and interaction of genotype and tillage for dry matter at different growth stages (spring barley, 2021)

	Dry matter (p-value)		
Factor	**Booting**	**Flowering**	**Maturity**
Genotype	0.0001 ***	0.0292 *	0.030 *
Tillage	0.001 **	0.0004 ***	0.005 **
Genotype × Tillage	0.2179	0.4541	0.259
Genotype			
Sydney	0.000 ***	0.056	0.041 *
Salome	0.422	0.018 *	0.553
Eunova	0.010 *	0.021 *	0.224

Table S3.6: Main effects and interaction of genotype and tillage for dry matter at different growth stages (winter wheat, 2020)

	Dry matter (p-value)		
Factor	**Booting**	**Flowering**	**Maturity**
Genotype	0.002 **	0.063	0.427
Tillage	0.465	0.389	0.708
Genotype × Tillage	0.147	0.522	0.951
Genotype			
Capo	0.108	0.309	0.823
Milaneco	0.725	0.813	0.653
Trebelir	0.272	0.588	0.486

Table S3.7: Main effects and interaction of genotype and tillage for nutrient uptake at different growth stages (spring barley, 2019)

		Booting		
	Carbon	Nitrogen	Phosphorus	Potassium
Genotype	0.0495*	0.2514	0.4477	0.6264
Tillage	0.0001***	0.0001***	0.0005***	0.0004***
Genotype × Tillage	0.7182	0.9027	0.9214	0.9925
		Flowering		
Genotype	0.567	0.1320	0.4597	0.3259
Tillage	0.0001***	0.0001***	0.0001***	.0001***
Genotype × Tillage	0.848	0.4873	0.8665	0.9254
		Maturity		
Genotype	0.9879	0.9248		
Tillage	0.0002**	0.0011**		
Genotype × Tillage	0.3521	0.2075		

Table S3.8: Main effects and interaction for yield parameters (spring barley 2019)

	p-value			
Factor	**Grain yield**	**Harvest index**	**Tillers count**	**Spike count**
Genotype	0.4903	0.0182*	0.0005***	0.0001***
Tillage	0.0001***	0.1140	0.1330	0.0008***
Genotype × Tillage	0.4770	0.8386	0.3442	0.2631

Table S3.9: Main effects and interaction for yield parameters (winter wheat 2020)

	p-value		
Factor	**Grain yield**	**Harvest index**	**Tillers count**
Genotype	0.232	0.421	0.083
Tillage	0.402	0.980	0.854
Genotype × Tillage	0.819	0.622	0.946

Table S3.10: Main effects and interaction for yield parameters (spring barley 2021)

	p-value			
Factor	**Grain yield**	**Harvest index**	**Thousand grain weight**	**Spike count**
Genotype	0.002 **	0.000 ***	0.000 ***	0.255
Tillage	0.057	0.009 **	0.084	0.038 *
Genotype × Tillage	0.677	0.203	0.193	0.368

Table S3.11: Main effects and interaction for yield parameters winter wheat 2022.

	p-value			
Factor	**Grain yield**	**Harvest index**	**Thousand grain weight**	**Spike count**
Genotype	0.002 **	0.000 ***	0.000 ***	0.255
Tillage	0.057	0.009 **	0.084	0.038 *
Genotype × Tillage	0.677	0.203	0.193	0.368

Chapter 4

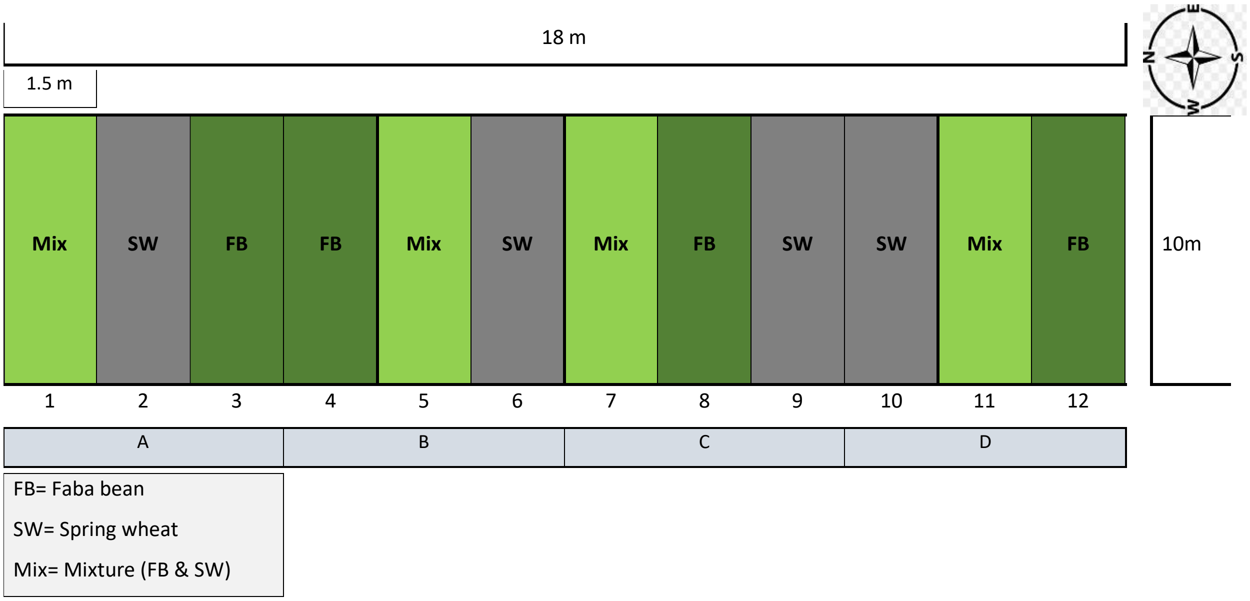


Figure S4.1: Layout of field experiment at Campus Klein-Altendorf, consisting of four blocks/replicates (A, B, C and D) and three treatments of spring wheat (SW), faba bean (FB) and mixture (Mix).

Figure S4.2: Trench across the experimental field measuring 1.5 m long and 1 m deep.

Figure S4.3: 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)

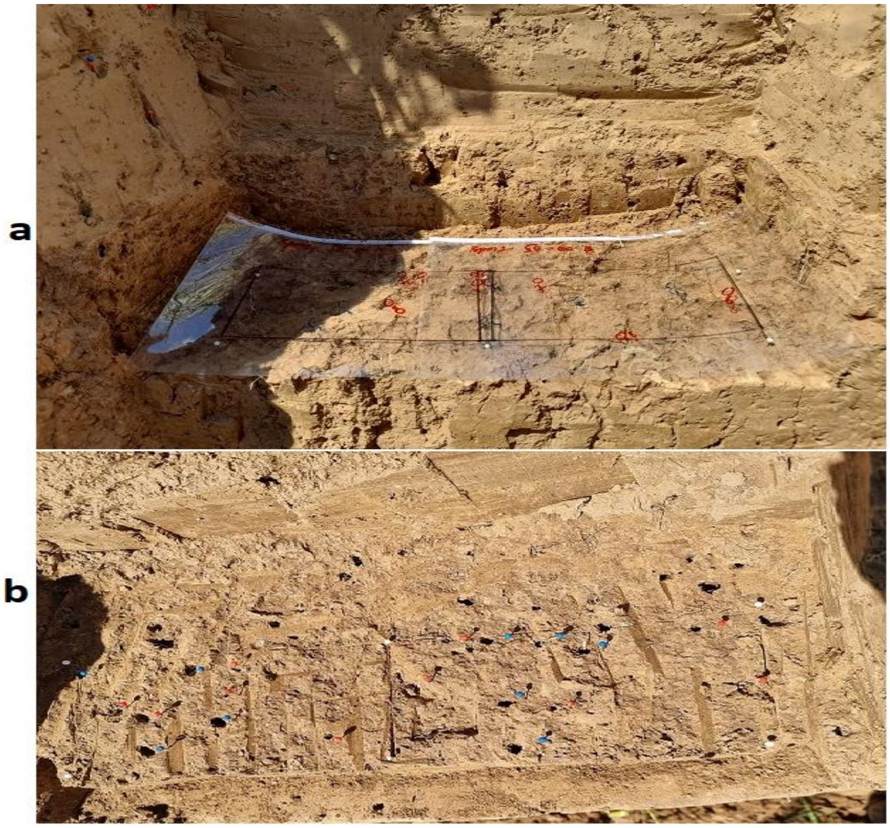


Figure S4.4: (a) The smoothened surface measuring 10 x 40 cm at a depth of 40 cm was covered with transparent plastic and labeled for the small and large biopores (2 replications of 10 x 20 cm). (b) Biopores in the soil were labeled with red (>5 mm) and blue (3-5 mm) needles representing the size of biopore.

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