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Root growth and belowground interactions and plasticity of field crops

Responses to nutrient availability and intercropping

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Summary

Understanding the growth of roots and belowground interactions of field crops constitutes a pathway to enhancing the performance of field crops. Global challenges related to climate change and a growing population are driving a more rational use of agricultural inputs to reduce soil and water ressources degradation. Roots play a central role in this process, as shown by research that has provided valuable insights into their development, functions, diversity, and adaptations to the environmental conditions.

To contribute to this understanding, the present thesis aims to investigate the root growth and interactions occuring in intercropping systems as well as the effects of nutrient omission on root traits of field crops.

For intercropping, a field experiment was conducted using one faba bean cultivar and two spring wheat cultivars sown at three sowing densities, defining three intercropping designs. Destructive root coring was conducted (0–100 cm) in the intercrops and sole crops at two development stages. FTIR spectroscopy was used to discriminate the species' root masses. In intercrops, sowing density affected more than the cultivar choice the root growth and belowground interactions. The highest sowing density led to a decrease of root biomass and more competitive interaction between faba bean and winter wheat. The lowest sowing densities promoted deeper root growth of wheat. Regarding the cultivar choice, the early root growth in depth and in density of one spring wheat cultivar lowered faba bean root growth. The findings highlight the importance of plant density and root co-occurrence in belowground interactions of intercrops.

To investigate the nutrient availability´s effects on root growth and plasticity, root and shoot sampling was conducted in 2019 for sugar beet, 2019/20 and 2020/21 for winter wheat and 2021/2022 for winter rye at the long-term fertilizer experiment (LTFE) Dikopshof. Various fertilizer treatments were chosen in the three studies including: fully fertilized including manure (m) and supplemental mineral fertilizer (s) (NPKCa+m+s), fully fertilized without manure (NPKCa), N omitted (_PKCa), and P omitted (N_KCa) for winter rye and additionally lime omitted (NPK_) and no fertilization for winter wheat and sugar beet. N availability affected root morphology and plasticity: N omission reduced root growth in winter rye and winter wheat, with stage-specific effects on root diameter, root length density and P omission significantly impacts root traits of field crops, demonstrating the plasticity of root systems in adapting to nutrient-limited conditions. Sustained Ca and K omission affected to a less extent root morphological traits. The Results found in this thesis suggest that nutrient availability as well as intercropping system may affect the root growth and plasticity of field crops. However, the responses are species specific, and affected by growth stage. The results provide valuable insights into potential root traits that can be considered in breeding programs and agronomically relevant insights that serve to design sustainable cropping systems.

Zusammenfassung

Das Verständnis des Wurzelwachstums und der Wechselwirkungen im Boden von Feldfrüchten ist ein wichtiger Schritt zur Steigerung ihrer Ertragskraft. Globale Herausforderungen wie der Klimawandel und das Bevölkerungswachstum erfordern einen effizienten Einsatz von Betriebsmitteln in der Landwirtschaft, um die Degradation von Boden- und Wasserressourcen zu reduzieren. Wurzeln spielen dabei eine zentrale Rolle, wie Forschungsergebnisse belegen, die wertvolle Einblicke in ihre Entwicklung, Funktionen, Diversität und Anpassung an Umweltbedingungen liefern.

Um zu diesem Verständnis beizutragen, untersucht die vorliegende Dissertation das Wurzelwachstum und die Wechselwirkungen in Mischkultursystemen sowie die Auswirkungen von Nährstoffmangel auf die Wurzeleigenschaften von Feldfrüchten.

Für die Mischkultur wurde ein Feldversuch mit einer Ackerbohnensorte und zwei Sommerweizensorten durchgeführt, die in drei verschiedenen Aussaatdichten angebaut wurden, wodurch drei Mischkulturdesigns entstanden. In den Mischkulturen und den Reinkulturen wurden in zwei Entwicklungsstadien Wurzelkernproben (0–100 cm) entnommen. Die Wurzelmassen der verschiedenen Arten wurden mittels FTIR-Spektroskopie bestimmt. Bei Mischkulturen beeinflusste die Aussaatdichte das Wurzelwachstum und die Interaktionen im Boden stärker als die Sortenwahl. Die höchste Aussaatdichte führte zu einer geringeren Wurzelbiomasse und verstärkter Konkurrenz zwischen Ackerbohne und Winterweizen. Die niedrigsten Aussaatdichten förderten ein tieferes Wurzelwachstum des Weizens. Hinsichtlich der Sortenwahl zeigte sich, dass das frühe Wurzelwachstum einer Sommerweizensorte in Tiefe und Dichte das Wurzelwachstum der Ackerbohne hemmte. Die Ergebnisse unterstreichen die Bedeutung von Pflanzendichte und Wurzelkollokation für die Interaktionen im Boden.

Um die Auswirkungen der Nährstoffverfügbarkeit auf Wurzelwachstum und -plastizität zu untersuchen, wurden im Langzeitdüngungsversuch (LTFE) Dikopshof 2019 Wurzel- und Sprossproben für Zuckerrüben, 2019/20 und 2020/21 für Winterweizen und 2021/22 für Winterroggen entnommen. In den drei Studien wurden verschiedene Düngungsbehandlungen gewählt: Volldüngung mit Stallmist (m) und ergänzendem Mineraldünger (s) (NPKCa+m+s), Volldüngung ohne Stallmist (NPKCa), Stickstoffmangel (_PKCa) und Phosphormangel (N_KCa) für Winterroggen sowie zusätzlich Kalkmangel (NPK_) und keine Düngung für Winterweizen und Zuckerrüben. Die Stickstoffverfügbarkeit beeinflusst die Wurzelmorphologie und -plastizität stark: Stickstoffmangel kann die Wurzelbiomasse, -länge und -verzweigung verringern. Stickstoffmangel reduzierte das Wurzelwachstum und den Kornertrag bei Winterroggen und Winterweizen, mit stadienabhängigen Auswirkungen auf Wurzeldurchmesser und Wurzellängendichte.

Phosphormangel beeinflusst die Wurzeleigenschaften signifikant und verdeutlicht die Plastizität der Wurzelsysteme bei der Anpassung an nährstofflimitierte Bedingungen. Anhaltender Kalzium- und Kaliummangel beeinträchtigte die Wurzelmorphologie in geringerem Maße.

Die Ergebnisse dieser Arbeit deuten darauf hin, dass sowohl die Nährstoffverfügbarkeit als auch das Anbausystem das Wurzelwachstum und die Wurzelplastizität von Feldfrüchten beeinflussen können. Die Reaktionen sind jedoch artspezifisch und vom Wachstumsstadium abhängig. Die Ergebnisse liefern wertvolle Erkenntnisse über potenzielle Wurzelmerkmale, die in Züchtungsprogrammen berücksichtigt werden können, sowie agronomisch relevante Einblicke für die Entwicklung nachhaltiger Anbausysteme.

Abbreviations, acronyms and units

°C	degree Celsius
Al	aluminium
ANOVA	analysis of variance
Ca	calcium (used for liming)
cm	centimetre
cv.	cultivar
e.g.	exempli gratia
FTIR	Fourier transform infrared
g	gram
ha	hectare
K	potassium
kg	kilogram
kg ha^{-1} a^{-1}	kilogram per hectare per annum (year)
LAI	Leaf area index
LER	land equivalent ratio
LTFE	long term fertilizer experiment
m	metre
mg	milligram
Mn	manganese
N	nitrogen
n	sample size
N_{min}	mineral nitrogen
N_t	Total nitrogen
NUE	nitrogen use efficiency
P	phosphorus
R/S	root to shoot ratio
RLD	root length density
RMD	root mass density
SD	sowing density
TSD	total sowing density

1 General introduction

Crop production plays a central role in agriculture, directly influencing food production, food security, as well as agroecosystem stability. In light of the rising global population alongside incessant resource scarcity and significant challenges related to climate change, including shifts in global temperature, changes in precipitation patterns and increased occurrences of extreme weather events (Pachauri et al. 2014; Mbow et al. 2019; Lee et al. 2023), there is a need to ensure sustainable crop production to meet the global food demand. This makes it crucial to study the mechanisms influencing crop production, aiming to develop crops with greater stress tolerance and reduced dependence on external resources (irrigation and fertilizers) and thus, improving the efficiency and sustainability of crop production.

Crop production is directly linked to plant growth. Plant growth has various facets ranging from cell to organs, plants, and ecosystem levels (Hilty et al. 2021). Plant growth at the ecosystem level encompasses both above- and belowground biomass production. Most breeding studies on field crops focus on the phenotyping of aboveground plant parts, but the belowground parts are often neglected (Maqbool et al. 2022). Roots are the primary interface between the soil and the plant and affect nutrient and water resource acquisition from the soil. Within breeding strategies, the selection for optimum nutrient and water uptake requires the understanding of root traits in field crops, including how these traits interact with each other and with other genetic and environmental factors, to a similar extent as aboveground traits (Lynch 2007; Comas et al. 2013; Maqbool et al. 2022).

While the characterization and measurement of aboveground biomass can be done easily based on either destructive or indirect measurement (such as Airborne or space-borne LiDAR image analysis) (Jin et al. 2020), measuring belowground (root) traits over large areas remains difficult (Freschet, Roumet, et al. 2021) As a result our understanding of belowground mechanisms and interactions involved in resource uptake, especially for field crops and at the ecosystem level, remains limited. Recent advances in computer vision applications have allowed image-based approaches for high-throughput phenotyping of root traits (Lobet et al. 2015). Non-invasive methods like X-ray computer tomography and magnetic resonance imaging can facilitate the exploration of root systems (Shao et al. 2021), root simulation modelling can also support the study of root physiology and ecology and the relationship with crop productivity (Hinsinger et al. 2011; Seidel et al. 2022).

Enhancing root adaptation, called plasticity, to resource scarcity through breeding and management practices can be a key strategy to improve the sustainability and performance of low-input agricultural systems (Lynch 2019). Schneider and Lynch (2020) argued that in low-input systems, plasticity may be advantageous by enabling plants to exploit resource patches through increased lateral root spread, potentially conferring a competitive advantage (Lynch 2018). So, understanding how roots grow and function in low-input cropping systems, such as in intercropping systems and in low-nutrient monocultures, is essential for developing ideotypes and management practices that enhance resource use efficiency of agricultural systems.

1.1 Belowground phenotyping: Approach for studying root function, structure, and crop performance

Belowground phenotyping, or root phenotyping, consists of the characterization of functional and structural root traits called *phenes*. Root *phenes* characterize root architecture, morphology, anatomy, or physiology (York et al. 2013). These characteristics influence agricultural performance such as biomass and yield. Because of its importance, root phenotyping has been incorporated into breeding programs for many years (Tracy et al. 2020). Breeders have selected for various root traits, both in controlled environments and at the field scale. They start with trait identification, followed by root and shoot phenotyping, and then phenotype introgression into elite germplasm. This process serves to identify varieties that allow for enhanced crop productivity under limited growth conditions (Tracy et al. 2020). So, there is a need to improve and breed crop cultivars with a favourable root system but the lack of high-throughput root phenotyping tools for characterizing root traits at the field scale remains a barrier to breeding for root system improvement (Li et al. 2022). Advances in high-throughput phenotyping of roots can improve the selection process for root ideotypes with improved crop nutrient acquisition and to understand the influence of inter- and intraspecific root system variation at the ecosystem level (York et al. 2013).

At the field scale, root phenotyping remains a time-consuming and labour-intensive task. Many methods were developed in recent years to study roots under field conditions. Monolith methods (Majdi et al. 1992; Buman et al. 1994; Henry et al. 2012; Kemper et al. 2022), soil coring, needle boards, rhizotron and minirhizotron methods (Majdi et al. 1992), profile wall methods (Böhm 1979) as well as root excavation methods. Soil coring and profile wall methods are rather discontinuous measurement methods that allow for assessing root traits under field conditions in a specific time and space. Rhizotrons, minirhizotrones, and in-growth bags are continuous measurement methods and enable the assessment of root traits over time. The method selection depends on the aim of the study, the root trait investigated, the degree of detail needed, and the availability of the resources (Freschet, Pagès, et al. 2021).

1.2 Root traits as an indicator of the interactions of crops with their environment

1.2.1 Root traits identification

The characterization of plant root systems includes many types of traits. Bardgett et al. (2014) categorized these into four categories: architectural, morphological, physiological, and biotic:

- Architectural root traits include traits such as root length density (RLD), rooting depth, and root branching. They allow for the characterization of the spatial configuration of the entire root system of an individual plant;

- Morphological traits include root diameter, specific root length, and root dry matter content. They describe features of individual roots;
- Physiological root traits give insights into the nutrient uptake kinetics, root respiration, and release of root exudates;
- Biotic traits involve direct interactions between roots and soil biota that affect nutrient capture, such as associations with mycorrhizal fungi and rhizobia (in legumes), but also interactions with pathogens.

It is widely recognized that enhanced root mass has many roles: a plant with higher root mass can better anchor to the soil, as well as for carbon storage and its sequestration (Kätterer et al. 2011; Poeplau and Don 2015). RLD is linked to aggregate stability (Hudek et al. 2022), water uptake, and nutrient acquisition from the soil (Tajima 2021). Root diameter is also considered an important trait affecting macropores and nutrient acquisition (Yanai et al. 1995; Perkons et al. 2014; Hudek et al. 2022). Roots with a large diameter, such as the taproots of dicotyledonous plants, can explore compacted soil more easily than roots with small diameters (Materechera et al. 1992), enhancing the nutrient acquisition efficiency of the plant grown under suboptimal edaphic conditions. Another important root morphological trait is specific root length (SRL), defined as root length per root mass and represents belowground economics (Freschet, Pagès, et al. 2021). SRL can also be linked with nutrient uptake efficiency (Eissenstat 1992; Isaac and Borden 2019; Kemper et al. 2023). Crops with increased SRL have long and thin roots and are less expensive to produce (Ostonen et al. 2007). In breeding programs, many traits were already incorporated into new germplasm; a list of those is provided by Tracy et al. (2020). A definition of root traits measured generally in root research is provided in Table 1.1.

Table 1.1: Most commonly investigated plant root traits and their functions (adapted from Atkinson (2000) and Bardgett et al. (2014).

Traits/phenes	Description	Functions/significance
Root length (RL)	Length of all root segments present in the investigated soil layers	Absorption of nutrients or water from soil; Indicator of baseline soil microbial activity, especially the arbuscular mycorrhizal fungal (AMF) activity in soil and of microbial functioning, e.g., organic phosphorus catabolism.
Root length density (RLD)	Root length in a defined volume of soil	Soil formation and structural stability (Bardgett et al. 2014).
Root mass	Oven-dry weight of the total roots present in the investigated soil layers (and attached micro-organisms)	Carbon cycling (Bardgett et al. 2014), ressource allocation.
Root mass density (RMD)	Root mass per unit soil volume	Resource allocation (Wilson et al. 1999; Birouste et al. 2014).
Specific root length (SRL)	Length-to-mass ratio (L/M) of a root fragment	Characterization of the economic aspects of the root system; indication of environmental changes (Ostonen et al. 2007).
Root to shoot ratio (R/S)	The ratio of the dry weight of root to the dry weight of leaves + stem (branches)	Relative allocation strategy of the crop. Atkinson (2000)
Root diameter	The diameter of an average individual root, usually assumed to be a plain cylinder.	Potential for mycorrhizal development; regulation of water stress; influences and responses to soil physical condition; soil formation and structural stability (Bardgett et al. 2014).
Root topology	Branching pattern of the individual root axes.	Branching increases the volume of soil that can be accessed by roots and the number of root tips, which are key sites for resource acquisition (York et al. 2016).
Root links	Root part between two forks or a fork and a tip	Indicator of root branching (see above).
Root angle	Angle to soil surface	Subsoil exploration, water capture, and drought tolerance (Lynch 2022).

1.2.2 Root plasticity

Root traits are highly plastic in response to environmental gradients and species-species interactions (Chapman et al. 2012; Valverde-Barrantes et al. 2013; Bardgett et al. 2014; Chen et al. 2021). Plasticity is the ability of an organism to change its phenotype in response to environmental conditions (Nicotra et al. 2010). Plasticity does not explicitly improve plant performance or survival. Plastic responses may be of short or long duration. For example, the final diameter of a root is established after tissue growth, and while growing tissues may respond to the local environment, mature tissue does not. In contrast, expression of nitrate transporters may change to track environmental signals that fluctuate on short time scales (Lobet et al. 2019). Various plastic responses of plants to edaphic conditions have already been reported, showing roots to grow according to the distribution of available water in soil (Dinneny 2019), with the production of finer and deeper roots being important traits enabling acquisition of water (Bardgett et al. 2014). Plants alter their architecture to forage for nutrients (Gonzalez et al., 2021; Uga

2021). Studies implying various plant species from different cropping systems are needed to better place the plasticity of root traits in the context of sustainable agricultural strategies (Bardgett et al. 2014).

1.3 Root traits in intercropping systems

Intensive agriculture is based on the use of high fertilization inputs and of irrigation systems to increase the yield per unit of land area, but it has failed to assure agricultural sustainability because of the creation of ecological imbalance and degradation of natural resources (Tsiafouli et al. 2015). Sustainable agriculture, on the other hand, aims at the use of low external inputs while maintaining sufficient food production. Intercrops, also known as mixed cropping or polyculture, are a traditional farming practice with diversified crop cultivation, use low inputs, and improve the quality of the agroecosystem. Intercrops are defined as the practice of cultivating two or more crops with different rooting abilities, canopy structure, height, and nutrient requirements simultaneously (Hauggaard-Nielsen et al. 2008; Lithourgidis et al. 2011).

Intercrops present numerous benefits: yield enhancement, environmental security, production sustainability, and greater ecosystem services (Maitra et al. 2021). Benefits of intercropping could not only be associated with low-input systems, but also under favourable cultivation conditions (Homulle et al. 2022). Intercropping can increase crop production and maintain soil organic matter levels in soil. It is known that the underlying mechanisms are associated with above- and belowground nutrient (e.g., nitrogen) availability, uptake, and use efficiency (Xu et al. 2019). These benefits, however, are not always achieved, partly due to incomplete knowledge about the plant characteristics that optimize interactions between intercropped plants, particularly root-root interactions (Homulle et al. 2022). Studies on roots in intercropping showed that intercrops enhance the root mass (Ma and Chen 2016). The enhanced root mass production in intercrops was observed in the case of faba bean/maize mixtures (Li et al. 2006; Xia et al. 2013) and clover/ryegrass mixtures (Davidson and Robson 1990). However, there is also evidence that a higher plant species richness leads to decreased root biomass (Bessler et al. 2009). Besides root biomass, horizontal and vertical root distribution is also altered by the presence of a mixing partner. Li et al. (2006) found that the roots of maize intercropped with wheat were limited laterally, but had a greater RLD than sole-cropped maize. Also, Ren et al. (2017), in a maize/soybean intercrop, found an enhanced root length density (RLD) in both maize and soybean compared to the corresponding sole crop RLD..

The modification of spatiotemporal root distribution and enhanced root biomass in intercrops might lead to an increased nutrient uptake and eventually to higher yields (Hauggaard-Nielsen et al. 2001). This highlights the necessity to study the roots of intercrops, especially with regard to varying management practices such as sowing density and cultivar choice. Brooker et al. (2015) emphasized that the main challenge for research on mixed cropping systems is to understand their underlying processes, such as competition and facilitation.

1.4 Roots of field crops under nutrient limitation

In light of the current environmental challenge of modern agriculture, sustainable agriculture strategies should focus more on the acquisition of resources in field crops grown in sole cropping systems. Besides water stress, nutrient availability is the most limiting factor affecting crop production. In European agriculture, the "Farm to Fork" Strategy aims to reduce nutrient losses from fertilizers by at least 50% by 2030, while ensuring no deterioration in soil fertility. This reduction is targeted for both organic and mineral fertilizers. The strategy also aims to reduce the overall use of fertilizers by 20% (European Commission 2020). Hence, the study of root characteristics under nutrient limitation is becoming crucial to improve the yield of field crops. Atkinson (2000) previously stated that when nutrients are abundant and edaphic conditions are beneficial for their availability, besides the need for a minimum root length, more complex characteristics are likely to be unimportant. But, when the nutrients are limited due to nutrient scarcity, to the intensity of external supply, or where spatial distribution is particularly complex due to edaphic properties, then the root system and its various characteristics become critically important.

1.4.1 Root traits of cereals under low nutrient conditions

Cereal grains play a central role in maintaining food security worldwide. Around the world, rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and to a lesser degree, barley (*Hordeum vulgare L.*), sorghum (*Sorghum bicolor*.), and millet (*Pennisetum glaucum*) are staple food crops that are essential to the survival of billions of people. Over 50% of the world's caloric intake comes directly from cereal consumption (Sarwar 2013). Another source of fibre is rye (*Secale cereale*), which is widely produced in Europe. Several studies have reported that cereal yields can be achieved by selective breeding for deeper root architecture, beneficial for nitrate capture, and greater water use efficiency. The steep, cheap, and deep root ideotype is associated with root depth, N uptake, growth, and yield under N stress (Lynch 2013, 2019). A meta-analysis by Lopez et al. (2023) revealed that root length and biomass, in most cases, decreased with increasing N, P, and K deficiency. For instance, in wheat, studies showed that there is a decrease of root biomass in response to N deficiency (Barraclough et al. 1989; Xue et al. 2014; Mehrabi et al. 2021), whereas Wang et al. showed a variable response of winter wheat to nutrient deficiency. Another important effect of N availability is an increase in root branching (Belford et al. 1987), which occurs especially in N-enriched soil patches for barley (Drew and Saker 1975).

1.4.2 Root traits of sugar beet under low nutrient conditions

Sugar beet (*Beta vulgaris*) is a biennial plant, mainly cultivated for its taproot (tuber), a sucrose storage organ. Sugar beet is a common source of succrose in temperate latitudes. Along with sugarcane, it constitutes the main sugar crop worldwide. In 2019, sugar beet was cultivated on 409,000 ha in Germany, or 3.5% of the agricultural area (German Federal Statistical Office DESTATIS). Kutschera (1960) described the root system of sugar beet as consisting of a classic taproot that grows vertically and produces several lateral branches, which then branch out, forming an extensive fibrous root system

that gradually colonizes the deeper soil layers. Due to its deep-rooted system and its long vegetation period, sugar beet root system impacts the soil health, improves soil structure, and contributes to lower nitrate leaching.

The effects of nutrient deficiency on sugar beet shoots and taproots have been described in several studies (Ulrich and Hills 1969; Terry and Ulrich 1973; Christenson and Draycott 2006; Otto et al. 2009). While much attention has traditionally been focused on the sugar beet's taproot, its primary sucrose storage organ, the fine roots consist an equally vital yet often overlooked component of the root architecture. It has been shown that sugar beet roots exceed 1m of depth, but water uptake can be restricted by physiological properties of the roots (Brown and Biscoe 1985; Brereton et al. 1986; Fitters et al. 2017). Those physiological properties may be linked to fine root morphology. So, a deep knowledge of fine root distribution and turnover in sugar beet can help optimize fertilizers application, minimizing losses and environmental pollution. Fine roots also play a significant role in carbon cycling and soil structure formation. Their decay contributes to organic matter pool in the soil, promoting soil health and long-term fertility. From an agronomic perspective, the fine root system is critical for sugar beet's role in crop rotations. Its deep and fibrous rooting pattern improves soil porosity, reduces compaction, and decreases nitrate leaching by capturing residual nutrients from previous crops. Therefore, studying fine root dynamics provides valuable insights for sustainable agricultural practices and for breeding more efficient and resilient sugar beet varieties.

1.5 Interspecific and intraspecific root-root interactions

The importance of the study of root-root interactions in sole crops, called intraspecific interactions, and in mixture species, called interspecific interactions (Atkinson 2000), was highlighted in several studies (Faget et al. 2013). These studies have shown that when plants of the same or of different species interact, a whole range of root properties are drastically changed. For instance, competition between apple trees influenced radial spread, distribution with depth, total root length, root length density, the development of woody roots, and root survival. Competition between apple trees and grass swards also influenced many of these, but changed root system branching, the periodicity of new growth, AMF infection and root activity (Atkinson 2000). In cropping systems, intercropped maize kept high root mass density (RMD) for a longer time span in the soil profile when intercropped with wheat or faba bean (Li et al. 2011). However, studies of interspecific root-root interactions have been limited by the ability to identify the roots of the different species. Several methods for root species identification in mixtures have been applied. Methods based on DNA, ^{13}C, or root morphology are time-consuming and need extensive training (Rewald et al. 2012). The monolith excavation method combined with visual distinction (Li et al. 2011; Yu et al. 2022) is rather simple and cheap but less accurate. Infrared spectroscopy has been used to discriminate roots of different species such as corn/soybean (White et al. 2011), pea/oat (Naumann et al. 2010), pea/oat and maize/barnyard grass (Legner et al. 2018), faba bean/wheat (Streit et al. 2019), and blue lupin/winter rye (Kemper et al. 2022).

Numerous studies have identified different types of interactions occurring in interspecific cropping systems on both levels aboveground and belowground. These interactions include: competition, facilitation (or cooperation), complementarity, and compensation. According to Justes et al. (2021), competition occurs when one species has a greater ability to use limiting resources (e.g., nutrients, water, space, light) than others. Complementarity occurs when intercropped plants have different requirements for abiotic resources in space, time, or form. Cooperation (or facilitation) is observed when the modification of the environment by one specie is beneficial to the other(s). Compensation occurs when the failure of one specie is compensated by the other(s) because they differ in their sensitivity to abiotic or biotic stress (Justes et al. 2021; Döring and Elsalahy 2022). Focusing on belowground interactions, Yu et al. (2022) describe the mechanisms that drive interspecific belowground competition (e.g. driven by resource depletion) and facilitation (e.g. due to nutrient or water enrichment or enrichment of beneficial microbiome) in intercropping. Broadly, root-root interactions have been studied mainly from the perspective of resource availability, with only few studies focusing on the non-resource-driven processes such as allelopathy (Faget et al. 2013).

Root-root interactions can also occur in intraspecific conditions such as in sole crops (Faget et al. 2013). Roots of different individuals are attracted to one another despite a nutrient patch being positioned elsewhere (Cahill Jr et al. 2010), or more lateral roots developed if individuals were not related to one another suggesting the so called kin recognition- defined as the organism's ability to distinguish between close genetic kin and non-kin (Hepper 1986). In fact, plants use a ‘chemical radar’ (e.g., by sensing the extent of diffusion of root-secreted ethylene) to detect belowground obstacles, and regulate the level of root production as well as above-ground growth in response to available soil volume (Chen et al. 2021; Pandey et al. 2021; Wheeldon et al. 2021). This means an increased root production as a response to neighbours. Those findings could be linked to other studies that investigate the effect of sowing density on root production. For example, Hecht et al. (2016) found that root dry weight in the cores increased with sowing density. However, Chen et al. (2021) showed that neighbour-induced root over-proliferation is not a prevalent feature in plants, but rather depends on genotype or environmental factors.

1.6 Thesis objectives and structure

Increasing the productivity of cropping systems while reducing the amount of resources and input requirements in terms of water, fertilizers, and land can optimize resource use efficiency in agricultural practices, thus allowing a sustainable cropping system. Recent research has highlighted the study of roots as a central component in optimizing cropping systems. The overarching aim of this study is to combine destructive root measurements with methods such as Fourier Transform Infrared (FTIR) spectroscopy (in case of intercropping) and image analysis of roots to study roots of field crops under intercropping, including the factors genotype and sowing density, and under nutrient omission, including N, P, K, as well as, under lime omission.

Chapter 2 is intended to answer three research questions related to cereal/legume intercropping: (1) how do different sowing densities and spring wheat genotypes affects root traits in faba bean/spring wheat in-row intercropping, (2) what are the types of interactions occurring belowground (3) what are the key differences in terms of root traits between sole crops and intercrops?

For this, a field experiment was conducted with one faba bean cultivar and two spring wheat cultivars sown at three sowing densities. Destructive root coring was conducted (0–100 cm) in the intercrops and sole crops at two development stages. FTIR spectroscopy was used to discriminate the species' root masses. We define a plant-plant interaction index of belowground root growth.

Chapter 3: Here, the focus lies on the study of winter rye roots grown under Nitrogen (N) and Phosphorus (P) fertilizer omission focusing on the field scale. To the best of my knowledge, the characterization of root traits of winter rye grown in the field, especially when cultivated as a main crop and not only as a cover crop, and under nutrient deficiencies, has not been studied. To fill this research gap, we designed a field experiment in the long-term fertilizer experiment (LTFE) Dikopshof in 2022 (Germany). We considered 5 dates of root sampling in the topsoil layer (0-30cm).

The study aims to answer the research questions: (1) How do root traits of winter rye cultivated as the main crop in the field respond to nutrient deficiencies in the topsoil? (2) Which plastic trends are to be observed across different growing stages?

Chapter 4: Another cereal was explored for its root plasticity to nutrient deficiencies. Winter wheat shoot and root traits were destructively evaluated under N, P, K, and lime omission as well as for the fully fertilized with manure, fully fertilized and unfertilized treatments in the LTFE Dikopshof over two growing periods in 2020 and 2021 and considering not only the topsoil but also the deeper soil layers (0-100 cm). The study aims to answer the research questions: How do root traits of winter wheat respond to nutrient omission in the topsoil, subsoil, and deep subsoil layer?

Chapter 5: In this study, the effects of N, P, K, and Ca omission on shoot growth as well as on topsoil root biomass, growth, and morphology of the fine roots of sugar beet were investigated. Classical shoot observation methods were combined with root phenotyping using the destructive excavation method, combined with image analysis. The study aims to answer the research questions: How do root traits of sugar beet respond to nutrient omission? Which root architectural shifts could be observed across different sugar beet growth stages?

By combining these complementary studies, the research seeks to identify crop-specific and ecosystem-level responses to nutrient constraints and intercropping systems. Ultimately, this knowledge aims to inform crop breeders to identify traits presenting plastic responses to nutrient limitations and better design intercropping systems, planning nutrient management and present reference values needed for improving agronomic practices and modelling exercises. All together to support sustainable agricultural practices in the context of reduced input use and environmental challenges.

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

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Abstract

Background and Aims: Intercrops offer multiple advantages over sole crops. The aim of our study was to characterize root growth and interactions in spring wheat/faba bean intercrops to better understand belowground interactions that govern resource capture.

Material and Methods: A field experiment was conducted with one faba bean cultivar and two spring wheat cultivars sown at three sowing densities, defining three intercropping designs. Destructive root coring was conducted (0-100 cm) in the intercrops and sole crops at two development stages. FTIR spectroscopy was used to discriminate the species' root masses. The plant-plant interaction index was calculated to represent the belowground interactions.

Results: A negative impact of intercropping on total root mass was observed in the treatment with high sowing density in both stages. For the fully and partial replacement design treatments, plant-plant facilitation was more pronounced than competition in all layers. Competition dominated root growth in the treatment with high sowing density in both stages. Lower sowing densities encouraged deep root growth of wheat (both cultivars) in intercropping. The early root growth in depth and in density of one spring wheat cultivar impacted negatively faba bean root growth. Intercropping resulted in a grain yield advantage in both fully and only one partial replacement design treatment.

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

Keywords: FTIR spectroscopy, intercropping, crop mixture, land equivalent ratio, root carbon and nitrogen.

2.1 Introduction

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

The mixture mechanisms that affect intercrop performance are (resource use) complementarity (e.g. through different rooting habits/structures), competition (for light, soil water, and nutrients), and facilitation (e.g. of phosphorus and micronutrient acquisition via root-root interactions)(Vandermeer 1992; Brooker et al. 2015; Stomph et al. 2020; Zhang et al. 2021).

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

To optimize the intercrop cultivation (e.g. choice of partners, sowing density) and to enhance ecosystem services (e.g. root-based C input for enhanced C sequestration), a better understanding of the underlying mechanisms responsible for belowground growth and interactions in species mixtures and of other

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

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

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

Currently, knowledge of the root systems contribution to intercrop yield advantage and the related effects of cultivar choice and sowing density is scarce. Specific belowground processes between the species should be considered to improve interspecific facilitation in future species mixture designs (Yu et al. 2022). The aim of this study was therefore to investigate the effect of faba bean and spring wheat

intercropping on root and shoot growth as a first step to understand root interactions in intercrops and to study the effects of different sowing densities and cultivars on belowground growth and interaction

2.2 Materials and Methods

2.2.1 Site description, field design, and crop management

The research facility Campus Klein-Altendorf (CKA) of the University of Bonn, Germany, is located in Rheinbach near Bonn (50° 37' 31'' N, 6° 59' 21'' E). The soil at the experimental station was classified as Haplic Luvisol, derived from loess and characterised by a silty-loamy texture with clay accumulation in the subsoil between about 45 and 95 cm soil depth (Barej et al. 2014). The climate at the experimental station can be described as moderately humid with maritime influences. The mean annual air temperature and precipitation are 10.3 °C and 669 mm (1991 to 2020), respectively. In 2021, an in-row mixture trial of spring wheat (Triticum aestivum L.) and faba bean (Vicia faba L.) with two spring wheat and one faba bean cv. and three total sowing densities (TSD) representing three types of intercropping designs was established. Each cultivar was also sown as a sole crop. In a subset of these plots, the presented root observations were conducted (Table 2.1). The sowing densities of sole crops considered in this study are higher than the usually applied densities in Germany, but as the emergence rate is not well known we kept them to better reflect the interactions in intercrops. The sowing densities in grain/m2 and in % are given in Table 2.2.

Table 2.1: Treatments with spring wheat (cv. SU Ahab, cv. Anabel) and faba bean (cv. Fanfare) and the respective sowing densities at Campus Klein-Altendorf in 2021. The total sowing density (TSD) is the sum of both sowing densities.

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

The experiment presented in this study of a large in-row mixture experiment. Due to a sowing error, the intended field design could not be fully implemented and there were therefore less than four field replicates available for the current study (Table S2.1). Therefore, root sampling was repeated four times in the selected plots (one plot for each treatment). The plot size was 15m2 (1.5 x 10 m) with a row distance of 21 cm and 6 rows per plot.

Table 2.2: Sowing density considered for each treatment and the corresponding number of sown grains per m2.

Sowing density (rate) in %	**Spring wheat (grains per m2)**	**Faba bean (grains per m2)**
33	160	18
50	240	27
100	480	54

The preceding crop in 2020 was spring barley. On 30/03/2021, the soil was harrowed to 10 cm soil depth. Soil mineral N was 98 kg ha-1 (16 kg ha-1 from 0-30, 27 kg ha-1 from 30-60 cm and 55 kg ha-1 from 60-90 cm) on 17/02/2021. Spring wheat cultivars SU Ahab and Anabel and faba bean cultivar Fanfare were sown on 30-31/03/2021. The cultivars are described in Paul et al. (2024). Spring wheat emerged mid-April (BBCH 11/12 on 19/04/2021) and faba bean emerged about one week later. Hand harvest took place on 13/08/2021 (BBCH 99) and machine harvest on 25 August 2021, when both crops were fully ripened. No fertilizers or pesticides were applied.

2.2.2 Root sampling

Root samples were taken with a soil auger with an inner diameter of 9 cm down to 100 cm soil depth in the selected plots on 09/06/2021 and on 05-06/07/2021. The root sampling in the intercrop treatments covered always one faba bean and one wheat plant and the core was placed not exactly above a row but next to the row (from the row to 1.5 cm from the middle of the row) (see Fig. S2.1). On 09/06/2021, the BBCH stages of wheat and faba bean were 39 (end of shooting) and 63 (full flowering), respectively. On 05-06/07/2021, the BBCH stages of wheat and faba bean were 69 (end of flowering) and 71 (approx. 10% of the pods have a species or variety-specific size achieved), respectively. Samples were taken in eight plots (three sole crops and five intercrops) replicated four times per plot (Table 2.1). Soil cores were split into ten centimetre sections and stored separately in plastic bags and dried under a plastic crop tunnel before sample preparation and evaluation performed at the University of Göttingen, Germany.

2.2.3 Quantification of root biomass, root carbon and nitrogen contents

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

ultra-centrifugal mill (Retsch, ZM 200, Haan, Germany) and stored in glass vials for the next analysis (see Section 2.4).

Due to low absolute weights in deeper soil layers, the root mass samples of the subsoil layers were pooled for weighing and for the C and N content determination (after the FTIR analyses) resulting in samples soil depths of 0-10 cm, 10-20 cm, 20-30 cm, 30-60 cm, and 60-100 cm. Root C and N were measured according to ISO 13878 and ISO 10694 standards with an elemental analyzer VarioMAX cube (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.2.4 Discrimination between species

2.2.4.1 Fourier Transform Infrared Spectroscopy (FTIR)

The roots of the sole crops of the two spring wheat cultivars (SU Ahab and Anabel) and one faba bean cultivar (Fanfare) were used to evaluate the species' root proportion in the intercrop samples. Absorption spectra of the ground root samples of the sole crops, as well as of the intercrops, were measured by the FTIR-ATR spectrometer (Alpha-P with a diamond crystal attenuated total reflection (ATR) device, Bruker Optics, Ettlingen, Germany) with a resolution of 4 cm-1 and 32 scans in the spectral range of 4000-400 cm-1. Each sample was measured 3 to 5 times. The evaluation of the FTIR-ATR spectra was conducted with the Opus software Quant 2 (version 7.2, Bruker Optics, Ettlingen, Germany). The FTIR spectra of the sole crop sample species were used for a cluster analysis (Opus software, version 7.2, Bruker Optics) to allow for species discrimination. For the cluster analyses, the spectra were pre-processed by second derivative and vector normalization, the frequency range was reduced and the Euclidian's distance and Ward's algorithm was applied (Fig. S2.2, S2.3 and S2.4). The interspecific heterogeneity for both species was higher than the intraspecific heterogeneity permitting a separation of the two species. Both spring wheat cultivars separately but also combined were clearly separable from faba bean via cluster analysis (Fig. S2.5). Since the average FTIR spectra of both spring wheat cultivars were very similar, both spring wheat cultivars were combined for the second sampling date analyses (Fig. S2.5 and S2.6).

2.2.4.2 Model establishment

For the quantification of the root proportion of each species in the intercrops root samples, the FTIR spectra of the single species samples were used to generate a model. For establishing a two-species model, a calibration set of 35 "artificial mixtures" was generated in 3 % steps from 0 % to 100 % for spring wheat and faba bean, respectively. These mixtures covered the complete calibration range. 20 additional "artificial mixtures" with known species composition were generated to be used for external calibration of the model. With the FTIR spectra of these calibration mixtures, a model was calculated on the basis of multivariate calibrations with the method of partial least square (PLS) regression using the software Quant 2 (Opus, version 7.2, Bruker Optics, Ettlingen, Germany). The absorption of infrared radiation is correlated to the concentration of compounds in a multi-compound system. The established

model was evaluated by an internal validation (cross validation) and was subsequently optimized by the Quant 2 software. This optimization process detected the best data preparation and the best frequency range to explain the actual mixtures of the calibration samples. Six to eight of the proposed optimized models were verified by an external calibration (20 additional "artificial mixtures"). Both internal validation and external calibration were compared with the calculated statistical parameters of each calibration. For the first sampling date for each wheat cultivar, a separate model was generated. The statistical parameters of the model (calibration/internal validation and external calibration) are shown in Tables S2 (date one) and S3 (date two). With the chosen model, the FTIR spectra of the mixed species samples were evaluated with the associated model. The output of this evaluation was the percent share of each species within the mixed species root mass samples which were used for further calculations. Values outside the calibration range (below 0% or above 100%) were corrected to 0 % and 100%.

2.2.5 Data analysis and statistics

2.2.5.1 Root parameters and indexes

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

$$RLD = \frac{Root\ length\ per\ layer}{Soil\ volume\ of\ the\ layer} \quad (2.1)$$

The soil volume of each layer is equal to 636 cm3 (core diameter: 9 cm, sample height: 10 cm).

Root mass (t ha-1) was calculated according to the equation (2.2)

$$Root\ mass = \frac{Root\ mass\ for\ the\ corresponding\ layer}{Surface\ area\ of\ cylinder} \quad (2.2)$$

The surface area of cylinder (core auger) is equal to 63.6 cm2.

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

$$SRL = \frac{Root\ length\ per\ layer}{Root\ mass\ for\ the\ corresponding\ layer} \quad (2.3)$$

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

Various terminologies for characterizing the yield advantages in intercrops exist in the literature, namely, 'mixing effect' (Hof-Kautz and Rauber 2003), 'overyielding' (Li, Zhang, et al. 2013; Streit et al. 2019; Nelson et al. 2021; Yang et al. 2022) or 'Relative Yield Total' (Willey and Osiru 1972), which is identical to 'Land Equivalent Ratio' (LER) defined by De Wit and Van den Bergh (1965). In the context of our study, we use the term root mass advantage to characterize the positive effect of intercrops on root biomass.

So, the LER for the faba bean and spring wheat mixtures was calculated for aboveground biomass (LER_{AGB}) at the the two growing stage and at harvest as well as for belowground biomass (LER_{Root}) according to Equations 2.4-2.6. The LER was only calculated for the treatments with fully replacement

designs. The LER for bean and wheat in intercrops is the sum of the partial LER for bean ($pLER_{Bean}$) and wheat ($pLER_{Wheat}$):

$$LER = pLER_{Bean} + pLER_{Wheat} \quad (2.4)$$

$$pLER_{Bean} = \frac{Biomass\ bean\ in\ intercrop}{Biomass\ bean\ in\ sole\ cropping} \quad (2.5)$$

$$pLER_{Wheat} = \frac{Biomass\ wheat\ in\ intercrop}{Biomass\ wheat\ in\ sole\ cropping} \quad (2.6)$$

The expected values of grain yield, root mass, RLD and SRL were estimated based on the equation (2.7):

$$Yexpected = p * M \quad (2.7)$$

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

We applied an adapted version of the 4C approach of Justes et al.(2021) to find out when and where =facilitation or competition dominate. Here, instead of using the pLER as presented in Justes et al.(2021), the calculation is being adapted by dividing the root biomass by the plant density (Equations 2.8 -2.10). The novel index is called plant-plant interaction index (PPII), where:

$$PPII = PPII_{Wheat} + PPII_{Bean} \quad (2.8)$$

with

$$PPII_{Bean} = \frac{Root\ mass\ of\ bean\ in\ intercrops}{Root\ mass\ of\ bean\ in\ sole\ crops} \quad (2.9)$$

$$PPII_{Wheat} = \frac{Root\ mass\ of\ wheat\ in\ intercrops}{Root\ mass\ of\ wheat\ in\ sole\ crops};$$

PPII is compared with the ratio of plant density DR (with density in plants per m^2);

$$DRWheat = \frac{Density\ of\ wheat\ in\ intercrops}{Density\ of\ wheat\ in\ sole\ crops} \quad (2.10)$$

$$DRBean = \frac{Densitiy\ of\ bean\ in\ intercrops}{Density\ of\ bean\ in\ sole\ crops}$$

And finally we compared PPII with DR by dividing PPII by DR. If PPII/DR=1, neutral effect. If PPII/DR<1, net competition. If PPII/DR>1, net facilitation.

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

2.2.5.2 The statistical analyses

The statistical analyses were performed using the programme R version 4.2.1 (23/06/2022) (R Core Team2018).

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

2.2.6 Shoot sampling, soil water, and nutrient derivation

Shoot biomass, plant height, number of plants per m2 and volumetric soil water content at 0, 30, 45, 60 and 90 cm soil depth were measured in the days preceding the two dates when the root sampling took place. Shoot samples for estimation of shoot dry weight were collected destructively with one sample per plot on 06.06 and 06-08/07/2021. Hand harvest of 2 row meters took place on 13/08/2021 in which 1m from both the 3rd and 4th rows (2m in total per plot) were harvested and ensuring that cuts were made a minimum of 1m from the plot boundary to reduce boundary effects. Wheat and faba bean were separated manually in case of intercrop treatments. The fresh biomass samples were weighed and (in case of large samples only aliquots) then oven-dried (105° C) until constant weight was reached and weighed again to estimate shoot, straw or grain dry matter. Due to lack of replicates regarding shoot biomass and yield at harvest, the aboveground dataset is only presented as supplementary (Table S2.4).

The soil water content was measured at soil depth of 0, 30, 45, 60, and 90 cm with a mobile FDR probe (ThetaProbe ML3, ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany) on 07/06/2021 and 05/07/2021. Soil samples from 0-30, 30-60, and 60-90 cm soil depth were collected to estimate soil mineral nitrogen (Nmin) before sowing (17/02/2021, pooled samples over field) and one day after harvest (26/08/2021, pooled samples per plot) using a Pürckhauer auger. Nitrate-N and ammonium-N were determined photometrically using a continuous flow analyser (Seal QuAAtro 39, Norderstedt, Germany) after K_2SO_4 extraction of the soil sample.

2.2.7 General characteristics of the growth period

The growing season in 2021 can be characterized as chilly in April and May with a normal rainfall pattern, however, a storm with a heavy rainfall occurred on 14-15/07/2021 with about 120 mm of rainfall. In the growth period from 30/03/2021 to 25/08/2021, total rainfall was 395 mm and the mean air temperature was 14°C (Fig. S2.7).

2.3 Results

2.3.1 Aboveground overyielding in intercrops

Total dry matter grain yield in intercrops varied from 4.5 t ha-1 to 5.6 t ha-1 (Table S2.4). In intercrops with cv. SU Ahab, the grain yield attained values were higher for the treatments of the partial replacement design and fully replacement design but lower that for the additive design (Table 2.3). For intercrops with the cv. Anabel the lower sowing density of the partial replacement treatment (TSD=66%) resulted in grain yield value lower than the expected one. However, for that same cultivar, a value of grain yield attained higher than the expected one was found under fully replacement design (FB_50_SW_Ana_50, TSD=100%).

Table 2.1: Attained and expected values (n=1) of grain yield in intercrops at harvest (13/08/2021).Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%))

	SW_SUAh_100	**SW_Ana_100**	**FB_100**	**FB_33_SW_Ana_33**	**FB_33_SUAh_33**	**FB_50_Ana_50**	**FB_50_SUAh_50**	**FB_100_SUAh_100**
SW GY	4	5.2		2.3	3	2.5	2.7	2.2
expected SW GY				1.716	1.32	2.6	2	4
FB GY			3.4	2.9	2.6	2	2.7	3.1
expected FB GY				1.122	1.122	1.7	1.7	3.4
Total GY	4	5.2	3.4	5.2	5.6	4.5	5.4	5.4
expected Total GY				6.916	2.442	4.3	3.7	7.4

In intercrops, LER could only be calculated for the fully replacement design treatments (FB_50_SW_Ana_50 and FB_50_SW_SUAh_50), the shoot LER values ranged from 1.03 to 1.42 (Table S2.5) with a mean across both varieties of 1.28±0.20 at the first sampling date and 1.10±0.10 at the second sampling date. At harvest, the wheat contributed less (lower pLERBean) than the faba bean to the positive grain yield overyielding (1.27±0.28, mean across both cultivars). The comparison between both wheat varieties revealed that the grain yield LER of the intercrops with SU Ahab was higher than in intercrops with Anabel. The cv. SU Ahab seems to be more advantageous for mixtures (higher LER for grains and higher absolute grain yield in mixture) than the cultivar Anabel (Table S2.5).

2.3.2 Root growth in intercrops

2.3.2.1 Characterisation of root mass

The cumulated root mass over the soil profile (all soil depths measured) increased from the first to second date by 19% (mean of the two cultivars) for the sole crop wheat and 34% for the sole crop faba bean (Table S2.5). For the intercrops, the greatest increase between the two sampling dates were estimated in treatments FB_50_SW_Ana_50 (46%) and FB_100_SW_SUAh_100 (41%) and the lowest were estimated for the treatments FB_50_SW_SUAh_50 (21%) and FB_33_SW_Ana_33 (20%). On sampling date one (09/06/2021), the significantly highest mean values of total root mass (0-1 m) were observed in the intercrop with wheat cv. SU Ahab with TSD=66% (FB_33_SW_SUAh_33) and 100% (FB_50_SW_SUAh_50) TSD with 2.11 t ha-1 and 2.03 t ha-1, respectively (Table S2.6).

At the first sampling date (Fig. 2.1), the lowest root mass values in the topsoil (0-30 cm) were determined for the wheat sole crops. The highest sowing density (TSD=200%) showed lower total root mass as compared to the two other sowing densities in intercropping. For the upper subsoil (30-60 cm), the sole wheat root mass was significantly higher than all intercrop treatments. The intercropping of faba bean with the wheat cv. Anabel at the lowest sowing density achieved the lowest root mass value, while the faba bean sole crop achieved the second lowest total root mass at this soil depth. For the deeper subsoil layers (60-100 cm), the faba bean sole crop presented the lowest value. At the first sampling date, spring wheat cv. Anabel developed more roots in deeper soil layers as a sole crop and in intercropping in comparison to cv. SU Ahab (Fig. 2.1).

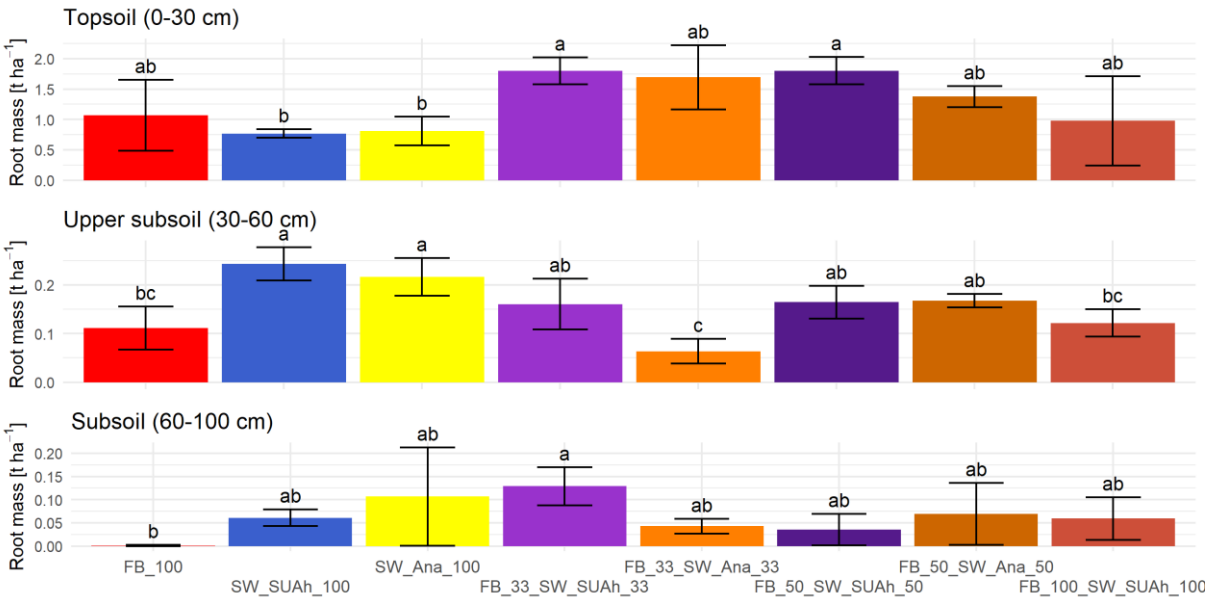

Figure 2.1: Mean (n=4) total root mass (sum of both crops) in t ha-1 at the first sampling date (09/06/2021) for three soil layers. Different letters indicate significant differences (Anova and Tukey post-hoc test, α=0.05). Error bars refer to the standard deviation. Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel,

FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

At the second sampling date, no significant differences between the treatments with regard to topsoil root mass were observed (Fig. 2.2). The intercrops with low sowing density (FB_33_SW_SUAh_33 and FB_33_SW_Ana_33) achieved the significantly lowest values of root mass cultivars in the upper subsoil (30-60 cm). In the deeper soil layer (60-100 cm), faba bean reached the lowest root mass. Results of a two-way Anova (α=0.05) indicated that the cultivar choice had no significant effect on root mass but sowing density had. Also, no significant interactions between the sowing density and cultivar for root mass were found (Table S2.7).

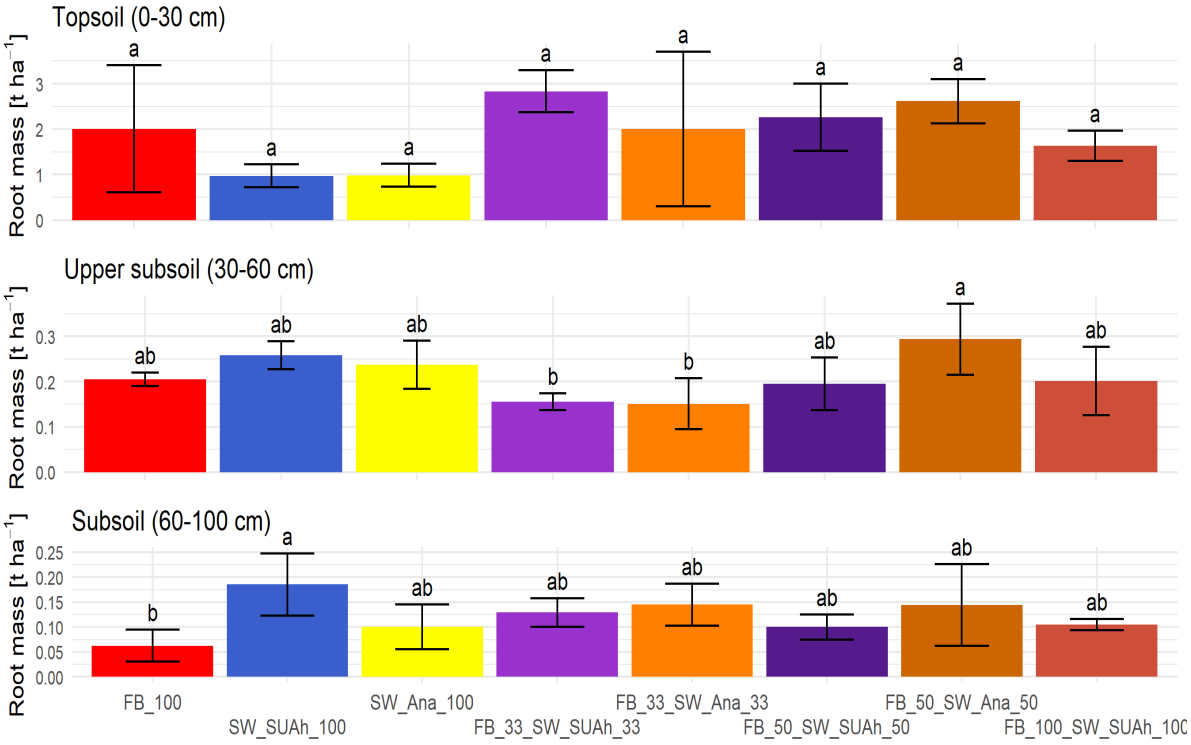

Figure 2.2: Total root mass (sum of both crops) in t ha-1 of the second sampling date (05-06/07/2021) for three soil layers. Different letters indicate significant differences (Anova and Tukey post-hoc test, α=0.05). Error bars refer to the standard deviation. Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

2.3.2.2 Proportion of faba bean and spring wheat root in intercrops

The results of discrimination between species using the FTIR showed that wheat root mass dominated in the subsoil (20/30-100 cm, Fig. 2.3). In general, there were no significant differences in faba bean root mass proportions between the different treatments. Only in the first sampling date significant

differences in 0-10 cm (the very high sowing density led to low faba bean root proportions) and in 60-100 cm depth (the intercrop treatments with wheat cv. Anabel had low faba bean root proportions) were observed. The quick and deep rooting ability of the cv. Anabel in comparison to cv. SU Ahab is illustrated by the greater proportion of faba in intercrops with cv. SU Ahab in the deeper soil depths (60-100 cm) at both sampling dates (although the differences were only significant at the first sampling date).

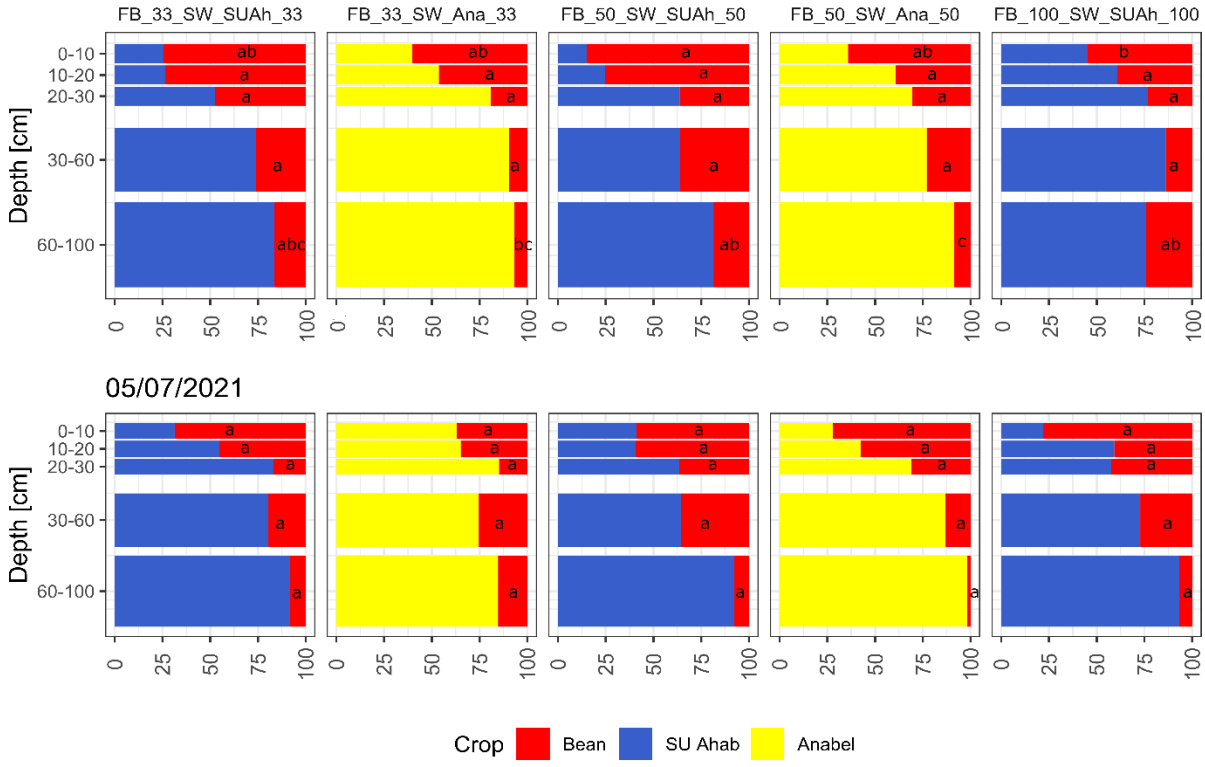

Figure 2.3: Mean values (n = 4) of species proportion of root mass (%) of sprC MHZing wheat and faba bean in five intercrops. Different letters indicate significant differences (Anova and Tukey post-hoc test, α=0.05) between proportion of root mass of faba bean within each soil layer (0-10 cm, 10-20 cm, 20-30 cm, 30-60 cm, 60-90 cm) in 09/06/2021 (top panel) and 05/07/2021 (bottom panel). Treatment abbreviations: FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50=Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)

2.3.2.3 Root mass advantage in intercropping

At the first sampling date (09/06/2021) in the topsoil and upper subsoil layers (0-40 cm) for intercrops with wheat cv. Anabel and 0-30 cm for intercrops with wheat cv. SU Ahab), a positive root mass LER was observed (Table 2.4). A the second sampling date (05/07/2021), the root mass LER was above one for the layers 0-20 cm for the intercrop with cv. SU Ahab and above one from the layers 0-60 cm for the intercrops with cv. Anabel (Table 2.4).

Table 2-3: Mean values ± standard deviation of root partial land equivalent ratio of bean (pLER Bean, n = 4), wheat (pLER Wheat, n =4) and root land equivalent ratio (LER, n = 4) based on root mass of the intercrops with wheat for two sampling dates for the replacement treatment with cv. SU Ahab FB_50_SW_Ana_50 and with cv. Anabel FB_50_SW_SUAh_50. For the first sampling date (60-100 cm), no values were provided for the treatment FB_50_SW_SUAh_50 due to absence or low root mass in all replicates. No standard deviation was provided for the treatment FB_50_SW_Ana_50 due low number of replicates (n=1). * refers to Significant differences for pLER from 0.5, for LER from 1 ($p \leq 0.05$, t-test).

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

2.3.3 Effect of sowing density on root mass of intercrops

The analysis based on the comparison between the attained and the expected values of root mass revealed that, on both sampling dates, under high sowing density (TSD=200%, additive design) the expected values of root mass in 0-1m soil depth were higher than the attained values (Fig. 2.4). In contrast, for the lower sowing densities (TSD=66%, partial replacement design and TSD=100%, full replacement design), the attained values were higher than the expected one.

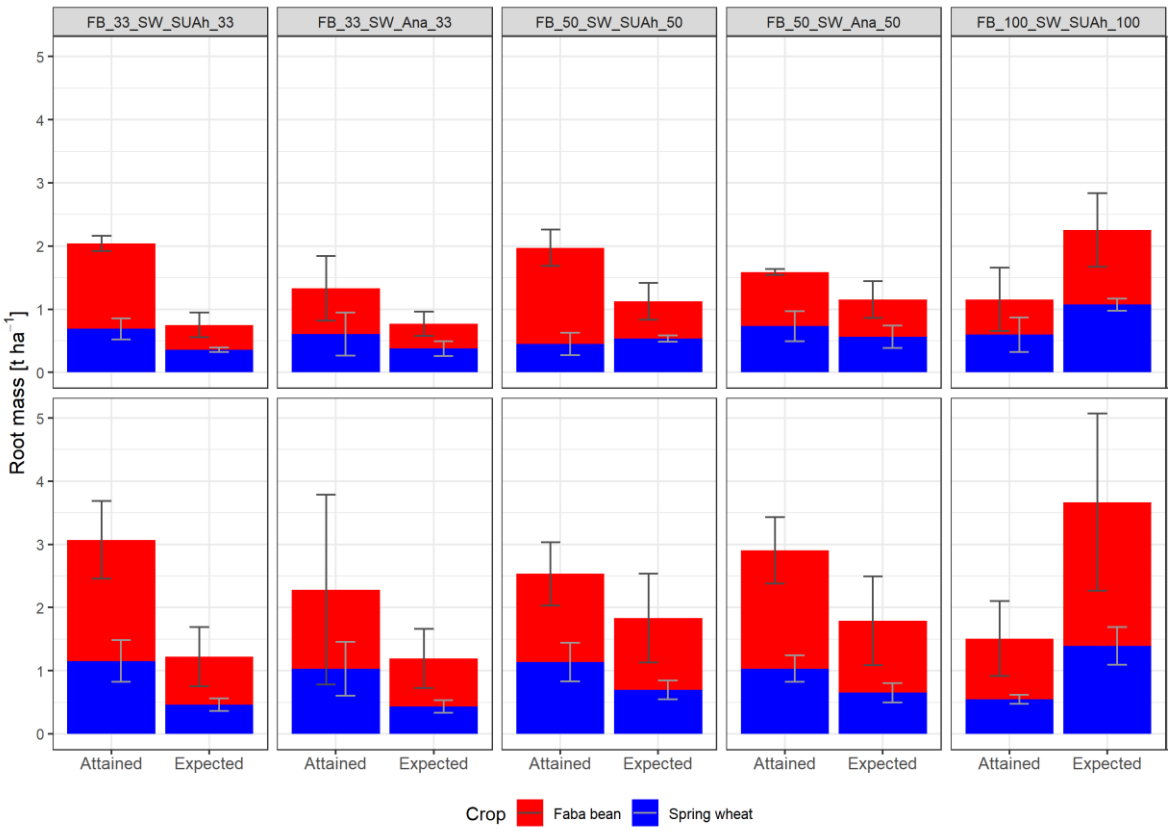

Figure 2.4: Expected vs. attained values of mean root mass (t ha-1, n=4) over 0-1m soil depth in intercrops on 09/06/2021 (top panels) and on 5/07/2021 (bottom panels). The error bars refer to the standard deviation. Treatment abbreviations: FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

2.3.3.1 Root length density

On both sampling dates and in all soil layers, the RLD of the tap rooted sole faba bean was lowest (Fig. 2.5 and 2.6). In the upper subsoil (30-60 cm), mostly significant differences were found between RLD of faba bean and spring wheat in sole cropping. For the mixed cropping treatments, the RLD in the upper subsoil was higher for the fully replacement treatments (TSD=100%) as compared to the partial replacement ones (TSD=66%) and vice versa in the deeper subsoil 0-100 cm). Thus, lower sowing densities encouraged deep rooting in mixtures.

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

For deep subsoil (60-100 cm) and for all treatments, the RLD decreased with soil depth. However, the mean RLD for the subsoil (60-100 cm) was found to be highest in the 33%-33% mixture with the wheat cv. SU Ahab. Additionally, in both treatments with TSD 66%, the mean RLD from 60-100 cm was higher in comparison to the mean RLD of 30-60 cm. Both the intercrops and the spring wheat sole crops attained slightly higher cumulative RLD values than the faba bean, with a mean value over all intercrops

and sole crop spring wheat treatments of around 18 cm cm-3 compared to 5 cm cm-3 for the faba bean (0-1m soil depth) (Table S2.8).

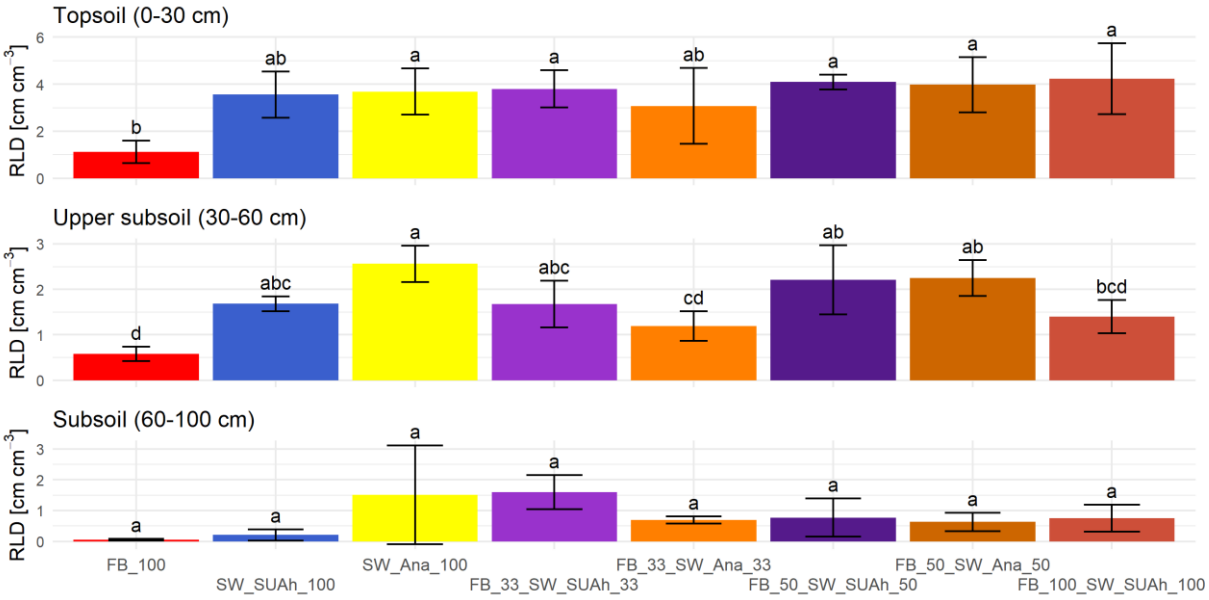

Figure 2.5: Mean values ± standard error (n = 4) of root length density (RLD, not crop-specific) in cm cm-3, for sole faba bean and sole spring wheat, as well as for the mixtures treatments for cumulated three soil layers in 09/06/2021. Different letters indicate significant differences at each soil depth (Anova and Tukey post-hoc test, α=0.05). Error bars refer to the standard deviation. Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

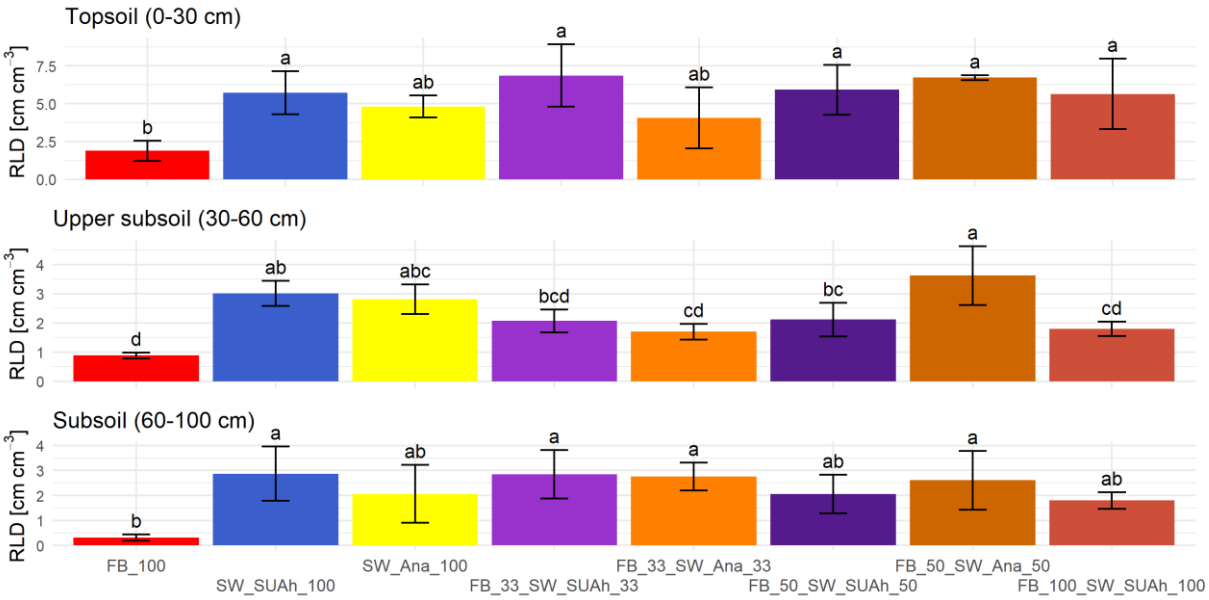

Figure 2.6: Mean values ± standard error (n = 4) of root length density (not crop-specific) in cm cm-3 (RLD), for sole faba bean and sole spring wheat, as well as for the mixtures treatments for cumulated three soil layers in 05/07/2021. Different letters indicate significant differences (Anova and Tukey post-hoc test, α=0.05). Error bars refer to the standard deviation. Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100 _SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

2.3.3.2 Specific root length

On both sampling dates, the mean SRL (all depths) was lower in faba bean compared to spring wheat (Table S2.9). An enhanced SRL (more fine roots in 0-100 cm) in intercrops as compared with the expected SRL from sole crops was observed. A trend for decreasing mean SLR values with increasing TSD in the mixtures was observed.

Generally, the mean PPII decreased from the topsoil to the subsoil. The analysis of PPII showed that under fully replacement design (TSD=100%) and partial replacement design (TSD=66%), the facilitation were the most dominant interaction. In contrast, the competition between the species was more pronounced in the additive design (Fig. 2.7) in both growing stages.

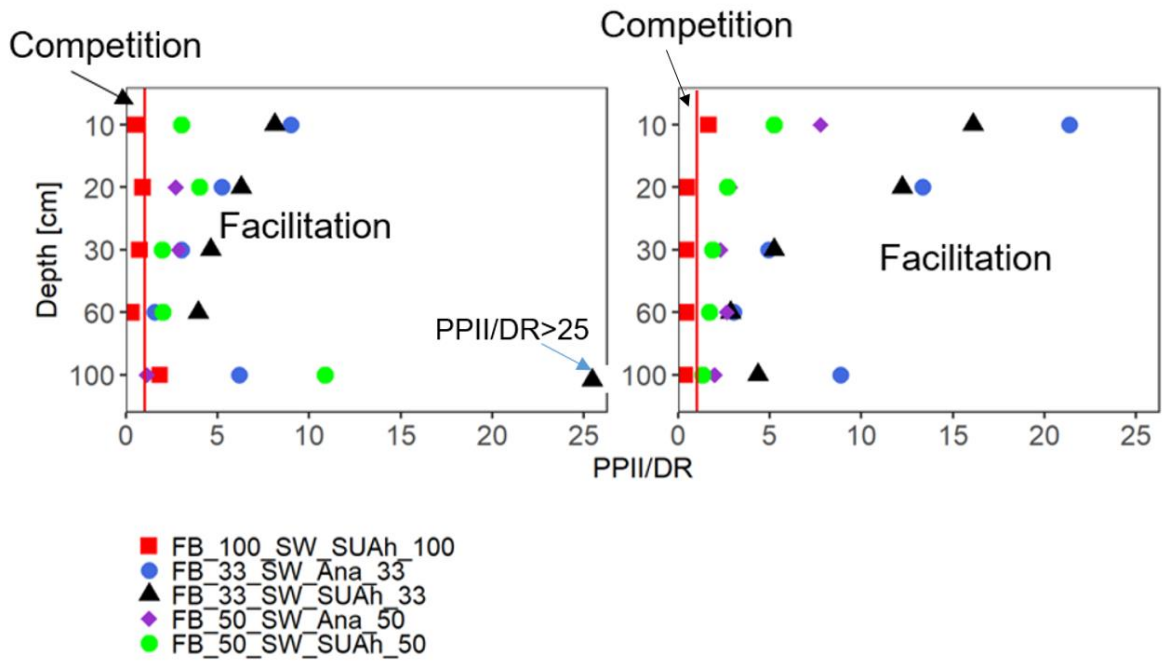

Figure 2.7: The mean PPII/DR is shown for each soil depth. The area where PPII/DR>1 indicates facilitation between the two species. The area where PPII/DR<1 indicates competition between the two species. The red line shows PPII/DR=1, indicating a neutral effect. The mean PPII/DR was calculated as the mean of PPII/DR across treatment´s replicates (n=4) for the sampling dates in 09/06/2021 (left panel) and 05/07/2021 (right panel). X axis was cut the value 25, data points>25 are displaced. Treatment abbreviations: FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

2.3.3.3 Root carbon content

The root C content, calculated as C concentrations (mean: 45%) multiplied by root dry matter, did not change significantly across the treatments for both sampling dates. However there was a trend of higher root C contents in the intercrop treatments compared with the sole crops, with the exception of the treatment with TSD=200% (Fig. S2.8). For the intercrop treatments with wheat cv. SU Ahab, there was a decrease of root C content with increasing TSD. The opposite trend was observed for the wheat cv. Anabel.

2.3.3.4 Root nitrogen content

The mean root N content were 2.3% (sole faba bean), 0.7% (sole wheat), and 1.2% (intercrop). As expected, the lowest values of root N content were estimated in sole spring wheat (Fig. S2.9). Root N content in several intercrop treatments was comparable to the sole crop faba bean treatment. On the second sampling date, no significant differences were observed between the intercropping treatments and sole faba bean. However, in faba bean, the root N content was also found to be higher in the deeper soil layers (20-60 cm).

2.3.4 Soil mineral N

Before the establishment of the crops, the initial N_{min} was 16 kg ha-1 in the topsoil (0-30 cm), 27 kg ha-1 in the upper subsoil (30-60 cm) and 55 kg ha-1 in the deeper soil (60-90 cm). After harvest, lower N_{min} values over the whole soil layers were found in the spring wheat sole crop treatments. The topsoil N_{min} values were lower in sole cropping (wheat and bean) as compared to the intercropping treatments (Fig. S2.10). The highest topsoil value (25 kg ha-1) was determined in the treatment FB_100_SW_SUAh_100. In the upper subsoil 30-60 cm, the lowest value of 7.7 kg ha-1 was measured in the intercropping treatment with highest total grain yield and with lowest sowing density (FB_33_SW_Ana_33) followed by both spring wheat sole treatments. Again, the highest value in 30-60 cm soil depth of 18 kg ha-1 was measured in the treatment with the highest sowing density FB_100_SW_SUAh_100. In the deeper subsoil (60-90 cm), soil Nmin was lowest in the intercrop treatments FB_100_SW_SUAh_100 and FB_50_SW_SUAh_50.

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

2.3.5 Soil volumetric water content

In general, the soil volumetric water content around the flowering of spring wheat in July (second sampling date) was higher than at the early sampling date in June (first sampling date). Soil volumetric water content for the spring wheat cultivar Anabel, which indicates the potential to root quickly and deeply, was lower in the sole crop treatment and in mixtures compared to the cultivar SU Ahab at the second sampling date, particularly at deeper soil depths (Fig. S2.11). However, in the treatment with the cultivar SU Ahab as a sole crop and as intercrop (TSD=100%) the lowest soil water content values were measured at 30-60 cm soil depth. In general, soil water depletion was lower for the low density (FB_33_SW_SUAh_33) as compared to the very high density intercrop treatment (FB_100_SW_SUAh_100) (sampling date 2, 30-90 cm).

2.4 Discussion

2.4.1 Root mass, root length density and belowground interactions

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

Root system extension of wheat often exceeds the one of legumes like faba bean (Gregory et al. 1995; Turpin et al. 2002), though under field conditions, factors such as phenology, sampling technique and sampling depth may influence root growth. The faba bean root mass at flowering (2.3 t ha-1) observed in our study is higher than the values reported in the studies from Rengasamy and Reid (1993), who reported average root mass over years and treatments of approximately 1.4 t ha- 1 for a sampling depth of 70 cm. These values are also higher than the values reported by Streit et al. (2019) who found values of around 0.7 t ha-1 for a sampling depth up to 60 cm. This difference can be attributed to the higher sowing density considered in our study for the sole cropping treatments and also the sampling technique as we always considered a faba bean in the soil core which overrepresented the faba bean compared to the study of Streit et al. (2019), for instance. Literature revealed high variability for spring wheat root masses ranging from 0.8 t ha-1 to 1.4 t ha-1 at flowering (Wechsung et al. 1995; Gan et al. 2009). In our study, a spring wheat root mass of 1.4 t ha-1 was reached at flowering over the soil depth of 0 to 1 m. This rather high value can be partly attributed to the enhanced sowing density considered for the sole crops compared to the optimal sowing density recommended for spring wheat.

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

A combination of tap rooted and fibrous rooted crops is widely recognized as being one of the mechanisms of overyielding in intercrops due to belowground complementarity which may increase water and nutrient acquisition by niche differentiation and due to resource partitioning (Yu et al. 2022). In line with this finding, the attained values of root mass in the intercrop treatments for both wheat and faba bean (0-1m soil depth) were mostly higher than the expected values (Fig. 2.4). This applied for both the low density (TSD=66%) and the nearly optimal sowing density (TSD=100%), but not for the very high sowing density (TSD=200%).

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

2.4.2 Sowing density effect on root growth advantage and facilitation and competition

The spatial arrangement in intercropping is an important factor for the above- and belowground growth (Wang et al. 2018; Homulle et al. 2022). In our study, the spatial arrangement was represented by the sowing density that characterized the designs considered in the study, as well as by the completely mixed design or adjacent row design which permitted a high interaction between the species (Homulle et al., 2022; Li et al., 2006). The high sowing density in the additive design resulted in low root biomass over the whole soil profile (Table S2.6) and enhanced plant-plant competition between faba bean and spring wheat in both growing stages.

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

2.4.3 Cultivar effect on belowground growth and interactions in intercrops

Although statistically there was no significant effect of the cultivar on the root mass, we observed a difference in rooting ability between both spring wheat cultivars (Fig. 2.1 and 2.2, Fig. 2.5 and 2.6). The ability of cv. Anabel to root quickly and deeply around faba bean flowering as compared to cv. SU Ahab resulted in lower root mass proportions of faba bean intercropped with cv. Anabel compared to

intercropped with cv. SU Ahab. . Moreover, comparing the root growth patterns in intercrops and sole crops in two different growth stages (flowering of wheat and flowering of bean), permitted to better understand the cultivar effect of root growth dynamics in intercrops. Other studies only considered studying root growth around flowering (Streit et al. 2018), where it is assumed that the species reach their maximum root mass (Chirinda et al. 2012). In our study, we found that the early dominance of one spring wheat cultivar (cv. Anabel) impacted negatively faba bean root growth in intercrops.

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

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

Plant diversity also affects soil organic C stocks in deeper soil which is more stable and difficult to access for microbes (Chen et al. 2020). Hence, root-based C inputs in deeper soil layers is the major source of soil organic carbon (Yu et al. 2022). We observed no significant effect of intercropping on root C in the deep soil layer (60-100 cm, date 05/07/2021, Fig. S2.11). In the deeper soil layers (30-100 cm), total C in roots in the mixtures was on average 22% greater than the average root C in sole faba bean and 18% lower than average root C in sole spring wheat (mean of both cultivars), providing a possible mechanism for the divergence in soil C sequestration between sole crops and intercropping systems. Similar trends were observed by Cong et al.(2015).

Characterization of soil water depletion at different soil layers below the root zone is important in evaluating water use pattern and its linkage to the RLD (Moroke et al. 2005). Our results didn´t confirm the positive correlation between RLD and soil water depletion already reported in other studies (Moroke et al. 2005; Zhang, Whalley, et al. 2020).This can be explained by to the non-significant differences between the intercrop treatments in term of RLD, found in our study (Fig.2.5 and 2.6).

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

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

2.5 Conclusion

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

Also, we suggest to conduct a similar study under limited growth conditions and with several sampling dates to better assess the relationship between above- and belowground overyielding and support the

generalization of the obtained results. Moreover, there is a need to explore the effects of mixtures on soil C and N sequestration to mitigate climate change.

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Statements and Declarations

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Competing Interests

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

Author Contributions

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

Data availability

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

3 Winter rye root growth and plasticity in response to nitrogen and phosphorus omission under field conditions.

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Abstract

Aims. We investigated the effects of N and P omission on winter rye growth and root traits under field conditions.

Methods. Sampling was conducted during the 2022 season at the long-term fertilizer experiment Dikopshof, Germany. Four fertilizer treatments were chosen: (1) fully fertilized including manure (m) and supplemental mineral fertilizer (s) (NPKCa+m+s), (2) fully fertilized without manure (NPKCa), (3) N omitted (_PKCa), and (4) P omitted (N_KCa). Shoot biomass, number of tillers and root traits: topsoil root biomass, nodal root number, root angle, root length density (RLD), specific root length (SRL), and root diameter were assessed at five growth stages from tillering to flowering.

Results. Shoot and root biomass showed significant interaction between fertilizer treatments and sampling date. The NPKCa+m+s treatment led to the highest shoot biomass up to early boot stage. Around flowering the nutrient omission treatments resulted in the lowest shoot biomass compared with the NPKCa+m+s treatment. In contrast, root biomass showed non-significant differences among treatments at the beginning of the growing season and around early boot stage. All other root traits showed a non-significant interaction between fertilizer treatments and sampling dates, and while there was a significant impact of sampling dates, fertilizer treatments often led to non-significant differences. The N and P omission treatments showed the lowest RLD.

Conclusions. These findings demonstrate the strong impact of nutrient omission and developmental stage on shoot and root growth of winter rye.

Keywords: shoot growth, root growth, root:shoot dry mass ratio, crop yield, nutrient stress

3.1 Introduction

Nitrogen (N) and phosphorus (P) are critical nutrients that are central to crop productivity, with N being key for amino acid and protein synthesis, while P is essential for energy transfer, photosynthesis, and structural development (Uga et al. 2015). However, the availability of these nutrients is often limited in agricultural soils, which can result in reduced crop yields and grain quality (Hammer et al. 2009). Addressing these constraints requires improved crop nutrient use efficiency to tackle the challenges of global food security and environmental sustainability. Enhancing the functional plasticity of root systems emerges as a key approach to improve nutrient acquisition and crop performance under resource-limited conditions. The root system architecture—comprising traits such as root biomass, length, diameter and angle—is key to exploring soil and acquiring nutrients (Lynch 1995a, 2019). Environmental factors, including microbiome activity, soil water availability, soil compaction, salinity, nutrient distribution but also the crop management further influence root growth dynamics, modulating root system depth, root angle, and nutrient and water uptake efficiency (Oyanagi et al. 1993, p. 2021; Manschadi et al. 1997; Hecht et al. 2016; Correa et al. 2019; Yu et al. 2021).

Recent studies highlight strategic adjustments in root growth patterns that enable plants to optimize resource use efficiency in sub-optimal environments (Lynch 2019). For instance, the root angle is a main determinant of root placement within the soil profile. Steeper root angles promote deeper soil penetration, enhancing access to mobile resources like nitrate and water under drought or low N conditions (Trachsel et al. 2013; Schneider et al. 2022). Conversely, shallower root angles facilitate topsoil exploration, improving the acquisition of immobile nutrients such as phosphorus (Bonser et al. 1996; Liao et al. 2001; Lynch and Brown 2001). Moreover, root mass is an important trait for carbon storage and its sequestration (Kätterer et al. 2011; Poeplau and Don 2015). A recent study showed that under N deficiency, root length and root biomass decreased by 9% and 7%, respectively, but root length per shoot biomass increased by 33%, alongside a 44% enhancement in the root:shoot (RS) ratio, reflecting carbon allocation strategies for nutrient foraging (Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, and Rachmilevitch 2023). Root length density is linked to aggregate stability (Hudek et al. 2022) as well as water and nutrient acquisition (Tajima 2021). Root diameter is also considered an important trait affecting nutrient acquisition (Perkons et al. 2014), for instance, in dicotyledonous plants, taproots with thicker diameters can penetrate compacted soil more easily than smaller root diameters (Materechera et al. 1992), enhancing the nutrient acquisition efficiency under sub-optimal conditions. Root diameter can be a proxy for root function, as finer roots generally have higher specific surface area and are more efficient in nutrient and water uptake. Several studies have reported varying effects of nutrient deficiency on root diameter, but the direction and magnitude of these responses differ across crops and conditions. Another important root morphological trait is the specific root length (SRL), defined as root length per root mass, which is an indicator of the root soil exploration capacity (Freschet, Pagès, et al. 2021). The SRL can also be linked with nutrient uptake efficiency

(Eissenstat 1992; Danso et al. 2018; Kemper et al. 2023). Research shows that crops with increased SRL have long and thin roots and are less expensive to produce (Ostonen et al. 2007) . Root phenotyping is often conducted under controlled environments, as it provides a greater likelihood of reproducible root phenotypes compared to field phenotyping. However, transferability of plant responses from controlled environments to field conditions remains a challenge(Langstroff et al. 2022). Therefore, field phenotyping remains a critical component in particular for root traits that are expressed at later stages of plant growth or in deeper soil layers (Tracy et al. 2020).

Long-term fertilizer experiments (LTFE) serve as an important platform for research (Seidel et al. 2021); however, studies mostly focus on the above-ground traits as affected by fertilizer omission, often neglecting the specific impacts of nutrient omissions on root system architecture under field conditions (Siddiqui et al. 2021; Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, and Rachmilevitch 2023). Renowned for its adaptability to nutrient-poor soils and challenging growing environments, winter rye (*Secale cereale*) serves as an excellent model for investigating root-shoot interactions under nutrient stress (Arsova et al. 2020). Compared to wheat, rye has demonstrated more vigorous early vegetative growth (Paponov et al. 1999), higher radiation use efficiency (RUE) (Sieling et al. 2016), and greater frost tolerance (Griffith et al. 1992; Limin and Fowler 1991), all of which contribute to its resilience under sub-optimal conditions. A key factor behind the superior performance of rye is its highly developed root system, which facilitates efficient nutrient and water uptake, allowing it to thrive in marginal soils (Dittmer 1937; Kaye et al. 2019). Despite this potential, the role of rye roots in nutrient acquisition and stress adaptation remains largely unexplored. Given that rye is a widely produced crop in Europe (European-Commission 2024), the lack of studies on root phenotyping highlights a significant research gap (Comas et al. 2013; Takahashi and Pradal 2021). Investigating rye's root system architecture is a unique opportunity to understand how root traits respond to varying nutrient availability during the growth period. This study aims to fill this knowledge gap on rye responses to nutrient deficiency by investigating the effects of N and P deficiency on root and shoot traits of winter rye cultivated at a LTFE. By examining morphological root and shoot trait adaptations on various dates from tillering to anthesis, the study seeks to provide valuable insights for developing crop management and breeding strategies to improve productivity and resilience in nutrient-limited environments.

3.2 Materials and Methods

3.2.1 Experimental design

A sampling campaign was conducted in 2022 at the long-term fertilizer experiment Dikopshof near Cologne, Germany (50.8079 N, 6.9529 E, 62 m a.s.l.). The experiment was established in 1904, with a 5-year crop rotation currently including sugar beet (*Beta vulgaris*), winter wheat (*Triticum aestivum* L.), winter rye, Persian clover (*Trifolium resupinatum* L.), and potato (*Solanum tuberosum* L.). The soil type is classified as a Haplic Luvisol derived from loess above sand with a silty loam (topsoil) and (silty)

clay loam texture (below 30 cm soil depth). The experiment is a non-randomized block design without replicates and comprises seven treatments: NPKCa+m+s ("Ca" stands for lime, "+m" stands for farmyard manure fertilization and "+s" stands for supplemental mineral fertilization), NPKCa, _PKCa, N_KCa, NP_Ca, NPK_, and no fertilizer (the "_" stands for the omission of the corresponding nutrient). After harvesting the preceding crop, cattle farmyard manure is supplied on sugar beet, potato, and winter rye plots at a total rate of 60 t ha^{-1} per five-year rotation (fresh matter, treatments "+m"). The fertilizer management has not changed since 1953, except for a slight increase of the N fertilizer treatment (+ 30 kg N ha^{-1}) on winter wheat in some treatments, which occurred in the 1980s. For further details about the field experiment refer to Seidel et al. (2021). For the current study, four treatments were considered: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa), and P omission (N_KCa).

3.2.2 Crop management and biomass sampling dates

Crop management activities are presented in table 3-1. Root and shoot sampling were performed during the vegetative stage and at the end of flowering (Table 3.1). Winter rye shoot biomass was estimated destructively by cutting four times 50 cm of a row for each treatment. Shoot biomass was oven-dried (105 °C) until constant weight was reached and weighed again to estimate shoots dry weight. Crop phenology was monitored by using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und CHemical Industry (Meier 2018)).

Table 3.1: Crop management, crop growth stages and sampling dates for winter rye during the 2022 season.

Date	**Activity**	**Description**
11/07/2021	Ploughing	--
11/09/2021	Sowing	Cultivar EternoKWS, at 12 cm row distance
31/10/2021	Organic amendment	Farm yard manure application
28/03/2022	Fertilization	Mineral P and N application
10/05/2022	Fertilization	N-fertilization, only for NPKCa+m+s treatment (~BBCH 55)
16/03/2022	Shoot and root sampling	2-4 tillers detectable (BBCH 22-24)
04/04/2022	Shoot and root sampling	Beginning of stem elongation (BBCH 30)
29/04/2022	Shoot and root sampling	Early to mid-boot stage (BBCH 41-43)
27/05/2022	Shoot and root sampling	Inflorescence emerged (BBCH 56-58)
21/06/2022	Shoot and root sampling	End of flowering (BBCH 69)
27/07/2022	Harvest	Conducted at physiological maturity

Simultaneously, manual soil coring using a 1 m auger with a 9 cm inner diameter, was conducted in each of the cut rows to sample the roots in the ploughed soil top layer (0-30 cm). Roots per auger were then carefully cleaned with tap water. For the current study, we measured shoot and root traits included number of tillers, number of nodal roots, root angle, the specific root length (SRL), root length density

(RLD), average diameter and root length per diameter class. The nodal root angles, number of tillers, and the number of nodal roots emerging from shoot tissue (root number) were estimated manually for all plants. The angular spread of the roots was defined as the deviation angle of the two most horizontally distant shoot roots (180° would be roots at soil surface, Figure S3.1). The samples were then sieved (2 mm and 0.63 mm) and sorted to remove the debris. The roots were then scanned with a flat-bed scanner (Expression 12000XL, Epson, Suwa, Japan). To avoid overlapping during the scanning, samples with abundant roots were divided into sub-samples. Images were then analyzed with WinRhizo 2016a software (Régent Instruments Inc., Quebec, QC; Canada) to estimate the SRL (m g^{-1}), the RLD, (cm cm^{-3} soil), average diameter (mm) and root length (cm) for each diameter class. Table 2 presents the equation for the SRL and RLD calculations. The RLD was calculated for the top 30 cm soil as the ploughed layer (Table 3.2). The roots were then oven-dried (50 °C) until constant weight was reached. Afterwards, the dry matter root biomass (g m^{-2}) was calculated using the equation in Table 3.2 by considering the surface area of the auger cylinder. While specific root length (SRL) was also calculated based on the top 30 cm soil sample (Table 3.2).

Table 3.2: Equations and units for the calculated root morphology parameters for winter rye fertilizer omission experiment at Dikopshof.

Root parameter	Unit	Equation
Root biomass[1]	g m^{-2}	$Root\ biomass = \frac{Root\ mass\ for\ the\ ploughed\ layer}{Surface\ area\ of\ cylinder}$
Specific root length	m g^{-1}	$SRL = \frac{Root\ length\ in\ the\ ploughed\ layer}{Root\ mass\ for\ the\ corresponding\ layer}$
Root length density	cm cm^{-3}	$RLD = \frac{Root\ length\ in\ the\ ploughed\ layer}{Soil\ volume\ of\ the\ layer}$

[1] Depth of ploughed layer = 30 cm.

After scanning and image analysis the roots were then dried in the oven at 50°C and weighted using Sartorius ENTRIS 4231 fine scale with 0.001 g level of precision (Sartorius Lab Instrument GmbH & Co, Goettingen, Germany) to derive dry matter root biomass After scanning and image analysis the roots were then dried in the oven at 50°C and weighted using Sartorius ENTRIS 4231 fine scale with 0.001 g level of precision (Sartorius Lab Instrument GmbH & Co, Goettingen, Germany) to derive dry matter root biomass. After weighing, the root samples were milled, sieved, and analyzed for total C and total N using an elemental analyzer (Euro-EA, HEKAtech GmbH, Wegberg, Germany).

To facilitate analysis, the root length was additionally categorized into six classes ranging from < 0.15 mm up to > 0.75 mm diameter, with equal intervals of 0.15 mm, where L1 was the lowest and L6 was the highest root diameter, respectively (Table 3.3). This step size was selected as a balance between resolution and interpretability as it allows us to distinguish finer roots while keeping the number of classes manageable. The minimum interval possible in WinRhizo is 0.10 mm, but using 0.15 mm steps provided sufficient detail to detect shifts in the fine-root fraction without creating excessive class fragmentation.

Table 3.3: Root diameter classification ranges.

Diameter class	Description
L1	< 0.15 mm
L2	0.15 to 0.30 mm
L3	0.30 to 0.45 mm
L4	0.45 to 0.60 mm
L5	0.60 to 0.75 mm
L6	> 0.75 mm

3.2.3 Soil nutrient and water sampling and monitoring

Four soil samples per treatment at each sampling date were collected from the ploughed topsoil (0-30 cm) with a Pürkhauer auger with an 18 mm-diameter. The samples per treatment were then pooled together and frozen. After thawing, the soil was analyzed for mineral N content (N_{min}) by extraction with potassium sulfate solution (VDLUFA 1991). The N_{min} concentrations in the extracts were measured by a Skalar Continuous Flow Analyser (Skalar Analytical B.V., Breda, Netherlands). Further, the calcium acetate calcium lactate extract method (Schüller 1969) was used to quantify the plant-available phosphorus (P_{cal}) and potassium (K_{cal}). The P concentration in the extracts was determined colorimetrically following molybdenum blue reaction (Murphy and Riley 1962) on a spectrophotometer (Specord 205, Analytik Jena, Germany). K concentration in the extracts was determined by atomic absorption spectroscopy (novAA 400 P, Analytik Jena, Germany). Around flowering, when root systems of cereals commonly achieve their maximum root growth, volumetric soil water content at 3, 30 and 60 cm soil depth was measured using the FDR moisture sensor HH2 within ML3 Theta Probe (ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany) at winter rye flowering on 27/05/2022.

3.3 Statistical analysis

The collected data were analyzed using the R software (version 4.1.1). Due to the old experimental set up, we used the sub samples as pseudo replicates. Data normality was tested by sampling date by using the Shapiro wilk test. To test the main and interaction effects for the intermediate measurements, a repeated measures analysis of variance (ANOVA) was performed by using the **anova_test** function in the **rstatix** package (Kassambara, 2023), by specifying the argument "**within**" for treatment and sampling date as factor variables. The multiple mean comparison was performed by applying the Bonferroni test ($P \leq 0.05$), using the **pairwise_t_test** function also part of the **rstatix** package. For the final grain yield, a one-way ANOVA was performed using the **aov** function within the base R **stats** package. Finally, the **ggcorr** function within the **ggplot2** (Wickham 2016) R package by using the GGally extension (Schloerke et al., 2018) was used to visualize the correlation coefficients between the shoot and root variables in a correlation matrix. To assess the relationships between key aboveground

and belowground traits, we performed a Pearson correlation analysis. This analysis was conducted using the **cor.test** function also in the **stats** package. To visualize the correlation matrix and identify significant relationships, we used the **corrplot** package (Wei et al. 2013) for graphical representation of the correlation coefficients.

3.4 Results

Overall, N and P omission treatments significantly affected root and shoot biomass as well as root traits during the season. Fertilizer treatments significantly affected root morphological traits, though large variation was observed among the different treatments.

3.4.1 Soil conditions

The NPKCa+m+s resulted in the highest soil N_{min}, K_{cal}, and P_{cal} contents during all sampling dates (Figure 3.1). While soil P_{cal} and K_{cal} content were the lowest in the N_KCa treatment. As expected, the soil N_{min} was the highest in the NPKCa+m+s treatment with the highest values observed in the first (29.14 mg kg^{-1}) and last (27.61 mg kg^{-1}) sampling dates, but declined in the second and third sampling dates (Figure 3.1). The rest of treatments showed considerably lower N_{min} values as well as a tendency to decrease as the season progressed. The soil N_{min} values were lowest for _PKCa in all dates.

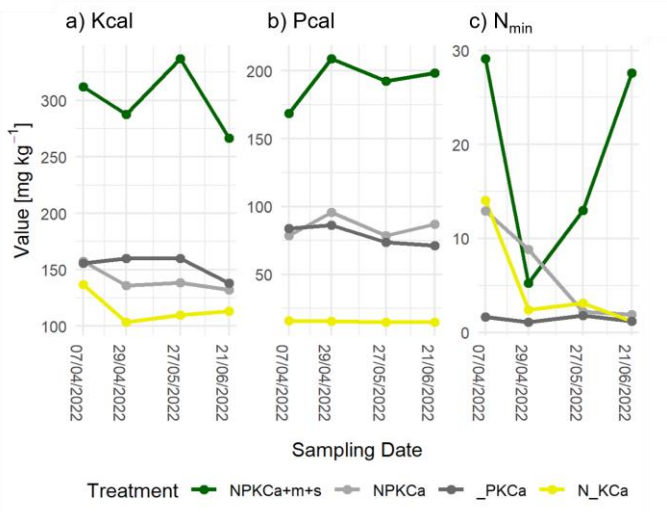

Figure 3.1: Soil P_{cal}, K_{cal} and N_{min} content for four sampling dates during the 2022 winter rye growing season as affected by N and P omission treatments. Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa); Soil P and K content measured using the calcium acetate-lactate extract method; Soil mineral nitrogen content (N_{min}) measured by extraction with potassium sulfate solution. One pooled sample collected by treatment and sampling date.

For soil water content, the volumetric water content data collected around flowering on 27/05/2022, showed non-significant differences at the top 3 cm (Figure S3.2). However, at 30 cm, the N omission treatment resulted in the highest soil moisture at ~20%, followed by the P omission and NPKCa

treatment. At 60 cm, most treatments resulted in non-significant differences, except for NPKCa+m+s, which showed the strongest decrease in soil moisture.

3.4.2 Shoot biomass

Fertilizer treatments and sampling date resulted in significant interactions (Table 3.4). Shoot biomass significantly increased over the season. The shoot biomass for the NPKCa+m+s treatment was significantly higher than the rest of treatments during the first three sampling dates (up to BBCH ~56-58), with values ranging from 32.94 g m^{-2} at the first sampling date (BBCH 22-24) to 442.59 g m^{-2} at sampling date 3 (BBCH 41-43, Figure 3.2). As the season progressed, the N omission treatment showed significantly lower biomass than the other treatments. At the last sampling date, right after flowering (BBCH 69), the NPKCa+m+s showed significantly higher biomass than the N and P omission treatments, which showed non-significant differences between them and the NPKCa treatments.

3.4.3 Root biomass

Root biomass showed a significant interaction between treatment and sampling dates with root biomass significantly increasing over time, up to BBCH 56-58, and remaining the same till the end of flowering (Table 3.4). Contrary to the shoot biomass, non-significant differences were observed at the first and the third sampling dates (Figure 3.2). In sampling date 2 (BBCH 30), the NPKCa+m+s treatment led to the highest root biomass. In the last sampling date, non-significant differences between the NPKCa+m+s with the N omission treatment were observed, though the N omission treatment showed significantly lower root biomass than the P omission treatment and the NPKCa treatments (Figure 3.2). The NPKCa+m+s treatment showed a decline in root biomass, most probably due to a beginning of root decay (Figure 3.2).

3.4.4 Root:shoot ratio

The root:shoot ratio showed a non-significant interaction between treatments and sampling dates (Table 4). Fertilizer treatments resulted in non-significant differences, though with large within treatment variation, with an average of 0.52 root:shoot ratio across all treatments. But root:shoot ratio significantly varied over the season with the highest values up to sampling date 2 (BBCH 30), but then significantly decreasing in the last sampling dates till the end of flowering (sampling date 5, Table 3.4).

Table 3.4: Repeated measures analysis of variance (ANOVA) for winter rye above and below ground dry biomass and root:shoot ratio as affected by N and P omission treatments during the 2022 season.

Treatments[1]	Above ground dry biomass (g m^2)			Root dry biomass (g m^2)			root:shoot ratio (-)		
_PKCa	221.83	± 226.30[3]	a	33.94	± 13.97	a	0.53	± 0.52	NS
N_KCa	372.58	± 385.69	ab	55.79	± 32.67	ab	0.66	± 0.72	NS
NPKCa	542.83	± 568.85	ab	61.24	± 35.62	b	0.48	± 0.50	NS
NPKCa+m+s	757.86	± 885.84	b	73.01	± 38.52	b	0.42	± 0.44	NS
Sampling dates[2]									
16/03	21.45	± 8.38	a	22.89	± 10.46	a	1.26	± 0.52	a
04/04	43.67	± 19.08	a	36.56	± 10.97	ab	0.92	± 0.31	b
29/04	277.30	± 131.53	a	55.22	± 24.03	b	0.24	± 0.11	c
27/05	808.15	± 329.11	b	83.06	± 37.04	c	0.11	± 0.04	c
21/06	1218.31	± 758.99	c	82.25	± 29.81	c	0.08	± 0.04	c
Treatment (T)[4]	*			*			NS		
Date (D) [4]	*			*			*		
T × D[4]	*			*			NS		

1 Treatments: NPKCa+m+s ("+m" stands for farmyard manure fertilization and "+s" stands for supplemental mineral fertilization), NPKCa, _PKCa, N_KCa, NP_Ca, NPK_, and no fertilizer (the "_" stands for the omission of the corresponding nutrient, "Ca" stands for lime).

[2] Sampling dates: see table 3.1 for BBCH at sampling date.

[3] mean ± standard deviation for four pseudo replicates.

[4] Significant (*) or non-significant (NS) according to the repeated ANOVA test. Values followed by the same letter do not differ according to Bonferroni test at $P \leq 0.05$.

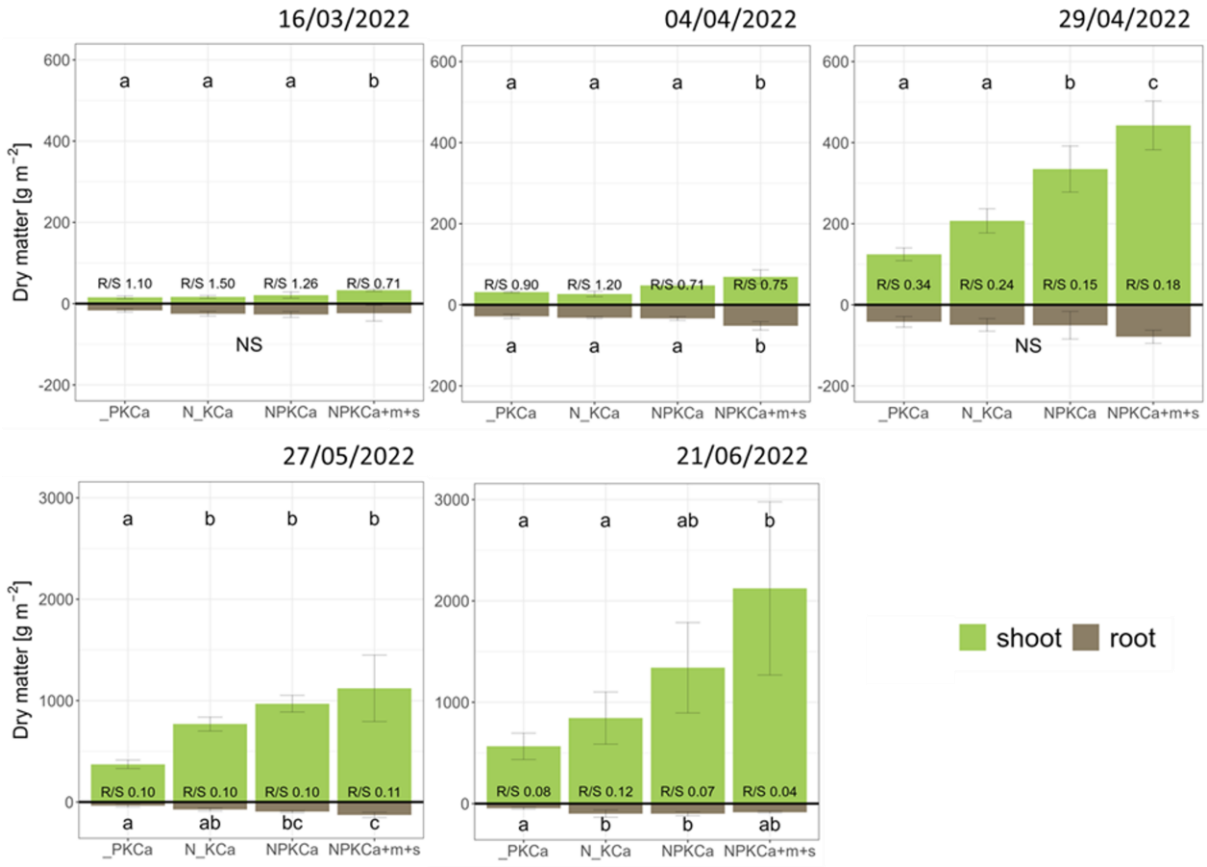

Figure 3.2: Average winter rye shoot and root dry biomass (0–30 cm, see table S3.1 for absolute values) and root:shoot ratio (R/S, unitless) under N and P omission treatments across five sampling dates (top right, see Table 3.1 for BBCH stages) during the 2022 season. Treatments: N omission (_PKCa), P omission (N_KCa), fully fertilized with (NPKCa+m+s) and without (NPKCa) manure. Within each date, treatments sharing the same letter are not significantly different (Bonferroni test, 5%); NS = not significant. Error bars show standard deviation (n=4 pseudo replicates). Letters above bars indicate shoot biomass differences; letters below, root biomass differences. R/S ratios did not differ significantly across treatments.

3.4.5 Root and shoot traits

In general, all root traits showed a non-significant interaction between fertilizer treatments and sampling dates, fertilizer treatments often led to non-significant differences but root traits significantly changed over time (Table 3.5).

Number of nodal roots. Fertilizer treatments resulted in non-significant effects on number of nodal roots, with large variation within treatments observed. The number of nodal roots significantly increased as the season progressed with the highest number of nodal roots shown in sampling date 4, which was maintained till the end of flowering (Table 3.5).

Root angle. Root angles significantly increased over the season, from lower than 90° at the beginning of the growth period (date 1) to the largest value of 110°, observed at date 3 (BBCH 41-43, Table 3.5). However, later in the season, root angle was significantly lower, reaching 97° by the end of flowering (BBCH 69).

RLD. Even though the interaction of fertilizer treatment and sampling date were not significant, treatments and sampling date significantly affected RLD (Table 3.5). The N and P omission treatments resulted in the lowest RLD among treatments, while both the fully fertilized treatments resulted in the highest RLD among treatments. As for sampling dates, RLD was the lowest at the beginning of the growing seasons, and the highest in sampling dates 4 (BBCH 56-58) till the end of flowering in sampling date 5 (BBCH 69).

SRL. The SRL was not significantly different among treatments but the sampling date significantly affected it, with the highest values in the early sampling dates up to BBCH 41-43 (sampling date 3), varying from 150.5 to 181.87 m g^{1}, and then significantly decreasing later in the season, reaching 124.91 m g^{-1} by the end of flowering (Table 3.5).

Average diameter and length per diameter classes. Our data showed that the root diameter was consistent across treatments with non-significant differences among them (Table 3.5). But root diameters significantly increased over sampling dates from the lowest value early in the season (0.23 mm), to the highest values between 0.26 and 0.27 mm in the last two sampling dates, respectively (Table 5). As for the proportion of the different diameter classes to the total root length, all treatments showed a considerably higher proportion of roots in the L1 and L2 classes (Figure 3.3). The share of very fine roots (L1, less than 0.15 mm) of the N omission treatment was the highest among treatments at date 2 (BBCH 30) and 4 (BBCH 56-58). At the late stage, the share of medium to coarse roots tended to increase in all treatments and was highest for the fully fertilized treatments NPKCa+m+s and NPKCa. Also, in the P omission treatment the share of very fine and fine roots was enhanced (Figure 3.3).

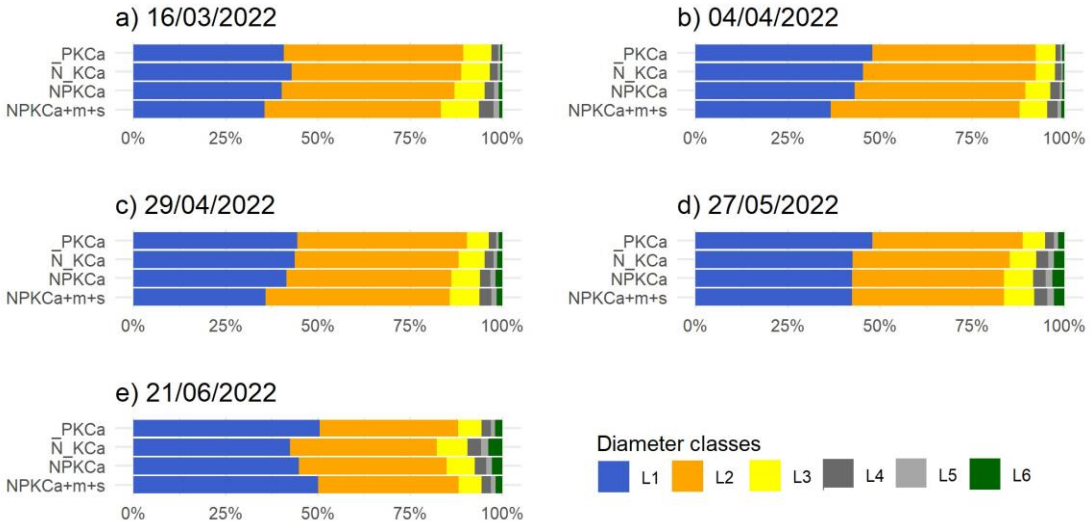

Figure 3.3: Proportion (%) for each diameter class to total root length as affected by N and P omission treatments over five sampling dates (See table 3.1 for BBCH at samplings date). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Average values of root length per root diameter class were used to represent the proportion. L1: < 0.15 mm, L2: 0.15 to 0.30 mm, L3: 0.30 to 0.45 mm, L4: 0.45 to 0.60 mm, L5: 0.60 to 0.75 mm, L6: > 0.75 mm.

Table 3.5: Repeated measures analysis of variance (ANOVA) and treatment comparison for winter root rye traits and tiller number as affected by N and P omission treatments during the 2022 season.

Treatments[1]	**Nodal root number (-)**		**Root angle (º)**		**Root length density (cm cm^{-3})**		**Specific root length (m g^{-1})**		**Root diameter (cm)**		**Tiller number (-)**	
_PKCa	32.63 ± 24.15[3]	NS	98.27 ± 24.30	NS	1.95 ± 0.64 a		185.28 ± 63.63	NS	0.23 ± 0.02	NS	4.68 ± 2.24	NS
N_KCa	38.75 ± 25.77	NS	104.34 ± 18.89	NS	2.51 ± 1.03 ab		154.67 ± 50.03	NS	0.25 ± 0.04	NS	5.20 ± 2.02	NS
NPKCa	33.30 ± 25.19	NS	106.97 ± 18.83	NS	3.00 ± 1.37 bc		149.17 ± 43.17	NS	0.25 ± 0.03	NS	5.65 ± 2.35	NS
NPKCa+m+s	33.10 ± 18.02	NS	110.97 ± 21.96	NS	3.54 ± 1.54 c		145.28 ± 37.84	NS	0.25 ± 0.03	NS	6.00 ± 2.58	NS
Sampling dates[2]												
16/03	11.19 ± 2.23	a	78.76 ± 12.89	a	1.20 ± 0.38 a		150.50 ± 37.97	abc	0.23 ± 0.02	ab	4.18 ± 1.78	NS
04/04	18.88 ± 8.65	a	109.73 ± 20.93	bc	2.27 ± 0.59 b		193.71 ± 53.32	a	0.22 ± 0.01	a	5.41 ± 2.03	NS
29/04	35.38 ± 11.90	b	121.32 ± 13.96	b	3.52 ± 1.00 c		181.87 ± 24.25	ab	0.24 ± 0.02	bc	6.00 ± 2.80	NS
27/05	56.31 ± 24.74	c	105.66 ± 19.47	c	3.43 ± 1.21 c		142.00 ± 64.43	bc	0.26 ± 0.03	c	6.19 ± 2.88	NS
21/06	52.60 ± 17.33	c	97.46 ± 19.08	c	3.33 ± 1.35 c		124.91 ± 36.16	c	0.27 ± 0.04	c	5.33 ± 1.50	NS
Treatment (T)[4]	NS		NS		*		NS		NS		NS	
Date (D) [4]	*		*		*		*		*		NS	
T × D[4]	NS		NS		NS		NS		NS		*	

[1] Treatments: NPKCa+m+s ("+m" stands for farmyard manure fertilization and "+s" stands for supplemental mineral fertilization), NPKCa, _PKCa, N_KCa, NP_Ca, NPK_, and no fertilizer (the "_" stands for the omission of the corresponding nutrient, "Ca" stands for lime).

[2] Sampling dates: see table 3.1 for BBCH at sampling date.

[3] mean ± standard deviation for four pseudo replicates.

[4] Significant (*) or non-significant (NS) according to the repeated ANOVA test. Values followed by the same letter do not differ according to Bonferroni test at $P \leq 0.05$.

Tiller number. A significant interaction between fertilizer treatment and sampling date was observed (Table 3.3). Overall treatment, differences were observed only for sampling dates 3 (BBCH 41-43) and 5 (BBCH 56-58), but not for the rest of sampling dates. A trend to decreased tiller number in N and P omission treatments compared to the NPKCa+m+s treatment was observed, though not significant for dates 1 (BBCH 22-24) and 2 (BBCH 30), but significant for date 3 (BBCH 41-43). However, in date 5 (BBCH 69), the opposite trend was observed where the P omission treatment resulted in higher tiller numbers compared to the NPKCa+m+s treatment (Figure 3.4).

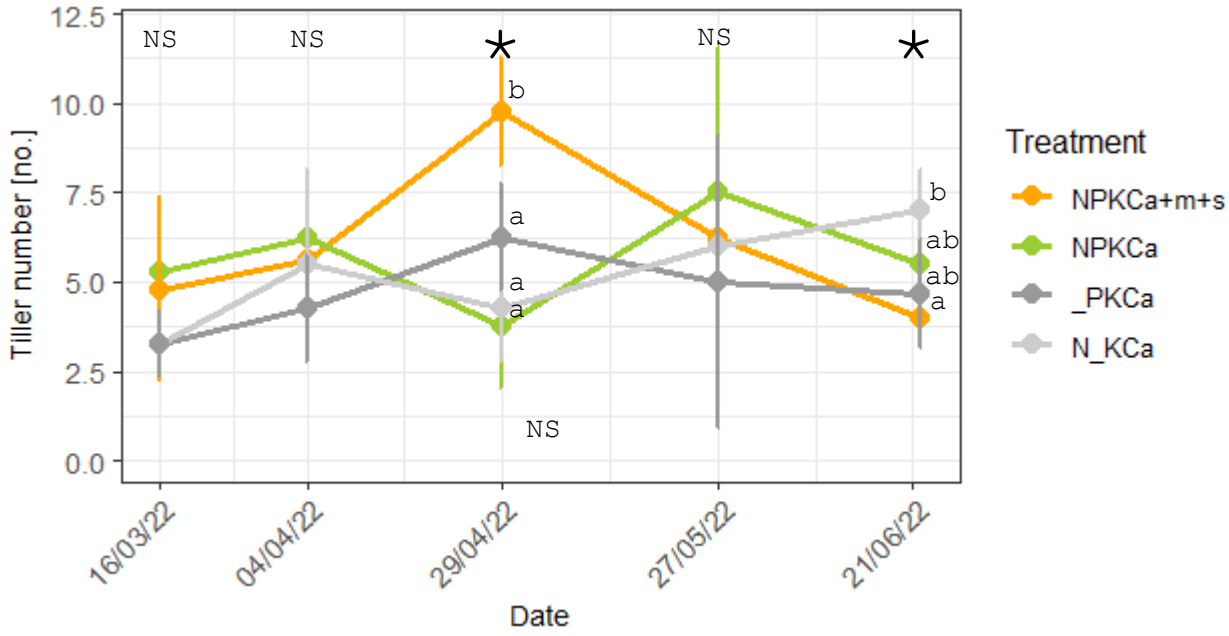

Figure 3.4: Tiller number for winter rye as affected by nitrogen (N) and (P) omission treatments over five sampling dates, during the 2022 season. Treatments: N omission (_PKCa), P omission (N_KCa), fully fertilized with (NPKCa+m+s) and without (NPKCa) manure. Within each date, treatments sharing the same letter are not significantly different (Bonferroni test, $P \leq 0.05$); NS = not significant. Error bars show standard deviation (n=4 pseudo replicates). Letters above (significant (*) or non-significant (NS) at 5% confidence level). Treatments followed by the same letter do not differ according to the Bonferroni test at 5% confidence level.

***C and N tissue content*.** Table S3.2 summarizes fertilizer treatments and sampling date effects on shoot and root C and N contents and C:N ratio. Fertilizer treatments affected root and shoot C and N tissue contents and tissue C:N ratio. Shoot C and N contents showed a significant interaction between fertilizer treatment and sampling date, the same was observed for root N content and root C:N ratio. While the shoot C:N ratio and root N content resulted in non-significant interactions between treatment and sampling date. On average, the shoot C content was slightly higher (44.0%) in comparison to the root C content (39.0%) and significantly increased at the end of the season (Figure S3.3). Treatment effects on shoot C content were similar among treatments (Figure S3.3), except for the last sampling date, where the P omission treatment showed the highest shoot C content among treatments (Figure S3.3). By contrast, root C content showed non-significant differences among treatments and sampling dates. For

Shoot N content, although significant treatment effects were observed across the season, no significant differences among treatments were detected at the final sampling date, corresponding to the end of flowering. Though, a general decline was observed as the season progressed (Table S3.2, Figure S3.4). The shoot and root C:N ratio was mostly not affected by treatment application, except for the root C:N ratio at the end of flowering, where the P omission treatments resulted in higher C:N ratio than the N omission treatment (Table S3.2, Figure S3.5).

Grain yield. Significant differences were observed among all treatments, with the NPKCa+m+s treatment showing the highest yield with 6.8 t ha^{-1}, even though the NPKCa resulted in considerably lower yield of 3.8 t ha^{-1}. The P and N omission treatments resulted in the lowest yields, with 53% and 80% yield reduction, respectively, compared to the fully fertilized treatment with manure (Figure 3.5).

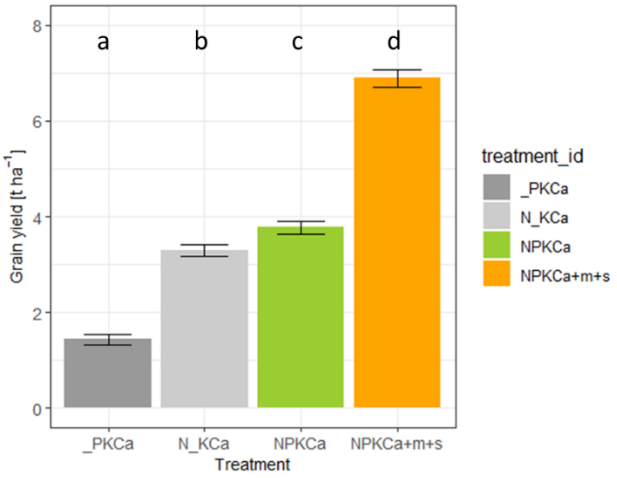

Figure 3.5: Winter rye grain yield as affected by N and P omission treatments during the 2022 season. Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Treatments followed by the same letter do not differ according to Tukey high significant difference at 5% confidence level. Error bars refer to the standard deviation (n=3 pseudo replicates).

3.4.6 Correlation of measured variables

Figure S3.6 shows the correlation coefficients for the collected root and shoot variables for all sampling dates. When correlating the root and shoot variables (all dates), a positive correlation (0.7) was observed (Figure S3.6a). The RLD and root biomass showed the highest correlation coefficient with yield (0.4), among all the variables, including shoot biomass (0.3). As for the temporal differences, the root biomass in dates 1 to 4 was strongly associated with yield (0.6-0.8), but not on date 5 (0.3) (Figure S3.6b-S3.6e). The tiller number was positively associated with yield in most dates (0.1-0.7), except in date 5. The correlation of root angle with yield was generally positive, except in date 4. The number of nodal roots relationship with yield varied widely in direction and magnitude by dates. The SRL was negatively associated with yield during most dates, except at date 5 (21/06/2022), where a weak positive relationship was observed (0.2). Root biomass showed a strong correlation with RLD (06-0.9) in all sampling dates. In the later sampling dates 3 to 5, RLD showed a strong positive correlation with root angle (0.6-0.7).

3.5 Discussion

3.5.1 Temporal effects of N and P omission on aboveground growth

Shoot Biomass. Our results demonstrated that N and P omission led to a decrease in shoot biomass over time, with stronger reduction in the N omission treatment particularly in the last two sampling dates (BBCH: 56-58 and 69). These findings align with well-established research showing that N and P availability strongly influence biomass accumulation in rye (Mirsky et al., 2017). In cereals, N application typically leads to substantial shoot biomass growth, whereas P fertilization effects are often less pronounced (Bélanger et al. 2015; Kostić et al. 2021). The reduction in shoot biomass observed in our N and P omission treatments suggests that winter rye, like other cereals, relies heavily on adequate N and P supply for optimal aboveground growth. The significant interaction between omission treatment and sampling date indicates the dynamic effect of nutrient availability on shoot biomass over time, meaning that the omission effect is not consistent across all growing stages. Results suggest that N availability at the beginning of the season had lasting effects on the biomass production of winter rye. These findings may inform on the timing and application of fertilizers to optimize crop production.

Tiller number. P omission tends to decrease the tiller number (Graham et al. 1983; Rodríguez et al. 1999), a trend that we also observed in our results in booting stage (BBCH 41-43), but not at the end of flowering, where the opposite was observed with the P omission treatment showing the highest tiller number among treatments. The decline in tiller number in the fully fertilized treatment may be associated with the onset of reproductive development, where optimal nutrient conditions support fewer but more dominant reproductive tillers.

3.5.2 Dynamic effects of N and P omission on winter rye root traits

Among the measured root traits, only root biomass showed a significant interaction between treatment and sampling date, indicating that nutrient effects changed across growth stages. In contrast, other root traits varied mainly with time, reflecting developmental changes rather than treatment effects. In the following, we discuss the observed patterns for each trait and their possible physiological implications.

Root biomass. N omission led to lower root biomass values than the P omission treatment. Lopez et al., (2023) also reported reduced root biomass due to nutrient omission in field studies, though, P omission led to a more detrimental effect on root biomass with N and P omission leading to a decrease in root biomass by 7% and 25%, respectively (Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, and Rachmilevitch 2023). Therefore, the pattern in our results likely reflects differences in rooting depth rather than total root production. Because sampling was limited to the 0–30 cm ploughed layer, deeper root growth typical of N-limited plants (Lynch 2013) may not have been successfully captured, whereas under P deficiency root proliferation usually concentrates in the upper soil layers where P is more available (Kumar et al., 2020). The soil in our experiment contained relatively high levels of available P (> 50 mg kg^{-1}), which may also have reduced the contrast between the P-omitted and fertilized treatments. Overall, both nutrients influence root development, P deficiency

mainly restricts shoot growth, whereas N limitation tends to affect both shoot and root biomass. The small decline in root biomass of the fully fertilized with manure treatment at the final sampling date may likely reflect a post-flowering decay (Gregory et al. 1978).

Root to shoot ratio. Consistent with previous studies, we observed an increase in the root:shoot ratio under N and P omission resulting in enhanced biomass partitioning to the roots, which is a well-documented adaptive mechanism in plants facing nutrient scarcity (Amanullah 2015; Lopez et al. 2023). The magnitude of this adjustment can vary depending on species-specific root plasticity and soil nutrient distribution (Kumar et al. 2020).

Root angles. In general, under P omission, roots tend to develop wider angles, leading to increased lateral expansion within the top soil, resulting in shallower and broader rooting system (Bonser et al. 1996; Niu et al. 2013). However, this trend was not observed in our results, as P omission treatment showed non-significant differences when compared to the fully fertilized treatment. The N omission treatment showed steeper root angles around flowering (21/06/2022), this shift suggests a plastic response of winter rye roots to N omission. Similar plastic responses have been described in maize, where under N-limited conditions, some genotypes with initially shallow root angles developed steeper ones, enabling exploration of deeper soil layers (Trachsel et al. 2013; Schneider et al. 2022). Overall, N deficiency tends to promote steeper, deeper-rooting systems, while P deficiency typically results in shallower roots, as shown in species such as common bean (Bonser et al., 1996; Liao et al., 2001).

Root length density. Our findings on RLD reduction under N omission align with previous studies, which report that N deficiency leads to a decrease in total root length and RLD (particularly within the topsoil (0–30 cm) across multiple crops, including winter wheat, maize, cotton, and sugar beet (Anderson 1987; Barraclough et al. 1989b; Xue et al. 2014; Hadir et al. 2021; Mehrabi et al. 2021b; Fang et al. 2022) . Root morphology responses to P deficiency can be genotype dependent, leading to variable root system adjustments among cultivars (Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, and Rachmilevitch 2023). Although our study did not find a significant decrease in RLD under P omission, RLD values were consistently lower than those of the fully fertilized treatment across all sampling dates. Similar reductions under P limitation have been reported in maize (Sheng et al. 2012; Zhang, Peng, et al. 2012; Deng et al. 2014), oilseed rape (Duan et al. 2020), sugar beet (Hadir et al. 2021a), soybean (Otani and Ae 1996; Ao et al. 2010), common bean (Ho et al. 2005; Ochoa et al. 2006; Miguel et al. 2013), wheat (Teng et al. 2013), and other crops such as buckwheat, castor, peanut, and sorghum (Otani and Ae 1996). The lack of a significant P effect in our results may be due to sampling being restricted to the ploughed layer (0–30 cm), where root length density is typically highest in winter rye (Kemper et al. 2023).

Average diameter. Under P deficiency, plants exhibit a plastic response characterized by increased branching density and decreased lateral root diameter. This plasticity enhances foraging efficiency in the topsoil but may limit root penetration into deeper or compacted soil layers (Materechera et al. 1992). In our study, the response in average root diameter to nutrient omission was more pronounced at the

beginning of the growing period where we observed a negative impact of both N and P omission on the root diameter of winter rye. However, around flowering and in later growth stages, we did not find significant differences in average root diameter under either N or P omission suggesting that these nutrient deficiencies may not strongly influence this trait in winter rye. Similar findings have been reported in other crops, such as potato under N deficiency (Sharifi et al. 2005) and maize under P omission (Li et al. 2017). However, the literature presents conflicting results, with some studies showing an increase in root diameter under N deficiency (Anderson 1987), while others report a decrease (Eghball et al. 1993; Hadir et al. 2021). Likewise, P deficiency has been linked to reduced root diameter in maize at specific growth stages (Sheng et al. 2012; Zhang, Peng, et al. 2012). These inconsistencies suggest that root diameter plasticity to nutrient availability may be species-specific, influenced by environmental conditions, plant developmental stage, or genotype.

***SRL*.** Our repeated measures ANOVA showed no significant differences in SRL among treatments over the season, indicating that N and P omission had little effect compared with full fertilization. Although not significant, SRL tended to be higher in the P omission treatment (N_KCa) early in the season, while later the NPKCa+m+s treatment showed slightly higher values. This shift may reflect changes in carbon allocation under prolonged nutrient stress, as plants reduce fine-root growth to preserve resources (Eissenstat 1992; Ke et al. 2024). In contrast to our results, previous studies reported higher SRL under low-nutrient conditions (Ostonen et al. 2007) and under N deficiency in winter wheat (Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, and Rachmilevitch 2023). Poorter and Ryser (2015a) described SRL as an adaptive trait analogous to specific leaf area (SLA), with SRL showing less consistent responses across environments. When root types were analyzed separately, they found that lateral roots (the most active in nutrient acquisition) tended to exhibit higher SRL under nutrient limitation. Taken together, these findings suggest that SRL responses to nutrient stress are variable across species and experimental conditions, and that in winter rye, SRL adjustments are modest and phenological stage-dependent.

3.5.3 Relationships between root traits and grain yield

A positive but only moderate correlation between the number of nodal roots and grain yield was observed. Also, during the last two sampling dates, the number of nodal roots had a tendency to be higher in the P omission treatment, compared to the fully fertilized treatment with manure. A meta-analysis from (Niu et al. 2013), reported similar findings, where P omission promoted lateral root growth in cereal crops. Grando and Ceccarelli (1995) also compared modern barley cultivars, landraces and wild barley and showed that there was a significant increase in the number of seminal roots during domestication, suggesting that there may be a relationship between seminal root number and crop productivity.

3.5.4 Root plasticity to nutrient omission: quantitative insights and agronomic relevance

Our results highlight condition-specific root trait responses to nutrient deficiency. For example, under N deficiency, we observed significantly steeper root angles and a trend toward fewer nodal roots at later growth stages, patterns consistent with the root ideotypes proposed by Uga (2021) for improved nitrogen uptake. In contrast, under P deficiency, root angle did not differ significantly from the fully fertilized treatment, but we observed a consistent trend of increased nodal root number in later stages. These responses align with Uga's ideotype for low-P conditions, which favors shallower axial roots and numerous adventitious (nodal) roots to enhance topsoil P capture. However, trade-offs must be considered: while thicker roots may be beneficial under high nutrient availability, in nutrient-limited soils, finer roots, root hairs, or increased lateral branching may be more effective for nutrient acquisition (Gonzalez et al. 2021). The study characterizes different responses of root traits to either N or P omission. This suggests different strategies for soil nutrient foraging and demonstrate that even within a single genotype, root traits adjust in measurable ways to long-term nutrient omission. Despite the challenges of belowground measurements, the findings provide field-based insights of winter rye root phenotypic plasticity that can inform modelling efforts and guide experimental design. As it provides reference values for improving process-based crop models (Seidel et al. 2022) and root architectural models (Schnepf et al. 2018), and guides future sampling strategies especially with regard to linking the timing of sampling with its agronomic relevance.

3.5.5 Limitations of the study

The current study was conducted within the framework of the Dikopshof LTFE, which was established in 1904, before modern experimental design and statistical replication became standard. Each fertilizer treatment is represented by a single field plot. Therefore, sub-samples taken within a plot served as pseudo-replicates to capture within-plot variability but do not provide a full estimate of the experimental error. Adding spatial replication is not possible without compromising over a century of accumulated treatment effects. As a result, the statistical analysis supports a case study perspective. Still, the consistent ranking of treatments across multiple traits contributes to the long-term dataset and provides a basis for comparison in future seasons.

The study was limited to a single growing season and one winter rye genotype. Due to the five-year crop rotation, temporal replication is not immediately possible. These constraints mean that genotype-by-environment interactions remain untested, and the generalization of results across environments or cultivars should be made with caution. Nevertheless, the observed patterns across all root and shoot traits offer useful insights into the effects of nutrient supply. The magnitude of these traits can serve as a reference for future modelling and as a basis for designing broader multi-season, multi-genotype experiments.

Methodological limitations also need to be considered. Root sampling was restricted to the ploughed layer (0–30 cm) to avoid disturbing subsoil conditions that are critical to the integrity of the long-term

experiment. This sampling strategy likely underestimates deeper root responses, but most fine roots typically concentrate in the topsoil. Future studies could make use of non-invasive technologies, such as spectral electrical impedance tomography (Michels et al. 2024), to explore root development below 30 cm without altering the soil structure.

Shoot P concentration was not measured due to limited instrument availability at the time of analysis and the later unavailability of the samples. This prevents a direct link between shoot P status and the P omission treatment. Addressing this limitation should be a focus in future campaigns by ensuring complete elemental analysis.

Despite these constraints, the study offers a quantitative snapshot of root phenotypic responses to long-term nutrient omission in winter rye. The results provide effect sizes that can inform modelling efforts and supply a reference point for evaluating future genotypes, seasons, and deeper sampling strategies.

3.6 Conclusion

We conclude that fertilizer management and crop developmental stage at sampling exert a strong influence on winter rye root phenotypic plasticity. The effects of nutrient omission on the shoot were not only easier to quantify but also clearer in direction and treatment ranking, resulting in an approximately 80 % yield reduction under N omission and 53 % under P omission. However, assessing roots in the field proved more challenging yet equally essential, as they play a central role in plant adaptation to abiotic stress through soil exploration and nutrient acquisition. In our experiment, N deficiency promoted deeper root growth (steeper angles), whereas P deficiency enhanced surface foraging through increased nodal roots later in the season. Although the study was limited to one growing season and a single winter rye genotype within a fixed long-term experiment (making it a quantitative case study), the consistent trends across traits provide valuable baseline data. These field-based insights contribute to the development of crop models that better integrate root dynamics and inform the design of multi-season, multi-genotype experiments. Ultimately, optimizing root growth patterns, through targeted breeding or site-specific cultivar selection, will be key to improving resource use efficiency in suboptimal environments.

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4 Winter wheat shoot and root phenotypic plasticity under fertilized and nutrient-deficient field conditions

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Abstract

While it is widely recognized that nitrogen (N), phosphorus (P), potassium (K), and lime (Ca) are crucial for high agricultural yields and soil fertility, field studies examining shoot-root responses to nutrient deficiencies remain limited. Understanding crop adaptation strategies to nutrient-poor soils is crucial for optimizing low-input agriculture. In this study, we investigated the shoot and root responses of winter wheat cultivated in two growing periods (2019/20 and 2020/21) at the long-term fertilizer experiment in Dikopshof, Germany, under no fertilization, N, P, K, and Ca omission with two fully fertilized treatments (NPKCa without and with farmyard manure). We analyzed crop phenology, leaf area index (LAI), plant height, shoot biomass, grain and straw yield, root biomass, root length, root length density (RLD), root-to-shoot ratio (R/S), specific leaf area (SLA), specific root length (SRL) and root length per shoot biomass. Our results showed that unfertilized and N omission treatments led to a decrease in shoot biomass, LAI, plant height, yield, root length, and root biomass while leading to an increase in SRL, R/S, and root length per shoot biomass. The fully fertilized treatment with manure consistently had the highest values for shoot traits, root length, and root biomass in both growing periods. Limitations of P, K, and Ca reduced the straw yield but increased root length and root length per shoot biomass. Our findings highlight the complex interactions between nutrient deficiencies, shoot and root performance, and environmental factors, emphasizing the need for further research on cultivar differences and higher temporal resolution sampling to enhance crop productivity and nutrient management strategies.

Keywords: root length density, root biomass, specific root length, specific leaf area, leaf area index, long-term experiment

4.1 Introduction

The primary function of both above and belowground structures in plants is to obtain resources for their growth and protection. Although there is general coordination between the allocation and morphology of leaf and root biomass above and below ground across different species (Enquist and Niklas 2002), these associations can be significantly altered due to plant phenotypic modifications induced by changing environmental conditions (Laughlin et al. 2010; Freschet et al. 2015). Plants can adapt to their environment by making changes at various levels of integration, such as adjusting the partitioning of biomass among leaves, stems, and roots, modifying the anatomical structures of each organ, and changing the physiological characteristics of the cells that constitute these organs (Freschet et al. 2015; Poorter and Ryser 2015). Plants likely adjust at all three levels (Poorter and Ryser 2015). For example, nutrient supply alterations typically result in modifications to functional traits that determine a plant's capacity to obtain resources and overcome the restrictions imposed by the scarce resource (Lynch, 1995).

The aboveground adaptations to nutrient deficiencies in cereals have been widely studied. Studies have reported a decrease in the cereal production of biomass during the growing period and a reduction in yield (and its stability) at harvesting (about 60%) when nitrogen (N) and phosphorus (P) were deficient (Liang, 2022; Macholdt et al., 2019; Sawyer, 2010). Other studies also reported decreased aboveground biomass for maize (Pandey et al. 2000; Plénet et al. 2000), barley (Hansson et al. 1987), and winter wheat (Wang et al., 2014) when the plants grew in nutrient-poor soils. Specifically, winter wheat yield was affected by about 30% reduction under N deficiency (Zhang et al., 2018), about 55% under P deficiency (Rodriguez et al., 1998), and about 30% under potassium (K) deficiency (Zhang et al., 2020). In the long-term fertilizer experiment (LTFE), Dikopshof, Seidel et al. (2021) reported a 7% yield loss for winter wheat due to P omission, averaged over the period from 1906 to 2018.

The current knowledge about the effects of nutrient deficiencies on aboveground and, especially, belowground traits, including their ratios, is based mainly on seedling experiments or controlled conditions experiments such as greenhouses or pot experiments (Rich and Watt 2013a; Kravchenko et al. 2017). Due to the nature of sampling in field experiments, these studies are rarely carried out exhaustively. Therefore, there is a knowledge gap in some critical aspects, like the effect of P, K, and lime (Ca) deficiency in plants growing in fields (Lopez et al. 2023). In a recent literature review, Lopez et al. (2023) identified only 50 field-scale studies that explored the effect of nutrient deficiencies on root traits (26 for N, 19 for P, and 5 for K). However, not all of these studies assessed a comprehensive set of root traits; in some cases, only RLD was examined. Among their findings, they showed that N, P, and K deficiency led to a decrease in absolute root length, root length density (RLD), and root biomass in common arable crops. On the other hand, an increase in the root-to-shoot ratio (R/S) and root length per shoot biomass ratio under N and P deficiency was reported (Lopez et al., 2023). Specifically, roots of winter wheat showed a decrease in root length and root biomass under N and P deficiency. Deficiency of N resulted in a reduction of root length by approximately 10% and root biomass by about 3%.

(Barraclough et al., 1989; Comfort et al., 1988; Mehrabi et al., 2021; Wang et al., 2014; Xue et al., 2014), while P deficiency led to a decrease of about 20% in root length and 10% in root biomass (Teng et al. 2013). Wheat R/S and root length per shoot biomass were higher in nutrient-deficient soils (Lopez et al., 2023).

Field-scale studies, particularly those conducted over multiple years, often encounter variability in their results. This variability can arise from various factors, such as weather conditions, soil characteristics, and management practices, which can fluctuate yearly (Kravchenko et al. 2017). While these factors also vary across years in long-term field experiments (LTFEs), the major advantage of LTFEs lies in their ability to provide long time series of data. These data are crucial for interpreting the results of short-term studies, understanding trends, and assessing the long-term build-up of treatment or management effects (Johnston and Poulton 2018). Furthermore, the treatments used in LTFEs allow for the detailed study of specific management practices and their interactions, making them useful for understanding crop responses and refining agricultural practices over time.

To the best of our knowledge, only six studies have investigated root adaptations to nutrient deficiency in long-term field experiments (Hadir et al., 2021; Pellerin et al., 2000; Sheng et al., 2012; Steingrobe et al., 2001; Zhang et al., 2012; Zhao et al., 2016). Among their findings, Pellerin et al. (2000) found that P-deficient maize plants exhibited a lower and delayed emergence of adventitious roots. Sheng et al. (2012) found that P fertilization reduced arbuscular mycorrhizal fungal colonization while increasing RLD and the percentage of fine roots in maize. Zhang et al. (2012) observed that P-deficient maize exhibited reduced growth rate, increased P use efficiency, and developed more thin roots with a diameter less than 0.6 mm compared to maize treated with sufficient P. Regarding K deficiency, Zhao et al. (2016) discovered that the K-tolerant maize line had significantly higher root length, volume, and surface area compared to the K-sensitive line under -K treatment. Steingrobe et al. (2001) found that winter barley shoot development and grain yield were reduced under P deficiency, but the standing root system size remained similar to the P-fertilized treatment (although total root production was higher in the P-deficient treatment, root mortality was also higher). In sugar beet, a decrease of RLD was found under N, P and K deficiency (Hadir et al. 2021).

We aim to answer how the above and belowground traits of winter wheat react to the lack of nutrients. Specifically, we are interested in the aboveground traits such as: shoot biomass, leaf area index (LAI), plant height and grain and straw yield. And belowground traits such as: root biomass, total root length, RLD, root mass density (RMD), average root diameter, root length distribution, and link connectivity. Also, we are interested in the main ratios to evaluate the performance of the crop under nutrient-poor conditions, such as specific leaf area (SLA), specific root length (SRL), R/S, and root length per shoot biomass. Beyond examining individual traits, we aim to assess the strength and nature of correlations between shoot and root traits. We also aim to assess the correlations between shoot traits, root traits, and soil nutrient content through Pearson correlation analysis, as well as to evaluate multivariate patterns among traits using PCA to identify how nutrient treatments shape trait interactions.

In line with existing literature, we hypothesize that nutrient deficiencies will lead to reductions in shoot biomass, LAI, root biomass, and root length while increasing the ratios (SLA, SRL, R/S, and root length per shoot biomass) as adaptive responses to nutrient stress.

For this purpose, we have conducted a two-year field campaign in the LTFE Dikopshof , established in 1904, which includes plots maintained under different fertilization treatments: complete fertilizer application (NPKCa), the complete fertilizer treatment plus manure, N fertilizer omission, P fertilizer omission, K fertilizer omission, Ca omission, as well as unfertilized plots, creating soil conditions with varying nutrient deficiencies.

4.2 Materials and Methods

4.2.1 Site description

The field study was carried out in the LTFE Dikopshof during the growing period of winter wheat 2019/20 (growing period 1) and 2020/21 (growing period 2). The LTFE Dikopshof was established in 1904 near Cologne, Germany (50 48' 21" N, 6 59' 9" E, altitude: 61 m), located at the intermediate terrace of the Rhine river. This LTFE is the tenth oldest in the world (Körschens 1997). The groundwater table is about 20 m below the surface. The Atlantic climate with mild winters and summers results in a mean annual temperature of 10.1 °C and a mean annual precipitation of 630 mm. The general soil type is classified as a Haplic Luvisol derived from loess above sand (Holthusen et al. 2012). The depth of the loess layer in the experimental field varies from 1.1 to 1.3 m and is followed by gravel layers. The soil texture can be described as silty loam (topsoil) and (silty) clay loam (below 30 cm soil depth). Soil bulk density ranged from about 1.4 g cm^{-3} in the topsoil to about 1.5-1.6 g cm^{-3} below 30 cm soil depth.

4.2.2 Experimental design and management

The five-year crop rotation at the LTFE Dikopshof comprises sugar beet (*Beta vulgaris*), winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.), a fodder legume, and oat/potato (*Avena sativa* L./*Solanum tuberosum* L., potato replaced oat in 1953). The fodder crop initially used was red clover (*Trifolium pratense*), then lucerne (*Medicago sativa*), and, after 1967, mainly persian clover (*Trifolium resupinatum* L.). In each of the five strips (A to E), one of the five crops of the rotation was grown (Fig. 4.1).

The experiment is a non-randomized block design without replicates (see experimental design in Fig. 4.1) and comprises six core treatments: NPKCa, PKCa, N_KCa, NP_Ca, NPK, and unfertilized (_ stands for the omission of the corresponding nutrient). In addition to these core treatments, three additional variants exist for each: (1) with farmyard manure (FMY) application (+m), (2) with supplemental synthetic fertilizer application introduced since 1953 (+s), and (3) with both FMY and supplemental synthetic fertilizer application (+m+s). The nutrient amounts applied in the +m and +s variants are equivalent, with the +s treatment designed to compensate for the nutrients supplied by FMY in the +m treatment. The inclusion of the NPKCa+m+s treatment represents the highest nutrient input, reflecting

fertilization practices comparable to those currently used in surrounding farms. The fertilizer inputs of the other treatments are lower (and below common amounts applied in conventional farming) as they were fixed in 1906 (partly adapted in 1953) and kept static since then. A total of 60 t ha^{-1} of fresh cattle manure is applied per rotation (20 t ha^{-1} before sugar beet, winter rye, and potato), distributed after harvesting the preceding crop (five-year rotation mean since 1953, treatments "+m"). In total, the experiment consists of five strips with 24 treatments per strip (120 plots). The fertilization management has not changed since 1953, except for a slight increase of the N fertilizer application (+ 30 kg N ha^{-1}) on winter wheat in some treatments, which occurred in the 1980s. The fertilizer amounts applied in winter wheat and in the whole rotation can be seen in Table 4.1. Crop residues and stubble are removed during the entire period, except for roots and senesced potato leaves. Since 1909, the regular depth of plowing has been 30 cm. The plot size is 18.5 × 15 m with a core plot of 10 × 9 m. For more specifics and a detailed list of all crops per strip and year until 2018, is provided in the supplementary information of the study conducted by Seidel et al. (2021). The considered plots cultivated with winter wheat are plots B7 to B13 in strip B (2019/20) and A7 to A13 in strip A (2020/21) shown in Figure 4.1.

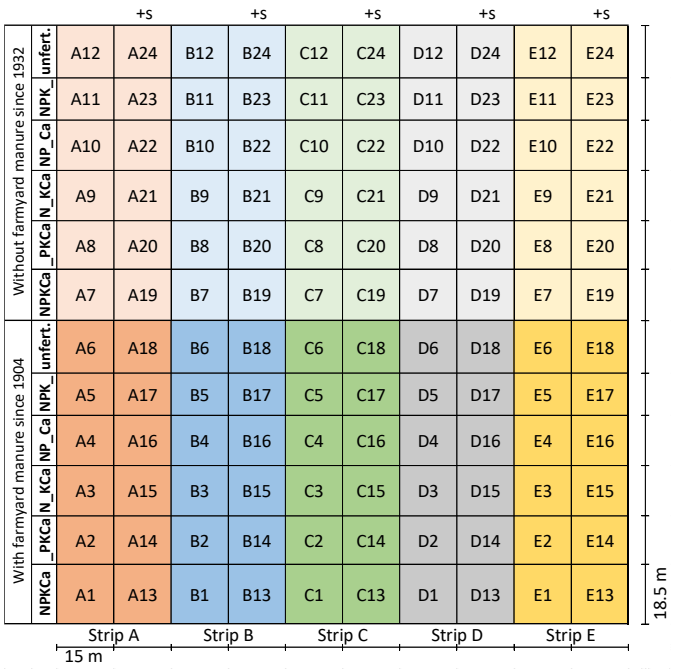

Figure 4.1: Experimental setup of the long-term fertilizer experiment at LTFE Dikopshof after 1953 (strips A to E). The Arabic numbers stand for the treatments. Since 1932, no replicates are available. The experiment is not randomized. Treatment ID 7: NPKCa, 8: _PKCa, 9: N_KCa, 10: NP_Ca, 11: NPK_, 12: unfertilized and 13: NPKCa+m+s. Each color represents a strip. Darker colors represent the plots where farmyard manure is applied, and lighter colors represent the plots without farmyard manure application.

Table 4.1: Considered fertilization treatments and fertilizer application rates (N, P, K, and Ca) in winter wheat and in the whole crop rotation (rotation sum) in the LTFE Dikopshof. Base and supplemental fertilizers are mineral fertilizers. +m stands for manure (nutrients applied via cattle farmyard manure).

		Application rate per element							
		Winter Wheat (kg ha^{-1} yr^{-1})				**Rotation Sum (kg ha^{-1})**			
Treatment	**Type of fertilizer**	**N**	**P**	**K**	**Ca**	**N**	**P**	**K**	**Ca**
	Base	120	31	116	0	290	155	580	1143
NPKCa+m+s	+m					120*	66	249	150
	+s					120	66	249	0
NPKCa	Base	60	31	116	0	230	155	580	1143
_PKCa	Base	0	31	116	0	0	155	580	1143
N_KCa	Base	60	0	116	0	230	0	580	1143
NP_Ca	Base	60	31	0	0	230	155	0	1143
NPK_	Base	60	31	116	0	230	155	580	0
unfertilized	Base	0	0	0	0	0	0	0	0

* N in manure refers to total N

4.2.3 Crop management and sampling

In this study, winter wheat was grown under various treatments: NPKCa+m+s, NPKCa, _PKCa, N_KCa, NP_Ca, NPK_, and an unfertilized treatment. Winter wheat (variety Boss) was sown on 25th November 2019 and harvested on 4th August 2020 in the growing period 1 (2019/20) and sown on 6th November 2020 and harvested on 13th August 2021 in the growing period 2 (2020/21).

The dates of the field campaigns (growing periods 1 and 2), along with the sampled crop traits and the soil characteristics measured during each campaign, are presented in Table 4.2. Each sampling within each plot was measured four times due to the non-randomized block design of the experiment.

Table 4.2: Dates of field campaigns and phenological traits measured during the winter wheat growing period 1 (2019/20) and 2 (2020/21)*.

	Date	BBCH	Phenology	Height (cm)	S_Biomass (t ha^{-1})	LAI (m^2 m2)	N_Till_ Ears	Yield (t ha^{-1})	Soil	Soil_ Moist (%)	Roots
Growing period1 (2019/20)	17/03/2020	23	tillering	x	x	x			x		x
	30/03/2020	23-24	tillering	x							
	7/04/2020	24-25	tillering		x	x					
	22/04/2020	31	stem elongation	x	x	x					
	7/05/2020	32-37	stem elongation	x	x	x					
	19/05/2020	39-45	booting	x	x	x			x	x	x
	26/05/2020									x	
	2/06/2020	65-69	flowering	x						x	
	9/06/2020									x	
	17/06/2020	83-84	ripening	x			x			x	
	23/06/2020									x	
	04/08/2020	89	ripening					x			
Growing period 2 (2020/21)	5/05/2021	24-28	tillering	x	x	x				x	
	19/05/2021									x	
	2/06/2021									x	
	8/06/2021	57-59	heading	x	x	x					
	16/06/2021									x	
	28/06/2021	69	flowering	x	x	x	x		x		x
	13/08/2021	89		x				x			

*BBCH = phenological stage, Height = plant height, S_Biomass = shoot biomass, LAI = leaf area index, N_Till_Ears = number of tillers or ears, Yield = grain and straw yield, Soil = soil nutrient content (C, N, Nmin, Pcal, Kcal, pH), Soil_Moist = soil moisture, Roots = roots traits that comprise RLD, RMD, R/S, SRL, root diameter classes, and root link analysis.

The presented crop, soil and root data comprise:

- Soil total C (%), total N (N_t) (%), soil mineral N (N_{min}) (kg ha^{-1}), plant available P (P_{cal}) (mg kg^{-1}) and K (K_{cal}) (mg kg^{-1}), and pH values
- Volumetric soil water content (%)
- Phenological development stages (BBCH stage)
- Plant height (cm)
- Leaf area index (LAI) (m^2 m^2)
- Dry matter shoot biomass (t ha^{-1})
- Number of tillers and ears per m^2
- Grain and straw yield (t ha^{-1})
- Total root length (cm)
- Average root diameter (mm)
- Root length density by soil layer (RLD) (cm cm^{-3})
- Total root biomass (t ha^{-1})
- Root mass density by soil layer (RMD) (mg cm^{-3})
- Distribution of root length by diameter classes (%)
- Link connectivity (cm cm^{-1})
- Root-to-shoot ratio (R/S)
- Specific leaf area (SLA) (m^2 kg^{-1})
- Specific root length (SRL) (m g^{-1})
- Root length per shoot biomass (km kg^{-1})

The growing period 1 (2019/20) was drier and warmer compared with the growing period 2 (2020/21) (Fig. S4.1 in supplementary information (SI)). The rainfall during growing period 1 (2019/20) was

scarce even in the months where it is usually more abundant (July), otherwise the growing period 2 (2020/21) had greater amounts of rainfall and lower mean temperatures. On 14th July 2021, a heavy rainfall event occurred with 120 mm of precipitation.

4.2.4 Soil sampling and analysis

Soil samples were collected using a Pürckhauer auger, with four replicates per treatment, and divided by layer (0–30 cm, 30–50 cm, and 50–100 cm). The samples were then pooled per treatment and frozen. After thawing, the soil was analyzed for N_{min} by extraction with potassium sulfate solution. N_{min} concentrations in the extracts were measured by a Skalar Continuous Flow Analyser (Skalar Analytical B.V., Breda, Netherlands). Moreover, the soil samples were prepared by drying and sieving, and the contents of C_{org}[1] and N_t were determined using elemental analysis (Euro-EA elemental analyzer from HEKAtech GmbH). P_{cal} and K_{cal} were determined using a calcium acetate-lactate extract as described in Schüller (1969). P concentration in the extracts was determined colorimetrically following molybdenum blue reaction (Murphy and Riley 1962) on a spectrophotometer (Specord 205, Analytik Jena, Germany). K concentration in the extracts was determined by atomic absorption spectroscopy (novAA 400P, Analytik Jena, Germany). In addition, the pH of the soil samples was determined using a $CaCl_2$ solution and a pH Meter Multi 3630 IDS from WTW and Sentix 940P electrode.

Soil moisture content was determined using the FDR moisture sensor HH2 within ML3 Theta Probe (ecoTech Umwelt-Meßsysteme GmbH, Bonn, Germany). Measurements were taken at various soil depths up to 90 cm soil depth. The measurements were conducted directly on the rows after the biomass sampling. See Table 4.2 for the days of sampling.

4.2.5 Shoot observations

4.2.5.1 BBCH

The BBCH-scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) was used to visually identify the phenological development stages of winter wheat per treatment.

4.2.5.2 Plant height

Plant height was measured in the field using a ruler, with measurements taken from the soil surface to the top of the plant without applying any force to stretch the leaves upwards. For each treatment, plant height was measured on 10 plants, and the mean value was calculated.

4.2.5.3 LAI and shoot biomass

The LAI was determined destructively. For that, the plants (entire shoot) of an area of 1 m^2 (four replicates per treatment) were cut and separated into specific organs, including leaves, stems, and ears. The separated parts were then brought to the laboratory and scanned using the LI-3100C Area Meter (Li-Cor). The shoot LAI was calculated for each 1 m^2. The shoot dry matter was determined by oven-

[1] Total carbon was analyzed, and since the soil contains no carbonates, the total C measurement corresponds entirely to organic carbon (C_{org})

drying (at 105 °C) the samples used to estimate the LAI. The dry biomass was then scaled up to tons per hectare.

4.2.5.4 Grain and straw yield

Yield was determined by harvesting three 15 m² areas using a machine that threshed the wheat and separated the straw from the grains. The straw and grains were weighed, and a subsample was taken to the laboratory to estimate moisture content and adjust the yield weight. Both the straw and grain yields were then converted to tons per hectare.

4.2.5.5 Number of tillers and ears

Plants, tillers, and ears per plant were enumerated manually in four separate locations within each treatment, each conducted in a 1 m^2 area.

4.2.5.6 Specific leaf area (m^2 g^{-1})

SLA was calculated based on Equation 4.1. For this calculation, only the leaf area and biomass of the leaves were considered due to the previous separation of the organs while processing.

$$SLA = \frac{Leaf\ surface\ area\ (m^2)}{Dry\ leaf\ biomass\ (g)} \quad 4.1$$

4.2.6 Root sampling, preparation and scanning

Roots of winter wheat plants were extracted twice during growing period 1 (on March 17 and May 19, 2020) and once during growing period 2 (on June 28, 2021) using a root auger with an inner diameter of 9 cm and a length of 1 m. Soil cores were divided into 10 cm segments (0-10 cm, 10-20 cm, ..., 90-100 cm) and placed in plastic bags. Four measurements were collected from each plot, consisting of two samples (measurements 1 and 2) taken within the row (in-row) and two samples (measurements 3 and 4) taken between adjacent rows (between-row). Before sampling, the surrounding area of each sampling point was visually inspected to ensure the absence of any weeds.

The soil samples containing winter wheat roots (280 samples per date) were refrigerated and processed sequentially. To separate the roots from the soil, the samples were soaked in tap water and hand-washed using sieves with mesh sizes of 1 mm, 0.83 mm, and 0.5 mm to remove the coarsest soil and debris. The use of multiple sieve sizes ensured that fine roots were included while allowing the larger sieves to retain most of the roots, making the cleaning process more efficient. Subsequently, the roots were sorted with tweezers, to remove the smallest particles and dead roots. After cleaning, all roots from the different sieves were combined and stored in tap water at 3 °C until scanning.

Roots were scanned using an EPSON scanner (HP Expression 1100XL). Each sample's roots were arranged in an acrylic glass platter filled with tap water, scanned, and analyzed using WinRHIZO (see scanning settings in Fig. S4.2 in SI) software (version Pro 2020a, Regent Instruments, Quebec, Canada).

4.2.7 Total root length (cm) and average root diameter (m)

The total root length and average root diameter per sample were directly estimated using WinRHIZO software.

4.2.8 Root length density by soil layer (cm cm^{-3})

To calculate RLD, the total root length (in cm) was divided by the volume of the core sample, which is 636.17 cm^3 (calculated based on a radius of 4.5 cm and height of 10 cm):

$$RLD = \frac{Root\ length\ in\ the\ soil\ sample\ (cm)}{Volume\ of\ the\ soil\ sample\ (cm^3)} \quad 4.2$$

4.2.9 Root biomass (t ha^{-1})

After analysis, the root weight per soil layer and replicate was determined by drying the roots at 60 °C and weighing the dried roots. Then, the root biomasses per layer were added to calculate the total root biomass for the core profile (1 m depth). The total root biomass was divided by the area of the core (63.52 cm^2) converted to tons per hectare, and then averaged across the four sampling points within each treatment:

$$Root\ biomass = \frac{Dry\ root\ biomass\ throughout\ the\ depth\ of\ the\ soil\ core\ (t)}{Area\ of\ the\ soil\ core\ (ha)} \quad 4.3$$

4.2.9.1 Root mass density by soil layer (mg cm^{-3})

To calculate RMD, the root biomass per layer was divided by the volume of the core sample (636.17 cm^3) and converted to mg per cm^3:

$$RMD = \frac{Dry\ root\ biomass\ in\ the\ soil\ sample\ (mg)}{Volume\ of\ the\ soil\ sample\ (cm^3)} \quad 4.4$$

4.2.9.2 Root-to-shoot ratio

The R/S was calculated by dividing the total root biomass (t ha^{-1}) by the shoot biomass (t ha^{-1}):

$$R/S = \frac{Dry\ root\ biomass\ (t\ ha^{-1})}{Dry\ shoot\ biomass\ (t\ ha^{-1})} \quad 4.5$$

4.2.9.3 Specific root length (m g^{-1})

SRL was calculated by dividing the total root length (m) by the total dry root biomass (g):

$$SRL = \frac{Total\ root\ length\ (m)}{Total\ dry\ root\ biomass\ (g)} \quad 4.6$$

4.2.9.4 Root length per shoot biomass (km^2 kg^{-1})

It was calculated by dividing the total root length (km) by the shoot biomass (kg):

$$Root\ length\ per\ shoot\ biomass = \frac{Total\ root\ length\ (km)}{Dry\ shoot\ biomass\ (kg)} \quad 4.7$$

4.2.9.5 Distribution of root length by diameter classes

WinRHIZO software was used to estimate the sample's root length per diameter class. Based on these results, the root length (per sample) was calculated for three classes: very fine (diameter < 0.15 mm),

fine (diameter between 0.15 - 0.6 mm), and medium (diameter > 0.6 mm). The length per diameter class was calculated and plotted as the length of a diameter class per total length in the sample.

4.2.9.6 Link connectivity

Five root systems within a top- and subsoil layer were selected and processed with WinRHIZO for link analysis. Link analysis categorizes root segments (links) into three different groups: exterior-exterior (EE), exterior-interior (EI), and interior-interior (II) (see a sketch of the link types in Fig. S4.3). The length per link type divided by the total length (in the five root systems) was calculated and plotted. More details on the method can be found in Fitter & Stickland (1991).

4.2.10 Statistical data analyses

The data obtained from the experiment were analyzed using R software (version 4.0.2). Due to the lack of true replication in the experimental setup, we present the data as descriptive statistics, reporting only the mean and standard deviation to show the variability within each treatment. Our analysis focuses on comparing each nutrient-deficient treatment with the fully fertilized treatment (NPKCa) to evaluate the effects of nutrient deficiencies.

Also, for the days when shoot and root were sampled, Pearson's correlation coefficient was calculated to find the linear association between the soil parameters (pH, C_{org}, N_t, N_{min}, P_{cal}, and K_{cal}), aboveground and belowground (only total root length, root biomass, and average root diameter) variables. Pearson analysis was conducted for the entire soil profile (average), topsoil (0-30 cm depth), and subsoil (below 30 cm depth). A detailed description of this statistical analysis can be found in Kirch (2008). For the entire soil profile approach, the average of the soil values were used, except for the first sampling date in 2020 (March 17th, 2020), where only data from the 0-30 cm depth range were available.

In order to compare the differences between treatments, we calculated the relative changes in shoot and root traits using the mean values of the sampling points within each treatment, with the NPKCa treatment as the baseline. The included traits were: grain yield, straw yield, shoot biomass, total root biomass, total root length, SLA, SRL, R/S, and root length per shoot biomass. We compared the treatments within the same growing period and BBCH stage. In summary, we used graphical indicators: green arrows indicating higher values compared to the baseline, yellow arrows representing similar values (ranging from -5% to 5%)[2], and red arrows denoting lower values compared to the baseline.

For the days when shoot and root samples were taken (two in growing period 1 and one in growing period 2), we included a Principal Component Analysis (PCA) to evaluate the relationships and variability among traits measured. PCA reduces the dataset's dimensionality while preserving its variability, enabling the identification of patterns, correlations, and treatment clustering. The analysis incorporated key variables: pH, C_{org}, N_t, N_{min}, K_{cal}, P_{cal}, plant height, LAI, shoot biomass, root biomass, total root length, RLD, RMD, average root diameter, R/S, root length per shoot biomass, SRL, and SLA.

[2] The choice of 5% as the threshold for categorizing changes was made to strike a balance between capturing meaningful variations while avoiding the overinterpretation of minor differences that may arise from inherent variability in the data.

PCA was conducted using the *FactoMineR* package in R. Visualization of the PCA results was performed with the *factoextra* package.

4.3 Results

4.3.1 Below ground parameters and their response to soil nutrients

4.3.2 Soil water content

Throughout both growing periods, the NPKCa treatment exhibited moderate soil water content, with lower values in 2019/20 (11.8% for topsoil and 16.2% for subsoil) than in 2020/21 (18% for topsoil and 18.2% for subsoil). In both years, the unfertilized and _PKCa treatments consistently showed higher soil water content across the soil profile compared to NPKCa. In contrast, the NPKCa+m+s treatment exhibited lower soil water content levels. The N_KCa, NP_Ca, and NPK_ treatments displayed fluctuating soil water content, with variations between increases and decreases relative to NPKCa depending on the measurement date and soil depth (Tables S4.1 and S4.2).

4.3.3 Soil nutrient content

Across all soil layers and sampling dates, the NPKCa+m+s treatment consistently showed the highest nutrient levels (N_{min}, K_{cal}, and P_{cal}). In contrast, the unfertilized treatment displayed the lowest nutrient content and pH levels. Specific deficiencies were observed in particular treatments: N_KCa often had the lowest P_{cal} levels, NP_Ca frequently had the lowest K_{cal} levels (Tables S4.3, S4.4 and S4.5)

4.3.4 Total root length and root length density

Throughout all growing periods and BBCH stages, the total root length of the _PKCa and unfertilized treatments was consistently lower than that of the NPKCa treatment. Additionally, the NPKCa+m+s and NPK_ treatments showed similar or even greater total root lengths compared to the NPKCa treatment (see Fig. 4.2 and for detailed information, refer to Table S4.6, S4.7, and S4.8). The differences are also illustrated in Figure 4.3, which displays the distribution of RLD throughout the entire soil profile.

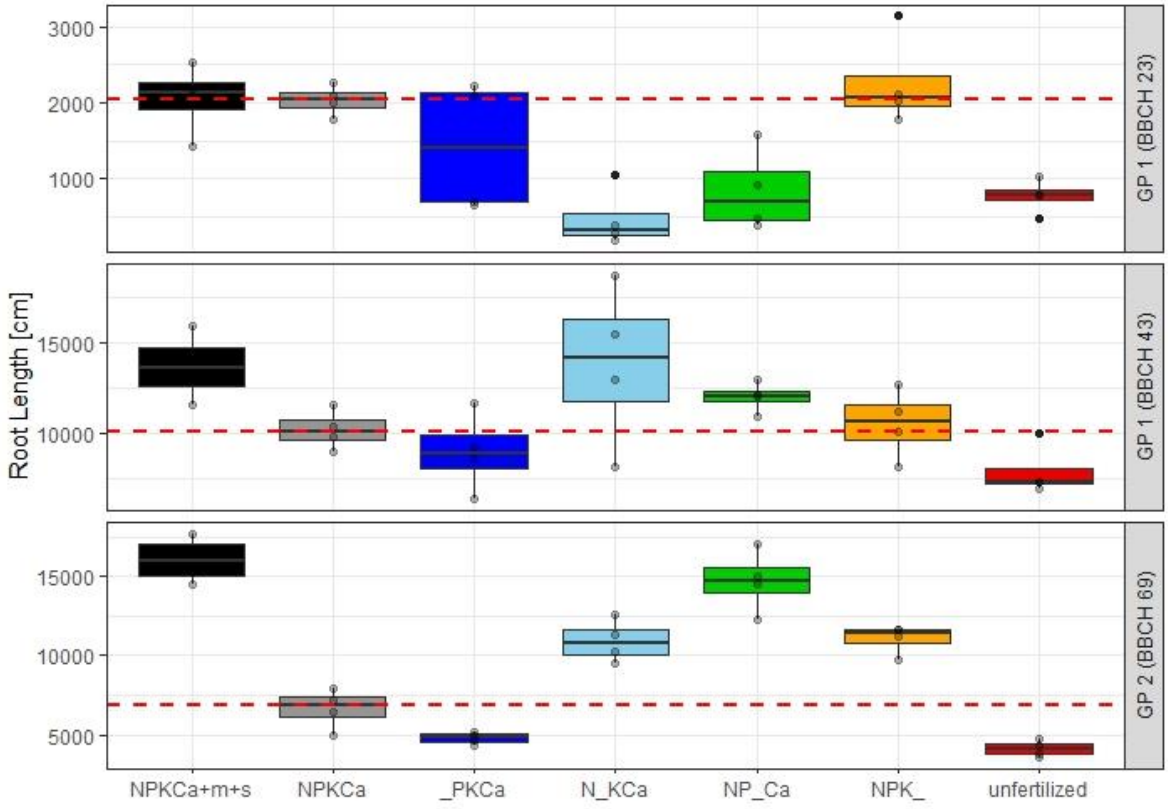

Figure 4.2: Total root length (cm) across different treatments during GP1: growing period 1 (2019/20 - BBCH 23), growing period 1 (2019/20 - BBCH 43) and GP2: growing period 2 (2020/21 - BBCH 69). The red dashed line represents the median root length of the NPKCa treatment as a baseline for comparison. Boxplots indicate the variability and median root length within each treatment.

At tillering in growing period 1 (2019/20 – BBCH 23), the RLD of the NPKCa treatment was 0.98 cm cm^{-3} in the topsoil and 0.07 cm cm^{-3} in the subsoil. Only the NPK_ treatment exhibited a greater RLD, showing a 12% increase in both the top and subsoil compared to NPKCa. Most of the other treatments, including NPKCa+m+s, _PKCa, N_KCa, NP_Ca, and the unfertilized treatments, displayed lower RLD values, varying from 6% to 78% in both topsoil and subsoil.

At booting of growing period 1 (2019/20 – BBCH 43), the root length density (RLD) for the NPKCa treatment was 2.29 cm cm^{-3} in the topsoil and 1.30 cm cm^{-3} in the subsoil. At flowering of growing period 2 (2020/21 – BBCH 69), these values were 2.12 cm cm^{-3} in the topsoil and 0.58 cm cm^{-3} in the subsoil. Treatments such as NPKCa+m+s, N_KCa, NP_Ca, and NPK_ exhibited higher total root lengths and RLDs than NPKCa in both soil layers. Increases ranged from 1% to 62% at booting and from 37% to 106% at flowering in the topsoil. In the subsoil, the increases were between 5% and 27% at booting and between 82% and 209% at flowering. In contrast, the unfertilized and _PKCa treatments showed lower RLDs compared to NPKCa, with reductions of 20% to 30% in the topsoil and 25% to 47% in the subsoil across both growth stages.

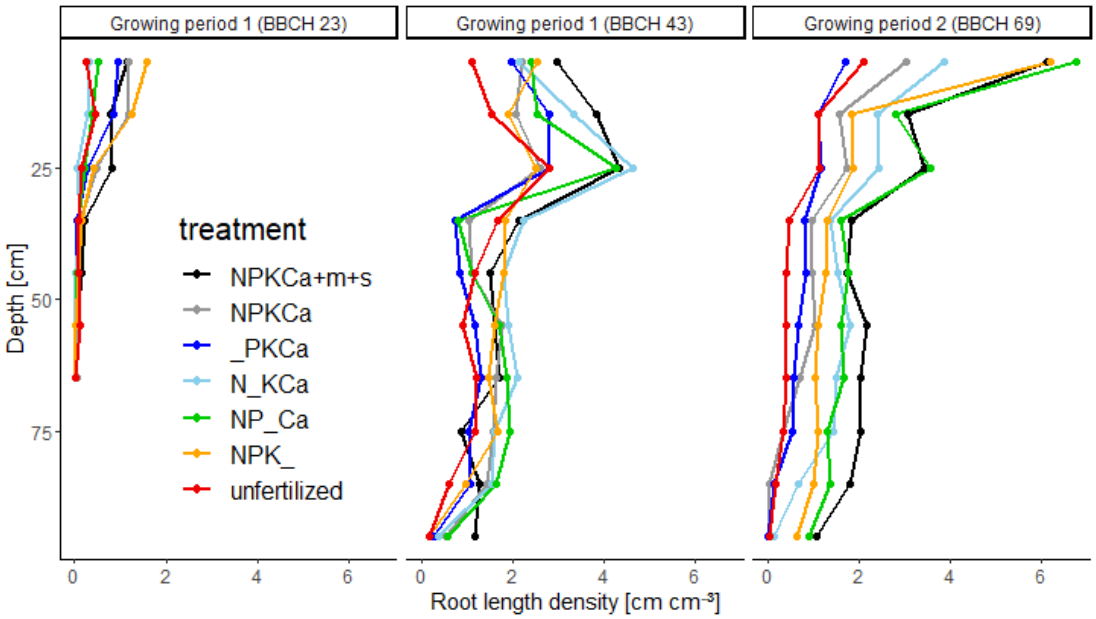

Figure 4.3: Observed mean (n=4) root length density (cm cm^{-3}) in seven treatments of the LTFE Dikopshof, Germany, for growing period 1 (2019/20 - BBCH 23), growing period 1 (2019/20 - BBCH 43) and, growing period 2 (2020/21 - BBCH 69). Standard deviation within treatment is shown in SI in Table S4.6, S4.7 and S4.8.

4.3.5 Root biomass and root mass density

Throughout all growing periods and BBCH stages, the _PKCa and unfertilized treatments consistently showed lower total root biomass compared to the NPKCa treatment. In contrast, the NPKCa+m+s treatment exhibited greater root biomass values relative to NPKCa. The N_KCa, NP_Ca, and NPK_ treatments displayed variable results (see Fig. 4.4 for reference and detailed information in Tables S4.6, S4.7, and S4.8). The differences are also illustrated in Figure 4.5, which displays the distribution of RMD throughout the entire soil profile. Additionally, a figure showing both aboveground and belowground biomass is presented in Fig. S4.4 of the SI.

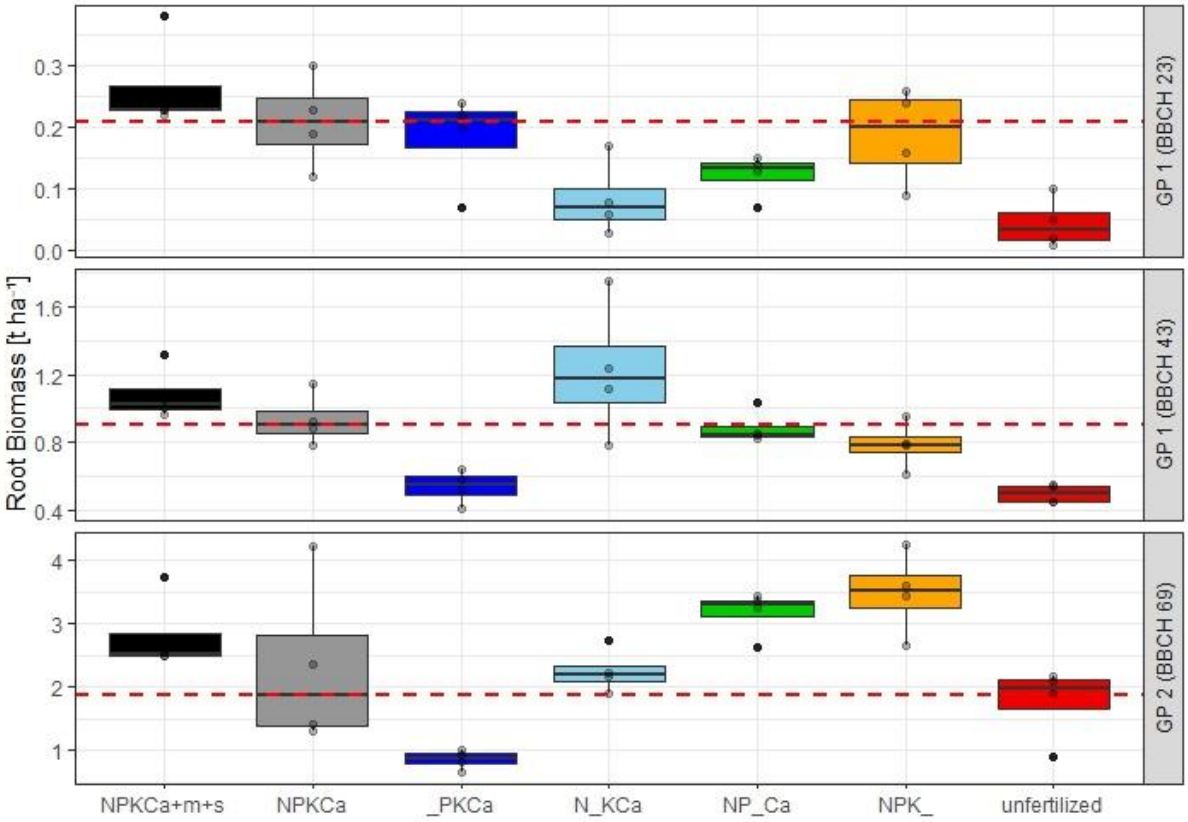

Figure 4.4: Total root biomass (t ha^{-1}) across different treatments during GP1: growing period 1 (2019/20 - BBCH 23), growing period 1 (2019/20 - BBCH 43) and GP2: growing period 2 (2020/21 - BBCH 69). The red dashed line represents the median root biomass of the NPKCa treatment as a baseline for comparison. Boxplots indicate the variability and median root biomass within each treatment.

When examining the different soil profiles (Fig. 4.5), at tillering in growth period 1 (2019/20 – BBCH 23), at booting in growth period 1 (2019/20 – BBCH 43), and at flowering in growth period 2 (2020/21 – BBCH 69), the RMD of NPKCa+m+s was greater (by between 2% and 44%) than the RMD of NPKCa in the topsoil. The treatments with _PKCa and the unfertilized areas exhibited lower RMD in both the topsoil and subsoil across all growth periods. Additionally, N_KCa, NP_Ca, and NPK_ treatments showed higher RMD at booting (only topsoil) and at flowering (only subsoil).

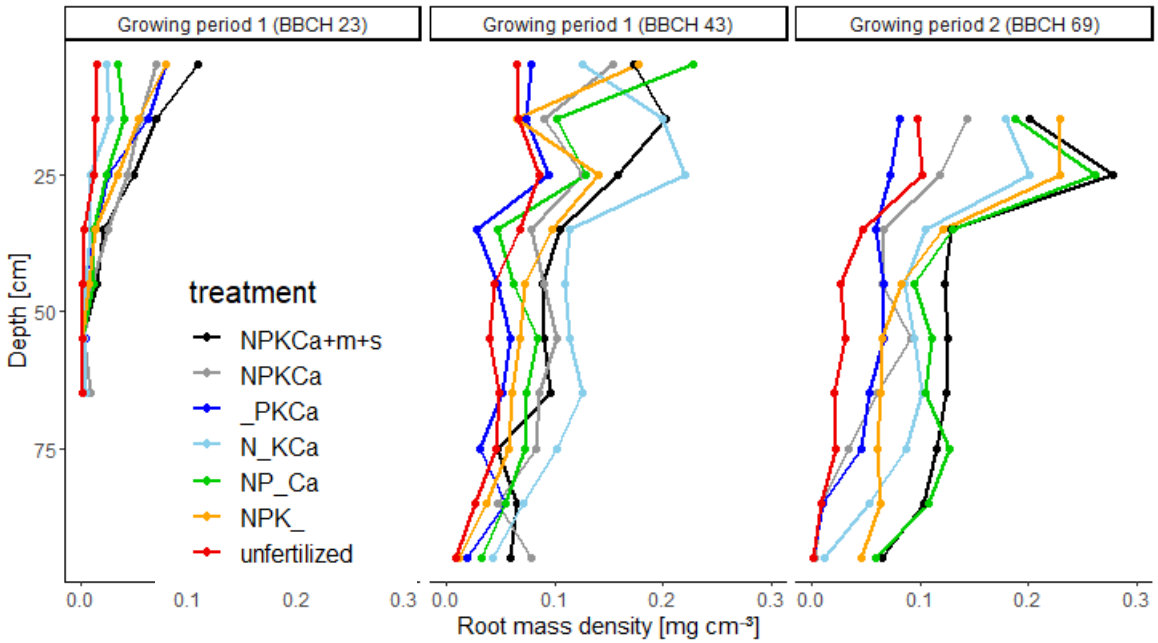

Figure 4.5: Observed mean (n=4) root mass density (mg cm^{-3}) in seven treatments of the LTFE Dikopshof, Germany for growing period 1 (2019/20 - BBCH 23), growing period 1 (2019/20 - BBCH 43) and growing period 2 (2020/21 - BBCH 69). The values of RMD of the first layer (0-10 cm) in growing period 2 (BBCH 69) were omitted to avoid distorting the graph and allow a comparison between treatments. However, the values can be observed in Table S4.8 of the supplementary material. Standard deviation within treatment is shown in SI in Tables S4.6, S4.7, and S4.8.

4.3.6 Root length distribution based on average root diameter

During growing period 1 (2019/20 - BBCH 23), treatments N_KCa and unfertilized showed that more than 50% of the total root length consisted of very fine roots (diameter less than 0.15 mm). The remaining treatments had approximately 30-40% of very fine roots. For growing period 1 (2019/20 - BBCH 43) and growing period 2 (2020/21 - BBCH 69), the root length distribution was similar across all treatments, with around 50% of very fine roots in growing period 1 (2019/20 - BBCH 43) and around 20% in growing period 2 (2020/21 - BBCH 69) (see Fig. S4.5 in the supplementary material for details).

4.3.7 Link analysis

At both sampling dates (BBCH 23 and 43) during growing period 1, the treatments _PKCa, N_KCa, NP_Ca, and unfertilized led to more external-external (EE) links in the topsoil roots than NPKCa (Fig. S6a, S6b in supplementary material). In the subsoil (60-70 cm depth) at BBCH 23, all treatments developed less EE links than NPKCa (Fig. S4.6a and Table S4.9). In contrast, at BBCH 43, the link types were similar among all treatments in the subsoil (80-90 cm) (Fig. S4.6b and Table S4.10).

In growing period 2 (2020/21, BBCH 69), the proportion of EE, EI, and II links were similar among treatments in the topsoil (Fig. S4.6c). However, in the subsoil, roots in treatments NPKCa, _PKCa, and the unfertilized treatment developed more EE links than the remaining treatments (Fig. S4.6c and Table S4.11).

4.3.8 Above ground parameters

4.3.8.1 Shoot biomass, leaf area index, and plant height

Throughout growing periods 1 (2019/20) and 2 (2020/21), the NPKCa+m+s treatment exhibited greater shoot dry biomass, LAI, and plant height in comparison with the NPKCa treatment, as indicated in Tables 4.3 and 4.4. During growing period 1 (2019/20), the unfertilized and _PKCa treatments consistently yielded the lowest values on all sampling dates. On the other hand, NPKCa, N_KCa, NP_Ca, and NPK_ resulted in similar shoot dry matter, LAI, and plant height across most of the sampling dates in growing period 1 (2019/20) (see Table 4.3 and Table S4.12).

Table 4.3: Observations of the mean phenological development stage (BBCH stage), shoot dry biomass, leaf area index (m^2 m^{-2}), and plant height (cm) of winter wheat cultivated in seven treatments of the LTFE Dikopshof, Germany, in growing period 1 (2019/20). Means with standard deviation within treatment are presented. Some dates were excluded in this table but are shown in Table S4.12 in the SI.

Samp. Date	Treatment	BBCH	Shoot dry biomass (t ha^{-1})	LAI (m^2 m^{-2})	Plant height (cm)
17/03/2020	NPKCa+m+s	23	0.25 ± 0.02	0.31 ± 0.03	6.2 ± 0.6
	NPKCa	23	0.23 ± 0.06	0.26 ± 0.06	5.3 ± 0.5
	_PKCa	23	0.16 ± 0.01	0.21 ± 0.02	5.1 ± 0.4
	N_KCa	23	0.18 ± 0.01	0.22 ± 0.01	4.6 ± 1.0
	NP_Ca	23	0.16 ± 0.03	0.19 ± 0.04	5.0 ± 0.5
	NPK_	23	0.17 ± 0.01	0.21 ± 0.01	6.2 ± 0.6
	unfertilized	23	0.07 ± 0.02	0.09 ± 0.03	5.0 ± 0.2
7/04/2020	NPKCa+m+s	25	0.83 ± 0.11	0.99 ± 0.17	
	NPKCa	24	0.54 ± 0.08	0.56 ± 0.03	
	_PKCa	24	0.43 ± 0.03	0.44 ± 0.03	
	N_KCa	24	0.52 ± 0.04	0.54 ± 0.03	
	NP_Ca	24	0.58 ± 0.04	0.61 ± 0.05	
	NPK_	25	0.61 ± 0.12	0.59 ± 0.16	
	unfertilized	24	0.22 ± 0.08	0.21 ± 0.07	
22/04/2020	NPKCa+m+s	31	1.86 ± 0.09	1.86 ± 0.14	20.7 ± 2.8
	NPKCa	31	1.16 ± 0.11	1.07 ± 0.12	14.5 ± 1.2
	_PKCa	31	0.86 ± 0.08	0.82 ± 0.07	11.5 ± 1.0
	N_KCa	31	1.29 ± 0.05	1.22 ± 0.06	13.2 ± 1.5
	NP_Ca	31	1.12 ± 0.15	1.08 ± 0.15	12.3 ± 1.8
	NPK_	31	1.14 ± 0.29	0.98 ± 0.26	14.9 ± 1.8
	unfertilized	31	0.45 ± 0.18	0.41 ± 0.16	9.2 ± 1.4
7/05/2020	NPKCa+m+s	37	4.87 ± 0.36	3.89 ± 0.29	43.0 ± 4.0
	NPKCa	37	2.77 ± 0.37	1.84 ± 0.25	33.4 ± 0.3
	_PKCa	37	2.06 ± 0.12	1.35 ± 0.05	26.7 ± 1.3
	N_KCa	37	3.35 ± 0.45	2.17 ± 0.26	30.2 ± 2.5
	NP_Ca	37	2.89 ± 0.61	1.96 ± 0.49	31.7 ± 2.5
	NPK_	37	2.77 ± 0.31	1.72 ± 0.18	34.6 ± 1.1
	unfertilized	32	0.58 ± 0.21	0.34 ± 0.12	20.9 ± 1.8
19/05/2020	NPKCa+m+s	43	5.11 ± 0.89	2.28 ± 0.36	54.1 ± 4.3
	NPKCa	43	4.40 ± 0.80	1.68 ± 0.25	45.5 ± 1.4
	_PKCa	43	3.51 ± 0.54	1.15 ± 0.21	36.8 ± 0.9
	N_KCa	45	4.83 ± 0.42	1.82 ± 0.11	45.5 ± 3.4
	NP_Ca	43	4.38 ± 0.81	1.72 ± 0.25	43.6 ± 1.1
	NPK_	43	4.91 ± 0.87	1.94 ± 0.31	48.7 ± 2.8
	unfertilized	39	1.91 ± 0.22	0.74 ± 0.05	35.4 ± 2.8

In growing period 2 (2020/21) across all sampling dates, the NP_Ca, NPKCa+m+s, N_KCa, and NPK_ treatments exhibited higher values for shoot biomass and LAI than NPKCa treatment while the _PKCa and unfertilized treatments produced lower values for these traits in comparison with NPKCa (refer to Table 4.4).

Table 4.4: Observations of the mean phenological development stage (BBCH stage), shoot dry biomass, leaf area index (m^2 m^{-2}), and plant height (m) of winter wheat cultivated at seven treatments at the LTFE Dikopshof, Germany, in growing period 2 (2020/21). Means with standard deviation within treatment are presented.

Samp. Date	Treatment	BBCH	Dry biomass (t ha^{-1})	LAI (m^2 m^{-2})	Plant height (m)
5/05/2021	NPKCa+m+s	28	0.91 ± 0.11	0.85 ± 0.14	21.6 ± 1.9
	NPKCa	27	0.85 ± 0.27	0.85 ± 0.36	19.5 ± 1.4
	_PKCa	26	0.29 ± 0.06	0.30 ± 0.05	16.1 ± 0.4
	N_KCa	26	0.99 ± 0.15	0.91 ± 0.09	22.3 ± 0.7
	NP_Ca	25	1.24 ± 0.16	1.18 ± 0.14	25.5 ± 0.9
	NPK_	26	0.95 ± 0.09	0.86 ± 0.10	22.6 ± 0.7
	unfertilized	24	0.28 ± 0.07	0.23 ± 0.05	16.9 ± 0.8
8/06/2021	NPKCa+m+s	59	5.08 ± 0.64	2.12 ± 0.22	73.6 ± 1.0
	NPKCa	58	4.27 ± 0.44	1.71 ± 0.12	72.1 ± 2.6
	_PKCa	57	1.04 ± 0.11	0.47 ± 0.03	49.6 ± 2.2
	N_KCa	57	5.11 ± 0.66	1.90 ± 0.30	69.1 ± 1.5
	NP_Ca	59	6.55 ± 0.68	2.27 ± 0.20	73.5 ± 1.5
	NPK_	59	5.77 ± 0.34	2.53 ± 0.17	71.6 ± 0.3
	unfertilized	58	0.77 ± 0.19	0.35 ± 0.07	44.4 ± 1.0
28/06/2021	NPKCa+m+s	69	10.33 ± 0.78	1.82 ± 0.07	78.4 ± 1.1
	NPKCa	69	8.02 ± 1.10	1.35 ± 0.25	74.0 ± 1.4
	_PKCa	69	1.98 ± 0.16	0.32 ± 0.02	51.2 ± 1.1
	N_KCa	69	8.20 ± 0.45	1.32 ± 0.12	73.2 ± 1.2
	NP_Ca	69	11.89 ± 0.70	1.64 ± 0.10	75.2 ± 1.6
	NPK_	69	10.11 ± 0.83	1.50 ± 0.11	74.9 ± 1.0
	unfertilized	69	1.30 ± 0.24	0.21 ± 0.04	49.0 ± 3.5

4.3.9 Plants and ears per m^2

During growing period 1 (2019/20) at BBCH 23-28, the NPKCa treatment recorded the highest plant density, reaching 151 plants per m^2. In comparison, all the other treatments had lower densities (the lowest was the unfertilized treatment with only 54 plants per m^2). Conversely, in the second growing period (2020/21) at BBCH 69, the NPKCa treatment had one of the lowest plant densities, with only 251 plants per m^2. This was just above the _PKCa treatment (135 plants per m^2), and the unfertilized treatment (167 plants per m^2). The NPKCa+m+s treatment exhibited the highest plant and ear density. Detailed data on plant and ear counts for all treatments during both growing periods are presented in Table S4.13.

In growing period 1 (2019/20), the unfertilized treatment consistently exhibited delayed growth, as indicated by retarded BBCH stages, in comparison to the other treatments. However, in growing period 2 (2020/21), both the _PKCa and unfertilized treatments inhibited the plant development, particularly during the tillering and early flowering stages.

4.3.10 Grain and straw yield

The omission of N and the absence of any fertilization resulted in a reduction in both straw and grain yield during both growing periods. Conversely, the straw and grain yield of the NPKCa, N_KCa, NP_Ca, and NPK_ treatments were similar between each other during either growing period. The

NPKCa+m+s treatment exhibited particularly high grain yield, as well as straw yield, during both growing periods (as depicted in Fig. 4.6, Fig. 4.7 and Fig. S4.7).

In the growing period 2 (2020/21), the mean grain yield exhibited an average reduction of 27% as compared to the growing period 1 (2019/20). However, the decline was more pronounced in the unfertilized and N_KCa treatments, with a reduction of 52% and 34%, respectively. NPK_ and NP_Ca treatments showed a relatively mild decline, with a reduction of 10% and 16%, respectively (Fig. 4.5). In contrast, the straw yield behaved differently, with most treatments showing an increase in the growing period 2 (2020/21) compared to the growing period 1 (2019/20). NPK_, NP_Ca, and NPKCa treatments showed an increase in straw yield (60%, 43%, and 43%, respectively), whereas N_KCa exhibited a slight increase of 5%. However, the _PKCa and unfertilized treatments were severely affected, showing a reduction of 81% and 75%, respectively (Fig. 4.7).

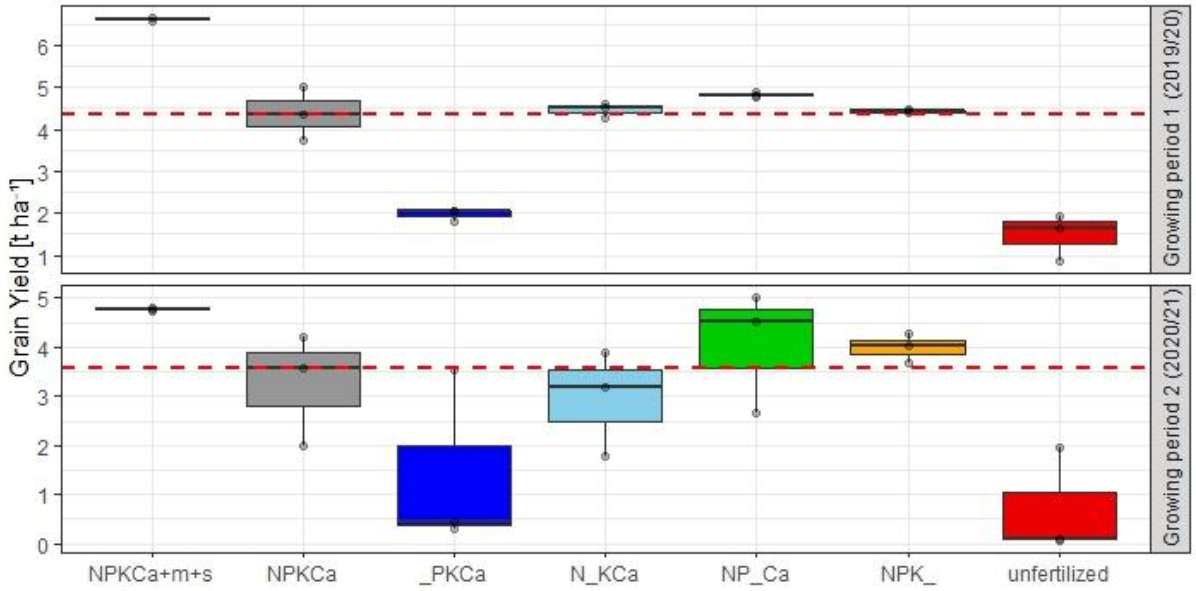

Figure 4.6: Observed dry matter grain yield of winter wheat cultivated in seven treatments of the LTFE Dikopshof, Germany during growing period 1 (2019/20) and growing period 2 (2020/21). The dashed red line represents the median of NPKCa treatment.

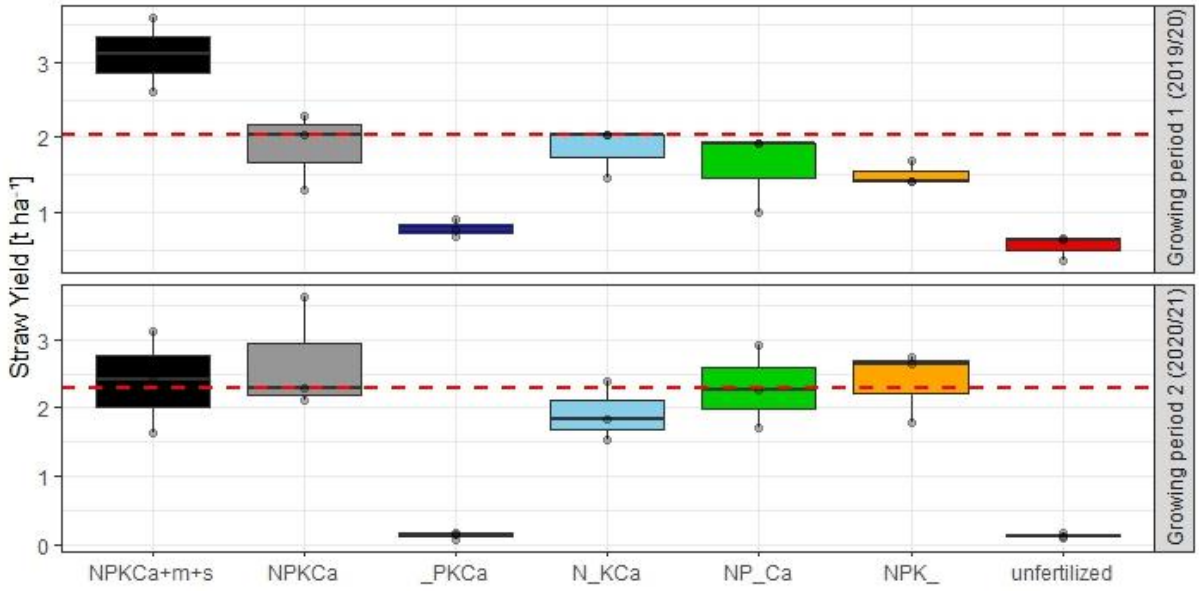

Figure 4.7: Observed dry matter straw yield of winter wheat cultivated in seven treatments of the LTFE Dikopshof, Germany during growing period 1 (2019/20) and growing period 2 (2020/21). The dashed red line represents the median of NPKCa treatment.

4.3.11 Performance-related ratios for above and below ground traits: SLA, SRL, R/S and Root length per shoot biomass.

The performance-related ratios during the two root sampling dates in growing period 1 (2019/20 – BBCH 23 and BBCH 43) and the one root sampling date in growing period 2 (2020/21 -BBCH 69) are shown in Table 4.5

Table 4.5: Specific leaf area (m^2 kg^{-1}), specific root length (m g^{-1}), root-to-shoot ratio and root length per shoot biomass (km kg^{-1}) during the two root sampling dates in growing period 1 (2019/20 – BBCH 23 and BBCH 43) and the one root sampling date in growing period 2 .(2020/21 -BBCH 69).

Period	treatment	Specific leaf area (m2 kg-1)			Specific root length (m g-1)			Root-to-shoot ratio			Root length per shoot biomass (km kg-1)		
Growing period 1 (2019/20) Tillering	NPKCa+m+s	12.7	±	12.7	124.5	±	29	1.08	±	0.3	130.4	±	130.4
	NPKCa	11.7	±	11.7	158.4	±	56.6	0.93	±	0.26	146.5	±	146.5
	_PKCa	12.8	±	12.8	119.1	±	44.9	1.12	±	0.49	134.7	±	134.7
	N_KCa	12.2	±	12.2	80.9	±	20.1	0.46	±	0.32	39.9	±	39.9
	NP_Ca	12.4	±	12.4	102	±	44.4	0.79	±	0.22	79.3	±	79.3
	NPK_	12.1	±	12.1	201.9	±	61.1	1.09	±	0.47	207.1	±	207.1
	unfertilized	11.4	±	11.4	304.3	±	177.1	0.68	±	0.74	177.2	±	177.2
Growing period 1 (2019/20) Booting	NPKCa+m+s	12.5	±	12.5	198.3	±	15.7	0.21	±	0.02	42.7	±	42.7
	NPKCa	10.8	±	10.8	172.1	±	12.2	0.21	±	0.03	36.9	±	36.9
	_PKCa	9.1	±	9.1	273.6	±	98.1	0.16	±	0.06	39.8	±	39.8
	N_KCa	10.6	±	10.6	177.5	±	26.5	0.26	±	0.1	45.3	±	45.3
	NP_Ca	11.1	±	11.1	214.4	±	25.1	0.21	±	0.04	44.8	±	44.8
	NPK_	11.1	±	11.1	212.1	±	37.4	0.16	±	0.02	33.9	±	33.9
	unfertilized	10.8	±	10.8	248.8	±	35.4	0.26	±	0.06	66.2	±	66.2
Growing period 2 (2020/21) Flowering	NPKCa+m+s	11.1	±	11.1	91.2	±	13.1	0.28	±	0.07	24.5	±	24.5
	NPKCa	12.9	±	12.9	52.8	±	19.1	0.28	±	0.13	13.2	±	13.2
	_PKCa	9.7	±	9.7	90.1	±	21.3	0.44	±	0.1	38.2	±	38.2
	N_KCa	10.8	±	10.8	78.2	±	20.6	0.28	±	0.13	20.9	±	20.9
	NP_Ca	8.3	±	8.3	72.6	±	12.4	0.27	±	0.04	19.5	±	19.5
	NPK_	8.3	±	8.3	50.4	±	6.7	0.35	±	0.09	17.2	±	17.2
	unfertilized	8.9	±	8.9	40.7	±	17.2	1.36	±	0.41	50.4	±	50.4

4.3.12 Relative changes of the traits and indices under nutrient deficiency compared to full fertilization

The relative change of shoot and root traits across different treatments with NPKCa as the baseline (Fig. 4.8) showed that the NPKCa+m+s treatment consistently enhances most parameters such as grain and straw yield, shoot and root biomass, root length, SLA, SRL, and root length per shoot biomass. The R/S was similar to the baseline. Additionally, N deficiency and unfertilized conditions reduce grain and straw yield, root and shoot biomass, and root length but increased SRL, R/S, and root length per shoot biomass, with some exceptions. Furthermore, P, K, and Ca deficiency reduced straw yield but increased root length and the root length per shoot biomass, with some exceptions.

	Growing Period	BBCH	NPKCa+m+s	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
Grain yield (t ha^{-1})	1 (2019/20)	89	51%	-55%	2%	10%	1%	-66%
	2 (2020/21)	89	46%	-56%	-9%	25%	22%	-78%
Straw yield (t ha^{-1})	1 (2019/20)	89	65%	-58%	-2%	-14%	-20%	-70%
	2 (2020/21)	89	-11%	-95%	-28%	-14%	-10%	-95%
Shoot biomass (t ha^{-1})	1 (2019/20)	23	9%	-27%	-20%	-31%	-23%	-67%
	1 (2019/20)	43	16%	-20%	10%	0%	12%	-56%
	2 (2020/21)	69	29%	-75%	2%	48%	26%	-84%
Total root biomass (t ha^{-1})	1 (2019/20)	23	26%	-13%	-60%	-42%	-11%	-79%
	1 (2019/20)	43	16%	-42%	31%	-5%	-16%	-47%
	2 (2020/21)	69	21%	-63%	-3%	36%	50%	-24%
Total root length (m)	1 (2019/20)	23	1%	-30%	-77%	-59%	11%	-62%
	1 (2019/20)	43	34%	-12%	36%	18%	3%	-23%
	2 (2020/21)	69	142%	-28%	65%	122%	66%	-38%
Specific leaf area (m^2 kg^{-1})	1 (2019/20)	23	8%	9%	4%	6%	3%	-2%
	1 (2019/20)	43	16%	-15%	-2%	3%	3%	1%
	2 (2020/21)	69	-13%	-25%	-16%	-36%	-35%	-31%
Specific root length (m g^{-1})	1 (2019/20)	23	-21%	-25%	-49%	-36%	27%	92%
	1 (2019/20)	43	15%	59%	3%	25%	23%	45%
	2 (2020/21)	69	73%	71%	48%	37%	-5%	-23%
Root-to-shoot ratio	1 (2019/20)	23	16%	20%	-50%	-15%	18%	-27%
	1 (2019/20)	43	0%	-26%	20%	-3%	-25%	23%
	2 (2020/21)	69	-3%	56%	-1%	-5%	24%	378%
Root length per shoot biomass (km kg^{-1})	1 (2019/20)	23	-11%	-8%	-73%	-46%	41%	21%
	1 (2019/20)	43	16%	8%	23%	21%	-8%	79%
	2 (2020/21)	69	85%	188%	58%	47%	30%	280%

Figure 4.8: Relative change of shoot and root trait across different treatments. The baseline for the analysis is the values of NPKCa treatment. Green arrows indicate higher values, yellow arrows represent similar values (from -5% to 5%), and red arrows denote lower values. Comparisons were made between treatments within the same growing period and BBCH.

4.3.13 Correlation of traits

Data used in Pearson correlation is included in Table S4.14. In the entire profile, LAI and shoot biomass consistently showed a moderate positive correlation with C_{org}, total soil N_t, and N_{min} at all sampling dates. Root biomass consistently exhibited a moderate positive correlation with LAI and shoot biomass in both growing periods and all BBCH stages. Root length consistently displayed a positive moderate correlation with root biomass in all three sampling dates in both growing periods (Fig. 4.9).

Additionally, during growing period 1 (2019/20) at BBCH 23, LAI and shoot biomass displayed a moderate positive correlation with soil pH, K_{cal}, and P_{cal} (which is not observed in growing period 2). Average root diameter displayed a moderate positive correlation with soil nutrients, root length and root biomass (Fig. 9a). However, in the same growing period at BBCH 43, average root showed no significant correlation with the other analyzed parameters (Fig. 9b). In growing period 2 (2020/21) at BBCH 69, the average root diameter displayed a moderate negative correlation with soil pH, C_{org}, total soil N_t, K_{cal}, and P_{cal} (Fig. 4.9c).

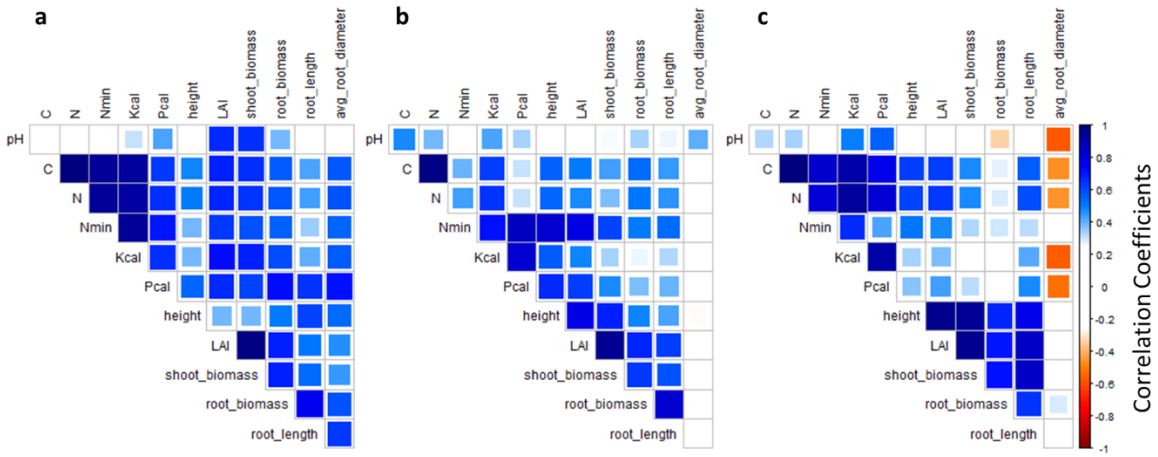

Figure 4.9: Pearson correlation for soil properties, above and below ground traits during the shoot-root sampling dates: a) First sampling date in growing period 1 (2019/20, BBCH=23), b) Second sampling date in growing period 1 (2019/20, BBCH=43), and, c) First sampling date in growing period 2 (2020/21, BBCH=69). P-values between -0.3 to 0.3 are not significant.

Across all sampling dates, there are strong positive correlations between C_{org}, N_t, N_{min}, K_{cal}, and P_{cal} in both the topsoil and subsoil (Fig. S4.8 and S4.9). Aboveground traits such as height, LAI, and shoot biomass correlate strongly with each other. Belowground traits (root traits) generally show positive correlations with shoot traits.

In the topsoil (Fig. S4.8), LAI, shoot, and root biomass were positively correlated with soil nutrients during growing period 1 (2019/20 - BBCH 23), particularly with P_{cal}. Furthermore, N_{min} was positively correlated with LAI, shoot biomass, root biomass, and root length during growing period 1 (2019/20 - BBCH 43). Average root diameter exhibited a strong negative correlation with other traits in growing period 2 (2020/21 - BBCH 69).

In the subsoil (Fig. S4.9), specifically, N_{min} positively correlates with LAI and shoot biomass during growing period 1 (BBCH 43). Furthermore, C_{org} and N_t positively correlate with plant height, LAI, shoot and root biomass, and root length during growing period 2 (2020/21 - BBCH 69).

4.3.14 PCA analysis

The PCA analysis was conducted to highlight the variability among treatments based on key shoot and root variables. The scree plot (Fig. S4.10 in SI) shows that the first two principal components (PC1 and PC2) account for a combined 67.2% of the total variance, with PC1 explaining 47.2% and PC2 20%.

PC1 primarily separates treatments based on variables such as root biomass, shoot biomass, LAI, and plant height, with positive associations along the positive PC1 axis (Fig. 4.10). PC2 captures secondary variability, driven by traits such as SLA and root length.

The treatments exhibited distinct responses in growth performance. The NPKCa+m+s treatment formed a separate cluster with positive associations on PC1 and PC2, indicating increased root and shoot biomass (Fig. 4.10). In contrast, the unfertilized treatment aligned negatively with PC1, reflecting poor performance across the analyzed traits. The NPK_ and NP_Ca treatments overlapped, suggesting similar

moderate responses. Other treatments, such as NPKCa, N_KCa and _PKCa, varied along PC1 and PC2 based on their specific trait contributions.

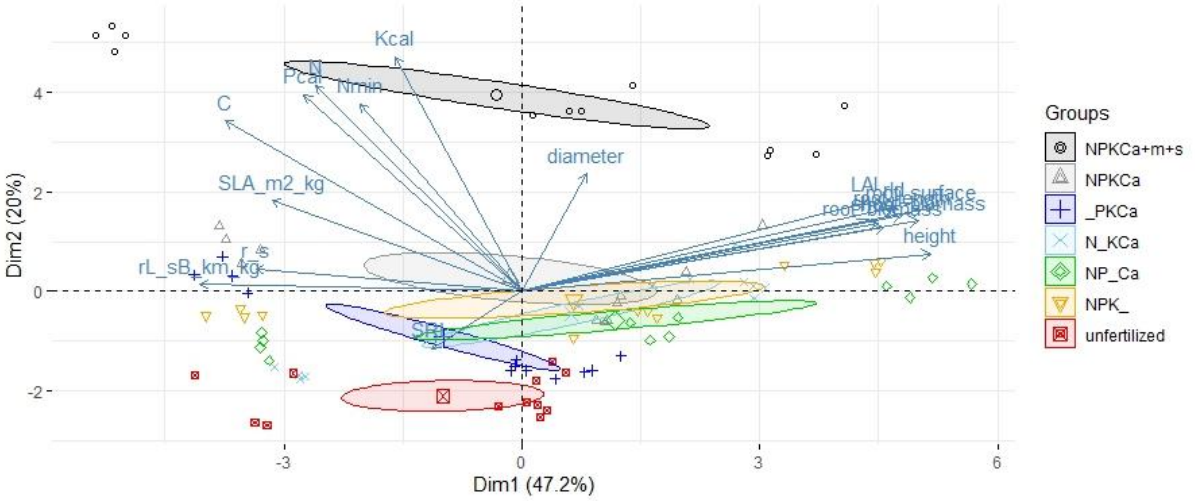

Figure 4.10: PCA biplot illustrating the relationships between trait variables (arrows) and treatments (symbols) across the first two principal components (Dim1 = PC1 and Dim 2 = PC2). The arrows represent the contributions of variables, with their direction and length indicating the strength and influence on the principal components. Treatments are plotted based on their scores along PC1 and PC2, showing the variability and separation of responses to different treatments

4.4 Discussion

The aim of this study was to investigate the response of above and belowground traits to nutrient deficiencies and evaluate the performance of the crop under nutrient-poor conditions. Specifically, we examined aboveground traits such as shoot biomass, LAI, and plant height, as well as belowground traits including root biomass, total root length, RLD, and various ratios that provide insights into plant performance. We expected to observe a decrease in shoot biomass, LAI, root biomass, and root length under nutrient deficiencies while anticipating an increase in the examined ratios as indicators of nutrient stress. Some of our results align with previous studies and support these expectations.

4.4.1 Belowground traits and their responses to soil nutrients

As expected, total root biomass, and length were highest in the most nutrient-rich treatment (NPKCa+m+s) and lowest in treatments with low nutrient availability, particularly under severe N deficiency (_PKCa) and in the unfertilized treatment. This decline aligns with findings that nutrient deficiencies reduce root growth due to limited resource availability for cell division and elongation (Giehl and von Wirén 2014).

While severe N deficiency negatively impacted absolute root biomass, root length (especially in the subsoil), and RLD in our study, previous research has shown that mild N deficiency can stimulate root growth as plants increase their nutrient foraging efforts (NaNagara et al. 1976; Comfort et al. 1988; Peng et al. 2012; Jia and von Wirén 2020). This discrepancy could be attributed to varying degrees of N limitation severity. The studies reporting enhanced root growth generally investigated moderate N

limitations and focused on early growth stages, during which plants may exhibit compensatory root growth. In contrast, severe N deficiency, especially in later growth stages, typically reduces root biomass and length due to insufficient resources for cell division and elongation. For example, Lopez et al. (2023) reported in a meta-analysis a mean decrease in root length and biomass (about 18%) under N deficiency.

Further, P deficiency can stimulate lateral root growth and fine root development (Liu, 2021; Zhang et al., 2012) as a compensatory mechanism to enhance P acquisition by increasing root surface area in contact with the soil (Williamson et al. 2001; Bonifas and Lindquist 2009). This effect was observed in our study, where P omission resulted in similar or greater root biomass and length compared with the NPKCa treatment during booting and flowering. This adaptive response, regulated by hormone signaling involving auxin and ethylene (Hermans et al. 2006), may provide an advantage in nutrient-deprived conditions by promoting root foraging into the topsoil and subsoil. Similar root elongation and biomass increases under P deficiency have been reported in common bean (Lynch, 2011), though contrasting findings in wheat indicate root length and biomass reductions (Teng et al. 2013).

As for the effect of K deficiency on winter wheat root traits, the lack of field studies limits our ability to draw definitive conclusions. Nevertheless, a decrease in root length in K-poor soils has been observed in studies with cotton (Mullins et al. 1994), maize (Zhao, Yu, et al. 2016), and sugar beet (Hadir et al. 2021). Our findings show that K deficiency lowered root biomass only at an early stage (tillering) but increased root length at booting and flowering. This variability highlights the complexity of root responses to K deficiency based on the genotype and soil conditions (Rengel and Damon 2008) and may show that root length increases at later stages as a compensatory response.

While the root biomass in NPK_ was slightly lower than in the NPKCa treatment, root length was similar or greater. This may suggest that a slightly reduced pH, as observed in our experiment, facilitates the mobilization of certain cations such as iron, zinc, and manganese. These micronutrients are essential for root metabolic processes and cell elongation, potentially supporting root growth. This phenomenon was demonstrated in a previous experiment conducted at the same site (LTFE Dikopshof), where sugar beet leaves treated with NPK_ displayed an increase in zinc and nickel content (Yi et al. 2020).

Regarding other investigated root traits, such as the link analysis, the literature suggests that root systems grown in low nutrient conditions tend to have longer link lengths, particularly interior (II and EI) links. This distinguishes them from classic herringbone root systems where branching is predominantly confined to a main axis, typically found in dicots with tap roots. Herringbone-type root systems are generally more efficient in acquiring mobile nutrients such as N or K, although they are more costly for the crop in terms of production and maintenance (Fitter and Stickland 1991; Hadir et al. 2021). In line with the result of Fitter & Stickland (1991) who observed that dicots exhibited the tendency described above while monocots did not, our experiment involving winter wheat, a monocot plant, did not show large differences.

4.4.2 Aboveground traits

The grain yield during the growing period 2 (2020/21) was lower compared to the growing period 1 (2019/20), despite experiencing higher total precipitation. This could be attributed to the contrasting weather patterns between 2020 and 2021. In 2020, although it was warmer overall, there were significant rainfalls in February/March and June. On the other hand, in 2021, there was low rainfall from March to May, and even though it was colder in general, the temperature in June (during flowering) was quite high. These differences in temperature and rainfall patterns likely influenced the grain yield. In contrast, the straw yield was lower in the growing period 1 (2019/20), which could be attributed to the prevailing drought conditions. The lower harvest index in 2021, as well as the high mean air temperatures in June (Fig. S4.1), indicate heat and drought stress during flowering, which has a significant impact on winter wheat yield (Yu et al., 2018).

The treatment with the highest soil nutrient content (NPKCa+m+s) consistently exhibited greater aboveground traits, including shoot dry biomass, LAI, and plant height, while the unfertilized and _PKCa treatments consistently yielded the lowest values across all sampling dates. This aligns with studies showing that nutrient deficiencies, particularly N deficiency, significantly reduce grain and straw yields due to its essential role in chlorophyll formation, photosynthesis, and protein synthesis (Hermans et al. 2006a; Fageria 2009; Qin et al. 2019). Overall, fertilizer omission led to declines in grain yield, straw yield, and shoot biomass, as nutrient limitations restrict the availability of essential elements required for photosynthesis and biomass production, ultimately reducing yield (Liu et al., 2020).

Furthermore, N_KCa treatment resulted in similar shoot dry matter, LAI, and plant height in comparison with NPKCa, with no severe decrease observed. This suggests that the omission of P may not be critical for the development of shoot biomass on this site with a relatively nutrient-rich soil, which is supported by previous research on long term data (various crops) under P fertilizer omission (Gransee and Merbach 2000; Deubel et al. 2002; Kunzová and Hejcman 2010; Zicker et al. 2018; Seidel et al. 2021). These findings indicate that the impact of P deficiencies on wheat shoot biomass may be relatively small.

In our study, K omission led to an increase in grain yield but a decrease in straw yield compared with full fertilizer treatment, which contrasts with other research showing that K deficiency generally reduces both grain and straw yields due to its crucial role in carbohydrate metabolism and transport (Pettigrew 2008; Brhane et al. 2017). Shoot biomass, LAI, and plant heights in K omission were similar (to NPKCa) in one period but greater in another, potentially due to very low shoot performance in NPKCa in the latter year. Typically, K is crucial for activating enzymes involved in photosynthesis and for the translocation of photosynthates from source to tissues (Tränkner et al. 2018). Its deficiency harms these processes, leading to inhibited growth and reduced biomass (Hasanuzzaman et al. 2018). Additionally, K regulates stomatal opening and closing, which controls water use efficiency and drought tolerance. K deficiency often results in poor water regulation, contributing to reduced plant vigor (Schroeder and Hagiwara 1990; Perez et al. 2016).

In our study, the total shoot biomass was similar to NPKCa, which is consistent with the study of (Ahrends et al. 2020), who analyzed long-term yield data from four crops in the LTFE. They reported similar mean yields for the NPKCa and NPK_ treatments, with a slight decline observed in the NPK_ treatment (e.g., winter wheat yield of 4.7 t ha^{-1} for NPKCa vs. 4.51 t ha^{-1} for NPK_). While Ca is generally not limiting, the absence of additional lime application in NPK_ could contribute to competitive interactions among base cations (e.g., Mg, K) or other indirect effects on nutrient availability and uptake.

4.4.3 Performance-related ratios for above and belowground

Some studies suggest that lower SLA, indicating thicker leaves, may be a response to reduced water loss under nutrient stress (Poorter et al., 2009). However, our findings did not show substantial changes in SLA under N deficiency, suggesting that other adaptive mechanisms may be involved. Similarly, we did not observe a consistent trend in SLA under K deficiency. Environmental conditions, particularly in the growing period 1 (2019/20), which was warmer and drier with limited rainfall even during typically wetter months like July, may have influenced plant responses. This could explain why SLA under K deficiency was greater than that of the NPKCa treatment early during this period. However, at BBCH 43, SLA was comparable to the NPKCa treatment. Some studies have also reported no significant changes in SLA under moderate K deficiency (Pettigrew 2008; Zhang, Thornburg, et al. 2020). In contrast, severe nutrient stress, as seen in unfertilized treatments, can sometimes alter SLA, but this varies with the severity of the deficiency and plant adaptation strategies (Xu and Mou 2016). In our study, SLA under unfertilized conditions was similar to NPKCa in 2019/20 but lower in 2020/21.

While SLA responses to nutrient deficiencies varied, root system adaptations provided further insights into plant strategies for coping with limited nutrient availability. Our study showed that N deficiency and unfertilized conditions led to increases in SRL, R/S, and root length per shoot biomass, indicating a shift in resource allocation towards root development to enhance nutrient acquisition. Higher SRL suggests the development of finer roots, which are more efficient in nutrient uptake, while increased R/S and root length per shoot biomass reflect an strategy to optimize nutrient acquisition under stress (Hermans et al. 2006). In contrast, no consistent pattern was observed for SRL and R/S under P deficiency, though previous research suggests that wheat plants often respond by increasing R/S to enhance P uptake efficiency (de Souza Campos et al. 2019). Similarly, increased root length per shoot biomass under P deficiency indicates a strategic allocation of resources toward root development to enhance nutrient uptake while ensuring reproductive success (Junaidi et al. 2018; Lopez, Ahmadi, Amelung, Athmann, Ewert, Gaiser, Gocke, Kautz, Postma, Rachmilevitch, et al. 2023).

Beyond N and P deficiencies, root responses to K and Ca omissions revealed distinct adaptation patterns. K deficiency in our study resulted in a decrease in R/S, contrasting with studies reporting an increase as plants prioritize root growth to explore more soil for nutrients (Andrews et al. 1999). In the Ca omission

treatment, SRL, R/S, and root length per shoot biomass generally increased, enhancing nutrient uptake efficiency by expanding the root surface area and optimizing resource allocation (Eissenstat 1992).

4.4.4 Correlation of traits

Our findings show relationships between plant traits and soil nutrient dynamics. LAI and shoot biomass were moderately correlated with C_{org}, N_t, and N_{min} across all sampling dates, consistent with studies linking nutrient availability to biomass production and soil carbon inputs (Ziter and MacDougall 2013). Root biomass correlated with LAI and shoot biomass, reflecting its role in plant growth and yield (Shamuyarira et al. 2022; Bektas et al. 2023). Similarly, root length and root biomass were consistently correlated, though root length is more sensitive to environmental and management changes and is closely linked to aboveground biomass (Ma et al. 2019). Aboveground traits such as height, LAI, and shoot biomass showed strong correlations, as reported by Gleason et al. (2018). Positive correlations between above and belowground traits, including root biomass and root length with shoot biomass, align with studies on shoot-root biomass allocation influenced by species traits and growth stages (Mokany and Ash 2008; Husáková et al. 2018; Bektas et al. 2023).

4.4.5 Other insights of our study

Roots contribute to soil C_{org} through root turnover, exudation, and decomposition of dead roots (Tresder et al. 2005). Under optimal nutrient conditions (e.g., NPKCa+m+s), increased root biomass may enhance carbon inputs, improving soil structure, fertility, and overall health. Conversely, nutrient deficiencies (e.g., N, P, K, Ca) reduce root biomass, leading to lower carbon inputs and potentially affecting C_{org} levels and soil quality (Rasse et al. 2005). Fine roots, which decompose more rapidly than coarse roots, play a crucial role in carbon cycling. The observed increase in fine roots under P deficiency in our study suggests a potentially higher contribution to C_{org} despite overall reductions in root biomass, as fine roots release carbon into the soil more efficiently (Gill and Jackson 2000).The ability of plants to allocate resources to root growth, particularly under nutrient stress, indicates an adaptive strategy to maintain nutrient uptake efficiency and contribute to soil carbon dynamics. This adaptation can be crucial for long-term soil health and fertility, as continuous root inputs are vital for sustaining C_{org} levels (Wang et al., 2023, 2024).

In this regard, Long-term experiments (LTEs) are important in agricultural research, particularly for studying root dynamics and soil-plant interactions under varying nutrient levels while maintaining the same climate and soil type (Grosse et al. 2021; Körschens 2021). The LTE at Dikopshof has provided valuable insights into the long-term effects of nutrient management on shoot development and now on root growth. LTEs allow us to observe how plants evolve over multiple growing seasons, providing a comprehensive understanding of shoot and root dynamics. Nutrient application, soil changes, and crop rotation practices can have cumulative effects on soil properties and root development. These LTEs, such as Dikopshof (which has the same fertilizer management over decades), help in understanding how these cumulative effects influence root growth and function over time, offering a more holistic view of

plant-soil interactions. By covering multiple years, LTEs give an understanding of varying weather conditions, and other environmental factors. This variability is key to understanding the resilience and adaptability of root systems under different climatic scenarios. These insights are essential for developing sustainable agricultural practices that optimize root growth and nutrient uptake while maintaining soil health (Grosse et al. 2021; Körschens 2021). However, one limitation of the Dikopshof LTE is the absence of spatial replicates for its treatments, which complicates the application of standard statistical analyses, particularly for short-term studies. Despite this limitation, the advantages of an LTE, including long-term data collection and insights into plant-soil dynamics, make it invaluable for understanding the effects of treatments over time.

Our findings drew a picture of the complex interactions between nutrient deficiencies and shoot and root performance. The variations in response observed in different treatments highlight the importance of considering above and belowground traits and environmental conditions when evaluating nutrient stress. Further research is justified to explore the mechanisms driving these responses and to elucidate the potential implications for crop productivity and nutrient management strategies.

4.5 Summary and conclusions

This study aimed to investigate above- and belowground trait responses to nutrient deficiencies and crop performance under nutrient-poor conditions. As expected, shoot and root biomass decreased under nutrient deficiencies, while the treatment with the highest nutrient content consistently exhibited superior aboveground traits. In contrast, unfertilized and low-nutrient treatments showed the lowest values. Weather patterns, particularly drought, likely influenced lower grain yield in the growing period 2 (2020/21). Absolute root biomass and length increased with fertile soils, while reductions under P and K deficiencies were primarily observed at early growth stage. Soil pH reductions had minimal effects on root traits. Unexpected trends in above and belowground trait ratios may be linked to resource competition. These findings highlight the complex interactions between nutrient availability, plant performance, and environmental factors, underscoring the need for further research on crop productivity and nutrient management strategies. Future studies should explore cultivar-specific responses and incorporate higher temporal resolution sampling for a more detailed analysis.

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Author contributions

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Declaration of conflict of interest

All authors of this manuscript declare no potential sources of conflict of interest.

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5 Sugar beet shoot and Root Phenotypic Plasticity to Nitrogen, Phosphorus, Potassium and Lime Omission

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Abstract

In low input agriculture, a thorough understanding of the plant-nutrient interactions plays a central role. This study aims to investigate the effects of nitrogen (N), phosphorus (P), and potassium (K) and liming omission on shoot growth as well as on topsoil root biomass, growth and morphology (tuber and fibrous roots) of sugar beet grown under field conditions at the Dikopshof long-term fertilizer experiment (Germany). Classical shoot observation methods were combined with root morphology and link measurements using an image analysis program. Omission of the nutrients N, P and K as well as of liming led to a significant decrease in shoot growth. Tuber yield was lowest for the unfertilized and the K omission treatment. The root shoot ratio was highest in the N deficient treatment. In the K omission treatment, a strategic change from a less herringbone root type (early stage) to a more herringbone root type (late stage), which is more efficient for the acquisition of mobile nutrients, was observed. By contrast, a change from a more herringbone (early stage) to a less herringbone root type (late stage) which is less expensive to produce and maintain was observed in the unfertilized treatment. We conclude that sugar beet alters its root morphology as a nutrient acquisition strategy.

Keywords: nutrient omission; root coring; specific root length; root link analysis; root to shoot ratio; leaf area index; fibrous roots

5.1 Introduction

A deeper understanding of effects of nutrient limitation on shoot and root growth is of value for plant breeding and cultivar selection, organic farming and low-input agriculture (Goulding et al. 2008), and improvement of crop models (Craufurd et al. 2013; Warren et al. 2015; McCormack et al. 2017). Crop production is considered as an integration of processes occurring in both root and shoot systems (Bingham 2001). The shoot part of the plant is responsible for light interception and biomass allocation (Monteith 1977). The root part comprises many other functions essential for crop production, including water and nutrient uptake, improvement of soil organic matter, carbon sequestration in form of root debris, root exudates and root respiration (Allmaras et al. 2004; Benjamin et al. 2010), plants anchoring in the soil and symbiosis with soils microorganisms. In general, the above-ground part of plants has been intensively studied, however, the below-ground parts have largely been neglected, in the past (Fageria 2012). The reasons behind this are difficulties of observation of root systems related to the time and labor requirements. Two types of observation methods can be distinguished: destructive methods using an auger or shovel, implicating a big loss of soil volume and less insight into the architecture of the root systems; and non-destructive methods, such as the rhizotrons, which besides faster root morphology characterization, permit to have insight into root growth dynamics (Van Noordwijk 1993; Smit et al. 2000), but measurements with these methods are often carried out under controlled (non-field) conditions and do not allow estimation of root biomass or root nutrient concentrations. The choice of methods depends on the crop studied, the soil properties, labor availability and the objective of the study (Otto et al. 2009).

Recently, the study of below ground mechanisms have received a growing interest (Hoffmann 2017), focusing on fine roots traits, mycorrhizal associations and nutrient acquisition (Klimešová et al. 2018). Effective nutrient acquisition largely depends on the ability of root systems to explore the soil (Li et al. 2016) and on the source-sink relationship (Hoffmann 2019). Especially in organic farming where mineral fertilizers are not applied, high efficiency of acquisition of nutrients by roots is important to secure yields. In general, root systems with either a more herringbone topology (branches more or less confined to the main axis) or with longer links (either interior or exterior) should be favoured in conditions where soil-derived resources limit growth (Fitter and Stickland 1991). Long interior and exterior links are associated with more efficient exploitation of soils with low nutrient conditions (Fitter and Stickland 1991) but are more expensive to produce. According to the authors, root systems of dicots became more herringbone and link length generally increased under low nutrient conditions.

According to Carvalho and Foulkes (2018), root morphology refers to the surface features of a single root axis as an organ, including characteristics of the epidermis such as root hairs, root diameter, the root cap, the pattern of appearance of daughter roots, undulations of the root axis, and cortical senescence, root topology describes the branching pattern of the individual root axes. Parameters such as specific root length (SRL), root diameter, and surface area of roots are key root morphological traits and permit to study the response of root systems as affected by different edaphic factors (Ostonen et al.

2007). Root length and root diameter distribution may be obtained in two ways: by microscopic measurements, which are laborious, or by computerized image analysis which is fast (Berntson 1992; Bouma et al. 2000). An advanced level of the morphology analysis constitutes the "link analysis". It represents the study of each link (root part between two forks or a fork and a tip) regarding the morphology and the basic connectivity (Fitter 1987). The link analysis delivers the following parameters for each link: length, average diameter, projected area, surface area and basic connectivity analysis. Whereas root morphology and link analysis can be done on incomplete roots, the root topology requires a complete root system.

The sugar beet is the most important sugar plant of the temperate latitudes. In 2019, sugar beet was cultivated on 409,000 ha in Germany, which is 3.5% of the agricultural area (German Federal Statistical Office DESTATIS). Sugar beet is a biennial plant, mainly cultivated for its taproot (tuber), a storage organ of sucrose. It constitutes with sugar cane the main sugar crops in the world. Kutschera (1960) described the root system of sugar beet as being constituted from a classical taproot that grows vertically and produces several lateral branches, which subsequently branch further, forming an extended fibrous root system which progressively colonizes deep soil layers (Vamerali et al. 2009).

The effects of nutrient deficiency on sugar beet shoots and tap roots were described in several studies (Ulrich and Hills 1969; Terry and Ulrich 1973; Christenson and Draycott 2006; Otto et al. 2009). N is considered as the most important nutrient limiting sugar beet crop production. N deficiency results in low yield, but also, a high level of N leads to lower sugar beet yield quality (Shaw et al. 2002; Hergert 2010). P is considered as the second most limiting factor in sugar beet production, given its structural role, being part of the ADP and nucleic acid, and also given its role in energy transfer. In organic farming, P is most often delivered in form of manure. K was also shown to be an important nutrient in sugar beet growth due to its importance in photosynthesis and respiration (Terry and Ulrich 1973). Abdel-Motagally (Abdel-Motagally and Attia 2009) reported that low K inputs led to a decrease in the photosynthetic activity of the sugar beet. However, few studies investigated the effect of nutrients on the fibrous root system of the sugar beet (Hodge 2004), although uptake of soil nutrients and water depends on the extension and functionality of laterals, fine roots and root hairs (Hetrick 1991; Vamerali et al. 2009). In particular, in sugar beet, the fibrous root system may be one of the most important factors affecting not only the biomass production but also the sugar production (Theurer 1993).

This study aims to investigate the effects of N, P, K and liming omission on sugar beet shoot growth (biomass, leaf area index (LAI), and yield) as well as on sugar beet root biomass and growth of both the tuber and the topsoil fibrous root system under field conditions. For that, we analyze field data of sugar beet grown in 2019 in a long-term fertilizer experiment and applied the image analysis software WinRhizo Pro.

5.2 Materials and Methods

5.2.1 Experimental site

The Dikopshof long-term fertilizer field experiment was established in 1904 near Cologne, Germany (50 48′ 21″ N, 6 59′ 9″ E, altitude: 61 m), located at the intermediate strath terrace of the Rhine river. This long-term field experiment is the tenth oldest long-term field experiment in the world (Körschens 1997). The groundwater table is about 20 m below the surface. The Atlantic climate with mild winters and summers results in a mean annual temperature of 10.1 °C and a mean annual precipitation of 630 mm. The general soil type is classified as a Haplic Luvisol derived from loess above sand (Holthusen et al. 2012). The depth of the loess layer in the experimental field varies from about 1.1 to 1.3 m. The soil texture can be described as silty loam (topsoil) and (silty) clay loam (below 30 cm soil depth). The clay-depleted topsoil horizon (Al) is concordant with the plowed Ap horizon (0–30 cm), followed by an illuvial Bt horizon down to about 80 cm, which is characterized by an increase in clay content. The subsequent cambic horizon is 20 cm thick, followed by a layer of loess that is present until the sand and gravel layers starts (Holthusen et al. 2012). Soil bulk density increased from about 1.4 g cm−3 in the topsoil (0–30 cm) to about 1.5–1.6 g cm−3 below 30 cm soil depth.

5.2.2 Experimental design and fertilizer management

The experiment is a non-randomized block design and comprises 24 treatments and five strips. In this study we focus on the following six treatments: NPKCa, _PKCa, N_KCa, NP_Ca, NPK_, and no fertilizer applied (_ stands for the omission of the corresponding nutrient). Cattle farmyard manure was supplied on sugar beet, potato and winter rye plots after harvesting of the preceding crop at an average rate of 60 t ha−1 year−1 (fresh matter manure), with a dry matter content of 20–30% and a C:N ratio of ~25:1 (treatments with “+m”). Moreover, treatments without application of manure (“ ”) and, since 1953, with (“+s”) and without (“ ”) supplemental mineral fertilizer application were established. This procedure aimed to compensate for the amount of nutrients previously supplied by manure. The fertilization management has not changed since 1953, except for a slight increase of the N fertilizer application (+30 kg N ha−1) on winter wheat, which occurred in the 1980s. The five-year crop rotation was performed with sugar beet (Beta vulgaris), winter wheat (Triticum aestivum L.), winter rye (Secale cereale L.), a fodder legume, and oat/potato (Avena L./Solanum tuberosum L., potato replaced oat in 1953). The fodder crop initially used was red clover (Trifolium pratense), then lucerne (Medicago sativa) and, after 1967, persian clover (Trifolium resupinatum L.) was mainly used. In each of the five strips, one of the crops of the rotation was grown. Thus, the experiment consists of five strips with 24 treatments per strip (120 plots, Figure 5.1). Crop residues were removed during the entire period, except for roots and senesced potato leaves. Since 1909, the depth of plowing before sugar beet was regularly about 30 cm. The plot size is 15 m × 18.5 m with a core plot for final harvest of 9 m × 10 m.

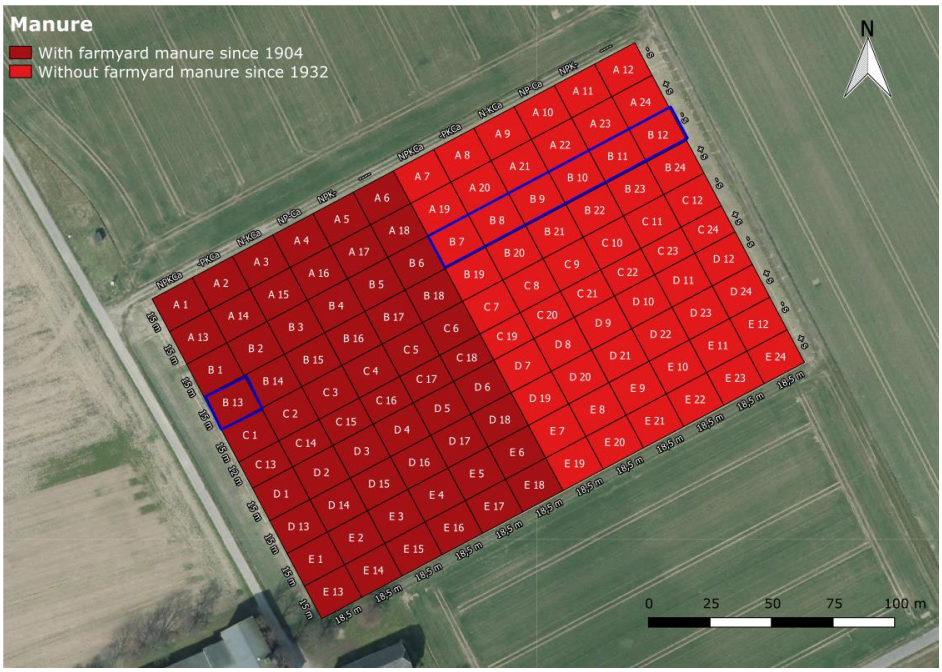

Figure 5.1: Experimental setup of the long-term fertilizer experiment Dikopshof, Germany after 1953 (strips A to E). In the current study, the considered plots are plots B7 to B13 in strip B (marked in blue).

5.2.3 Crop management and field data collection

In this study, sugar beet grown in the treatments NPKCa + m + s (B13), NPKCa (B7), _PKCa (B8), N_KCa (B9), NP_Ca (B10), NPK_ (B11) and the unfertilized control (B12) of strip B were considered (Figure 5.1). In the framework of crop rotation at the Dikopshof, the crop that preceded the sugar beet was potato. In autumn 2018, the soil was tilled with a cultivator and then ploughed with a plough to 30 cm depth. In February, 20 t ha−1 of cattle farmyard manure was applied in treatment NPKCa + m + s. Before sowing of sugar beet, the soil was tilled with a cultivator (15 cm depth) and a harrow (8 cm depth). Sugar beet (variety BTS 7300 N, BETASEED, Frankfurt am Main, Germany) was sown on April 8, 2019 with a sowing density of 10 plants per m2. Amount of fertilizers applied in per treatment and in the whole 5-year crop rotation are shown in Table 5.1. Insects and diseases were controlled with pesticides according to standard grower practice.

Table 5.1: Nutrients and fertilizer type applied at sugar beet in kg ha^{-1} a^{-1} and at the five year rotation in kg ha^{-1} since 1953 at the long term fertilizers experiment Dikopshof

		Application Rate per Element							
		Sugar Beet (kg ha−1 a−1)				Rotation (kg ha−1)			
Treatment	Type of Fertilizer	N	P	K	Ca	N	P	K	Ca
NPKCa+m+s	Synthetic	120	53	199	0	530	287	1078	1293
	Manure	40	22	83	50				
NPKCa	Synthetic	80	31	116	0	230	155	580	1143
_PKCa	Synthetic	0	31	116	0	0	155	580	1143
N_KCa	Synthetic	80	0	116	0	230	0	580	1143
NP_Ca	Synthetic	80	31	0	0	230	155	0	1143
NPK_	Synthetic	80	31	116	0	230	155	580	0
unfertilized		0	0	0	0	0	0	0	0

5.2.4 Field data collection

The Dikopshof field trial was established more than a century ago without field repetitions of the treatments. Consequently we collected internal repetitions within one plot per treatment. The collection of field data was conducted on 16/05/2019, 13/06/2019, 10/07/2019 and 10/09/2019. Harvest started in November. The presented plant and soil data from 2019 comprise leaf area index (LAI), dry matter shoot weight (DM shoot), dry matter root weight (DM root), shoot and root C, N, P, K concentrations, root morphology and root link analysis, topsoil mineral N, P, K and pH values (Table S5.1), and yield at harvest (fresh matter sugar beet tuber).

5.2.5 Leaf area index

The leaf area index (LAI) was determined destructively on sampling dates two, three and four using the cut sugar beet plants and a LI-3100C Area Meter (LI-COR Biosciences GmbH, Bad Homburg, Germany). The same plants were then dried and weighted.

5.2.6 Shoot and root fresh and dry weight and nutrient concentrations

The shoot fresh matter was oven-dried (105 °C) and weighed to estimate shoot dry matter. Root weight was determined after analysis by drying (105 °C) and weighing using Sartorius ENTRIS 4231 fine scale with 0.001 g level of precision (Sartorius Lab Instrument GmbH & Co, Goettingen, Germany). For dates two to four, the root and shoot samples were milled, sieved and analyzed for total C and total N with an elemental analyzer (Euro-EA, HEKAtech GmbH, Wegberg, Germany) and for P and K with a flame photometer (ELEX 6361, Eppendorf, Hamburg, Germany). Only one mixed sample per treatment and date was analyzed for total C, total N, P and K (no replicates). The fresh matter tuber yield was collected in the core plot and weighed at harvest in November.

5.2.7 Root sampling, preparation and scanning

The sugar beets were excavated with a shovel with the surrounding soil to minimize loss of roots on four dates during the growth period (5 plants per treatment). The extraction of the whole tuber with taproot was only possible at the first sampling date. Later, in sampling date 2, 3 and 4 the tuber and the first part of the taproot was harvested and analysed (about first 30 cm) but not the whole taproot.

Due to severe drought and very dry soil conditions at sampling date 3, we decided to not present the root morphology data from that date. The sampling procedure at that date proved to be difficult as large dry chunks of soil were loosened when the plants were removed and probably many roots were torn off. The results of the analyses also differed fundamentally from those of the other dates.

The sugar beet roots were processed directly after harvest. To bare roots from soil, the soil was soaked in tap water and washed by hand using a sieve with 0.55 mm mesh size until rudest soil and debris was cleared away. On sampling dates two, three and four, large roots (tuber) had to be cut for scanning (Figure 5.2). Subsequently, roots were sorted by hand, filtering out smallest particles and dead roots. Afterwards, cleaned roots were scanned directly using an EPSON scanner (HP Expression 1100XL,

Epson America, Inc., Los Alamitos, CA, USA). For that, the roots of each sample (one plant) were laid preferably without overlaps into an acrylic glass platter filled with tap water and scanned with a resolution of 800 dpi as black-and-white picture (Figure 5.2). All samples were scanned and finally analyzed via the root image software WinRHIZO version Pro 2020a (Regent Instruments, Québec, QC, Canada).

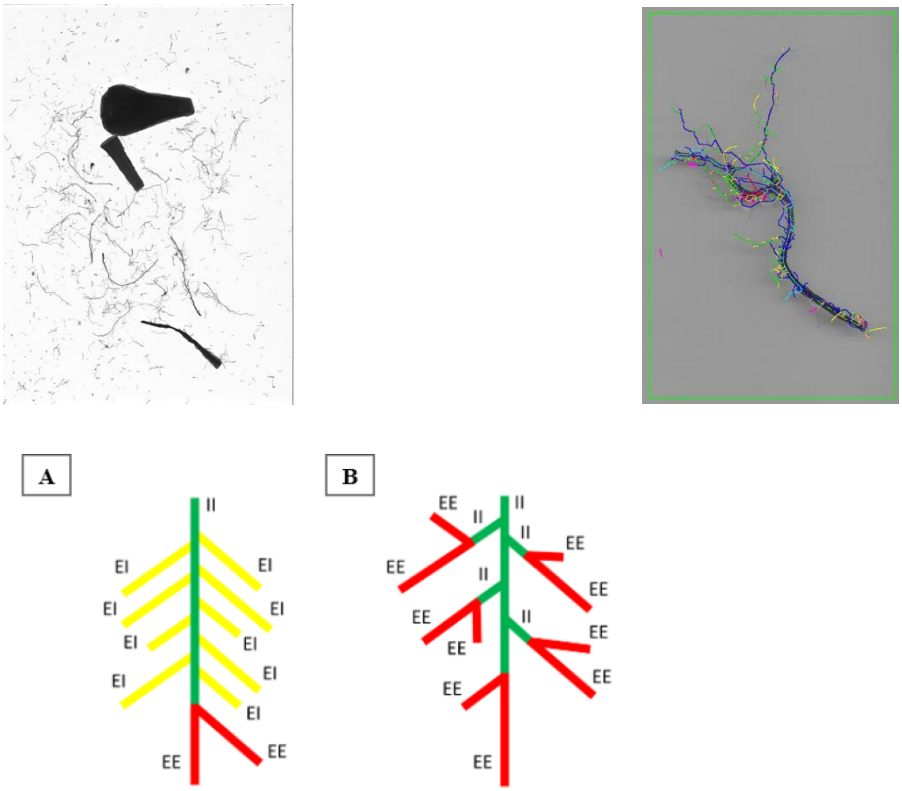

Figure 5.2: Example scan of sugar beet roots (left) and an example for the root analysis with Software WinRHIZO Pro 2020a (mid) from treatment NPKCa + m+s and sampling date 10 July. The diagram on the right shows the distinction between extreme branching patterns ((A) herringbone; (B) dichotomous), and the definition of terms used in the analysis, namely exterior-interior (El), interior-interior (II) and exterior-exterior (EE) links; unlabeled links are interior (based on Fitter and Stickland (1991)).

5.2.7.1 Image analysis of root length, root diameter, and analysis of basic link connectivity

The root scans were analyzed using the software WinRHIZO. Topsoil root length was calculated per plant. Root diameter classes of very fine (<0.1 mm), fine (0.1–0.5 mm), medium to coarse (>0.5 mm) roots were defined and analyzed (Table 5.2). Besides measuring total root length (cm) and root diameter (mm), a link analysis was performed to investigate root basic link connectivity Fitter and Stickland (1991).

Table 5.2: Parameters used to define root morphology and link basic connectivity.

Parameter	Definition	Unit
Root length	Total length of all roots present per plant	cm
Average root diameter	Average diameter of all roots of one sample	mm
L1	Root length (cm) of very fine roots (diameter < 0.1 mm)	cm
L2	Root length (cm) of fine roots (diameter 0.1–0.5 mm)	cm
L3	Root length (cm) of medium fine roots (diameter > 0.5 mm)	cm
EE	Length in cm of exterior-exterior links per cm of total root length	cm
EI	Length in cm of exterior-interior links per cm of total root length	cm
II	Length in cm of interior-interior links per cm of total root length	cm

Classic herringbone root systems (tap root and primary laterals only and thus many interior links) can be distinguished from the highly branched dichotomous architecture type root systems with many exterior links (Fitter and Stickland 1991). Via link analysis, root segments (links) were classified into basic connectivity classifications by categorizing them into different link groups (EE, EI, and II; E stands of exterior and I for interior links). Exterior links which end in a meristem (EE and EI) can be distinguished from interior links (II) (Figure 5.2). For every link, data of the basic connectivity classification was analyzed (Table 5.2). To compare connectivity across the different treatments, the absolute number of each link group (EE, EI and II) per centimeter root length was calculated. For the statistical analysis, the mean across plants segment replicates was calculated, resulting in the mean absolute number of links per centimeter root length.

5.2.7.2 Soil observations and analysis

The frozen soil samples (one per treatment, depth and date) were thawed and the mineral nitrogen concentration (Nmin) was determined. After preparation of the samples (drying and sieving), the concentrations of P and K (PCAL and KCAL) available to plants were determined with a calcium-acetate-lactate extract (Schüller 1969a). Additionally, the pH value of the respective soil samples was determined (CaCl solution, with a Multi 3630 IDS pH Meter and a SenTix 940P electrode, both from WTW, Weilheim, Germany). Those samples were taken from the topsoil with a hand shovel.

5.2.7.3 Data analyses

The data was analyzed using programming language R (version 1.3.959). Means and standard deviation of shoot dry matter, LAI, and mean values of root dry matter, root length over all replicates for each treatment and each sampling date were calculated. Due to the sampling procedure, the final number of replicates was different between dates, between treatments and between traits (Table S5.2).

A one-way univariate analysis of variances (ANOVA, $\alpha = 0.05$) followed by a post hoc analysis of significance (Tukey-test) was conducted using the number of samples (plants) per treatment as replicates to assess the differences between treatments in affecting the root dry matter and shoot dry matter, LAI, SRL, root shoot ratio, total root length and average root diameter. Shapiro-Wilk test was used to assess the normality of all groups' (defined by the treatment and sampling date). All groups were normally distributed and no transformations were needed. The experimental design at the Dikopshof is a non-randomized block design. So, due to the lack of plot replicates at this old trial we used measurement

replicates within the plots for this analysis. The number of replicate for each analysis is presented in Table S5.2. The treatments with (n=2) were excluded from the statistical analysis using ANOVA.

To have a better understanding of the proportion of root length classes within each single root system, we chose the sample with the median total root length within one treatment and per sampling date. The topological index (TI) of the roots was calculated as the ratio between log(altitude) and log(magnitude) (Fitter, 1985).

5.3 Results

5.3.1 Growing conditions during the growth period

The growth period in 2019 can be characterized as exceptionally dry and hot (Figure 5.3). Especially in the months June, July, and August, above average air temperatures were observed. Rainfall was below average from June to September. Especially in the months of late June, July and August, the plants suffered from drought stress and leaves were wilted. The plants recovered in autumn after some rainfall events and when the temperature dropped.

The impact of nutrient or liming omission was clearly reflected by the soil analysis data (Table S5.1). The highest mineral topsoil N concentrations were observed in treatment NPKCa + m + s and the lowest ones in the N omission treatment. Nutrient omission (N, P, K) led to low values of the respective nutrient in the topsoil. Liming enhanced the pH value from mean value of 5.7 (NPK_) to 6.5 (mean of all other treatments).

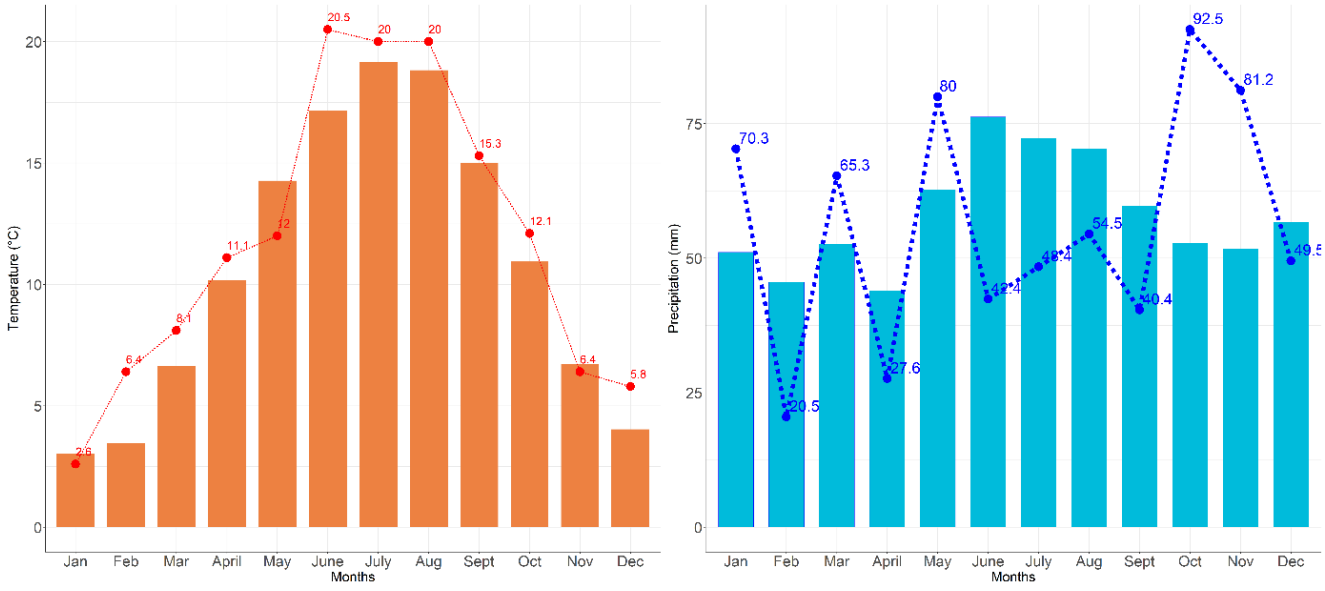

Figure 5.3: Mean monthly air temperature (left panel) and precipitation (right panel) from 1980 to 2019 (bars) and for 2019 (lines) at Dikopshof (source: interpolated data from the German Weather Service, DWD).

5.3.2 Shoot growth

The shoot dry matter weight increased from sampling date 1 to sampling date 3 for all treatments (Table 5.3, Figure 5.4). On sampling date 4, the measured shoot dry matter weight decreased in comparison to

the values measured on sampling date 3 for all treatments except for the unfertilized and the treatment NPKCa + m + s, where shoot dry matter weight increased compared to sampling date 3. The LAI increased from date 2 to date 3. From date 3 to date 4 it decreased again for all treatments except for the treatment NPKCa + m + s. The decline of shoot dry matter and LAI from date 3 to 4 can be attributed to the dry spell and related visible wilting and drop of leaves.

The omission of the nutrients N, P and K as well as of liming led to a significant decrease in shoot growth (Table 5.3, Figure 5.4). In June, the shoot biomass of the N limited and the unfertilized treatment were significantly lower than the fully fertilized treatment NPKCa + m+s. In July, significant differences in shoot biomass were observed following the order: fully fertilized > P omission and no liming > N and K omission > unfertilized treatment. On sampling date 4, the shoot biomass of treatment NPKCa + m + s was significantly higher compared to the other treatments with nutrient omission or the unfertilized treatment, but no significant differences were observed between those treatments.

In general, the highest LAI value was observed for the fully fertilized treatment NPKCa + m + s. Treatment NPKCa experienced decreases in shoot growth parameters after sampling date 2. Although treatment NPKCa showed very similar values for LAI as well as for dry matter at the two first sampling dates compared to treatment NPKCa + m + s, its performance decreased significantly on the third and fourth sampling dates.

5.3.3 Root dry matter weight

The root dry matter weight increased from sampling date 1 to sampling date 4 for all treatments (Table 5.3) and thus did not decline from date 3 to 4 such as the shoot traits (biomass and LAI) did for almost all treatments. The highest value of root dry matter was measured in the treatment NPKCa + m + s and the lowest one in the unfertilized treatment for all sampling dates. Among the nutrient omission plots, the K omission treatment showed the lowest values of root dry matter weight on sampling date 2. On sampling date 3, the significantly highest value of root dry matter was observed in the treatment NPKCa + m + s and the N omission treatment. On sampling date 4, the lowest value of root dry matter was measured in the P omission and treatment NPKCa.

5.3.4 Root shoot ratio

The root shoot ratio provides insights on where the sugar beet allocates the most carbon in the current phase of its growth. While a ratio of below 1 was achieved in all treatments on the two first sampling dates, this changed at the third sampling date with values above 1 (Table 5.3). On the fourth sampling date, the ratio increased again. On sampling dates 2, 3 and 4, outstanding high values of root shoot ratio were observed in the N omission treatment. The lowest root shoot ratios on the fourth sampling date were measured in the P and the K omission treatment.

Table 5.3: Mean values (and standard deviation if replicates were available) of the observed shoot and root variables of sugar beet at the four sampling dates (three in case of leaf area index) and fresh matter tuber yield of the core plot at harvest in 2019 at the long-term fertilizer experiment Dikopshof, Germany. Different letters indicate significant differences (ANOVA, $\alpha = 0.05$).

	NPKCa + m + s	**NPKCa**	**_PKCa**	**N_KCa**	**NP_Ca**	**NPK_**	**No Fertilization**
				Shoot Dry Matter Weight (g·plant^{-1})			
16/05	1.86	1.28	0.91	1.03	0.71	0.39	0.54
13/06	25.80 ± 1.27 ab	27.39 ± 5.13 a	14.18 ± 4.93 c	17.56 ± 5.34 bc	16.01 ± 2.27 bc	17.82 ± 5.91 bc	10.73 ± 5.90 c
10/07	58.15 ± 1.79 a	47.62 ± 6.43 ab	39.09 ± 9.91 bc	47.85 ± 5.54 ab	42.28 ± 2.75 bc	50.08 ± 1.46 ab	33.06 ± 6.32 c
10/09	79.41 ± 31.30 a	25.25 ± 8.16 b	34.25 ± 1.30 b	42.20 ± 3.64 b	43.02 ± 6.12 b	41.63 ± 8.03 b	37.47 ± 1.48 b
				Leaf area index (m−2 m−2)			
13/06	2.28 ± 0.42 b	2.89 ± 0.21 a	1.28 ± 0.16 cd	1.53 ± 0.19 c	0.99 ± 0.33 de	1.66 ± 0.07 c	0.73 ± 0.24 e
10/07	3.96 ± 0.56 a	3.38 ± 0.39 ab	2.88 ± 0.77 bc	2.93 ± 0.13 bc	2.85 ± 0.50 bc	3.06 ± 0.07 abc	2.27 ± 0.40 c
10/09	4.31 ± 2.33 a	0.91 ± 0.34 b	1.55 ± 0.47 b	1.84 ± 0.39 b	1.35 ± 0.31 b	1.83 ± 0.35 b	1.59 ± 0.14 b
				Root dry matter weight (g·plant−1)			
16/05	0.3	0.22	0.17	0.18	0.14	0.07	0.1
13/06	9.39 ± 0.31 a	9.02 ± 5.05 a	5.01 ± 0.69 ab	5.50 ± 0.78 ab	2.75 ± 1.25 b	6.09 ± 0.76 ab	2.72 ± 0.39 b
10/07	83.19 ± 14.17 a	69.34 ± 6.42 ab	81.82 ± 7.70 a	66.51 ± 17.83 ab	59.37 ± 2.22 ab	70.54 ± 1.75 ab	51.35 ± 10.55 b
10/09	259.55 ± 100.98 a	121.79 ± 46.65 b	193.48 ± 5.93 ab	133.57 ± 5.93 b	155.35 ± 11.28 ab	178.18 ± 32.99 ab	167.41 ± 25.80 ab
				Root-shoot ratio			
16/05	0.16	0.17	0.19	0.17	0.2	0.17	0.19
13/06	0.42 ± 0.11 a	0.36 ± 0.24 a	0.39 ± 0.13 a	0.29 ± 0.05 a	0.21 ± 0.08 a	0.37 ± 0.12 a	0.30 ± 0.05 a
10/07	1.54 ± 0.16 b	1.51 ± 0.13 b	2.07 ± 0.27 a	1.40 ± 0.38 b	1.42 ± 0.07 b	1.55 ± 0.20 b	1.55 ± 0.14 b
10/09	3.29 ± 0.27 bc	4.04 ± 0.65 abc	5.00 ± 0.27 a	3.22 ± 0.17 c	3.27 ± 0.20 bc	4.29 ± 0.12 ab	3.83 ± 0.69 bc
				Fresh matter tuber yield at harvest (t ha−1)			
	93.4	63.6	66.96	56.65	51.11	66.09	50.42

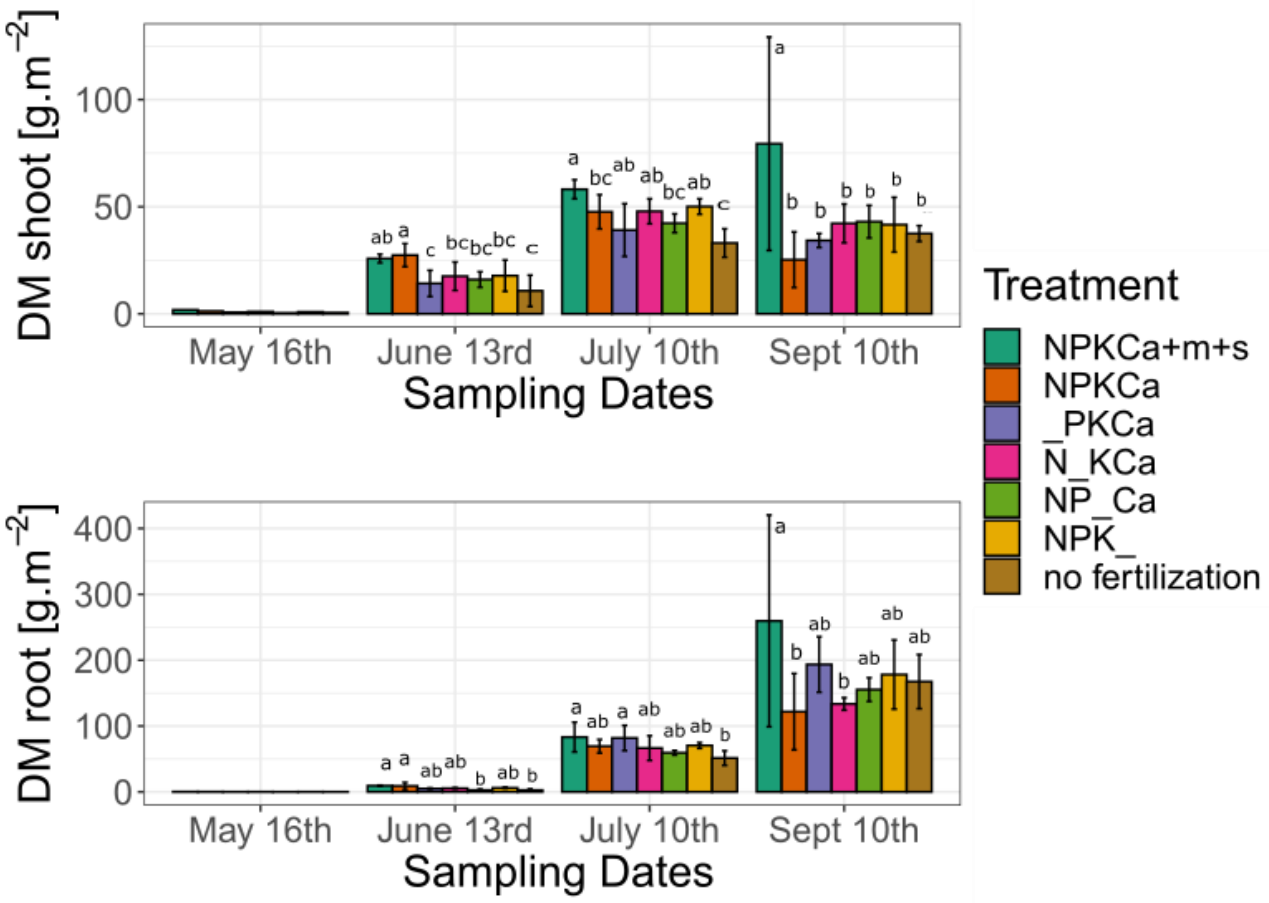

Figure 5.4: Mean values of the observed shoot and root dry matter weight of sugar beet at the four sampling dates in 2019 at the long-term fertilizer experiment Dikopshof, Germany. The error bars correspond to the 95% interval of confidence. Different letters indicate significant differences between treatments (ANOVA, $\alpha = 0.05$).

The analysis results of root and shoot nutrient concentrations (Table S5.3) showed that N omission resulted in lowest shoot and root N concentrations similar to the unfertilized treatment especially at the last sampling date where the shoot biomass was the highest. This trend could be also seen when investigating the results of the P and K root and shoot analysis. The unfertilized treatment showed higher N concentrations at sampling date 2 and 3 compared to the fully fertilized treatment. At sampling date 2 and 3, the NPKCa + m + s treatment showed low shoot N concentration as compared to the other treatment with N fertilizers, root N did not follow this trend. Low values of root N in all dates were given in the N omitted treatment and in the unfertilized treatment.

When P was omitted, low values of shoot P and root P were measured. When K was omitted, the values of K in shoot did not show a lower value comparable to the unfertilized treatment at the 2nd and 3rd sampling date, but at sampling date 4, the value of K in shoot was even lower than in the unfertilized treatment. The root K concentration was at all sampling dates affected by the omission of K.

The shoot and root total C and N analysis clearly showed that the C/N ratio of the N deficient treatment was higher in both shoot and root compared to the other treatments. Due to the lower N availability in

the soil, sugar beet has consumed less N, which results in an increased value of the C/N ratio both in the shoots and in the roots. This was also observed in the unfertilized treatment at the fourth sampling date.

3.5. Root morphology

The root morphology parameters root length, average root diameter and root length within each root diameter class were estimated for each treatment and each sampling date (Table 4). The values of root length increased between the two first sampling dates and decreased between the sampling date 2 and 3. The values increased again between the third and the fourth sampling dates. This trend could be also seen in the respective root classes.

The total root length was significantly highest in treatment NPKCa+ m+ s and the unfertilized treatment followed by treatment in May and July, however there was no significance differences between the treatments in late growing stage (July and September). The average root diameter was also not significantly different between treatment on sampling dates 1, 3 and 4. The only significant differences between the treatments could be detected on sampling date 2, where the treatment with no liming shows a higher value of average root diameter as compared to the treatment with no K.

The SRL differed only significantly between the treatments at the sampling date 4. The highest values were observed in the treatment NPKCa, and the lowest value in the fully fertilized with manure and the unfertilized treatment.

Table 5.4: Effect of fertilization on the root morphology parameters of sugar beet on three sampling dates in 2019 at the long-term fertilizer experiment Dikopshof, Germany. Mean values of total root length are given in cm, average root diameter in mm and the specific root length in m g−1. Different letters indicate significant differences between treatments (ANOVA, $\alpha = 0.05$). * excluded from the statistical test.

	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	No Fertilization
				Total root length (cm)			
16/05	731.00 ±150.15 a	559.71±281.50 ab	225.91±42.63 b	432.79±233.54 ab	194.57±91.13 b	358.86±296.45 ab	606.51±107.24 ab
13/06	1096.59 ± 458.37a	904.53 ± 143.88 ab	652.03 ±90.55 bc	810.76±152.92 abc	606.93± 41.35 bc	578.67 ± 98.54 bc	432.64 ±158.07 c
10/09	1658.6 ± 1107.65 a	1921.9 ± 156.65 a	1851.31±249.68 a	2047.73 ± 394.91 a	2476.75 ± 696.71 a	1475.9 ± 103.33 *	1305.11±150.79 a
				Average root diameter (mm)			
16/05	0.41 ± 0.04 a	0.44 ±0.07 a	0.46 ±0.06 a	0.44 ±0.05a	0.50 ±0.17 a	0.50 ±0.08 a	0.34 ±0.00 a
13/06	0.74 ± 0.19 ab	0.80 ±0.03 ab	0.68 ±0.04 ab	0.72 ±0.10ab	0.58 ±0.11 b	0.85 ±0.17 a	0.82 ±0.00 ab
10/09	2.23 ± 1.23 a	1.14 ±0.13 a	1.08 ±0.36 a	1.11 ±0.22a	0.93 ± 0.26 a	0.93 ±0.44 a	1.29 ±0.24 a
				Topological specific root length (m g−1)			
16/05	23.73	43.14	15.89	31.84	29.62	16.87	62.55
13/06	1.29 ±0.21 a	6.15 ±10.23 a	1.32 ± 0.28 a	1.49 ± 0.38 a	2.94 ± 1.56 a	0.96 ±0.21 *	1.81 ±0.69 a
10/09	0.06 ±0.04 b	0.22 ± 0.09 a	0.09 ± 0.01 ab	0.14 ± 0.00 *	0.16 ± 0.03 ab	0.08 ±0.01 *	0.08 ±0.02 b

To have a better comparison between the root classes as well as between treatments, the results were presented as proportion of each class from the total root length for each treatment for dates 2 and 4 (Figure 5.5). The root diameter classes change their distributions across the sampling dates. The share of very fine and fine roots for the unfertilized treatment was low at date 2 but high at date 4. At the late stage, the share of medium to coarse roots was highest for the fully fertilized treatments NPKCa + m + s and NPKCa, also in P deficient treatment the share of very fine and fine roots was enhanced.

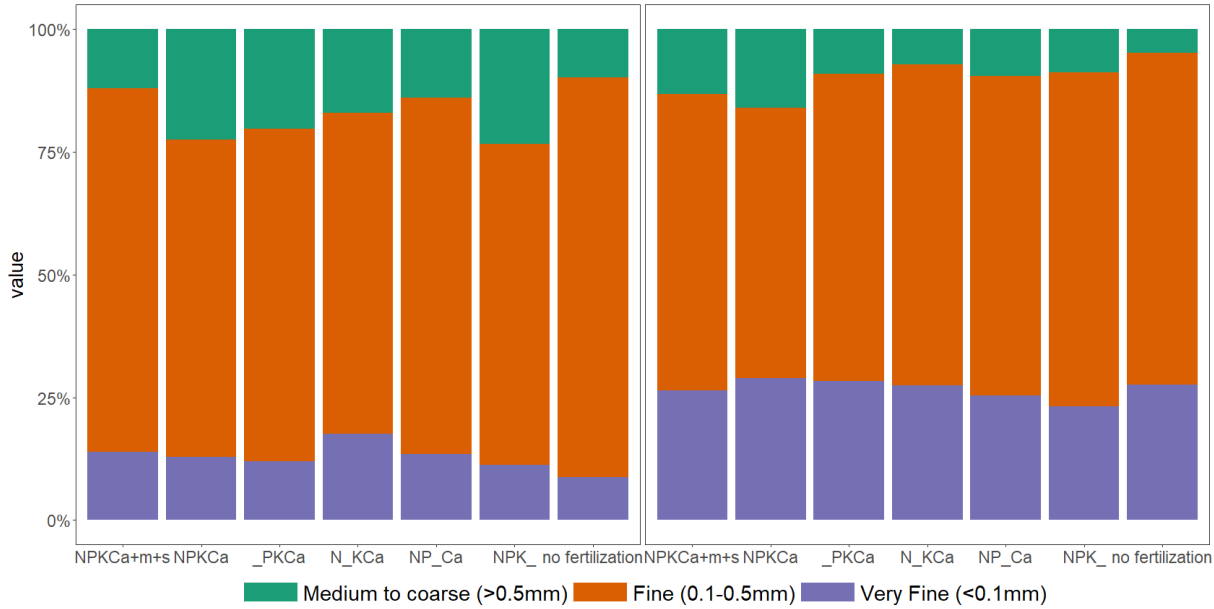

Figure 5.5: Proportion of fine root diameter class to total root length in % at early growth stage (13 June, left panel) and late growth stage (10 September, right panel) of sugar beet for seven fertilizer treatments grown in 2019 at the long-term fertilizer experiment Dikopshof, Germany. Median values of root length per root diameter class were used to represent the proportion.

3.6. Root link analysis

The share of interior root length from total root length ranged from 50 to 70% (Table S5.4). In the early growth stage, the share of interior root length was low for the P omission treatment and high for the fully fertilized treatment. On sampling date 4, the share of interior root length was higher in the K omission and the no liming treatments compared to the other treatments. On the same date, the share of interior root length of total root length was highest in the P omission treatment and lowest in the unfertilized treatment. There was no clear trend observed for the unfertilized treatment.

The number of root links per cm root (branching) shows that in the K omission treatment, root branching was lowest on sampling date 1 but highest on date 4 (Table S5.5). Interestingly, branching is similar in the fully fertilized treatment NPKCa + m + s and the unfertilized treatment.

The topological index (TI) refers to the exploitation efficiency of plants, a higher TI characterize a more herringbone root system and lower TI refers to a more dichotomous root system. The TI of all treatments is higher than 1 (Figure 5.6). The topological index was highest for the liming omission (1.36) and the fully fertilized treatment (1.26) on date 1. On sampling dates 2 the topological index was highest for the

K omission treatment. The TI was low for the N omission treatment and fully fertilized treatment without manure on sampling date 1. The P deficiency results in higher TI compared to the fully fertilized treatment.

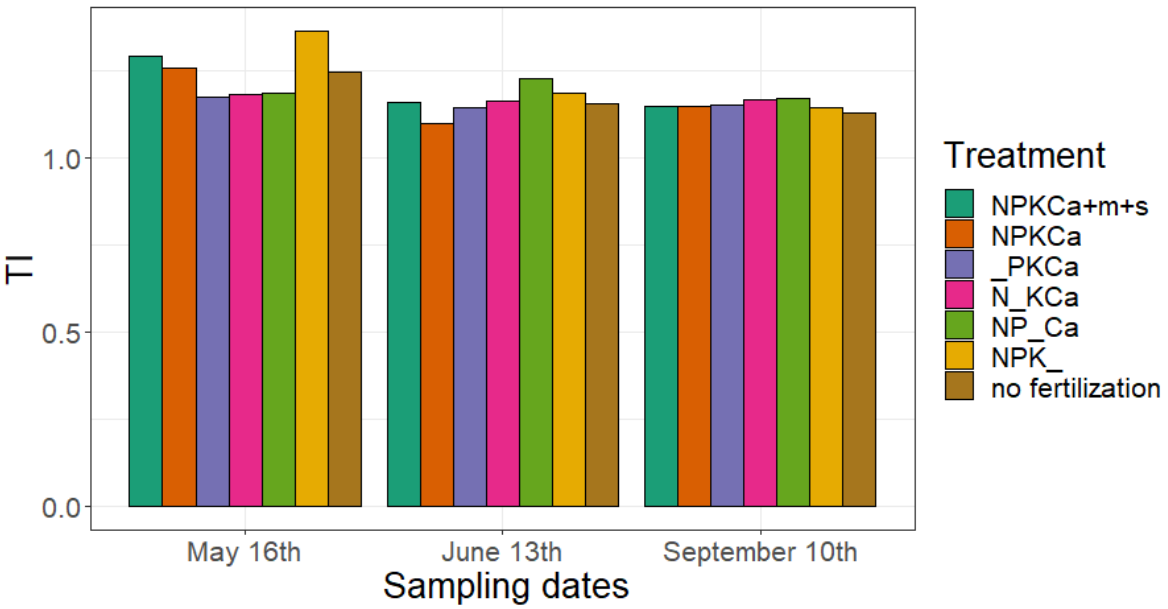

Figure 5.6: Topological index (TI) of sugar beet topsoil root system for all considered treatments at three sampling dates in 2019 at the long term fertilizer experiment Dikopshof, Germany.

The most outstanding and significant results of this study showing different impacts of nutrient limitation on shoot and root growth, root topology and root morphology are represented in Table 5.5.

Table 5.5: The matrix presents the observed strategies of shoot and root growth of sugar beet under optimally fertilized conditions and nutrient omission of sugar beet at the long-term fertilizer experiment Dikopshof in 2019. The symbol/divides into strategy in the early growth stages from strategy in the late growth stage. Significant differences are marked with *, others are non-significant trends.

	Full Fertilization	**No N**	**No P**	**No K**	**No Liming**	**No Fertilization**
Shoot DM	high */high *	low */low				low */low
Leaf area index	high */high *					low */
Root DM	high */high *	/high	/low *			low */low
Root-shoot ratio		high */high *	/low	/low		/low
Tuber yield	high			low		low
Total root length	high */	low *		low */high		low */low
Root diameter				/low *		/high
Specific root length	/low *			high/high	low/	high/low *
Share of very fine & fine roots	high/		/high	low/		high/high
Share of interior links	high/		low/high	low/high		/low
Number of total links		low/		low/high	/high	/low
Topological index		Low/	/high	/high	high/	

5.4 Discussion

5.4.1 Effect of N, P, and K omission and omission of liming on shoot growth, root shoot ratio and yield

Sugar beet growth was affected by the omission of every single nutrient and the omission of liming. Our results showed that all shoot growth traits were significantly reduced as a result of nutrient omission compared to the fully fertilized treatment. A decrease of sugar beet shoot growth under low nutrient conditions was already reported (Shaw et al. 2002). Traits such as leaf area index, shoot dry matter and root dry matter are reacting differently to nutrient limitation type depending on the growth stage. Our findings show that, depending on the growth stage, nutrient omission can affect also N, P and K concentrations of the aboveground and belowground plant organs in different ways.

Based on tuber yields of different treatments, our study showed that K was the most limiting factor in sugar beet grown under field conditions at that site. In general, it is largely recognized that the nutrient most limiting sugar beet growth is N (Tsialtas and Maslaris 2005). However, in our trial, N omission still resulted in the second highest fresh tuber yield among all treatments. Similar as described in (Davidson 1969; Vamerali et al. 2009), N omission caused a sharp decline in shoot biomass.

Plants respond to nutrient limitations by changing their root shoot ratio. In low nutrient conditions, the allocation of biomass to the roots is often favored (Hermans et al. 2006b; Yan et al. 2019). Our results in the late growth stage (July and September) confirmed that in low nutrient conditions, the root shoot ratio—but not the absolute tuber yield of sugar beet—was enhanced in the N omission treatment. An increase of the root shoot ratio under P deficiency was not observed, this was also already reported by (Claassen et al. 1990). The highest root shoot ratio was achieved by the N omission treatment. According to the concept of functional equilibrium between above and below ground parts of plants, under N deficiency the greater part of the N taken up is used to ensure the root growth, and thus diminishing the translocated part to the shoot. Due to this, shoot growth is depressed earlier and to a much greater extent than root growth (Holthusen et al. 2012). This this goes in line with Hoffmann (Hoffmann 2019) who stated that sugar yield is more determined by dry matter partitioning (sink) than by canopy formation (source). Also, the low but considerable topsoil mineral N values of 2 to 10 kg ha^{-1} (Yi et al. 2020) let us assume that N sources such as mineralization and atmospheric N deposition may have contributed to a low but sufficient N supply at that site.

5.4.2 Impact of nutrient or liming omission on root morphology and link basic connectivity

Root plasticity might be important factors in the acquisition of immobile resources such as P. In the late growing stage the shares of very fine roots and fine roots were higher under P deficiency compared to the other treatment, this plasticity was already reported for other (Zhang, Yu, et al. 2012; Kumar et al.

2019). Conversely, in Wheat, lower share of very fine roots under increased P stress was reported (Shen et al. 2018).

The root length was enhanced compared to the treatment with no P deficiency, a greater root length in deficient P plots compared to the fully fertilized was observed. Similar results were already reported for sugar beet (Steingrobe 2001). However, the opposite was reported in Wheat (Shen et al. 2018).

Herringbone type root systems are characterized by many interior root links, long links, and a higher TI. They are more efficient in exploiting soils with low nutrient concentrations, but more expensive to produce and maintain by the plant (Fitter and Stickland 1991). The herringbone root topology is more efficient for the acquisition of mobile nutrients such as N or K due to the reduced incidence of depletion zone overlap, whereas the dichotomous-type root systems (highly branched, lower TI) were judged to be preferable for the uptake of immobile ions such as P (Fitter 1987; Fitter and Stickland 1991; Dunbabin et al. 2003). However, the results of the root analysis presenting the share of II links of total link length in P, showed a shift from a less herringbone root architecture (56.5%) at the early stage to a more herringbone type root architecture (70.4%) with more interior links in the late stage (Table S4). The roots were particularly affected by K deficiency, as well as by non-fertilization and full fertilization. The roots in the fully fertilized treatments can be characterized with a high total root length, a high share of fine and very fine roots and many interior links at the early stage as well as a high average root diameter and a low SRL at late growth stages. Likewise, Hodge (Hodge 2004) reported of greater root length of thinner roots and low SRL in nutrient-rich zones and Song et al. (Song et al. 2019) reported of significantly lower root length in the low nutrient (N, P, K) treatments than those of the two highest nutrient treatments in Pistacia chinensis Bunge seedlings. In the unfertilized treatment the share of very fine roots, SRL and TI were high compared to the other treatments in the early growth stage. In the later growth stages, average root diameter was high and total root length, SLR, share of interior links, number of total links, and TI were low In contrast to no fertilization, the K omission treatment shows high total RL, high SRL in the late growth stage, i.e., highly acquisitive traits, which makes sense if considering that at the loess site, K can be mineralized from the solid phase. Our results confirm the hypothesis of Mollier and Pellerin (Mollier and Pellerin 1999) that P deficiency mainly affects the root system morphology through its effect on the C budget with no additional specific effect of P deficiency on root morphogenesis.

The SRL provides insights into the crop investment in biomass. Plants with high SRL build more root length for a given dry-mass investment and are generally considered to have higher rates of nutrient and water uptake per dry mass (Ostonen et al. 2007). However, other studies report increasing, decreasing or constant SRL values as response to nutrient limitation (Ryser 1998, 2006). In Our study, no significant differences between the treatments for the first sampling dates were observed but there was a tendency for high SRL in the unfertilized treatment (date 1). During the late growing stage, SRL differed

significantly between the treatments with the lowest values observed in the fully fertilized and the unfertilized treatments and highest values observed in the K omission treatment.

5.4.3 Other factors influencing root growth and observations

Many factors influence root architecture such as water supply (Fitters et al. 2020; Maurel and Nacry 2020), soil texture (Rogers et al. 2016), soil structure (Watt et al. 2003), temperature (Nagel et al. 2009; Rich and Watt 2013), micro-organisms (Hetrick 1991) and the selected variety (Fitters et al. 2020). In particular, investigation of plant nutrient uptake should consider also the water supply (Gleeson and Good 2010). On the one hand, because water in the soil is a main factor in the mobility of nutrients, and on the other hand, because it is largely recognized that water is an essential component of plant growth and forms with nutrients N, P and K the two most limiting factors to plant growth.

Both link analysis and other root morphology parameters behave differently as a function of the sampling dates. These changes are firstly related to the growth stage but also to the impact of climate parameters occurring at the respective sampling date. The growth period in 2019 was characterized by extreme drought and above-average temperatures. Precipitation in June, July and August 2019 was so low it can, be assumed that drought stress was an important factor for sugar beet deriving the differences in the response to nutrient deficiency. Moreover, the method of sampling only allowed to examine the roots located in the topsoil (about 0–30 cm) and the topsoil is usually most affected by in-season drought events. When comparing the root length over the four dates in the field, one can observe large differences between the measured total root lengths. Especially at sampling date 3 the crops already had suffered from drought for some weeks, leaves wilted and dropped off, and shoot and root growth was limited. Root sampling with the shovel was difficult at that date and may have led to a demolition of fine roots. Thus, the root morphology traits of that date were not presented in this study.

Sampling of fibrous sugar beet roots is complicated due to the root structure and depth. For instance, root augers cannot be used for sampling tubers with a diameter of more than 10 cm. The use of other methods for easier extraction of fine roots with a defined soil volume such as monoliths (Perkons et al. 2014) should therefore be adopted when examining the root architecture of sugar beet.

5.5 Conclusions

Studies investigating the impact of nutrient deficiency are often carried out under controlled conditions such as pot experiments. To our knowledge no studies were carried out to investigate sugar beet root and shoot growth, root morphology and topology under various nutrient omissions and under field conditions. Our study reports that nutrient omission negatively impacted shoot growth parameters of sugar beet. All treatments but the fully fertilized one showed a decline in the shoot growth parameters shoot dry weight and LAI from date three to four due to the dry spell. In contrast, root weight increased from sampling date one to four. Shoot growth depression in the N omission treatment was high but root

growth depression rather low. In September, the root-shoot ratio was highest for the N omission treatment and lowest for the K, P omission and fully fertilized treatments.

One of the main drivers of SRL is the shoot biomass and the carbon that can be allocated from the shoot to the roots. TI and SRL were highest for the K omission treatment which reveals the effort of the plants to exploit the soil with the low K concentrations. The findings also clearly underline the general importance of root plasticity including a shift towards the one or the other root type to maintain resource acquisition capacity in low input agriculture.

The study provides general insights into the effects of nutrient deficiency on root and shoots growth, root morphology and root branching of sugar beet under field conditions. A better understanding of the impact of nutrient limitation and low soil pH values on shoot and root growth is especially important in organic farming or in low-input agriculture systems. Further studies should be carried out under field conditions taking into consideration also crop water uptake and the deeper root system. Use of other methods such as monoliths should permit and easier extraction of fine roots in the context of studies that investigate the root architecture of sugar beet or other tuber crops.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S5.1: Soil analysis data. Topsoil mineral N, plant available soil P and K (PCAL and KCAL) extracted with a calcium-acetate-lactate extract in kg ha-1 and topsoil pH value for the seven treatments and four sampling dates in 2019 at the long-term fertilizer experiment Dikopshof (taken from Yi et al. (2020)), Table S5.2: Number of analyzed replicates per sampling date and treatment for LAI, DM root, DM shoot, Root morphology (total root length, root diameter) and link basic connectivity. For link basic connectivity, the replicates correspond to segments of one sample per treatment and per sampling date; Table S5.3. Means of shoot and root C, N, P, K parameters as well as shoot C/N ration and root C/N ratio; Table S5.4. Share of interior root length (II, in %) exterior-interior (EI, in %) and exterior (EE, in %) of total root length (II, EI and EE) for the seven treatments and three sampling dates in 2019 at the long-term fertilizer experiment Dikopshof; Table S5.5. Number of interior (II), exterior-interior (EI) and exterior-exterior (EE) links per cm root length for the seven treatments and three sampling dates in 2019 at the long-term fertilizer experiment Dikopshof.

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6 General discussion

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

The benefits of cereal/legume intercrops to increase grain yield have been reported in several studies (Yu et al. 2016; Demie et al. 2022; Li et al. 2023; Paul et al. 2024). In our study, LER in the fully replacement design indicated that intercropping was favourable to increase the aboveground biomass and yield. We found that the response to intercropping varied depending on cultivar choice (chapter 2). The results emphasize the importance of the study of cultivar choice in maximizing the benefits of intercropping. Similar conclusions were drawn in a similar context, but only focusing on aboveground biomass production in intercrops conducted by Demie et al. (2022) and Paul et al. (2024). So, Paul et al. (2024) concluded that selecting a site-specific combination of cereals and legume cultivars along with appropriate management practices, namely, the sowing density, is key to realizing the benefits of intercrops.

6.1.1 Effect of plant density on root growth

In our study, the effect of sowing density on root growth of cereal/legume intercrops was evaluated using three experimental designs, each corresponding to a different sowing density. Specifically, we compared: (1) a partial replacement design, with a lower sowing density compared to the optimally used sowing density in sole crops; (2) a fully replacement design, nearly matching the optimal sowing density of sole crops; and (3) an additive design, with a very high sowing density. Data from early and late growth stages indicate that total root mass (0-1m) was reduced under very high sowing density. The novel intercrops interaction index PPII was introduced in Chapter 2. It provides insights into the mechanisms underlying our findings. In both the partial and full replacement design treatments, plant-plant facilitation was more pronounced than competition across all soil layers. In contrast, competition dominated root growth in the high-density (additive) treatment at both growth stages. These results highlight the impact of interspecific root-root interactions on root growth in intercropping systems.

Several studies, such as Ma and Chen (2016), have reported enhanced root growth in intercrops compared to sole cropping systems. In our study, this enhancement was observed only in the partial and fully replacement design treatments (corresponding to low and nearly optimal sowing densities).

Results of Chapter 2 show that root mass in intercrops also increases with sowing density up to a certain point, after which further increases lead to a decline in root biomass. In line with this pattern, Weiner and Freckleton (2010) demonstrated that aboveground biomass yield increases linearly with plant density until a critical threshold, beyond which total yield per area plateaus. This is an outcome referred to as the final constant yield. This reallocation of biomass between above- and belowground components

highlights the importance of optimizing sowing density to achieve the objectives of sustainable crop production in intercropping systems.

With regard to investigating the effect of sowing density on RLD, there is only a limited number of relevant studies in the literature across different species (Hecht et al. 2016). In the grape vine, Archer and Strauss (1985) observed steeper and greater RLD in denser stands of grapevine. Similarly, Azam-Ali et al. (1984) observed faster and deeper root growth at higher sowing densities of pearl millet. Manschadi et al. (1997) found an increase in RLD with increasing sowing density and over time for faba bean, although for the high sowing densities, RLD decreased after pod setting. Although those studies were done on sole crops, they can serve as a basis for analysing the effect of total sowing density on roots in intercrops. However, they are not sufficient to clearly understand the whole processes as the interspecific interactions are in sole crops neglected, and processes such as facilitation could rather inhibit or foster the proliferation of roots in one or another direction. In intercropping conditions, to our knowledge, only Wang et al. (2018) evaluated the effect of increasing total sowing density in a maize/spring wheat strip intercropping system on root growth. They found that with increasing sowing density of maize in species mixtures, root growth of the intercropped maize was increased significantly in comparison to the maize sole crop. But the effect on RLD was not well studied in intercropping. In In our study, we found that increased sowing density promoted root length density (RLD) only at the first sampling date and solely within the 0–10 cm soil depth. In contrast, lower sowing densities of spring wheat and faba bean enhanced deep root growth of wheat (in both cultivars) under intercropping.

6.1.2 Effect of cultivar choice

In the intercrops (Chapter 2), total root mass and plant-plant interactions were affected more by sowing density than by the spring wheat cultivar. Discrepancies between the two wheat cultivars were evident in their influence on faba bean root growth. The early root growth in depth and in the density of one spring wheat cultivar impacted negatively faba bean root growth. Indeed, although there was no statistically significant effect of the cultivar on the root mass, we observed differences in rooting ability between the two spring wheat cultivars. Cultivar Anabel exhibited faster and deeper rooting around the time of faba bean flowering compared to cv. SU Ahab. This led to lower root mass proportions of faba bean in intercrops with cv. Anabel than in those with cv. SU Ahab. Similarly, Wang et al. (2025)found for soybean/maize intercrops that soybean varieties determined the total root length of maize in intercrops. Also, in an experiment conducted by Streit et al. (2019) wheat/faba bean intercrops root growth were highly dependent on the bean genotype. The results highlight the importance of cultivar choice in designing intercropping systems and encourage deeper investigations using multiple genotypes, to better understand the mechanisms underlying the observed discrepancies.

6.1.3 Types of interactions occurring belowground in spring wheat/faba bean intercrops

The findings of the study (Chapter 2) show clear patterns in how sowing density and cultivar choice affect belowground interactions. While competition is consistently observed at shallow depths (10–20 cm) across all treatments, likely due to limited resources and high root overlap near the surface. However, deeper soil layers (30–100 cm) exhibit increasing facilitation, particularly under low and nearly optimal TSD. Notably, the partial replacement design with *cv*. SU Ahab shows the strongest facilitative response, suggesting that this combination may optimize root resource sharing between faba bean and spring wheat at depth. In contrast, the additive design with *cv*. SU Ahab shows persistent competition regardless of depth. These findings suggest that both plant density and root co-occurrence, defined by cultivar choice, are critical factors for intercrops management.

The results from aboveground overyielding and interspecific interactions, assessed using the PPII, showed a positive correlation between facilitation, enhanced root growth, and overyielding, particularly in intercrops grown at low and optimal sowing density. However, due to the lack of true field replicates, it was not possible to statistically validate the relationship between belowground interactions and aboveground yield performance.

Nevertheless, the observed complementary interactions could be leveraged to optimize nutrient and water uptake in intercropping systems, and may help to reduce the need for synthetic fertilizers and enhance crop productivity under suboptimal conditions.

6.1.4 Key differences in terms of root traits between sole crops and intercrops

The analysis of the root traits: RLD, SRL, as well as of root mass, shows that under low and nearly optimal sowing density, the total root growth (0-1m) was enhanced in intercropping compared to sole cropping. These findings support the idea that integrating legumes with cereals can improve root traits and resource use efficiency, ultimately improving the productivity of cropping systems in low-input agriculture. However, they also underscore the importance of selecting appropriate sowing densities and genotypes to fully realize these benefits

6.2 Effect of N omission on root traits and plasticity of field crops

It is widely recognized that root growth can vary in response to N supply (Campbell et al. 1977). An important effect of N availability is an increase in root branching which occurs especially in N enriched soil patches for barley (Drew and Saker 1975) and positive effects of N supply on root mass, root length density, and root specific area of winter wheat (Xue et al. 2014). Gruber et al. (2013) indicates for Arabidopsis plants that reduced N availability stimulates primary and particularly lateral root elongation but not lateral root initiation (Linkohr et al. 2002; López-Bucio et al. 2003). However, under extreme N deficiency, lateral root growth is almost inhibited, suggesting that plants require a certain level of N to maintain an active foraging strategy. These examples indicate that the availability of N in the soil can

lead to various effects on root phenotypic plasticity depending on the concentration of the supplied nutrient and the timing of the supplementation This could be explained by the fact that important developmental processes, such as root hair formation, primary root growth and lateral root formation, are particularly sensitive to changes in the internal and external concentration of nutrients implying sensing processes (López-Bucio et al. 2003; Krouk et al. 2010).

Such plasticity is well documented in cereals. Wheat, for example, exhibits broad physiological, genetic, and morphological adjustments under N deficiency, including altered photosynthesis, increased expression of N-related genes, changes in shoot morphology, and modifications of root architecture (Li et al. 2013; Curci et al. 2017; Wang et al. 2021). In winter rye (Chapter 3), N omission reduces the grain yield, shoot biomass, and root biomass. The RLD exhibits lower value at early tillering (BBCH 22–24), onset of stem elongation (BBCH 30), early booting (BBCH 41–43), and mid- to late heading **(**BBCH 56–58), respectively. At the beginning of stem elongation, N resulted in a significant reduction in average root diameter, and caused steeper root angles around flowering. This shift already reported for maize suggests a strategy to explore more soil volume and thus, allow more resource acquisition (Trachsel et al. 2013). This aspect is interconnected with the already reported absence of lateral root branching as a result of extreme N deficiency (Gruber et al. 2013).

Our findings demonstrate the strong impact of N deficiency on winter rye root phenotypic plasticity but also highlight the importance of growth stages on the variability of the observations. For instance, the response of average diameter to N omission was more pronounced at the beginning of the growing period, where we observed a negative impact of N omission on the root diameter of winter rye. However, around flowering and in later growth stages, we did not find significant differences in average root diameter under either N omission, suggesting that these nutrient deficiencies may not strongly influence this trait in winter rye at these growing stages. Similar findings have been reported in other crops, such as potato under N deficiency (Sharifi et al. 2005). It is indeed noteworthy that this study focuses only on the topsoil layer. Studies including subsoil layers should be conducted to better understand winter rye root plasticity to nutrient omission, especially of mobile nutrients such as N.

In the study of winter wheat (Chapter 4), N omission treatments led to a decrease in root length and root biomass while leading to an increase in SRL, R/S, and root length per shoot biomass. Optimal fertilisation with manure led to the highest values for shoot traits, root length, and root biomass in both growing periods and lowest in treatments with low nutrient availability, particularly under N omission and in the unfertilized treatment. This decline aligns with findings that nutrient deficiencies reduce root growth due to limited resource availability for cell division and elongation (Giehl and von Wirén 2014). The N deprived treatments (_PKCa and unfertilized) consistently show lower root length and RLD across depths and growth stages. Several studies show that N deficiency reduces the total root length and root length density of winter wheat (Barraclough et al. 1989; Xue et al. 2014; Mehrabi et al. 2021).

In a meta-analysis, Lopez et al. (2023) reported a mean decrease in root length and biomass (about 18%) under N deficiency.

While N omission negatively impacted root length (especially in the subsoil) and RLD in winter rye and winter wheat, previous research on Maize has shown that mild N deficiency can stimulate root growth as plants increase their nutrient foraging efforts in the early growth stage This stimulation is mostly done at the cost of shoot growth (Peng et al. 2012) but N over application inhibited early root growth and failed to increase shoot dry weight and grain yield of maize plant (Peng et al. 2012). Also, Comfort et al. (1988) reported an increase in root length in the topsoil layer (0-30 cm) under a moderate level of N deficiency, but application of high amounts of N suppressed root growth in subsoil (below 30 cm). Similarly, Odone et al. (2024) found a slightly less deep root growth when more N was applied.

These studies together with ours show that optimal N supply is key for increasing NUE of cereals by considering both the temporal and spatial pattern of root growth.

6.3 Linking root traits to N foraging and belowground N and C dynamics across cropping systems

6.3.1 N uptake and N_{min} content in the soil

Deep roots are crucial for limiting the losses of nutrients and also making use of nutrients present in the subsoil. So, deep-rooted crops can support the regulation of nutrient cycling and groundwater flow, pedogenesis, as well as soil carbon sequestration and storage in deep soil layers (Pierret et al. 2016).

Particularly for N, characteristics of root architecture influence the N uptake and foraging from soil (Odone et al. 2024). The 'deep, steep & cheap' ideotype is expected to increase water and N acquisition (Lynch 2013). Wacker et al. (2022) demonstrate the higher uptake of ^{15}N-labelled deep nitrogen due to deeper rooting in winter wheat. The results from the intercropping study (Chapter 2) help us to draw insights on the relationship between the N_{min} and root length. In this study, we have almost the same starting conditions, but we compare various cropping systems settings. In the subsoil layers (60–100), N_{min} below faba bean sole crops was higher than that below spring wheat sole crops (both cultivars). This could be attributed to the low RLD of faba bean in deeper layers, which decreased the N uptake (Kage 1997), but also to the biological fixation by faba bean that enriched the soil with N. There was a difference in N uptake rate between intercrops and sole crops. Similarly, in long-term experiment, an increase of topsoil organic N content by 11% was observed in intercropping as compared to sole cropping, indicating that increased biological N fixation contributed to increased soil N content (Cong et al. 2015). Moreover, it is widely recognized that N uptake is mainly performed by the fine roots (McCormack et al. 2017). This was also indicated by the intercropping study (Chapter 2), where for the low density treatments with the high SRL (higher fine roots), N uptake was greater than in the high density treatments. Conversely, in the study of N_{min} in winter wheat (Chapter 4), there was a positive

correlation between N_{min} present in the soil and root length. However, it should be noted that N_{min} sampling in all experiments was not replicated, which limits the strength of our conclusions regarding the effect of root traits on N uptake. In addition, root N content is not a reliable indicator of N uptake (Gent and Forde 2017), as roots primarily serve as transport organs for nitrogen to the shoot. Therefore, similar studies incorporating both root and shoot N uptake, and their relationships with root traits, should be conducted.

6.3.2 Carbon turnover

Root biomass constitutes an important part of the soil C and N pool (Tresder et al. 2005). In agriculture, and especially for food and fodder crops, while the aboveground biomass is harvested to feed humans or cattle, or to produce fibre, roots remain essentially in the soil and through multiple mechanisms like decomposition fill the soil C and N pool. Especially, fine roots decompose more rapidly than coarse roots. Therefore, enhancing root biomass could introduce additional organic carbon into agricultural soils and thus enhance soil C sequestration (Heinemann et al. 2023). In the winter wheat study (Chapter 4), it was shown that C content of roots was higher in the fully fertilized with manure, hence enhancing carbon inputs. Conversely, nutrient deficiencies (e.g., N, P, K) reduce root biomass, leading to lower carbon inputs and potentially affecting C_{org} levels and soil quality (Rasse et al. 2005). In intercrops (Chapter 2), in the deeper soil layers (30–100 cm), total C in roots in the mixtures was on average 22% greater than the average root C in sole faba bean and 18% lower than average root C in sole spring wheat (mean of both cultivars) providing a possible mechanism for the divergence in soil C sequestration between sole crops and intercrops. Similar trends were observed by Cong et al. (2015).

The C/N ratio is a critical indicator of nutrient dynamics and plays a central role in determining soil nitrogen availability. In the study on sugar beet (Chapter 5), total carbon and nitrogen analyses of both shoot and root tissues revealed that plants under nitrogen-deficient conditions exhibited significantly higher C/N ratios compared to other treatments. This increase resulted from reduced nitrogen uptake due to limited soil availability, while carbon accumulation continued. As a result, both shoots and roots showed elevated C/N ratios, reflecting an imbalance between carbon assimilation and nitrogen acquisition, especially under N omission in sugar beet. The results highlight the importance of optimal fertilisation, cropping systems in increasing root biomass, promoting soil C sequestration and maintaining favourable C/N ratios. In addition, a genotype selection can be an option for increasing root biomass C input to soil while maintaining or even enhancing yield (Heinemann et al. 2023). This highlight how optimizing root traits can directly support sustainable soil management and long-term agroecosystem productivity.

6.4 Effect of P omission on root traits of field crops

Several plastic responses of plants to P deficiency have been listed in the literature. These include changes in the spatial organization of primary roots and lateral roots (Richardson et al. 2009; Lynch

2011) in order to increase soil exploration (Hinsinger 2001; Richardson et al. 2011) adaptation of total root length (Smith and De Smet 2012), promotion of lateral root growth and fine root development (Zhang et al. 2012; Liu 2021) as a compensatory mechanism to enhance P acquisition by increasing root surface area (Bonifas and Lindquist 2009), increasing the branching density and thus decreasing the lateral root diameters, the decreased root diameter limits root penetration through the soil (Materechera et al. 1992). So, under N deficiency plants often allocate more root biomass to deeper soil layers, while under P deficiency, root proliferation tends to be concentrated in the upper soil layers where P is more available (Kumar et al. 2019). In the winter rye study (Chapter 3), at the beginning of stem elongation, P omission led to a significant reduction in average root diameter, with a similar reduction observed for N omission. However, around flowering and in later growth stages, we did not find significant differences in average root diameter under P omission, suggesting that these nutrient deficiencies may not strongly influence this trait in winter rye in later growing stages. Similar findings have been reported in other crops, such as maize under P omission (Li et al. 2017). These observations were also mirrored in the SRL. SRL is inversely correlated to root diameter. Winter rye tends to increase its surface area and thus increase the SRL in order to acquire more belowground resources. Although not significant, we observe a trend where the treatments with P omission (N_KCa) resulted in higher SRL than the two fully fertilized treatments at the beginning of the growing stage, but in late growing stage, NPKCa+m+s treatment showed significantly higher SRL than the P omission treatment. This suggests a shift in resource allocation under prolonged nutrient stress, as the plant may reduce fine root growth to preserve carbon resources (Eissenstat 1992; Ke et al. 2024). For the winter wheat study (Chapter 4), P omission resulted in similar or greater root biomass and length compared to the NPKCa treatment during booting and flowering. Similar root elongation and biomass increases under P deficiency have been reported in common bean (Lynch 2011), though contrasting findings in wheat indicate root length and biomass reductions (Lopez et al. 2023).

In spring wheat (Chapter 4), no consistent pattern was observed for SRL and R/S under P deficiency, although previous research suggests that wheat plants often respond by increasing R/S to enhance P uptake efficiency (de Souza Campos et al. 2019). Similarly, increased R/S under P deficiency indicates a strategic allocation of resources toward root development to enhance nutrient uptake (Lopez et al. 2023). In sugar beet (Chapter 5), at the late stage, in the P-deficient treatment, the share of very fine and fine roots was enhanced compared to the fully fertilized treatment with manure. This suggests an increase in fine root biomass under P deficient treatment in sugar beet. Reflecting an adaptation to better acquire nutrients like P. The adaptation was also reflected in the comparison between the topological indexes of sugar beet under P omission and under fully fertilization with manure. The P deficiency results in a more herringbone root system compared to the fully fertilization treatment.

So, across all three crop species, P omission caused root system adjustment, but the magnitude, direction and timing of these responses varied substantially. Highlighting crop specific root plasticity and developmental stage- dependent strategies to cope with nutrient stress.

6.5 Effect of K and Ca omission on root traits of field crops

6.5.1 K omission

Potassium is an essential macronutrient that has been overshadowed in root research by nitrogen and phosphorus (Sustr et al. 2019). K plays a crucial role in various aspects of root growth and development (Sustr et al. 2019). Adequate cytoplasmic K^+ levels are essential for protein synthesis and enzyme activity in root cells, contributing to cytoplasmic pH homeostasis (Walker et al. 1998) and maintenance of the anionic charge of proteins (Maathuis and Sanders 1996). Cell expansion in the elongation zone requires turgor pressure, which builds up via osmotically active substances, including K^+ (Pritchard 1994; Dolan and Davies 2004). In the maturation zone, root hairs grow apically via the action of K^+ fluxes (Rigas et al. 2001; Desbrosses et al. 2003; Zhao et al. 2016). Furthermore, K^+ affects the R/S ratio via phloem transport (Cakmak et al. 1994a, 1994b). Adaptive changes of root system architecture and root length have been observed in plants to enhance K^+ uptake in K limiting conditions (Wang and Wu 2010, 2013). For example, increased root surface area has been observed in soils with low K availability compared to well-supplied soils (Høgh-Jensen and and Pedersen 2003). In the sugar beet study (Chapter 5), K omission resulted in the lowest root branching early in the growing season, but increased branching and SRL in the late growing stage compared to the fully fertilized. Overall, sugar beet roots exhibited considerable phenotypic plasticity in response to the omission of N, P, K, and Ca. The most pronounced changes occurred in response to N and P omissions, with considerable plastic responses, including increased root branching and changes in biomass allocation to roots. K and Ca omissions also induced plastic responses, though to a lesser extent. Root mass was particularly affected by K. The root length in K omitted was significantly lower than the fully fertilized treatment at the beginning of the growing season, but became the highest at the late growing stages. K omission also resulted in significantly low root diameter. This was already reported in a resistant rice genotype where higher abundance of fine roots was observed under K limitation (Jia et al. 2008; Jordan-Meille et al. 2018; Sustr et al. 2019). For winter wheat (Chapter 3), K omission lowered root biomass only at an early stage (tillering) but increased root length at booting and flowering. This variability highlights the complexity of root responses to K omission based on the specie and growing stage.

6.5.2 Lime omission

Liming is well known to increase the physical and chemical properties of soil by increasing soil pH of alleviating aluminium (Al) and Manganese (Mn) toxicity, maximizing nutrient availability for plants, and decreasing P immobilization (Li et al. 2019). Lime also affects the physical properties of the soil by improving its stability (Frank et al. 2019). While these effects on soil are well documented, few studies

have focused on the effect of liming on root traits. Our results together with previous studies suggest that Ca can affect root architecture, however the effects appear species- and context-dependent. For example, the incorporation of high lime doses increased maize root growth in the deep subsoil (de Moraes et al. 2023). It has also been shown that plants growing in acidic pH had a higher SRL compared with those grown at alkaline or neutral pH. High pH inhibited root elongation of narrow-leaf lupine, with a reduction of up to 90% in surface area between plants growing at pH 7.5 and pH 6.5, comparable to results previously observed by Tang et al. (1992), presenting a strategy to increase the SRL and therefore increase nutrient uptake (Robles-Aguilar et al. 2019). However, in sugar beet (Chapter 5), this effect was not pronounced. A trend to have lower SRL at the early growth stage was observed, but couldn´t be statistically confirmed. We also observed lower values of root length per tuber. These differences may be partly attributed to the sampling strategy, which focuses only on the soil surrounding sugar beet tuber and do not allow for detailed conclusions regarding morphological root traits. For winter wheat (Chapter 4), Ca omission increased RLD, indicating potential response to explore more soil volume and cope with nutrient unavailability caused by Ca omission. These contrasting findings suggest that Ca omission can impact root growth and development, but the magnitude and direction of the response may vary depending on the crop species, growth stage and type of experimental conditions

6.6 Root plasticity to nutrient omission: quantitative insights and agronomic relevance

Across the four studies, both crop-specific and general patterns of root plasticity emerged in response to N, P, K, Ca omission and to intercropping. The observed plasticity varied by species, growth stage, and methodological approach. Specific root traits demonstrated clear plastic responses. Indeed, winter rye showed decrease in root diameter under P and N omission, accompanied by increases SRL, reflecting a shift toward finer roots under N and P omission at early growth stages but not at late growth stages. Winter wheat exhibited increased root biomass and length under P omission during booting and flowering, while SRL and R/S responses were variable depending on growth stage. In sugar beet, P deficiency increased the proportion of very fine and fine roots at late stages, whereas K omission reduced root branching early but promoted it later, along with higher SRL and lower root diameter. Ca omission induced trends toward lower SRL in sugar beet and higher root length density in winter wheat, illustrating nutrient-specific and species-specific plasticity. So, an important factor influencing the findings was the temporal aspect of root sampling. Discrepancies between the observed responses across growing stages highlight the plastic adjustments of roots to nutrient stress and the shifts in resource allocation between the early and late growing stages. These factors emphasize the need for continuous sampling approaches when evaluating root traits and their adaptation to nutrient limitations and to intercropping. The insights gained here can inform future research programs focused on improving nutrient use efficiency and optimizing the belowground interactions and hence the resilience of different

cropping systems. Despite the challenges of belowground measurements, the findings provide field-based insights of root phenotypic plasticity that can inform modelling efforts and guide experimental design. As it provides reference values for improving process-based crop models (Seidel et al. 2022; Demie et al. 2025) and root architectural models (Schnepf et al. 2018), and guides future sampling strategies especially with regard to linking the timing of sampling with its agronomic relevance.

6.7 Limitations and future research needs

While the findings of this study provide valuable insights, there are several limitations to consider. One major challenge is the difficulty in accurately observing and measuring root growth, particularly under field conditions, where the root systems of crops can be highly complex and temporally and spatially variable. Additionally, this research did not fully explore the interaction between nutrient stress and other factors, such as water availability, soil parameters, or intercropping system, which may further influence root plasticity (Gleeson and Good 2010; Rogers et al. 2016). Hence, future research should address these gaps by conducting multi-year and multi-location experiments to examine the long-term effects of nutrient stress and intercropping strategies. Also, only the complete omission of nutrients was evaluated in the studies of this thesis. However, it was also shown that the degree and timing of nutrient supply may affect the plasticity of roots. Future studies should focus on integrating multiple levels of nutrient supply to further understand the response of crops´ rooting systems to nutrient stress and better design fertilisation strategies. Furthermore, integrating root trait data into crop models (Demie et al. 2025) and exploring the genetic basis of root plasticity would provide valuable insights for breeding programs aimed at developing crops for low-input and climate-resilient farming systems.

6.8 Conclusion

The thesis presents four complementary research studies conducted under field conditions. Across multiple crops (winter rye, winter wheat and sugar beet), we showed that long-term nutrient deficiency impacted root growth and plasticity. The magnitude and type of effects differed across crops and especially across growth stages. Intercropping revealed complementary root growth interactions in low and normal sowing density but not under high sowing density. Together, these studies provide important insights into root growth and plasticity under field conditions. The methodology used in all studies was based on real observations rather than pot or controlled chambers, providing a realistic understanding on how roots grow in complex field conditions. This strengthens the relevance of the findings for agronomic practices and modelling exercises. Our research highlights the importance of selecting genotypes with strong root plasticity to cope with nutrient deficiency, adopting diversified cropping systems such as cereal-legume intercrops, and applying nutrient more precisely through targeted management. Together, these approaches can enhance crop productivity and resilience under low input conditions.

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APPENDIX A: SUPPLEMENTARY MATERIAL FROM CHAPTER 2

Table S2.1: Number of replicates in the field experiment.

Treatment	Number of replicates
FB_100	1
FB_100_SW_SUAh_100	1
FB_33_SW_SUAh_33	2
FB_33_SW_Ana_33	1
FB_50_SW_SUAh_50	3
FB_50_SW_Ana_50	1
SW_SUAh_100	1
SW_Ana_100	3

Figure S2.1: Picture showing the placement of the core when sampling the roots in intercrops. This covered always one faba bean and one wheat plant and the core was placed not exactly above a row but next to the row (from the row to 1.5 cm from the middle of the row. Picture taken on 09/06/2021.

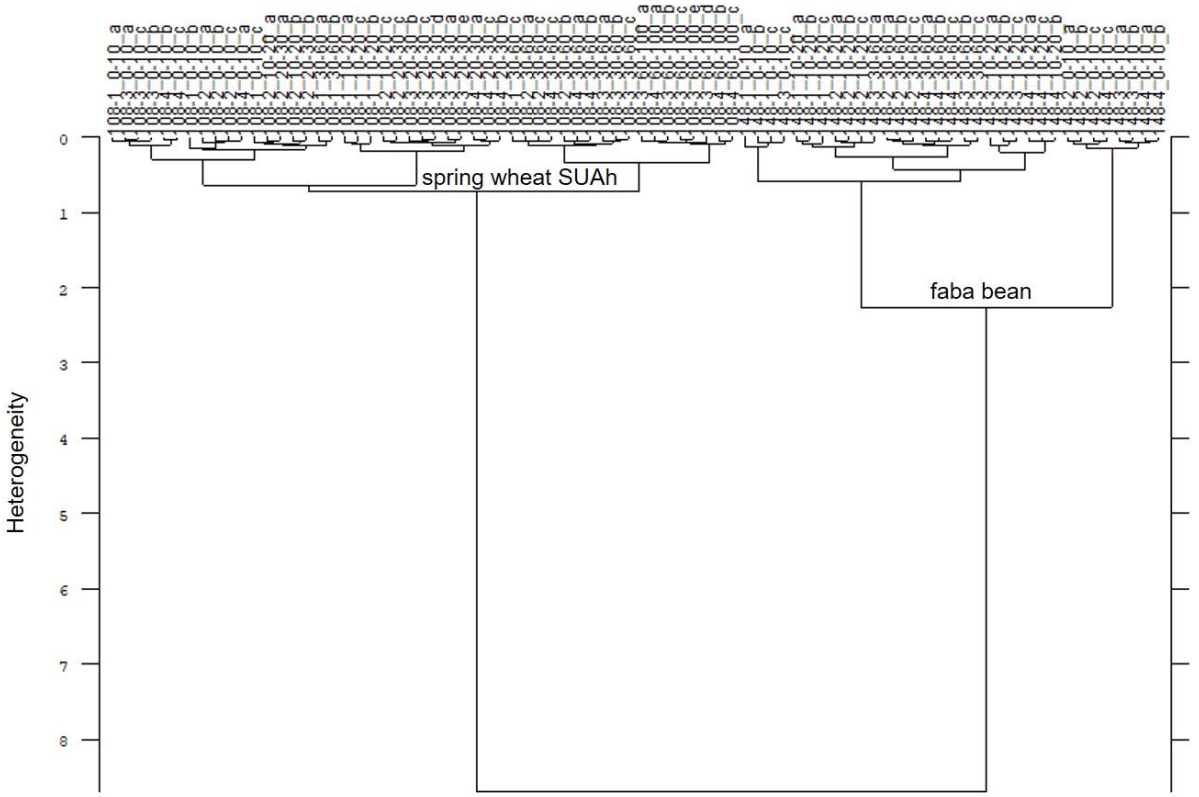

Figure S2.2: Cluster analysis of FTIR spectra of single species samples of dried and ground roots of spring wheat (*cv*. SU Ahab) and faba bean of the first sampling date. Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (4000-3630 cm^{-1}, 1840-1480 cm^{-1} and 1120-760 cm^{-1}), Ward's algorithm and Euclidian distance.

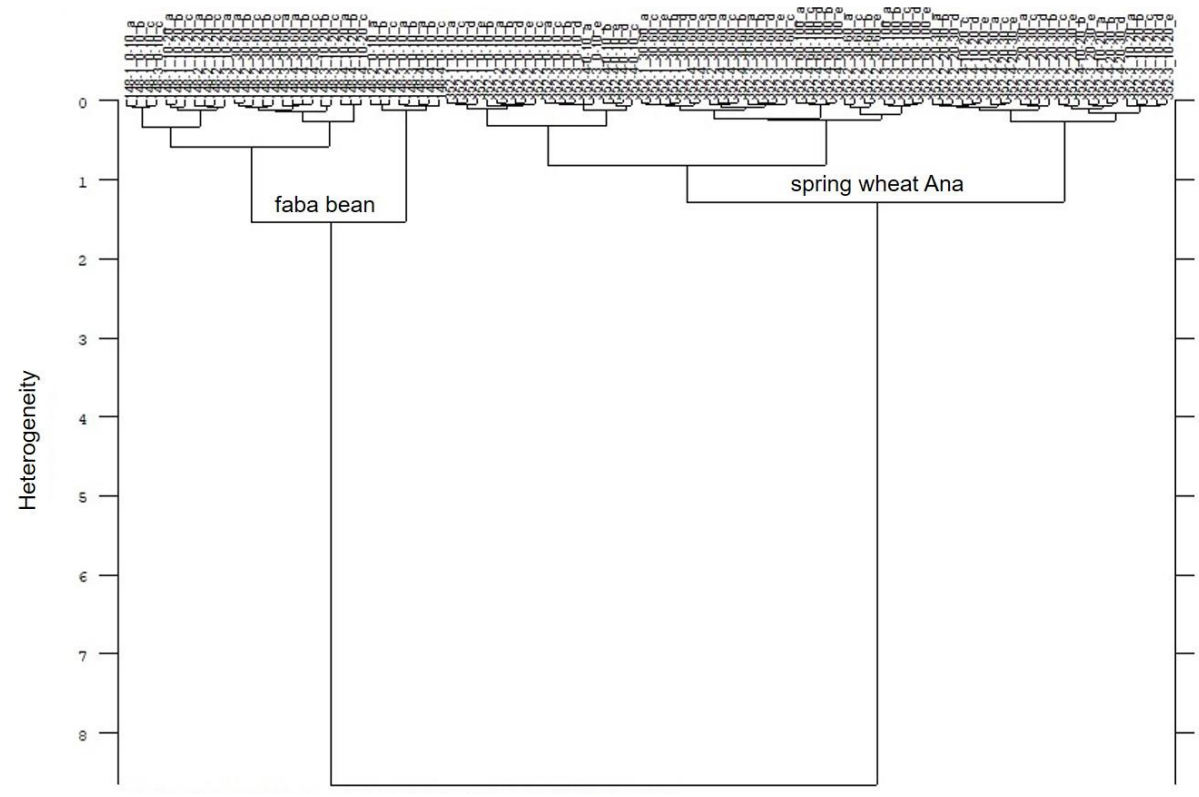

Figure S2.3: Cluster analysis of FTIR spectra of single species samples of dried and ground roots of spring wheat (*cv*. Anabel) and faba bean (*cv*. Fanfare) of the first sampling date. Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (3700-3330 cm^{-1}, 1550-1300 cm^{-1} and 1200-850 cm^{-1}), Ward's algorithm and Euclidian distance.

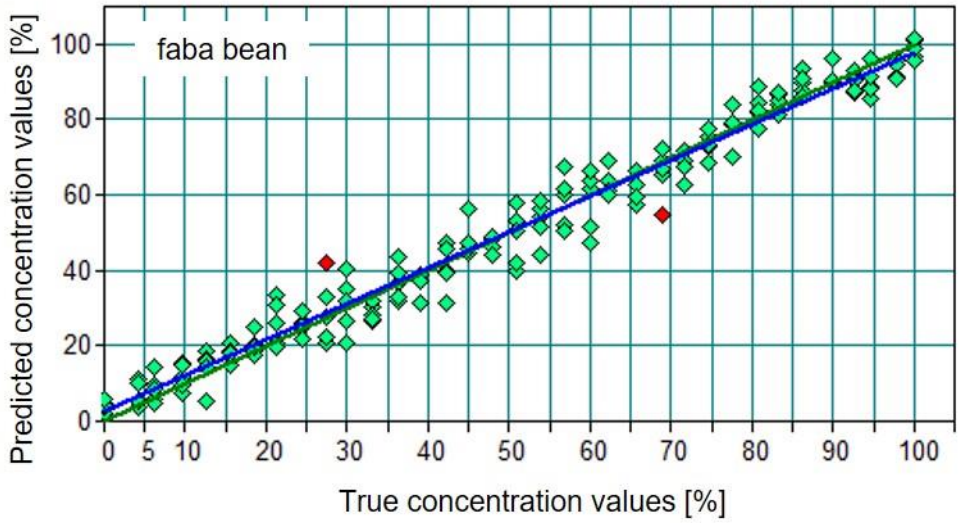

Figure S2.4: Predicted concentration values of faba bean (blue regression line) in comparison to the true (known) concentration values (green dots and green regression line) of the "artificial mixtures" of the model Ana – faba bean of the first sampling date. The red dots are outlier calculated by the model. The corresponding figure of spring wheat for the model SUAh – faba bean of the first sampling date as well as spring wheat – faba bean model of the second sampling date are very similar and therefore not shown.

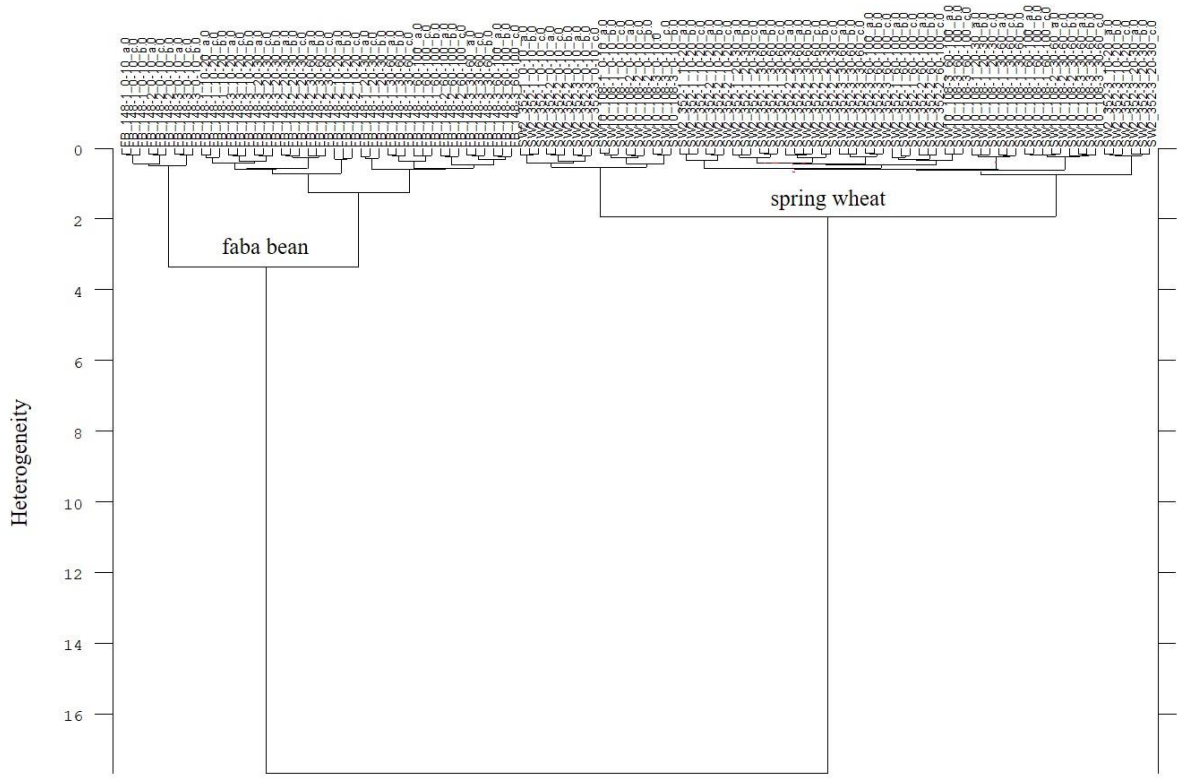

Figure S2.5: Cluster analysis of FTIR spectra of single species samples of dried and ground roots of both spring wheat varieties (Ana, SUAh) and faba bean of the second sampling date. Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (3750-2750 cm^{-1} and 1800-850 cm^{-1}), Ward's algorithm and Euclidian distance.

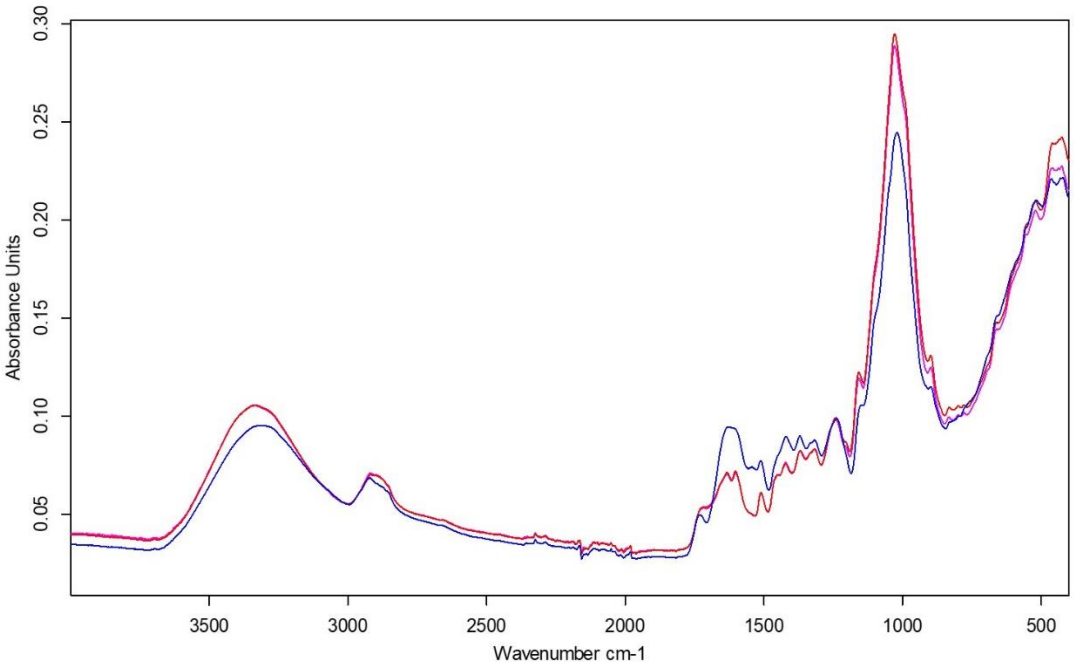

Figure. S2.6: Average FTIR spectra of faba bean (blue line) and spring wheat *cv*. SU Ahab (pink line) and spring wheat *cv*. Anabel (red line) at the second sampling date. The red and pink average FTIR spectra of the two spring wheat varieties coincide nearly in every wave number.

Table S2.2: Model details and statistical values for the models of spring wheat Ahab (SUAh) and faba bean (FB), as well as spring wheat Anabel (Ana) and faba bean at the first sampling date. Abbreviation: 1st der. – first derivative, SNV – vector normalization, R^2 - coefficient of determination (%), RPD – residual prediction deviation, RMSECV – root mean square error of cross validation, offset – offset of the regression line "predicted

concentration values vs. true concentration values" for both species, slope– slope of the regression line "predicted concentration values vs. true concentration values", RMSEP – root mean square error of prediction, SW – spring wheat.

first sampling date		SU Ahab - FB	Anabel - FB
Data pre-processing		1st der. + SNV	1st der. + SNV
Frequency range [cm^{-1}]		3998 - 3636	3702 - 3328
		1841 - 1477	1547 - 1300
		1120 - 759	1202 - 849
Calibration	R^2	93.72	97.26
internal validation	RPD	3.99	6.04
	RMSECV	7.49	4.94
	Offset SW	3.048	2.257
	Offset FB	3.377	2.404
	Slope	0.936	0.953
External validation	RPD	3.33	4.23
	RMSEP	8.45	6.55
	Offset SW	0.309	5.906
	Offset FB	3.903	2.948
	Slope	0.958	0.911

Table S2.3: Model details and statistical values for the model of both spring wheat varieties (SW) and faba bean (FB) at the second sampling date. Abbreviation: 2nd der. – second derivative, SNV – vector normalization, R^2 - coefficient of determination (in %), RPD – residual prediction deviation, RMSECV – root mean square error of cross validation, offset – offset of the regression line "predicted concentration values vs. true concentration values" for both species, slope– slope of the regression line "predicted concentration values vs. true concentration values", RMSEP – root mean square error of prediction, SW – spring wheat.

second sampling date		SW - FB
Data pre-processing		2nd der. + SNV
Frequency range [cm^{-1}]		3638 - 3277
		2920 - 2557
		2200 - 1839
		1479 - 759
Calibration	R^2	95.89
internal validation	RPD	4.94
	RMSECV	6.02
	Offset SW	2.802
	Offset FB	2.426
	Slope	0.948
External validation	RPD	4.73
	RMSEP	5.67
	Offset SW	2.077
	Offset FB	1.587
	Slope	0.963

Table S2.4. Spring wheat (wheat) and faba bean (bean) shoot traits (n=1) measured on two sampling dates and at harvest. cv. Stands for cultivar. The species and the sowing density (SD) is given in %. Crop height is given in cm. Dry matter above- ground biomass (AGB), grain yield (GY), and straw are given in t ha^{-1}. Bean and Wheat nr. stand for number of plants per m^2.

abbr.	SW_SUAh_100	SW_Ana_100	FB_100	FB_33_SW_Ana_33	FB_33_SUAh_33	FB_50_Ana_50	FB_50_SUAh_50	FB_100_SUAh_100
				6-8/06/2021				
SW height	48	49		56	55	57	56	54
FB height			58	62	55	64	59	68
SW AGB	2.6	3.1		1.8	1.7	2.6	2	2.3
FB AGB			1.9	0.8	0.4	1.1	0.7	1.6
				5-8/07/2021				
Wheat nr.	398	395		105	119	221	190	436
Bean nr.			48	19	17	26	26	45
Wheat height	79	75		75	80	78	83	85
Bean height			105	92	88	94	95	100
Wheat AGB	**8**	10		5.3	5.9	5.8	4.8	5.5
FB AGB			6.6	2.7	2.6	3	3.7	4.7
				Harvest (13/08/2021)				
SW GY	4	5.2		2.3	3	2.5	2.7	2.2
FB GY			3.4	2.9	2.6	2	2.7	3.1
Total GY	4	5.2	3.4	5.2	5.6	4.5	5.4	5.4
Proportion of FB of total GY, in %	0	0	100	56	47	45	50	58
SW straw	4.6	5.5		3	3.7	3.4	3.5	NA
FB straw			3.5	2.2	1.9	1.6	2.1	NA
SW AGB	8.6	10.7		5.3	6.7	5.9	6.2	NA
FB AGB			6.9	5.1	4.5	3.6	4.8	NA
Proportion of FB of total AGB, in %			100	49.0	40.2	37.9	43.6	NA

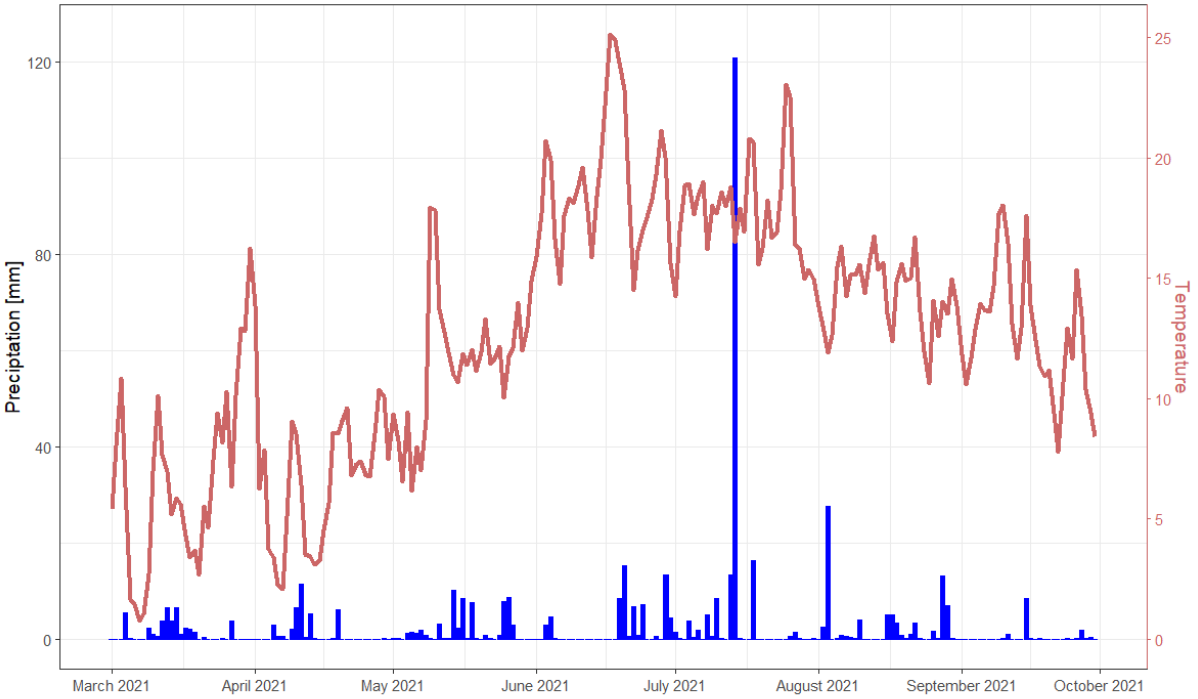

Figure S2.7: Daily precipitation in mm (blue bars) and mean daily air temperature in °C in the study site at Campus Klein Altendorf, University of Bonn, Germany, from 03/2021 to 10/2021.

Table S2.5: Shoot partial land equivalent ratio of bean ($pLER_{Bean}$, n = 1) and wheat ($pLER_{Wheat}$, n = 1) and the land equivalent ratio (LER, n = 1) of the intercrops for two sampling dates (**1**: 6-8/06/2021 and **2**:5-8/07/2021) and of grain yield at harvest (13/08/2021). SD represent the sowing densities for the the fully replacement treatments FB_50_SW_Ana_50 and FB_50_SW_SUAh_50. The mean and standard deviation (sd) were calculated based on the (n=2) values obtained for FB_50_SW_Ana_50 and FB_50_SW_SUAh_50 in each of the sampling dates.

		LER					
Sampling date	Treatment	Wheat SD	Bean SD	pLERBean	pLERWheat	LER	Mean±sd
1	FB_50_SW_Ana_50	50	50	0.58	0.84	1.42	1.28±0.20
1	FB_50_SW_SUAh_50	50	50	0.37	0.77	1.14	
2	FB_50_SW_Ana_50	50	50	0.45	0.58	1.03	1.10±0.10
2	FB_50_SW_SUAh_50	50	50	0.56	0.6	1.16	
Harvest	FB_50_SW_Ana_50	50	50	0.59	0.48	1.07	1.27±0.28
	FB_50_SW_SUAh_50	50	50	0.79	0.68	1.47	

Table S2.6: Sum of the mean root mass (t ha^{-1}) for the soil depth 0-1 m, as well as calculated increase in root mass, on sampling dates one (09/06/2021, flowering of spring wheat) and two (05/07/2021, flowering of faba bean).

Treatment	Sampling date 1	Sampling date 2	Increase (%)
FB_100	1.50	2.28	34
FB_100_SW_SUAh_100	1.17	1.98	41
FB_33_SW_Ana_33	1.83	2.30	20
FB_33_SW_SUAh_33	2.11	3.14	33
FB_50_SW_Ana_50	1.64	3.07	46
FB_50_SW_SUAh_50	2.03	2.57	21
SW_Ana_100	1.17	1.35	13
SW_SUAh_100	1.09	1.45	25

Table S2.7: Results of a two way Anova (alpha=0.05) including degrees of Freedom (Df), sum of squares (Sum Sq), Mean square (Mean Sq), F value and P value.

	Df	Sum Sq	Mean Sq	F value	P (>F)
SD	2	1.6316	0.8158	4.313	0.0331
Cultivar	1	0.2848	0.2848	1.506	0.2387
SD:Cultivar	1	0.1023	0.1023	0.541	0.4734
Residuals	15	2.8372	0.1891		

Table S2.8: Mean (n=4) root length density (RLD, cm^3 cm^{-3}) per layer and cumulated for topsoil, upper and deeper subsoil on A) 09/06/2021) and B) 05/07/2021.

A)

mean RLD	**Depth (cm)**													
Treatment	10	20	30	Sum 0-30	40	50	60	Sum 30-60	70	80	90	100	Sum 60-100	Sum 0-100
FB_100	0.8	0.3	0.2	1.3	0.3	0.3	0.0	0.6	0.0	0.0	0.0	0.0	0.1	2
SW_SUAh_100	2.3	0.9	0.9	4.1	0.6	0.7	0.7	2.0	0.2	0.0	0.0	0.0	0.3	6.4
SW_Ana_100	2.4	0.8	1.1	4.3	1.0	0.9	0.9	2.8	0.9	0.4	0.1	0.1	1.5	8.6
FB_33_SW_Ana_33	2.0	0.8	0.9	3.8	0.4	0.4	0.5	1.3	0.3	0.2	0.1	0.1	0.7	5.8
FB_33_SW_SUAh_33	2.2	1.0	0.8	4.0	0.6	0.5	0.5	1.7	0.7	0.5	0.3	0.1	1.6	7.3
FB_50_SW_Ana_50	2.4	1.1	1.1	4.6	0.8	1.0	0.6	2.4	0.5	0.1	0.0	0.0	0.7	7.7
FB_50_SW_SUAh_50	2.2	1.1	0.8	4.1	0.9	0.9	0.4	2.2	0.4	0.2	0.1	0.0	0.8	7.1
FB_100_SW_SUAh_100	2.4	0.7	1.1	4.2	0.6	0.6	0.3	1.5	0.3	0.2	0.2	0.1	0.8	6.5

B)

Mean RLD	**Depth (cm)**													
Treatment	10	20	30	Sum 0-30	40	50	60	Sum 30-60	70	80	90	100	Sum 60-100	Sum 0-100
FB_100	1.9	0.7	0.7	3.3	0.6	0.6	0.2	1.5	0.2	0.2	0.1	0.0	0.4	5.2
SW_SUAh_100	5.7	2.3	2.2	10.2	1.9	1.7	1.5	5.0	1.5	0.9		0.4	2.8	18
SW_Ana_100	5.2	2.2	1.8	9.3	2.1	1.9	1.7	5.6	1.6	1.0	0.5	0.4	3.6	18.5
FB_33_SW_Ana_33	5.6	1.9	1.6	9.0	1.1	0.8	1.2	3.2	1.3	1.1	0.7	0.4	3.5	15.7
FB_33_SW_SUAh_33	6.5	2.4	2.0	10.9	1.4	1.3	1.2	4.0	1.7	1.3	0.9	0.7	4.6	19.5
FB_50_SW_Ana_50	6.7	2.3	2.3	11.3	2.0	2.5	1.6	6.0	1.4	0.9	0.5	0.5	3.3	20.6
FB_50_SW_SUAh_50	6.4	2.1	1.8	10.3	1.7	1.7	1.0	4.3	1.0	0.8	0.7	0.3	2.8	17.4
FB_100_SW_SUAh_100	6.6	1.5	1.8	9.9	1.3	1.2	0.9	3.4	0.9	1.0	0.8	0.4	3.0	16.3

Table S2.9: Observed and expected specific root length SRL (m g-1) per layer and mean values for topsoil, upper and deeper subsoil on A) 09/06/2021) and B) 05/07/2021.

A)

Mean of SRL	**Depth (cm)**														
Treatment	10	20	30	mean 0-30	40	50	60	mean 30-60	70	80	90	100	mean 60-100	mean 0-100	Expected 0-100
FB_100	8	25.3	43.4	25.6	44.1	72.6	52.8	56.5	124.3				124.3	52.9	
SW_SUAh_100	45	118.6	105.8	89.8	79.3	99.4	86.9	88.5	60.3	53.9	43.6	NA	52.6	85	
SW_Ana_100	42.5	112.6	126.1	93.7	106.9	124.7	116.9	116.2	135.1	117.5	120.8	104.6	119.5	109.3	
FB_33_SW_SUAh_33	13.8	81.7	110.4	68.6	95.2	114	110.6	106.6	127.8	132.5	107.9	84.1	113.1	93.4	45.507
FB_33_SW_Ana_33	13	95.5	141.8	83.4	121.4	207.2	219.6	182.7	201.1	207.1	151.3	108.3	166.9	142.8	53.526
FB_50_SW_SUAh_50	14.3	69.5	107.7	63.8	97.2	151.7	116.8	121.9	165.7	204	149	NA	172.9	103.3	68.95
FB_50_SW_Ana_50	16	107.7	114.3	79.3	131.4	131.4	141.8	134.9	163.1	133	98.1	75.6	117.4	115.1	81.1
FB_100_SW_SUAh_100	60.3	65.8	92.9	73	97	128.4	134.9	120.1	112.9	143.4	131.5	134.1	130.5	98.9	137.9

B)

Mean of SRL	**Depth (cm)**														
Treatment	10	20	30	mean 0-30	40	50	60	mean 30-60	70	80	90	100	mean 60-100	mean 0-100	Expected 0-100
FB_100	19.4	31.7	38.4	29.8	37	51.6	52.5	47	70.5	73.3	91	97.6	83.1	56.3	
SW_SUAh_100	48.1	130.8	124	101	111.5	118.4	125.7	118.5	131.7	150.1	193.5	175.4	162.7	130.92	
SW_Ana_100	35.8	124.9	103.4	88	113.8	128.5	124	122.1	171.9	205.9	261.8	195.6	208.8	146.56	
FB_33_SW_SUAh_33	17.7	75.3	129.3	74.1	121.1	132.6	156.3	136.7	173.9	218.7	205.1	218.4	204	144.84	61.78
FB_33_SW_Ana_33	52.1	104.5	114.7	90.4	105.8	160.2	235.1	167	192.4	175.6	234.3	215.5	204.5	159.02	66.94
FB_50_SW_SUAh_50	21.1	69.8	102.8	64.6	86.3	112.6	142.8	113.9	173.2	190.5	202.4	178.6	186.2	128.01	93.61
FB_50_SW_Ana_50	18.8	74.3	125.9	73	112.9	125.1	158.4	132.1	161.8	199.1	229.1	226	204	143.14	101.43
FB_100_SW_SUAh_100	32.8	64.8	79.2	58.9	82.7	127.4	153	121	172	209.6	187.4	147.1	179	125.6	187.22

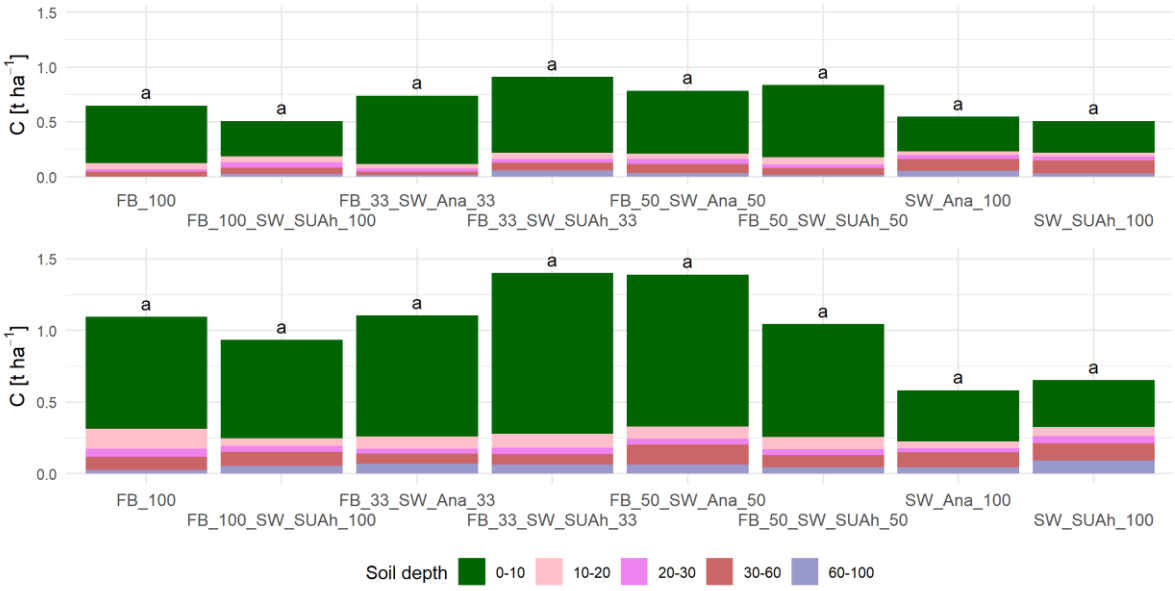

Figure S2.8: Root C content (not crop-specific) in t ha-1, for sole faba bean and sole spring wheat, as well as for the intercrop treatments for three soil layers on 09/06/2021 (top panel) and 05/07/2021 (bottom panel). Different letters are significant differences comparing the cumulative root C content over all soil layers (Anova and Tukey post-hoc test, α=0.05). FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

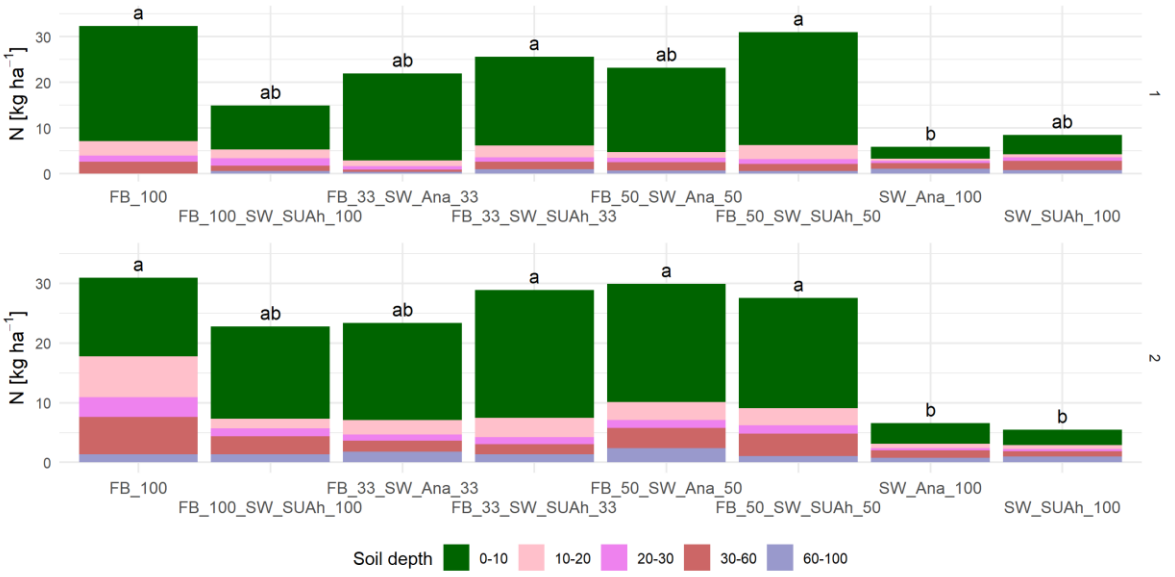

Figure S2.9: Root nitrogen (N) content in kg ha-1, for sole faba bean and sole spring wheat, as well as for the intercrop treatments (not crop-specific) for three soil layers sampled on 09/06/ 2021 (top panel) and on 05/07/ 2021 (bottom panel). Different letters indicate significant differences comparing the cumulative root N over all soil layers (Anova and Tukey post-hoc test, α=0.05). FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop

Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

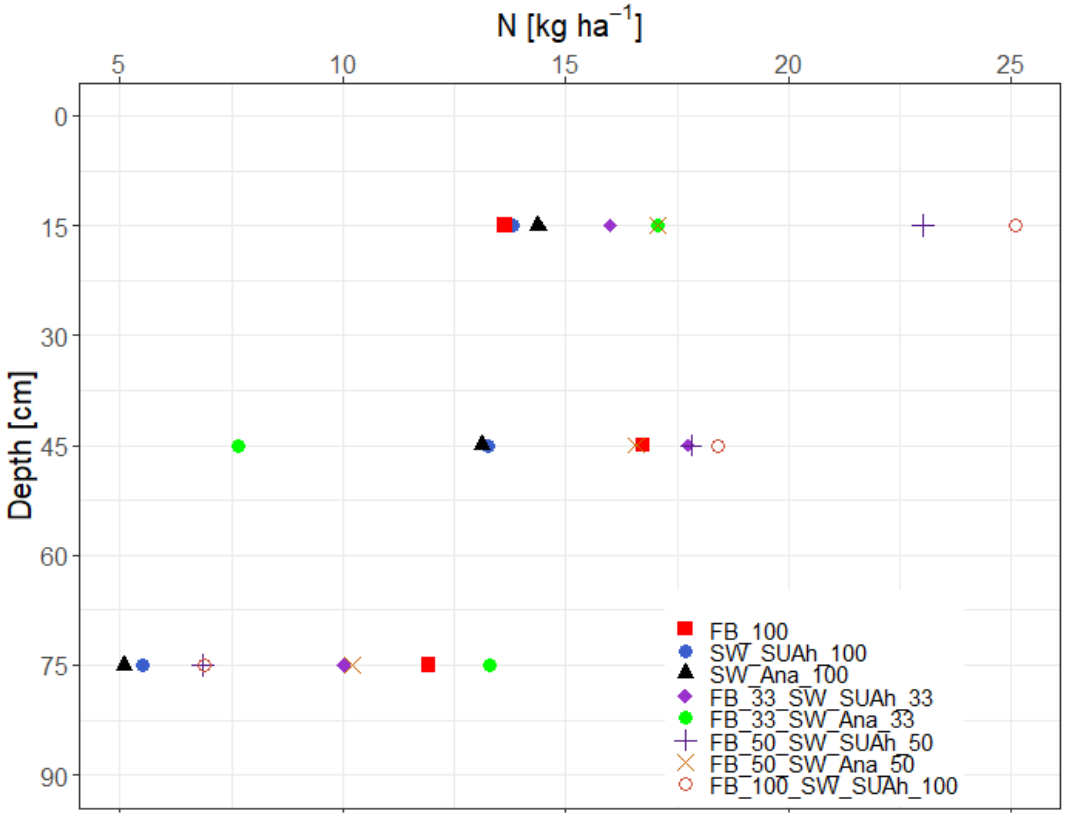

Figure S2.10: Soil mineral nitrogen (Nmin) in kg ha-1 at different depth (0-30, 30-60, 60-90 cm soil depth) after the harvest (26/08/2021). FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel, (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)). Points refer to middle point of the investigated soil layer depth (e.g. 15 cm in case of 0-30 cm).

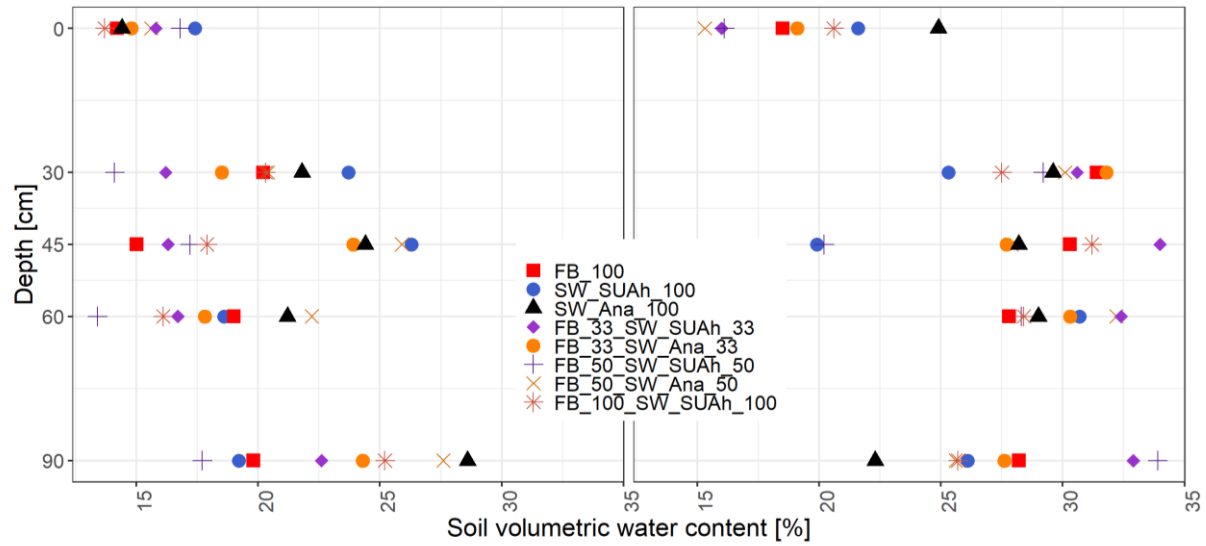

Figure S2.11: Soil volumetric water contents at different soil depths (0, 30, 60, 90 cm) measured at sampling date 06/06/2021 (left panel) and 05/07/2021 (right panel). FB_100=Sole crop faba bean Fanfare, SW_SUAh_100=Sole crop spring wheat SU Ahab, SW_Ana_100=Sole crop spring wheat Anabel, FB_33_SW_SUAh_33=Intercrop Fanfare (SD=33%) x SU Ahab (SD=33%), FB_33_SW_Ana_33=Intercrop Fanfare (SD=33%) x Anabel (SD=33%), FB_50_SW_SUAh_50=Intercrop Fanfare (SD=50%) x SU Ahab (SD=50%), FB_50_SW_Ana_50 =Intercrop Fanfare (SD=50%) x Anabel (SD=50%), FB_100_SW_SUAh_100= Intercrop Fanfare (SD=100%) x SU Ahab (SD=100%)).

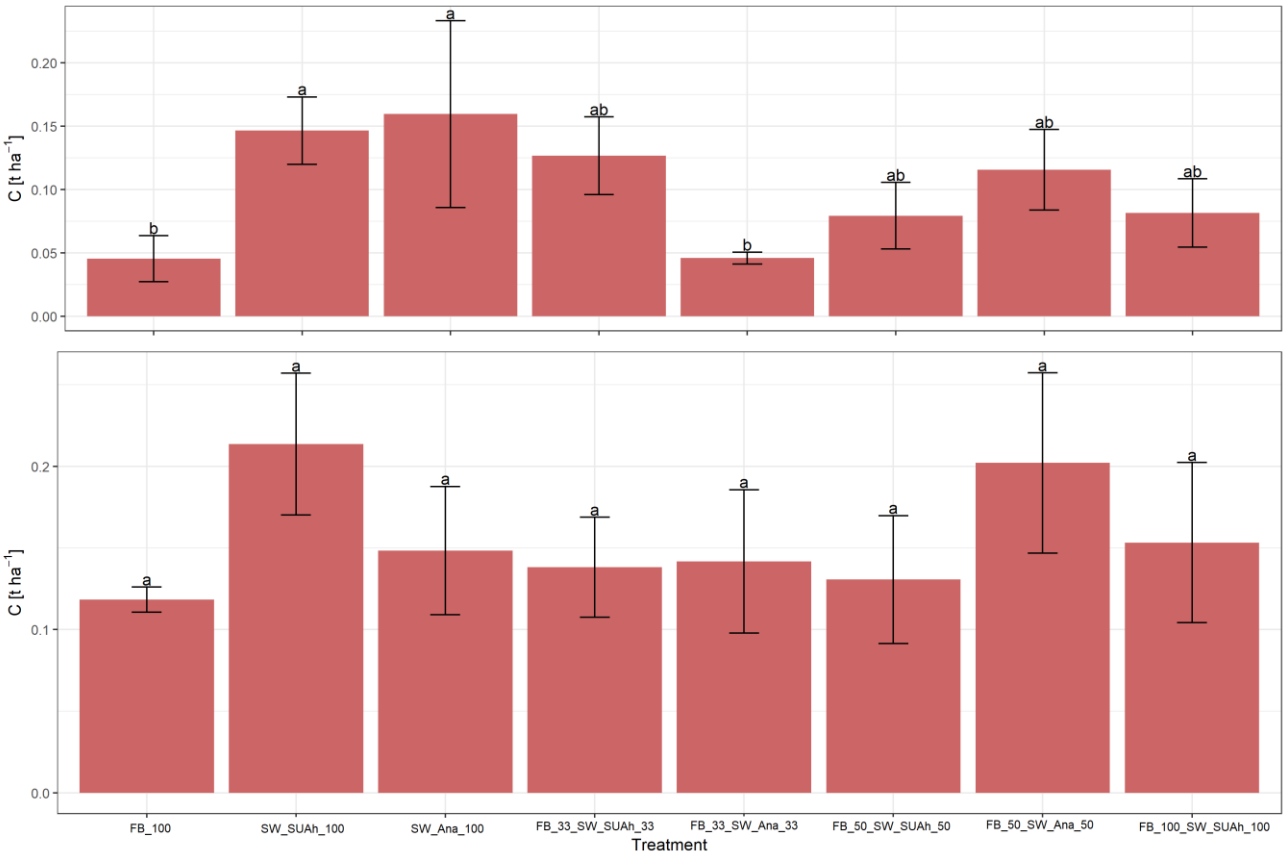

Figure S2.12: Deep soil (30-100 cm) root C (mean (n=4), not crop-specific) in t ha-1, for sole faba bean and sole spring wheat, as well as for the intercrop treatments for three soil layers on date one (top panel) and date two (bottom panel). Different letters indicate significant differences comparing the cumulative root C over 30-60 cm and 60-100 cm soil layers (Anova, alpha=0.05, Tukey test).

APPENDIX B: SUPPLEMENTARY MATERIAL FROM CHAPTER 3

Table S3.1. Average ± standard deviation of root biomass in the topsoil 30 cm layer in g m-2 as affected by N and P omission treatments over five sampling dates (16/03/2022 (2-4 tillers detectable), 04/04/2022 (beginning of stem elongation), 29/04/2022 (early to mid-boot stage), 27/05/2022 (60-80% of inflorescence emerged) and 5: 21/06/2022 (end of flowering)). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission fertilized (_PKCa) and P omission fertilized (N_KCa).

Sampling date	NPKCa+m+s	NPKCa	_PKCa	N_KCa
16/3/2022	23.50+19.41	26.49±7.15	16.86±3.95	24.72±5.78
04/04/2022	51.87±10.43	33.91±4.59	28.41±5.26	32.03±2.48
29/04/2022	78.87±16.24	50.38±34.07	42.13±13.12	49.51±15.54
27/05/2022	126.50±25.7	95.37±10.24	36.51±13.13	73.84±14.13
21/06/2022	84.33±9.52	100.05±19.27	45.78±11.53	98.83±35.80

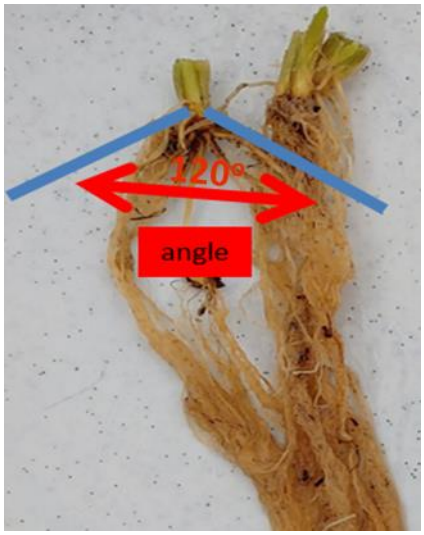

Figure S3.1. Depiction of root angle measurements (blue line) in rye plants, root angle is measured in the nodal roots.

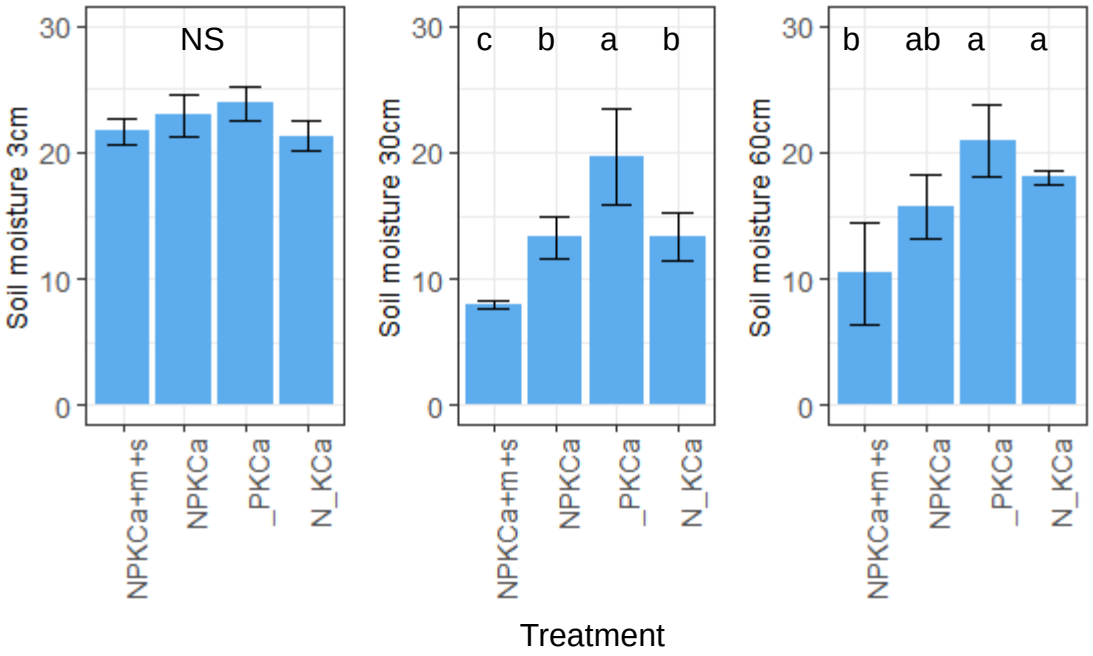

Figure S3.2. Soil moisture content (%) at 3, 30, and 60 cm soil depth on 27/05/2022, around flowering as affected by N and P omission treatments during the 2022 season, over five sampling dates (1: 16/03/2022, 2: 04/04/2022, 3: 29/04/2022,4: 27/5/2022 and 21/06/2022). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Values followed by the same letter do not differ according to Tukey high significant difference at 5% confidence level. NS= not significant at 5% confidence level.

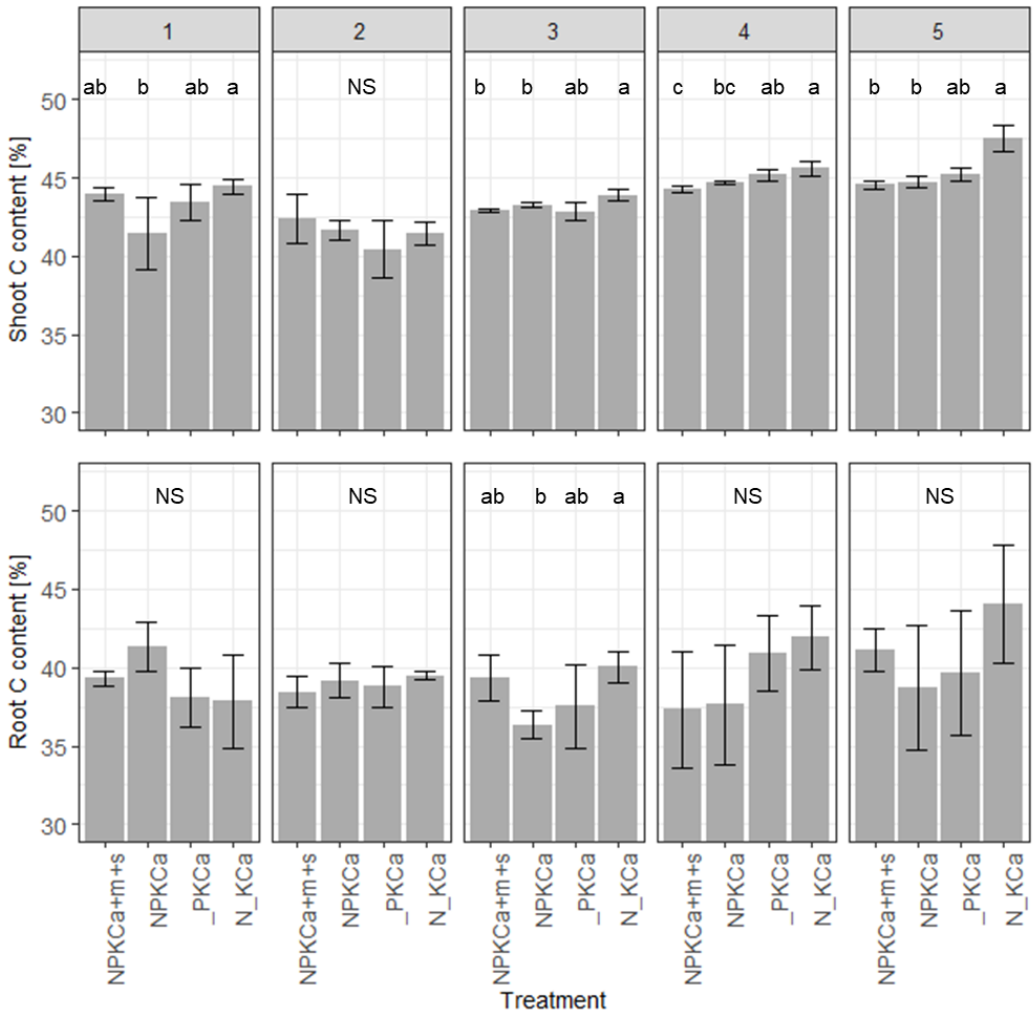

Figure S3.3: Shoot (a) and root (b) C content (%) as affected by N and P omission treatments during the 2022 season, over five sampling dates (1: 16/03/2022, 2: 04/04/2022, 3: 29/04/2022,4: 27/5/2022 and 21/06/2022). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Values followed by the same letter do not differ according to Tukey high significant difference at 5% confidence level. NS= not significant at 5% confidence level. For shoot N content in date 5, an Aligned rank transform for nonparametric factorial ANOVA was implemented.

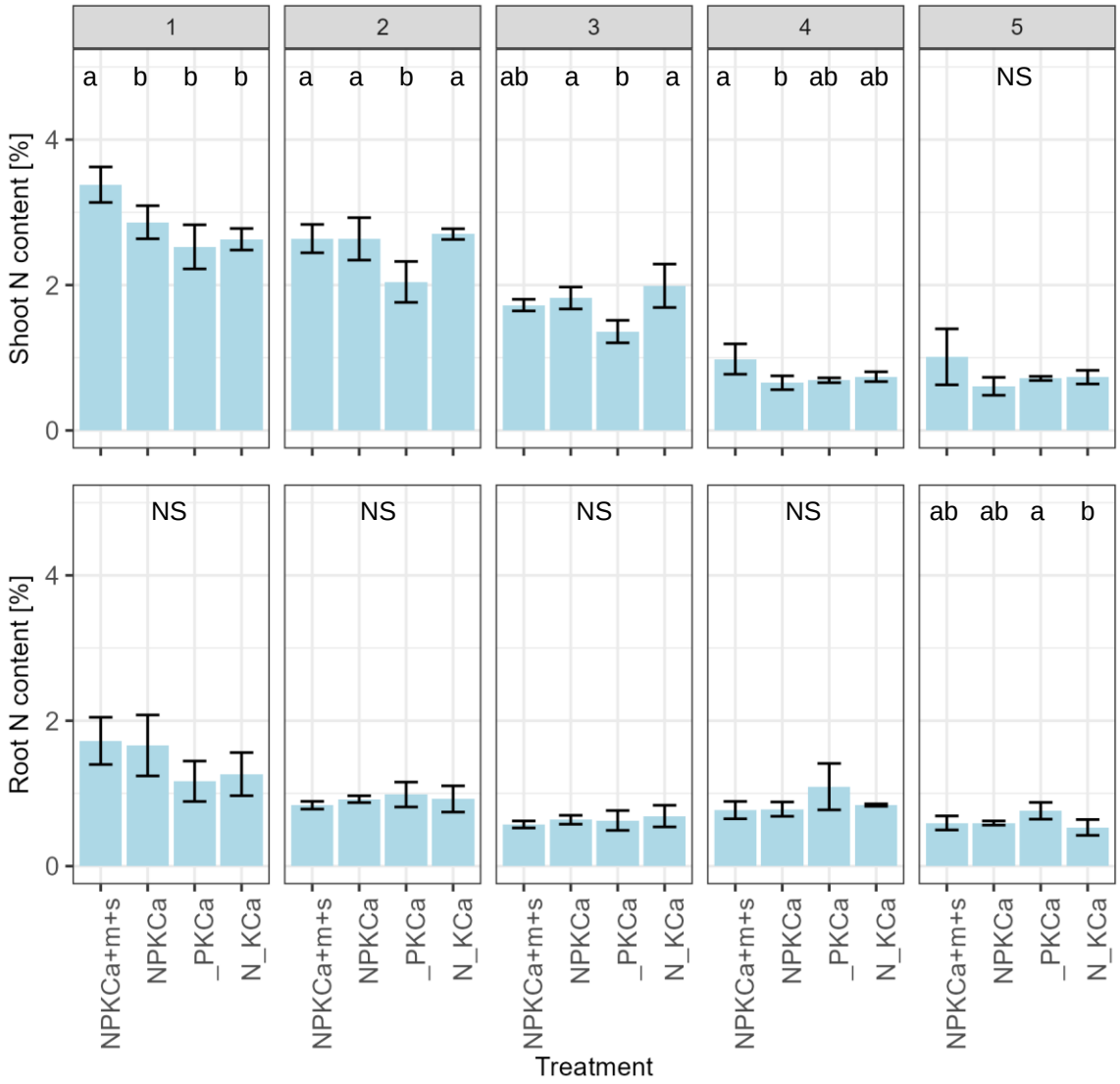

Figure S3.4. Shoot (a) and root (b) N content (%) as affected by N and P omission treatments during the 2022 season, over five sampling dates (1: 16/03/2022, 2: 04/04/2022, 3: 29/04/2022,4: 27/5/2022 and 21/06/2022). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Values followed by the same letter do not differ according to Tukey high significant difference at 5% confidence level. NS= not significant at 5% confidence level. For root N content in date 4, an Aligned rank transform for nonparametric factorial ANOVA was implemented.

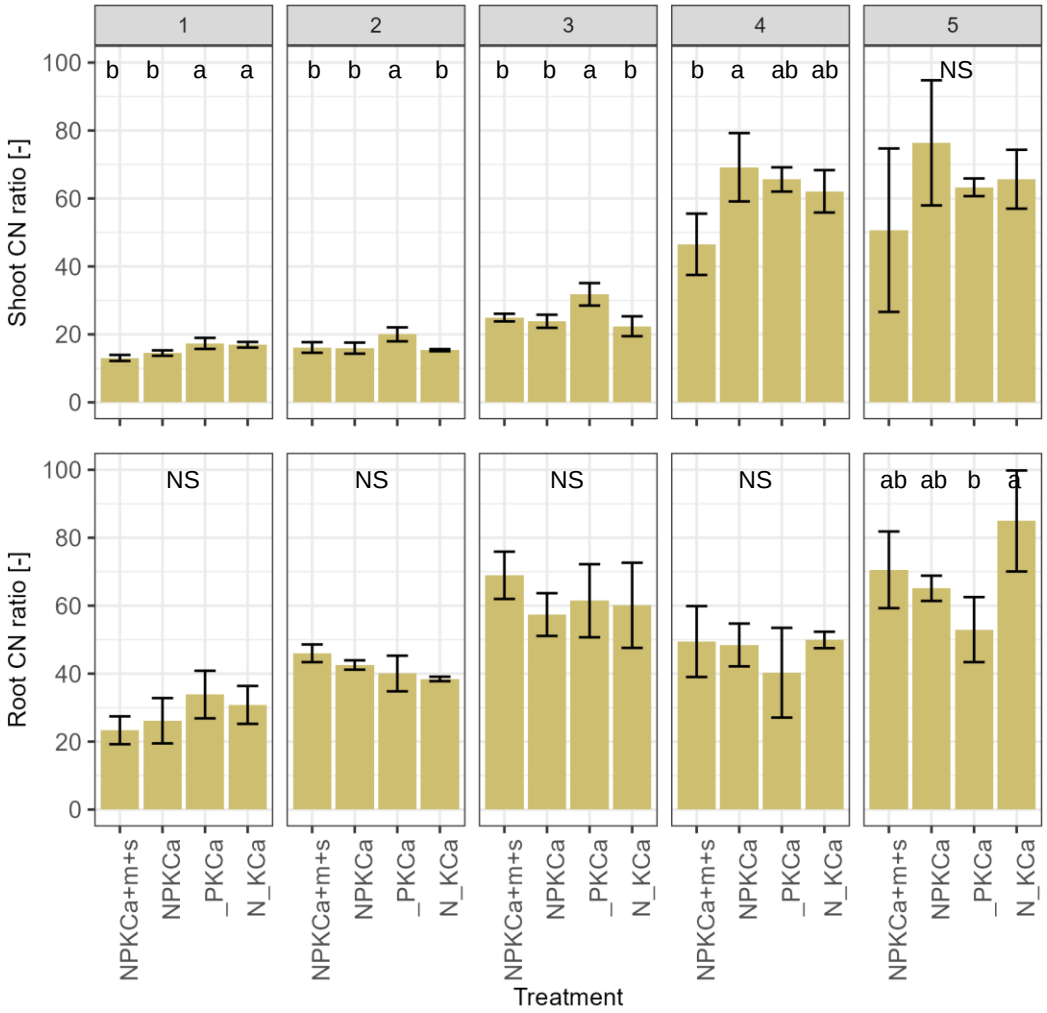

Figure S3.5. Shoot (a) and root (b) C:N ratio as affected by N and P omission treatments during the 2022 season, over five sampling dates (1: 16/03/2022, 2: 04/04/2022, 3: 29/04/2022,4: 27/5/2022 and 21/06/2022). Treatments: Fully fertilized plus manure (NPKCa+m+s), fully fertilized with mineral fertilizer only (NPKCa), N omission (_PKCa) and P omission (N_KCa). Values followed by the same letter do not differ according to Tukey high significant difference at 5% confidence level. NS= not significant at 5% confidence level.

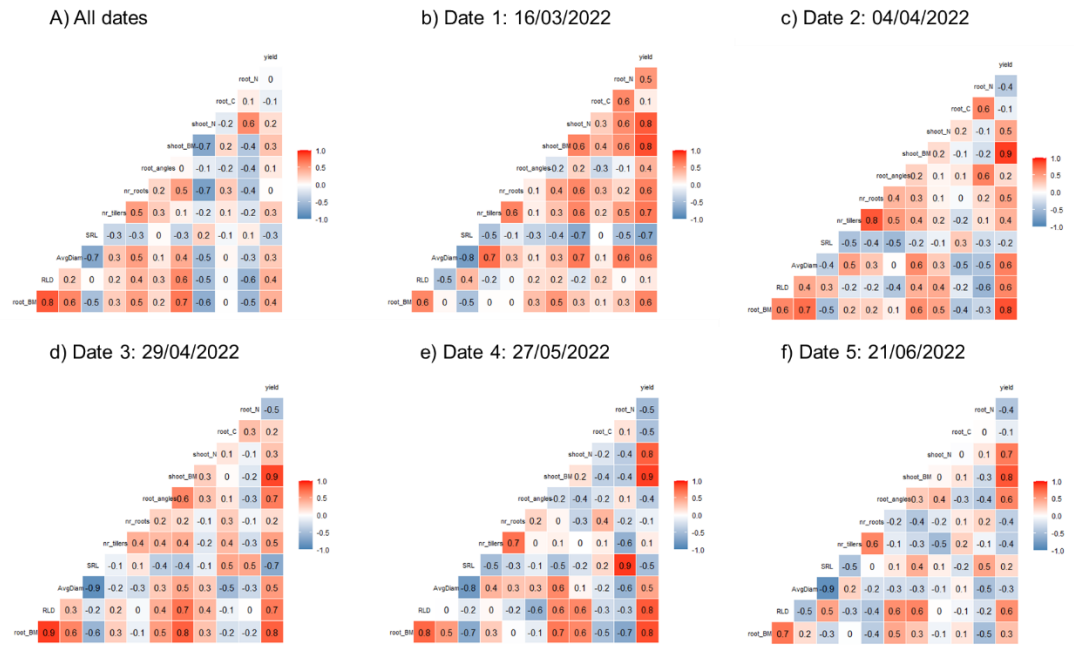

Figure S3.6: Correlation coefficients for different root and shoot variables for a) all five sampling dates (1: 16/03/2022, 2: 04/04/2022, 3: 29/04/2022, 4: 27/05/2022 and 21/06/2022) and, b to f) by sampling date. Abbreviations= nr_tillers= number of tillers (no.), shoot_BM= shoot biomass (g m-2), shoot_N= shoot nitrogen content (%), shootCN= Shoot C:N ratio (-), nr_roots= number of seminal roots (no.), root_angle= root angle (no.), root_BM= root biomass (g m-2), root_C= root C content (%), root_N= root N content (%), root_CN (%), yield (g m-2).

APPENDIX C: SUPPLEMENTARY MATERIAL FROM CHAPTER 4

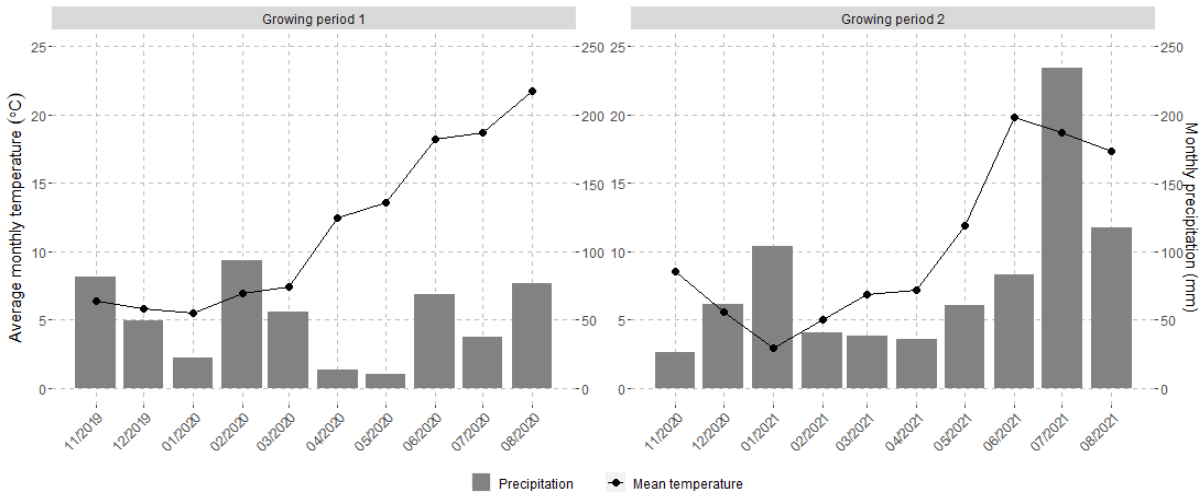

Figure S4.1: Monthly rainfall and mean temperature during the winter wheat growing period 1 (2019/20) and growing period 2 (2020/21) at the LTFE Dikopshof (source: interpolated data from the German Weather Service, DWD).

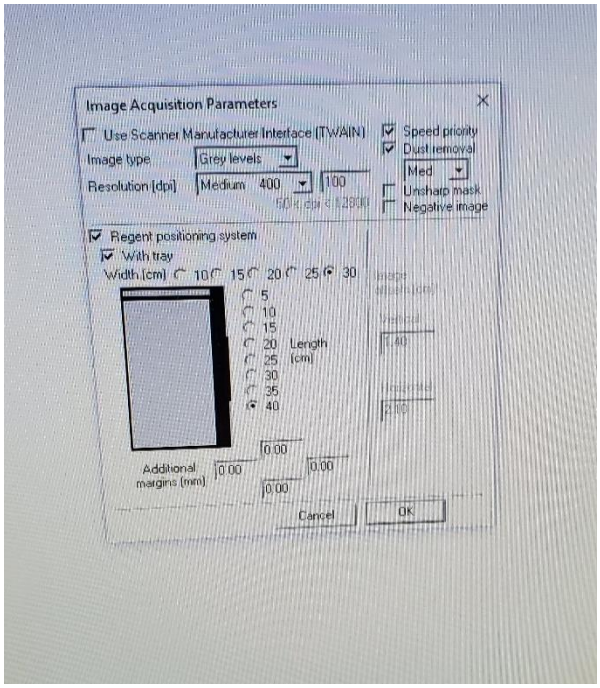

Figure S4.2: Settings for scanning using WinRHIZO

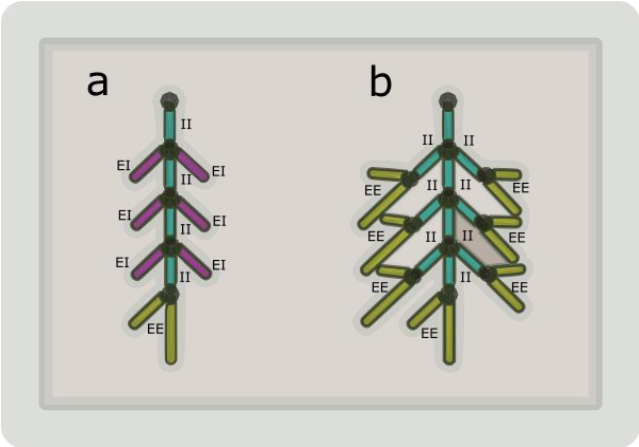

Figure S4.3: Type of links in roots: exterior-exterior (EE) in yellow, exterior-interior (EI) in magenta, and interior-interior (II) in blue. According to the predominant type of links the roots could be: a) Herringbone, or b) dichotomous type. Picture source: adapted from Hadir et al. (2021) and Fitter (1987)

Table S4.1. Mean soil water content (%) ± sd - Growing period 1 (2019/20)

Date	Depth (cm)	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
19/05/2020	**15**	11.6 ± 0.4	13.4 ± 1.1	13.8 ± 1.2	12.3 ± 4.1	13.2 ± 1.3	14.7 ± 1.6	18.3 ± 1.9
	40	15.7 ± 0.8	16.6 ± 2.3	20.9 ± 5.3	15.1 ± 1.0	15.5 ± 2.6	17.6 ± 2.6	25.0 ± 4.7
	65	19.6 ± 3.0	22.2 ± 3.4	24.9 ± 7.5	18.6 ± 1.6	21.6 ± 2.7	25.4 ± 2.4	26.3 ± 5.8
	90	19.8 ± 3.0	21.4 ± 4.6	20.6 ± 3.4	18.1 ± 7.2	18.4 ± 3.6	20.2 ± 1.2	10.7 ± 5.1
26/05/2020	**15**	9.3 ± 1.1	9.3 ± 1.8	10.3 ± 3.8	11.4 ± 0.8	10.2 ± 1.8	12.0 ± 1.9	13.2 ± 2.4
	40	9.6 ± 1.5	12.8 ± 0.5	13.2 ± 2.9	11.6 ± 1.3	12.5 ± 1.1	11.8 ± 1.7	21.3 ± 2.8
	65	18.2 ± 1.8	16.5 ± 3.1	18.3 ± 5.8	17.0 ± 2.0	16.7 ± 2.1	17.7 ± 2.4	24.3 ± 3.4
	90	16.6 ± 2.0	16.3 ± 1.3	13.5 ± 3.4	16.1 ± 2.1	13.9 ± 4.5	14.5 ± 2.3	15.7 ± 3.8
02/06/2020	**15**	7.5 ± 1.0	8.3 ± 0.7	10.5 ± 1.6	10.3 ± 1.6	7.3 ± 3.9	9.5 ± 0.7	9.8 ± 2.8
	40	8.9 ± 1.4	9.2 ± 2.9	12.7 ± 5.1	10.8 ± 0.9	11.7 ± 2.7	10.7 ± 0.9	20.1 ± 3.9
	65	11.7 ± 1.2	14.9 ± 2.9	18.6 ± 3.8	14.4 ± 2.0	13.0 ± 3.7	14.1 ± 1.1	19.6 ± 1.1
	90	16.3 ± 3.6	17.1 ± 2.7	19.3 ± 2.7	12.5 ± 1.0	13.0 ± 3.1	13.9 ± 0.7	15.4 ± 6.1
09/06/2020	**15**	10.4 ± 0.8	10.7 ± 1.6	11.0 ± 1.6	9.8 ± 0.8	12.1 ± 2.4	12.3 ± 1.4	14.5 ± 3.2
	40	10.4 ± 0.9	11.6 ± 0.7	12.1 ± 3.1	8.8 ± 1.6	10.4 ± 1.5	11.6 ± 0.9	19.5 ± 1.1
	65	12.2 ± 0.8	12.7 ± 0.8	15.8 ± 1.8	11.8 ± 1.2	13.2 ± 1.0	14.0 ± 1.9	22.8 ± 2.6
	90	14.7 ± 0.6	14.8 ± 0.9	14.0 ± 1.5	13.5 ± 3.0	14.4 ± 0.6	11.5 ± 4.1	15.2 ± 4.0
16/06/2020	**15**	11.2 ± 1.3	13.1 ± 2.0	13.6 ± 1.8	11.0 ± 1.1	11.9 ± 0.7	12.4 ± 0.8	15.3 ± 2.6
	40	11.5 ± 1.6	11.6 ± 2.1	14.6 ± 2.2	13.3 ± 1.1	11.6 ± 0.6	12.4 ± 0.7	17.4 ± 2.3
	65	13.8 ± 1.8	15.7 ± 3.3	18.8 ± 2.1	15.8 ± 1.0	15.0 ± 1.0	14.2 ± 1.5	18.4 ± 1.8
	90	13.7 ± 0.5	15.5 ± 1.6	18.5 ± 1.9	16.4 ± 2.0	16.3 ± 0.9	15.8 ± 0.5	17.5 ± 2.9
23/06/2020	**15**	11.8 ± 0.8	11.4 ± 0.6	13.0 ± 0.6	10.3 ± 0.3	9.9 ± 0.7	10.2 ± 1.1	14.1 ± 2.6
	40	10.5 ± 0.6	11.5 ± 0.8	11.7 ± 1.8	10.6 ± 0.3	10.0 ± 0.9	9.9 ± 1.9	16.5 ± 3.7
	65	11.8 ± 1.5	11.5 ± NA	19.9 ± 3.5	13.8 ± 2.2	13.3 ± 4.1	14.6 ± 1.5	20.1 ± 2.1
	90	14.4 ± 1.4	0.0 ± NA	16.5 ± 6.3	11.2 ± 1.4	15.2 ± 1.0	14.7 ± 3.7	15.8 ± 2.7

Table S4.2. Mean soil water content (%) ± sd - Growing period 2 (2020/21)

Date	Depth (cm)	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
05/05/2021	0	14.4 ± 2.9	17.1 ± 0.6	17.2 ± 0.5	18.6 ± 1.2	16.6 ± 1.2	18.6 ± 1.7	16.0 ± 0.7
	30	22.7 ± 4.6	17.6 ± 3.6	25.9 ± 7.4	25.6 ± 4.4	23.2 ± 8.5	24.0 ± 6.6	24.6 ± 9.7
	60	24.6 ± 3.0	22.9 ± 6.8	30.2 ± 3.6	29.2 ± 7.5	29.4 ± 8.8	31.9 ± 6.0	35.0 ± 1.5
	90	20.1 ± 2.1	17.7 ± 6.5	14.9 ± 4.4	9.6 ± 1.8	29.8 ± NA	15.6 ± 3.5	13.7 ± NA
19/05/2021	0	30.6 ± 4.5	32.5 ± 0.8	30.2 ± 2.5	29.7 ± 2.2	28.4 ± 1.4	30.8 ± 0.5	28.7 ± 1.6
	30	17.2 ± 2.3	20.7 ± 5.0	16.9 ± 3.0	19.0 ± 0.7	14.4 ± 6.2	15.6 ± 0.4	21.0 ± 2.2
	60	23.2 ± 3.6	23.9 ± 3.1	22.6 ± 2.0	22.3 ± 3.1	19.4 ± 1.7	22.1 ± 3.6	25.3 ± 4.0
	90	21.6 ± 8.0	7.6 ± 2.3	10.8 ± NA	12.4 ± 6.4	9.8 ± 0.7	17.6 ± 3.6	15.6 ± 2.6
02/06/2021	0	15.5 ± 4.2	13.8 ± 2.2	18.6 ± 1.2	15.7 ± 0.3	18.0 ± 0.9	17.3 ± 2.4	17.4 ± 0.3
	30	19.3 ± 1.0	16.3 ± 3.6	25.0 ± 5.5	19.8 ± 2.7	15.9 ± 2.5	12.3 ± 2.1	20.0 ± 4.1
	60	20.2 ± 2.3	22.1 ± 1.2	23.1 ± 4.8	24.0 ± 2.7	20.7 ± 0.6	22.0 ± 3.4	22.7 ± 6.4
	90	21.2 ± 5.4	20.0 ± 9.7	14.3 ± 2.7	10.1 ± 4.1	16.5 ± 3.5	14.8 ± 2.6	13.8 ± 0.7
16/06/2021	0	6.1 ± 1.0	9.6 ± 1.7	12.8 ± 2.1	12.6 ± 0.8	9.7 ± 1.0	10.9 ± 2.6	12.7 ± 2.4
	30	6.9 ± 0.9	16.4 ± 1.2	14.1 ± 0.6	16.4 ± 2.3	15.0 ± 1.5	14.7 ± 3.7	20.2 ± 4.6
	60	11.8 ± 1.0	22.8 ± 2.1	22.2 ± 0.7	21.5 ± 1.4	23.3 ± 5.2	18.1 ± 1.4	23.9 ± 5.9
	90	16.1 ± 0.0	8.5 ± 3.4	16.8 ± 1.2	20.4 ± 0.7	6.6 ± NA	8.0 ± 1.2	10.1 ± 2.0

Table S4.3. Topsoil nutrient content at the first soil-root sampling campaign during 2019/20 (BBCH 23).

Treatment	Layer	NPKCa+m+s	NPKCa	_PKCa	N_Ca	NP_Ca	NPK_	unfertilized
pH	0-30	6.6	6.8	6.8	6.8	6.8	6.5	5.8
Nmin (kg ha-1)	0-30	13.8	6.8	7	4.7	5.6	3.9	4.8
Kcal (mg kg-1)	0-30	354	164	161	111	38	62	48
Pcal (mg kg-1)	0-30	174	119	129	25	110	114	30

Table S4.4. Topsoil and subsoil nutrient concentrations at the second soil-root sampling campaign during winter wheat booting in 2020 (BBCH 43).

Treatment	Layer	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
pH	0-30	6.6	6.7	6.8	6.8	6.1	5.5	5.9
	30-50	6.7	6.7	7	6.8	6.3	6	6.1
	50-100	7	7	7	6.9	6.6	6.3	6.2
C (%)	0-30	1.3	0.8	0.8	0.8	0.6	0.8	0.7
	30-50	0.8	0.6	0.5	0.8	0.3	0.6	0.5
	50-100	0.3	0.4	0.3	0.7	0.3	0.4	0.3
N (%)	0-30	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	30-50	0.1	0.1	0.1	0.1	0	0.1	0.1
	50-100	0	0	0	0.1	0	0.1	0
Nmin (kg ha-1)	0-30	26	13	5.1	11.8	18.4	12.1	4.6
	30-50	4.9	1.8	1.4	2.9	3.8	3	1.5
	50-100	10	2.4	2	2	3.9	8.3	2.4
Kcal (mg kg-1)	0-30	417	112	206	90	61	79	41
	30-50	351	84	97	47	52	58	51
	50-100	144	56	40	48	42	42	43
Pcal (mg kg-1)	0-30	201	117	61	43	116	63	19
	30-50	152	69	34	9	85	21	0
	50-100	25	0	0	2	0	0	0

Table S4.5. Topsoil and subsoil nutrient concentrations at the first soil-root sampling campaign at flowering 2020/21 (BBCH 69).

Treatment	**Layer**	**NPKCa+m+s**	**NPKCa**	**_PKCa**	**N_Ca**	**NP_Ca**	**NPK_**	**unfertilized**
pH	0-30	6.6	6.1	6.7	6.1	6.1	5.6	6.1
	30-60	6.6	6.2	6.7	5.9	6.2	5.5	6
	60-90	7.1	6.9	6.9	6.7	6.6	6.3	6.2
C (%)	0-30	1.1	0.8	0.7	0.8	0.7	0.7	0.7
	30-60	0.7	0.6	0.5	0.7	0.6	0.7	0.5
	60-90	0.3	0.3	0.3	0.3	0.3	0.4	0.3
N (%)	0-30	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	30-60	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	60-90	0.1	0.1	0.1	0.1	0.1	0.1	0.1
N_{min} (kg ha^{-1})	0-30	6.7	3.6	2.6	3.9	2.3	6.5	2.9
	30-60	1.2	3.5	1	3.2	0.8	2.6	2.3
	60-90	0.7	1.9	0.3	1.5	0.3	1.5	1.8
NO_3-N (mg kg^{-1})	0-30	5.8	2.4	1.9	1.3	0.9	2	0.8
	30-60	1.2	1.8	0.7	0.8	0.3	0.4	0.5
	60-90	0.3	0.4	0.1	0	0	0	0
NH_4-N (mg kg^{-1})	0-30	1	1.2	0.7	2.6	1.4	4.5	2
	30-60	0.1	1.7	0.3	2.4	0.5	2.2	1.9
	60-90	0.4	1.6	0.2	1.5	0.3	1.5	1.8
K_{cal} (mg kg^{-1})	0-30	283	114	126	110	31	125	38
	30-60	202	79	82	89	29	75	39
	60-90	72	57	44	41	46	47	42
P_{cal} (mg kg^{-1})	0-30	205	75	76	15	74	46	31
	30-60	140	46	36	12	46	44	22
	60-90	11	11	9	4	8	12	10

Table S4.6. Roots traits (BBCH 23) - Period 1 (2019-2020)

Trait	Layer	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
Root Biomass (ton ha-1)	0-10	0.11 ± 0.01	0.07 ± 0.06	0.08 ± 0.05	0.03 ± 0.01	0.04 ± 0.05	0.08 ± 0.04	0.02 ± 0.02
	10-20	0.07 ± 0.05	0.06 ± 0.02	0.06 ± 0.04	0.03 ± 0.02	0.04 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
	20-30	0.05 ± 0.03	0.05 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.02
	30-40	0.02 ± 0.02	0.03 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.01
	40-50	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
	50-60	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	60-70	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Topsoil (0-30cm)	0.23 ± 0.07	0.17 ± 0.07	0.17 ± 0.08	0.06 ± 0.04	0.10 ± 0.05	0.17 ± 0.07	0.04 ± 0.07
	Subsoil (30-70cm)	0.04 ± 0.03	0.04 ± 0.03	0.02 ± 0.02	0.02 ± 0.03	0.03 ± 0.03	0.02 ± 0.02	0.00 ± 0.02
	Total (0-70cm)	0.27 ± 0.08	0.21 ± 0.08	0.18 ± 0.08	0.09 ± 0.06	0.12 ± 0.04	0.19 ± 0.08	0.05 ± 0.04
Total Root Length (cm)	0-10	741.4 ± 169.4	748.2 ± 201.2	603.4 ± 495.7	197.4 ± 192.9	324.2 ± 489.7	1015.4 ± 341.0	158.3 ± 165.5
	10-20	490.3 ± 228.8	760.5 ± 113.5	540.6 ± 289.3	188.0 ± 209.9	251.8 ± 186.6	792.5 ± 189.1	292.4 ± 157.3
	20-30	526.0 ± 182.0	361.3 ± 148.8	185.1 ± 96.4	27.6 ± 22.4	148.9 ± 97.6	268.9 ± 113.0	105.6 ± 54.8
	30-40	137.9 ± 99.2	85.0 ± 52.0	41.3 ± 16.8	19.4 ± 14.1	55.9 ± 27.4	97.3 ± 57.0	52.0 ± 26.2
	40-50	99.1 ± 72.2	37.4 ± 46.9	19.3 ± 5.6	14.7 ± 9.2	29.7 ± 10.8	50.8 ± 44.2	55.2 ± 50.5
	50-60	27.3 ± 10.2	20.7 ± 17.8	18.4 ± 15.2	12.2 ± 8.8	11.1 ± 6.3	16.7 ± 12.1	83.5 ± 47.6
	60-70	35.9 ± 5.7	23.9 ± 38.9	10.8 ± 13.8	7.5 ± 3.9	17.8 ± 10.6	21.2 ± 2.7	19.6 ± 7.7
	Topsoil (0-30cm)	1757.8 ± 346.8	1870.0 ± 260.4	1329.1 ± 862.7	413.0 ± 407.3	724.9 ± 572.2	2076.8 ± 631.9	556.2 ± 214.1
	Subsoil (30-70cm)	300.2 ± 117.6	167.0 ± 81.1	89.8 ± 25.1	53.9 ± 21.4	114.5 ± 28.2	186.1 ± 75.4	210.3 ± 99.1
	Total (0-70cm)	2058.0 ± 457.5	2037.0 ± 205.9	1418.8 ± 855.8	466.9 ± 389.3	839.4 ± 545.2	2262.8 ± 602.1	766.6 ± 224.9
RLD (cm cm-3)	0-10	1.17 ± 0.27	1.18 ± 0.32	0.95 ± 0.78	0.31 ± 0.31	0.51 ± 0.77	1.60 ± 0.53	0.25 ± 0.26
	10-20	0.77 ± 0.36	1.20 ± 0.18	0.85 ± 0.45	0.30 ± 0.33	0.40 ± 0.29	1.25 ± 0.30	0.46 ± 0.25
	20-30	0.83 ± 0.29	0.57 ± 0.23	0.29 ± 0.15	0.05 ± 0.03	0.23 ± 0.16	0.43 ± 0.18	0.17 ± 0.09
	30-40	0.22 ± 0.16	0.14 ± 0.08	0.07 ± 0.03	0.03 ± 0.02	0.09 ± 0.04	0.15 ± 0.09	0.08 ± 0.04
	40-50	0.16 ± 0.11	0.06 ± 0.08	0.03 ± 0.01	0.02 ± 0.02	0.05 ± 0.02	0.08 ± 0.07	0.09 ± 0.08
	50-60	0.04 ± 0.02	0.03 ± 0.03	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.13 ± 0.07
	60-70	0.06 ± 0.01	0.04 ± 0.06	0.02 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.03 ± 0.01

	Topsoil (0-30 cm)	0.92 ± 0.181	0.98 ± 0.137	0.70 ± 0.452	0.22 ± 0.213	0.38 ± 0.301	1.09 ± 0.331	0.29 ± 0.113
	Subsoil (30-70 cm)	0.12 ± 0.047	0.07 ± 0.033	0.04 ± 0.011	0.02 ± 0.008	0.05 ± 0.012	0.07 ± 0.029	0.08 ± 0.040
	Total (0-70 cm)	0.46 ± 0.10	0.46 ± 0.05	0.32 ± 0.19	0.11 ± 0.09	0.19 ± 0.12	0.51 ± 0.13	0.17 ± 0.05
RMD (mg cm-3)	0-10	0.11 ± 0.01	0.07 ± 0.05	0.08 ± 0.05	0.02 ± 0.01	0.03 ± 0.05	0.08 ± 0.04	0.02 ± 0.02
	10-20	0.07 ± 0.05	0.06 ± 0.02	0.06 ± 0.04	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.01 ± 0.00
	20-30	0.05 ± 0.03	0.05 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.00	0.03 ± 0.02	0.01 ± 0.01
	30-40	0.02 ± 0.02	0.03 ± 0.03	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
	40-50	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.02	0.01 ± 0.01	0.00 ± 0.00
	50-60	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	60-70	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Topsoil (0-30 cm)	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	0.01 ± 0.01
	Subsoil (30-70 cm)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
	Total (0-70 cm)	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00
Average Diameter (mm)	0-10	0.26 ± 0.08	0.23 ± 0.06	0.25 ± 0.09	0.12 ± 0.01	0.16 ± 0.06	0.23 ± 0.02	0.17 ± 0.06
	10-20	0.24 ± 0.05	0.23 ± 0.03	0.28 ± 0.14	0.17 ± 0.06	0.18 ± 0.07	0.25 ± 0.03	0.17 ± 0.04
	20-30	0.28 ± 0.04	0.40 ± 0.09	0.25 ± 0.13	0.13 ± 0.03	0.16 ± 0.04	0.30 ± 0.05	0.22 ± 0.05
	30-40	0.36 ± 0.06	0.29 ± 0.16	0.22 ± 0.10	0.15 ± 0.05	0.21 ± 0.12	0.27 ± 0.06	0.18 ± 0.10
	40-50	0.37 ± 0.05	0.28 ± 0.10	0.15 ± 0.05	0.15 ± 0.03	0.21 ± 0.07	0.33 ± 0.10	0.28 ± 0.17
	50-60	0.15 ± 0.04	0.27 ± 0.15	0.23 ± 0.07	0.16 ± 0.01	0.18 ± 0.03	0.17 ± 0.11	0.15 ± 0.03
	60-70	0.13 ± 0.01	0.17 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	0.15 ± 0.04	0.11 ± 0.01	0.15 ± 0.03
	Topsoil (0-30cm)	0.26 ± 0.04	0.29 ± 0.025	0.26 ± 0.08	0.14 ± 0.01	0.16 ± 0.04	0.26 ± 0.02	0.19 ± 0.02
	Subsoil (30-70cm)	0.25 ± 0.07	0.25 ± 0.045	0.18 ± 0.04	0.15 ± 0.00	0.19 ± 0.04	0.22 ± 0.05	0.19 ± 0.06
	Total (0-70cm)	0.26 ± 0.03	0.27 ± 0.03	0.22 ± 0.03	0.14 ± 0.00	0.18 ± 0.02	0.24 ± 0.03	0.19 ± 0.03

Table S4.7. Roots traits (BBCH 43) - Period 1 (2019-2020)

Trait	Layer	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
Root Biomass (ton ha^{-1})	0-10	0.17 ± 0.10	0.15 ± 0.09	0.08 ± 0.02	0.13 ± 0.09	0.23 ± 0.14	0.18 ± 0.09	0.06 ± 0.05
	10-20	0.20 ± 0.05	0.09 ± 0.01	0.07 ± 0.03	0.20 ± 0.03	0.10 ± 0.02	0.06 ± 0.02	0.07 ± 0.03
	20-30	0.16 ± 0.07	0.13 ± 0.05	0.09 ± 0.04	0.22 ± 0.06	0.13 ± 0.06	0.14 ± 0.11	0.09 ± 0.03
	30-40	0.11 ± 0.07	0.08 ± 0.03	0.03 ± 0.02	0.11 ± 0.05	0.05 ± 0.02	0.10 ± 0.05	0.07 ± 0.02
	40-50	0.09 ± 0.01	0.09 ± 0.05	0.05 ± 0.01	0.11 ± 0.06	0.06 ± 0.02	0.07 ± 0.03	0.04 ± 0.01
	50-60	0.09 ± 0.03	0.10 ± 0.04	0.06 ± 0.03	0.11 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.04 ± 0.01
	60-70	0.10 ± 0.02	0.09 ± 0.02	0.05 ± 0.03	0.13 ± 0.06	0.07 ± 0.04	0.06 ± 0.04	0.05 ± 0.03
	70-80	0.05 ± 0.06	0.08 ± 0.04	0.03 ± 0.01	0.10 ± 0.07	0.07 ± 0.02	0.06 ± 0.03	0.05 ± 0.03
	80-90	0.06 ± 0.05	0.05 ± 0.03	0.05 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
	90-100	0.06 ± 0.04	0.08 ± 0.04	0.02 ± 0.01	0.04 ± 0.03	0.03 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
	Topsoil (0-30cm)	0.53 ± 0.12	0.37 ± 0.06	0.25 ± 0.05	0.55 ± 0.15	0.46 ± 0.11	0.38 ± 0.16	0.22 ± 0.03
	Subsoil (30-100cm)	0.55 ± 0.10	0.57 ± 0.14	0.29 ± 0.06	0.68 ± 0.25	0.43 ± 0.11	0.40 ± 0.14	0.28 ± 0.08
	Total (0-100cm)	1.08 ± 0.16	0.93 ± 0.15	0.54 ± 0.10	1.23 ± 0.40	0.89 ± 0.10	0.79 ± 0.14	0.50 ± 0.05
Total Root Length (cm)	0-10	1884 ± 723	1402 ± 693	1263 ± 98	1352 ± 1063	1531 ± 883	1611 ± 383	714 ± 449
	10-20	2448 ± 932	1314 ± 310	1794 ± 239	2123 ± 322	1625 ± 35	1208 ± 362	987 ± 87
	20-30	2751 ± 766	1662 ± 778	1774 ± 1262	2947 ± 1520	2706 ± 719	1602 ± 350	1778 ± 820
	30-40	1358 ± 987	667 ± 101	475 ± 76	1434 ± 805	504 ± 136	1173 ± 304	1056 ± 196
	40-50	968 ± 236	696 ± 290	535 ± 206	1160 ± 469	704 ± 239	1150 ± 292	747 ± 66
	50-60	1032 ± 285	1128 ± 382	755 ± 328	1210 ± 402	1088 ± 286	1022 ± 320	580 ± 124
	60-70	1079 ± 549	1036 ± 200	830 ± 329	1344 ± 474	1182 ± 426	945 ± 379	777 ± 367
	70-80	563 ± 620	1000 ± 211	672 ± 143	1019 ± 447	1239 ± 148	1064 ± 624	749 ± 310
	80-90	812 ± 518	909 ± 249	681 ± 195	974 ± 270	1046 ± 166	622 ± 372	379 ± 100
	90-100	750 ± 638	355 ± 341	169 ± 88	234 ± 140	374 ± 110	102 ± 83	107 ± 110
	Topsoil (0-30cm)	7084 ± 1593	4378 ± 284	4831 ± 1465	6423 ± 2401	5861 ± 709	4421 ± 362	3480 ± 1089
	Subsoil (30-100cm)	6563 ± 677	5790 ± 1086	4117 ± 720	7375 ± 2266	6136 ± 735	6078 ± 1811	4395 ± 881
	Total (0-100cm)	13646 ± 1885	10168 ± 1114	8949 ± 2173	13798 ± 4484	11998 ± 834	10499 ± 1916	7875 ± 1423

RLD (cm cm^{-3})								
	0-10	2.96 ± 1.14	2.20 ± 1.09	1.99 ± 0.15	2.12 ± 1.67	2.41 ± 1.39	2.53 ± 0.60	1.12 ± 0.71
	10-20	3.85 ± 1.47	2.06 ± 0.49	2.82 ± 0.37	3.34 ± 0.51	2.55 ± 0.06	1.90 ± 0.57	1.55 ± 0.14
	20-30	4.33 ± 1.20	2.61 ± 1.22	2.79 ± 1.98	4.63 ± 2.39	4.25 ± 1.13	2.52 ± 0.55	2.80 ± 1.29
	30-40	2.13 ± 1.55	1.05 ± 0.16	0.75 ± 0.12	2.25 ± 1.27	0.79 ± 0.21	1.84 ± 0.48	1.66 ± 0.31
	40-50	1.52 ± 0.37	1.09 ± 0.46	0.84 ± 0.32	1.82 ± 0.74	1.11 ± 0.37	1.81 ± 0.46	1.17 ± 0.10
	50-60	1.62 ± 0.45	1.77 ± 0.60	1.19 ± 0.52	1.90 ± 0.63	1.71 ± 0.45	1.61 ± 0.50	0.91 ± 0.20
	60-70	1.70 ± 0.86	1.63 ± 0.31	1.31 ± 0.52	2.11 ± 0.74	1.86 ± 0.67	1.49 ± 0.60	1.22 ± 0.58
	70-80	0.89 ± 0.97	1.57 ± 0.33	1.06 ± 0.22	1.60 ± 0.70	1.95 ± 0.23	1.67 ± 0.98	1.18 ± 0.49
	80-90	1.28 ± 0.81	1.43 ± 0.39	1.07 ± 0.31	1.53 ± 0.42	1.64 ± 0.26	0.98 ± 0.58	0.60 ± 0.16
	90-100	1.18 ± 1.00	0.56 ± 0.54	0.26 ± 0.14	0.37 ± 0.22	0.59 ± 0.17	0.16 ± 0.13	0.17 ± 0.17
	Topsoil (0-30cm)	3.71 ± 0.83	2.29 ± 0.15	2.53 ± 0.77	3.37 ± 1.26	3.07 ± 0.37	2.32 ± 0.19	1.82 ± 0.57
	Subsoil (30-100cm)	1.47 ± 0.15	1.30 ± 0.24	0.92 ± 0.16	1.66 ± 0.51	1.38 ± 0.17	1.36 ± 0.41	0.99 ± 0.20
	Total (0-100cm)	2.15 ± 0.30	1.60 ± 0.18	1.41 ± 0.34	2.17 ± 0.70	1.89 ± 0.13	1.65 ± 0.30	1.24 ± 0.22
RMD (mg cm^{-3})								
	0-10	0.17 ± 0.10	0.15 ± 0.09	0.08 ± 0.02	0.13 ± 0.09	0.23 ± 0.14	0.18 ± 0.09	0.06 ± 0.05
	10-20	0.20 ± 0.05	0.09 ± 0.01	0.07 ± 0.03	0.20 ± 0.03	0.10 ± 0.02	0.06 ± 0.02	0.07 ± 0.03
	20-30	0.16 ± 0.07	0.13 ± 0.05	0.09 ± 0.04	0.22 ± 0.06	0.13 ± 0.06	0.14 ± 0.11	0.09 ± 0.03
	30-40	0.11 ± 0.07	0.08 ± 0.03	0.03 ± 0.02	0.11 ± 0.05	0.05 ± 0.02	0.10 ± 0.05	0.07 ± 0.02
	40-50	0.09 ± 0.01	0.09 ± 0.06	0.05 ± 0.01	0.11 ± 0.06	0.06 ± 0.02	0.07 ± 0.03	0.04 ± 0.01

50-60	0.09 ± 0.03	0.10 ± 0.04	0.06 ± 0.03	0.11 ± 0.02	0.08 ± 0.02	0.07 ± 0.03	0.04 ± 0.01
60-70	0.10 ± 0.02	0.09 ± 0.02	0.05 ± 0.03	0.13 ± 0.06	0.07 ± 0.04	0.06 ± 0.04	0.05 ± 0.03
70-80	0.05 ± 0.06	0.08 ± 0.04	0.03 ± 0.01	0.10 ± 0.07	0.07 ± 0.02	0.06 ± 0.03	0.05 ± 0.03
80-90	0.06 ± 0.05	0.05 ± 0.03	0.06 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.01
90-100	0.06 ± 0.04	0.08 ± 0.04	0.02 ± 0.01	0.04 ± 0.03	0.03 ± 0.00	0.01 ± 0.01	0.01 ± 0.01
Topsoil (0-30cm)	0.18 ± 0.04	0.12 ± 0.02	0.08 ± 0.02	0.18 ± 0.05	0.15 ± 0.04	0.13 ± 0.05	0.07 ± 0.01
Subsoil (30-100cm)	0.08 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	0.10 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.01
Total (0-100cm)	0.11 ± 0.02	0.09 ± 0.02	0.05 ± 0.01	0.12 ± 0.04	0.09 ± 0.01	0.08 ± 0.01	0.05 ± 0.01

Average Diameter (mm)	0-10	0.24 ± 0.07	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.23 ± 0.03	0.22 ± 0.04	0.26 ± 0.11
	10-20	0.21 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.03
	20-30	0.19 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.00	0.19 ± 0.02
	30-40	0.20 ± 0.03	0.25 ± 0.02	0.26 ± 0.01	0.23 ± 0.03	0.25 ± 0.03	0.21 ± 0.01	0.21 ± 0.02
	40-50	0.21 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.19 ± 0.01	0.20 ± 0.01
	50-60	0.22 ± 0.03	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.20 ± 0.01
	60-70	0.21 ± 0.02	0.23 ± 0.01	0.22 ± 0.02	0.21 ± 0.02	0.19 ± 0.01	0.21 ± 0.02	0.20 ± 0.02
	70-80	0.18 ± 0.05	0.22 ± 0.01	0.24 ± 0.02	0.22 ± 0.05	0.21 ± 0.01	0.20 ± 0.00	0.20 ± 0.01
	80-90	0.22 ± 0.06	0.21 ± 0.00	0.23 ± 0.03	0.23 ± 0.03	0.22 ± 0.02	0.20 ± 0.00	0.21 ± 0.01
	90-100	0.23 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.24 ± 0.01	0.24 ± 0.03	0.22 ± 0.02	0.22 ± 0.03
	Topsoil (0-30cm)	0.22 ± 0.03	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.22 ± 0.04
	Subsoil (30-100cm)	0.21 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
	Total (0-100cm)	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.20 ± 0.00	0.21 ± 0.01

Table S4.8. Roots traits (BBCH 69) - Period 2 (2020-2021).

Trait	Layer	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
Root Biomass (ton ha^{-1})	0-10	1,56 ± 0,52	1,74 ± 1,33	0,41 ± 0,17	1,34 ± 0,53	2,00 ± 0,23	2,54 ± 0,69	1,41 ± 0,58
	10-20	0,20 ± 0,04	0,14 ± 0,05	0,08 ± 0,03	0,18 ± 0,04	0,19 ± 0,10	0,23 ± 0,05	0,10 ± 0,01
	20-30	0,28 ± 0,03	0,12 ± 0,01	0,07 ± 0,03	0,20 ± 0,03	0,26 ± 0,09	0,23 ± 0,06	0,10 ± 0,04
	30-40	0,13 ± 0,04	0,07 ± 0,00	0,06 ± 0,02	0,10 ± 0,03	0,13 ± 0,04	0,12 ± 0,05	0,05 ± 0,03
	40-50	0,12 ± 0,03	0,06 ± 0,02	0,07 ± 0,03	0,09 ± 0,06	0,09 ± 0,01	0,08 ± 0,02	0,03 ± 0,01
	50-60	0,13 ± 0,06	0,09 ± 0,03	0,07 ± 0,01	0,09 ± 0,02	0,11 ± 0,02	0,07 ± 0,03	0,03 ± 0,03
	60-70	0,12 ± 0,05	0,06 ± 0,02	0,05 ± 0,02	0,10 ± 0,03	0,10 ± 0,01	0,06 ± 0,02	0,02 ± 0,02
	70-80	0,11 ± 0,05	0,03 ± 0,04	0,05 ± 0,03	0,09 ± 0,02	0,13 ± 0,02	0,06 ± 0,02	0,02 ± 0,03
	80-90	0,10 ± 0,02	0,01 ± 0,01	0,01 ± 0,01	0,05 ± 0,03	0,11 ± 0,01	0,06 ± 0,03	0,01 ± 0,01
	90-100	0,06 ± 0,05	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,01	0,06 ± 0,03	0,05 ± 0,03	0,00 ± 0,00
	Topsoil (0-30cm)	2,04 ± 0,52	2,00 ± 1,33	0,56 ± 0,18	1,72 ± 0,51	2,44 ± 0,34	3,00 ± 0,78	1,61 ± 0,55
	Subsoil (30-100cm)	0,78 ± 0,16	0,33 ± 0,09	0,30 ± 0,04	0,53 ± 0,16	0,73 ± 0,05	0,50 ± 0,14	0,16 ± 0,11
	Total (0-100cm)	2,82 ± 0,61	2,33 ± 1,36	0,86 ± 0,15	2,26 ± 0,35	3,17 ± 0,37	3,50 ± 0,66	1,77 ± 0,58
Total Root Length (cm)	0-10	3889 ± 619	1931 ± 1053	1081 ± 157	2462 ± 615	4295 ± 1023	3947 ± 1197	1332 ± 351
	10-20	1957 ± 348	1004 ± 384	705 ± 43	1534 ± 294	1770 ± 546	1168 ± 265	701 ± 162
	20-30	2173 ± 127	1102 ± 199	749 ± 333	1549 ± 199	2256 ± 775	1193 ± 353	724 ± 213
	30-40	1169 ± 175	619 ± 152	502 ± 312	875 ± 180	1020 ± 159	833 ± 448	292 ± 241
	40-50	1093 ± 195	613 ± 211	525 ± 252	984 ± 702	1028 ± 372	798 ± 76	254 ± 81
	50-60	1375 ± 572	660 ± 139	421 ± 132	1149 ± 240	1012 ± 205	695 ± 172	248 ± 201
	60-70	1293 ± 126	449 ± 106	370 ± 135	952 ± 84	1063 ± 200	663 ± 189	244 ± 183
	70-80	1284 ± 649	217 ± 173	338 ± 351	908 ± 158	817 ± 557	705 ± 188	217 ± 210
	80-90	1133 ± 203	21 ± 11	85 ± 39	418 ± 111	860 ± 109	637 ± 261	98 ± 102
	90-100	670 ± 514	15 ± 8	10 ± 4	89 ± 61	579 ± 301	397 ± 190	12 ± 5
	Topsoil (0-30cm)	8019 ± 718	4037 ± 1249	2535 ± 477	5544 ± 721	8320 ± 1943	6307 ± 1693	2757 ± 1693
	Subsoil (30-100cm)	8018 ± 1680	2594 ± 390	2253 ± 381	5376 ± 1246	6380 ± 1674	4728 ± 1125	1365 ± 1125
	Total (0-100cm)	16037 ± 1463	6631 ± 1262	4787 ± 365	10921 ± 1320	14699 ± 1966	11036 ± 914	4122 ± 526
RLD (cm cm^{-3})	0-10	6,11 ± 0,97	3,04 ± 1,65	1,70 ± 0,25	3,87 ± 0,97	6,75 ± 1,61	6,20 ± 1,88	2,09 ± 0,55
	10-20	3,08 ± 0,55	1,58 ± 0,60	1,11 ± 0,07	2,41 ± 0,46	2,78 ± 0,86	1,84 ± 0,42	1,10 ± 0,25

Trait	Layer	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
	20-30	3,42 ± 0,20	1,73 ± 0,31	1,18 ± 0,52	2,43 ± 0,31	3,55 ± 1,22	1,87 ± 0,56	1,14 ± 0,33
	30-40	1,84 ± 0,28	0,97 ± 0,24	0,79 ± 0,49	1,38 ± 0,28	1,60 ± 0,25	1,31 ± 0,70	0,46 ± 0,38
	40-50	1,72 ± 0,31	0,96 ± 0,33	0,83 ± 0,40	1,55 ± 1,10	1,62 ± 0,58	1,25 ± 0,12	0,40 ± 0,13
	50-60	2,16 ± 0,90	1,04 ± 0,22	0,66 ± 0,21	1,81 ± 0,38	1,59 ± 0,32	1,09 ± 0,27	0,39 ± 0,32
	60-70	2,03 ± 0,20	0,71 ± 0,17	0,58 ± 0,21	1,50 ± 0,13	1,67 ± 0,31	1,04 ± 0,30	0,38 ± 0,29
	70-80	2,02 ± 1,02	0,34 ± 0,27	0,53 ± 0,55	1,43 ± 0,25	1,28 ± 0,88	1,11 ± 0,30	0,34 ± 0,33
	80-90	1,78 ± 0,32	0,03 ± 0,02	0,13 ± 0,06	0,66 ± 0,17	1,35 ± 0,17	1,00 ± 0,41	0,15 ± 0,16
	90-100	1,05 ± 0,81	0,02 ± 0,01	0,02 ± 0,01	0,14 ± 0,10	0,91 ± 0,47	0,62 ± 0,30	0,02 ± 0,01
	Topsoil (0-30cm)	4,20 ± 0,38	2,12 ± 0,65	1,33 ± 0,25	2,91 ± 0,38	4,36 ± 1,02	3,30 ± 0,89	1,44 ± 0,23
	Subsoil (30-100cm)	1,80 ± 0,38	0,58 ± 0,09	0,51 ± 0,09	1,21 ± 0,28	1,43 ± 0,31	1,06 ± 0,25	0,31 ± 0,19
	Total (0-100cm)	2,52 ± 0,23	1,04 ± 0,20	0,75 ± 0,06	1,72 ± 0,21	2,31 ± 0,31	1,73 ± 0,14	0,65 ± 0,08
RMD (mg cm^{-3})	0-10	1,56 ± 0,52	1,74 ± 1,33	0,41 ± 0,17	1,34 ± 0,54	2,00 ± 0,23	2,55 ± 0,69	1,42 ± 0,58
	10-20	0,20 ± 0,04	0,14 ± 0,05	0,08 ± 0,03	0,18 ± 0,04	0,19 ± 0,10	0,23 ± 0,05	0,10 ± 0,01
	20-30	0,28 ± 0,03	0,12 ± 0,01	0,07 ± 0,03	0,20 ± 0,03	0,26 ± 0,09	0,23 ± 0,06	0,10 ± 0,04
	30-40	0,13 ± 0,04	0,07 ± 0,00	0,06 ± 0,02	0,10 ± 0,03	0,13 ± 0,04	0,12 ± 0,05	0,05 ± 0,03
	40-50	0,12 ± 0,03	0,06 ± 0,02	0,07 ± 0,03	0,09 ± 0,06	0,09 ± 0,01	0,08 ± 0,02	0,03 ± 0,01
	50-60	0,13 ± 0,06	0,09 ± 0,03	0,07 ± 0,01	0,09 ± 0,02	0,11 ± 0,02	0,07 ± 0,03	0,03 ± 0,03
	60-70	0,12 ± 0,05	0,06 ± 0,02	0,05 ± 0,02	0,10 ± 0,03	0,10 ± 0,01	0,06 ± 0,02	0,02 ± 0,02
	70-80	0,11 ± 0,05	0,03 ± 0,04	0,05 ± 0,03	0,09 ± 0,02	0,13 ± 0,02	0,06 ± 0,02	0,02 ± 0,03
	80-90	0,10 ± 0,02	0,01 ± 0,01	0,01 ± 0,01	0,05 ± 0,03	0,11 ± 0,01	0,06 ± 0,03	0,01 ± 0,01
	90-100	0,06 ± 0,05	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,03	0,06 ± 0,03	0,05 ± 0,03	0,00 ± 0,00
	Topsoil (0-30cm)	0,68 ± 0,17	0,67 ± 0,44	0,19 ± 0,06	0,57 ± 0,17	0,82 ± 0,11	1,00 ± 0,26	0,54 ± 0,18
	Subsoil (30-100cm)	0,11 ± 0,02	0,05 ± 0,01	0,04 ± 0,01	0,08 ± 0,02	0,10 ± 0,01	0,07 ± 0,02	0,02 ± 0,02
	Total (0-100cm)	0,28 ± 0,06	0,23 ± 0,14	0,09 ± 0,02	0,23 ± 0,04	0,32 ± 0,04	0,35 ± 0,07	0,18 ± 0,06
Average Diameter (mm)	0-10	0,30 ± 0,04	0,39 ± 0,12	0,31 ± 0,05	0,34 ± 0,05	0,35 ± 0,05	0,38 ± 0,02	0,46 ± 0,12
	10-20	0,21 ± 0,01	0,23 ± 0,00	0,21 ± 0,01	0,23 ± 0,01	0,22 ± 0,01	0,28 ± 0,01	0,23 ± 0,02
	20-30	0,22 ± 0,02	0,23 ± 0,02	0,21 ± 0,02	0,23 ± 0,01	0,23 ± 0,01	0,29 ± 0,03	0,24 ± 0,02
	30-40	0,22 ± 0,01	0,23 ± 0,01	0,22 ± 0,02	0,24 ± 0,02	0,22 ± 0,02	0,25 ± 0,01	0,28 ± 0,05
	40-50	0,21 ± 0,01	0,21 ± 0,02	0,20 ± 0,01	0,23 ± 0,02	0,21 ± 0,01	0,22 ± 0,01	0,21 ± 0,03
	50-60	0,21 ± 0,02	0,22 ± 0,01	0,21 ± 0,01	0,22 ± 0,03	0,22 ± 0,02	0,21 ± 0,02	0,20 ± 0,03

Trait	Layer	NPKCa+m+s			NPKCa			_PKCa			N_KCa			NP_Ca			NPK_			unfertilized		
	60-70	0,22	±	0,01	0,22	±	0,01	0,23	±	0,01	0,22	±	0,02	0,25	±	0,01	0,21	±	0,02	0,22	±	0,01
	70-80	0,22	±	0,01	0,22	±	0,01	0,22	±	0,01	0,22	±	0,03	0,23	±	0,01	0,21	±	0,02	0,20	±	0,02
	80-90	0,21	±	0,02	0,23	±	0,01	0,22	±	0,02	0,22	±	0,03	0,26	±	0,03	0,21	±	0,01	0,21	±	0,01
	90-100	0,21	±	0,02	0,26	±	0,05	0,25	±	0,01	0,23	±	0,02	0,27	±	0,02	0,23	±	0,03	0,31	±	0,13
	Topsoil (0-30cm)	0,24	±	0,02	0,28	±	0,04	0,24	±	0,01	0,27	±	0,02	0,26	±	0,02	0,32	±	0,01	0,31	±	0,04
	Subsoil (30-100cm)	0,21	±	0,00	0,23	±	0,01	0,22	±	0,00	0,23	±	0,02	0,24	±	0,01	0,22	±	0,01	0,23	±	0,03
	Total (0-100cm)	0,22	±	0,01	0,24	±	0,01	0,23	±	0,01	0,24	±	0,01	0,24	±	0,00	0,25	±	0,01	0,26	±	0,03

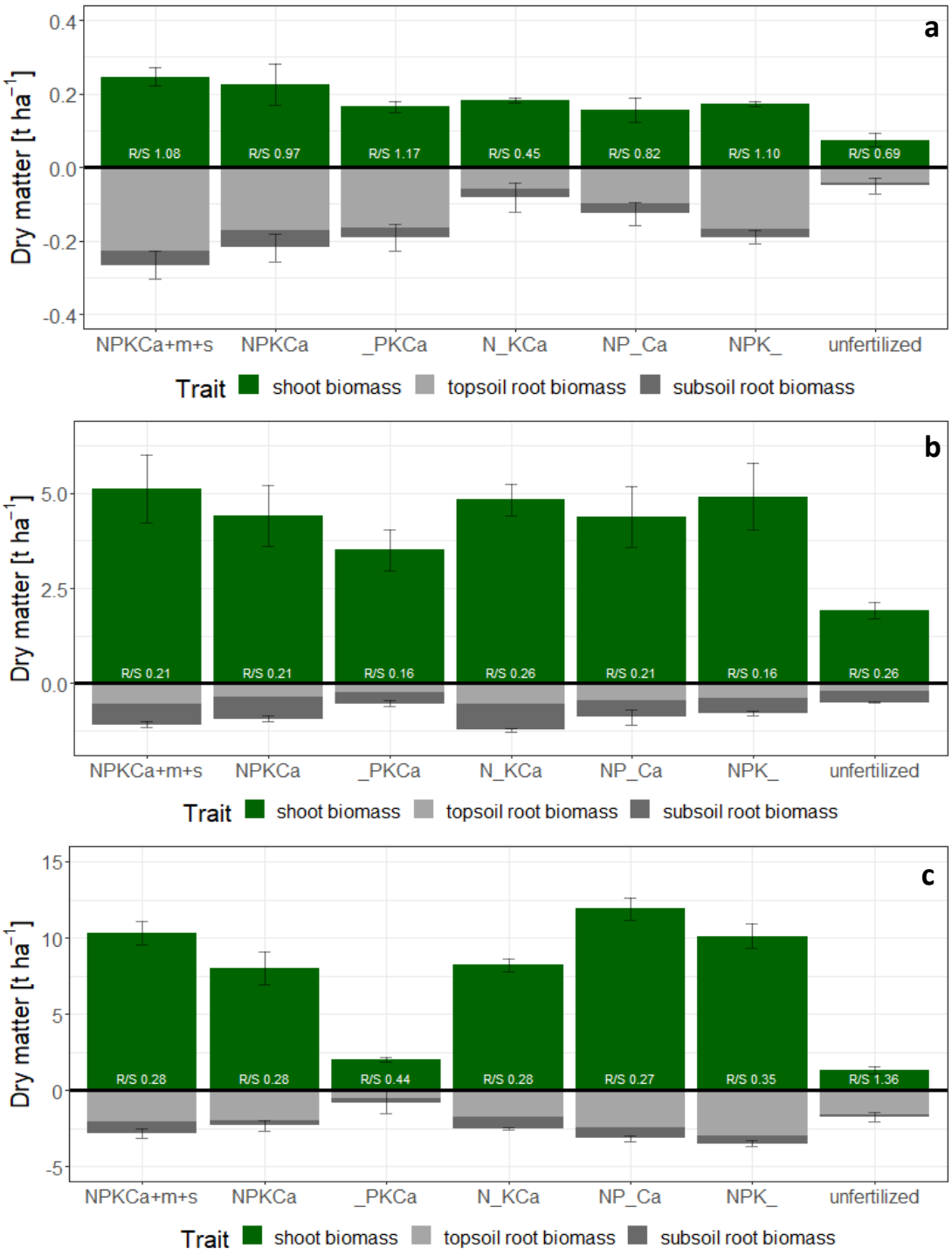

Figure S4.4. Mean (n=4) shoot and root biomass (topsoil: 0-30 cm; subsoil: 30-100cm), root-to-shoot-ratio (R/S) at seven treatments at the LTFE Dikopshof, Germany. a) growing period 1 (2019/20 - BBCH 23). b) growing period 1 (2019/20 - BBCH 43) and, c) growing period 2 (2020/21 - BBCH 69). Error bars refer to the standard error.

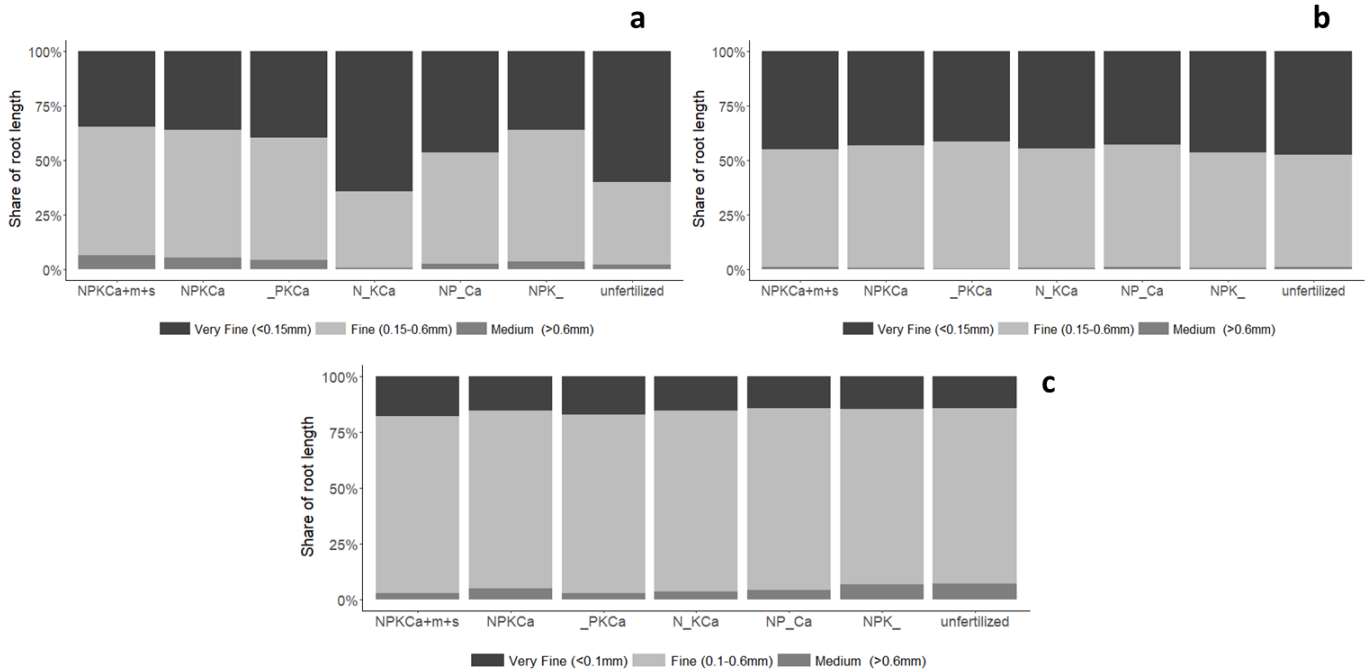

Figure S4.5:Observed mean (n=4) root length distribution based on average diameter in seven treatments of the LTFE Dikopshof, Germany in a) growing period 1 (2019/20 - BBCH 23), b) growing period 1 (2019/20 - BBCH 43) and, c) growing period 2 (2020/21 - BBCH 69).

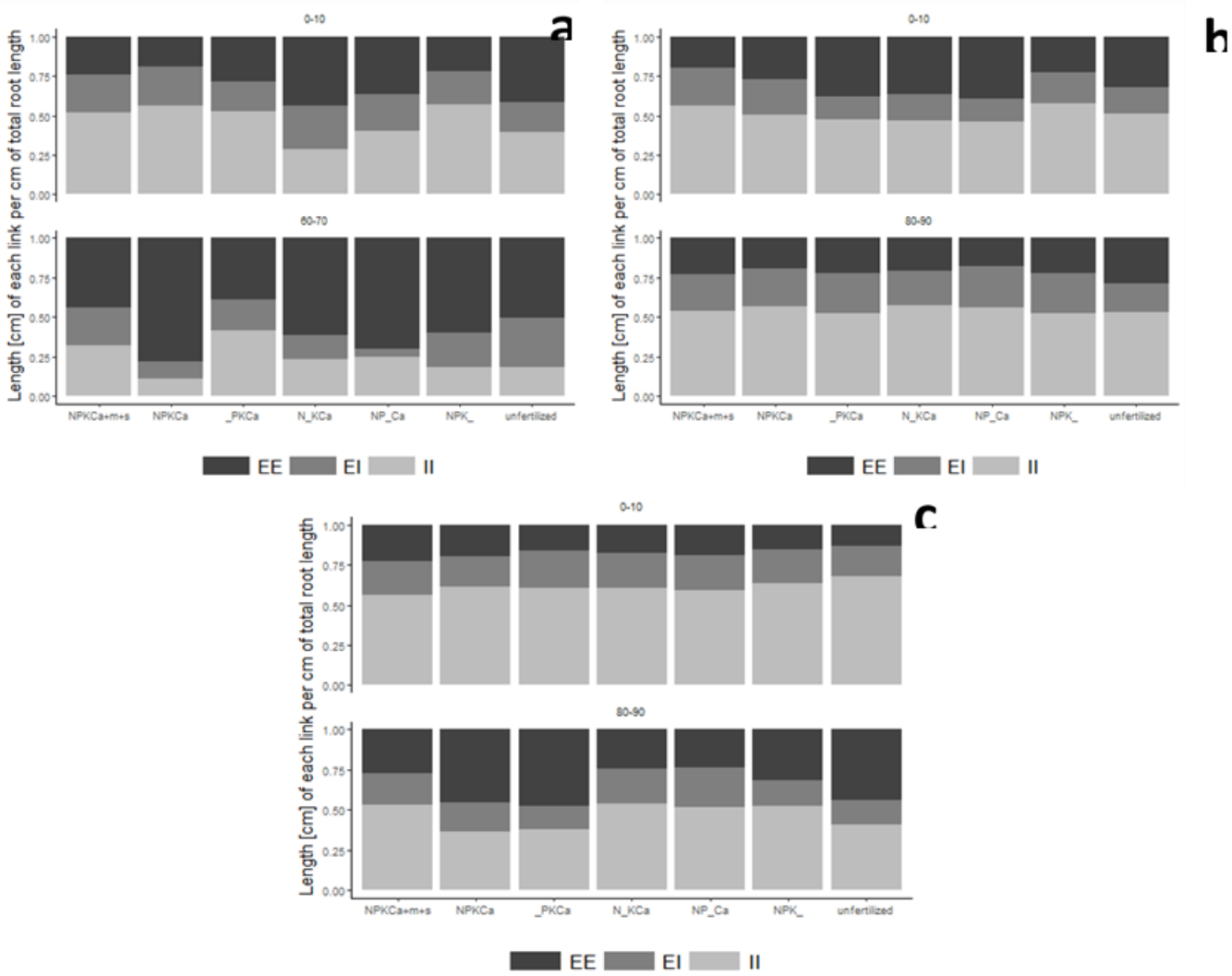

Figure S4.6. Link analysis: Share of root link y at the topsoil (0-10 cm) and subsoil (either 60-70cm at BBCH23 or 80-89cm at BBCH43) in seven treatments of the LTFE Dikopshof, Germany in growing period 1 (2019/20) at a) BBCH 23, b) BBCH 43, and growing period 2 (2020/21) at c) BBCH 69. Link types EE: external-external, EI: external-internal, II: internal-internal.

Table S4.9. Link analysis (BBCH 23) - Period 1 (2019-2020)

Layer	Root Type	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
	%EE	0.24 ± 0.10	0.19 ± 0.06	0.29 ± 0.23	0.44 ± 0.14	0.37 ± 0.13	0.22 ± 0.08	0.42 ± 0.19
0-10	%EI	0.24 ± 0.01	0.25 ± 0.03	0.19 ± 0.02	0.27 ± 0.12	0.23 ± 0.05	0.21 ± 0.03	0.19 ± 0.07
	%II	0.52 ± 0.10	0.56 ± 0.08	0.52 ± 0.24	0.29 ± 0.06	0.40 ± 0.16	0.57 ± 0.06	0.39 ± 0.13
	%EE	0.44 ± 0.38	0.78 ± 0.36	0.39 ± 0.44	0.61 ± 0.31	0.70 ± 0.30	0.60 ± 0.28	0.50 ± 0.35
60-70	%EI	0.24 ± 0.26	0.11 ± 0.21	0.20 ± 0.17	0.15 ± 0.26	0.05 ± 0.06	0.22 ± 0.21	0.32 ± 0.38
	%II	0.32 ± 0.12	0.11 ± 0.15	0.41 ± 0.30	0.24 ± 0.09	0.25 ± 0.23	0.18 ± 0.17	0.18 ± 0.11

Table S4.10. Link analysis (BBCH 43) - Period 1 (2019-2020)

Layer	Root Type	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
	%EE	0.20 ± 0.06	0.27 ± 0.07	0.38 ± 0.10	0.37 ± 0.21	0.39 ± 0.19	0.23 ± 0.08	0.32 ± 0.09
0-10	%EI	0.24 ± 0.05	0.23 ± 0.04	0.15 ± 0.06	0.17 ± 0.07	0.15 ± 0.08	0.20 ± 0.03	0.17 ± 0.04
	%II	0.57 ± 0.06	0.51 ± 0.08	0.47 ± 0.07	0.47 ± 0.14	0.46 ± 0.14	0.58 ± 0.09	0.51 ± 0.09
	%EE	0.23 ± 0.14	0.20 ± 0.07	0.23 ± 0.08	0.21 ± 0.05	0.18 ± 0.07	0.22 ± 0.04	0.29 ± 0.08
80-90	%EI	0.24 ± 0.09	0.24 ± 0.09	0.25 ± 0.06	0.22 ± 0.04	0.26 ± 0.08	0.26 ± 0.09	0.18 ± 0.06
	%II	0.54 ± 0.10	0.57 ± 0.04	0.52 ± 0.07	0.58 ± 0.05	0.56 ± 0.04	0.53 ± 0.05	0.54 ± 0.03

Table S4.11. Link analysis (BBCH 69) - Period 2 (2020-2021).

Layer (cm)	Root Type	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	unfertilized
0-10	%EE	0.23 ± 0.08	0.20 ± 0.02	0.16 ± 0.09	0.18 ± 0.07	0.20 ± 0.11	0.16 ± 0.06	0.14 ± 0.04
	%EI	0.21 ± 0.03	0.19 ± 0.01	0.23 ± 0.02	0.22 ± 0.02	0.21 ± 0.01	0.21 ± 0.03	0.19 ± 0.04
	%II	0.56 ± 0.07	0.61 ± 0.02	0.61 ± 0.09	0.61 ± 0.05	0.60 ± 0.11	0.64 ± 0.08	0.67 ± 0.07
10-20	%EE	0.28 ± 0.08	0.26 ± 0.12	0.31 ± 0.12	0.39 ± 0.12	0.33 ± 0.16	0.26 ± 0.07	0.31 ± 0.17
	%EI	0.27 ± 0.03	0.22 ± 0.05	0.24 ± 0.09	0.16 ± 0.06	0.23 ± 0.05	0.21 ± 0.04	0.19 ± 0.07
	%II	0.45 ± 0.09	0.53 ± 0.10	0.45 ± 0.10	0.45 ± 0.07	0.44 ± 0.13	0.53 ± 0.05	0.50 ± 0.11
20-30	%EE	0.26 ± 0.07	0.43 ± 0.20	0.42 ± 0.21	0.30 ± 0.13	0.24 ± 0.09	0.28 ± 0.07	0.38 ± 0.10
	%EI	0.28 ± 0.04	0.20 ± 0.10	0.23 ± 0.15	0.24 ± 0.03	0.25 ± 0.06	0.22 ± 0.04	0.19 ± 0.07
	%II	0.46 ± 0.07	0.38 ± 0.12	0.35 ± 0.10	0.47 ± 0.10	0.51 ± 0.05	0.50 ± 0.07	0.43 ± 0.12
30-40	%EE	0.30 ± 0.12	0.36 ± 0.16	0.26 ± 0.03	0.43 ± 0.06	0.38 ± 0.17	0.28 ± 0.14	0.41 ± 0.11
	%EI	0.17 ± 0.03	0.20 ± 0.08	0.23 ± 0.07	0.14 ± 0.06	0.15 ± 0.13	0.22 ± 0.10	0.17 ± 0.02
	%II	0.49 ± 0.04	0.45 ± 0.10	0.52 ± 0.06	0.44 ± 0.04	0.47 ± 0.11	0.50 ± 0.06	0.42 ± 0.09
40-50	%EE	0.25 ± 0.05	0.43 ± 0.11	0.45 ± 0.24	0.35 ± 0.13	0.33 ± 0.08	0.37 ± 0.10	0.32 ± 0.09
	%EI	0.20 ± 0.10	0.18 ± 0.04	0.17 ± 0.08	0.19 ± 0.13	0.22 ± 0.04	0.15 ± 0.02	0.25 ± 0.05
	%II	0.50 ± 0.06	0.39 ± 0.11	0.39 ± 0.17	0.46 ± 0.06	0.46 ± 0.09	0.49 ± 0.08	0.44 ± 0.06
50-60	%EE	0.26 ± 0.08	0.27 ± 0.04	0.40 ± 0.10	0.31 ± 0.08	0.25 ± 0.04	0.40 ± 0.15	0.36 ± 0.15
	%EI	0.27 ± 0.11	0.16 ± 0.05	0.18 ± 0.05	0.21 ± 0.08	0.23 ± 0.03	0.17 ± 0.03	0.20 ± 0.07
	%II	0.44 ± 0.13	0.57 ± 0.08	0.43 ± 0.07	0.48 ± 0.05	0.52 ± 0.05	0.44 ± 0.16	0.44 ± 0.09
60-70	%EE	0.22 ± 0.07	0.30 ± 0.09	0.37 ± 0.06	0.36 ± 0.15	0.25 ± 0.07	0.35 ± 0.13	0.36 ± 0.17
	%EI	0.25 ± 0.04	0.18 ± 0.04	0.20 ± 0.02	0.16 ± 0.12	0.19 ± 0.03	0.18 ± 0.07	0.20 ± 0.12
	%II	0.52 ± 0.10	0.52 ± 0.12	0.43 ± 0.06	0.49 ± 0.05	0.56 ± 0.05	0.47 ± 0.08	0.44 ± 0.06
70-80	%EE	0.31 ± 0.09	0.37 ± 0.12	0.45 ± 0.11	0.31 ± 0.08	0.23 ± 0.12	0.30 ± 0.12	0.36 ± 0.15
	%EI	0.14 ± 0.01	0.20 ± 0.04	0.14 ± 0.04	0.18 ± 0.05	0.25 ± 0.14	0.21 ± 0.06	0.15 ± 0.05
	%II	0.53 ± 0.03	0.43 ± 0.09	0.41 ± 0.11	0.52 ± 0.08	0.52 ± 0.05	0.49 ± 0.08	0.50 ± 0.11
80-90	%EE	0.26 ± 0.12	0.45 ± 0.19	0.48 ± 0.04	0.24 ± 0.09	0.24 ± 0.05	0.32 ± 0.06	0.44 ± 0.19
	%EI	0.19 ± 0.08	0.18 ± 0.08	0.15 ± 0.07	0.22 ± 0.07	0.25 ± 0.11	0.16 ± 0.06	0.15 ± 0.10
	%II	0.52 ± 0.06	0.37 ± 0.13	0.38 ± 0.08	0.54 ± 0.03	0.52 ± 0.10	0.52 ± 0.04	0.41 ± 0.12
90-100	%EE	0.23 ± 0.11	0.59 ± 0.24	0.69 ± 0.35	0.49 ± 0.32	0.32 ± 0.13	0.25 ± 0.10	0.68 ± 0.39
	%EI	0.27 ± 0.05	0.16 ± 0.12	0.07 ± 0.10	0.15 ± 0.11	0.16 ± 0.05	0.22 ± 0.02	0.14 ± 0.22
	%II	0.49 ± 0.08	0.26 ± 0.16	0.24 ± 0.26	0.36 ± 0.23	0.52 ± 0.11	0.53 ± 0.11	0.18 ± 0.19

Table S4.12. Shoot traits - Period 1 (2019-2020)

Sampling Date	Treatment	BBCH	Dry biomass (t ha-1)	LAI (m2 m-2)	Plant height (cm
17/03/2020	NPKCa+m+s	23	0.25 ± 0.02	0.31 ± 0.03	6.2 ± 0.6
	NPKCa	23	0.23 ± 0.06	0.26 ± 0.06	5.3 ± 0.5
	_PKCa	23	0.16 ± 0.01	0.21 ± 0.02	5.1 ± 0.4
	N_KCa	23	0.18 ± 0.01	0.22 ± 0.01	4.6 ± 1.0
	NP_Ca	23	0.16 ± 0.03	0.19 ± 0.04	5.0 ± 0.5
	NPK_	23	0.17 ± 0.01	0.21 ± 0.01	6.2 ± 0.6
	unfertilized	23	0.07 ± 0.02	0.09 ± 0.03	5.0 ± 0.2
30/03/2020	NPKCa+m+s	24			8.2 ± 1.0
	NPKCa	24			6.4 ± 0.6
	_PKCa	23			6.0 ± 0.8
	N_KCa	24			6.0 ± 0.8
	NP_Ca	24			6.1 ± 0.9
	NPK_	24			7.2 ± 1.2
	unfertilized	23			5.3 ± 0.8
07/04/20	NPKCa+m+s	25	0.83 ± 0.11	0.99 ± 0.17	
	NPKCa	24	0.54 ± 0.08	0.56 ± 0.03	
	_PKCa	24	0.43 ± 0.03	0.44 ± 0.03	
	N_KCa	24	0.52 ± 0.04	0.54 ± 0.03	
	NP_Ca	24	0.58 ± 0.04	0.61 ± 0.05	
	NPK_	25	0.61 ± 0.12	0.59 ± 0.16	
	unfertilized	24	0.22 ± 0.08	0.21 ± 0.07	
22/04/2020	NPKCa+m+s	31	1.86 ± 0.09	1.86 ± 0.14	20.7 ± 2.8
	NPKCa	31	1.16 ± 0.11	1.07 ± 0.12	14.5 ± 1.2
	_PKCa	31	0.86 ± 0.08	0.82 ± 0.07	11.5 ± 1.0
	N_KCa	31	1.29 ± 0.05	1.22 ± 0.06	13.2 ± 1.5
	NP_Ca	31	1.12 ± 0.15	1.08 ± 0.15	12.3 ± 1.8
	NPK_	31	1.14 ± 0.29	0.98 ± 0.26	14.9 ± 1.8
	unfertilized	31	0.45 ± 0.18	0.41 ± 0.16	9.2 ± 1.4
07/05/2020	NPKCa+m+s	37	4.87 ± 0.36	3.89 ± 0.29	43.0 ± 4.0
	NPKCa	37	2.77 ± 0.37	1.84 ± 0.25	33.4 ± 0.3
	_PKCa	37	2.06 ± 0.12	1.35 ± 0.05	26.7 ± 1.3
	N_KCa	37	3.35 ± 0.45	2.17 ± 0.26	30.2 ± 2.5
	NP_Ca	37	2.89 ± 0.61	1.96 ± 0.49	31.7 ± 2.5
	NPK_	37	2.77 ± 0.31	1.72 ± 0.18	34.6 ± 1.1
	unfertilized	32	0.58 ± 0.21	0.34 ± 0.12	20.9 ± 1.8
19/05/2020	NPKCa+m+s	43	5.11 ± 0.89	2.28 ± 0.36	54.1 ± 4.3
	NPKCa	43	4.40 ± 0.80	1.68 ± 0.25	45.5 ± 1.4
	_PKCa	43	3.51 ± 0.54	1.15 ± 0.21	36.8 ± 0.9
	N_KCa	45	4.83 ± 0.42	1.82 ± 0.11	45.5 ± 3.4
	NP_Ca	43	4.38 ± 0.81	1.72 ± 0.25	43.6 ± 1.1
	NPK_	43	4.91 ± 0.87	1.94 ± 0.31	48.7 ± 2.8
	unfertilized	39	1.91 ± 0.22	0.74 ± 0.05	35.4 ± 2.8
02/06/2020	NPKCa+m+s	69			65.2 ± 3.6
	NPKCa	69			56.3 ± 1.3
	_PKCa	69			46.7 ± 0.6
	N_KCa	69			59.9 ± 2.3
	NP_Ca	69			57.3 ± 2.2
	NPK_	69			60.5 ± 4.0
	unfertilized	65			48.8 ± 5.2
17/06/2020	NPKCa+m+s	83			67.1 ± 1.5
	NPKCa	83			56.3 ± 1.3
	_PKCa	83			44.7 ± 3.7
	N_KCa	84			58.4 ± 1.1
	NP_Ca	84			58.2 ± 1.9
	NPK_	83			60.7 ± 3.3
	unfertilized	84			50.2 ± 1.9

Table S4.13. Plant (plants/m²) and ear (ears/m²) densities recorded at different growth stages (BBCH 23-28, BBCH 65, and BBCH 69) for two growing periods (GP 1: 2019/20 and GP 2: 2020/21).

	GP 1 (2019/20)		**GP 2 (2020/21)**	
	BBCH 23-28	**BBCH 65**	**BBCH 69**	**BBCH 69**
	plants/m²	**ears/m²**	**tillers/m²**	**ears/m²**
NPKCa+m+s	131	467	345	345
NPKCa	151	420	251	246
_PKCa	119	267	135	133
N_KCa	126	427	295	283
NP_Ca	135	520	342	318
NPK_	102	454	294	292
unfertilizer	54	158	167	130

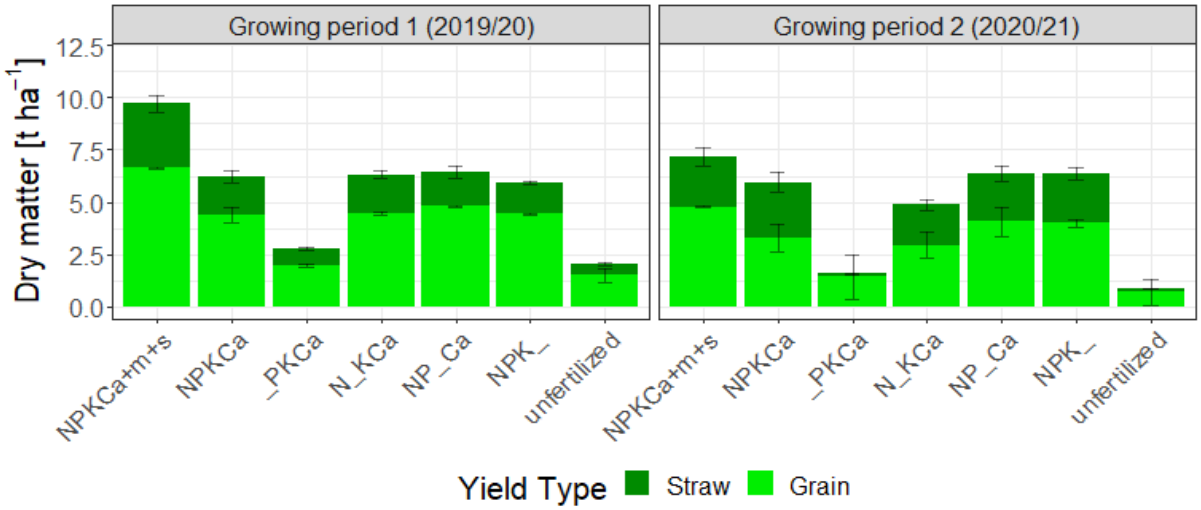

Figure S4.7. Observed dry matter grain and straw yield of winter wheat cultivated in the seven treatments of the LTFE Dikopshof, Germany, in growing period 1 (2019/20) and growing period 2 (2020/21). Error bars refer to the standard error.

Table S4.14. Data used for Pearson Correlation test: For readability purpose the table iss divided into Part1, Part2, Part3, Part4)

Part 1/4

treatment	year	bbch	N_ level	P_ level	K_ level	Ca_ level	pH_ topsoil	C_ topsoil	N_ topsoil	Nmin_ topsoil	Kcal_ topsoil	Pcal_ topsoil	pH_ subsoil	C_subsoil	N_subsoil	Nmin_ subsoil	Kcal_ subsoil	Pcal_ subpsoil
NPKCa+m+s	2020	23	120	31	116	1	6.64	1.23	0.11	13.80	353.53	173.51						
NPKCa+m+s	2020	23	120	31	116	1	6.64	1.23	0.11	13.80	353.53	173.51						
NPKCa+m+s	2020	23	120	31	116	1	6.64	1.23	0.11	13.80	353.53	173.51						
NPKCa+m+s	2020	23	120	31	116	1	6.64	1.23	0.11	13.80	353.53	173.51						
NPKCa	2020	23	60	31	116	1	6.76	0.87	0.08	6.80	163.79	119.01						
NPKCa	2020	23	60	31	116	1	6.76	0.87	0.08	6.80	163.79	119.01						
NPKCa	2020	23	60	31	116	1	6.76	0.87	0.08	6.80	163.79	119.01						
NPKCa	2020	23	60	31	116	1	6.76	0.87	0.08	6.80	163.79	119.01						
_PKCa	2020	23	0	31	116	1	6.82	0.77	0.07	7.00	161.21	128.89						
_PKCa	2020	23	0	31	116	1	6.82	0.77	0.07	7.00	161.21	128.89						
(part_PKCa	2020	23	0	31	116	1	6.82	0.77	0.07	7.00	161.21	128.89						
_PKCa	2020	23	0	31	116	1	6.82	0.77	0.07	7.00	161.21	128.89						
N_KCa	2020	23	60	0	116	1	6.84	0.80	0.07	4.73	110.70	25.44						
N_KCa	2020	23	60	0	116	1	6.84	0.80	0.07	4.73	110.70	25.44						
N_KCa	2020	23	60	0	116	1	6.84	0.80	0.07	4.73	110.70	25.44						
N_KCa	2020	23	60	0	116	1	6.84	0.80	0.07	4.73	110.70	25.44						
NP_Ca	2020	23	60	31	0	1	6.77	0.75	0.07	5.62	38.09	110.45						
NPK_	2020	23	60	31	116	0	6.52	0.79	0.07	3.92	62.16	114.33						
NPK_	2020	23	60	31	116	0	6.52	0.79	0.07	3.92	62.16	114.33						
NPK_	2020	23	60	31	116	0	6.52	0.79	0.07	3.92	62.16	114.33						
NPK_	2020	23	60	31	116	0	6.52	0.79	0.07	3.92	62.16	114.33						
unfertilized	2020	23	0	0	0	0	5.81	0.74	0.07	4.82	48.00	30.35						
unfertilized	2020	23	0	0	0	0	5.81	0.74	0.07	4.82	48.00	30.35						
unfertilized	2020	23	0	0	0	0	5.81	0.74	0.07	4.82	48.00	30.35						
unfertilized	2020	23	0	0	0	0	5.81	0.74	0.07	4.82	48.00	30.35						
NPKCa+m+s	2020	43	120	31	116	1	6.57	1.27	0.12	25.99	417.09	201.09	6.83	0.53	0.06	7.46	247.38	88.60
NPKCa	2020	43	60	31	116	1	6.70	0.78	0.07	12.96	111.79	116.90	6.83	0.45	0.05	2.10	69.99	34.64
NPKCa	2020	43	60	31	116	1	6.70	0.78	0.07	12.96	111.79	116.90	6.83	0.45	0.05	2.10	69.99	34.64

NPKCa	2020	43	60	31	116	1	6.70	0.78	0.07	12.96	111.79	116.90	6.83	0.45	0.05	2.10	69.99	34.64
NPKCa	2020	43	60	31	116	1	6.70	0.78	0.07	12.96	111.79	116.90	6.83	0.45	0.05	2.10	69.99	34.64
_PKCa	2020	43	0	31	116	1	6.84	0.81	0.07	5.10	205.69	61.49	6.96	0.41	0.05	1.69	68.71	17.12
_PKCa	2020	43	0	31	116	1	6.84	0.81	0.07	5.10	205.69	61.49	6.96	0.41	0.05	1.69	68.71	17.12
_PKCa	2020	43	0	31	116	1	6.84	0.81	0.07	5.10	205.69	61.49	6.96	0.41	0.05	1.69	68.71	17.12
_PKCa	2020	43	0	31	116	1	6.84	0.81	0.07	5.10	205.69	61.49	6.96	0.41	0.05	1.69	68.71	17.12
N_KCa	2020	43	60	0	116	1	6.76	0.80	0.08	11.77	89.72	42.89	6.86	0.73	0.07	2.47	47.84	5.28
N_KCa	2020	43	60	0	116	1	6.76	0.80	0.08	11.77	89.72	42.89	6.86	0.73	0.07	2.47	47.84	5.28
N_KCa	2020	43	60	0	116	1	6.76	0.80	0.08	11.77	89.72	42.89	6.86	0.73	0.07	2.47	47.84	5.28
N_KCa	2020	43	60	0	116	1	6.76	0.80	0.08	11.77	89.72	42.89	6.86	0.73	0.07	2.47	47.84	5.28
NP_Ca	2020	43	60	31	0	1	6.11	0.56	0.06	18.37	60.88	116.34	6.44	0.29	0.04	3.85	47.15	42.50
NP_Ca	2020	43	60	31	0	1	6.11	0.56	0.06	18.37	60.88	116.34	6.44	0.29	0.04	3.85	47.15	42.50
NP_Ca	2020	43	60	31	0	1	6.11	0.56	0.06	18.37	60.88	116.34	6.44	0.29	0.04	3.85	47.15	42.50
NP_Ca	2020	43	60	31	0	1	6.11	0.56	0.06	18.37	60.88	116.34	6.44	0.29	0.04	3.85	47.15	42.50
NPK_	2020	43	60	31	116	0	5.51	0.75	0.07	12.09	79.09	63.17	6.15	0.49	0.06	5.61	49.95	10.49
NPK_	2020	43	60	31	116	0	5.51	0.75	0.07	12.09	79.09	63.17	6.15	0.49	0.06	5.61	49.95	10.49
NPK_	2020	43	60	31	116	0	5.51	0.75	0.07	12.09	79.09	63.17	6.15	0.49	0.06	5.61	49.95	10.49
NPK_	2020	43	60	31	116	0	5.51	0.75	0.07	12.09	79.09	63.17	6.15	0.49	0.06	5.61	49.95	10.49
unfertilized	2020	43	0	0	0	0	5.88	0.71	0.07	4.62	40.80	19.30	6.14	0.38	0.05	1.95	47.30	0.00
unfertilized	2020	43	0	0	0	0	5.88	0.71	0.07	4.62	40.80	19.30	6.14	0.38	0.05	1.95	47.30	0.00
unfertilized	2020	43	0	0	0	0	5.88	0.71	0.07	4.62	40.80	19.30	6.14	0.38	0.05	1.95	47.30	0.00
unfertilized	2020	43	0	0	0	0	5.88	0.71	0.07	4.62	40.80	19.30	6.14	0.38	0.05	1.95	47.30	0.00
NPKCa+m+s	2021	69	120	31	116	1	6.47	1.18	0.11	8.54	329.39	215.21	6.72	0.48	0.06	2.05	157.45	70.15
NPKCa+m+s	2021	69	120	31	116	1	6.47	1.18	0.11	8.54	329.39	215.21	6.72	0.48	0.06	2.05	157.45	70.15
NPKCa+m+s	2021	69	120	31	116	1	6.47	1.18	0.11	8.54	329.39	215.21	6.72	0.48	0.06	2.05	157.45	70.15
NPKCa+m+s	2021	69	120	31	116	1	6.47	1.18	0.11	8.54	329.39	215.21	6.72	0.48	0.06	2.05	157.45	70.15
NPKCa	2021	69	60	31	116	1	6.35	0.83	0.09	4.68	127.67	95.02	6.71	0.45	0.06	2.66	66.92	32.49
NPKCa	2021	69	60	31	116	1	6.35	0.83	0.09	4.68	127.67	95.02	6.71	0.45	0.06	2.66	66.92	32.49
NPKCa	2021	69	60	31	116	1	6.35	0.83	0.09	4.68	127.67	95.02	6.71	0.45	0.06	2.66	66.92	32.49
NPKCa	2021	69	60	31	116	1	6.35	0.83	0.09	4.68	127.67	95.02	6.71	0.45	0.06	2.66	66.92	32.49
_PKCa	2021	69	0	31	116	1	6.64	0.73	0.08	2.21	152.12	95.28	6.76	0.42	0.06	1.83	71.99	32.32
_PKCa	2021	69	0	31	116	1	6.64	0.73	0.08	2.21	152.12	95.28	6.76	0.42	0.06	1.83	71.99	32.32
_PKCa	2021	69	0	31	116	1	6.64	0.73	0.08	2.21	152.12	95.28	6.76	0.42	0.06	1.83	71.99	32.32
_PKCa	2021	69	0	31	116	1	6.64	0.73	0.08	2.21	152.12	95.28	6.76	0.42	0.06	1.83	71.99	32.32

N_KCa	2021	69	60	0	116	1	6.25	0.81	0.08	4.41	107.00	20.66	6.45	0.46	0.06	2.99	59.21	12.87
N_KCa	2021	69	60	0	116	1	6.25	0.81	0.08	4.41	107.00	20.66	6.45	0.46	0.06	2.99	59.21	12.87
N_KCa	2021	69	60	0	116	1	6.25	0.81	0.08	4.41	107.00	20.66	6.45	0.46	0.06	2.99	59.21	12.87
N_KCa	2021	69	60	0	116	1	6.25	0.81	0.08	4.41	107.00	20.66	6.45	0.46	0.06	2.99	59.21	12.87
NP_Ca	2021	69	60	31	0	1	6.06	0.74	0.08	2.26	30.60	73.69	6.47	0.43	0.06	1.55	36.60	33.02
NP_Ca	2021	69	60	31	0	1	6.06	0.74	0.08	2.26	30.60	73.69	6.47	0.43	0.06	1.55	36.60	33.02
NP_Ca	2021	69	60	31	0	1	6.06	0.74	0.08	2.26	30.60	73.69	6.47	0.43	0.06	1.55	36.60	33.02
NP_Ca	2021	69	60	31	0	1	6.06	0.74	0.08	2.26	30.60	73.69	6.47	0.43	0.06	1.55	36.60	33.02
NPK_	2021	69	60	31	116	0	5.46	0.75	0.08	5.43	111.08	54.04	5.87	0.50	0.06	2.52	57.34	26.09
NPK_	2021	69	60	31	116	0	5.46	0.75	0.08	5.43	111.08	54.04	5.87	0.50	0.06	2.52	57.34	26.09
NPK_	2021	69	60	31	116	0	5.46	0.75	0.08	5.43	111.08	54.04	5.87	0.50	0.06	2.52	57.34	26.09
NPK_	2021	69	60	31	116	0	5.46	0.75	0.08	5.43	111.08	54.04	5.87	0.50	0.06	2.52	57.34	26.09
unfertilized	2021	69	0	0	0	0	5.79	0.67	0.07	3.39	39.34	27.32	5.95	0.38	0.06	2.29	41.16	12.71
unfertilized	2021	69	0	0	0	0	5.79	0.67	0.07	3.39	39.34	27.32	5.95	0.38	0.06	2.29	41.16	12.71
unfertilized	2021	69	0	0	0	0	5.79	0.67	0.07	3.39	39.34	27.32	5.95	0.38	0.06	2.29	41.16	12.71
unfertilized	2021	69	0	0	0	0	5.79	0.67	0.07	3.39	39.34	27.32	5.95	0.38	0.06	2.29	41.16	12.71

Part 2/4

treatment	year	bbch	pH	C	N	Nmin	Kcal	Pcal
NPKCa+m+s	2020	23	6.64	1.23	0.11	13.8	353.53	173.51
NPKCa+m+s	2020	23	6.64	1.23	0.11	13.8	353.53	173.51
NPKCa+m+s	2020	23	6.64	1.23	0.11	13.8	353.53	173.51
NPKCa+m+s	2020	23	6.64	1.23	0.11	13.8	353.53	173.51
NPKCa	2020	23	6.76	0.87	0.08	6.8	163.79	119.01
NPKCa	2020	23	6.76	0.87	0.08	6.8	163.79	119.01
NPKCa	2020	23	6.76	0.87	0.08	6.8	163.79	119.01
NPKCa	2020	23	6.76	0.87	0.08	6.8	163.79	119.01
_PKCa	2020	23	6.82	0.77	0.07	7	161.21	128.89
_PKCa	2020	23	6.82	0.77	0.07	7	161.21	128.89
_PKCa	2020	23	6.82	0.77	0.07	7	161.21	128.89
_PKCa	2020	23	6.82	0.77	0.07	7	161.21	128.89
N_KCa	2020	23	6.84	0.8	0.07	4.73	110.7	25.44
N_KCa	2020	23	6.84	0.8	0.07	4.73	110.7	25.44
N_KCa	2020	23	6.84	0.8	0.07	4.73	110.7	25.44
N_KCa	2020	23	6.84	0.8	0.07	4.73	110.7	25.44
NP_Ca	2020	23	6.77	0.75	0.07	5.62	38.09	110.45
NP_Ca	2020	23	6.77	0.75	0.07	5.62	38.09	110.45
NP_Ca	2020	23	6.77	0.75	0.07	5.62	38.09	110.45
NP_Ca	2020	23	6.77	0.75	0.07	5.62	38.09	110.45
NPK_	2020	23	6.52	0.79	0.07	3.92	62.16	114.33
NPK_	2020	23	6.52	0.79	0.07	3.92	62.16	114.33
NPK_	2020	23	6.52	0.79	0.07	3.92	62.16	114.33
NPK_	2020	23	6.52	0.79	0.07	3.92	62.16	114.33
unfertilized	2020	23	5.81	0.74	0.07	4.82	48	30.35
unfertilized	2020	23	5.81	0.74	0.07	4.82	48	30.35
unfertilized	2020	23	5.81	0.74	0.07	4.82	48	30.35

treatment	year	bbch	pH	C	N	Nmin	Kcal	Pcal
unfertilized	2020	23	5.81	0.74	0.07	4.82	48	30.35
NPKCa+m+s	2020	43	6.74	0.77	0.08	13.63667	303.95	126.0967
NPKCa+m+s	2020	43	6.74	0.77	0.08	13.63667	303.95	126.0967
NPKCa+m+s	2020	43	6.74	0.77	0.08	13.63667	303.95	126.0967
NPKCa+m+s	2020	43	6.74	0.77	0.08	13.63667	303.95	126.0967
NPKCa	2020	43	6.78	0.56	0.06	5.72	83.92	62.05667
NPKCa	2020	43	6.78	0.56	0.06	5.72	83.92	62.05667
NPKCa	2020	43	6.78	0.56	0.06	5.72	83.92	62.05667
NPKCa	2020	43	6.78	0.56	0.06	5.72	83.92	62.05667
_PKCa	2020	43	6.92	0.54	0.06	2.823333	114.3667	31.90667
_PKCa	2020	43	6.92	0.54	0.06	2.823333	114.3667	31.90667
_PKCa	2020	43	6.92	0.54	0.06	2.823333	114.3667	31.90667
_PKCa	2020	43	6.92	0.54	0.06	2.823333	114.3667	31.90667
N_KCa	2020	43	6.82	0.75	0.07	5.566667	61.79667	17.81333
N_KCa	2020	43	6.82	0.75	0.07	5.566667	61.79667	17.81333
N_KCa	2020	43	6.82	0.75	0.07	5.566667	61.79667	17.81333
N_KCa	2020	43	6.82	0.75	0.07	5.566667	61.79667	17.81333
NP_Ca	2020	43	6.33	0.38	0.05	8.686667	51.72333	67.11
NP_Ca	2020	43	6.33	0.38	0.05	8.686667	51.72333	67.11
NP_Ca	2020	43	6.33	0.38	0.05	8.686667	51.72333	67.11
NP_Ca	2020	43	6.33	0.38	0.05	8.686667	51.72333	67.11
NPK_	2020	43	5.93	0.57	0.06	7.77	59.66333	28.05
NPK_	2020	43	5.93	0.57	0.06	7.77	59.66333	28.05
NPK_	2020	43	5.93	0.57	0.06	7.77	59.66333	28.05
NPK_	2020	43	5.93	0.57	0.06	7.77	59.66333	28.05
unfertilized	2020	43	6.05	0.49	0.06	2.84	45.13	6.433333
unfertilized	2020	43	6.05	0.49	0.06	2.84	45.13	6.433333
unfertilized	2020	43	6.05	0.49	0.06	2.84	45.13	6.433333
unfertilized	2020	43	6.05	0.49	0.06	2.84	45.13	6.433333

treatment	year	bbch	pH	C	N	Nmin	Kcal	Pcal
NPKCa+m+s	2021	69	6.64	0.71	0.08	4.21226	214.7617	118.5017
NPKCa+m+s	2021	69	6.64	0.71	0.08	4.21226	214.7617	118.5017
NPKCa+m+s	2021	69	6.64	0.71	0.08	4.21226	214.7617	118.5017
NPKCa+m+s	2021	69	6.64	0.71	0.08	4.21226	214.7617	118.5017
NPKCa	2021	69	6.59	0.58	0.07	3.331238	87.17	53.33333
NPKCa	2021	69	6.59	0.58	0.07	3.331238	87.17	53.33333
NPKCa	2021	69	6.59	0.58	0.07	3.331238	87.17	53.33333
NPKCa	2021	69	6.59	0.58	0.07	3.331238	87.17	53.33333
_PKCa	2021	69	6.72	0.52	0.06	1.957542	98.6975	53.305
_PKCa	2021	69	6.72	0.52	0.06	1.957542	98.6975	53.305
_PKCa	2021	69	6.72	0.52	0.06	1.957542	98.6975	53.305
_PKCa	2021	69	6.72	0.52	0.06	1.957542	98.6975	53.305
N_KCa	2021	69	6.38	0.58	0.07	3.464773	75.14083	15.46333
N_KCa	2021	69	6.38	0.58	0.07	3.464773	75.14083	15.46333
N_KCa	2021	69	6.38	0.58	0.07	3.464773	75.14083	15.46333
N_KCa	2021	69	6.38	0.58	0.07	3.464773	75.14083	15.46333
NP_Ca	2021	69	6.39	0.5	0.06	1.696858	35.401	41.154
NP_Ca	2021	69	6.39	0.5	0.06	1.696858	35.401	41.154
NP_Ca	2021	69	6.39	0.5	0.06	1.696858	35.401	41.154
NP_Ca	2021	69	6.39	0.5	0.06	1.696858	35.401	41.154
NPK_	2021	69	5.74	0.58	0.07	3.490095	75.25167	35.40667
NPK_	2021	69	5.74	0.58	0.07	3.490095	75.25167	35.40667
NPK_	2021	69	5.74	0.58	0.07	3.490095	75.25167	35.40667
NPK_	2021	69	5.74	0.58	0.07	3.490095	75.25167	35.40667
unfertilized	2021	69	5.9	0.48	0.06	2.659133	40.55333	17.57833
unfertilized	2021	69	5.9	0.48	0.06	2.659133	40.55333	17.57833
unfertilized	2021	69	5.9	0.48	0.06	2.659133	40.55333	17.57833
unfertilized	2021	69	5.9	0.48	0.06	2.659133	40.55333	17.57833

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treatment	year	bbch	height	LAI	shoot_biomass	root_biomass	root_length	rld	rmd	diameter	root_surface	r_s
NPKCa+m+s	2020	23	6.7	0.27	0.21	0.23	1435.98	0.32	0.03	0.32	119.27	1.1
NPKCa+m+s	2020	23	6.8	0.32	0.26	0.23	2080.26	0.47	0.03	0.29	198.73	0.88
NPKCa+m+s	2020	23	5.65	0.33	0.25	0.38	2531.89	0.57	0.06	0.25	197.07	1.5
NPKCa+m+s	2020	23	5.7	0.33	0.26	0.22	2183.75	0.49	0.03	0.24	175.11	0.84
NPKCa	2020	23	5.6	0.22	0.21	0.19	2275.44	0.51	0.03	0.23	140.07	0.89
NPKCa	2020	23	5.65	0.35	0.31	0.3	2097.46	0.47	0.04	0.24	166.9	0.97
NPKCa	2020	23	4.65	0.25	0.2	0.12	1992.24	0.45	0.02	0.28	166.77	0.61
NPKCa	2020	23	5.45	0.23	0.18	0.23	1782.71	0.4	0.03	0.3	154.11	1.25
_PKCa	2020	23	4.9	0.24	0.19	0.22	2097.32	0.47	0.03	0.25	200.76	1.19
_PKCa	2020	23	4.85	0.19	0.15	0.24	2219.84	0.5	0.04	0.2	185.49	1.56
_PKCa	2020	23	5.6	0.21	0.16	0.07	656.81	0.15	0.01	0.19	33.26	0.43
_PKCa	2020	23	4.9	0.2	0.15	0.2	701.38	0.16	0.03	0.21	69.59	1.31
N_KCa	2020	23	4	0.22	0.19	0.08	376.08	0.08	0.01	0.14	16.22	0.43
N_KCa	2020	23	4.15	0.2	0.17	0.06	264.49	0.06	0.01	0.14	10.81	0.35
N_KCa	2020	23	4.2	0.22	0.18	0.03	187.67	0.04	0.01	0.14	8.57	0.17
N_KCa	2020	23	6.1	0.23	0.19	0.17	1039.21	0.23	0.02	0.15	61.03	0.91
NP_Ca	2020	23	5.25	0.17	0.14	0.07	479.56	0.11	0.01	0.15	21.83	0.51
NP_Ca	2020	23	4.9	0.15	0.13	0.13	378.41	0.08	0.02	0.17	18.26	1.03
NP_Ca	2020	23	5.6	0.25	0.2	0.15	1576.09	0.35	0.02	0.18	112.81	0.75
NP_Ca	2020	23	4.35	0.2	0.16	0.14	923.47	0.21	0.02	0.21	69.25	0.88
NPK_	2020	23	5.55	0.21	0.17	0.24	2023	0.45	0.03	0.25	163.86	1.38
NPK_	2020	23	6.9	0.2	0.17	0.26	3142.32	0.71	0.04	0.2	234.09	1.55
NPK_	2020	23	6.05	0.2	0.17	0.16	2104.24	0.47	0.02	0.24	170.22	0.95
NPK_	2020	23	6.1	0.22	0.18	0.09	1781.68	0.4	0.01	0.27	126.45	0.5
unfertilized	2020	23	5.3	0.12	0.1	0.05	470.67	0.11	0.01	0.22	30.7	0.51
unfertilized	2020	23	4.85	0.06	0.06	0.1	1017.98	0.23	0.01	0.21	64.67	1.77
unfertilized	2020	23	4.95	0.08	0.07	0.01	791.35	0.18	0	0.16	45.05	0.14

treatment	year	bbch	height	LAI	shoot_biomass	root_biomass	root_length	rld	rmd	diameter	root_surface	r_s
unfertilized	2020	23	4.75	0.08	0.07	0.02	786.29	0.18	0	0.16	38.05	0.29
NPKCa+m+s	2020	43	58.7	2.05	4.79	1.05	14272.12	2.24	0.11	0.21	916.74	0.22
NPKCa+m+s	2020	43	56.25	2.42	5.55	1	11513.05	1.81	0.1	0.22	844.21	0.18
NPKCa+m+s	2020	43	52.6	1.93	4.03	0.96	12886.22	2.03	0.1	0.2	877.28	0.24
NPKCa+m+s	2020	43	48.9	2.73	6.08	1.32	15914.41	2.5	0.13	0.21	1056.25	0.22
NPKCa	2020	43	44.65	1.48	3.71	0.93	9776.87	1.54	0.09	0.21	630.5	0.25
NPKCa	2020	43	46.95	1.71	4.45	0.79	8929.52	1.4	0.08	0.22	598.95	0.18
NPKCa	2020	43	43.9	1.53	3.93	0.88	10386.01	1.63	0.09	0.21	673.27	0.22
NPKCa	2020	43	46.45	2.02	5.5	1.15	11578.82	1.82	0.11	0.22	804.34	0.21
_PKCa	2020	43	38.15	0.85	2.74	0.64	6358.82	1	0.06	0.22	418.99	0.24
_PKCa	2020	43	36.5	1.17	3.56	0.58	8548.11	1.34	0.06	0.21	536.64	0.16
_PKCa	2020	43	36.05	1.27	3.79	0.52	11632.56	1.83	0.05	0.23	790.1	0.14
_PKCa	2020	43	36.55	1.31	3.96	0.41	9254.53	1.45	0.04	0.23	624.97	0.1
N_KCa	2020	43	49.6	1.85	4.7	0.79	8087.96	1.27	0.08	0.21	513.76	0.17
N_KCa	2020	43	42.4	1.8	4.89	1.24	12950.16	2.04	0.12	0.21	826.94	0.25
N_KCa	2020	43	42.85	1.68	4.37	1.76	18736.13	2.95	0.18	0.23	1302.94	0.4
N_KCa	2020	43	47	1.95	5.38	1.12	15417.12	2.42	0.11	0.2	977.15	0.21
NP_Ca	2020	43	44.05	1.4	3.17	0.85	12961.6	2.04	0.08	0.23	884.69	0.27
NP_Ca	2020	43	44.05	2.01	4.93	1.03	11978.81	1.88	0.1	0.23	826.91	0.21
NP_Ca	2020	43	44.4	1.7	4.72	0.84	12121.42	1.91	0.08	0.22	795.3	0.18
NP_Ca	2020	43	41.9	1.76	4.7	0.82	10928.2	1.72	0.08	0.21	722.15	0.18
NPK_	2020	43	45.1	2.13	5.28	0.96	10063.44	1.58	0.1	0.21	656.48	0.18
NPK_	2020	43	51.9	1.5	3.76	0.61	8120.56	1.28	0.06	0.2	531.51	0.16
NPK_	2020	43	48.2	1.96	4.81	0.78	12681.55	1.99	0.08	0.2	803.56	0.16
NPK_	2020	43	49.6	2.17	5.8	0.79	11131.12	1.75	0.08	0.2	699.04	0.14
unfertilized	2020	43	37.7	0.71	1.99	0.45	6941.26	1.09	0.05	0.21	461.29	0.23
unfertilized	2020	43	36.35	0.81	2.17	0.45	7304.06	1.15	0.05	0.21	521.34	0.21
unfertilized	2020	43	31.3	0.75	1.83	0.55	7258.74	1.14	0.06	0.22	485.37	0.3
unfertilized	2020	43	36.2	0.69	1.66	0.54	9995.68	1.57	0.05	0.19	579.89	0.32

treatment	year	bbch	height	LAI	shoot_biomass	root_biomass	root_length	rld	rmd	diameter	root_surface	r_s
NPKCa+m+s	2021	69	77.8	1.75	10.27	2.55	14484.83	2.28	0.26	0.22	1049.38	0.25
NPKCa+m+s	2021	69	79.3	1.87	9.87	2.5	15203.74	2.39	0.25	0.22	1085.41	0.25
NPKCa+m+s	2021	69	77.1	1.76	11.45	2.49	16743	2.63	0.25	0.22	1189.9	0.22
NPKCa+m+s	2021	69	79.4	1.89	9.74	3.74	17715.2	2.78	0.37	0.24	1398.63	0.38
NPKCa	2021	69	75.3	1.66	9.28	4.24	7201.16	1.13	0.42	0.25	725.44	0.46
NPKCa	2021	69	73.2	1.43	8.56	1.31	4960.11	0.78	0.13	0.23	368.32	0.15
NPKCa	2021	69	72.4	1.23	7.35	2.35	7907.34	1.24	0.24	0.25	701.51	0.32
NPKCa	2021	69	74.9	1.08	6.87	1.41	6455.69	1.01	0.14	0.25	490.21	0.21
_PKCa	2021	69	49.8	0.31	1.91	0.93	5153.55	0.81	0.09	0.23	381.11	0.49
_PKCa	2021	69	51.7	0.32	1.9	1.02	4340.63	0.68	0.1	0.23	320.82	0.54
_PKCa	2021	69	52.4	0.3	1.88	0.83	4651.85	0.73	0.08	0.22	323.82	0.44
_PKCa	2021	69	50.9	0.34	2.21	0.66	5003.7	0.79	0.07	0.23	381.67	0.3
N_KCa	2021	69	72.6	1.14	8	2.75	9550.79	1.5	0.27	0.22	787.94	0.34
N_KCa	2021	69	74.9	1.41	7.85	2.17	11288.35	1.77	0.22	0.24	867.37	0.28
N_KCa	2021	69	73	1.34	8.08	2.22	10256.57	1.61	0.22	0.26	875.74	0.27
N_KCa	2021	69	72.3	1.38	8.86	1.9	12587.05	1.98	0.19	0.23	950.68	0.21
NP_Ca	2021	69	77	1.63	11.49	3.35	12280.5	1.93	0.34	0.25	1045.74	0.29
NP_Ca	2021	69	76.1	1.71	11.82	3.27	14997.7	2.36	0.33	0.25	1258.49	0.28
NP_Ca	2021	69	73.4	1.72	12.9	2.62	14454.45	2.27	0.26	0.24	1147.46	0.2
NP_Ca	2021	69	74.4	1.51	11.36	3.45	17064.27	2.68	0.35	0.24	1374.16	0.3
NPK_	2021	69	74.2	1.44	9.86	3.61	11656.91	1.83	0.36	0.26	1129.29	0.37
NPK_	2021	69	74.4	1.37	9.21	4.27	11191.81	1.76	0.43	0.25	1042.21	0.46
NPK_	2021	69	74.6	1.63	10.15	2.67	9699.69	1.52	0.27	0.26	862.22	0.26
NPK_	2021	69	76.4	1.54	11.2	3.45	11593.73	1.82	0.35	0.24	1000.84	0.31
unfertilized	2021	69	50.9	0.23	1.42	1.89	4308.29	0.68	0.19	0.28	446.03	1.33
unfertilized	2021	69	47.7	0.15	1.05	0.92	3830.09	0.6	0.09	0.23	301.61	0.87
unfertilized	2021	69	52.6	0.21	1.15	2.17	3580.38	0.56	0.22	0.24	392.29	1.88
unfertilized	2021	69	44.7	0.25	1.57	2.1	4768.07	0.75	0.21	0.27	450.9	1.33

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treatment	year	bbch	rL_sB_km_kg	root_length_topsoil	root_biomass_topsoil	rld_topsoil	rmd_topsoil	root_length_subsoil	root_biomass_subsoil	rld_subpsoil	rmd_subsoil	SRL	SLA_m2_kg
NPKCa+m+s	2020	23	107.77	1304.06	0.22	0.68	0.07	131.92	0.01	0.05	0	97.69	12.7
NPKCa+m+s	2020	23	125.72	1773.64	0.21	0.93	0.07	306.62	0.02	0.12	0.01	138.85	12.47
NPKCa+m+s	2020	23	156.98	2148.32	0.32	1.12	0.11	383.57	0.06	0.15	0.02	103.13	12.94
NPKCa+m+s	2020	23	131.04	1805.02	0.16	0.94	0.05	378.72	0.06	0.15	0.01	158.24	12.66
NPKCa	2020	23	166.78	2114.05	0.16	1.11	0.06	161.39	0.03	0.06	0.01	175.43	10.24
NPKCa	2020	23	106.99	2042.29	0.26	1.07	0.09	55.17	0.04	0.02	0.01	109.7	11.36
NPKCa	2020	23	159.91	1778.94	0.11	0.93	0.04	213.31	0.01	0.09	0	230.88	12.8
NPKCa	2020	23	152.3	1544.56	0.14	0.81	0.05	238.15	0.09	0.1	0.02	117.42	12.43
_PKCa	2020	23	178.02	2041.25	0.22	1.07	0.07	56.06	0	0.02	0	143.65	13.14
_PKCa	2020	23	226.33	2110.17	0.22	1.1	0.07	109.67	0.02	0.05	0.01	140.94	12.39
_PKCa	2020	23	63.03	571.44	0.06	0.3	0.02	85.36	0.01	0.04	0	140.01	13.06
_PKCa	2020	23	71.47	593.36	0.16	0.31	0.06	108.02	0.04	0.04	0.01	51.76	12.64
N_KCa	2020	23	31.81	330.16	0.08	0.17	0.02	45.93	0	0.02	0	72.32	11.97
N_KCa	2020	23	24.31	185.59	0.03	0.1	0.01	78.9	0.03	0.03	0.01	72.48	11.97
N_KCa	2020	23	16.3	126.03	0.03	0.07	0.01	61.64	0	0.03	0	68.05	12.42
N_KCa	2020	23	87.38	1010.2	0.11	0.53	0.03	29.01	0.06	0.01	0.01	110.9	12.25
NP_Ca	2020	23	55.04	349.21	0.05	0.18	0.02	130.35	0.02	0.06	0.01	110.24	12.36
NP_Ca	2020	23	47.34	246.27	0.07	0.13	0.02	132.13	0.06	0.05	0.02	44.81	12.3
NP_Ca	2020	23	123.52	1503.49	0.15	0.79	0.05	72.6	0	0.03	0	152.62	12.59
NP_Ca	2020	23	91.14	800.45	0.12	0.42	0.04	123.02	0.02	0.05	0.01	100.38	12.55
NPK_	2020	23	182.38	1754.84	0.19	0.92	0.06	268.16	0.05	0.11	0.01	133.09	11.96
NPK_	2020	23	295.2	3015.94	0.25	1.58	0.08	126.38	0.01	0.05	0	186.49	12.23
NPK_	2020	23	195.73	1872.37	0.15	0.98	0.05	231.87	0.01	0.09	0	207.31	12.01
NPK_	2020	23	155.1	1663.9	0.08	0.87	0.03	117.78	0.01	0.05	0	280.58	12.23
unfertilized	2020	23	75.45	350.74	0.05	0.18	0.02	119.93	0	0.05	0	134.48	12.51
unfertilized	2020	23	283.3	839.55	0.09	0.44	0.03	178.43	0.01	0.07	0	169.66	11.35
unfertilized	2020	23	170.7	439.92	0.01	0.23	0.01	351.43	0	0.14	0	476.14	10.7
unfertilized	2020	23	179.41	594.69	0.02	0.31	0.01	191.59	0	0.08	0	436.83	11.14
NPKCa+m+s	2020	43	46.85	6861.75	0.41	3.6	0.14	7410.37	0.64	1.66	0.09	213.02	11.98

treatment	year	bbch	rL_sB_km_kg	root_length_topsoil	root_biomass_topsoil	rld_topsoil	rmd_topsoil	root_length_subsoil	root_biomass_subsoil	rld_subpsoil	rmd_subsoil	SRL	SLA_m2_kg
NPKCa+m+s	2020	43	32.59	5277.1	0.55	2.77	0.18	6235.94	0.45	1.4	0.06	180.74	12.22
NPKCa+m+s	2020	43	50.22	7039.75	0.48	3.69	0.16	5846.47	0.48	1.31	0.07	210.11	13.37
NPKCa+m+s	2020	43	41.15	9156.53	0.69	4.8	0.23	6757.88	0.63	1.52	0.09	189.46	12.55
NPKCa	2020	43	41.46	4431.28	0.46	2.32	0.15	5345.59	0.47	1.2	0.07	165.71	11.15
NPKCa	2020	43	31.54	4092.44	0.31	2.14	0.1	4837.08	0.47	1.09	0.07	178.59	10.75
NPKCa	2020	43	41.58	4749.1	0.33	2.49	0.11	5636.92	0.55	1.27	0.08	185.46	10.9
NPKCa	2020	43	33.09	4237.46	0.38	2.22	0.13	7341.36	0.77	1.65	0.11	158.61	10.28
_PKCa	2020	43	36.52	3016.87	0.27	1.58	0.09	3341.95	0.38	0.75	0.05	155.09	8.69
_PKCa	2020	43	37.78	4725.79	0.28	2.48	0.09	3822.32	0.3	0.86	0.04	231.03	9.18
_PKCa	2020	43	48.29	6595.84	0.27	3.46	0.09	5036.72	0.25	1.13	0.04	352.5	9.37
_PKCa	2020	43	36.75	4986.97	0.17	2.61	0.06	4267.56	0.24	0.96	0.03	355.94	9.24
N_KCa	2020	43	27.07	3657.76	0.39	1.92	0.13	4430.19	0.39	0.99	0.06	161.76	11.01
N_KCa	2020	43	41.63	6191.36	0.53	3.24	0.18	6758.8	0.71	1.52	0.1	163.93	10.32
N_KCa	2020	43	67.35	9518.95	0.75	4.99	0.25	9217.18	1.01	2.07	0.14	167.29	10.75
N_KCa	2020	43	45.08	6321.95	0.5	3.31	0.17	9095.16	0.61	2.04	0.09	217.14	10.17
NP_Ca	2020	43	64.28	6867.73	0.53	3.6	0.18	6093.88	0.31	1.37	0.04	240.03	12.38
NP_Ca	2020	43	38.19	5257.27	0.53	2.75	0.18	6721.54	0.51	1.51	0.07	182.05	11.41
NP_Ca	2020	43	40.37	5505.41	0.31	2.88	0.1	6616.01	0.53	1.49	0.08	226.99	10.08
NP_Ca	2020	43	36.57	5815.27	0.47	3.05	0.16	5112.93	0.35	1.15	0.05	208.55	10.47
NPK_	2020	43	29.95	4861.96	0.6	2.55	0.2	5201.48	0.36	1.17	0.05	164.97	11.31
NPK_	2020	43	33.93	4080.83	0.39	2.14	0.13	4039.73	0.22	0.91	0.03	207.69	11.19
NPK_	2020	43	41.43	4567.43	0.27	2.39	0.09	8114.12	0.51	1.82	0.07	255.68	11.43
NPK_	2020	43	30.17	4174.6	0.26	2.19	0.09	6956.52	0.53	1.56	0.08	219.98	10.5
unfertilized	2020	43	54.85	2968.39	0.21	1.56	0.07	3972.87	0.24	0.89	0.03	241.02	9.95
unfertilized	2020	43	52.97	3939.49	0.25	2.06	0.08	3364.57	0.2	0.76	0.03	253.61	10.42
unfertilized	2020	43	62.18	2263.4	0.18	1.19	0.06	4995.34	0.37	1.12	0.05	207.39	11.42
unfertilized	2020	43	94.66	4747.29	0.22	2.49	0.07	5248.38	0.31	1.18	0.04	293.13	11.57
NPKCa+m+s	2021	69	22.16	8799.49	1.99	4.61	0.66	5685.34	0.56	1.28	0.08	89.14	10.84
NPKCa+m+s	2021	69	24.22	7061.98	1.72	3.7	0.57	8141.75	0.78	1.83	0.11	95.56	11.39
NPKCa+m+s	2021	69	22.98	8158.54	1.66	4.27	0.55	8584.46	0.83	1.93	0.12	105.57	9.42

treatment	year	bbch	rL_sB_km_kg	root_length_topsoil	root_biomass_topsoil	rld_topsoil	rmd_topsoil	root_length_subsoil	root_biomass_subsoil	rld_subpsoil	rmd_subsoil	SRL	SLA_m2_kg
NPKCa+m+s	2021	69	28.57	8056.17	2.79	4.22	0.93	9659.03	0.95	2.17	0.14	74.37	12.95
NPKCa	2021	69	12.19	4325.39	3.87	2.27	1.29	2875.78	0.38	0.65	0.05	26.67	14.6
NPKCa	2021	69	9.11	2718.19	1.09	1.42	0.37	2241.92	0.21	0.5	0.03	59.69	12.25
NPKCa	2021	69	16.91	5630.96	2.04	2.95	0.68	2276.38	0.31	0.51	0.04	52.82	12.37
NPKCa	2021	69	14.77	3472.92	1	1.82	0.33	2982.77	0.41	0.67	0.06	71.97	12.27
_PKCa	2021	69	42.35	3218.98	0.68	1.69	0.23	1934.56	0.25	0.43	0.04	86.91	10.13
_PKCa	2021	69	35.92	2426.73	0.73	1.27	0.24	1913.9	0.29	0.43	0.04	66.88	8.65
_PKCa	2021	69	38.93	2112.34	0.48	1.11	0.16	2539.51	0.35	0.57	0.05	87.94	9.78
_PKCa	2021	69	35.56	2381.03	0.35	1.25	0.12	2622.67	0.31	0.59	0.04	118.57	10.16
N_KCa	2021	69	18.77	5962	2.44	3.12	0.81	3588.79	0.31	0.81	0.04	54.61	10.14
N_KCa	2021	69	22.6	5603.4	1.58	2.94	0.53	5684.95	0.59	1.28	0.08	81.56	11.03
N_KCa	2021	69	19.96	4510.16	1.64	2.36	0.55	5746.41	0.58	1.29	0.08	72.69	11.34
N_KCa	2021	69	22.32	6102.22	1.23	3.2	0.41	6484.83	0.67	1.46	0.1	104.11	10.8
NP_Ca	2021	69	16.8	6089.16	2.61	3.19	0.87	6191.33	0.74	1.39	0.11	57.52	9.64
NP_Ca	2021	69	19.95	10405.64	2.58	5.45	0.86	4592.07	0.69	1.03	0.1	68.5	8.82
NP_Ca	2021	69	17.62	7393.23	1.93	3.87	0.65	7061.22	0.69	1.59	0.1	86.5	7.81
NP_Ca	2021	69	23.62	9390.59	2.66	4.92	0.89	7673.68	0.79	1.72	0.11	77.71	6.83
NPK_	2021	69	18.59	8033.93	3.24	4.21	1.08	3622.99	0.37	0.81	0.05	50.68	7.62
NPK_	2021	69	19.1	7271.98	3.87	3.81	1.29	3919.82	0.4	0.88	0.06	41.18	7.62
NPK_	2021	69	15.02	4231.66	2	2.22	0.67	5468.03	0.67	1.23	0.1	56.99	10.63
NPK_	2021	69	16.27	5692.42	2.89	2.98	0.96	5901.31	0.56	1.33	0.08	52.77	7.4
unfertilized	2021	69	47.58	3163.38	1.77	1.66	0.59	1144.91	0.12	0.26	0.02	35.75	7.97
unfertilized	2021	69	57.4	2856.95	0.82	1.5	0.27	973.14	0.1	0.22	0.01	65.58	9.17
unfertilized	2021	69	48.86	2859.04	2.09	1.5	0.7	721.34	0.08	0.16	0.01	25.91	8.37
unfertilized	2021	69	47.75	2147.95	1.77	1.13	0.59	2620.12	0.32	0.59	0.05	35.74	9.96

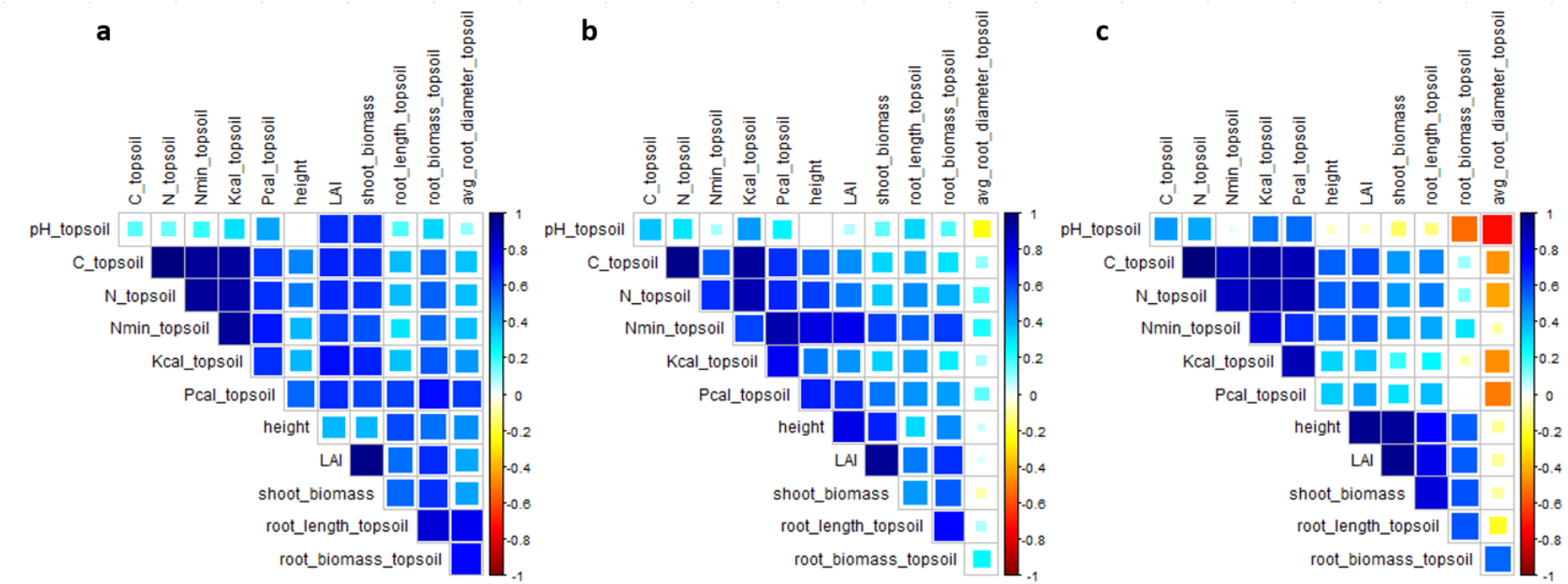

Figure S4.8. Pearson correlation for TOPSOIL soil properties, above and below ground TOPSOIL traits during the shoot-root sampling dates: a) Frist sampling date in growing period 1 (2019/20, BBCH=23), b) Second sampling date in growing period 1 (2019/20, BBCH=43), and, c) First sampling date in growing period 2 (2020/21, BBCH=69). P-values between -0.3 to 0.3 are not significant.

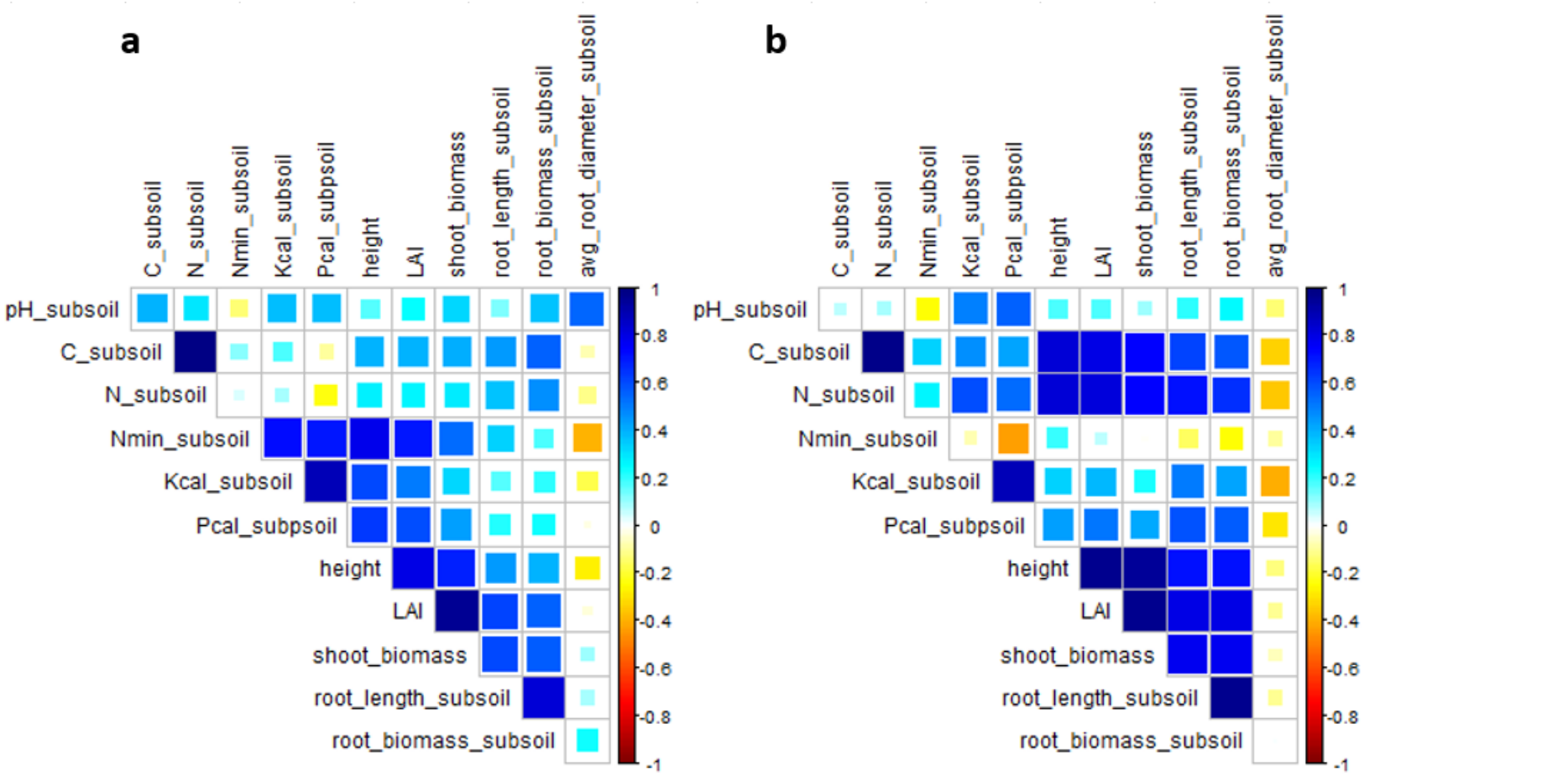

Figure S4.9. Pearson correlation for SUBSOIL soil properties, above and below ground TOPSOIL traits during the shoot-root sampling dates: a) Second sampling date in growing period 1 (2019/20, BBCH=43), and, b) First sampling date in growing period 2 (2020/21, BBCH=69). P-values between -0.3 to 0.3 are not significant.

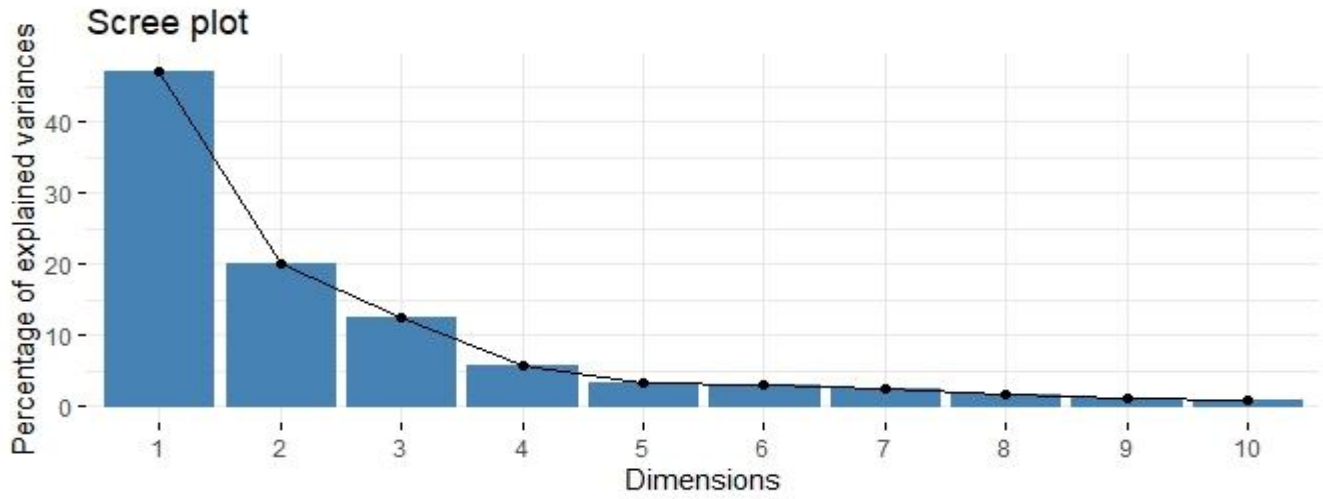

Figure S4.10. Scree plot showing the percentage of explained variance for each principal component. The first two components explain the majority of the variance, with subsequent components contributing progressively less.

APPENDIX D: SUPPLEMENTARY MATERIAL FROM CHAPTER 5

Table S5.1: Soil analysis data. Topsoil mineral N, plant available soil P and K (PCAL and KCAL) extracted with a calcium-acetate-lactate extract in kg ha-1 and topsoil pH value for the seven treatments and four sampling dates in 2019 at the long-term fertilizer experiment Dikopshof (taken from Yi et al. 2020).

Sampling	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	no fertilization
			Mineral N				
May, 16	80.9	22.8	10.1	76.5	74.0	109.5	13.5
June, 13	32.5	9.4	5.2	18.8	26.6	36.2	20.7
July, 10	4.5	6.8	3.1	2.7	3.4	18.2	6.8
September,	4.9	4.1	1.8	4.0	8.1	3.6	3.2
			P_{CAL}				
May, 16	392	142	168	30	90	57	18
June, 13	344	158	182	36	88	67	35
July, 10	369	84	166	34	61	82	47
September,	292	147	147	28	91	65	18
			K_{CAL}				
May, 16	655	219	228	226	121	162	108
June, 13	563	211	243	198	100	144	126
July, 10	531	275	239	216	117	137	122
September,	576	462	320	285	84	117	76
			pH value				
May, 16	6.4	6.5	6.9	6.4	6.6	5.5	5.8
June, 13	6.5	6.8	7.0	6.7	6.8	5.8	5.6
July, 10	6.7	6.3	6.9	6.4	6.8	5.8	5.5
September,	6.5	6.6	6.8	6.7	6.7	5.5	5.7

Table S5.2: Number of analyzed replicates per sampling date and treatment for LAI, DM root, DM shoot, Root morphology (total root length, root diameter) and link basic connectivity. For link basic connectivity, the replicates correspond to segments of one sample per treatment and per sampling date.

	LAI			**DM root**				**DM shoot**				**SRL**			**Root morphology**				**Link basic connectivity**		
Sampling date	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**4**
NPKCa+m+s	5	5	4	1	5	5	4	1	5	5	4	1	3	3	5	4	3	3	5	3	3
NPKCa	6	5	5	1	6	5	5	1	6	5	5	1	4	3	6	5	3	3	6	1	3
_PKCa	5	5	5	1	5	5	5	1	5	5	5	1	5	3	5	5	3	3	5	3	2
N_KCa	5	6	5	1	5	6	5	1	5	6	5	1	4	2	5	4	4	3	5	4	3
NP_Ca	5	5	5	1	5	5	5	1	5	5	5	1	4	3	6	5	3	3	6	4	3
NPK_	5	5	4	1	5	5	4	1	5	5	4	1	5	2	6	5	3	2	6	3	2
no fertilization	5	6	5	1	5	6	5	1	5	6	5	1	5	3	5	4	4	3	5	3	3

Table S5.3: Means of shoot and root C, N, P, K parameters as well as shoot C/N ration and root C/N ratio.

Sampling date	NPKCa+m+s	NPK_	_PKCa	N_KCa	NP_Ca	NPKCa	no fertilization
			Shoot N (%)				
June, 13	4.41	4.03	4.83	4.71	4.69	4.66	4.6
July, 10	2.05	2.72	3.03	3.13	3.18	2.89	3.32
September, 10	2.57	1.89	2.82	2.11	2.22	1.99	1.88
			Shoot C (%)				
June, 13	36.38	37.08	36.87	37.1	37.06	36.64	39.04
July, 10	39.56	38.82	40.87	39.29	40.67	39.75	41.45
September, 10	33.96	32.89	37.47	35.2	36.28	34.6	32.56
			Shoot C/N ratio				
June, 13	8.24	7.9	9.2	7.87	7.64	7.86	8.48
July, 10	27.1	12.78	14.25	12.54	13.47	13.78	12.5
September, 10	13.23	16.31	17.41	16.7	13.29	17.36	17.33
			Root C/N ratio				
June, 13	18.83	20.02	26.25	18.52	18.6	18.33	21.76
July, 10	29.74	51.76	65.55	45.03	55.31	50.22	56.97
September, 10	44.7	48.04	77.9	45.11	43.38	43.56	63.55
			Root C (%)				
June, 13	41.1	42.83	42.31	41.84	42.68	43.03	42.44
July, 10	35.91	43.6	42.9	43.53	43.52	44.04	43.72
September, 10	50.15	50.95	47.01	49.5	47.46	50.2	49.03
			Root N (%)				
June, 13	2.23	2.14	1.61	2.26	2.3	2.35	1.95
July, 10	1.47	0.842	0.65	0.97	0.787	0.88	0.77
September, 10	1.12	1.06	0.6	1.1	1.09	1.15	0.77
			Shoot N (%)				
June, 13	4.41	4.69	4.03	4.71	4.83	4.66	4.6
July, 10	2.05	3.18	2.72	3.13	3.03	2.89	3.32
September, 10	2.57	2.22	1.89	2.11	2.82	1.99	1.88
			Shoot P (%)				
June, 13	0.37	0.41	0.4	0.35	0.43	0.42	0.37
July, 10	0.2	0.26	0.19	0.16	0.21	0.24	0.21
September, 10	0.18	0.15	0.18	0.1	0.14	0.13	0.09
			Shoot K (%)				
June, 13	10.13	8.37	7.12	7.89	5.49	8.15	4.76
July, 10	9.16	6.34	4.29	5.61	3.7	5.58	3.18
September, 10	6.62	3.61	0.75	2.88	2.92	3.67	1.68
			Root P (%)				
June, 13	0.35	0.37	0.39	0.3	0.34	0.37	0.3
July, 10	0.21	0.19	0.12	0.1	0.17	0.21	0.11
September, 10	0.16	0.09	0.12	0.06	0.14	0.15	0.07
			Root K (%)				
June, 13	4.26	2.82	2.38	3.79	2.78	4.01	2.54
July, 10	2	1.1	0.66	1.18	0.72	1.12	0.79
September, 10	1.41	0.65	0.44	0.76	0.86	0.88	0.52

Table S5.4: Share of interior root length (II, in %) exterior-interior (EI, in %) and exterior (EE, in %) of total root length (II, EI and EE) for the seven treatments and three sampling dates in 2019 at the long-term fertilizer experiment Dikopshof.

Sampling date	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	no fertilization
Share of interior (II) root length of total root length (%)							
May, 16	70.0	63.1	65.3	56.5	59.4	64.6	64.8
June, 13	60.7	58.0	60.7	55.1	59.9	57.5	62.2
September, 10	65.6	67.0	66.8	70.4	68.9	66.4	61.7
Share of exterior-interior (EI) root length of total root length (%)							
May, 16	20.6	24.9	18.8	27.1	26.5	20.8	26.7
June, 13	26.8	25.0	21.7	32.9	27.8	26.6	26.5
September, 10	20.7	19.7	20.4	17.1	19.1	21.3	23.8
Share of exterior-exterior (EE) root length of total root length (%)							
May, 16	9.4	12.0	15.9	16.5	14.1	14.6	8.5
June, 13	12.5	17.0	17.6	12.1	12.3	15.9	11.4
September, 10	13.7	13.3	12.8	12.5	12.0	12.3	14.4

Table S5.5: Number of interior (II), exterior-interior (EI) and exterior-exterior (EE) links per cm root length for the seven treatments and three sampling dates in 2019 at the long-term fertilizer experiment Dikopshof.

Sampling date	NPKCa+m+s	NPKCa	_PKCa	N_KCa	NP_Ca	NPK_	no fertilization
Number of interior (II) links per cm root length							
May, 16	3.5	3.6	1.9	2.1	1.9	2.8	2.9
June, 13	2.8	3.1	3.0	2.8	2.8	2.5	2.7
September, 10	5.8	6.0	6.6	6.2	7.1	6.6	5.7
Number of exterior-interior (EI) links per cm root length							
May, 16	0.6	0.8	0.5	0.6	0.7	0.5	0.7
June, 13	0.9	1.0	0.9	1.0	0.9	0.8	0.9
September, 10	1.3	1.3	1.3	1.2	1.3	1.5	1.3
Number of exterior-exterior (EE) links per cm root length							
May, 16	0.3	0.4	0.4	0.3	0.3	0.3	0.2
June, 13	0.5	0.7	0.7	0.5	0.5	0.4	0.5
September, 10	0.9	0.8	0.8	0.7	0.8	0.9	0.9

APPENDIX E: List of Co- author contributions

Ahmadi, S. H., Seidel, S. J., Lopez, G., Kamali, B., Gaiser, T., Hadir, S., Demie, D. T., Andersen, M. N., Ewert, F. and Ochoa, I. H., 2025. Root: shoot ratio of field crops under conventional and conservation tillage: A meta analysis. Soil Use and Management, 41 (1), e70026. https://doi.org/10.1111/sum.70026.

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