Institut für Nutzpflanzenwissenschaften und Ressourcenschutz
Fachgebiet Agrarökologie und Organischer Landbau

Root growth of sole and mixed cover crops in organic farming

Dissertation

zur
Erlangung des Grades
Doktor der Agrarwissenschaften (Dr. agr.)

der Landwirtschaftlichen Fakultät
der Rheinischen Friedrich-Wilhelms-Universität Bonn

von
Roman Kemper
aus
Soest

Bonn 2024

Referent: Prof. Dr. Thomas F. Döring

Korreferentin: Prof. Dr. Miriam Athmann

Tag der mündlichen Prüfung: 18.12.2023

Angefertigt mit Genehmigung der Landwirtschaftlichen Fakultät der Universität Bonn

CONTENTS

List of publications

Parts of this thesis have been published in peer-reviewed journals:

Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. https://doi.org/10.3390/agriculture10110503

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

Kemper R, Döring TF, Legner N, Meinen C, Athmann M (2023) Oil radish, winter rye and crimson clover: root and shoot performance in cover crop mixtures. Plant and Soil. https://doi.org/10.1007/s11104-023-06240-y

Kemper R, Döring T, Athmann M (2023) Schaffen Zwischenfrüchte großlumige Bioporen in Unterböden? In: Bibic, V.; Schmidtke, K. (Hrsg.) One Step Ahead - einen Schritt voraus! Beiträge zur 16. Wissenschaftstagung Ökologischer Landbau, Frick (CH), 07. bis 10. März 2023, Verlag Dr. Köster, Berlin. https://orgprints.org/id/eprint/50540

Bublitz TA, Kemper R, Müller P, Kautz T, Döring TF, Athmann M (2022) Relating Profile Wall Root-Length Density Estimates to Monolith Root-Length Density Measurements of Cover Crops. Agronomy 12:48. https://doi.org/10.3390/agronomy12010048

Summary

Cover crops are an important tool in arable farming systems. They provide many different agroecosystem services. For many of these functions, such as nutrient uptake, erosion prevention and soil structure formation, the roots of cover crops are of central importance. In recent years, cover crop mixtures have become increasingly popular in agricultural practice. Mixtures can have advantages over sole crops in terms of productivity and agroecosystem services. In agricultural practice and consulting, the advantages of mixtures are often mentioned with regard to the rooting of cover crop stands, and complementarity in the root zone as well as increased rooting across the entire soil profile are postulated.

The aim of the present thesis is to test different cover crop species for their rooting intensity and, in a further step, to investigate the root traits in cover crop mixtures and possible belowground mixture effects. In addition, the potential of cover crops to create large-sized biopores is assessed. Different methods for determining root length density are compared.

In 2018/19 and 2019/20, sole and mixed cover crops were investigated for shoot and root traits in the field. The latter were determined using the monolith and profile wall method. Species-specific root masses in mixtures were analysed by Fourier transform infrared spectroscopy.

The results showed that winter hardy cover crop species increased root length over winter. Oil radish, winter turnip rape and phacelia rooted intensively into large-sized biopores in the subsoil. Positive mixture effects partly occurred in the shoot, whereby these were higher for nutrient content than for biomass. There were no significant mixing effects for root mass. Weak partners were suppressed in root mass. In mixtures with oil radish, there were positive mixture effects for root length and root mass density in the subsoil. For the specific root length, positive mixture effects were found in all mixtures. Neither a vertical niche differentiation for root mass density nor complementarity effects for root mass could be clearly found. The biopore genesis potential of cover crops was low, but highest for blue lupin.

Compared to the monolith method, the root length density estimated with the profile wall method was underestimated, especially in highly rooted zones. High agreement between both methods was found for the cumulative root distribution. The influence of soil depth was very decisive for the comparison of both methods.

Overall, it was shown that for the cover crop mixtures tested in this thesis mixture effects of root traits were rather small. Complementarity in the root zone and increased rooting over the entire soil profile could not be found. In addition, future perspectives for root research on cover crop mixtures as well as agronomic management options for cover cropping are discussed.

Zusammenfassung

Zwischenfrüchte sind ein wichtiges pflanzenbauliches Werkzeug in Ackerbausystemen. Sie erfüllen viele unterschiedliche Ökosystemdienstleistungen. Für viele dieser Funktionen, zum Beispiel Nährstoffaufnahme, Erosionsvermeidung und Bodenstrukturbildung, sind die Wurzeln der Zwischenfrüchte von zentraler Bedeutung. In den vergangenen Jahren haben sich zunehmend Zwischenfruchtmischungen in der landwirtschaftlichen Praxis durchgesetzt. Mischungen können gegenüber Reinsaaten Vorteile in Produktivität und Ökosystemdienstleistungen haben. In der landwirtschaftlichen Praxis und Beratung werden auch hinsichtlich der Durchwurzelung von Zwischenfruchtbeständen die Vorteile von Mischungen immer wieder angeführt und Komplementarität im Wurzelraum sowie erhöhte Durchwurzelung über das gesamte Bodenprofil postuliert.

Ziel der vorliegenden Arbeit ist, es unterschiedliche Zwischenfruchtarten auf ihre Durchwurzelungsintensität zu prüfen und in einem weiteren Schritt die Wurzeleigenschaften in Zwischenfruchtmischungen sowie mögliche unterirdische Mischungsvorteile zu untersuchen. Darüber hinaus wird das Potential von Zwischenfrüchten zur Bildung großlumiger Bioporen abgeschätzt. Unterschiedliche Methoden zur Bestimmung der Wurzellängendichte werden verglichen.

In den Jahren 2018/19 und 2019/20 wurden Zwischenfrucht-Reinsaaten und -Mischungen auf Spross- und Wurzeleigenschaften im Feld untersucht. Letztere wurden mit der Monolith- und der Profilwandmethode ermittelt. Die Wurzelmassenanteile in Mischungen wurden mittels Fourier-Transform-Infrarotspektroskopie analysiert.

Die Ergebnisse zeigen, dass winterharte Zwischenfruchtarten über den Winter an Wurzellänge zunahmen. Ölrettich, Winterrübsen und Phacelia durchwurzelten großlumige Bioporen im Unterboden intensiv. Positive Mischungseffekte traten im Spross teilweise auf, wobei diese für die Nährstoffgehalte höher waren als für die Biomasse. Für die Wurzelmasse gab es keine signifikanten Mischungseffekte. Schwache Partner wurden in der Wurzelmasse unterdrückt. In Mischungen mit Ölrettich zeigten sich positive Mischungseffekte für Wurzellängen- und -massendichte im Unterboden. Für die spezifische Wurzellänge zeigten sich in allen Mischungen positive Mischungseffekte. Weder eine vertikale Nischendifferenzierung für die Wurzelmassendichte noch Komplementaritätseffekte für die Wurzelmasse konnten eindeutig gefunden werden. Das Bioporengenese-Potential von Zwischenfrüchten ist gering und am höchsten noch für die Blaue Lupine zu bewerten.

Im Vergleich zur Monolithmethode wurde die mit der Profilwandmethode geschätzte Wurzellängendichte deutlich unterschätzt, insbesondere in stark durchwurzelten Zonen. Hohe Übereinstimmung zwischen beiden Methoden zeigte sich für die kumulative Wurzelverteilung. Der Einfluss der Bodentiefe war für den Vergleich beider Methoden sehr entscheidend.

Insgesamt zeigte sich, dass für die in dieser Arbeit getesteten Zwischenfruchtmischungen die Mischungseffekte der Durchwurzelung eher gering waren und Komplementarität im Wurzelraum sowie erhöhte Durchwurzelung über das gesamte Bodenprofil nicht nachgewiesen werden konnten. Zudem werden Zukunftsperspektiven für die Wurzelforschung zu Zwischenfruchtmischungen sowie agronomische Bewirtschaftungsmaßnahmen für den Zwischenfruchtanbau diskutiert.

Abbreviations, acronyms and units

°C	degree celsius
AME	absolute mixture effect
Anova	Analysis of variance
CC	crimson clover
CE	complementarity effect
cm	centimetre
DM	dry mass
DNA	deoxyribonucleic acid
e.g.	exempli gratia
et al.	et alii
Fig.	Figure
FTIR	Fourier transform infrared
g	gram
ha	hectare
HSD	honest significant difference
i.e.	id est
K	potassium
kg	kilogram
LER	land equivalent ratio
LU	blue lupin
m	metre
mg	milligram
MSC	multiplicative scattering correction
N	nitrogen
n	sample size

NE	net effect
N_{min}	mineral nitrogen
OR	oil radish
P	phosphorus
pLER	partial land equivalent ratio
PLS	partial least square
qPCR	quantitative polymerase chain reaction
R^2	coefficient of determination
RLD	root length density
RMD	root mass density
RME	relative mixture effect
RMSECV	root mean square error of cross validation
RPD	residual prediction deviation
SE	selection effect
SNV	Standard Normal Variate (vector normalization)
SRL	specific root length
var.	varietas
vs.	versus
WR	winter rye

1 General Introduction

1.1 Functions of cover crops and their roots in arable cropping systems

Cover crops are crops that are cultivated after the harvest of the main crop and before the establishment of the next main crop to provide numerous ecosystem services (Justes et al. 2017). Although they are typically not intended for harvesting (Justes et al. 2017), they can be utilised as forage (Lütke Entrup 1996). When cover crops are primarily cultivated to catch soil mineral nitrogen (N), they are commonly referred to as 'catch crops'; they are also called 'green manures' when their primary purpose is to deliver nutrients to the subsequent main crop (Thorup-Kristensen et al. 2003). Ecosystem services provided by cover crops in arable farming systems are soil coverage, weed suppression (Brust et al. 2014), protection of soil erosion, an increase of soil organic matter content, nutrient capture and water retention as well as soil structure remediation, suppression of soil pathogens and biological nitrogen fixation (Griffiths et al. 2022). Blanco-Canqui et al. (2015) provide an overview of the diverse ecosystem services offered by cover crops demonstrating their multifunctionality. An adapted version of their findings is presented in Table 1.1.

Table 1.1 Ecosystem services of cover crops (Blanco-Canqui et al. 2015, adapted).

Ecosystems services of cover crops	Mechanism of ecosystem service
improvement in soil physical properties	▪ increase of soil aggerate stability ▪ alleviation of soil compaction ▪ improvement of soil macroporosity ▪ increase in water infiltration ▪ moderation of soil temperature
improvement in chemical and biological properties	▪ increase of soil organic carbon stocks ▪ improvement of nutrient cycling - fixation of nitrogen (legumes) - uptake of residual nitrogen - reduction of nitrate leaching - scavenging nutrients from deeper soils ▪ increase in earthworm population ▪ enhancement of microbial activity and biomass
soil conservation	▪ reduction of wind and water erosion ▪ decrease of runoff
weed suppression	▪ competition for water, nutrients, light ▪ allelochemicals
pest or disease control	▪ diversification of crop rotations ▪ interruption of life cycles of pests and diseases
increase or maintenance of crop yield	▪ interaction of several ecosystem services
provision of forage for livestock	▪ production of biomass
provision of biomass for biofuel	▪ production of biomass
maintenance or improvement of biodiversity and wildlife habitat	▪ provision of cover, nesting area, and food

Nitrogen retention and green manuring are major aims of cultivating cover crops (Thorup-Kristensen et al. 2003). Many studies verified the importance of cover crop roots, especially fast and deep growing roots, for nutrient uptake, soil mineral nitrogen depletion and prevention of nitrogen leaching (Herrera et al. 2010; Kristensen and Thorup-Kristensen 2004; Sainju et al. 1998; Sapkota et al. 2012; Thorup-Kristensen 2001). Besides, rhizodeposition is meaningful for the retention of nitrogen by cover cropping (Kanders et al. 2017). Moreover, carbon released by the roots of cover crops promotes nitrogen immobilisation by soil microorganisms (in‘t Zandt et al. 2018). Thus, the meaning of cover crops and especially their roots is indisputable for the reduction of nitrate leaching and the catch crop service. However, the green manure service of cover crops is less clear, especially for non-legumes (Thorup-Kristensen et al. 2003). Hence, the transfer of nitrogen taken up by the cover crop to the subsequent main crop is challenging, as nitrogen release from residues and the demand of the main crop often do not match (Sieling 2019).

Cover crops are also discussed to mitigate climate change due to carbon sequestration, reduced fertiliser use after legume cover crops and change in surface albedo (Kaye and Quemada 2017). The carbon sequestration potential of cover crops in arable soil was investigated in meta-analyses by Poeplau and Don (2015) and Jian et al. (2020). Poeplau and Don (2015) reported a mean carbon sequestration rate of cover cropping of 0.32 Mg ha^{-1} year^{-1} and Jian et al. (2020) of 0.56 Mg ha^{-1} year^{-1}. Roots are of central importance for carbon sequestration through cover crops as they contribute more effectively to the soil carbon pool than the shoot (Kätterer et al. 2011). Poeplau et al. (2021) showed the high importance of root-derived organic carbon to enhance the mean residence time of organic carbon. Thus, maximising root biomass input in soils is very crucial for carbon sequestration in arable soils.

Cover crop roots can change soil physical properties, as coarse roots can increase macropores and fine roots increase micropores (Bodner et al. 2014). Cover crops with coarse root axes and high rooting density have the potential to improve soil hydraulic conductivity and thus diminish surface runoff (Yu et al. 2016). Root channels left by cover crops with coarse root axes might be used by roots of the subsequent crop (Williams and Weil 2004). Moreover, aggregate stability and soil macroporosity are reported to be correlated with total root length (Hudek et al. 2022). Especially winter hardy grass cover crops were found to improve soil aggregate stability (Liu et al. 2005).

A further major aim of cover cropping in arable farming systems is weed suppression. In field experiments in southern Germany, cover crops were able to reduce weed dry matter by more than 90 % (Brust et al. 2014) compared to a no cover crop control. Moreover, in that study, it was emphasised that cover crops need fast growth and soil coverage to achieve successful weed suppression. Weed biomass and weed density decreased with increasing cover crop biomass (Osipitan et al. 2019). Thus, maximising cover crop biomass and surface cover is a central objective when growing cover crops for weed suppression. This can be managed by species choice, sowing time, seeding rate, termination method and timing (Osipitan et al. 2019). Cover crops also effectively suppress weeds in the early development of succeeding main crops (Osipitan et al. 2018). Brust et al. (2014) showed that well-established cover crops reached soil coverage of nearly 100 % after only six weeks. Cover crops are effective in erosion control. Roots are decisive in preventing erosion, especially when the shoot is removed or destroyed (Baets et al. 2011). Furthermore, Baets et al. (2011) showed that grasses or cereals are effective in erosion control due to their fine-branched roots (Baets et al. 2011).

Due to limitations of additional fertilisers and reliance on cultural and mechanical measures for weed control, the agroecosystem services of cover crops are particularly important in organic farming systems. Wittwer et al. (2017) showed that cover crops can potentially reduce the yield gap between organic and conventional arable farming due to biological nitrogen fixation by legume cover crops and by reduction of weed pressure. For the same reasons, Grosse and Heß (2018) demonstrated the importance of cover crops for weed and nitrogen management in organic cropping systems with reduced tillage. Likewise, Halde et al. (2014) reported that cover crop mulches with hairy vetch enable no-till practices in organic cropping systems due to weed suppression and nitrogen supply.

For the above-mentioned functions of cover crops in arable systems, especially nutrient uptake, soil erosion control, soil organic carbon enhancement and soil structure improvement, roots are of central importance. These below-ground agroecosystem services of cover crop are reviewed by Griffiths et al. (2022) and demonstrated in figure 1.1. Although these agroecosystem services of cover crop roots are already described, open questions remain. Especially, questions of the link between root traits and functions and how they behave in mixtures need further investigations.

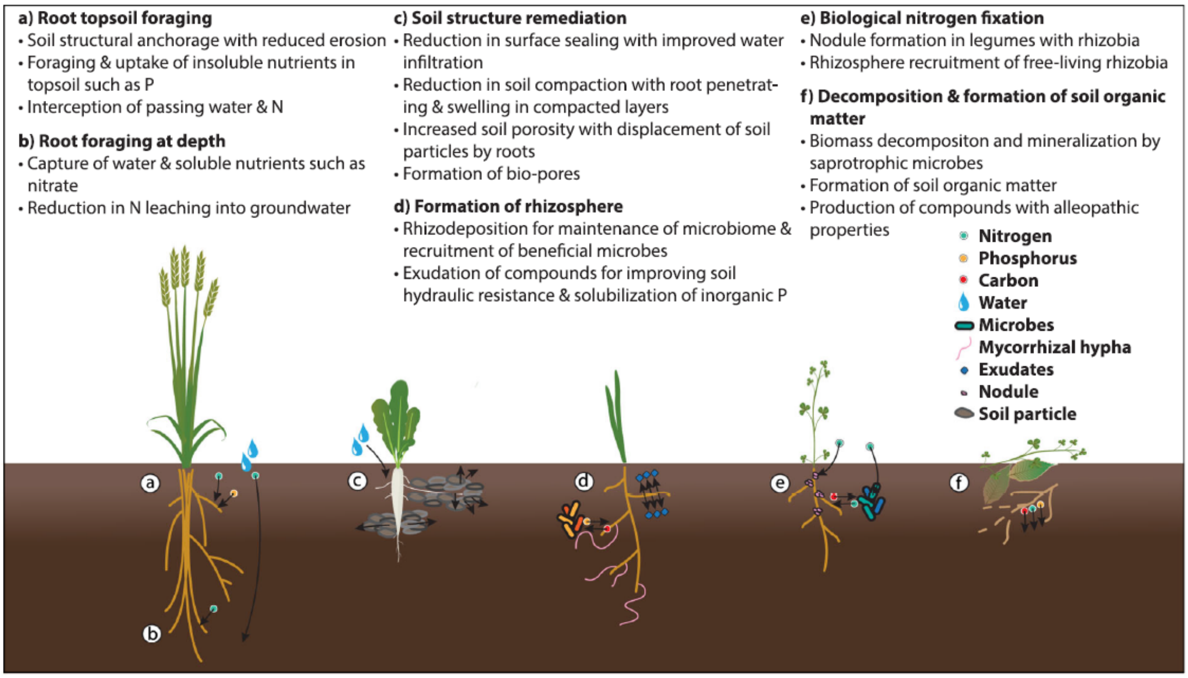

Fig. 1.1 Agroecosystem services of cover crop roots and their mechanisms (Griffiths et al. 2022).

1.2 Cover crop species and their root morphology

Root systems can be classified as fibrous (homorhizous) root systems or taproot (allorhizous) systems. Freschet et al. (2021) defined fibrous roots as “basal and shoot-borne roots of monocotyledonous plants” and taproots as the “first root to emerge from the seed that usually forms the central axis of the root system”. When comparing the root systems of different crops in detail, various root traits are used. A root trait is defined as any morphological, physiological or phenological feature of the root system measurable at the individual level, without reference to the environment or any other level of organisation (Poirier et al. 2018; Violle et al. 2007). Root traits can be divided into the following categories: architectural, morphological, physiological, biotic and chemical (Bardgett et al. 2014; Poirier et al. 2018). Architectural root traits characterise the spatial configuration of the entire root system of an individual plant, such as root length density (RLD) and root mass density (RMD); morphological traits describe features of individual roots, such as specific root length (SRL) (Bardgett et al. 2014; Poirier et al. 2018). RLD refers to length of root per unit soil volume measured in cm cm^{-3}; RMD represents the root mass per soil volume, measured in mg cm^{-3}; SRL is the root length per root mass in m g^{-1} (Freschet et al. 2021). Further, the cumulative share of root length can describe the vertical root distribution. It is the proportion of root length down to a specific soil depth in relation to the total root length of the soil profile. Root growth can not only be studied in topsoil but also in subsoil, which is the soil below the tilled or formerly tilled soil (Kautz et al. 2013), as well as in large-sized biopores which are vertical pores formed by soil inhabiting organisms, such as earthworms or plant roots (Kautz 2015). Several methods with various advantages and disadvantages are available for studying roots, of which (mini)rhizotrones, auger methods, monolith methods and profile wall methods are very common (Böhm 1979; Freschet et al. 2021). The two latter methods were used and compared in this thesis.

The cover crop species studied for root growth in the field experiments of this thesis are crimson clover, blue lupin, winter rye, bristle oat, oil radish, winter turnip rape and phacelia and will be described in the following (see Fig. 1.2).

crimson clover | blue lupin | winter rye | bristle oat

oil radish | winter turnip rape | phacelia

Fig. 1.2 Cover crop species used in the field experiments at the end of October

Crimson clover (*Trifolium incarnatum* L.) is an annual winter hardy fodder legume with a low regrowth capacity (Kolbe et al. 2004). Originating from the Mediterranean region, its suitability for temperate climates was improved by crop breeding, selecting for improved winter hardiness. It is used as a winter cover crop, often in mixtures for fodder purposes such as ‘Landsberger

Gemenge' (Lütke Entrup 1996). Crimson clover has a taproot or allorhizous root system (Kutschera et al. 2009), see Fig. 1.3. The root excavations by Kutschera et al. (2009) revealed the following root morphology of crimson clover: The thickened part of the taproot is about 10 cm long and has a diameter of more than 1.8 mm. Crimson clover has some stronger and several thinner lateral roots of first order. The stronger laterals grow vertically and have many fine laterals up to fourth order. Nodules of crimson clover are often two-branched. Kutschera et al. (2009) found root depth of up to 98 cm.

Blue or narrow-leafed lupin (*Lupinus angustifolius* L.) is an annual legume with a strong taproot that originated from the Mediterranean region (Kolbe et al. 2004). Already Schultz-Lupitz (1895) conducted field experiments with blue lupin and described it as a deep-rooting and well nitrogen-fixing cover crop. Alkaloid-containing bitter lupins and alkaloid-free sweet lupins exist, which can be used for green manuring or feeding purposes, respectively (Kolbe et al. 2004). The blue lupin has a taproot system (Fig. 1.3) with a taproot diameter of about 7.5 mm and lateral roots up to third order and spherical root nodules (Kutschera et al. 2009). In their root examinations, Kutschera et al. (2009) found root depth of blue lupin up to 166 cm depth.

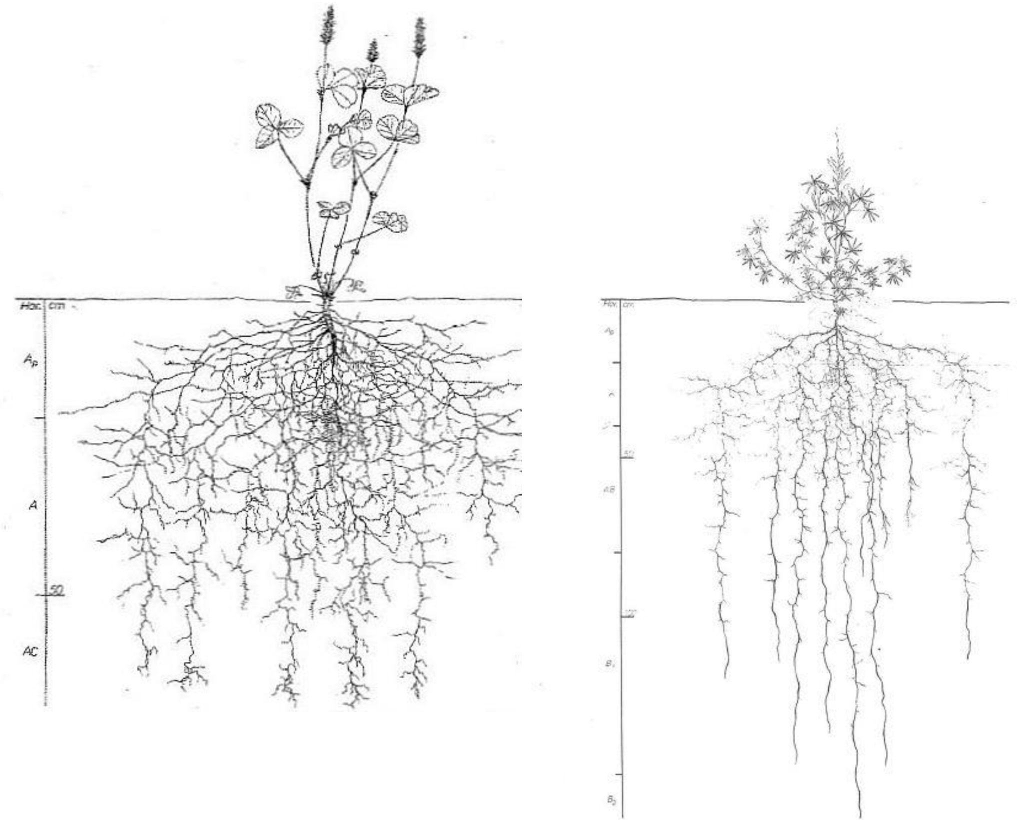

Fig. 1.3 Root drawing of crimson clover (*Trifolium incarnatum* L.), left, and of blue lupin (*Lupinus angustifolius* L.), right, from Kutschera et al. (2009), reprinted with permission from the publisher.

Winter rye (*Secale cereale* L.), a typical winter cover crop, is a winter hardy, undemanding, fast-growing and intensively rooting cereal (Kolbe et al. 2004), which is commonly used for fodder purposes in spring (Lütke Entrup 1996). Winter rye is a fibrous or homorhizous rooted crop with laterals up to third order and a diameter of the seminal roots of up to 1.8 mm (Kutschera et al. 2009). Root depth up to 122 cm was found in the studies of Kutschera (1960).

Bristle oat (*Avena strigosa* Schreb.) is a cereal and a close relative of the common *Avena sativa* L. It is a relatively new cover crop species in Central Europe that is characterised by high root biomass and a pronounced weed suppressive ability (Brust and Gerhards 2012). Bristle oat has an allorhizous root system similar to oat (*Avena sativa* L.); see Fig. 1.4.

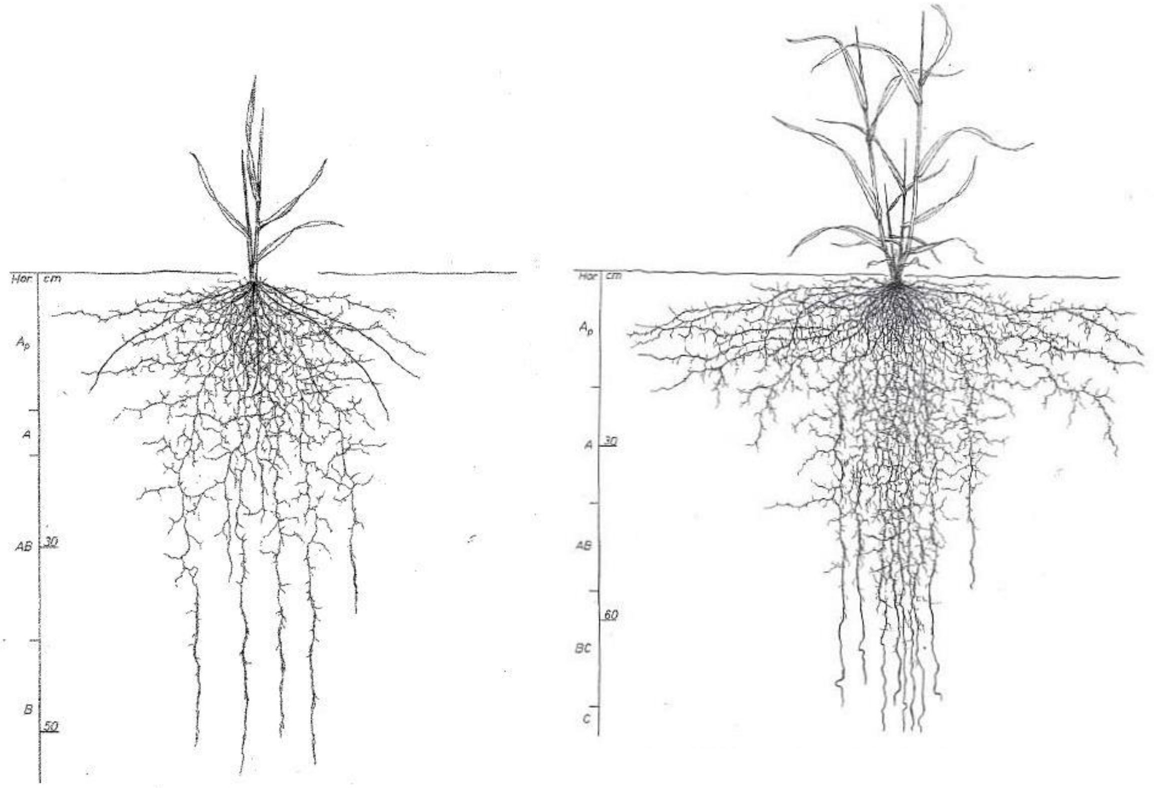

Fig. 1.4 Root drawing of winter rye (*Secale cereale* L.), left, and of oat (*Avena sativa* L.) as a representative for bristle oat (*Avena strigosa* Schreb.), right, from Kutschera et al. (2009), reprinted with permission from the publisher.

Oil radish (*Raphanus sativus* L. var. *oleiformis* Pers.) is an annual plant that belongs to the Brassicaceae family and is a widely used cover crop species, which is characterised by late sowing tolerance, rapid emergence, fast juvenile development, strong root growth (Kolbe et al. 2004) as well as competitiveness and high crop growth rates (Tribouillois et al. 2015). Oil radish is considered a non-winter hardy cover crop; however, it is only frost-killed at temperatures below -10°C (Lütke Entrup 1996). Due to its high content of glucosinolates and high amount of stems, oil radish is less suitable for forage purposes, and the use as green manure is the main focus (Kolbe et al. 2004). Oil radish is an important cover crop in sugar beet cultivation as there are oil radish cultivars that suppress the sugar beet cyst nematode (*Heterodera schachtii*) (Lütke Entrup 1996). Kutschera et al. (2009) described the following root morphology of oil radish: A taproot forms a spherical or cone-shaped turnip up to 50 mm in diameter; the taproot diameter at the end of the turnip is more than 2 mm. Lateral roots are up to fourth order. Roots of oil radish were found up to a depth of 153 cm by Kutschera et al. (2009).

Winter turnip rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs) is a winter hardy Brassicaceae and a close relative of oilseed rape (Diepenbrock et al. 1999). Winter turnip rape is a typical winter cover crop that is used for forage purposes, especially sheep grazing (Kolbe et al. 2004). Winter turnip rape has an allorhizous root system similar to oilseed rape, see Fig. 1.5.

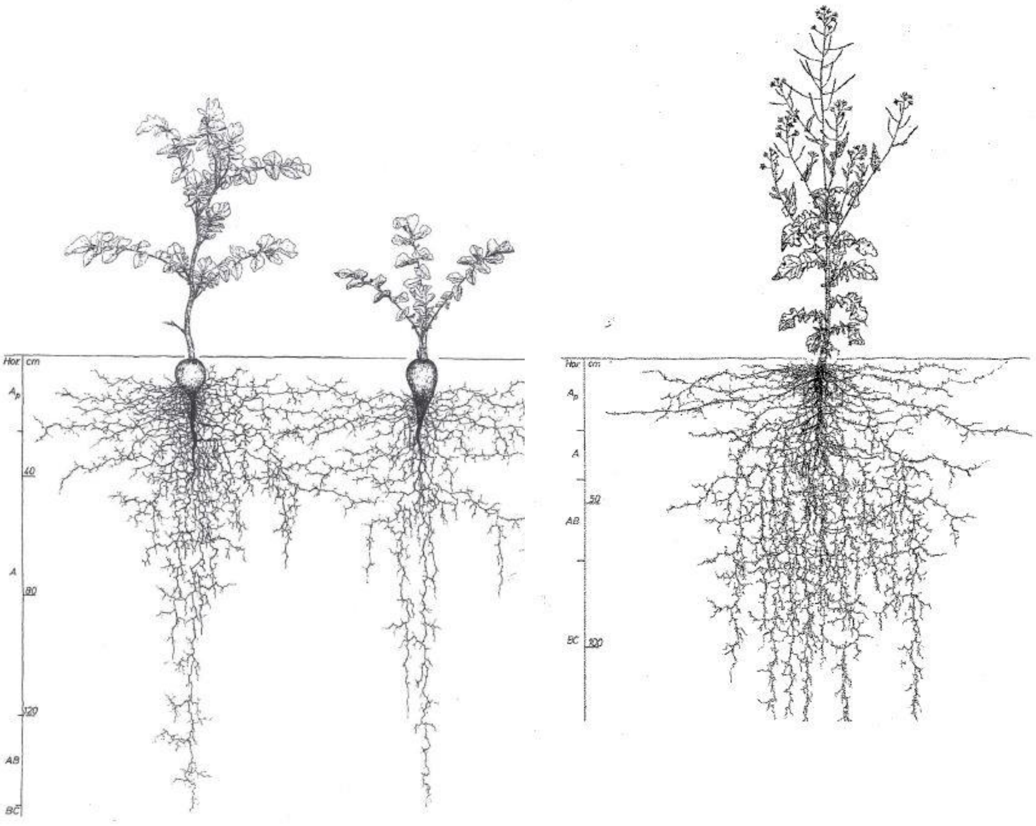

Fig. 1.5 Root drawing of oil radish (*Raphanus sativus* var. *oleiformis* L.), left, and of rapeseed (*Brassica napus* var. *napus* L.) as a representative for winter turnip rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs), right, from Kutschera et al. (2009), reprinted with permission from the publisher.

Phacelia (*Phacelia tanacetifolia* Benth.) is a fast-growing annual plant from the Boraginaceae family (Hansen et al. 2009) that originates from California and is used as green manure or bee pasture (Lütke Entrup 1996). Phacelia is non-winter hardy, drought tolerant, neutral against sugar beet cyst nematode (Kolbe et al. 2004), and has high potassium and phosphorus uptakes (Wendling et al. 2016). Phacelia is popular as a cover crop in conservation tillage systems before sowing of sugar beet or maize (Lütke Entrup 1996), as dry frost-killed plant material breaks easily (Kolbe et al. 2004). The investigations of Kutschera et al. (2009) showed the following root morphology of this species: Phacelia has an allorhizous root system (Fig. 1.6); the thickened part of the taproot is about 5-8 cm long and has a root diameter of 5 mm. Lateral roots are up to fourth order and roots were found up to a depth of 120 cm.

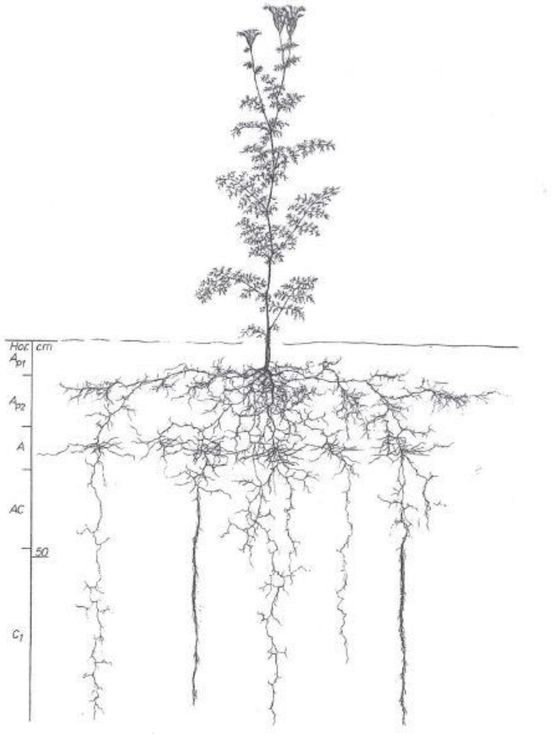

Fig. 1.6 Root drawing of Phacelia (*Phacelia tanacetifolia* Benth.) in Kutschera et al. (2009), reprinted with permission from the publisher.

1.3 Cover crop mixtures

Intercropping or mixed cropping is the simultaneous cultivation of two or more different crops on the same field (Vandermeer 1989; Willey 1979). Not only main crops, but also cover crops can be mixed. The primary agronomic reasons for sowing mixed cover crops are risk mitigation against crop failure (Wendling et al. 2019; Yachi and Loreau 1999), and the potential for overyielding in mixtures. Overyielding refers to the phenomenon where the combined yield of the mixed crops exceeds the average yield of the same crops grown separately as sole crops (Schmid et al. 2008). It can be quantified by absolute (AME) or relative mixture effects (RME), which is the absolute or relative difference between the observed yield in the mixture and the expected yield calculated from the sole crop yields and the relative density of the mixture partners. The net effect (NE) is the same as the absolute mixture effect (Loreau and Hector 2001). When the intercrop exceeds the highest yielding sole crop, the term transgressive overyielding is used (Li et al. 2023). Alternatively, the benefit of mixtures can be quantified by the concept of land use efficiency, which is expressed as the land equivalent ratio (LER). The LER is the relative land area under sole cropping that is required to harvest the same yields as in intercropping (Mead and Willey 1980), e.g., a LER > 1 indicates that intercropping provides a more efficient use of land compared to sole cropping.

Mechanisms of overyielding (Fig. 1.7) can be separated into two main effects: the complementarity and the selection or sampling effect (Loreau and Hector 2001). The selection effect occurs due to the dominance of one species (Loreau and Hector 2001; Tilman et al. 2001). The complementarity effect consists of both niche differentiation and facilitation (Loreau and Hector 2001). Niche differentiation may be temporal, such as different times of growth and development or demand of a specific resource, spatial, e.g., different root depth or shoot architecture, or in form, like use of different nitrogen sources (Homulle et al. 2022; Justes et al. 2021; Tilman et al. 2001), and thus, reduces interspecific compared to intraspecific competition (Mulder et al. 2001). Vertical root niche differentiation is an example of an underlying mechanism of spatial complementarity. In the case of facilitation, one species benefits from environmental modification by another species (Justes et al. 2021). Moreover, two other interspecific plant-plant interactions important in mixtures are competition and compensation. The former occurs when crop species use the same resources temporally and locally, and the latter is when one crop species compensates for the failure of the other as they respond differently to stress (Justes et al. 2021).

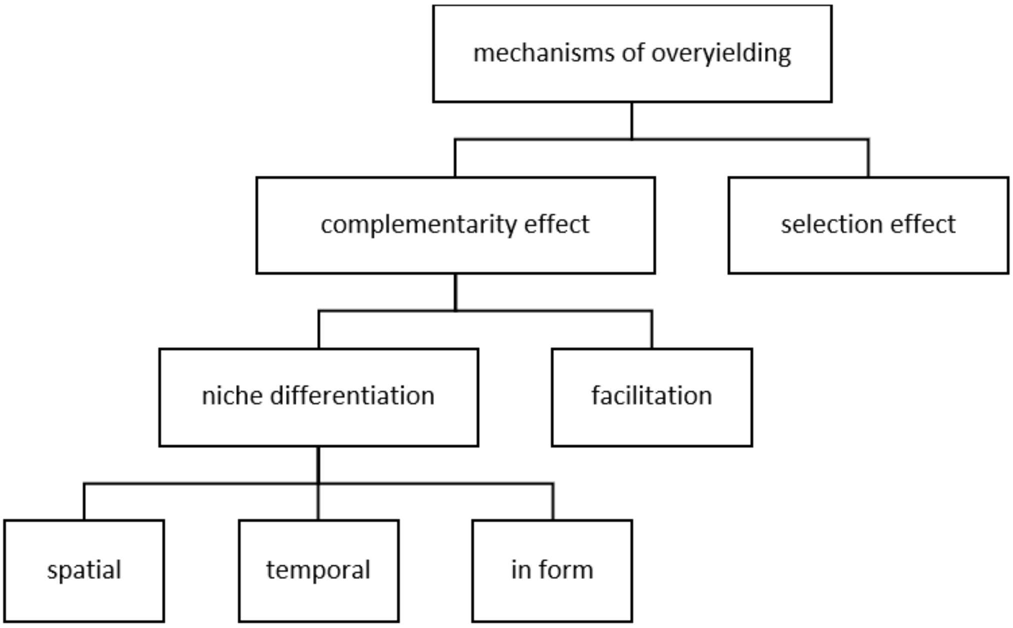

Fig. 1.7 Mechanisms of overyielding in crop mixtures

The mechanisms which may allow overyielding in cover crop mixtures may also lead to other benefits of mixtures compared to sole cover crops. Potential benefits of mixtures besides higher biomass include higher nutrient uptake (catch crop service), stronger weed suppression, higher soil carbon enrichment, and greater nutrient delivery to the subsequent crop (green manure service). Lavergne et al. (2021) reviewed these functions of cover crop mixtures and found inconsistent results when the services of mixtures were compared to those of sole cover crops. They emphasised the importance of the species functional group for the agroecosystem service: legume-based mixtures are more related to N supply for and yield effect of the subsequent crop, whereas non-legume mixtures perform better in weed suppression and N retention.

Several studies investigated cover crop mixture effects on N retention and N supply. Elhakeem et al. (2019) tested several mixtures of vetch, bristle oat and oil radish and showed that more N in the shoot was taken up by mixtures compared to sole crops. Cover crop mixtures of legumes and non-legumes are shown to provide a similar catch crop service and a higher green manure service as non-legume sole cover crops (Couëdel et al. 2018; Tosti et al. 2012; Tosti et al. 2014; Tribouillois et al. 2016). For cereal-legume cover crop mixtures, it was reported that the mixture decreased nitrate leaching to a similar level as the cereal sole crop (Tosti et al. 2014) and that introducing a legume in a cover crop mixture increased N accumulation and reduced the C/N ratio compared to sole crops of non-legumes (Tosti et al. 2012). This was similarly shown for crucifer-legume mixtures (Couëdel et al. 2018; Tribouillois et al. 2016). However, the seed density ratios of legumes and non-legumes in mixtures determine a trade-off between these two agroecosystem services (White et al. 2017). In their study, White et al. (2017) suggested some options for managing this trade-off: usage of very efficiently retaining non-legumes, usage of winter hardy legumes, and adaption of seed density ratios depending on nitrate content before sowing. Besides the N management, also other cover crop agroecosystem services have been compared for mixtures and sole crops. Ranaldo et al. (2020) showed that cover crop mixtures had higher shoot biomass and weed suppression than sole crops and that weed suppression increased with species diversity and functional diversity.

Compared to above-ground parameters, root traits in cover crop mixtures have not intensively been studied yet, especially in field experiments. To date, there is only a limited number of studies on this topic. In a study by Gentsch et al. (2020), rhizosphere carbon input and soil microbial biomass were enhanced in diverse cover crop mixtures compared to sole cover crops. Saleem et al. (2020) pointed out that at higher cover crop diversity, root architectural traits such as root area, root length, and root mass were enhanced. Amsili and Kaye (2021) investigated a cover crop mixture with complementary root traits and complementary nitrogen acquisition strategies of triticale (×*Triticosecale*), canola (*Brassica napus* L.) and crimson clover (*Trifolium incarnatum* L.), following the idea to explore a greater soil volume with mixtures and reduce root C/N ratio in comparison to the non-legume sole crop. Their mixture did not produce more root carbon than the triticale sole crop; however, crimson clover in the mixture led to a lower root C/N ratio. For a mixture of white mustard (*Sinapis alba* L.), Egyptian clover (*Trifolium alexandrinum* L.), phacelia (*Phacelia tanacetifolia* Benth.), and bristle oat (*Avena strigosa* Schreb.) Heuermann et al. (2019) applied a qPCR-based method to determine species-specific root biomass. They detected root biomass overyielding in the mixture, dominance of mustard and phacelia in root biomass as well as suppression of clover and oat in root biomass. In mixtures of hairy vetch (*Vicia villosa* Roth.) and cereal rye (*Secale cereale* L.), Bukovsky-Reyes et al. (2019) identified increased total root length and specific root length (SRL) for vetch. These initial findings highlight the need for further research on how the mixture of certain cover crops affects root traits.

The effect of species diversity and species identity on the performance of cover crop mixtures has been investigated in several studies (Finney and Kaye 2017; Freund et al. 2020; Lavergne et al. 2021; Wendling et al. 2019). Finney and Kaye (2017) revealed that in cover crop mixtures enhanced species richness positively affected the ecosystem services weed suppression, N retention, and above-ground biomass N, whereas species richness negatively affected N supply to the subsequent main crop, and it was not related to the yield of the subsequent main crop. Lavergne et al. (2021) reported that functions of cover crop mixtures are more connected with species functional groups rather than species richness. Also, other studies emphasised the importance of species identity and an associated high selection effect in cover crop mixtures. Freund et al. (2020) showed that species identity is more relevant than species diversity for biomass production and nutrient uptake of cover crops. Wendling et al. (2019) found no effect of species diversity and reported that cover crop biomass production was influenced by growing conditions and species identity. They also discussed that bispecies mixtures had higher biomass than multispecies mixtures. Moreover, Wendling et al. (2019) found overyielding in 81 % and transgressive overyielding in 37 % of the cover crop mixtures. Thus, above-ground biomass of mixed cover crops is often higher compared to the average of sole cover crops. However, when mixtures are compared to the best-performing sole crops (transgressive overyielding) above-ground biomass is similar (Elhakeem et al. 2021). This emphasises the importance of species identity in cover crop mixtures.

1.4 Thesis objectives and thesis outline

As demonstrated above, mixtures of cover crops may have several advantages compared to sole cover crops. Moreover, the roots of cover crops have multiple functions in agroecosystems. Thus, both mixtures and roots of cover crops have become an increasing focus of research in recent years. However, there is still a significant knowledge gap on how mixing cover crops influences the traits and functions of their roots.

This thesis aims to quantify different root profiles and to categorise root types of cover crops in a first step and to respond to the question: (1) Which root types of cover crops can be distinguished on the profile wall? Then, cover crops with different root types were combined in mixtures, by following the idea of exploiting the total soil profile more completely with roots of cover crop mixtures that consist of allorhizous and homorhizous species. Thus, regarding the root traits RLD, RMD, and SRL in cover crop mixtures and possible below-ground mixture effects this thesis aims at answering the following research questions: (2) How does the root mass differ between the partners in the cover crop mixtures across depth? (3) Are there below-ground mixture effects in mixed cover crops? (4) Can below-ground complementarity or vertical root niche differentiation be found in cover crop mixtures? By analysing the root pieces from root diameter and root length, it was the objective to answer the question: (5) Do cover crops have a potential for generating large-sized biopores? As in these cover crop field experiments both monolith and profile wall method were used, it was further intended to answer the question: (6) Does the root length density from the profile wall method agree with the monolith method?

In chapter 2, the cover crop species crimson clover, blue lupin, oil radish, winter turnip rape, winter rye, bristle oat and phacelia were screened for root length density, proportion of root length in large-sized biopores and vertical root distribution before and after winter with the profile wall method.

Cover crop mixtures of blue lupin and winter rye were analysed for mixture effects of the traits RMD, RLD and SRL, as well as shoot biomass and nutrient uptake in chapter 3. The monolith method was used for the investigation of root traits.

Based on this, in chapter 4, a two-species cover crop mixture of oil radish and winter rye and a three-species mixture of oil radish, crimson clover and winter rye were investigated regarding mixture effects of shoot and root traits as well as vertical root niche differentiation and complementarity using the monolith method.

In chapter 5, the density of large-sized biopores in subsoil after cultivation of cover crops was determined by photography and the potential of biopore genesis was assessed by calculating the numbers of root pieces per m² from root diameter classification of the monolith method.

A methodological comparison between the RLD estimation of the fast but less precise profile wall method and the RLD measurements of the accurate but laborious monolith method was performed in chapter 6. RLD and cumulative share of root length were related using linear regression and multiple linear regression, which considered depth differentiation.

The results of the previous chapters are synthesised and discussed in terms of root research methodology, mixture research on below-ground traits and the performance of cover crop roots in arable farming in chapter 7.

2 Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method

Roman Kemper [1], Tábata A. Bublitz [1], Phillip Müller [1], Timo Kautz[2], Thomas F. Döring [1], Miriam Athmann [1]

[1] Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Germany

[2] Department of Agronomy and Crop Science, Faculty of Life Sciences, Humboldt University of Berlin, Germany

---

This chapter has been published as Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. doi:10.3390/agriculture10110503.

Abstract

Many benefits of cover crops such as prevention of nitrate leaching, erosion reduction, soil organic carbon enhancement and improvement of soil structure are associated with roots. However, including root characteristics as a criterion for cover crop selection requires more knowledge on their root growth dynamics. Seven cover crop species (crimson clover, winter rye, bristle oats, blue lupin, oil radish, winter turnip rape and phacelia) were grown in a two-year organically managed field experiment in Germany to screen them for root intensity and vertical root distribution. Root length density (RLD) and proportion of root length in large-sized biopores were determined before and after winter with the profile wall method. RLD and cumulative root length were analysed using a three-parameter logistic function, and a logistic dose-response function, respectively. Fibrous rooted winter rye and crimson clover showed high RLD in topsoil and had a shallow cumulative root distribution. Their RLD increased further during winter in topsoil and subsoil. The crops with the highest RLD in the subsoil were taprooted oil radish, winter turnip rape and phacelia. Bristle oat had intermediate features. Blue lupin had low RLD in topsoil and subsoil. Phacelia, oil radish, winter turnip rape and bristle oat showed the highest share of root length in biopores. These complementary root characteristics suggest that combining cover crops of different root types in intercropping may be used to enhance overall RLD for maximising cover crop benefits.

Keywords

root length density, below-ground, biopore, catch crop, cumulative root distribution, legume

2.1 Introduction

Cover cropping is a multifunctional agroecological practice widely used in organic farming, aiming at higher crop diversity, reduced erosion and nitrate leaching [1]. Cover crops have the potential to reduce the yield gap between organic and conventional farming or between conservation agriculture and intensive tillage owing to reduction of weed pressure and additional nitrogen provided by legume cover crops [2]. Moreover, cover crops are being discussed as a measure to mitigate climate change due to carbon sequestration, reduced fertiliser use after legume cover crops and change in surface albedo [3].

Except for weed suppression [4], the main benefits of cover crops are associated with roots: prevention of nitrate leaching due to nutrient uptake [5], reduction of soil erosion [6], organic matter input to enhance soil organic carbon content [7,8] and improvement of soil structure [9,10]. Thus, it is important to know more about the root intensity, distribution and dynamics of cover crops in organic cropping systems.

Poeplau and Don [7] as well as Jian et al. [8] showed the potential of cover crops for carbon sequestration in arable soils. As roots contribute more effectively to the carbon pool than carbon input from above ground plant material [11], cover crop roots might have an important effect on carbon sequestration. Topsoil resistance against erosion can be increased due to a high root length density (RLD) in upper soil layers, as it was shown for a grass mixture by Baets et al. [6]. Moreover, surface runoff can be reduced by cover crops. Cover crops with coarse root axis, especially legumes, enhance hydraulic conductivity and thus diminish surface runoff [12]. Hence, soil physical properties can be managed by cover crop roots. Bodner et al. [13] showed with a range of different cover crops that coarse roots increase macropores whereas fine roots increase micropores. With their dense and ramified root system, the winter hardy cover crops annual ryegrass and rye increased aggregate stability in the topsoil [9].

On the other hand, roots of cover crops are important for nutrient uptake and therefore for nutrient saving in arable cropping systems, especially of highly mobile nutrients as nitrate, during winter. To highlight this property, they are also called catch crops. Sainju et al. [14] reported that higher RLD of cover crops correlated positively with shoot nitrogen uptake and negatively with nitrate concentration in soil. In contrast, Herrera et al. [15] found no relationship between nitrogen uptake and nitrate leaching for three different cover crops. However, they showed a relationship between rapid root establishment and reduction of nitrate leaching. In a study with three cover crops differentiated by rooting depth, Italian ryegrass, winter rye and oil radish, Kristensen and Thorup-Kristensen [16] described a linear relationship between nitrogen uptake and root intensity or root frequency. The deep rooting cover crops resulted in higher N uptake and lower residual soil nitrate. Similarly, Sapkota et al. [17] recorded that perennial ryegrass with a shallow but dense root system caused high nitrogen depletion in upper layers while the deep rooting oil radish depleted more mineral soil nitrogen in the total soil layer. Thus, deep rooting of cover crops is important to prevent nitrogen losses from the subsoil to deeper layers and to the groundwater. With a focus on deep rooting, Thorup-Kristensen [18] showed that residual subsoil nitrate is well correlated with root intensity and rooting depth, but not with above-ground measurements. For deep-rooted undersown cover crops it was shown that subsoil nitrate reduction was correlated with root growth in the subsoil, but not with root growth in the topsoil [19]. A high rooting depth was found for non-legume dicot species; temperature sum (growing degree-days) needed for these crops to reach one meter soil depth was much smaller than for grass species [18].

Consequently, cover crops with large root systems are desirable. For some beneficial effects such as improvement of topsoil structure with consequent reduction of surface runoff and erosion, intensive rooting in the topsoil is crucial. For other desired effects such as depletion of nitrate prone to be leached to the groundwater from deeper soil layers, deep rooting is the most important trait. This means high RLD in topsoil and deep rooting into the subsoil are efficient to explore the soil volume more completely, reduce erosion, improve soil structure and aggregate stability and to store organic carbon in arable soils. Thus, for optimizing cropping systems in an informed and targeted way, detailed knowledge on the root systems and their vertical distribution for different cover crops is indispensable.

Wendling et al. [20] gave detailed information on root traits of a wide range of cover crop species. However, root traits were analysed only until 50 cm soil depth and no function was used to illustrate the distribution of RLD by soil depth. Some information on RLD profiles can be found in Bodner et al. [21] for phacelia, rye, mustard and hairy vetch, and in Vos et al. [22] for winter rye and forage rape. Both studies used an exponential function for distribution of RLD by depth. However, they only examined RLD until 40 cm and 60 cm soil depth, respectively. To describe the exploration of the total soil volume, the cumulative root distribution of the RLD of different cover crops is of interest. In a meta-study, Fan et al. [23] modified a logistic dose-response curve for cumulative root distribution by depth for agricultural crops and showed different root distributions for different types of agricultural crops. However, cover crops were not included.

In the otherwise densely packed subsoil, tubular continuous biopores formed by plant roots or earthworms are sites of preferential flow for water and solutes and may thus potentially increase nitrate leaching, as shown in a microcosm study [24]. However, in the field, nitrate leaching in biopores may be counteracted by root growth, since these pores also provide paths of preferential root growth and improved nutrient and water uptake in the subsoil [25]. RLD of annual crops increased in the deeper subsoil when grown after a biopore density-increasing taproot precrop compared to a fibrous rooted precrop [26,27]. Roots use biopores to grow through compacted soil layers, and in less compacted soil layers roots can exit biopores and re-enter bulk soil [28]. In a study using *in situ* endoscopy to evaluate root growth of barley and oilseed rape in biopores, it was shown that in 85 % of the evaluated biopores both barley and oilseed rape established root-soil contact on the biopore wall. However, homorhizous barley had predominantly thin vertical or ingrowing roots contacting the pore wall, while allorhizous oilseed rape use mainly lateral roots to contact the pore wall [29]. Thus, it can be assumed that biopores are also used by cover crop species, possibly to different extents depending on their root system and rooting depth.

In this study we compared the RLD of seven different cover crops down to a depth of 1 m. We investigated allorhizous cover crops with a taproot-dominated root system (blue lupin, oil radish, winter turnip rape, phacelia), homorhizous cover crops with shoot-borne roots (winter rye, bristle oat) and crimson clover as an intermediate type which has a taproot with strong lateral roots. RLD was analysed with the profile wall method [30]. We differentiated between root length in large-sized biopores and root length in bulk soil and compared RLD before and after winter.

The objective of the present study was to evaluate rooting patterns of different cover crops. We hypothesized that (i) topsoil RLD is higher for fibrous rooted crops, (ii) subsoil RLD is higher for taprooted than fibrous rooted crops, (iii) 50 % of the cumulative root length is reached in lower soil depths for fibrous rooted compared to taprooted crops, (iv) non-winter hardy cover crops lose RLD during winter compared to winter hardy cover crops, and (v) allorhizous cover crop use large-sized biopores to a greater extent than homorhizous cover crops.

2.2 Materials and Methods

2.2.1 Experimental Site

The cover crop field experiments were performed on the research station for Organic Farming ‘Wiesengut’ of the University of Bonn in Hennef, Germany. Located at 50°48', N 7°17' E and an altitude of 65 m a.s.l. in the lowland of the river Sieg, the site is characterised by a Fluvisol with a silt loam texture on gravel layers with a soil thickness of 0.6 to 2.0 m and fluctuating groundwater level. Detailed information about soil parameters are given in Table 2.1 and Table 2.2.

Table 2.1. Soil parameters of the experimental fields in the topsoil (0-30 cm depth) in 2018/2019 and 2019/2020.

	2018/2019	**2019/2020**
pH ($CaCl_2$)	6.2	6.3
P_2O_5 [mg/100g]	5	8
K_2O [mg/100g]	21	15
MgO [mg/100g]	12	10
total organic carbon [%]	1.1	0.75
total nitrogen [%]	0.11	0.10

Table 2.2. Mineral nitrogen [kg/ha] at the time of sowing in 2018/2019 and 2019/2020.

	2018/2019	**2019/2020**
0-30 cm	17.1	53.9
30-60 cm	3.2	26.1
60-90 cm	3.4	11.7

The mean annual precipitation at the experimental site is 840 mm with a mean temperature of 10.3 °C. A comparison of the weather of the experimental years compared to the long-term mean (1981-2010) at the nearby weather station from the German Meteorological Service at Cologne Bonn Airport (Figure 2.1A) shows a dry summer and autumn in 2018 (July until November), a dry summer in 2019 (June and July) and shift of precipitation towards winter (December 2018, January 2019, February and March 2020). In 21 out of 24 months it was warmer in the experimental years compared to the long-term mean. The weather records in the experimental field (Figure 2.1B) are comparable to the weather data from the weather station at the Cologne Bonn Airport with dry summers and wet winters.

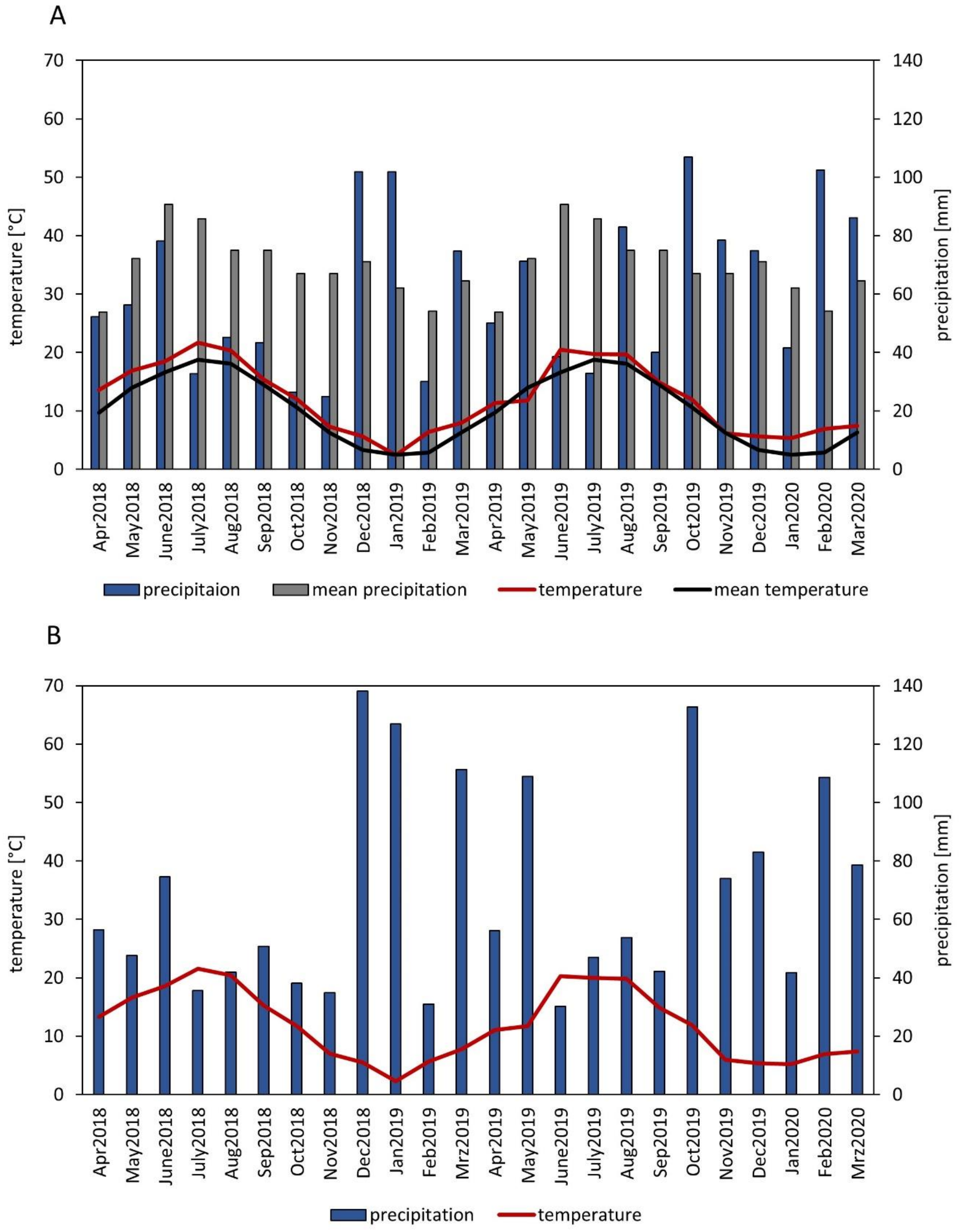

Figure 2.1. Precipitation [mm] and temperature [°C] of the experimental years compared to the long-term mean (1981-2010) at the nearby (12 km) weather station from the German Meteorological Service at Cologne Bonn Airport (A), Precipitation [mm] and temperature [°C] of the experimental years at the experimental station Wiesengut (B).

2.2.2 Experimental design and management practices

The field experiments were performed as a randomized complete block design with four blocks and a plot size of 1.5 m × 12.5 m in 2018/2019 and 1.5 m × 20 m in 2019/2020. The experimental field changed from 2018/2019 to 2019/2020 as the experiment rotated within crop rotation of the experimental farm. Three blocks (21 plots) were analysed for root traits. The following seven cover crops were sown in separate plots with a plot seeder with row distance of 17 cm on 12th August 2018 and on 14th August 2019: crimson clover, winter rye, bristle oat, blue lupin, oil radish, winter turnip rape, and phacelia (Table 2.3).

Table 2.3. Cover crops species of the field experiment.

cover crop species	cultivar	family	winter hardiness	recommended seed rate [kg/ha]
crimson clover (*Trifolium incarnatum* L.)	Linkarus	Leguminosae	winter hardy	30
winter rye (*Secale cereale* L.)	Bonfire	Poaceae	winter hardy	120
bristle oat (*Avena strigosa* Schreb.)	Pratex	Poaceae	non- winter hardy	80
blue lupin (*Lupinus angustifolius* L.)	Boruta	Leguminosae	non- winter hardy	120
oil radish (*Raphanus sativus* L. var. *oleiformis* Pers.)	Siletina	Brassicaceae	non- winter hardy	25
winter turnip rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs)	Jupiter	Brassicaceae	winter hardy	15
phacelia (*Phacelia tanacetifolia* Benth.)	Bee-happy	Boraginaceae	non- winter hardy	12

Pre-crop was faba bean (*Vicia faba* L.) in both years. After harvest of faba bean, tillage with a mouldboard plough with a ploughing depth of 23 cm was carried out. In 2018, all plots were irrigated with 50 mm in one application before sowing and 54 mm in three applications after sowing; in 2019 30 mm in one application were irrigated before sowing and 12 mm in one application after sowing. A sprinkler was used for irrigation with tap water. The seedbed was prepared with a rotary harrow. During crop establishment, mechanical hoeing as well as hand weeding was carried out to minimize weed occurrence so that roots would be attributable to cover crop species.

2.2.3 Profile wall method

RLD was analysed with the profile wall method [30], before winter (at the end of October 2018 and in mid-October 2019), as well as after winter (at the end of March 2019 and in mid-March 2020). With an excavator, a trench with a depth of 120 cm was established transversely to the short end of the plots (Figure A2, supplementary material). After root counting in October 2018, soil was refilled. In March 2019, the trench was re-opened 50 cm further into the plot. In October 2019 the trench could not be refilled because of wet conditions. Thus, the trench remained open during winter until March 2020. Soil beneath the area extending 50 cm further into the plot was removed to have an undisturbed profile wall in March 2020. A profile wall of 1 m × 1 m was smoothened with a spade and sharp blades for every plot. To expose the roots, a 5 mm thick soil layer was removed by using a toothed metal scraper and spraying water with a hand sprayer and a pressure of 400 kPa. A metal frame of 100 cm × 100 cm with a grid of 5 cm × 5 cm was fixed to the profile wall (Figure A3, supplementary material). Root length units equivalent to 5 mm root length were counted in each square of the grid. Root length units in large-sized biopores > 2 mm diameter and in the bulk soil were counted separately. RLD in biopores and share of root length in large-sized biopores was only presented in the 30-90 cm horizon as large-sized biopores in topsoil were destroyed by tillage and below 90 cm first gravel layers occurred. Root length density, which is root length per soil volume, was calculated for each square by dividing the root length by 12.5 cm^3.

2.2.4 Statistical analysis

Data were analysed with the programme R version 3.5.2 [31]. A non-linear regression for the RLD depending on soil depth was fitted with the function *nls*. A three-parameter logistic function was used:

$$RLD = \frac{a}{1 + be^{cx}} \tag{2.1}$$

where *RLD* is root length density in cm cm^{-3}, x is soil depth in cm, with $x>0$, while a, b, and c are parameters to be estimated from the data; a determines the asymptote, b shifts the curve horizontally and c controls the steepness of the function's slope. For graphical representation the diagram was rotated by 90 degrees so the vertical and horizontal axes indicate the soil depth and the RLD, respectively. As parameters a, b, and c are only partially helpful for interpretation, two points on the curve were chosen to outline the root distribution: RLD at a depth of 5 cm (RLD_5) and the depth at which RLD equals half the maximal RLD were calculated for each cover crop for a meaningful comparison of root distribution (Figure A1, supplementary material).

For the cumulative root distribution, a non-linear regression for the cumulative share of root length depending on soil depth was fitted with the function *nls* in R. A logistic dose-response curve [32] was used:

$$\frac{r}{r_{max}} = \frac{1}{1 + \left(\frac{x}{x_{50}}\right)^{d}} \tag{2.2}$$

where r is the cumulative root length, r_{max} is total length of roots, x is the soil depth in cm and x_{50} is the depth at which 50 % of total root length was found and d is a dimensionless shape-parameter.

Differences among cover crop species in rooting depth and in relative proportion of root length inside large-sized biopores, RLD in biopores and RLD in bulk soil in 30-90 cm were analysed by one-way analysis of variance with a Tukey's HSD test at a significance level of $\alpha=0.05$. If necessary, data were square root transformed to achieve homogeneity of variances and normal distribution. A t-test was performed to test for differences in RLD in large-sized biopores and in bulk soil in the 30-90 cm horizon.

2.3 Results

2.3.1 Root length density and rooting depth

In 2018/2019, the highest RLD before winter in the topsoil was detected for winter rye (up to 0.97 cm cm^{-3} at 5 cm depth) and for crimson clover (up to 0.82 cm cm^{-3} at 5 cm depth) (Figure 2.2A). A low RLD in the topsoil was found for taprooted species, whereas topsoil RLD of bristle oat was intermediate. Phacelia reached a high RLD in the subsoil. Winter rye and crimson clover reached a high RLD$_5$ (Table 2.4). Low RLD$_5$ was determined for blue lupin and phacelia. Half maximal RLD was found to be deep for bristle oat and phacelia and shallow for oil radish and crimson clover. Rooting depth of crimson clover was significantly lower than rooting depth of oil radish and winter turnip rape, rooting depth of other cover crops were intermediate.

An increase in RLD during winter was recorded for winter rye and crimson clover (Figure 2.2 C). After winter, highest RLD in the topsoil of up to 1.17 cm cm^{-3} at 5 cm depth for winter rye and up to 1.22 cm cm^{-3} at 5 cm depth for crimson clover was found. Highest RLD in the upper subsoil was determined for winter rye and crimson clover. A decrease in RLD during winter was detected for bristle oat, oil radish and phacelia. Blue lupin and winter turnip rape changed RLD only minimally over winter. As before winter, RLD_5 was high for winter rye and crimson clover and low for blue lupin and phacelia in March 2019 (Table 2.4). After winter, depth at half maximal RLD was high for bristle oat and phacelia and low for crimson clover and oil radish, as before winter. Rooting depth in March 2019 did not differ significantly between cover crops. Except for oil radish and blue lupin, all cover crops expanded their rooting depth from October 2018 to March 2019.

In the season 2019/2020, highest RLD in the topsoil in October with up to 3.90 cm cm^{-3} at 5 cm depth was found for winter rye (Figure 2.2B). RLD was low in the topsoil for blue lupin and phacelia with less than 0.50 cm cm^{-3}. An intermediate RLD was detected for crimson clover, oil radish, winter turnip rape and bristle oat. In the subsoil, winter turnip rape and oil radish reached high RLD. Low RLD in the subsoil was found for crimson clover, winter rye, bristle oat and blue lupin, and an intermediate RLD for phacelia. In October 2019, RLD_5 was high for winter rye and low for blue lupin and phacelia (Table 2.4). Half maximal RLD was recorded at greatest depth for phacelia, and most shallow for crimson clover, oil radish and winter turnip rape. Rooting depth in October 2019 did not differ significantly between cover crops. Except for crimson clover, bristle oat and blue lupin, all cover crops reached the observed 100 cm soil depth.

During winter in the season 2019/2020, RLD increased for all crops, slightly for bristle oat, blue lupin, oil radish, winter turnip rape and phacelia, stronger for crimson clover and winter rye (Figure 2.2D). Highest RLD in the topsoil was found for winter rye with more than 7.05 cm cm^{-3} at 5 cm depth, followed by crimson clover with more than 3.90 cm cm^{-3} at 5 cm depth. In the upper subsoil, winter rye reached the highest RLD. In the lower subsoil, highest RLD was found for winter turnip rape. As before winter, RLD_5 was high for winter rye and low for blue lupin and phacelia. Soil depth at half maximal RLD was high for bristle oat and oil radish and low for winter rye, phacelia and blue lupin. Rooting depth in March 2020 did not differ significantly between cover crops, as all cover crops reached the observed 100 cm soil depth.

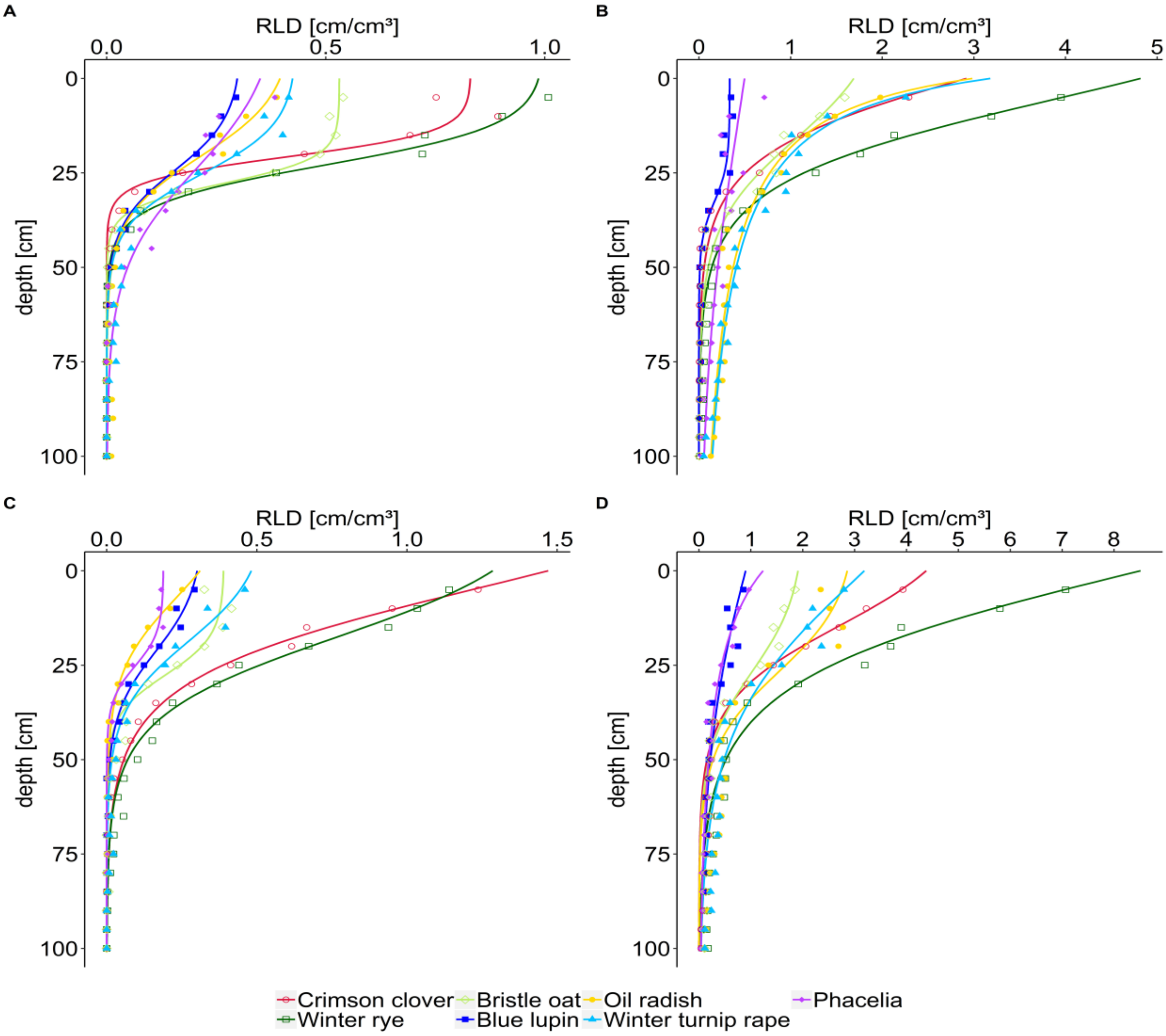

Figure 2.2. Root length density (RLD) of cover crops at different soil depth before winter (A, B) and after winter (C, D) in seasons 2018/2019 (A, C) and 2019/2020 (B, D). RLD was fitted by a non-linear regression using a three-parameter logistic function. Estimated parameters are shown on Table A1. Symbols show the means of the measured values (n=3).

Table 2.4. Mean values ± standard deviation (n=3) of rooting depth [cm], estimated RLD [cm cm^{-3}] at 5 cm depth (RLD$_5$), and estimated depth [cm] at half maximal RLD from a three-parameter logistic regression of root length density depending on soil depth for different cover crops before (October) and after winter (March) in two seasons (2018/2019 and 2019/2020). Different letters indicate significant differences (α=0.05) for rooting depth within one date.

year	date	cover crop	rooting depth [cm]	RLD$_5$ [cm cm-^{3}]	soil depth [cm] at half maximal RLD
2018/2019	October	crimson clover	42±13 b	0.82	21
		winter rye	57±13 ab	0.97	23
		bristle oat	65±5 ab	0.53	28
		blue lupin	62± 8 ab	0.29	25
		oil radish	77±20 a	0.37	22
		winter turnip rape	75±9 a	0.41	26
		phacelia	53±16 ab	0.33	28
	March	crimson clover	68±8	1.22	15
		winter rye	82±8	1.17	21
		bristle oat	73±20	0.39	28
		blue lupin	62±15	0.28	23
		oil radish	70±0	0.26	14
		winter turnip rape	78±6	0.44	21
		phacelia	55±13	0.19	25
2019/2020	October	crimson clover	82±12	2.22	11
		winter rye	100±0	3.97	14
		bristle oat	97±3	1.53	21
		blue lupin	80±30	0.33	33
		oil radish	100±0	2.00	10
		winter turnip rape	100±0	2.14	10
		phacelia	100±0	0.47	41
	March	crimson clover	100±0	3.90	18
		winter rye	100±0	7.05	16
		bristle oat	100±0	1.83	28
		blue lupin	100±0	0.81	15
		oil radish	100±0	2.75	28
		winter turnip rape	100±0	2.81	22
		phacelia	100±0	0.98	16

2.3.2 Cumulative distribution of root length

In October 2018, a shallower distribution for fibrous rooted crops and intermediate crimson clover than for taprooted crops was determined (Figure 2.3A, Table 2.5). The shallowest distribution was recorded for crimson clover, followed by winter rye and bristle oat. Oil radish and phacelia showed the deepest distribution. Blue lupin had the shallowest distribution among allorhizous crops. After winter, smaller differences in cumulative root distribution were recorded compared to October (Figure 2.3C, Table 2.5). The deepest distribution in March 2019 was found for winter rye.

Before winter in 2019, a shallower distribution of cumulative root length was found for fibrous rooted cover crops, intermediate crimson clover and blue lupin compared to taprooted oil radish, winter turnip rape and phacelia. Crimson clover had the shallowest distribution and phacelia the deepest (Figure 2.3B, Table 2.5). Root distribution of allorhizous cover crops was considerably deeper as compared to 2018/2019. After winter, in March 2020, crimson clover and winter rye showed the shallowest distribution of cumulative root length while phacelia and blue lupin showed the deepest distribution (Figure 2.3D, Table 2.5).

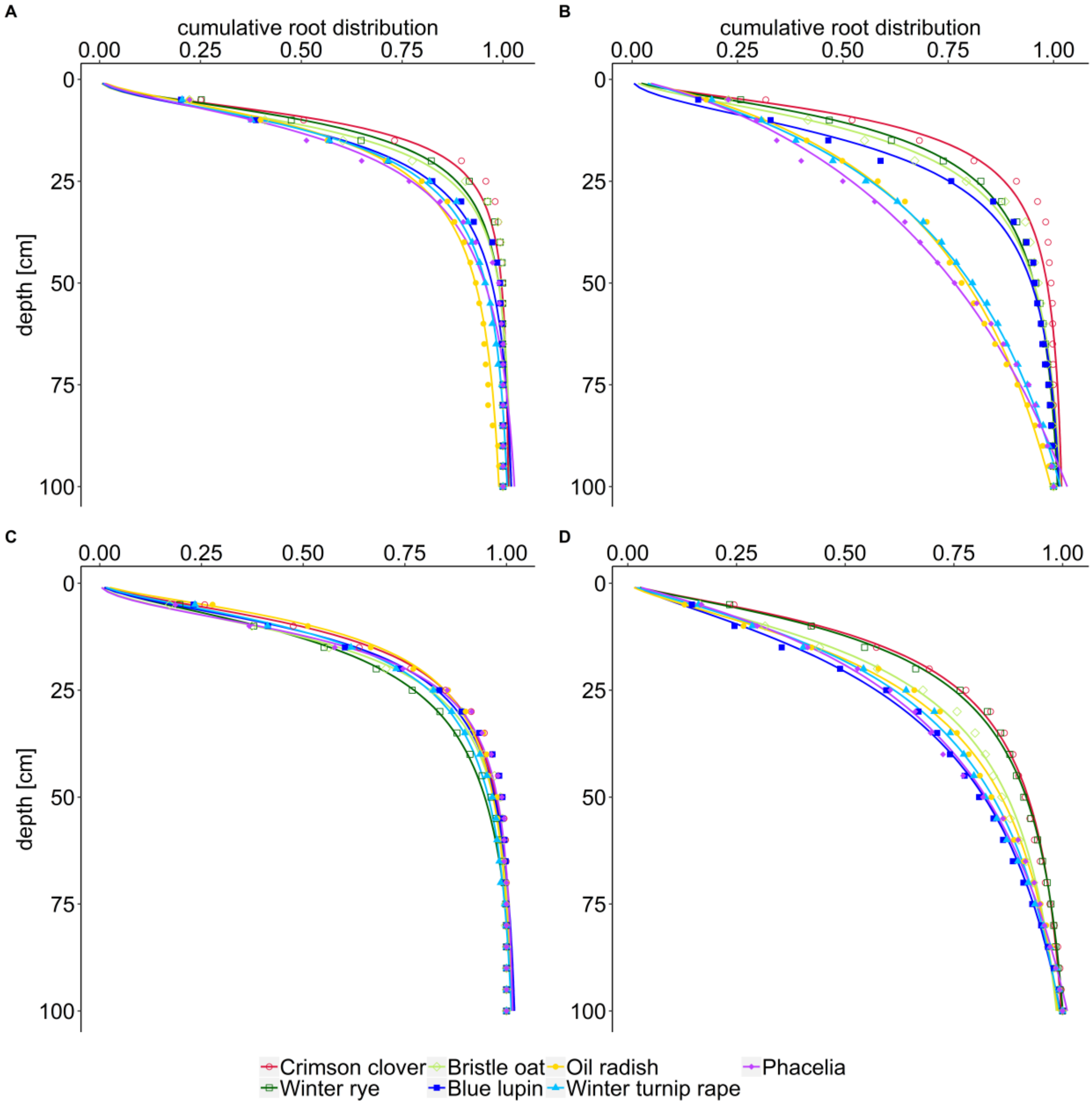

Figure 2.3. Cumulative distribution of root length of cover crops at different soil depth before winter (A, B) and after (C, D) in seasons 2018/2019 (A, C) and 2019/2020 (B, D). Cumulative distribution of root length was fitted by non-linear regression using a logistic dose-response curve. Symbols show the means of the measured values (n=3).

Table 2.5. Estimated parameters for a logistic curve of cumulative distribution of root length depending on soil depth and calculated x_{95} for different cover crops before (October) and after winter (March) in two seasons (2018/2019 and 2019/2020).

year	date	cover crop	c	x_{50} (cm)	x_{95} (cm)
2018/2019	October	crimson clover	-2.280	9.3	29.6
		winter rye	-2.029	10.2	34.9
		bristle oat	-2.184	11.5	36.6
		blue lupin	-1.955	12.7	42.6
		oil radish	-1.683	12.3	58.3
		winter turnip rape	-1.847	12.7	47.7
		phacelia	-1.710	14.1	48.6
	March	crimson clover	-1.738	10.6	41.4
		winter rye	-1.681	13.7	51.1
		bristle oat	-2.007	13.1	44,1
		blue lupin	-1.833	11.8	42.9
		oil radish	-1.624	9.9	43.3
		winter turnip rape	-1.687	11.8	48.0
		phacelia	-2.157	12.54	40.3
2019/2020	October	crimson clover	-1.749	9.1	36.4
		winter rye	-1.586	11.0	48.7
		bristle oat	-1.691	12.7	48.9
		blue lupin	-1.995	15.4	50.9
		oil radish	-0.962	34.6	84.7
		winter turnip rape	-1.005	35.8	79.1
		phacelia	-0.853	73.3	80.8
	March	crimson clover	-1.423	12.5	61.3
		winter rye	-1.403	13.3	62.1
		bristle oat	-1.460	17.6	75.3
		blue lupin	-1.302	25.8	80.7
		oil radish	-1.450	19.8	76.6
		winter turnip rape	-1.267	22.2	79.0
		phacelia	-1.104	28.0	77.5

2.3.3 Share of root length in large-sized biopores in the subsoil

In October 2018 and March 2019, no significant differences for the percentage of root length in large-sized biopores were detected (Table 2.6). RLD in biopores and bulk soil did not differ between species in October 2018. In March 2019 RLD in biopores did not differ between species, whereas RLD in bulk soil of crimson clover differed significantly from that of oil radish and phacelia. In the first experimental year RLD in biopores did not differ significantly from RLD in bulk soil. The highest share of root length in large-sized biopores in the subsoil were detected for oil radish (22.2 %), blue lupin (15.0 %), and winter turnip rape (9.7 %) in October 2018. After winter in March 2019 the highest proportion of root length in subsoil biopores was found for oil radish (9.7 %), bristle oat (9.6 %) and winter turnip rape (7.4 %).

In October 2019, the amount of root length in large-sized biopores was significantly higher for phacelia than for blue lupin and crimson clover (Table 2.6). Significant differences emphasised high RLD in biopores and in bulk soil for oil radish, winter turnip rape and phacelia. RLD in bulk soil was significantly higher than RLD in biopores for crimson clover, winter rye and oil radish. The highest percentage of root length in large-sized biopores between 30 and 90 cm soil depth were recorded for phacelia (31.3 %), bristle oat (17.5 %), oil radish (14.8 %), and winter turnip rape (11.3 %). In March 2020, no significant differences were found for the share of root length in biopores, whereas several differences in RLD in biopores and bulk soil were significant between cover crops. RLD in bulk soil was significantly higher than RLD in biopores for crimson clover, winter rye, bristle oat and winter turnip rape. The highest proportion of root length in large-sized biopores in the subsoil was found for phacelia (31.7 %), oil radish (21.8 %) and winter turnip rape (16.3 %).

Table 2.6. Mean values ± standard deviation (n=3) of share of root length in large-sized biopores and root length density (RLD) in large-sized biopores and in bulk soil in the subsoil (30- 90 cm) for different cover crops before (October) and after winter (March) in two seasons (2018/2019 and 2019/2020). Different letters indicate significant differences (α=0.05) for share of root length in large-sized biopores, RLD in biopores or RLD in bulk soil within one date. * indicates significant difference between RLD in biopores and in bulk soil (t-test, α=0.05) for one crop within on date.

year	date	cover crop	Share of root length in biopores (%)	RLD in biopores (cm cm^{-3})	RLD in bulk soil (cm cm^{-3})
2018/2019	October	crimson clover	0.0 ± 0.0	0.00000 ± 0.00000	0.00467 ± 0.00467
		winter rye	5.2 ± 6.6	0.00122 ± 0.00184	0.01203 ± 0.01064
		bristle oat	0.0 ± 0.0	0.00000 ± 0.00000	0.01037 ± 0.01230
		blue lupin	15.0 ± 22.7	0.00089 ± 0.00126	0.00974 ± 0.00721
		oil radish	22.2 ± 25.6	0.00378 ± 0.00568	0.01191 ± 0.00899
		winter turnip rape	9.7 ± 11.4	0.00189 ± 0.00201	0.02295 ± 0.02722
		phacelia	4.0 ± 3.9	0.00222 ± 0.00329	0.02900 ± 0.03786
	March	crimson clover	2.4 ± 4.2	0.00156 ± 0.00269	0.03617 ± 0.02756 ab
		winter rye	1.8 ± 1.6	0.00167 ± 0.00145	0.06958 ± 0.04422 a
		bristle oat	9.6 ± 8.5	0.00256 ± 0.00386	0.01844 ± 0.01600 ab
		blue lupin	0.0 ± 0.0	0.00000 ± 0.00000	0.01228 ± 0.01104 ab
		oil radish	9.7 ± 13.4	0.00044 ± 0.00038	0.00712 ± 0.00721 b
		winter turnip rape	7.4 ± 8.0	0.00156 ± 0.00139	0.02178 ± 0.01514 ab
		phacelia	4.8 ± 8.4	0.00022 ± 0.00038	0.00553 ± 0.00361 b
2019/2020	October	crimson clover	4.6 ± 8.0 b	0.00076 ± 0.00132 b	0.01746 ± 0.00443 c *
		winter rye	8.4 ± 8.5 ab	0.01139 ± 0.01136 ab	0.12561 ± 0.01412 b *
		bristle oat	17.5 ± 12.7 ab	0.01006 ± 0.00599 ab	0.05394 ± 0.02066 bc
		blue lupin	2.1 ± 2.2 b	0.00078 ± 0.00084 b	0.02637 ± 0.01559 c
		oil radish	14.8 ± 4.1 ab	0.04494 ± 0.02116 a	0.24978 ± 0.03510 a *
		winter turnip rape	11.3 ± 4.9 ab	0.03411 ± 0.00618 a	0.30151 ± 0.11987 a
		phacelia	31.3 ± 17.3 a	0.05650 ± 0.03817 a	0.10777 ± 0.03582 b
	March	crimson clover	6.1 ± 1.1	0.01228 ± 0.00086 ab	0.19456 ± 0.05342 abc *
		winter rye	6.2 ± 4.9	0.02656 ± 0.02384 ab	0.39517 ± 0.07584 a *
		bristle oat	5.6 ± 4.4	0.01089 ± 0.00746 ab	0.20400 ± 0.07336 abc*
		blue lupin	4.9 ± 1.9	0.00867 ± 0.00819 b	0.14786 ± 0.09526 bc
		oil radish	21.8 ± 8.2	0.09406 ± 0.06253 a	0.29670 ± 0.10020 ab
		winter turnip rape	16.3 ± 4.4	0.06130 ± 0.01706 ab	0.31382 ± 0.03824 ab *
		phacelia	31.7 ± 31.3	0.04767 ± 0.05273 ab	0.09699 ± 0.04619 c

2.4 Discussion

2.4.1 Characterisation of cover crop rooting: Root length density and rooting depth

In our study, crimson clover, the intermediate type between taprooted and fibrous rooted crops, rooted intensively in the topsoil, reaching up to 3.9 cm cm^{-3} in 5 cm depth. These values are in accordance with Sainju et al. [14], who measured an RLD of up to 10 cm cm^{-3} for crimson clover between 0-30 cm soil depth, which was significantly lower than RLD of rye.

In the topsoil, the homorhizous winter rye was the strongest rooting cover crop, increasing RLD over winter to more than 7.1 cm cm^{-3} in 5 cm depth. In several other field studies, winter rye was also the crop that rooted most intensively [21] and with higher RLD than for oilseed rape [22]. However, Bodner et al. [13] measured a significantly smaller RLD for winter rye than for oil radish and phacelia. This is contrary to our results with much higher RLD for winter rye compared to oil radish and phacelia in the surface near topsoil, and to the results of Vos et al. [22].

For the second homorhizous crop in our study, bristle oat, Wendling et al. [20] reported a higher RLD than for oil radish and phacelia, and bristle oat represented the highest RLD of all 20 tested cover crops. In their study, rye was not included. This shows the intensive rooting of *Poaceae*. However, in our experiment, bristle oat was intermediate in RLD with up to1.8 cm cm^{-3} in the 5 cm depth, i.e. not as intensive as winter rye and similar to or lower than the brassica cover crops. Results of Bodner et al. [33], reporting rather low total root length for bristle oat compared to other cover crops in a rhizobox experiment, support our findings that bristle oat is less intensively rooting than winter rye.

RLD of taprooted blue lupin was found to be low compared to all other crops, especially in the topsoil with up to 0.8 cm cm^{-3} in 5 cm. This result of low RLD of blue lupin is confirmed by a comparison of blue lupin as a main crop compared to cereals [34].

RLD of taprooted oil radish was intermediate in the topsoil with up to 2.8 cm cm^{-3} in 5 cm depth, and high in the subsoil. Similarly, in the study by Wendling et al. [20], RLD of oil radish was lower than that of fibrous rooted oats. However, in other cover crop comparisons, oil radish exhibited high RLD not only in the subsoil, but also in the topsoil [13]. In a minirhizotron study, oil radish RLD outscored winter rye RLD in both topsoil and subsoil [16]. Bodner et al. [33] reported oil radish to be the species with the highest total root length among 10 cover crop species in a rhizobox experiment, in which the only homorhizous species included was bristle oats. Munkholm and Hansen [35] determined a RLD of oil radish, which was slightly higher than that of homorhizous ryegrass in topsoil and subsoil. In the present study, RLD of taprooted winter turnip rape was very similar to that of oil radish, in both topsoil and subsoil. Wendling et al. [20] tested a summer turnip rape and reported a RLD of 6.00 cm cm^{-3} in the top 50 cm, which was also in the same range of oil radish (5.76 cm cm^{-3}).

RLD of taprooted phacelia was low in the topsoil compared to other crops, with up to 1.0 cm cm^{-3} in 5 cm depth. In the literature, higher RLD for phacelia are found [13,20,21]. In the rhizobox experiment of Bodner et al. [33], phacelia was among the crops with highest total root length and highest proportion in deep soil layers out of 10 cover crop species. RLD of phacelia in the subsoil might be underestimated by the profile wall method as proportion of RLD in large-sized biopores for phacelia is high and high root length in biopores are more difficult to count than in bulk soil as there can be several roots in one biopore – however this does not explain the lower values we found in the topsoil.

In our study, differences in rooting depth were not very pronounced. Rooting depth of crimson clover was significantly lower than that of brassica cover crops in October 2018, supporting the potential of brassica crops for capturing nitrate in deeper soil layers already early after cover crop establishment. Crops with low rooting depth in autumn caught up with rooting depth during winter. However, at our study site, root analysis was limited by gravel layers in about 1 m soil depth. Studies from sites with a larger soil volume available for roots compared to our experimental site showed that rye, ryegrass and oat needed more growing degree-days to reach one meter soil depth than radish, winter rape and phacelia [18], and that oil radish rooted much deeper, down to more than 2 m soil depth, as compared to winter rye with about 1 m soil depth [16].

In summary, from the results of our study, our hypothesis (i) that topsoil root length density is higher for fibrous rooted crops can only partly be confirmed. Winter rye had the highest RLD of all cover crops, but RLD of bristle oat was only sometimes higher than RLD of brassica cover crops. Crimson clover, an intermediate type between fibrous and taprooted cover crops, had the second highest RLD in the topsoil. Hypothesis (ii) that subsoil RLD is higher for taprooted than fibrous rooted crops is partly confirmed; namely, before winter and for the lower subsoil (>60 cm), for all taprooted crops except for lupin. In the upper subsoil (30-60 cm) winter rye reached higher RLD than the taprooted cover crops. Literature results at large support the notion of winter rye and crimson clover as crops with intensive rooting in the upper soil layers and bristle oats with less intensive rooting in the topsoil. In contrast to our study, in some other studies topsoil RLD of the taprooted crops oil radish and phacelia outscored topsoil RLD of fibrous rooted crops. However, in these studies differences in the subsoil between these two taprooted and the respective fibrous rooted crops tested were even more pronounced.

2.4.2 Cumulative distribution of root length

The cumulative distribution of root length is another approach to describe rooting characteristics. By comparing the cumulative percentage of root length with soil depth rather than the measured absolute values, this approach enables a rapid comparison of how the different species distribute their roots in the soil. As hypothesized (hypothesis iii), x_{50} and x_{95} values showed a shallow distribution of root length for fibrous rooted winter rye, bristle oat, and intermediate crimson clover, and a deep distribution for taprooted oil radish, phacelia, and winter turnip rape.

This result is confirmed by Thorup-Kristensen [18] who showed a constant root frequency up to 1.2 m soil depth for radish, winter rape and phacelia, while root frequency of rye, oat and ryegrass declined between 0.0 m and 1.2 m soil depth. In their rhizobox experiment, Bodner et al. [33] determined the highest proportion of roots in lower layers among ten species for oil radish and phacelia. Both studies confirm the high proportion of deep roots for allorhizous phacelia and brassica cover crops compared to homorhizous grasses. The intermediate position of blue lupin might be influenced by the low number of fine roots. The deeper root distribution of bristle oat compared to winter rye, especially after winter of the second experimental year, might be explained by its characteristics close to that of taprooted species, e.g. half maximal RLD at greater soil depths. Similarly, Bodner et al. [33] determined bristle oat out of all cover crops with low root length as the species with the highest proportion of root length in deeper layers.

2.4.3 Winter hardiness

Compared to other cover crops RLD of winter rye and crimson clover increased strongly during winter (Figure 2.2). The winter 2018/2019, esp. January 2019, was colder than the winter 2019/2020, (Figure 2.1). Thus, in 2018/2019, all non-winter hardy species except oil radish, which was only partially dead, were completely frost-killed, whereas in 2019/2020 oil radish was not frost-killed and the other non-winter hardy species were only partially frost-killed. This might explain the differences in RLD change of the non-winter hardy cover crops. Hence, winter-hardy cover crops increase RLD during winter, whereas non-winter hardy species decrease or slightly increase RLD depending on frost intensity and duration. Thus, our hypothesis (iv) that non winter-hardy cover crops lose RLD during winter compared to winter hardy cover crops can only be confirmed for winters with enough frost.

2.4.4 Share of root length in large-sized biopores in the subsoil

Up to 32 % of root length in large-sized biopores in the subsoil was determined (Table 2.6). The values are in a similar range as in studies with barley on a Haplic Luvisol, reporting up to 21 % [26] or 25 % [28] of RLD in large-sized biopores. The highest share of root length in large-sized biopores was found for the allorhizous species phacelia, oil radish, and winter turnip rape. Thus, taprooted crops seem to use biopores to a larger extent. This might be due to different strategies for exploring biopores by different rooting types. With *in situ* endoscopy, it was shown that homorhizous crops had thin vertical or ingrowing roots in biopores, while taproots of allorhizous crops grow centrally through biopores, contacting the pore wall with laterals [29] and that taprooted oilseed rape with thinner roots used biopores more intensively for root growth than taprooted faba bean with thick roots and only few laterals [36]. When supposing similarity of oilseed rape with winter turnip rape and oil radish and between faba bean and blue lupin, the results of Athmann et al. [36] could explain the differences in proportion of roots in biopores for brassica cover crops and blue lupin.

Bristle oat is the homorhizous crop using the pores most intensively. It is notable that bristle oat was the fibrous rooted species with characteristics closest to that of taprooted species, as can be seen from half maximal RLD at great soil depth and from cumulative root distribution. Blue lupin had a very low RLD throughout the soil profile, and many thick roots. Therefore, blue lupin had not enough fine roots to use the biopores to a large extent.

With the strong pore users phacelia, oil radish and winter turnip rape, our hypothesis (v) that allorhizous cover crop use large-sized biopores to a greater extent than homorhizous cover crops is confirmed.

To our knowledge this is the first study comparing cover crop species with respect to the use of large-sized biopores by roots. Future studies have to show if a larger share of roots in subsoil biopores is associated with higher nutrient uptake from nutrient rich biopore walls, which would likely imply higher nutrient uptake from the subsoil in total and higher prevention of nitrate leaching as biopores are preferential flow paths.

2.4.5 Cover crop rooting types

The information derived from analysis of root growth of the different cover crop species under study can be condensed to describe two basic cover crop rooting types: First, crops with dense root systems with many fine roots such as winter rye and crimson clover with high RLD in the topsoil which increases further during winter and a small x_{50} value. Second, taprooted oil radish, winter turnip rape and phacelia with high RLD in the subsoil, a greater x_{50} value, and a high percentage of root length in large-sized biopores. Blue lupin and bristle oat do not fit into either one of the categories. Bristle oat has intermediate features and blue lupin has low RLD in topsoil, subsoil and biopores.

Bodner et al. [33] and Bodner et al. [10] classified cover crop roots based on more detailed characteristics from root imaging and the Root Atlas [37,38], into four groups:

1. Topsoil-allocated with large diameters: e.g. legumes like vetch, faba bean, Egyptian clover
2. Topsoil-allocated with very dense root system and many fine roots: e.g. rye, buckwheat
3. Root density group with highly branched dense rooting systems: e.g. oil radish, camelina, phacelia
4. Low-order axes types like linseed and bristle oat with low-branched primary roots.

Out of the cover crops in our study, winter rye and crimson clover can be assigned to the second group, oil radish, winter turnip rape and phacelia to the third group, and bristle oat to the fourth group. According to the results of our study, lupin could be assigned to the first group. However, since lupin is well-known for deep rooting, following comparative studies have to verify this classification.

Considering these different rooting types suggests combining cover crops with different root characteristics in intercropping to enhance RLD in the total soil volume. Thus, combining intensive topsoil rooting cover crops with a biopore-using taprooted cover crops may be auspicious.

2.4.6 The profile wall method

Many studies with results of RLD for different cover crops can be found [12–14,16,20–22,33,35]. In contrast to our study, they mostly rely on roots washed out from the soil, and only some differentiated into different depth layers. Often only values for single or cumulated depth levels are presented, and no profile is shown. For example, soil cores were examined at a soil depth ranging from 2-7 cm [12,13] to 100 cm depth [35]. Segments of cores ranged between 5 cm [20] and 40 cm [35]. Diameter of soil cores ranged between 5.7 cm [20] and 8.7 cm [35]. In other studies, RLD was calculated from minirhizotron results reaching a depth of 2.42 m [16] or images were taken of rhizoboxes of 30 × 100 × 1 cm [33]. All these data provide valuable results. Different from the profile wall method, washed root samples enable determination of not only RLD, but also other root characteristics such as root mass, diameter, topology and chemical composition. In contrast, the strength of the profile wall method is that roots of a much larger number of plants enter into the assessment, that it is less time-consuming than root washing and therefore more depth levels and several assessment dates can be included to capture also root growth dynamics. RLD values are given in 5 cm steps for 1 m soil depth which allows more fine-grained regression analysis. Furthermore, as the position of the individual roots in the soil is maintained, it is possible to measure roots in biopores separately from roots in the bulk soil.

When comparing RLD results of the present study determined with the profile wall method with RLD results from the literature determined by washing roots from soil [13,14,20,21] it is conspicuous that RLD from the profile wall method is often smaller. However, relations of RLD between different cover crops are similar. That RLD determined by the profile wall method is lower than by washing roots from soil was already discussed by Böhm [30].

It is noticeable that absolute RLD values differ between 2018/2019 and 2019/2020. In 2018/2019 RLD_5 ranges between 0.19 and 1.22 cm cm^{-3}, whereas in 2019/2020 RLD_5 ranges between 0.33 and 7.05 cm cm^{-3}. Absolute RLD values can vary depending on the person counting the root length at the profile wall, and for our study, two different persons were counting in the two study years. Consequently, the two seasons cannot be compared in terms of absolute RLD values. However, the relation of RLD between crops is reliable and allows a relative comparison between different crops. To improve the profile wall method and minimize the human factor, we suggest taking images of the profile wall and using computer image analysis. Suitable software has recently become available, e.g. RootPainter [39].

2.5 Conclusions

The results of this study show that there are considerable differences between different cover crop species in RLD, root distribution between topsoil and subsoil and, in one of the two experimental years, also rooting depth. The notion that taprooted species explore the subsoil more and fibrous rooting species explore the topsoil more was confirmed for winter rye, oil radish, winter turnip rape and phacelia, but not for bristle oat and lupin. Fibrous rooted winter rye had a dense root system, a high topsoil RLD and a small x_{50} value. Similarly, crimson clover rooted very intensively in the topsoil despite not classified as a fibrous rooted crop. Taprooted oil radish, winter turnip rape and phacelia had a high subsoil RLD and a greater x_{50} value. Bristle oat has intermediate features between fibrous rooted and taprooted species. Blue lupin has low RLD in topsoil as well as in subsoil.

Therefore, the range of currently widely used cover crops includes crops with very different rooting types as a basis for including this criterion in management decisions. Further research will show if the different rooting types result in differences in nitrate uptake, reduction of soil erosion, soil organic carbon enhancement and soil structure improvement, and if combining cover crops of contrasting rooting types improve overall exploitation of the total soil volume. Also, the implications of differences in biopore use of the different cover crop species for nutrient uptake from the subsoil need further research.

The profile wall method is well suitable to present RLD and cumulative root distribution with non-linear regression as a function of soil depth. Further improvement of the method is necessary to increase comparability of different years, possibly via replacing manual countings with computer image analysis.

Supplementary Materials

Table A1 Estimated parameters for a three-parameter logistic regression of root length density depending on soil depth for different cover crops before (October) and after winter (March) in two seasons (2018/2019 and 2019/2020), Figure A1 Example of distribution of root length density depending on soil depth for two different crops. Two points on the curve show the RLD at a depth of 5 cm and the depth at half maximal RLD, Figure A2 Position of the plots and the trench relative to the plots, Figure A3 Photo of metal frame with a grid of 5 cm × 5 cm for manual root counting.

Author Contributions

M.A. and T.K. conceived the idea, planned the research and designed the experiments. R.K., P.M. and T.A.B. conducted the experiments in the field and collected the data. R.K. analysed the data and wrote the article. M.A., T.F.D. and T.K. contributed to data interpretation, writing and editing of the article. All authors have read and agreed to the published version of the manuscript.

Funding

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Acknowledgments

We thank the technical staff of the 'Wiesengut' organic farming research station and our students for help in the field.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Wezel, A.; Casagrande, M.; Celette, F.; Vian, J.-F.; Ferrer, A.; Peigné, J. Agroecological practices for sustainable agriculture. A review. *Agron. Sustainable Dev.* **2014**, *34*, 1–20, doi:10.1007/s13593-013-0180-7.

[2] Wittwer, R.A.; Dorn, B.; Jossi, W.; van der Heijden, M.G.A. Cover crops support ecological intensification of arable cropping systems. *Sci. Rep.* **2017**, *7*, 41911, doi:10.1038/srep41911.

[3] Kaye, J.P.; Quemada, M. Using cover crops to mitigate and adapt to climate change. A review. *Agron. Sustainable Dev.* **2017**, *37*, doi:10.1007/s13593-016-0410-x.

[4] Hartwig, N.L.; Ammon, H.U. Cover crops and living mulches. *Weed Sci.* **2002**, *50*, 688–699.

[5] Thorup-Kristensen, K.; Magid, J.; Jensen, L.S. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Adv. Agron.* **2003**, *79*, 227–302, doi:10.1016/S0065-2113(02)79005-6.

[6] Baets, S. de; Poesen, J.; Gyssels, G.; Knapen, A. Effects of grass roots on the erodibility of topsoils during concentrated flow. *Geomorphology* **2006**, *76*, 54–67, doi:10.1016/j.geomorph.2005.10.002.

[7] Poeplau, C.; Don, A. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. *Agric. Ecosyst. Environ.* **2015**, *200*, 33–41, doi:10.1016/J.AGEE.2014.10.024.

[8] Jian, J.; Du, X.; Reiter, M.S.; Stewart, R.D. A meta-analysis of global cropland soil carbon changes due to cover cropping. *Soil Biol. Biochem.* **2020**, *143*, 107735, doi:10.1016/j.soilbio.2020.107735.

[9] Liu, A.; Ma, B.L.; Bomke, A.A. Effects of Cover Crops on Soil Aggregate Stability, Total Organic Carbon, and Polysaccharides. *Soil Sci. Soc. Am. J.* **2005**, *69*, 2041–2048, doi:10.2136/sssaj2005.0032.

[10] Bodner, G.; Leitner, D.; Nakhforoosh, A.; Sobotik, M.; Moder, K.; Kaul, H.-P. A statistical approach to root system classification. *Front. Plant Sci.* **2013**, *4*, 292, doi:10.3389/fpls.2013.00292.

[11] Kätterer, T.; Bolinder, M.A.; Andrén, O.; Kirchmann, H.; Menichetti, L. Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. *Agric. Ecosyst. Environ.* **2011**, *141*, 184–192, doi:10.1016/j.agee.2011.02.029.

[12] Yu, Y.; Loiskandl, W.; Kaul, H.-P.; Himmelbauer, M.; Wei, W.; Chen, L.; Bodner, G. Estimation of runoff mitigation by morphologically different cover crop root systems. *J. Hydrol.* **2016**, *538*, 667–676, doi:10.1016/j.jhydrol.2016.04.060.

[13] Bodner, G.; Leitner, D.; Kaul, H.-P. Coarse and fine root plants affect pore size distributions differently. *Plant Soil* **2014**, *380*, 133–151, doi:10.1007/s11104-014-2079-8.

[14] Sainju, U.M.; Singh, B.P.; Whitehead, W.F. Cover Crop Root Distribution and Its Effects on Soil Nitrogen Cycling. *Agron. J.* **1998**, *90*, 511–518, doi:10.2134/agronj1998.00021962009000040012x.

[15] Herrera, J.M.; Feil, B.; Stamp, P.; Liedgens, M. Root growth and nitrate-nitrogen leaching of catch crops following spring wheat. *J. Environ. Qual.* **2010**, *39*, 845–854, doi:10.2134/jeq2009.0306.

[16] Kristensen, H.L.; Thorup-Kristensen, K. Root Growth and Nitrate Uptake of Three Different Catch Crops in Deep Soil Layers. *Soil Sci. Soc. Am. J.* **2004**, *68*, 529, doi:10.2136/sssaj2004.5290.

[17] Sapkota, T.B.; Askegaard, M.; Lægdsmand, M.; Olesen, J.E. Effects of catch crop type and root depth on nitrogen leaching and yield of spring barley. *Field Crops Res.* **2012**, *125*, 129–138, doi:10.1016/j.fcr.2011.09.009.

[18] Thorup-Kristensen, K. Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? *Plant Soil* **2001**, *230*, 185–195, doi:10.1023/A:1010306425468.

[19] Thorup-Kristensen, K.; Rasmussen, C.R. Identifying new deep-rooted plant species suitable as undersown nitrogen catch crops. *J. Soil Water Conserv.* **2015**, *70*, 399–409, doi:10.2489/jswc.70.6.399.

[20] Wendling, M.; Büchi, L.; Amossé, C.; Sinaj, S.; Walter, A.; Charles, R. Influence of root and leaf traits on the uptake of nutrients in cover crops. *Plant Soil* **2016**, *409*, 419–434, doi:10.1007/s11104-016-2974-2.

[21] Bodner, G.; Himmelbauer, M.; Loiskandl, W.; Kaul, H.-P. Improved evaluation of cover crop species by growth and root factors. *Agron. Sustainable Dev.* **2010**, *30*, 455–464, doi:10.1051/agro/2009029.

[22] Vos, J.; van der Putten, P.E.L.; Hassan Hussein, M.; van Dam, A.M.; Leffelaar, P.A. Field observations on nitrogen catch crops - II. Root length and root length distribution in relation to species and nitrogen supply. *Plant Soil* **1998**, *201*, 149–155, doi:10.1023/A:1004367530320.

[23] Fan, J.; McConkey, B.; Wang, H.; Janzen, H. Root distribution by depth for temperate agricultural crops. *Field Crops Res.* **2016**, *189*, 68–74, doi:10.1016/j.fcr.2016.02.013.

[24] Li, Y.; Ghodrati, M. Preferential Transport of Nitrate through Soil Columns Containing Root Channels. *Soil Sci. Soc. Am. J.* **1994**, *58*, 653–659, doi:10.2136/sssaj1994.03615995005800030003x.

[25] Kautz, T. Research on subsoil biopores and their functions in organically managed soils: A review. *Renew. Agric. Food Syst.* **2015**, *30*, 318–327, doi:10.1017/S1742170513000549.

[26] Perkons, U.; Kautz, T.; Uteau, D.; Peth, S.; Geier, V.; Thomas, K.; Lütke Holz, K.; Athmann, M.; Pude, R.; Köpke, U. Root-length densities of various annual crops following crops with contrasting root systems. *Soil Tillage Res.* **2014**, *137*, 50–57, doi:10.1016/j.still.2013.11.005.

[27] Han, E.; Kautz, T.; Perkons, U.; Uteau, D.; Peth, S.; Huang, N.; Horn, R.; Köpke, U. Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. *Biol. Fertil. Soils* **2015**, *51*, 847–856, doi:10.1007/s00374-015-1032-1.

[28] Kautz, T.; Perkons, U.; Athmann, M.; Pude, R.; Köpke, U. Barley roots are not constrained to large-sized biopores in the subsoil of a deep Haplic Luvisol. *Biol. Fertil. Soils* **2013**, *49*, 959–963, doi:10.1007/s00374-013-0783-9.

[29] Athmann, M.; Kautz, T.; Pude, R.; Köpke, U. Root growth in biopores—evaluation with in situ endoscopy. *Plant Soil* **2013**, *371*, 179–190, doi:10.1007/s11104-013-1673-5.

[30] Böhm, W. *Methods of Studying Root Systems;* Springer: Berlin, Heidelberg, 1979, ISBN 978-3-642-67284-2.

[31] R Core Team. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.*, 2018.

[32] Schenk, H.J.; Jackson, R.B. The Global Biogeography of roots. *Ecol. Monogr.* **2002**, *72*, 311–328, doi:10.1890/0012-9615(2002)072[0311:TGBOR]2.0.CO;2.

[33] Bodner, G.; Loiskandl, W.; Hartl, W.; Erhart, E.; Sobotik, M. Characterization of Cover Crop Rooting Types from Integration of Rhizobox Imaging and Root Atlas Information. *Plants* **2019**, *8*, doi:10.3390/plants8110514.

[34] Hamblin, A.P.; Tennant, D. Root length density and water uptake in cereals and grain legumes: how well are they correlated. *Aust. J. Agric. Res.* **1987**, *38*, 513, doi:10.1071/AR9870513.

[35] Munkholm, L.J.; Hansen, E.M. Catch crop biomass production, nitrogen uptake and root development under different tillage systems. *Soil Use Manage.* **2012**, *28*, 517–529, doi:10.1111/sum.12001.

[36] Athmann, M.; Sondermann, J.; Kautz, T.; Köpke, U. Comparing Macropore Exploration by Faba Bean, Wheat, Barley and Oilseed Rape Roots Using In Situ Endoscopy. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 689–700, doi:10.1007/s42729-019-00069-0.

[37] Kutschera, L.; Lichtenegger, E. *Wurzelatlas mitteleuropäischer Ackerunkräuter und Kulturpflanzen;* DLG-Verlag: Frankfurt am Main, Germany, 1960.

[38] Kutschera, L.; Lichtenegger, E.; Sobotik, M. *Wurzelatlas der Kulturpflanzen gemäßigter Gebiete mit Arten des Feldgemüsebaues;* DLG-Verlag: Frankfurt am Main, Germany, 2009.

[39] Smith, A.G.; Han, E.; Petersen, J.; Faircloth Olsen, N.A.; Giese, C.; Athmann, M.; Dresbøll, D.B.; Thorup-Kristensen, K. *RootPainter: Deep Learning Segmentation of Biological Images with Corrective Annotation*, 2020.

3 Root traits in cover crop mixtures of blue lupin and winter rye

Roman Kemper[1], Thomas F. Döring[1], Nicole Legner[2], Catharina Meinen[2], Miriam Athmann[3]

[1] Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Germany

[2] Division of Agronomy, Department of Crop Sciences, University of Göttingen, Germany

[3] Department of Organic Farming and Cropping Systems, University of Kassel, Germany

---

This chapter has been published as Kemper R, Döring TF, Legner N, Meinen C, Athmann M (2022) Root traits in cover crop mixtures of blue lupin and winter rye. Plant and Soil 475:309–328. doi:10.1007/s11104-022-05366-9.

Abstract

Purpose Mixtures of cover crops are gaining importance in farming systems. While complementarity between species is a potentially important mechanism underlying mixture benefits, there is little quantitative evidence about complementarity of root growth. Therefore, we aimed to gain detailed knowledge about mixture effects of specific root traits.

Methods Blue lupin (*Lupinus angustifolius* L.) and winter rye (*Secale cereale* L.) were grown as sole and intercrops in a two-year organically managed field experiment in Germany. Crops were analysed for shoot biomass and nutrient uptake as well as for root mass density (RMD), root length density (RLD) and specific root length (SRL). We used Fourier transform infrared (FTIR) spectroscopy to discriminate the root mass species-specifically in the mixtures.

Results In the shoot, overyielding was only found for N and P uptake in the 75:25 mixture of lupin and rye, whereas positive mixture effects of RLD, RMD, and root mass complementarity occurred only in the 50:50 mixture in one year. Consistently positive mixture effects of SRL were found. Root biomass proportion of rye was higher in topsoil, that of lupin higher in subsoil. Vertical niche differentiation in RMD between sole crops and mixtures was not observed.

Conclusion Among all root traits analysed, consistent mixture effects were observed only for SRL. Mechanisms behind mixture effects of root traits need further research. FTIR spectroscopy discriminated intercrop root mass well. Discrimination of mixture partners' root length would help to better understand complementarity of root growth in mixtures.

Keywords

intercrop, green manure, root length density, specific root length, vertical niche differentiation, complementarity

3.1 Introduction

Benefits of cover crops in arable farming systems such as nitrogen (N) retention and green manuring (Thorup-Kristensen et al. 2003), weed suppression (Hartwig and Ammon 2002), improvement of soil physical quality (Bodner et al. 2014), carbon sequestration (Jian et al. 2020) and climate change mitigation (Kaye and Quemada 2017) are widely understood and described. The significance of cover crop mixtures has increased in practical farming in the last years.

Benefits and synergistic interactions of crop mixtures (Bedoussac et al. 2015) and their management (Yu et al. 2016) are well understood, especially cereal-legume intercrops due to their complementary N use (Hauggaard-Nielsen et al. 2001a; Jensen 1996). Many studies examined mixture effects in shoot growth of cover crops (Couëdel et al. 2018; Elhakeem et al. 2019; Tosti et al. 2012; Tosti et al. 2014; Tribouillois et al. 2016; Wendling et al. 2017). Compared to pure stands, cover crop mixtures can have positive effects for shoot biomass and shoot N uptake (Elhakeem et al. 2019) and transgressive overyielding of shoot biomass is possible due to complementarity (Wendling et al. 2017).

Cover crop mixtures of legumes and non-legumes can effectively be used to minimize the trade-offs between N retention and N supply for the subsequent crop (Couëdel et al. 2018; Tosti et al. 2012; Tosti et al. 2014; Tribouillois et al. 2016). For crucifer-legume cover crop mixtures it was shown by Tribouillois et al. (2016) and Couëdel et al. (2018) that the catch crop service, i.e. the nitrate leaching reduction, of the mixtures is as high as of crucifer sole crop and that green manure service, i.e. the N availability for the next crop, is higher than for crucifer sole crop.

Despite the known benefits of cover crop mixtures and the importance of below-ground residues for soil quality, especially soil organic carbon (Poeplau et al. 2021), and the significance of root intensity for N retention (Thorup-Kristensen 2001), less is known about root traits of cover crop mixtures from field experiments. Heuermann et al. (2019) used a qPCR-based method to analyse root biomass in mixtures of white mustard (*Sinapis alba* L.), Egyptian clover (*Trifolium alexandrinum* L.), phacelia (*Phacelia tanacetifolia* Benth.), bristle oat (*Avena strigosa* Schreb.) and corresponding sole crops. They found root biomass overyielding in the mixture, root biomass dominated by mustard and phacelia which suppressed clover and oat as well as only low niche differentiation. Bukovsky-Reyes et al. (2019) investigated mixtures and sole crops of hairy vetch (*Vicia villosa* Roth.) and cereal rye (*Secale cereale* L.). They determined increased total root length and specific root length (SRL) in root traits in mixture compared to sole crop for vetch but not for rye.

More general knowledge about roots of intercrops is recognised from field experiments with main crops or from rhizotron studies. Using ^{32}P technique in a field experiment with a mixture of pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.), Hauggaard-Nielsen et al. (2001b) determined that barley root growth was faster than that of pea, and barley rooted deeper in mixture. This explains the competitive ability of cereals for soil inorganic N. Similarly, dominance of root growth of barley over vetch (*Vicia sativa* L.) was shown in a rhizotron experiment with a barley-vetch-mixture using microscopy for species identification (Ramirez-Garcia et al. 2015); moreover, they determined higher root intensity in deep layers in the mixture compared to barley pure stand. Streit et al. (2019) detected higher overyielding of root biomass than of shoot biomass in intercrops of winter wheat (*Triticum aestivum* L.) and winter faba bean (*Vicia faba* L.). Using Fourier transform infrared (FTIR) spectroscopy, which enables separation of root mass from intercropping partners, they did not find dominance of cereal biomass in root and shoot over legume biomass in contrast to other studies. Tosti and Thorup-Kristensen (2010) demonstrated in rhizotron experiment that legume roots were highly affected by interspecific competition with red beet (*Beta vulgaris* L.) and that fast initial root growth was important for competition. Similarly, Andersen et al. (2014) showed that more roots were observed in the mixtures than predicted, and that red clover (*Trifolium pratense* L.) root growth dominated in the topsoil, while red beet was dominant in deep layers.

In faba bean mixtures with oil crops root length density (RLD) in the subsoil increased in contrast to faba bean sole crop (Schröder and Köpke 2012). In intercrops of wheat and maize (*Zea mays* L.), wheat roots spread underneath maize mainly in deeper soil layers, RLD of wheat and maize was higher when intercropped than in sole crops (Li et al. 2006). Faba bean had shallow roots in mixture with maize, whereas roots of intercropped maize spread underneath faba bean. Thus, Li et al. (2006) concluded that greater soil exploration in the mixtures contributed to the grain yield advantage. The ‘three sisters’ maize, bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita pepo* L.) yielded higher in mixture compared to sole crops and their root volume density was higher in mixture than expected from the sole crops (Zhang et al. 2014). Grassland species mixtures showed root biomass overyielding and below-ground complementarity, but no evidence for vertical niche differentiation (Mommer et al. 2010).

As indicated in these studies, possible positive mixture effects on roots are higher root intensity, root length and volume density as well as root biomass in mixtures compared to sole crop. In some studies, also the spatial distribution of the roots was described, and in some cases complementary root distribution was observed with one mixture partner spreading mainly in the upper soil layers and the other partner mainly in the subsoil (Andersen et al. 2014; Li et al. 2006). Complementarity occurs when plants in a mixture require resources differently in space, time or form or one mixture partner facilitates the resource availability (Cardinale et al. 2007; Justes et al. 2021; Tilman et al. 2001), and thus, reduces interspecific compared to intraspecific competition (Mulder et al. 2001). Hence, complementarity consists of both niche differentiation and facilitation (Loreau and Hector 2001). Vertical root niche differentiation is a possible underlying mechanism of spatial complementarity. Besides, mixture effects of root traits, possible vertical niche differentiation of root growth which may allow below-ground complementarity is interesting to be investigated for cover crop mixtures. In our study, we attempted to maximise complementary root distribution by mixing homorhizous and allorhizous cover crops as suggested in Kemper et al. (2020), because different vertical rooting patterns may intensify niche differentiation. As a homorhizous crop we chose winter rye (*Secale cereale* L.) and blue lupin (*Lupinus angustifolius* L.) as an allorhizous crop. Besides vertical rooting patterns, roots of lupin and rye differently affect soil pore dynamics (Bodner et al. 2014) and access complementary nitrogen pools. Blue lupin can fix atmospheric N, thus, provide additional N input to the cropping system and may improve growth conditions for the subsequent crop. We chose a rye-lupin mixture since cover crop mixtures of non-legumes and legumes are known to minimize the trade-off between nitrate leaching reduction and N supply for the subsequent crop (Couëdel et al. 2018; White et al. 2017).

The aim of our study was to investigate cover crop mixtures of blue lupin and winter rye and its corresponding sole crops for the root traits root mass, root mass density, root length density, specific root length as well as shoot biomass and shoot nutrient uptake. We used FTIR spectroscopy to analyse the share of root mass of the mixture partners. We hypothesized that:

(1) cover crop mixtures of lupin and rye show a positive mixture effect for shoot biomass and shoot nutrient accumulation,

(2) cover crop mixtures of lupin and rye show a positive mixture effect for RLD and root mass,

(3) in cover crop mixtures root biomass proportion of rye in topsoil is higher compared to lupin and that root biomass proportion of lupin in subsoil is higher compared to rye,

(4) vertical RMD profile of lupin and rye differs between sole crops and mixture.

3.2 Materials and Methods

3.2.1 Experimental Site

The field trials were carried out on a Fluvisol with a silt loam texture on gravel layers beginning between 60 and 200 cm of soil depth and a shallow groundwater table at the experimental station for Organic Farming ‘Wiesengut’ of the University of Bonn in Hennef (50°48′ N, 7°17′ E), Germany, with a mean annual precipitation of 840 mm and a mean temperature of 10.3 °C. More detailed information about site, soil and climate are given in Kemper et al. (2020).

3.2.2 Experimental design and management practices

The field trials were laid out as a randomized complete block design with four replicates. Plot size was 1.5 m × 12.5 m in 2018 and 1.5 m × 20 m in 2019. Three replicates were examined for root traits. Pre-crop history of the trial in 2018 was spring wheat (*Triticum aestivum* L.) in 2015 (18 t ha^{-1} farmyard manure), potatoes (*Solanum tuberosum* L.) in 2016 (30 t ha^{-1} farmyard manure), spring wheat in 2017 (18 t ha^{-1} lime) and spring faba bean (*Vicia faba* L.) as a direct pre-crop in 2018. Pre-crop history of the trial in 2019 was spring oat (*Avena sativa* L.) in 2016 (18 t ha^{-1} farmyard manure), potatoes in 2017 (30 t ha^{-1} farmyard manure), spring oat in 2018 and spring faba bean as a direct pre-crop in 2019. After harvest of pre-crop, tillage, irrigation and mechanical and hand weeding were performed as described in detail in Kemper et al. (2020). Briefly, tillage was done with a mouldboard plough and a rotary harrow; 104 mm and 42 mm were irrigated in 2018 and 2019, respectively. The cover crops were sown with a plot seeder with row distance of 17 cm on 12th August 2018 and on 14th August 2019. Sole crops of fibrous rooted winter rye (WR, *Secale cereale* L., cv. Bonfire) and taprooted blue lupin (LU, *Lupinus angustifolius* L., cv. Boruta) were grown each with a seed rate of 120 kg/ha. Intercrops were sown as mixed and substitutive intercrops (Hauggaard-Nielsen et al. 2008) with proportions of 50:50 (LU_WR_50_50) in both years and additionally 75:25 (LU_WR_75_25) in 2019. The content of mineral nitrogen at the time of sowing for both experimental years is given in Table 3.1.

3.2.3 Shoot harvest and nutrient analysis

Shoot biomass was harvested on 15th November in 2018 and on 13th November in 2019 on 1.0 m² per plot and was dried at 60 °C for 24 hours and at 105 °C for further 24 hours. Dry matter samples were milled with a vibratory disc mill (RS 200 Retsch, Haan, Germany). Milled samples were analysed for N content with Dumas method using an Elemental analyser (Eurovector EA 3000, Pavia, Italy). After microwave digestion milled samples were analysed for phosphorous (P) content photometrically using a continuous flow analyser (Seal QuAAtro 39, Norderstedt, Germany) and for potassium (K) content with atomic absorption spectrometry using Analyst 200 (PerkinElmer, Waltham, USA).

Table 3.1 Mineral nitrogen [kg/ha] at the time of sowing in 2018/2019 and 2019/2020.

	2018/2019	**2019/2020**
0-30 cm	17.1	53.9
30-60 cm	3.2	26.1
60-90 cm	3.4	11.7
Total	23.7	91.7

3.2.4 Soil mineral nitrogen

Soil samples for analysis of soil mineral nitrogen (N_{min}) were taken using a Puerckhauer auger. Samples in soil depths 0-30 cm and 30-60 cm were taken in both years and in 2019 also for 60-90 cm. Four samples per plot were mixed for 30-60 cm and 60-90 cm, eight samples per plot were mixed for 0-30 cm. In 2018 soil samples were taken on 8th and 9th November and in 2019 on 13th November. Both nitrate and ammonium were determined photometrically using a continuous flow analyser (Seal QuAAtro 39, Norderstedt, Germany) after extraction with potassium sulphate.

3.2.5 Monolith method

In October 2018 and 2019 a trench (1.2 deep; 1.0 m wide) was established with an excavator transversely to the plots for three field replications. The monolith method by Böhm (1979) was used to extract monoliths down to a soil depth varying between 60 and 100 cm depending on the gravel layer appearing. Monoliths in 10 cm intervals of soil depth were collected. In 2018 two monoliths next to each other of 10 cm × 10 cm × 20 cm and in 2019 two monoliths next to each other in the topsoil (0-30cm) and three monoliths next to each other in the subsoil (30-100cm) of 10 cm ×10 cm × 17 cm were taken. With regard to the width of the plot, the monoliths were taken in the plot centre to minimize edge effects. After sampling in the field and before washing, monoliths were kept refrigerated at 5° C.

Subsequently, roots were washed from monoliths by soaking in water for easier disaggregation by hand. The water with the dissolved monolith was poured through a stack of sieves until the soil was completely dissolved. In 2018 sieves of 2.0 and 1.0 mm mesh size were used. In 2019 sieves of 4.0, 2.0, 1.0, 0.71, 0.63 and 0.5 mm mesh size were used. The roots recovered by the single sieves were washed off from the sieves and soil, stones and some particular organic matter were removed by repeated sedimentation and decantation. Until further processing, roots were kept refrigerated in water at 5° C. Roots were manually separated from remaining non-root material, especially particular organic matter, by tweezers. In 2019, roots recovered in the 0.5 mm sieve were not separated from particular organic matter. Thus, these roots were analysed for root length but not for root mass, to minimize the error by the mass of the non-root material, as roots recovered in sieves with smaller mesh size contribute to a high extent to root length, but their contribution to root mass is negligible (Livesley et al. 1998).

Washed roots were scanned with a photo scanner (Expression 12000XL, Epson, Suwa, Japan) at 800 dpi and root-length was analysed with WinRHIZO (Version WinRhizoPro 2019a 64bit, Regent Instruments, Quebec, Canada). For determination of root dry matter, roots were dried at 55 °C for 48 hours.

3.2.6 Fourier Transform Infrared Spectroscopy

The roots of winter rye (WR) and blue lupin (LU) of the years 2018 and 2019 were used to evaluate the species root proportion of the mixed species samples. The dried roots of the single species samples and mixed species samples harvested with the monolith method were ground to 0.2 mm (ultra-centrifugal mill, ZM 200, Retsch, Haan, Germany). Absorption spectra 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 4,000–400 cm^{-1}. Each sample was measured 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 single sample species were used for cluster analysis (Opus software, version 7.2, Bruker Optics) to separate the species. For the cluster analyses, the spectra were pre-processed by the second derivative and vector normalization, the frequency range was reduced to 1800–850 cm^{-1} and the Euclidian's distance and Ward's algorithm was applied (see Figs. B1 and B2, supplementary material). For 2018 and 2019, the interspecific heterogeneity for both species (9.426 and 4.303, respectively) was higher than the intraspecific heterogeneity (1.068 and 0.523 for WR, and 4.711 and 1.169 for LU, respectively), permitting separation of the two species.

For the quantification of the proportion of the species in the mixed species root samples, the FTIR spectra of the single species samples were used to generate a model. The details in establishing and evaluating the model were added in the supplementary material.

With the chosen model for each year, the FTIR spectra of the mixed species samples (of the corresponding year) were evaluated with the associated model. The output of this evaluation was the percental share of each species within the mixed species root samples. The percentage of the single species were used for further calculations.

3.2.7 Calculations

For each monolith, root length density (RLD), root mass density (RMD) and specific root length (SRL) was calculated according to equation 3.1, 3.2 and 3.3. Units of RLD, RMD and SRL are cm cm^{-3}, mg cm^{-3} and m g^{-1}, respectively.

$$RLD = \frac{root\ length}{soil\ volume} \tag{3.1}$$

$$RMD = \frac{root\ dry\ mass}{soil\ volume} \tag{3.2}$$

$$SRL = \frac{root\ length}{root\ dry\ mass} \tag{3.3}$$

For the statistical comparison of RLD, RMD and SRL the following combined soil layers were used: 0-10 cm, 10-30 cm, 30-60 cm and 60-100 cm.

Shoot performance and root mass of cover crop mixtures were analysed using the land equivalent ratio (*LER*) concept (Mead and Willey 1980). *LER* is the relative land area under sole cropping that is required to produce the same yields as under intercropping. The land use efficiency of an intercrop will be superior to that of corresponding sole crops if *LER* is > 1. *LER* is the sum of the partial land equivalent ratios *pLER* (equation 3.4). Y_i is shoot or root dry mass or shoot nutrient uptake (g/m²) in the intercrop of species *i* and M_i is shoot or root dry mass or shoot nutrient uptake (g/m²) of the sole crop *i*.

$$LER = \sum pLER_i = \sum \frac{Y_i}{M_i} \tag{3.4}$$

Root and shoot performance of cover crop mixtures was analysed using the relative mixture effect (*RME*) concept. *RME* (equation 3.5) was calculated for the root traits RMD, RLD and SRL as well as for shoot biomass and shoot nutrient uptake. *RME* is the percentage difference between the observed value in the cover crop mixture and expected value, which is the average of the sole cover crops weighted by relative density p_i. Relative density is the density in mixture divided by density in sole crop.

$$RME = \frac{\sum(Y_i - p_i * M_i)}{\sum p_i * M_i} * 100 \tag{3.5}$$

Additionally, we use the concept of Loreau and Hector (2001) partitioning the net biodiversity effect into complementarity and selection effect and the notation of Elhakeem et al. (2019). *NE* is the net biodiversity effect (g/m²) which is equal to the absolute mixture effect, i.e. the difference between observed and expected yield of the mixture. Y_i is the yield of species *i* in mixture (g/m²), M_i is the yield of species *i* as sole crop (g/m²) and p_i is the relative density (equation 3.6). *NE* is the sum of *CE* and *SE* (equation 3.7). *CE* is the complementarity effect (g/m²) and *SE* is the selection effect (g/m²).

$$NE = \sum(Y_i - p_i * M_i) \tag{3.6}$$

$$NE = CE + SE \tag{3.7}$$

$$CE = N * \Delta\bar{R}Y * \bar{M} \tag{3.8}$$

CE is calculated as the number of species (*N*) multiplied with the average relative yield gain ($\Delta\bar{R}Y$) and $\bar{M}$ which is the average yield of sole crops (equation 3.8). The relative yield gain (ΔRY) is the difference between observed and expected relative yield (equation 3.9).

CE indicates whether the mixed species benefit from facilitation or niche differentiation or whether species interfere with each other. CE is positive if the yield of both partners is higher than expected or the relative yield gain of one partner is higher than the relative yield loss of the other partner.

$$\Delta RY_i = RY_i - p_i = \frac{Y_i}{M_i} - p_i \tag{3.9}$$

SE is calculated by the number of species multiplied by the covariance between relative yield gain and sole crop yields (equation 3.10). SE shows the dominance by one species. It indicates the relation between the yield of sole crops and the relative yield gain in the mixture of the corresponding species. SE is positive if the relative yield gain of the species with the higher yield in sole crop is relatively higher. SE is negative if the relative yield gain of the species with the lower yield in sole crop is relatively higher.

$$SE = N * cov(\Delta RY_i, M_i) \tag{3.10}$$

3.2.8 Statistical analysis

Data were analysed with the programme R version 3.5.2 with R studio version 1.1.463 (R Core Team 2018).

Shoot biomass, root biomass, root-shoot-ratio, mean of RLD, RMD, SRL and N_{min} in combined soil layers were analysed by a one-factorial analysis of variance (Anova). Homogeneity of variances was checked with a plot of residuals versus fitted values and normal distribution was tested with a normal quantile-quantile (QQ) plot (Kozak and Piepho 2018). If necessary, data were square root or logarithm transformed. After Anova, mean values of treatments were compared with a Tukey test at a significance level of α=0.05 by using the agricolae package (Mendiburu 2019).

The percentage share of root mass and the difference between expected and observed shoot biomass, root biomass, RMD and pLER was tested with a two sample Welch t-test. The significance of LER > 1 was tested by a one sample t-test against 1. The significance of RME was tested by a one sample t-test against 0. Pearson’s correlation between RME of root traits were calculated.

3.3 Results

The growth period of 2018 was characterised by an extreme dry spell from July to November leading to extremely dry soil conditions in autumn. The summer of 2019 was dry as well, especially in June and July, but less extreme than 2018. Both growing periods were warmer compared to the long-term mean. Soil moisture content, recorded at a nearby weather station, was much higher in October and November in 2019 than in 2018 (Fig. 3.1).

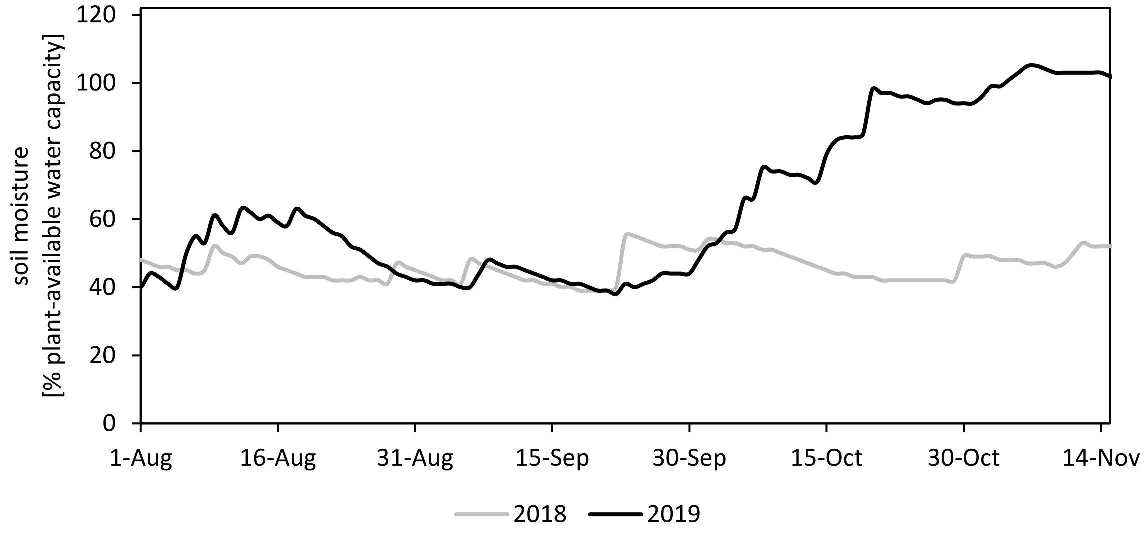

Fig. 3.1 Soil moisture in % of plant-available water capacity under grass on a sandy loam between 0 and 60 cm soil depth at the nearby (12 km) weather station from the German Meteorological Service at Cologne Bonn Airport in the period from two weeks before sowing until biomass sampling of cover crops for both experimental years.

3.3.1 Root and shoot biomass

In both years total shoot dry mass (DM) did not differ significantly between cover crops (Fig. 3.2). The shoot proportion of lupin was significantly lower than expected from sowing proportion in both years, whereas the shoot proportion of rye was significantly higher than expected in both years.

Total root DM did not differ significantly in 2018, but differed in 2019. For the 50:50-mixture, in 2018 root DM of winter rye was significantly higher than expected. In 2019 expected and observed root DM of mixtures did not differ significantly. Rye dominated root and shoot DM compared to lupin in both years and mixtures. The 75:25-mixture showed more balanced partners in shoot and root DM than the 50:50-mixture. In 2018, the root-shoot-ratios of rye and the 50:50-mixture were significantly higher than that of lupin. In 2019, the root-shoot-ratios of lupin and both mixtures were significantly lower than that of rye.

In the LER diagram (Fig. 3.3) all shoot values were below the diagonal dashed line for both mixtures. Thus, rye had competitive advantage over lupin in the shoot. For the 50:50-mixture, all shoot values for pLER of lupin were below 0.5 and for pLER of rye were above 0.5. For the 75:25-mixture all shoot values for pLER of lupin were below 0.75 and for pLER of rye were above 0.25. In the shoot blue lupin was suppressed by winter rye. For the shoot in 2018 and 2019, mean LER of the 50:50-mixture was 1.04 and 1.03, respectively and mean RME were -

0.3 % and -0.4 %, respectively. For the 75:25-mixture in 2019 mean shoot LER was 1.22 and mean shoot RME was 17.3 %. For the root mass, LER values for the 50:50 mixture in 2018 were all above 1 (mean 1.50), whereas in 2019 they were below 1 (mean 0.52). Two root LER values for the 75:25 mixture were below 1, one was above 1. The mean was 0.65.

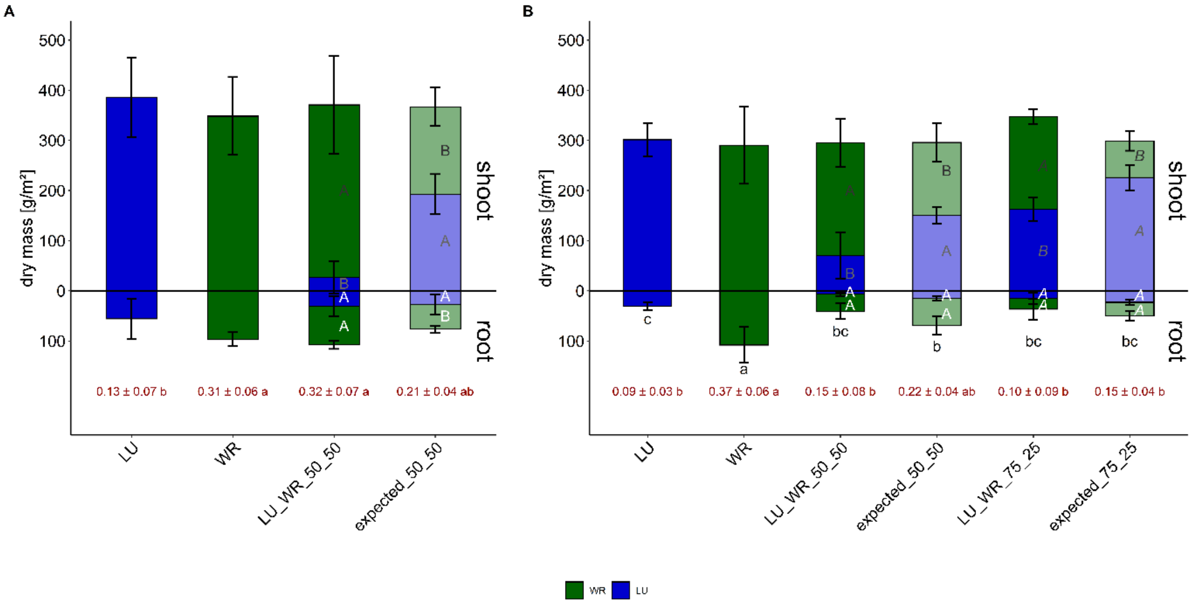

Fig. 3.2 Mean values ± standard deviation of shoot (n = 4) and root (n = 3) dry mass [g/m²] and root-shoot-ratio (n = 3) of sole cover crops blue lupin (LU), winter rye (WR), 50:50 and 75:25 cover crop mixtures of blue lupin and winter rye (LU_WR_50_50 and LU_WR_75_25) and of expected mixtures (corresponding proportions of sole crops) in 2018 (A) and 2019 (B). Bars above 0 represent shoot dry mass, bars below 0 show root dry mass. Bar colours refer to species: green for rye, blue for lupin. Transparent bars represent expected values. Red numbers below bars represent root-shoot-ratio. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different cover crops for total shoot or total root dry mass or root-shoot-ratio within one year. Different uppercase letters indicate significant differences ($p \leq 0.05$, t-test) between observed and expected dry mass for shoot or root of intercrop partner within one year.

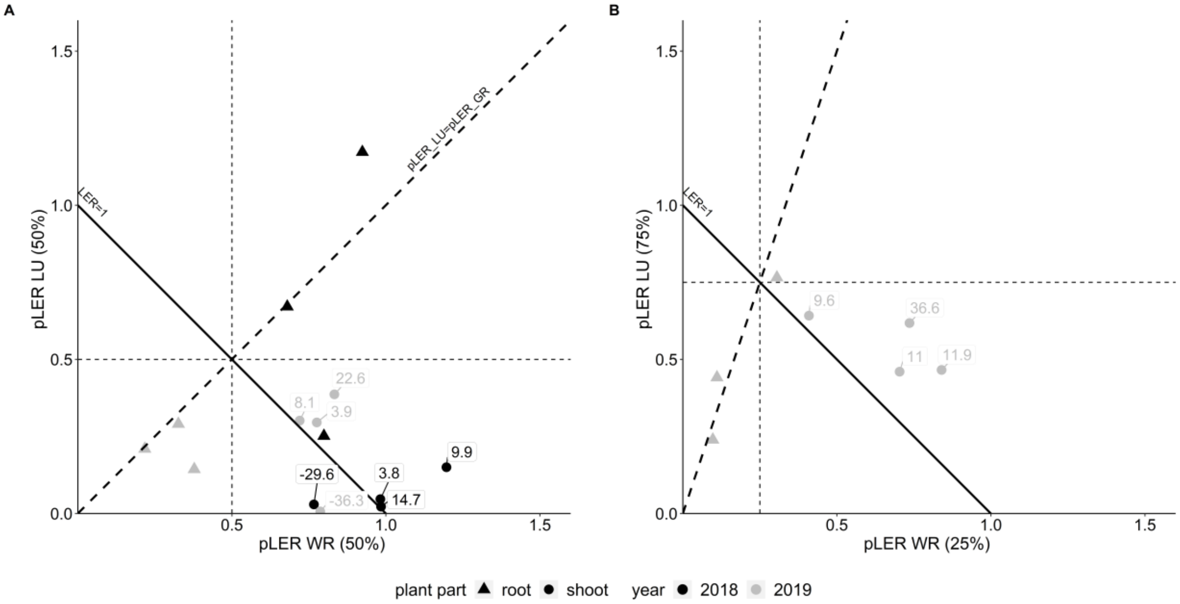

Fig. 3.3 Partial land equivalent ratio of shoot dry mass (circle) and root dry mass (triangle) of blue lupin (pLER LU) and winter rye (pLER WR) and relative mixture effect [%] (RME) of shoot dry mass in a 50:50-mixture (A) and in a 75:25-mixture (B) in two years 2018 (black symbols) and 2019 (grey symbols). RME of root mass is given in Fig. 3.6. The solid line corresponds to a land equivalent ratio of 1 (LER = pLER LU + pLER WR). The dashed horizontal and vertical lines represent the expected pLER for the mixture partners. Numbers adjacent to symbols represent RME.

3.3.2 Shoot nutrient uptake and soil mineral nitrogen depletion

LER and RME were only significant for N and P shoot uptake in the 75:25-mixture (Table 3.2). All pLER of shoot nutrient uptake were higher than expected for rye, lower than expected for lupin.

In 2018, N shoot uptake of lupin was significantly higher than that of rye. In 2019, N shoot uptake of the 75:25-mixture was significantly higher than that of rye. In both years, in the 50:50-mixture pLER of lupin for N shoot uptake was significantly lower than 0.5, and pLER of rye for N shoot uptake was significantly higher than 0.5. In 2019, in the 75:25-mixture pLER of rye for N shoot uptake was significantly higher than expected, LER (1.53 ± 0.16) was significantly > 1 and RME (29.60 % ± 9.88 %) significantly > 0.

In 2018, P shoot uptake was significantly lower in lupin than in rye and the 50:50-mixture. In 2019, a significantly higher P shoot uptake in the 75:25-mixture than in lupin was detected. In both years and for both mixtures, pLER of lupin for the P shoot uptake was significantly lower than expected and pLER of rye was significantly higher than expected. In 2019, in the 75:25-mixture LER (1.40 ± 0.17) was significantly > 1 and RME (50.53 % ± 12.86 %) significantly > 0.

K shoot uptake did not significantly differ between any of the cover crops in any of the years. In both years and for both mixtures, pLER of lupin for K shoot uptake was significantly lower than expected and pLER of rye was significantly higher than expected. None of the LER or RME values significantly differed from 1 or 0, respectively.

Cover crops significantly reduced soil N_{min} compared to the control by 80-130 kg/ha (Fig 3.4). Mixtures of blue lupin with winter rye reduced soil N_{min} significantly more compared to lupin sole crop and were as effective as rye sole crop in reduction of inorganic N. Differences were especially high in the soil layers of 30-60 cm and 60-90 cm.

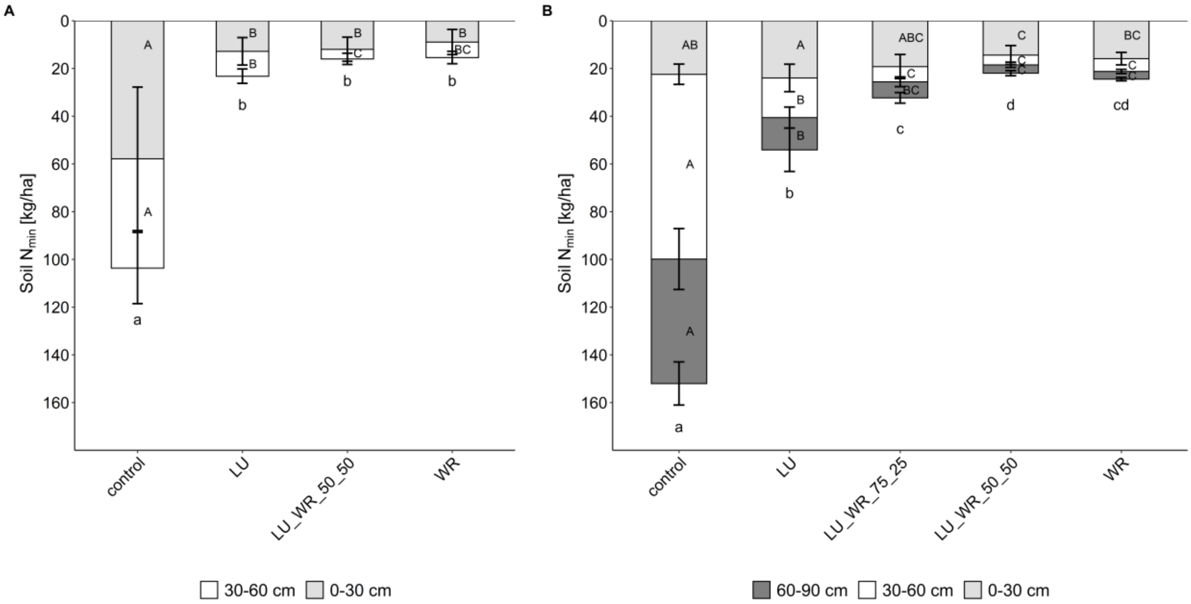

Fig. 3.4 Mean values ± standard deviation (n = 4) of soil mineral nitrogen [kg/ha] (N_{min}) under a weed-free control, sole cover crops blue lupin (LU), winter rye (WR), 50:50 and 75:25 cover crop mixtures of lupin and rye (LU_WR_50_50 and LU_WR_75_25) in 2018 (A) and 2019 (B). Bar colours refer to soil layer: light grey for 0-30 cm, white for 30-60 cm, dark grey for 60-90 cm. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different treatments for total N_{min} within one year. Different uppercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different treatments for N_{min} within each soil layer within one year.

Table 3.2 Mean values ± standard deviation of N-, P- and K-shoot uptake [g/m²] (n = 4) of sole cover crops blue lupin (LU), winter rye (WR), 50:50 and 75:25 cover crop mixtures of lupin and rye, partial land equivalent ratio (pLER), land equivalent ratio (LER) and relative mixture effect [%] (RME) in 2018 and 2019. Different lowercase letters indicate significant differences (p ≤ 0.05, HSD Tukey-test) between different cover crops for N-, P- or K-shoot uptake within one year. Significant differences for pLER from 0.5 or 0.75 and 0.25, for LER from 1 or for RME from 0 (p ≤ 0.05, t-test) are given in bold.

		2018						2019					
		N shoot uptake		P shoot uptake		K shoot uptake		N shoot uptake		P shoot uptake		K shoot uptake	
sole crop	LU [g/m²]	12.32 ± 2.406	a	1.05 ± 0.267	b	10.75 ± 2.153	a	10.45 ± 1.327	ab	1.01 ± 0.135	b	8.33 ± 1.249	a
	WR [g/m²]	7.62 ± 1.220	b	1.62 ± 0.314	a	11.85 ± 2.494	a	7.56 ± 2.475	b	1.39 ± 0.498	ab	9.74 ± 4.909	a
intercrop 50:50	LU [g/m²]	0.87 ± 1.073	ab	0.09 ± 0.122	a	0.71 ± 0.831	a	2.40 ± 1.566	ab	0.21 ± 0.138	ab	1.59 ± 1.028	a
	WR [g/m²]	8.90 ± 2.781		1.55 ± 0.449		13.03 ± 3.807		7.53 ± 1.855		1.29 ± 0.367		9.20 ± 2.729	
intercrop 75:25	LU [g/m²]	-		-		-		5.79 ± 0.954	a	0.52 ± 0.063	a	4.05 ± 0.509	a
	WR [g/m²]	-		-		-		6.78 ± 0.484		1.13 ± 0.045		8.31 ± 0.603	
pLER 50:50	LU	**0.06 ± 0.063**		**0.08 ± 0.078**		**0.06 ± 0.058**		**0.24 ± 0.155**		**0.22 ± 0.143**		**0.20 ± 0.136**	
	WR	**1.15± 0.189**		**0.96 ± 0.258**		**1.09 ± 0.113**		**1.01 ± 0.121**		**0.95 ± 0.135**		**1.01 ± 0.233**	
pLER 75:25	LU	-		-		-		0.56 ± 0.125		**0.52 ± 0.085**		**0.49 ± 0.051**	
	WR	-		-		-		**0.97 ± 0.287**		**0.88 ± 0.253**		**1.00 ± 0.384**	
LER 50:50		1.21 ± 0.218		1.04 ± 0.334		1.15 ± 0.153		1.25 ± 0.237		1.17 ± 0.244		1.21 ± 0.331	
LER 75:25		-		-		-		**1.53 ± 0.163**		**1.40 ± 0.174**		1.49 ± 0.374	
RME 50:50 [%]		-3.53 ± 20.858		21.48 ± 26,788		20.03 ± 25.652		10.03 ± 31.014		25.23 ± 29.445		22.05 ± 35.249	
RME 75:25 [%]		-		-		-		**29.60 ± 9.875**		**50.53 ± 12.861**		47.35 ± 30.198	

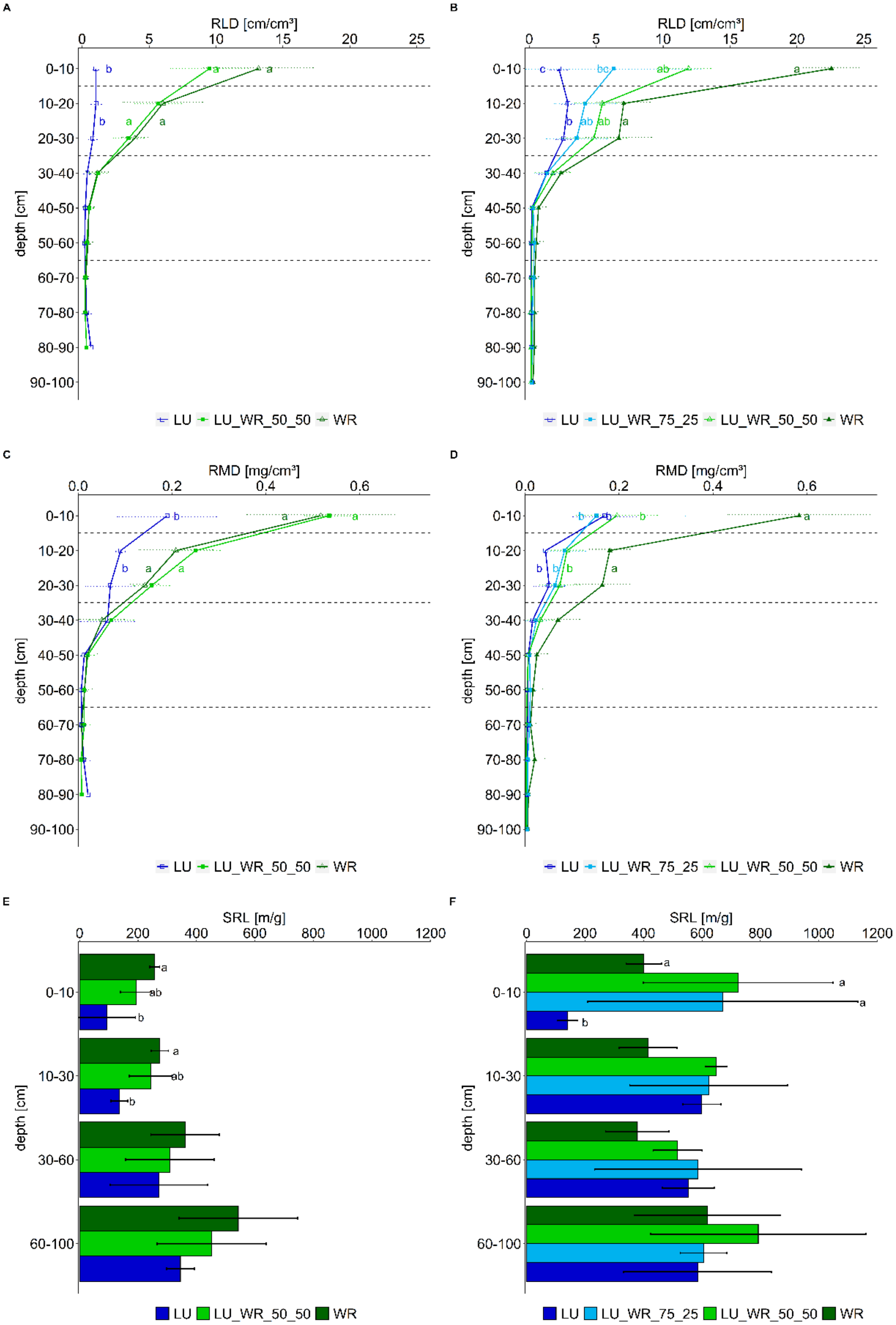
A
RLD [cm/cm³]
0
5
10
15
20
25
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
depth [cm]
b
a
a
b
a
a
LU
LU_WR_50_50
WR
B
RLD [cm/cm³]
c
bc
ab
a
b
ab
ab
a
LU
LU_WR_75_25
LU_WR_50_50
WR
C
RMD [mg/cm³]
0.0
0.2
0.4
0.6
b
a
a
b
a
a
D
RMD [mg/cm³]
b
b
b
a
b
b
b
a
E
SRL [m/g]
0
200
400
600
800
1000
1200
0-10
10-30
30-60
60-100
a
ab
b
a
ab
b
F
SRL [m/g]
a
a
a
b

Fig. 3.5 Mean values ± standard deviation (n = 3) of root length density [cm/cm³] (RLD) (A, B), root mass density [mg/cm³] (RMD) (C, D) and specific root length [m/g] (SRL) (E, F) of sole cover crops blue lupin (LU), winter rye (WR), 50:50- and 75:25-cover crop mixtures of lupin and rye (LU_WR_50_50 and LU_WR_75_25) in 2018 (A, C, E) and 2019 (B, D, F) at different soil depth. Colours refer to cover crops: dark green for rye, light green for 50:50-mixture, light blue for 75:25-mixture and dark blue for lupin. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different cover crops within the different soil depth layers (0-10 cm, 10-30 cm, 30-60 cm and 60-100 cm) within one year.

3.3.3 Root length density, root mass density and specific root length

Topsoil RLD was high for rye and low for lupin. In 2018, mixtures were similar to rye, and intermediate in 2019 (Fig. 3.5). Lupin had significantly lower RLD in 0-30 cm than rye and 50:50-mixture in 2018. In 2019, in 0-10 cm soil depth rye and 50:50-mixture had significantly higher RLD than lupin, whereas 75:25-mixture and lupin had significantly lower RLD than rye. In 10-30 cm soil depth rye had significantly higher RLD than lupin, both mixtures were intermediate. No significant differences for subsoil RLD were recorded.

Topsoil RMD was high for rye and low for lupin. In 2018, RMD of mixtures were similar to rye, and similar to lupin in 2019. In 2018, lupin had significantly lower RMD than rye and 50:50-mixture in 0-30 cm soil depth. In 2019, RMD of lupin and both mixtures was significantly lower than that of rye in topsoil. No significant differences for RMD below 30 cm soil depth were recorded.

In 2018, mean SRL ranged from 93 m/g in lupin to 543 m/g in rye, and in 2019 from 139 m/g in lupin to 793 m/g in 50:50-mixture. SRL in 2018 was significantly higher for rye than for lupin in 0-30 cm soil depth, the mixture was intermediate. In 2019, SRL of lupin was significantly lower than rye and both mixtures within 0-10 cm.

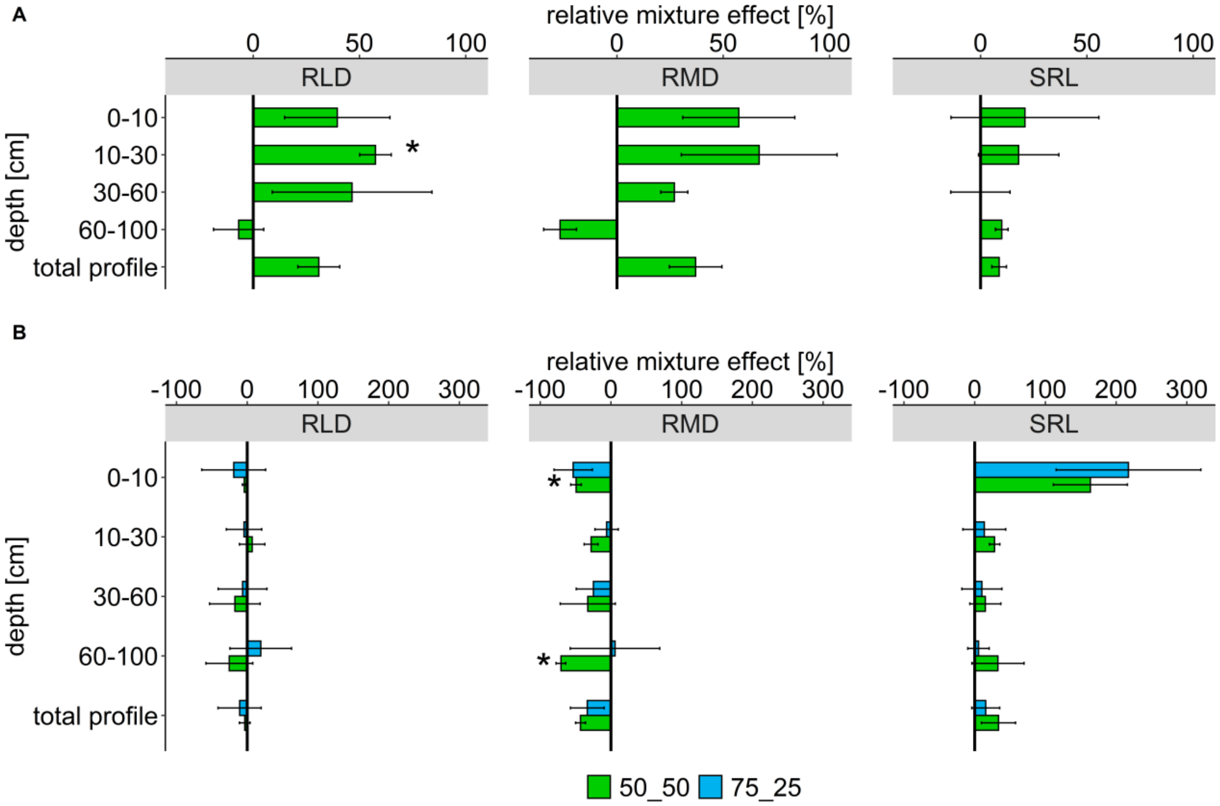

Fig. 3.6 Mean values ± standard error (n = 3) of relative mixture effect [%] for root length density (RLD), root mass density (RMD) and specific root length (SRL) for 50:50 and 75:25 cover crop mixtures of lupin and rye (50_50 and 75_25) in 2018 (A) and 2019 (B) at different soil depth and for the total soil depth profile. Colours refer to cover crop mixtures: light green for 50:50-mixture and light blue for 75:25-mixture. Asterisks indicate a significant difference in RME from 0 ($p \leq 0.05$, t-test).

3.3.4 Below-ground mixture effects

In 2018, relative mixture effects (RME) for all three root traits were positive in 0-60 cm, except SRL in 30-60 cm (-0.13 %). Positive RME for RLD was significantly different from 0 in 10-30 cm (Fig. 3.6). In 60-100 cm, RME for RLD and RME were negative, whereas RME for SRL was positive. Across the total profile, RME of all three root traits were positive.

In 2019, for the 50:50-mixture RME for RLD varied around 0, while RME for RMD were negative, and significantly lower than 0 in 0-10 cm and 60-100 cm. RME for RLD and RMD in the 75:25 were negative, except in 60-100 cm. Both mixtures showed positive RME for SRL in all depth layers. In the three mixtures RME of SRL was consistently positive and was significant for 0-10 cm across both mixtures and both years (t-test, df = 81, $p < 0.0001$). Positive

RME of RLD and of SRL were observed without trade-off with each other or RLD and SRL itself (Table B2, supplementary material).

Results based on the FTIR spectroscopy (Fig. 3.7) show that the share of rye root mass in the mixture was always significantly higher than of lupin in topsoil and share of root mass in mixture of lupin was significantly higher in subsoil in two out of three cases. In 2018 RMD of rye in topsoil (20-30 cm) was significantly higher than expected (Fig. 3.8). In all other cases there were no significant differences between observed and expected RMD of rye and lupin.

The calculation of the diversity effects gave the following results (Table 3.3): For the shoot DM and shoot nutrient uptake NE was clearly positive (75:25 in 2019) to slightly negative (shoot DM 50:50 in 2019 and N uptake in 2018). CE was always positive, whereas the SE was negative for shoot DM and N uptake and positive for P and K uptake. Significant positive diversity effects for shoot DM and shoot nutrient uptake were only found for the 75:25-mixture in 2019. For the root DM NE and CE were positive in 2018 and negative in 2019. SE behaved the other way around. Significant diversity effects for root DM were negative NE and CE for the 50:50-mixture in 2019.

Table 3.3 Mean values ± standard deviation of net biodiversity effect (NE), complementarity effect (CE) and selection effect (SE) of cover crop mixtures of blue lupin and winter rye (50:50 and 75:25) for shoot dry matter (DM) [g/m²] (n = 4), and shoot nutrient uptake [g/m²] (n = 4), and root dry matter (DM) [g/m²] (n = 3). Effects significant different from 0 ($p \leq 0.05$, t-test) are given in bold.

year	mixture	parameter	NE	CE	SE
2018	50:50	shoot DM	3.8 ± 69.67	21.5 ± 86.56	-17.7 ± 65.72
2018	50:50	root DM	31.6 ± 30.24	32.2 ± 33.58	-0.6 ± 3.34
2018	50:50	N uptake	-0.2 ± 1.95	2.3 ± 2.47	**-2.5 ± 1.37**
2018	50:50	P uptake	0.3 ± 0.39	0.1 ± 0.49	0.2 ± 0.17
2018	50:50	K uptake	2.4 ± 3.01	1.8 ± 1.99	0.6 ± 2.11
2019	50:50	shoot DM	-1.1 ± 71.44	6.6 ± 48.81	-7.7 ± 27.65
2019	50:50	root DM	**-28.5 ± 5.24**	**-31.8 ± 3.45**	3.3± 3.96
2019	50:50	N uptake	0.9 ± 2.75	2.2 ± 2.00	-1.3 ± 1.41
2019	50:50	P uptake	0.3 ± 0.34	0.2 ± 0.26	0.1 ± 0.15
2019	50:50	K uptake	1.8 ± 2.63	1.5 ± 2.51	0.3 ± 1.28
2019	75:25	shoot DM	49.2 ± 31.53	**61.3 ± 35.86**	-12.1 ± 27.80
2019	75:25	root DM	-13.4 ± 20.28	-19.7 ± 24.88	6.3 ± 5.02
2019	75:25	N uptake	**2.8 ± 0.71**	**4.6 ± 1.18**	-1.8 ± 1.72
2019	75:25	P uptake	**0.5 ± 0.08**	**0.5 ± 0.16**	0.1 ± 0.11
2019	75:25	K uptake	**3.7 ± 2.05**	3.6 ± 2.97	0.1 ± 1.29

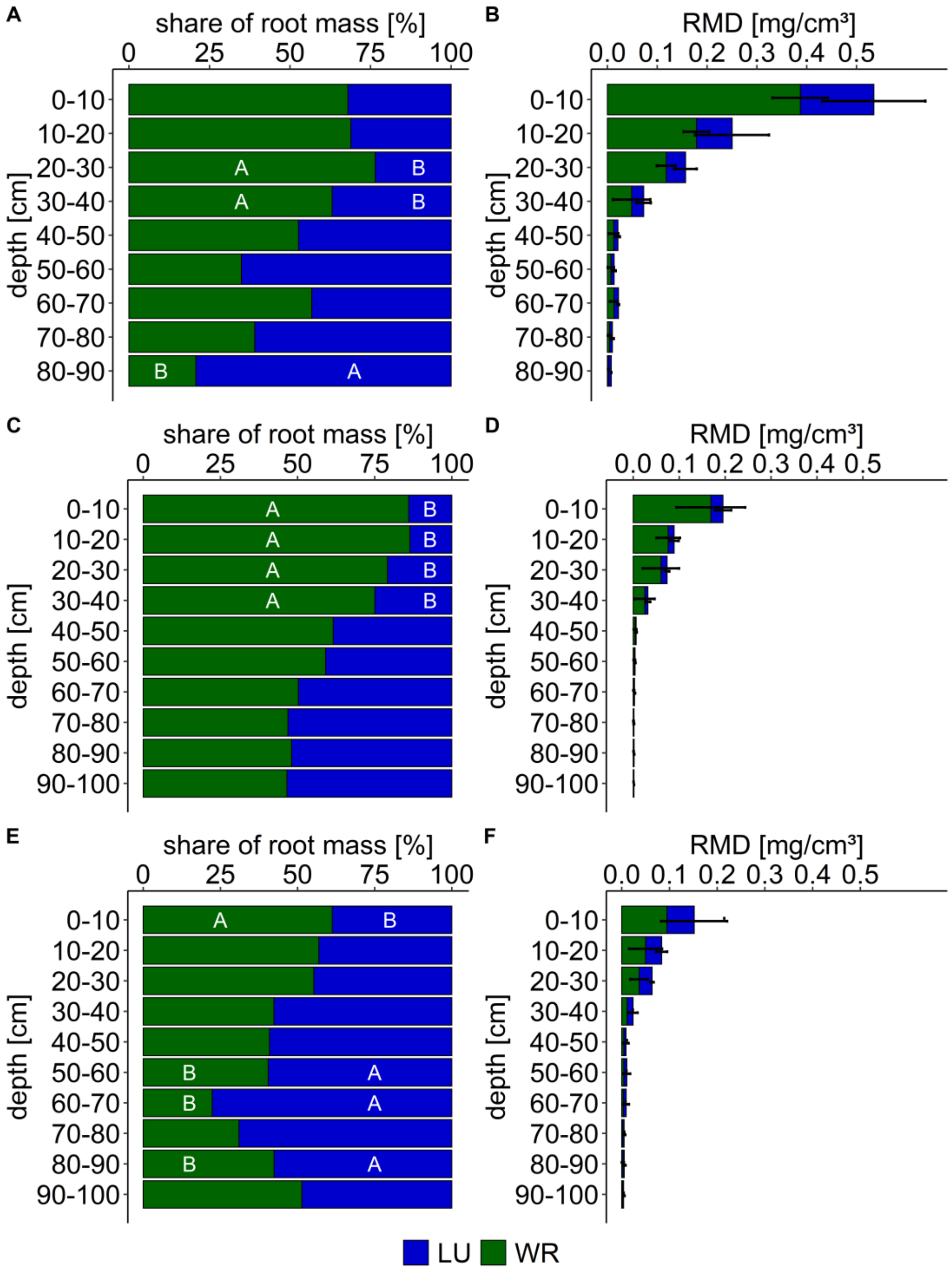

Fig. 3.7 Mean values (n=3) of share of root mass [%] of blue lupin and winter rye (A, C, E) and mean values ± standard deviation (n = 3) of root mass density [mg/cm³] (RMD) at different soil depth of blue lupin and winter rye (B, D, F) in a 50:50 cover crop mixtures of lupin and rye in 2018 (A, B), in a 50:50 cover crop mixtures of lupin and rye in 2019 (C, D) and for a 75:25 cover crop mixtures of lupin and rye in 2019 (E, F). Colours refer to species: green for rye, blue for lupin. Different uppercase letters indicate significant differences ($p \leq 0.05$, t-test) between share of root mass of blue lupin and winter rye within one soil depth layer for one crop partner.

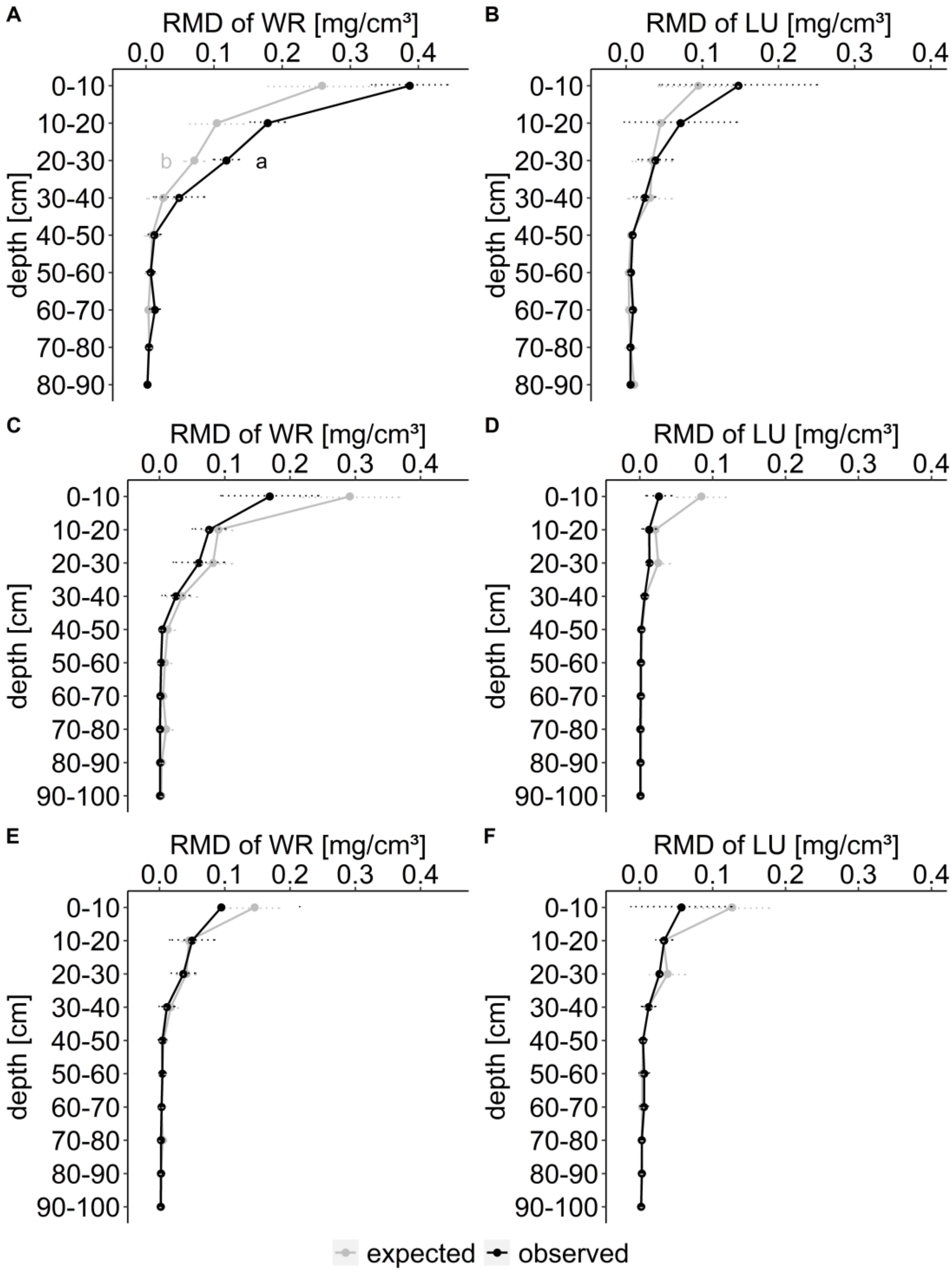

Fig. 3.8 Mean values ± standard deviation (n = 3) of observed (black) and expected (grey) RMD of rye (A, C, E) and lupin (B, D, F) in a 50:50 cover crop mixtures of lupin and rye in 2018 (A, B), in a 50:50 cover crop mixtures of lupin and rye in 2019 (B, C) and in a 75:25 cover crop mixtures of lupin and rye in 2019 (E, F). Different lowercase letters indicate significant differences ($p \leq 0.05$, t-test) between observed and expected RMD within one soil depth layer for one crop partner.

3.4 Discussion

3.4.1 Shoot biomass, shoot nutrient uptake and soil mineral nitrogen depletion

In the mixtures, our field experiment revealed lower shoot proportion and shoot nutrient uptake for lupin than expected from sole crops, while for rye these values were higher than expected from sole crops (Fig. 3.2; Table 3.2). Thus, rye had competitive advantage over the suppressed lupin. However, this interaction was examined three months after seeding while it may be useful to examine early competitive interactions on a single plant level as well, which may be recommended for further studies to understand competitiveness more in detail. Our field experiment confirmed results of other studies, showing the cereal to be dominant in cereal-legume intercrops (Hauggaard-Nielsen et al. 2001a; Yu et al. 2016). Complementarity of N resource use is discussed to be the driver of these intercrops (Corre-Hellou et al. 2006; Jensen 1996). This is shown in our study by a positive complementarity effect (CE) for shoot DM and shoot nutrient uptake (Table 3.3). Also, Elhakeem et al. (2019) reported positive CE for cover crop shoot mass and N uptake.

The best performing mixture and only one with significant overyielding for N- and P-uptake was the 75:25-mixture (Table 3.2). Likewise, the positive CE for shoot DM, N and P uptake were only significant in this mixture (Table 3.3). Moreover, for the shoot mass the mean LER of the 50:50-mixture was only slightly above 1 in both experimental years, whereas mean LER of the 75:25-mixture was 1.22 (Fig. 3.3). For N and P shoot uptake, LER and RME were only significant in the 75:25-mixture. The 75:25-mixture performed best as the proportion of the dominant partner was reduced for the benefit of the suppressed partner (Yu et al. 2016). Thus, our hypothesis (1) is confirmed only for the 75:25-mixture with more balanced partners. In the 50:50-mixture the biomass gain of rye in the mixture was countervailed by the biomass loss of lupin. However, in all mixtures LER was higher for shoot nutrient uptake than for shoot biomass. Thus, the hypothesis was also confirmed for shoot nutrient accumulation. With a view on nutrient retention in cover cropping LER of nutrient uptake might be more important than LER of biomass itself.

As shown in other studies with mixtures of legumes and non-legumes (Hauggaard-Nielsen et al. 2001a; Hauggaard-Nielsen et al. 2003), in our field experiments cover crops significantly reduced soil inorganic N compared to the control; and soil inorganic N was significantly reduced by mixtures compared to the legume sole crop (Fig. 3.4). Furthermore, they were as effective as the non-legume sole crop in N_{min}-reduction. This confirms the well-known advantage of non-legumes in cover crop mixtures with legumes to reduce the risk of nitrate leaching (Tosti et al. 2014; Tribouillois et al. 2016). We see here a link to increased SRL in mixtures, as high N retention was possible in mixtures although root mass was lower than in non-legume sole crop in 2019. Moreover, N retention and N supply to the subsequent crop present a trade-off which is minimized in mixtures compared to sole crops (Couëdel et al. 2018; White et al. 2017).

3.4.2 Root mass: Below-ground complementarity and vertical root niche differentiation

Topsoil root dry mass and RMD were dominated by rye compared to lupin in both years and mixtures (Fig. 3.2; Fig. 3.7 B, D, F). This could be expected from the relations in the shoot and from what is known from other studies showing competitiveness and dominance of cereals intercropped with legumes (Corre-Hellou et al. 2006; Hauggaard-Nielsen et al. 2001a). Beyond this, there is the question whether vertical niche differentiation of root growth, which may allow complementarity in soil resource use (Mommer et al. 2010), can be found.

FTIR spectroscopy analysis revealed a pattern of root mass allocation of cropping partners over soil depth within mixtures with higher RMD of rye in topsoil and higher RMD of lupin in subsoil (Fig. 3.7 A, C, E). Thus, our hypothesis (3) is confirmed. We assume that this results from the combination of the allorhizous lupin and the homorhizious rye. However, to examine vertical root niche differentiation we suggest not comparing root mass proportions of mixture partners, but rather comparing RMD of mixture partners in sole and intercrops. Thus, when the rooting profiles of sole and intercrops were contrasted, a clear pattern of vertical root niche differentiation was not found, as in all three mixtures observed and expected RMD was very similar, except for rye in topsoil in 2018 (Fig. 3.8). Thus, our hypothesis (4) cannot generally be confirmed. Hence, in our experiment the entire RMD profile of a cover crop was not changed when introducing this cover crop into a bispecific mixture.

Similar results were found by Mommer et al. (2010). For four grassland species they observed root biomass overyielding but no niche differentiation. Below-ground overyielding in their study mainly occurred in topsoil layers due to enhanced root growth of one species. Likewise, Heuermann et al. (2019) found rather low niche differentiation by comparing species-specific root mass distribution over depth compared for sole and intercrops. In their experiment they found root biomass overyielding in most cover crop mixtures, but dominating cover crops maintained the same niche as in sole crops, whereas suppressed cover crops were outcompeted for their niche.

Niche differentiation in our experiment might be not pronounced sufficiently to be clearly detected, because the growing time of two and a half month might have been too short for the differentiation to build up. This is similarly discussed for the grassland species of Mommer et al. (2010). Also, Heuermann et al. (2019) examined roots after a short growth period after sowing. In a meta-analysis Cardinale et al. (2007) showed that complementarity increases through time. Besides the impact of time, there is the question whether there is a need for vertical root niche differentiation. Possibly, cropping partners differentiate root growth in vertical niches not before strong competition occurs. Thus, due to the use of different N sources by legume and cereal, competition for N was reduced. The only indication for vertical root differentiation was found for RMD of rye in the topsoil in 2018, while no vertical root differentiation was seen in the second year. The mineral N content in 2019 at the time of sowing (Table 3.1) was more than three times higher than in 2018. Moreover, the soil moisture content, at least at a nearby weather station (Fig. 3.1), was much higher in 2019 than in 2018. Thus, growth conditions for cover crops were much better in the second years. We assume, at least in 2019, only moderate competition for water and N, so that vertical root niche differentiation was not a necessary strategy.

Alternatively to analysing rooting patterns for vertical niche differentiation, complementarity can be analysed on a more general level by the concept of Loreau and Hector (2001). For mixtures of grassland species, complementarity effects for root biomass were found (Mommer et al. 2010). In our study, indication of complementarity in root growth was found only in 2018 but not for the two mixtures in 2019, where CE for root mass was even negative (Table 3.3). These results reflect those for vertical root niche differentiation and may be explained by the soil conditions differing between years. Thus, the better above-ground performance of the 75:25 mixture in 2019 as compared to the 50:50 mixtures cannot be explained by root mass complementarity. Other explanatory approaches for the overyielding of N- and P-uptake are above-ground complementarity (Table 3.3) and below-ground facilitative interactions regarding N or other nutrients (Hauggaard-Nielsen and Jensen 2005; Li et al. 2014). Enhanced N and P concentrations in intercropped compared to sole cropped rye in 2019 could be a hint for this (data not shown). In faba bean-maize relay intercrop it was shown that overyielding was higher when roots intermingled compared to separating species roots (Li et al. 1999). Thus, even if we could not find root mass complementarity, below-ground effects are likely to be meaningful.

Contrary to the widespread view of complementary root growth and vertical root niche differentiation, we could not find a clear evidence of these concepts in our mixtures of rye and lupin. Thus, it may not be sufficient to measure vertical root niche differentiation or complementarity effects of root growth for root mass. Instead, we may need to run the calculations for root length, as it may have a different dynamic than root mass (Ryser 2006). Thus, techniques are needed to discriminate species-specific root length.

3.4.3 Relative mixture effects of root traits: RMD, RLD and SRL

We did not clearly observe overyielding of root biomass or RMD in our study. Only in 2018 we found higher than expected root mass and a positive RME of RMD (Fig. 3.6). The LER for root mass, which was only above 1 in 2018, reflects these results (Fig 3.3). This contrasts with other studies, where overyielding was found in cover crop mixtures (Heuermann et al. 2019) and in intercrops of winter wheat and winter faba bean (Streit et al. 2019). Unlike in our experiment Streit et al. (2019) examined winter crops, having a longer growth period to build up overyielding. The difference to the study of Heuermann et al. (2019) may be due to strong differences in the methodology and the absolute amount of root mass. In our understanding, the high root mass and root-shoot-ratios of Heuermann et al. (2019) need to be interpreted with caution.

Focusing on root length instead of root mass does not change the picture: RME of RLD was positive only in 2018 and transgressive overyielding of RLD was found in none of the mixtures (Fig. 3.5 and 3.6). More complete exploration of total soil volume by RLD of mixtures could thus not be confirmed. Hence, we cannot generally accept our hypothesis (2). In other mixture experiments with main crops, higher RLD in mixtures compared to sole crops were found in faba bean-oil crop mixtures (Schröder and Köpke 2012) and in maize-wheat intercrops (Li et al. 2006). The growing conditions of the cover crops in our study might be hardly comparable to those of the main crops in these studies, especially when relay intercropping is used (Li et al. 2006). Moreover, due to the extreme different RLD of sole crops, rye was not fully able to compensate the low RLD of lupin. Besides, the question remains whether it is necessary for the intercropped rye to invest in more RLD when rye and lupin can access complementary N resources.

Contrary to our expectations, in our study better above-ground crop performance could neither be related to increased RLD nor to increased root mass or vertical niche differentiation in mixtures. Rather, it was higher SRL which may have increased the efficiency of the rooting system by same or higher nutrient uptake with less root mass, as positive RME of SRL was found in both mixtures and years (Fig. 3.6). A positive mixture effect of SRL may be desirable to improve nutrient uptake efficiency of cover crop mixtures. Similar to our study, Bukovsky-Reyes et al. (2019) examined mixtures and sole crops of hairy vetch and cereal rye, and reported for vetches higher SRL in mixture than in sole crop. Hill et al. (2006) showed that SRL increased with nutrient deficiency, especially for P. Due to interspecific competition for nutrients mixture partners may invest more in acquisitive root traits (Bukovsky-Reyes et al. 2019) such as SRL to cope with competition. If SRL increases, as much as or even more nutrients can be taken up with less investment of assimilates in roots. Hence, ideally higher RLD for better soil exploitation and simultaneously increased SRL for improved nutrient acquisition is found in mixtures. For synergistic interactions in mixtures, it is important that trade-offs between positive mixture effects of different root traits are minimized. In our study, positive mixture effects of RLD and SRL were possible without trade-off with RME of another root trait or those root traits themselves (Table B2, supplementary material).

3.5 Conclusion

Cover crop mixtures of lupin and rye showed positive mixture effects for shoot biomass and shoot nutrient accumulation only for nutrient uptake in the 75:25-mixture of lupin and rye. This mixture performed best as the proportion of mixture partners in shoot and root mass was balanced. For the mixtures, FTIR spectroscopy revealed that rye had the highest proportion of root biomass in topsoil and that lupin had the highest proportion of root biomass in subsoil. However, a vertical differentiation in RMD between sole and intercrops was not found. Complementarity of root mass could not consistently be detected.

Mixture effects did occur on root-related variables, but they were not consistent across all depths or environments, and they could even be negative. Among the root traits measured, however, SRL had consistently positive mixture effects.

Future research may focus on mixture effects on root traits also for other intercrops and in other environments as we have conducted our experiments just on one site. Moreover, to enable optimization of cover cropping systems, research will need to elucidate the mechanisms leading to mixture effects of root traits and understand the conditions when mixture effects on root related variables occur.

FTIR spectroscopy gave valuable insights in the pattern of root mass allocation of mixture partners over soil depth. However, further research would profit tremendously from non-manual species-specific discrimination of root length and SRL to learn more about root growth of intercrops.

Acknowledgements

We thank the technical staff of the ‘Wiesengut’ organic farming research station and our students for help in the field.

Funding

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Author contributions

M.A. conceived the idea, planned the research and designed the experiments. R.K. conducted the experiments in the field, processed the root samples and collected the data. N.L. and C.M. performed the FTIR analysis. R.K. analysed the data and wrote the article. M.A., T.F.D. N.L. and C.M. contributed to data interpretation, writing and editing of the article. All authors read and approved the final manuscript.

Declarations

The authors declare no conflict of interest.

References

Andersen SN, Dresbøll DB, Thorup-Kristensen K (2014) Root interactions between intercropped legumes and non-legumes—a competition study of red clover and red beet at different nitrogen levels. Plant and Soil 378:59–72. doi:10.1007/s11104-013-2014-4

Bedoussac L, Journet E-P, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, Jensen ES, Prieur L, Justes E (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agronomy for Sustainable Development 35:911–935. doi:10.1007/s13593-014-0277-7

Bodner G, Leitner D, Kaul H-P (2014) Coarse and fine root plants affect pore size distributions differently. Plant and Soil 380:133–151. doi:10.1007/s11104-014-2079-8

Böhm W (1979) Methods of Studying Root Systems, Vol 33. Springer, Berlin, Heidelberg

Bukovsky-Reyes S, Isaac ME, Blesh J (2019) Effects of intercropping and soil properties on root functional traits of cover crops. Agriculture, Ecosystems & Environment 285:106614. doi:10.1016/j.agee.2019.106614

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences 104:18123–18128. doi:10.1073/pnas.0709069104

Corre-Hellou G, Fustec J, Crozat Y (2006) Interspecific Competition for Soil N and its Interaction with N2 Fixation, Leaf Expansion and Crop Growth in Pea–Barley Intercrops. Plant and Soil 282:195–208. doi:10.1007/s11104-005-5777-4

Couëdel A, Alletto L, Tribouillois H, Justes É (2018) Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agriculture, Ecosystems & Environment 254:50–59. doi:10.1016/j.agee.2017.11.017

Elhakeem A, van der Werf W, Ajal J, Lucà D, Claus S, Vico RA, Bastiaans L (2019) Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. Agriculture, Ecosystems & Environment 285:106627. doi:10.1016/j.agee.2019.106627

Hartwig NL, Ammon HU (2002) Cover crops and living mulches. Weed Science 50:688–699

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001a) Interspecific competition, N use and interference with weeds in pea–barley intercropping. Field Crops Research 70:101–109. doi:10.1016/S0378-4290(01)00126-5

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001b) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236:63–74. doi:10.1023/A:1011909414400

Hauggaard-Nielsen H, Ambus P, Jensen ES (2003) The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutrient Cycling in Agroecosystems 65:289–300. doi:10.1023/A:1022612528161

Hauggaard-Nielsen H, Jensen ES (2005) Facilitative root interactions in intercrops. In: Lambers H, Colmer TD (eds), Root Physiology: from Gene to Function, Vol 4. Springer, Dordrecht, pp 237–250

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES (2008) Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renewable Agriculture and Food Systems 23:3–12. doi:10.1017/S1742170507002025

Heuermann D, Gentsch N, Boy J, Schweneker D, Feuerstein U, Groß J, Bauer B, Guggenberger G, Wirén N von (2019) Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils. Scientific reports 9:11531. doi:10.1038/s41598-019-48060-0

Hill JO, Simpson RJ, Moore AD, Chapman DF (2006) Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. Plant and Soil 286:7–19. doi:10.1007/s11104-006-0014-3

Jensen ES (1996) Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant and Soil 182:25–38. doi:10.1007/BF00010992

Jian J, Du X, Reiter MS, Stewart RD (2020) A meta-analysis of global cropland soil carbon changes due to cover cropping. Soil Biology and Biochemistry 143:107735. doi:10.1016/j.soilbio.2020.107735

Justes E, Bedoussac L, Dordas C, Frak E, Louarn G, Boudsocq S, Journet E-P, Lithourgidis A, Pankou C, Zhang C, Carlsson G, Jensen ES, Watson C, Li L (2021) The 4 C approach as a way to understand species interactions determining intercropping productivity. Frontiers of Agricultural Science and Engineering 0:0. doi:10.15302/J-FASE-2021414

Kaye JP, Quemada M (2017) Using cover crops to mitigate and adapt to climate change. A review. Agronomy for Sustainable Development 37. doi:10.1007/s13593-016-0410-x

Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. doi:10.3390/agriculture10110503

Kozak M, Piepho H-P (2018) What’s normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. Journal of Agronomy and Crop Science 204:86–98. doi:10.1111/jac.12220

Li L, Sun J, Zhang F, Guo T, Bao X, Smith FA, Smith SE (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290. doi:10.1007/s00442-005-0256-4

Li L, Tilman D, Lambers H, Zhang F-S (2014) Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. The New phytologist 203:63–69. doi:10.1111/nph.12778

Li L, Yang S, Li X, Zhang F, Christie P (1999) Interspecific complementary and competitive interactions between intercropped maize and faba bean. Plant and Soil 212:105–114. doi:10.1023/A:1004656205144

Livesley SJ, Stacey CL, Gregory PJ, Buresh RJ (1998) Sieve size effects on root length and biomass measurements of maize (Zea mays) and Grevillea robusta. Plant and Soil 207:183–193. doi:10.1023/A:1026461107110

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76. doi:10.1038/35083573

Mead R, Willey RW (1980) The Concept of a ‘Land Equivalent Ratio’ and Advantages in Yields from Intercropping. Experimental Agriculture 16:217–228. doi:10.1017/S0014479700010978

Mendiburu F de (2019) Agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-0. Available online at: https://CRAN.R-project.org/package=agricolae. (accessed January 26, 2021)

Mommer L, van Ruijven J, Caluwe H de, Smit-Tiekstra AE, Wagemaker CAM, Joop Ouborg N, Bögemann GM, van der Weerden GM, Berendse F, Kroon H de (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117–1127. doi:10.1111/j.1365-2745.2010.01702.x

Mulder CP, Uliassi DD, Doak DF (2001) Physical stress and diversity-productivity relationships: the role of positive interactions. Proceedings of the National Academy of Sciences 98:6704–6708. doi:10.1073/pnas.111055298

Poeplau C, Don A, Schneider F (2021) Roots are key to increasing the mean residence time of organic carbon entering temperate agricultural soils. Global change biology 27:4921–4934. doi:10.1111/gcb.15787

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramirez-Garcia J, Martens HJ, Quemada M, Thorup-Kristensen K (2015) Intercropping effect on root growth and nitrogen uptake at different nitrogen levels. Journal of Plant Ecology 8:380–389. doi:10.1093/jpe/rtu024

Ryser P (2006) The mysterious root length. Plant and Soil 286:1–6. doi:10.1007/s11104-006-9096-1

Schröder D, Köpke U (2012) Faba bean (Vicia faba L.) intercropped with oil crops – a strategy to enhance rooting density and to optimize nitrogen use and grain production? Field Crops Research 135:74–81. doi:10.1016/j.fcr.2012.07.007

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

Thorup-Kristensen K (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant and Soil 230:185–195. doi:10.1023/A:1010306425468

Thorup-Kristensen K, Magid J, Jensen LS (2003) Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy 79:227–302. doi:10.1016/S0065-2113(02)79005-6

Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science (New York, N.Y.) 294:843–845. doi:10.1126/science.1060391

Tosti G, Benincasa P, Farneselli M, Pace R, Tei F, Guiducci M, Thorup-Kristensen K (2012) Green manuring effect of pure and mixed barley – hairy vetch winter cover crops on maize and processing tomato N nutrition. European Journal of Agronomy 43:136–146. doi:10.1016/j.eja.2012.06.004

Tosti G, Benincasa P, Farneselli M, Tei F, Guiducci M (2014) Barley–hairy vetch mixture as cover crop for green manuring and the mitigation of N leaching risk. European Journal of Agronomy 54:34–39. doi:10.1016/j.eja.2013.11.012

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

Tribouillois H, Cohan J-P, Justes E (2016) Cover crop mixtures including legume produce ecosystem services of nitrate capture and green manuring: assessment combining experimentation and modelling. Plant and Soil 401:347–364. doi:10.1007/s11104-015-2734-8

Wendling M, Büchi L, Amossé C, Jeangros B, Walter A, Charles R (2017) Specific interactions leading to trans-gressive overyielding in cover crop mixtures. Agriculture, Ecosystems & Environment 241:88–99. doi:10.1016/j.agee.2017.03.003

White CM, DuPont ST, Hautau M, Hartman D, Finney DM, Bradley B, LaChance JC, Kaye JP (2017) Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture, Ecosystems & Environment 237:121–133. doi:10.1016/j.agee.2016.12.016

Yu Y, Stomph T-J, Makowski D, Zhang L, van der Werf W (2016) A meta-analysis of relative crop yields in cereal/legume mixtures suggests options for management. Field Crops Research 198:269–279. doi:10.1016/j.fcr.2016.08.001

Zhang C, Postma JA, York LM, Lynch JP (2014) Root foraging elicits niche complementarity-dependent yield advantage in the ancient ‘three sisters’ (maize/bean/squash) polyculture. Annals of botany 114:1719–1733. doi:10.1093/aob/mcu191

4 Oil radish, winter rye and crimson clover: root and shoot performance in cover crop mixtures

Roman Kemper[1], Thomas F. Döring[1], Nicole Legner[2], Catharina Meinen[2], Miriam Athmann[3]

[1] Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Bonn, Germany

[2] Division of Agronomy, Department of Crop Sciences, University of Göttingen, Göttingen, Germany

[3] Department of Organic Farming and Cropping Systems, University of Kassel, Witzenhausen, Germany

---

This chapter has been published as Kemper R, Döring TF, Legner N, Meinen C, Athmann M (2023) Oil radish, winter rye and crimson clover: root and shoot performance in cover crop mixtures. Plant and Soil. doi:10.1007/s11104-023-06240-y

Abstract

Purpose The current knowledge of below-ground interactions in intercropping systems is limited due to methodological constraints. The current study aimed to investigate cereal-brassica and cereal-legume-brassica cover crop mixtures regarding mixture effects on root and shoot biomass as well as root traits, vertical root niche differentiation, and complementarity.

Methods Sole crops and two- and three-species-mixtures of winter rye (*Secale cereale* L.), crimson clover (*Trifolium incarnatum* L.), and oil radish (*Raphanus sativus* L. var. *oleiformis* Pers.) were grown in Germany in a two-year organic field experiment. Root traits were analysed using the monolith method. For discrimination of species root mass Fourier transform infrared (FTIR) spectroscopy was used.

Results Oil radish dominated mixtures above- and below-ground. Oil radish and its mixtures had highest root length density (RLD) and root mass density (RMD) in subsoil. Rye had highest root biomass and RLD in topsoil. Clover was uncompetitive and had low RLD and RMD. Large but non-significant mixture effects occurred in the shoot, especially for shoot nutrient uptake. Mixture effects were positive for RLD and RMD in subsoil and positive for specific root length (SRL) throughout the whole profile. There was no clear evidence for vertical root niche differentiation and root mass complementarity.

Conclusion Oil radish as a mixing partner increased rooting in subsoil. When comparing mixtures and sole crops, morphological changes, i.e. higher SRL in mixtures, were found. Contrary to expectations, changes in root allocation patterns, such as vertical niche differentiation or complementarity of root mass, were not observed.

Keywords

catch crop, intercrop, root mass density, specific root length, complementarity, vertical root niche differentiation

4.1 Introduction

Roots of cover crops provide several ecosystem services in arable soils. Griffiths et al. (2022) grouped these services into the main categories: soil structural remediation, soil resource capture, maintenance of the rhizosphere, and building soil organic matter content. To maximise the ecosystem services provided by roots, intercropping has been suggested as a potentially promising approach. Intercropping is defined as growing two or more different crops on the same field for at least some time during the growing period. Above-ground, intercropping has the potential to outperform sole crops due to overyielding (Wendling et al. 2017) and complementarity by niche differentiation (Justes et al. 2021) and to insure against crop failure by compensation (Döring and Elsalahy 2022). It is reasonable to assume that such mechanisms exist below-ground as well. Thus, the agroecosystem services of roots might be enhanced in mixtures. However, in their review, Homulle et al. (2022) underlined that there is still a knowledge gap in root-root interactions in intercropping systems. For mixtures of cover crops, Lavergne et al. (2021) reported that there are inconsistent results of studies comparing mixtures with pure stands, and advocate more research on root traits of cover crop mixtures.

Root mass of cover crops is important for carbon storage (Kätterer et al. 2011; Poeplau and Don 2015) and root length is relevant for aggregate stability (Hudek et al. 2022) and nutrient uptake (Sainju et al. 1998). Thus, the cover crop mixtures were investigated for enhanced architectural root traits root length density (RLD) and root mass density (RMD) which are root length per soil volume and root mass per soil volume, respectively (Freschet et al. 2021). Besides, the vertical RMD profiles of sole and mixed cover crops need to be compared to test whether mixtures achieve higher soil exploitation. Moreover, we analysed the morphological root trait specific root length (SRL), which is root length per root mass and represents below-ground economics (Freschet et al. 2021). SRL is also considered as an acquisitive root trait (Isaac and Borden 2019) that is discussed to be linked with nutrient uptake efficiency (Eissenstat 1992).

Below-ground interactions have been well-studied for cereal-legume intercrops (Esnarriaga et al. 2020; Hauggaard-Nielsen et al. 2001; Hauggaard-Nielsen and Jensen 2005), relay-strip-intercropping systems (Li et al. 2006) and perennial cropping systems like clover-ryegrass mixtures (Dirks et al. 2021) or grasslands (Mommer et al. 2010; Oram et al. 2018). However, cover crops differ from relay-strip-intercrops in temporal niche differentiation and from main crop or perennial crops in the cropping duration. For cover crop mixtures, less research on roots has been conducted so far. Heuermann et al. (2019) attempted to quantify root biomass distribution of a four-species cover crop mixture and found root mass overyielding but less clear results for niche differentiation. Amsili and Kaye (2021) tried to compose a cover crop mixture with complementary root traits and complementary nitrogen acquisition strategies, but did not find more root carbon in the mixture compared to the best sole crop.

In a previous study (Kemper et al. 2020), we screened RLD and vertical root distribution of several cover crops. Based on these findings we suggested intercropping differently rooting cover crops to maximise root exploitation throughout the soil profile. In a further study (Kemper et al. 2022), we analysed root traits in cover crop mixtures of tap-rooted blue lupin (*Lupinus angustifolius* L.) and fibrous rooted winter rye (*Secale cereale* L.) and found weak evidence for shoot overyielding and root mass complementarity and no vertical niche differentiation of root mass density (RMD) between sole crops and mixtures, but positive mixture effects for specific root length (SRL).

Our cover crop root type screening (Kemper et al. 2020) revealed that oil radish (*Raphanus sativus* L. var. *oleiformis* Pers.) and winter rye had very different rooting patterns. Winter rye had a high RLD in topsoil and a shallow cumulative root distribution, whereas tap-rooted brassicas like oil radish had high subsoil RLD, deep cumulative root distribution and intensive use of biopores. From other studies on roots of cover crops, it is known that legumes with larger root diameters and low root length allocation to deep layers strongly differ from brassicas with highly branched and dense rooting systems (Bodner et al. 2019). Thus, we expected more intensive root complementarity when intercropping rye with oil radish than with lupin. Hence, in the present study, we investigated mixture effects on root traits of two cover crop mixtures with complementary root traits: deep-rooting tap-rooted oil radish and shallow-rooting fibrous rooted winter rye (OR-WR) as well as oil radish, the leguminous shallow-rooting crimson clover and winter rye (OR-CC-WR). Based on our findings of vertical root distribution screening (Kemper et al. 2020) and the lupin-rye mixture (Kemper et al. 2022) we aimed to test the following hypotheses:

1. In both mixtures, mixture effects of shoot biomass and nutrient uptake are positive.
2. Oil radish and mixtures containing oil radish have higher RLD and RMD in the subsoil compared to rye and crimson clover.

(3) As a result of complementary root architecture, positive mixture effects for RLD and RMD occur in topsoil and subsoil.

(4) SRL is increased in mixtures compared to sole cover crops.

(5) Vertical root niche differentiation occurs for rye and oil radish as rye has higher RMD than expected from sole crops in topsoil, and oil radish has a higher RMD than expected from sole crops in subsoil.

4.2 Materials and Methods

4.2.1 Experimental Site

The field experiments were performed at the experimental farm for Organic Agriculture ‘Wiesengut’ of the University of Bonn in Hennef (50°48′ N, 7°17′ E), Germany. At this site mean (1981-2010) annual precipitation is 840 mm and the mean air temperature is 10.3 °C. Precipitation and mean temperature were 714 mm and 11.8 °C in 2018 and 814 mm and 11.4 °C in 2019. The soil of the experimental field is a Fluvisol with a silt loam texture on gravel layers with a soil thickness of 0.6 to 2.0 m. More details about climate and soil are given in a previous study (Kemper et al. 2020). Soil mineral nitrogen content in 0-90 cm at the time of sowing was 23.7 and 91.7 kg/ha in 2018 and 2019, respectively.

4.2.2 Experimental setup and management

Field trials were performed in a randomized complete block design with four replicates, three of which were investigated for root traits. The same three replications were used for the calculation of root-shoot-ratios. The size of the experimental plot was 1.5 m × 12.5 m in 2018 and 1.5 m × 20 m in 2019.

Sole cover crops of winter rye (WR, *Secale cereale* L., cv. Bonfire), crimson clover (CC, *Trifolium incarnatum* L., cv. Linkarus) and oil radish (OR, *Raphanus sativus* L. var. *oleiformis* Pers., cv. Siletina) were sown with a sowing density of 120 kg/ha, 30 kg/ha and 25 kg/ha, respectively. Cover crop mixtures of oil radish, crimson clover and winter rye were sown as mixed and substitutive intercrops (Hauggaard-Nielsen et al. 2008). There was a 50:50 mixture of oil radish and winter rye (OR-WR) and a 33:33:33 mixture of oil radish, crimson clover and winter rye (OR-CC-WR). Sowing of cover crops was performed with a plot seeder (Hege, Waldenburg, Germany) with a 17 cm row distance on 12th and 14th August in 2018 and 2019, respectively. Pre-crop was spring-sown faba bean (*Vicia faba* L.) in both years. Tillage after faba bean harvest was carried out with a mouldboard plough (23 cm deep) and a rotary harrow. The trial was irrigated to ensure crop establishment. More details on the pre-crop history of the experimental field, tillage, weeding, irrigation and soil moisture are given in Kemper et al. (2022).

4.2.3 Shoot biomass, nutrient content and soil mineral nitrogen

Shoot biomass samples of the cover crops were collected on 15th and 13th November 2018 and 2019, respectively. Samples were cut on 1.0 m² per plot, and dried at 105 °C until constant weight. After milling, samples were analysed for their nitrogen (N), phosphorous (P) and potassium (K) content.

On 8th and 9th November 2018 and 13th November 2019 soil samples were taken for analysis of soil mineral nitrogen (N_{min}) using a Puerckhauer auger (inside diameter 18 mm). In 0-30 cm and 30-60 cm, soil samples were taken in both years and additionally in 60-90 cm in 2019. Eight samples per plot were mixed for the topsoil layers, and for the subsoil layers four samples per plot were mixed. For further information on milling and chemical analysis of plant and soil samples see Kemper et al. (2022).

4.2.4 Monolith method

To gain information about root mass and root length the monolith method by Böhm (1979) was used. From a trench, dug in the transverse direction to the plots (Fig. C1, supplementary material), monoliths were extracted in 10 cm intervals of soil depth. Monolith size was 10 cm × 10 cm × 20 cm in 2018 and 10 cm × 10 cm × 17 cm in 2019. These root extractions were done in October 2018 and 2019. Subsequently, roots were washed from the soil, separated manually from organic residues, scanned at 800 dpi (photo scanner, Expression 12000XL, Epson, Suwa, Japan) and analysed with WinRHIZO (Version WinRhizoPro 2019a 64bit, Regent Instruments, Quebec, Canada) for root length and dried for root dry mass determination. For further details on the washing process, sieve sizes, separation from non-root material, scanning and drying please consider the detailed method description in Kemper et al. (2022).

4.2.5 Fourier Transform Infrared Spectroscopy

The roots of OR, CC and WR collected in 2018 and 2019 were used to evaluate the root mass proportion of the different species in the cover crop mixtures. The dried root samples of sole and mixed cover crops harvested with the monolith method were ground to 0.2 mm (ultra-centrifugal mill, ZM 200, Retsch, Haan, Germany). Absorption spectra 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 4,000-400 cm^{-1}. Each sample was measured five 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 single sample species were used for cluster analysis (Opus software, version 7.2, Bruker Optics) to confirm the species separation. For the quantification of the proportion of the species in the mixed species root samples, the FTIR spectra of the single species samples were used to generate a model. With the chosen model for each year, the FTIR spectra of the mixed species samples were evaluated. The output of this evaluation was the percental share of root mass of each species within the root samples of the cover crop mixture. The percentage of the single species was used for further calculations on the mixtures root mass. For details about establishing and evaluating the model see supplementary material and Kemper et al. (2022). Information about the species-specific proportions of root length in the cover crop mixtures could not be derived.

4.2.6 Calculations

Based on the root length and root mass obtained from the samples, root length density (RLD), root mass density (RMD) and specific root length (SRL) were calculated for the monoliths. RLD is root length per soil volume in cm cm^{-3}, RMD is root mass per soil volume in mg cm^{-3} and SRL is root length per root mass in m g^{-1}. Statistical comparisons of RLD, RMD and SRL were done in combined soil layers of 0-10 cm, 10-30 cm, 30-60 cm and 60-100 cm.

The land equivalent ratio (LER) (Mead and Willey 1980) was used to analyse the land use efficiency of cover crop mixtures for root and shoot mass and shoot nutrient uptake. An LER >1 indicates a higher land use efficiency of intercrops compared to sole crops. LER is calculated by adding up partial land equivalent ratios pLER (equation 4.1). Y_i and M_i are biomass (g/m²) or nutrient uptakes (g/m²) of intercrop and sole crop of species i, respectively.

$$\mathrm{LER} = \sum \mathrm{pLER_i} = \sum \frac{Y_\mathrm{i}}{M_\mathrm{i}} \tag{4.1}$$

The mixture performance of root and shoot traits was assessed by computing relative mixture effects (RME). RME is the relative gain observed in the mixture compared to the expected value, which is the sole crops average weighted by relative density p_i (equation 4.2). Relative density is the ratio of mixture and sole crop density. Here, the values for p_i were 1/2 and 1/3 for two-species and three-way mixtures, respectively.

$$\mathrm{RME} = \frac{\sum(Y_\mathrm{i} - p_\mathrm{i} * M_\mathrm{i})}{\sum p_\mathrm{i} * M_\mathrm{i}} * 100 \tag{4.2}$$

The overall diversity effects (g/m²) can be interpreted as a net biodiversity effect (NE), which can be decomposed into the complementarity effect (CE) and the selection effect (SE), according to Loreau and Hector (2001). Following this concept, we used the notation of Elhakeem et al. (2019). NE is the difference between observed and expected yield of the mixture and the sum of CE and SE (equations 4.3 and 4.4). CE indicates facilitation or niche differentiation in mixtures whereas SE shows species dominance. Details about the interpretation of complementarity and selection effect are discussed in Kemper et al. (2022). Y_i, M_i and p_i are used as in the equations above.

$$\mathrm{NE} = \sum(Y_\mathrm{i} - p_\mathrm{i} * M_\mathrm{i}) \tag{4.3}$$

$$\mathrm{NE} = \mathrm{CE} + \mathrm{SE} \tag{4.4}$$

CE is the species number (N) multiplied by the average relative yield gain ($\Delta\overline{\mathrm{R}}\mathrm{Y}$) and the sole crops average yield $\overline{\mathrm{M}}$ (equation 4.5).

$$\mathrm{CE} = \mathrm{N} * \Delta\overline{\mathrm{R}}\mathrm{Y} * \overline{\mathrm{M}} \tag{4.5}$$

ΔRY, the relative yield gain, is the observed minus the expected relative yield (equation 4.6).

$$\Delta\mathrm{RY_i} = \mathrm{RY_i} - p_\mathrm{i} = \frac{Y_\mathrm{i}}{M_\mathrm{i}} - p_\mathrm{i} \tag{4.6}$$

SE is the species number multiplied by the covariance between relative yield gains and sole crop yields (equation 4.7).

$$\mathrm{SE} = \mathrm{N} * \mathrm{cov}(\Delta\mathrm{RY_i}, \mathrm{M_i}) \tag{4.7}$$

4.2.7 Statistical analysis

Data analysis was performed with R (version 3.5.2) and R studio (version 1.1.463) (R Core Team 2018). A one-factorial analysis of variance was conducted for shoot biomass, root biomass, root-shoot-ratio and mean of RLD, RMD, SRL in combined soil layers. Subsequently, mean comparisons were done with a Tukey test at a significance level of α=0.05 with the *agricolae* package (Mendiburu 2019). The prerequisites for analysis of variance, homogeneity of variances and normal distribution, were visually checked with a plot of residuals versus fitted values and a normal quantile-quantile plot, respectively (Kozak and Piepho 2018). If necessary, data were square root or logarithm transformed to meet the above-mentioned prerequisites. A one-sample t-test against 1 was used to test the significance of LER and one sample t-test against 0 was used to test the significance of RME and diversity effects. With a two-sample Welch t-test the percentage share of root mass and the difference between expected and observed shoot biomass, root biomass, RMD and pLER were tested.

4.3 Results

In 2018 and 2019, the months June to September were characterised by above-average temperatures as well as below-average precipitation and thus dry soil conditions. However, drought intensity in 2018 was stronger than in 2019, as not only the summer but also the autumn was dry (Kemper et al. 2020).

4.3.1 Shoot biomass and nutrient uptake in sole and mixed cover crops

Total shoot dry mass (DM) did not differ significantly between cover crops in 2018 (Fig. 4.1). In 2019, shoot DM of crimson clover was significantly lower compared to cover crops containing oil radish. Shoot DM of rye was significantly lower than those of oil radish as sole crop and the OR-CC-WR mixture. When mixture proportions changed compared to expected values, proportions of clover and rye were reduced whereas proportions of oil radish increased (Fig. 4.1), indicating differences in competitiveness among these species. Partial LER values were mostly in line with these results; in both mixtures, pLER of rye was significantly increased in 2018 and decreased in 2019, while pLER of crimson clover was significantly reduced in both years and pLER of oil radish was higher than expected in the OR-CC-WR mixture in 2019

(Tables C1, C2, supplementary material). High pLERs in stronger partners were partly associated with low pLERs in weaker partners, especially in 2019. As a consequence, the LER values did not differ significantly from 1 for shoot biomass or shoot nutrient uptake (Tables C1, C2, supplementary material).

The analysis of the mixture effects into their components (complementarity and selection effects) yielded variable results. For the shoot dry matter and nutrient uptake, CE values were not significantly different from 0, except for N uptake which was positive in the two-species-mixture in 2018; significant SE values were found for both mixtures in 2019 (Table 4.1, Table C4, supplementary material). Mixtures with non-legumes significantly reduced soil mineral N in the soil before winter compared to legume sole crops and bare fallow (Fig. C2, supplementary material).

4.3.2 Root biomass in sole and mixed cover crops

Total root DM did not differ significantly among cover crops in 2018 (Fig. 4.1). In 2019, root DM of crimson clover was significantly lower than for all other cover crops.

When mixture proportions changed compared to expected value, proportions of clover were reduced whereas proportions of oil radish increased. LER values for root mass were < 1, except in the OR-CC-WR mixture which reached 1.35 in 2019 (Tables C1 and C2, supplementary material). However, LER values for root mass were not significantly different from 1. pLER of rye root mass was significantly reduced in both mixtures in 2019. As for shoot, complementarity effects (CE) of root mass were not significantly different from 0.

Root-shoot-ratio did not differ significantly among cover crops in 2018 (Fig. 4.1). In 2019, rye had the highest root-shoot-ratio and differed significantly from oil radish, crimson clover and mixtures.

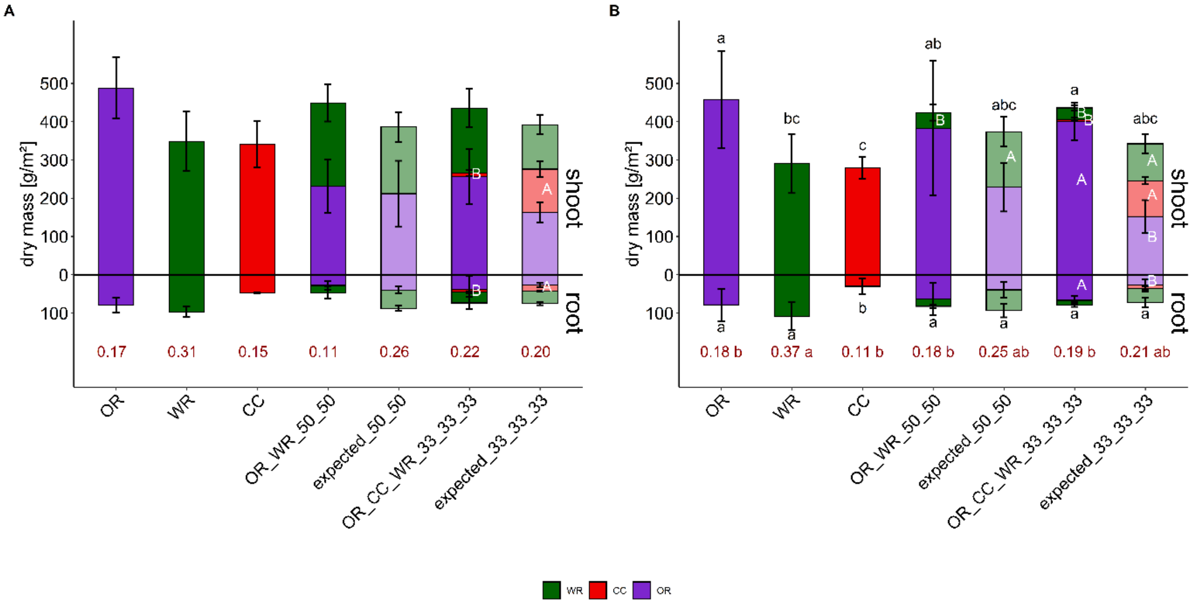

Fig. 4.1 Mean values ± standard deviation of shoot (n = 4) and root (n = 3) dry mass [g/m²] and mean values of root-shoot-ratio (n = 3) of sole cover crops oil radish (OR), winter rye (WR), crimson clover (CC), 50:50 cover crop mixtures of oil radish and winter rye (OR_WR_50_50), 33:33:33 cover crop mixtures of oil radish, crimson clover and winter rye (OR_CC_WR_33_33_33) and of expected mixtures (corresponding proportions of sole crops) in 2018 (A) and 2019 (B). Bars above 0 represent shoot dry mass, bars below 0 show root dry mass. Bar colours refer to species: violet for radish, green for rye, red for clover. Transparent bars represent expected values. Red numbers below bars represent root-shoot-ratio. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-Test) between different cover crops for total shoot or total root dry mass or root-shoot-ratio within one year. Different uppercase letters indicate significant differences ($p \leq 0.05$, t-test) between observed and expected dry mass for shoot or root of intercrop partner within one year.

Table 1.1 Mean values ± standard deviation of net biodiversity effect (NE), complementarity effect (CE) and selection effect (SE) of a 50:50 mixture of oil radish and winter rye (OR-WR) and a 33:33:33 mixture of oil radish, crimson clover and winter rye (OR-CC-WR) for shoot dry matter (DM) [g/m²] (n = 4) and root dry matter (DM) [g/m²] (n = 3). Effects significantly different from 0 ($p \leq 0.05$, t-test) are given in bold.

mixture	year	parameter	NE	CE	SE
OR-WR	2018	shoot DM	31.2 ± 65.3	41.4 ± 65.8	-10.2 ± 15.6
OR-WR	2018	root DM	-41.3 ± 23.7	-39.0 ± 26.6	-2.3 ± 4.7
OR-WR	2019	shoot DM	50.1 ± 93.5	-3.4 ± 64.6	**53.5 ± 32.1**
OR-WR	2019	root DM	-11.0 ± 12.0	-0.8 ± 13.2	-10.2 ± 10.4
OR-CC-WR	2018	shoot DM	43.4 ± 94.0	19.8 ± 83.9	23.6 ± 19.1
OR-CC-WR	2018	root DM	-1.2 ± 44.5	-2.0 ± 45.5	0.8 ± 2.3
OR-CC-WR	2019	shoot DM	93.8 ± 73.8	6.1 ± 965	**87.6 ± 27.2**
OR-CC-WR	2019	root DM	6.8 ± 33.8	11.0 ± 55.4	-4.1 ± 23.0

4.3.3 Root length density, root mass density and specific root length

Topsoil RLD in both years was highest for rye as a sole crop (Fig. 4.2A, B). In line with the root mass results, the RLD of crimson clover as a sole crop was generally low. In the lower subsoil (60-100 cm) RLD of crimson clover and rye as sole crops was lower than for the cover crop treatments with oil radish.

RMD of rye as a sole crop was highest between 10 and 30 cm in both years (Fig. 4.2C, D). In the lower subsoil (60-100 cm) RMD of sole crop crimson clover was lowest and RMD of sole crop oil radish and the OR-CC-WR mixture were highest.

Generally, SRL of rye tended to be lower than SRL of crimson clover, oil radish and the mixtures below 10 cm. SRL of oil radish was significantly higher than that of rye and crimson clover in the 10-30 cm soil layer in 2018 (Fig. 4.2E). In 2019, SRL of rye was significantly lower than that of oil radish and both mixtures in the 30-60 cm soil layer (Fig. 4.2F).

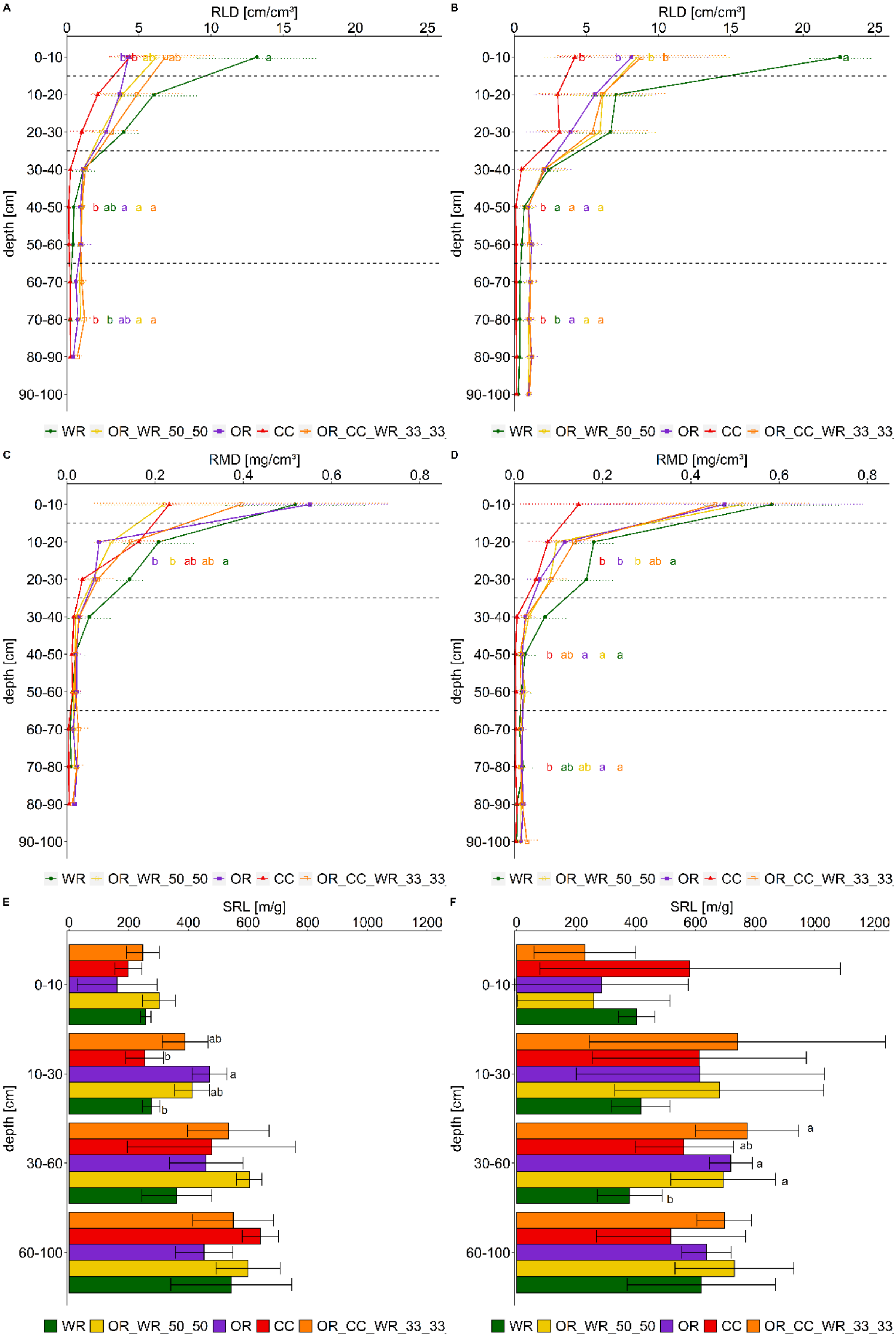
A
RLD [cm/cm³]
0
5
10
15
20
25
0-10
10-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
depth [cm]
b b ab ab a
b ab a a a
b b ab a a
WR
OR_WR_50_50
OR
CC
OR_CC_WR_33_33
B
RLD [cm/cm³]
b b b b a
b a a a a
b b a a a
C
RMD [mg/cm³]
0.0
0.2
0.4
0.6
0.8
b b ab ab a
D
RMD [mg/cm³]
b b b ab a
b ab a a a
b ab ab a a
E
SRL [m/g]
0
200
400
600
800
1000
1200
0-10
10-30
30-60
60-100
ab
b
a
ab
b
F
SRL [m/g]
a
ab
a
a
b

Fig. 4.2 Mean values ± standard deviation (n = 3) of root length density [cm/cm³] (RLD) (A, B), root mass density [mg/cm³] (RMD) (C, D) and specific root length [m/g] (SRL) (E, F) of sole cover crops oil radish (OR), winter rye (WR), crimson clover (CC), 50:50-cover crop mixtures of radish and rye (OR_WR_50_50), 33:33:33-cover crop mixtures of radish and rye (OR_CC_WR_33_33_33) in 2018 (A, C, E) and 2019 (B, D, F) at different soil depth levels. Colours refer to cover crops: green for rye, yellow for 50:50-mixture, orange for 33:33:33-mixture, red for clover and violet for radish. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different cover crops within the different soil depth layers (0-10 cm, 10-30 cm, 30-60 cm and 60-100 cm) within one year.

4.3.4 Below-ground mixture effects

Relative mixture effects of RLD in both mixtures were negative or positive in topsoil and total profile (Fig. 4.3). With increasing soil depth, RME of RLD increased. A significantly positive RME of RLD was found in the lower subsoil for the OR-CC-WR mixture in 2019.

RME of RMD was partly negative in the topsoil and total profile, whereas it was positive in the lower subsoil in both mixtures and years (Fig. 4.3). RME of RMD between 10-30 cm was significantly negative for the OR-WR mixture in 2019.

Relative mixture effects of SRL were positive for both mixtures and years in all soil layers except in 0-10 cm in 2019 (Fig. 4.3). RME of SRL between 10-30 cm were significantly positive for the OR-WR mixture in 2019.

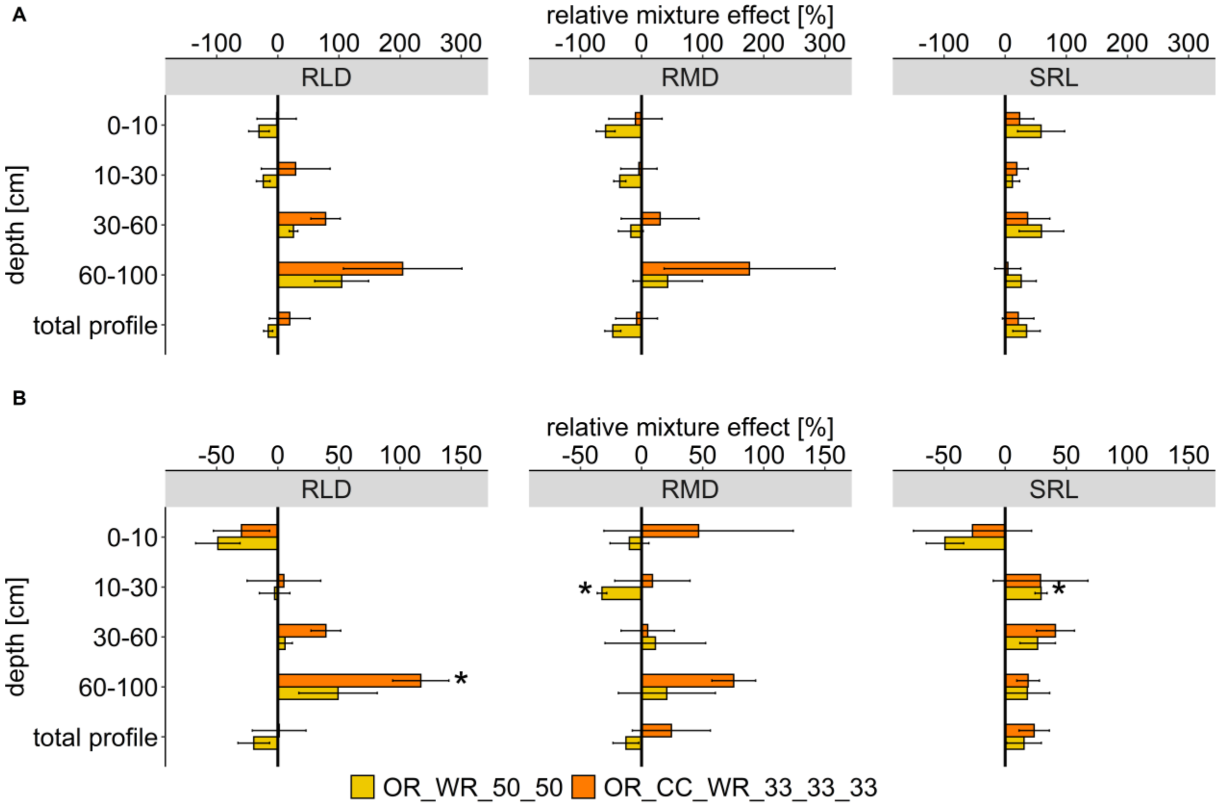

Fig. 4.3 Mean values ± standard error (n = 3) of relative mixture effect [%] for root length density (RLD), root mass density (RMD) and specific root length (SRL) for 50:50 cover crop mixtures of radish and rye (OR_WR_50_50) and 33:33:33 cover crop mixtures of radish, clover and rye (OR_CC_WR_33_33_33) in 2018 (A) and 2019 (B) at different soil depth levels and for the total soil depth profile. Colours refer to cover crop mixtures: yellow for 50:50-mixture and orange for 33:33:33-mixture. Asterisks indicate a significant difference in RME from 0 ($p \leq 0.05$, t-test).

4.3.5 Species-specific root mass proportions and vertical niche differentiation of RMD between sole and mixed cover crops

FTIR results revealed that the root mass density was dominated by oil radish below 30 cm in both mixtures and years (Fig. 4.4). In the topsoil winter rye reached shares of RMD comparable to oil radish except for the OR-CC-WR mixture in 2019. RMD shares for crimson clover were low across all depths in both years.

In the analysis of niche differentiation, observed and expected RMD of oil radish did not differ significantly in both mixtures and years (Fig. 4.5B, E, G, J). Observed RMD of winter rye was lower than expected RMD in single topsoil layers in both years in the OR-WR mixture (Fig.

4.5A, F) and in 2019 in the OR-CC-WR mixture (Fig. 4.5H). Crimson clover had a lower than expected RMD in topsoil in both years (Fig. 4.5D, I).

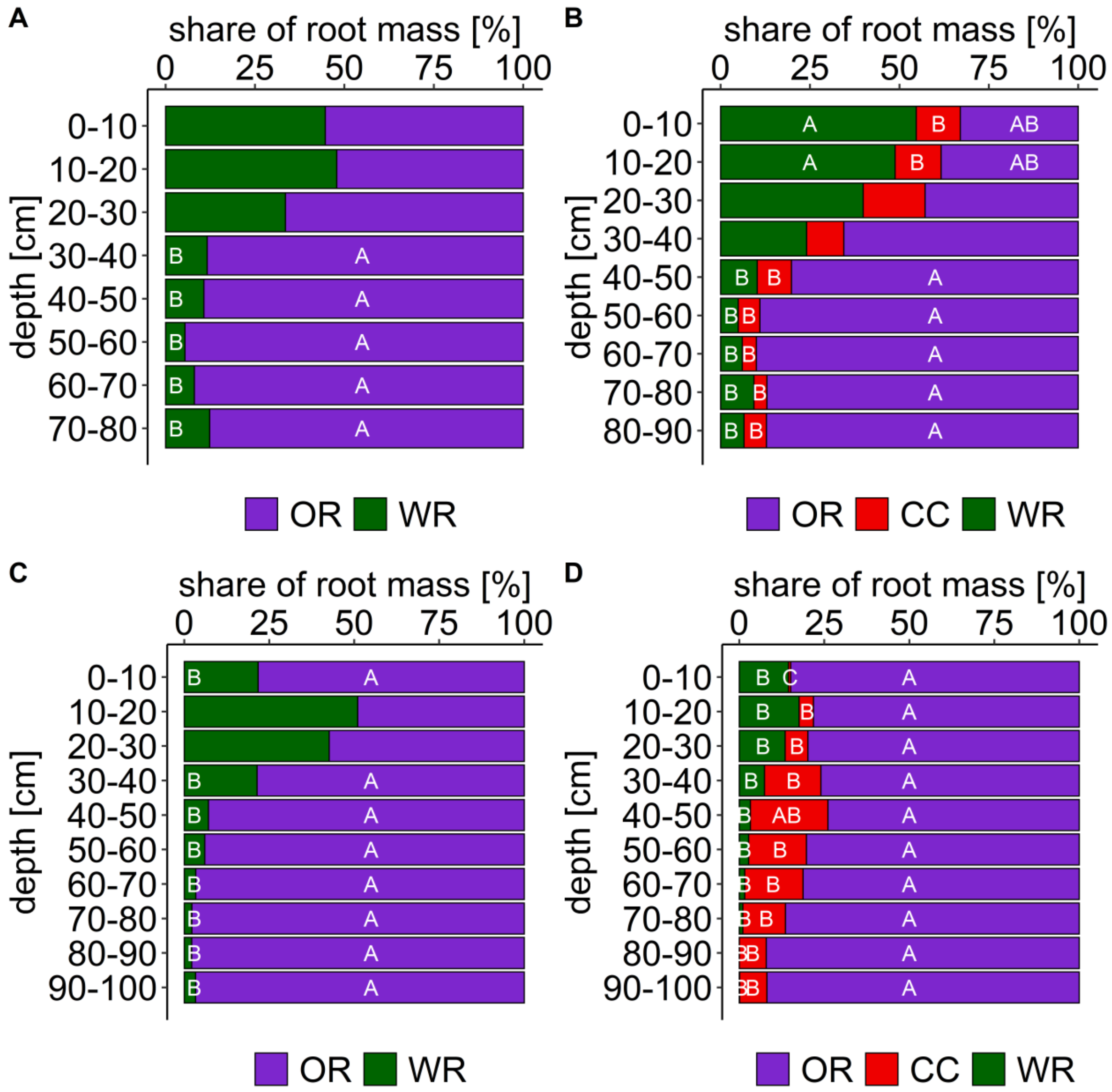

Fig. 4.4 Mean values (n=3) of root mass share [%] of oil radish (violet), crimson clover (red) and winter rye (green) at different soil depth in 2018 (A, B) and 2019 (C, D) in a 50:50 cover crop mixture of oil radish and winter rye (A, C) and in a 33:33:33 cover crop mixture of oil radish, crimson clover and winter rye (B, D). Different uppercase letters indicate significant differences ($p \leq 0.05$, t-Test) between proportions of root mass of mixture partners within one soil depth layer.

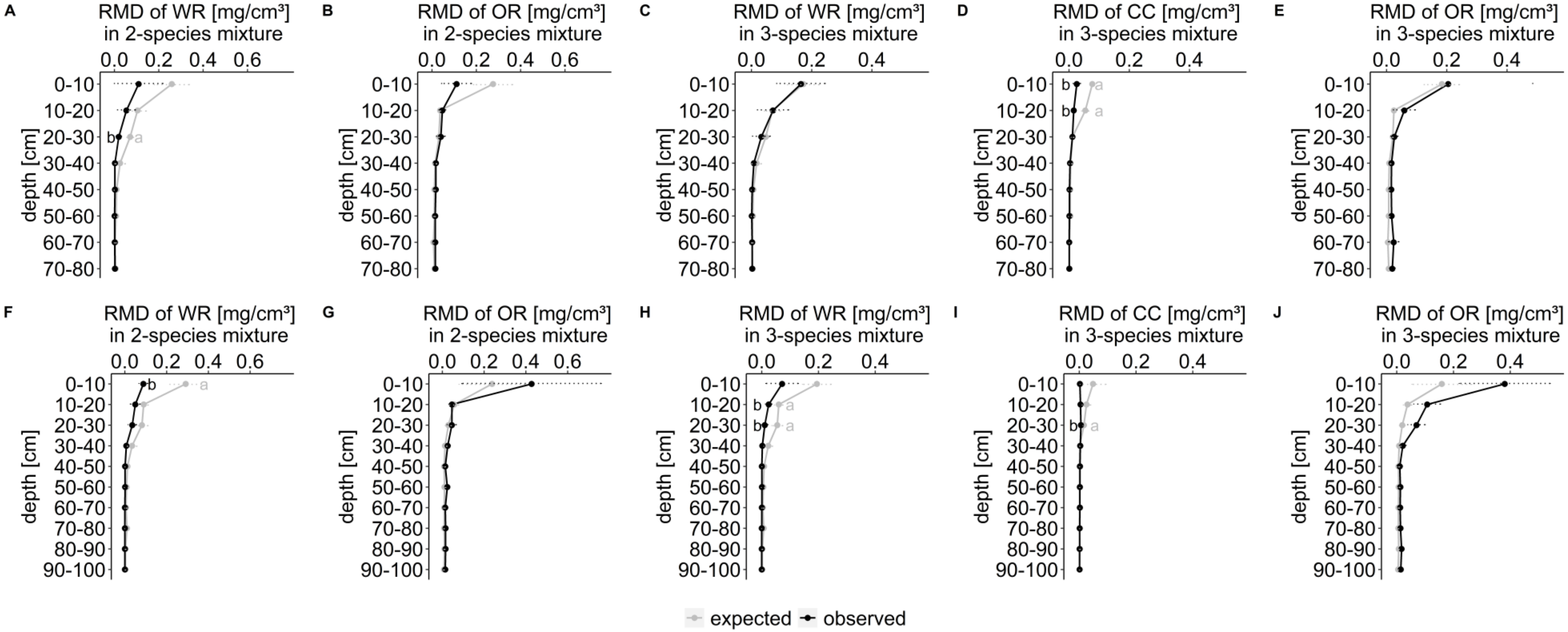

Fig. 4.5 Mean values ± standard deviation (n = 3) of observed (black) and expected (grey) RMD of winter rye (A, F) and oil radish (B, G) in a 50:50 cover crop mixture of oil radish and winter rye and of winter rye (C, H), crimson clover (D, I) and oil radish (E, J) in a 33:33:33 cover crop mixture of oil radish, crimson clover and winter rye in 2018 (A, B, C, D, E) and 2019 (F, G, H, I, J). Different lowercase letters indicate significant differences ($p \leq 0.05$, t-test) between observed and expected RMD within one soil depth layer for one crop partner.

4.4 Discussion

4.4.1 Biomass in sole and mixed cover crops

Unlike winter rye and crimson clover, oil radish completed its transition from vegetative to generative development before winter which is usually accompanied by a higher growth rate. This resulted in significantly higher shoot dry mass of cover crop treatments containing oil radish compared to the winter hardy sole crops rye and crimson clover (Fig. 4.1). Furthermore, crucifers have a high competitiveness for abiotic resources (Couëdel et al. 2018) in cover crop mixtures. Moreover, oil radish is known to be dominant due to its early development and horizontal leaf orientation, leading to a high light interception and shading of other mixture partners (Elhakeem et al. 2021b). Crimson clover was not able to compete with oil radish or winter rye, as its biomass share was consistently lower than expected from sole crops (Fig. 4.1). Crimson clover was not only weak in the mixture, root biomass was also low in the sole crop. This observation is in line with the results obtained by Amsili and Kaye (2021) in a five-species mixture with the lowest root biomass carbon found for crimson clover. In our study, winter rye had the highest root biomass in both years and the highest root-shoot-ratio (Fig. 4.1). Similarly, Amsili and Kaye (2021) observed the highest root biomass and root-shoot-ratio in triticale.

The LER values underlined the above-mentioned results. In the OR-WR mixture, root LER was below 1 in both years and shoot LER was only greater in 2018 than 1 (Table C1, supplementary material). In this year, there were two strong partners (oil radish and winter rye with significantly enhanced pLER), contributing equally to shoot performance of the mixture. In contrast, in 2019 oil radish dominated the mixture, while winter rye growth was weak, yielding an overall shoot LER < 1. Yu et al. (2016) discussed in their meta-analysis that a competitive balance in species mixtures is important for the performance of the mixture. In the OR-CC-WR mixture, pLER values for crimson clover were always low (Table C2, supplementary material). Thus, this mixture consisted of two strong (oil radish and winter rye) and one weak partner in 2018, and of one dominating (oil radish) and two weak partners in 2019. Overall, positive but non-significant mixture effects of shoot parameters occurred in the three-species mixtures. Our first hypothesis that there are positive mixture effects on shoot biomass and nutrient uptake was confirmed only for conditions resulting in balanced species composition.

Complementarity effects (CE) for N uptake were positive in 2018 (Table C3, supplementary material), showing that two strongly competing partners increased their N uptake by complementarity. The strong oil radish growth resulted in a positive and significant selection effect (SE) for shoot mass in both mixtures in 2019 illustrating the imbalance in mixture. The gain of oil radish was higher than the loss observed of winter rye and crimson clover. Likewise, other studies emphasised the importance of species identity and a concomitant high selection effect in cover crop mixtures (Freund et al. 2020; Wendling et al. 2019). In line with our results, complementarity effects in the literature are variable, but CE for root mass are mostly missing. In another study with cover crop mixtures (Elhakeem et al. 2019) positive CE were found for above-ground biomass. Grassland studies examined above-ground CE and the influence of root traits (Bakker et al. 2016; Felten and Schmid 2008). Felten and Schmid (2008) showed that rooting depth does not necessarily enhance CE of above-ground biomass in a short-duration grassland species pot experiment. Bakker et al. (2016) observed no relationship between root trait diversity and above-ground CE in a grassland biodiversity experiment.

Other grassland studies (Mommer et al. 2010; Oram et al. 2018) also reported positive CE for below-ground biomass. Oram et al. (2018) showed that root mass CE increased with species richness, but there was no evidence that functional diversity of rooting patterns was relevant for root mass CE. Instead, a weak but significant relationship between CE and deep rooting was found. However, it needs to be considered that they examined roots only until 40 cm soil depth. In our previous experiment, differences in rooting depth were only found for crimson clover and oil radish grown as sole crops in 2018 (Kemper et al. 2020). Detecting hardly positive CE for below-ground biomass in cover crop mixtures in the current study compared to grassland studies (Dirks et al. 2021; Mommer et al. 2010; Oram et al. 2018), may be due to the shorter growth period of cover crops compared to grassland species, as previously discussed by Kemper et al. (2022).

4.4.2 Root traits and their mixture effects

In our root trait analysis, the highest RLD values were observed for winter rye within the topsoil (0-30 cm, Fig. 4.2). RLD and RMD for crimson clover were low in top- and subsoil. For both RLD and RMD, high values were found in the subsoil for oil radish and mixtures containing oil radish. High values for RLD, root intensity and root growth rate of oil radish were previously shown by Wendling et al. (2016), Kristensen and Thorup-Kristensen (2004) and Thorup-Kristensen (2001), respectively. Based on these results, we can confirm the second hypothesis, that oil radish and mixtures containing oil radish differ in RLD and RMD in the subsoil compared to rye and crimson clover. Thus, positive RME for RLD and RMD were mainly found in the subsoil (Fig. 3), due to the taproot architecture of oil radish with a strong proliferation of roots in deeper soil layers (Bodner et al. 2019). In a previous experiment (Kemper et al. 2020), it was shown that oil radish intensively occupied biopores in the subsoil. This further explains higher root quantities observed in the subsoil in mixtures containing oil radish. Thus, mixtures with oil radish ensure rooting into the subsoil which may lead to enhanced nutrient uptake from the subsoil and prevention of leaching. This can be seen from the soil mineral N data in Fig. C2, supplementary material. Hence, our third hypothesis, that positive mixture effects for RLD and RMD occur for two- and three-species mixtures in topsoil and subsoil, can only be accepted for subsoil, not for topsoil.

Our results raise the question of why there is no strong mixture effect for RMD and RLD in the topsoil. For shoot biomass, Elhakeem et al. (2021a) detected that the average shoot biomass was greater in mixtures than in sole crops, but it was similar when mixtures were compared to the highest yielding pure stands. In another study, Elhakeem et al. (2021b) showed that mixture performance is governed by radiation interception of the dominant species. The more competitive partner was faster in ground cover and consequently contributed more to ground cover. Similar mechanisms of dominance may be true for below-ground interactions, as oil radish has a higher rooting depth penetration rate than winter rye (Thorup-Kristensen 2001). This might explain the higher soil volume and root mass dominance of oil radish. Thus, winter rye and crimson clover were outcompeted for root growth in mixtures, which become similar to pure stand of the dominant species.

In both years and mixtures, the RME of SRL was positive, except 0-10 cm in 2019. This confirms our fourth hypothesis and is in line with the results observed for lupin-rye mixtures (Kemper et al. 2022). In a situation with a competing mixture partner plants may increase root length for better nutrient and water acquisition without changing investment in root dry matter, as SRL is associated with below-ground economics (Freschet et al. 2021).

4.4.3 Vertical root niche differentiation

The root mass percentages, revealed by the FTIR analysis, did not show a clear pattern with homorhizous winter rye dominating the topsoil and allorhizous oil radish dominating the subsoil (Fig. 4.4). Oil radish dominated top- and subsoil rooting in 2019, while in 2018 winter rye was present to the same extent as oil radish at least in the topsoil. In both mixtures, there was no difference between expected and observed RMD for oil radish (Fig. 4.5), thus no indication for niche differentiation was found. Winter rye was outcompeted for its niche in upper soil layers by oil radish in the OR-CC-WR mixture in 2019 and in the OR-WR mixture in both years. The same was detected for crimson clover in both years. Thus, our data lead to the rejection of our fifth hypothesis regarding vertical root niche differentiation.

Likewise, in a mustard-phacelia-oat-clover mixture, the weaker components clover (*Trifolium alexandrinum* L.) and oat (*Avena strigosa* Schreb.) were outcompeted for their niche by phacelia (*Phacelia tanacetifolia* Benth.) and mustard (*Sinapis alba* L.), which occupied the same vertical niche as in sole crops (Heuermann et al. 2019). For white clover and perennial ryegrass, Dirks et al. (2021) found the same vertical niche of clover and grass root mass in two of three years. Mommer et al. (2010) reported that below-ground complementarity and overyielding were not due to vertical nice differentiation, but rather resulted from enhanced rooting in topsoil of one species.

Vertical niche differentiation and mixture effects might not have been established pronouncedly in our experiment due to a certain imbalance between the mixture partners. Murrell et al. (2017) showed that the productivity of mixtures is not higher than the best performing monoculture, especially when the mixture is dominated by this species. To achieve diverse and well-balanced mixtures, they suggested the following two strategies: (1) sow early and (2) reduce the sowing density of aggressive species. Elhakeem et al. (2021b) suggested that if species with slow initial growth or low radiation use efficiency are used as mixture components, the proportion of these species should be increased to facilitate their competitiveness. Bybee-Finley et al. (2022) provided a framework to adjust seed density ratios of cover crop mixtures with the objective to avoid dominating competitive species in the mixture and identifying relative seeding rates that produce high biomass with high species evenness. A well-designed mixture might more likely show vertical niche differentiation and mixture effects.

4.4.4 Below-ground interactions in cover crop mixtures of oil radish, winter rye and crimson clover

Different root traits represent specific functions of cover crop roots. Root intensity for improvement of aggregate stability can be represented by topsoil RLD (Hudek et al. 2022) and root intensity for nutrient uptake by subsoil RLD (Thorup-Kristensen 2001; Sainju et al. 1998). Nutrient uptake efficiency can be linked to SRL (Eissenstat 1992; Freschet et al. 2021). Carbon storage potential is determined by root mass (Kätterer et al. 2011; Poeplau and Don 2015) and rhizodeposition (Pausch and Kuzyakov 2018). The latter was not measured in the current study. N provision is linked to N_2-fixation. Having measured these traits in cover crop mixtures, we can assess the effects of below-ground functional diversity. In particular, there are potential effects of species identity and of mixing (Table 4.2). The mixture effect is the comparison of the mixture with the average of sole crops, called overyielding when positive. The identity effect shows that the performance of the mixture is determined due to the identity of the species in the mixture. Mixture effects were found for SRL and subsoil RLD. For both traits there was also an identity effect of oil radish, which was dominating the mixtures. The mixture effect of subsoil RLD occurred due to intensive subsoil rooting and biopore use of oil radish (Kemper et al. 2020). Topsoil structure may be improved by winter rye. N can be provided by N_2-fixing crimson clover, yet this species was suppressed in mixtures because of its low growth rate. Both winter rye and oil radish achieve high root biomass, but no root biomass overyielding in mixtures was found. Carbon storage potential is not only represented by root biomass, but also by rhizodeposition; while this was not measured in the current study, it might be important in cover crop mixtures (Gentsch et al. 2020).

Table 4.2 Identity and mixture effects of sole and mixed cover crops for different root traits representing specific functions of cover crop roots. The identity effect is given due to the species contained in the mixture: WR=winter rye, OR=oil radish, CC=crimson clover. The mixture effect, which is comparing the mixture with the mean of the sole crops, can be negative (-), positive (+), not existing (0) or was not analysed (?).

function of cover crop root	topsoil structure/ aggregate stability	subsoil nutrient uptake/ nitrate leaching prevention	nutrient uptake efficiency	N provision	carbon storage potential	
associated root trait	topsoil RLD	subsoil RLD	SRL	N_2-fixation	root dry mass	rhizodeposition
identity effect	WR	OR	OR	CC	WR/OR	?
mixture effect	0/-	+	+	-	0	?
evidence	Fig. 4.2 A, B Fig. 4.3	Fig. 4.2 A, B Fig. 4.3	Fig. 4.2 E, F Fig. 4.3	Fig. 4.4 B, D Fig. 4.5 D, I	Fig. 4.1 Fig. 4.3	-

Contrary to our assumptions, the mixing effects of the architectural root traits RLD and RMD were only found in the subsoil, whereas root biomass overyielding and a change in root allocation patterns (vertical niche differentiation of RMD between sole and mixed crops) were not detected. In contrast, there was a clear positive mixing effect for the morphological root trait specific root length (SRL). An increase in this acquisitive root trait SRL was previously found for lupin-rye mixtures (Kemper et al. 2022), faba bean-triticale mixtures (Esnarriaga et al. 2020) and vetch-rye mixtures (Bukovsky-Reyes et al. 2019). Changes in SRL might also result in altered root longevity and root turnover (Ostonen et al. 2007; Ryser 1996). For trees it was found that root exudation was increased with higher SRL (Meier et al. 2020; Yin et al. 2023). For herbaceous crop species, such a relationship has also been proposed, but could not be confirmed in a first attempt comparing whole root systems of different plant species (Roumet et al. 2008). To our knowledge, to date there are no studies attempting to relate root exudation to roots of different sizes or orders within the same species. Gentsch et al. (2020) found higher rhizodeposition in diverse cover crop mixtures compared to sole cover crops. Thus, it might be that for short term grown cover crops mixture effects are less found in change of root allocation patterns but mixture effects are rather shaped by morphological adaptions which might push rhizodeposition and microbial processes.

4.5 Conclusion

In mixtures, oil radish was dominant in shoot and root underlining the importance of species identity in cover crop mixtures. Positive mixture effects for RLD and RMD were more frequent in the subsoil due to a strong rooting of oil radish if integrated as a mixture partner. Consistently positive mixture effects were found for SRL, i.e. mixing makes roots thinner. Root mass complementarity, vertical root niche differentiation and mixture effects in topsoil might have not occurred pronouncedly because partners were not sufficiently balanced in mixtures. Thus, future research should focus on the balance when mixing different cover crop species. More conceptual planning of seed density ratios and mixture composition is advised to design well-balanced cover crop mixtures to exploit mixture effects above- and below-ground more effectively. Mixture effects seem to be more prominent for morphological than architectural root traits. Enhanced SRL in mixtures might be linked to higher root turnover and rhizodeposition which would influence cover crop functions such as carbon storage and microbial activity. Future research on roots of cover crop mixtures should consider root turnover, rhizodeposition, microbial processes as well as other acquisitive root traits such as root tips, branching and root N content.

We have given first insights into below-ground interactions of mixed cover crops and mixture effects on their root traits in autumn. Future root research will profit from methods that allow an understanding of below-ground interaction throughout the crop growth period.

Acknowledgements

We thank the technical staff of the 'Wiesengut' organic farming research station and our students for help in the field and laboratory.

Funding

The study was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Author contributions

M.A. conceived the idea, planned the research and designed the experiments. R.K. conducted the experiments in the field, processed the root samples and collected the data. N.L. and C.M. performed the FTIR analysis. R.K. analysed the data and wrote the article. M.A., T.F.D. N.L. and C.M. contributed to data interpretation, writing and editing of the article. All authors read and approved the final manuscript.

Declarations

The authors declare no conflict of interest.

References

Amsili JP, Kaye JP (2021) Root traits of cover crops and carbon inputs in an organic grain rotation. Renewable Agriculture and Food Systems 36:182–191. doi:10.1017/S1742170520000216

Bakker LM, Mommer L, van Ruijven J (2016) Can root trait diversity explain complementarity effects in a grassland biodiversity experiment? Journal of Plant Ecology 11:rtw111. doi:10.1093/jpe/rtw111

Bodner G, Loiskandl W, Hartl W, Erhart E, Sobotik M (2019) Characterization of Cover Crop Rooting Types from Integration of Rhizobox Imaging and Root Atlas Information. Plants 8. doi:10.3390/plants8110514

Böhm W (1979) Methods of Studying Root Systems, Vol 33. Springer, Berlin, Heidelberg

Bukovsky-Reyes S, Isaac ME, Blesh J (2019) Effects of intercropping and soil properties on root functional traits of cover crops. Agriculture, Ecosystems & Environment 285:106614. doi:10.1016/j.agee.2019.106614

Bybee-Finley KA, Cordeau S, Yvoz S, Mirsky SB, Ryan MR (2022) Finding the right mix: a framework for selecting seeding rates for cover crop mixtures. Ecological applications : a publication of the Ecological Society of America 32:e02484. doi:10.1002/eap.2484

Couëdel A, Alletto L, Tribouillois H, Justes É (2018) Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agriculture, Ecosystems & Environment 254:50–59. doi:10.1016/j.agee.2017.11.017

Dirks I, Streit J, Meinen C (2021) Above and Belowground Relative Yield Total of Clover–Ryegrass Mixtures Exceed One in Wet and Dry Years. Agriculture 11:206. doi:10.3390/agriculture11030206

Döring TF, Elsalahy H (2022) Quantifying compensation in crop mixtures and monocultures. European Journal of Agronomy 132:126408. doi:10.1016/j.eja.2021.126408

Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15:763–782. doi:10.1080/01904169209364361

Elhakeem A, Bastiaans L, Houben S, Couwenberg T, Makowski D, van der Werf W (2021a) Do cover crop mixtures give higher and more stable yields than pure stands? Field Crops Research 270:108217. doi:10.1016/j.fcr.2021.108217

Elhakeem A, van der Werf W, Ajal J, Lucà D, Claus S, Vico RA, Bastiaans L (2019) Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. Agriculture, Ecosystems & Environment 285:106627. doi:10.1016/j.agee.2019.106627

Elhakeem A, van der Werf W, Bastiaans L (2021b) Radiation interception and radiation use efficiency in mixtures of winter cover crops. Field Crops Research 264:108034. doi:10.1016/j.fcr.2020.108034

Esnarriaga DN, Mariotti M, Cardelli R, Arduini I (2020) The Importance of Root Interactions in Field Bean/Triticale Intercrops. Plants 9. doi:10.3390/plants9111474

Felten S von, Schmid B (2008) Complementarity among species in horizontal versus vertical rooting space. Journal of Plant Ecology 1:33–41. doi:10.1093/jpe/rtm006

Freschet GT, Pagès L, Iversen CM, Comas LH, Rewald B, Roumet C, Klimešová J, Zadworny M, Poorter H, Postma JA, Adams TS, Bagniewska-Zadworna A, Bengough AG, Blancaflor EB, Brunner I, Cornelissen JHC, Garnier E, Gessler A, Hobbie SE, Meier IC, Mommer L, Picon-Cochard C, Rose L, Ryser P, Scherer-Lorenzen M, Soudzilovskaia NA, Stokes A, Sun T, Valverde-Barrantes OJ, Weemstra M, Weigelt A, Wurzburger N, York LM, Batterman SA, Gomes de Moraes M, Janeček Š, Lambers H, Salmon V, Tharayil N, McCormack ML (2021) A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytologist 232:973–1122. doi:10.1111/nph.17572

Freund L, Mariotte P, Santonja M, Buttler A, Jeangros B (2020) Species identity, rather than species mixtures, drives cover crop effects on nutrient partitioning in unfertilized agricultural soil. Plant and Soil. doi:10.1007/s11104-020-04782-z

Gentsch N, Boy J, Batalla JDK, Heuermann D, Wirén N von, Schweneker D, Feuerstein U, Groß J, Bauer B, Reinhold-Hurek B, Hurek T, Céspedes FC, Guggenberger G (2020) Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. Biology and Fertility of Soils 56:943–957. doi:10.1007/s00374-020-01475-8

Griffiths M, Delory BM, Jawahir V, Wong KM, Bagnall GC, Dowd TG, Nusinow DA, Miller AJ, Topp CN (2022) Optimisation of root traits to provide enhanced ecosystem services in agricultural systems: A focus on cover crops. Plant, cell & environment 45:751–770. doi:10.1111/pce.14247

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236:63–74. doi:10.1023/A:1011909414400

Hauggaard-Nielsen H, Jensen ES (2005) Facilitative root interactions in intercrops. In: Lambers H, Colmer TD (eds), Root Physiology: from Gene to Function, Vol 4. Springer, Dordrecht, pp 237–250

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES (2008) Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renewable Agriculture and Food Systems 23:3–12. doi:10.1017/S1742170507002025

Heuermann D, Gentsch N, Boy J, Schweneker D, Feuerstein U, Groß J, Bauer B, Guggenberger G, Wirén N von (2019) Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils. Scientific reports 9:11531. doi:10.1038/s41598-019-48060-0

Homulle Z, George TS, Karley AJ (2022) Root traits with team benefits: understanding belowground interactions in intercropping systems. Plant and Soil 471:1–26. doi:10.1007/s11104-021-05165-8

Hudek C, Putinica C, Otten W, Baets S de (2022) Functional root trait-based classification of cover crops to improve soil physical properties. European Journal of Soil Science 73. doi:10.1111/ejss.13147

Isaac ME, Borden KA (2019) Nutrient acquisition strategies in agroforestry systems. Plant and Soil 444:1–19. doi:10.1007/s11104-019-04232-5

Justes E, Bedoussac L, Dordas C, Frak E, Louarn G, Boudsocq S, Journet E-P, Lithourgidis A, Pankou C, Zhang C, Carlsson G, Jensen ES, Watson C, Li L (2021) The 4 C approach as a way to understand species interactions determining intercropping productivity. Frontiers of Agricultural Science and Engineering 0:0. doi:10.15302/J-FASE-2021414

Kätterer T, Bolinder MA, Andrén O, Kirchmann H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agriculture, Ecosystems & Environment 141:184–192. doi:10.1016/j.agee.2011.02.029

Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. doi:10.3390/agriculture10110503

Kemper R, Döring TF, Legner N, Meinen C, Athmann M (2022) Root traits in cover crop mixtures of blue lupin and winter rye. Plant and Soil. doi:10.1007/s11104-022-05366-9

Kozak M, Piepho H-P (2018) What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. Journal of Agronomy and Crop Science 204:86–98. doi:10.1111/jac.12220

Kristensen HL, Thorup-Kristensen K (2004) Root Growth and Nitrate Uptake of Three Different Catch Crops in Deep Soil Layers. Soil Science Society of America Journal 68:529. doi:10.2136/sssaj2004.5290

Lavergne S, Vanasse A, Thivierge M-N, Halde C (2021) Using fall-seeded cover crop mixtures to enhance agroecosystem services: A review. Agrosystems, Geosciences & Environment 4. doi:10.1002/agg2.20161

Li L, Sun J, Zhang F, Guo T, Bao X, Smith FA, Smith SE (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290. doi:10.1007/s00442-005-0256-4

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76. doi:10.1038/35083573

Mead R, Willey RW (1980) The Concept of a 'Land Equivalent Ratio' and Advantages in Yields from Intercropping. Experimental Agriculture 16:217–228. doi:10.1017/S0014479700010978

Meier IC, Tückmantel T, Heitkötter J, Müller K, Preusser S, Wrobel TJ, Kandeler E, Marschner B, Leuschner C (2020) Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. New Phytologist 226:583–594. doi:10.1111/nph.16389

Mendiburu F de (2019) Agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-0. Available online at: https://CRAN.R-project.org/package=agricolae. (accessed January 26, 2021)

Mommer L, van Ruijven J, Caluwe H de, Smit-Tiekstra AE, Wagemaker CA, Joop Ouborg N, Bögemann GM, van der Weerden GM, Berendse F, Kroon H de (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117–1127. doi:10.1111/j.1365-2745.2010.01702.x

Murrell EG, Schipanski ME, Finney DM, Hunter MC, Burgess M, LaChance JC, Baraibar B, White CM, Mortensen DA, Kaye JP (2017) Achieving Diverse Cover Crop Mixtures: Effects of Planting Date and Seeding Rate. Agronomy Journal 109:259–271. doi:10.2134/agronj2016.03.0174

Oram NJ, Ravenek JM, Barry KE, Weigelt A, Chen H, Gessler A, Gockele A, Kroon H de, van der Paauw JW, Scherer-Lorenzen M, Smit-Tiekstra A, van Ruijven J, Mommer L (2018) Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. Journal of Ecology 106:265–277. doi:10.1111/1365-2745.12877

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of environmental change. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 141:426–442. doi:10.1080/11263500701626069

Pausch J, Kuzyakov Y (2018) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global change biology 24:1–12. doi:10.1111/gcb.13850

Poeplau C, Don A (2015) Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agriculture, Ecosystems & Environment 200:33–41. doi:10.1016/J.AGEE.2014.10.024

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. (accessed May 19, 2021)

Roumet C, Lafont F, Sari M, Warembourg F, Garnier E (2008) Root traits and taxonomic affiliation of nine herbaceous species grown in glasshouse conditions. Plant and Soil 312:69–83. doi:10.1007/s11104-008-9635-z

Ryser P (1996) The Importance of Tissue Density for Growth and Life Span of Leaves and Roots: A Comparison of Five Ecologically Contrasting Grasses. Functional Ecology 10:717. doi:10.2307/2390506

Sainju UM, Singh BP, Whitehead WF (1998) Cover Crop Root Distribution and Its Effects on Soil Nitrogen Cycling. Agronomy Journal 90:511–518. doi:10.2134/agronj1998.00021962009000040012x

Thorup-Kristensen K (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant and Soil 230:185–195. doi:10.1023/A:1010306425468

Wendling M, Büchi L, Amossé C, Jeangros B, Walter A, Charles R (2017) Specific interactions leading to transgressive overyielding in cover crop mixtures. Agriculture, Ecosystems & Environment 241:88–99. doi:10.1016/j.agee.2017.03.003

Wendling M, Büchi L, Amossé C, Sinaj S, Walter A, Charles R (2016) Influence of root and leaf traits on the uptake of nutrients in cover crops. Plant and Soil 409:419–434. doi:10.1007/s11104-016-2974-2

Wendling M, Charles R, Herrera J, Amossé C, Jeangros B, Walter A, Büchi L (2019) Effect of species identity and diversity on biomass production and its stability in cover crop mixtures. Agriculture, Ecosystems & Environment 281:81–91. doi:10.1016/j.agee.2019.04.032

Yin S, Wang C, Lv C, Zhou Z (2023) Short-term responses of root traits and carbon exudation to drought in a Larix gmelinii plantation. Plant and Soil 484:393–405. doi:10.1007/s11104-022-05800-y

Yu Y, Stomph T-J, Makowski D, Zhang L, van der Werf W (2016) A meta-analysis of relative crop yields in cereal/legume mixtures suggests options for management. Field Crops Research 198:269–279. doi:10.1016/j.fcr.2016.08.001

5 Schaffen Zwischenfrüchte großlumige Bioporen in Unterböden?

Roman Kemper[1], Thomas F. Döring[1], Miriam Athmann[2]

[1] Fachgebiet Agrarökologie und Organischer Landbau, Universität Bonn, Deutschland

[2] Fachgebiet Ökologischer Land- und Pflanzenbau, Universität Kassel, Deutschland

---

This chapter has been published as Kemper R, Döring T, Athmann M (2023) Schaffen Zwischenfrüchte großlumige Bioporen in Unterböden? In: Bibic, V.; Schmidtke, K. (Hrsg.) One Step Ahead - einen Schritt voraus! Beiträge zur 16. Wissenschaftstagung Ökologischer Landbau, Frick (CH), 07. bis 10. März 2023, Verlag Dr. Köster, Berlin.

Abstract

Perennial fodder crops are known to enhance the density of large-sized biopores in the subsoil, while studies on biopores created by cover crops are limited. The aim of the field experiments at the organic research farm Wiesengut was to determine the biopore density after growing cover crops and to assess the potential of biopore genesis. Biopore density was generally high in all treatments but did not differ from the control (bare fallow). The numbers of calculated root pieces from cover crops per m² were lower than the biopore densities measured. It can be assumed that biopores were not created by cover crops and that the potential of biopore genesis by cover crops is limited.

Keywords

Bioporendichte, Wurzelstücke, Pfahlwurzel, Lupine, Ölrettich

5.1 Einleitung und Zielsetzung

Zwischenfrüchten werden viele für Ackerbausysteme wichtige Funktionen zugeschrieben. So wird auch die Bildung großlumiger Bioporen genannt, Zwischenfrüchte werden für „bio-tillage“ empfohlen (Zhang und Peng, 2021). Großlumige Bioporen erfüllen wichtige Funktionen in Ackerböden wie verbesserter Gasaustausch, erhöhte Wasserinfiltration, Erleichterung der Unterboden-Durchwurzelung, Wasser- und Nährstoffaufnahme aus tiefen Bodenschichten sowie Nährstoffbereitstellung an der Bioporenwandung (Kautz, 2015; Xiong et al., 2022). Der Anbau von Futterpflanzen kann die Bioporendichte im Unterboden erhöhen und so die Durchwurzelung der Nachfrüchte in tieferen Bodenschichten fördern (Han et al., 2015). Studien zur Bioporengenese von Zwischenfrüchten sind rar. Ziel dieser Untersuchung war es, die Bioporendichte nach dem Zwischenfruchtbau zu erfassen, sowie das Bioporengenesepotenzial der Zwischenfrüchte anhand von Wurzelstücken zu berechnen.

5.2 Methoden

In den Jahren 2018 und 2019 wurden Feldversuche mit verschiedenen Zwischenfruchtarten und -mischungen (Tabelle 5.1) am Campus Wiesengut der Universität Bonn in Hennef (Sieg) angelegt. Die Auenböden auf Kies sind sandige bis schluffige Lehme mit einer Mächtigkeit von ca. 1 m. Details zu Standort und Versuchsanlage finden sich in Kemper et al. (2020). Die Zwischenfrüchte wurden mit einer Parzellensämaschine (Reihenabstand 17 cm) jeweils Mitte August nach Pflugeinsatz (23 cm tief) und Saatbettbereitung gesät. Zur sicheren Bestandesetablierung wurden die Versuche in beiden Jahren beregnet. Zur Erfassung der Bioporendichte wurden 0,25 m² Bodenfläche in 40 cm Tiefe im März 2019 und August 2020 freigelegt. Mit einem Spachtel wurde die Fläche aufgebrochen, loser Boden wurde abgesaugt. Mit einer Kamera (Nikon 7100D, 16 mm Brennweite, Blende 11) wurde die Fläche in einem abgedunkelten Rahmen in 60 cm Höhe fotografiert. Die Fotos wurden mit der Software RootPainter (Smith et al., 2022) analysiert, die Anzahl und Durchmesser der Bioporen erfasst.

Tabelle 5.1: Zwischenfruchtvarianten im Feldversuch

Zwischenfrucht	Sorte bzw. Mischungsverhältnis	Abkürzung	Saatstärke [kg ha^{-1}]
Kontrolle	Schwarzbrache	KO	-
Grünroggen	Bonfire	GR	120
Inkarnatklee	Linkarus	IK	30
Lupine	Boruta	LU	120
Ölrettich	Siletina	OR	25
Lupine-Grünroggen	50:50	LU_GR_50_50	60; 60
Ölrettich-Grünroggen	50:50	OR_GR_50_50	2,5; 60
Ölrettich-Inkarnatklee-Grünroggen	33:33:33	OR_IK_GR_33_33_33	8,3; 10; 40
Sandhafer	Pratex	SH	80
Winterrübsen	Jupiter	WRU	15
Phacelia	Beehappy	PH	12

Monolithe wurden im Oktober 2018 und 2019 wie in Kemper et al. (2022) beschrieben entnommen. Die Wurzeln wurden ausgewaschen, gescannt und mit WinRhizo nach Durchmesser klassifiziert. Die Berechnung der Wurzelstücke erfolgte wie bei Perkons (2018): die Wurzellängen wurden summiert und durch die Höhe des Monolithen (10 cm) geteilt, um die theoretische Anzahl an Wurzelstücken bei vertikalem Wurzelwachstum zu erhalten. Diese Maßzahl ermöglicht eine Bewertung des Bioporengenesepotenzials. Wurzelstücke von Phacelia, Sandhafer und Rübsen wurden nur 2018 erfasst. Für die Bioporendichte wurde eine einfaktorielle Varianzanalyse berechnet und mit dem Dunnett-Test wurden die Zwischenfrüchte gegen die Kontrolle getestet.

5.3 Ergebnisse

Sowohl in beiden Versuchsjahren als auch in allen drei Durchmesserklassen unterschied sich keine Zwischenfrucht-Variante in der Bioporendichte von der unbewachsenen Kontrolle (Abbildung 5.1). Bei der Anzahl der berechneten Wurzelstücke je m² wies das Jahr 2018/19 höhere Werte auf als 2019/20 (Tabelle 5.2). Wurzelstücke > 5 mm wurden in keinem Versuch gemessen. Insgesamt wiesen Grünroggen und Lupine sowie deren Mischung das höchste Bioporengenesepotenzial auf, wobei bei Poren > 2 mm nur die Lupine im ersten Versuchsjahr höhere Werte aufwies.

Tabelle 5.2: Berechnete Wurzelstücke je m² verschiedener Zwischenfrüchte in drei Durchmesserklassen in der Bodenschicht 40-50 cm 2018/19 sowie 2019/20.

	2018/19			2019/20		
Kultur	1-2 mm	2-5 mm	>5 mm	1-2 mm	2-5 mm	>5 mm
GR	149	12	0	38	6	0
IK	1	0	0	3	0	0
LU	136	44	0	1	0	0
LU_GR_50_50	154	13	0	0	0	0
OR	53	9	0	9	0	0
OR_GR_50_50	45	1	0	4	0	0
OR_IK_GR_33_33_33	19	0	0	2	0	0
PH	53	1	0			
SH	28	0	0			
WRU	51	4	0			

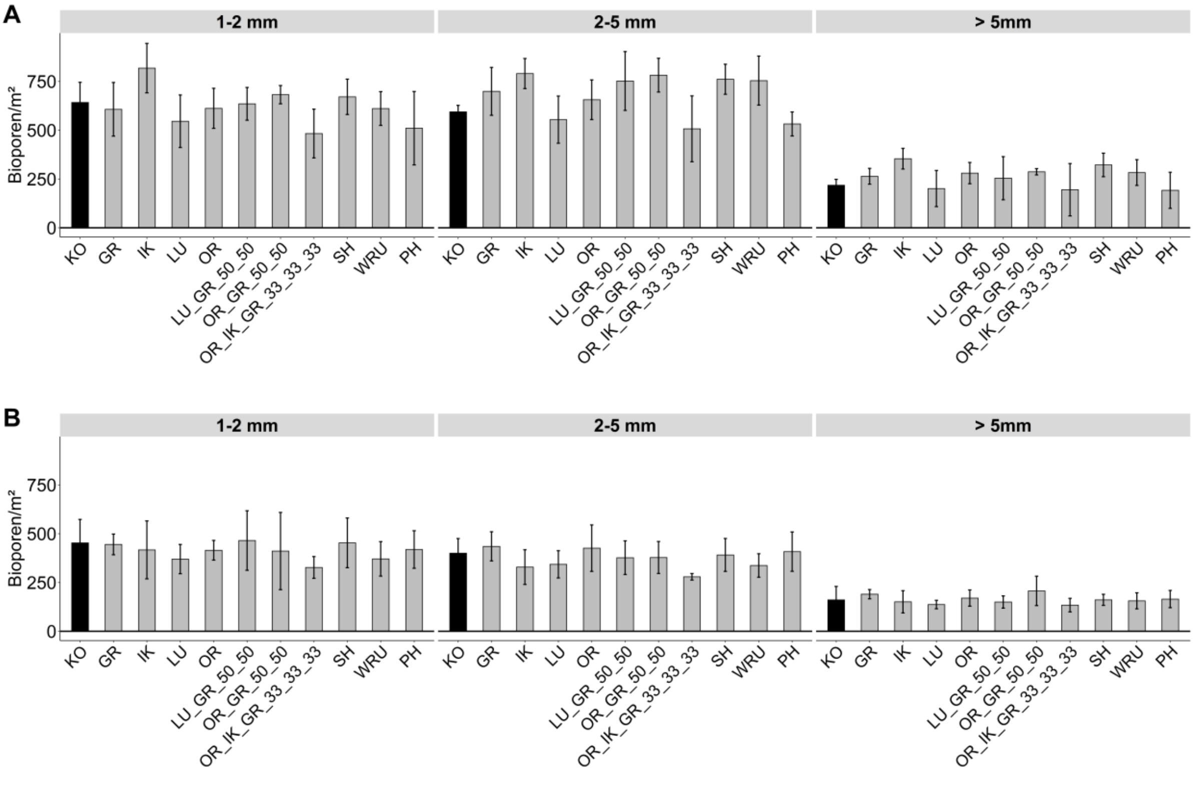

Abbildung 5.1: Bioporendichte (m^{-2}) nach Durchmesser unter Zwischenfrüchten in 2018/19 (A) und 2019/20 (B) in 40 cm Tiefe. Die Zwischenfrüchte (grau) wurden mit dem Dunnett-Test gegen die Kontrolle ohne Zwischenfrucht (schwarz) getestet.

5.4 Diskussion

Auffällig war die insgesamt hohe Bioporendichte am Wiesengut. Diese ist vermutlich Folge langjähriger ökologischer Bewirtschaftung und Bioporengenese durch Regenwürmer. Die nicht signifikanten Unterschiede zur unbewachsenen Kontrolle und die berechneten Wurzelstücke, insbesondere > 2 mm Durchmesser, sprechen gegen eine Bioporengenese durch Zwischenfrüchte in 40 cm Bodentiefe. Vermutlich ist deren Vegetationszeit von wenigen Monaten zu kurz, um hinreichend dicke Pfahlwurzeln zu bilden. Für > 2 mm wurden für Wegwarte 53 und für Luzerne 115 Wurzelstücke je m² in 45-55 cm Tiefe gefunden (Perkons, 2018). Mit den Ergebnissen aus 2018/19 scheint am ehesten die Lupine mit 44 Wurzelstücken je m² Bioporen generieren zu können. Studien mit kruziferen Zwischenfrüchten zeigten, dass diese Bioporen bilden und von nachfolgender Soja genutzt werden können (Williams und Weil, 2004). Es wurden jedoch nur einzelne Poren mit Minirhizotronen untersucht, nicht aber Bioporendichte und -durchmesser erfasst. Somit ist nicht auszuschließen, dass auch in unseren Versuchen einzelne Bioporen durch Zwischenfruchtwurzeln gebildet wurden, jedoch in der gesamten Bioporendichte von geringer Relevanz sind. Untersuchungen zur Wurzelmorphologie bei abruptem Wechsel der Lagerungsdichte von Burr-Hersey et al. (2017) zeigten, dass Ölrettich von einer dicken Pfahlwurzel zu mehreren dünnen Wurzeln in der verdichteten Zone wechselt, was die Bioporengenese im dichteren Unterboden beeinflussen würde.

5.5 Schlussfolgerungen

Es wurde deutlich, dass in einem bereits gut durchporten Boden Zwischenfrüchte mit einer kurzen Anbaudauer in 40 cm Tiefe kaum zusätzlich Bioporen schaffen können. Die Tendenz, dass in spezialisierten ökologischen Acker- und Gemüsebaubetrieben Zwischenfrüchte den mehrjährigen Feldfutterbau ersetzen sollen, ist daher im Hinblick auf die Bildung von Bioporen und damit die Verbesserung der Unterbodenstruktur kritisch zu sehen. Weitere Untersuchungen an anderen Standorten sind wichtig, um unsere Ergebnisse zu bestätigen.

Danksagung

Danke an das Team des Wiesenguts und die Studierenden für Hilfe in Feld und Labor. Bei der BLE und dem BÖLN bedanken wir uns für die Förderung des Projekts MIKODU.

Literatur

Burr-Hersey, J.E.; Mooney, S.J.; Bengough, A.G.; Mairhofer, S.; Ritz, K. (2017) Developmental morphology of cover crop species exhibit contrasting behaviour to changes in soil bulk density, revealed by X-ray computed tomography. PloS one 12:e0181872. DOI: https://doi:10.1371/journal.pone.0181872

Han, E.; Kautz, T.; Perkons, U.; Uteau, D.; Peth, S.; Huang, N.; Horn, R.; Köpke, U. (2015) Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. Biology and Fertility of Soils 51:847–856. DOI: https://doi:10.1007/s00374-015-1032-1

Kautz, T. (2015) Research on subsoil biopores and their functions in organically managed soils: A review. Renewable Agriculture and Food Systems 30:318–327. DOI: https://doi:10.1017/S1742170513000549

Kemper, R.; Bublitz, T.A.; Müller, P.; Kautz, T; Döring, T.F.; Athmann, M. (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. DOI: https://doi:10.3390/agriculture10110503

Kemper, R.; Döring, T.F.; Legner, N.; Meinen, C; Athmann, M. (2022) Root traits in cover crop mixtures of blue lupin and winter rye. Plant and Soil. DOI: https://doi:10.1007/s11104-022-05366-9

Perkons, U.K. (2018) Bioporengenese durch homo- und allorhize Kulturpflanzen: Einfluss auf das Wurzelwachstum der Nachfrüchte. Dissertation. Universität Bonn, Bonn

Smith; A.G.; Han; E.; Petersen J.; Olsen, N.A.F.; Giese, C.; Athmann, M.; Dresbøll, D.B.; Thorup-Kristensen, K. (2022) RootPainter: Deep Learning Segmentation of Biological Images with Corrective Annotation. New Phytologist. DOI: https://doi:10.1111/nph.18387

Williams, S.M.; Weil, R.R. (2004) Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop. Soil Science Society of America Journal 68:1403–1409. DOI: https.//doi:10.2136/sssaj2004.1403

Xiong, P.; Zhang, Z.; Peng, X. (2022) Root and root-derived biopore interactions in soils: A review. Journal of Plant Nutrition and Soil Science. DOI: https://doi:10.1002/jpln.202200003

Zhang, Z.; Peng, X. (2021) Bio-tillage: A new perspective for sustainable agriculture. Soil and Tillage Research 206:104844. DOI: https://doi:10.1016/j.still.2020.104844

6 Relating Profile Wall Root-Length Density Estimates to Monolith Root-Length Density Measurements of Cover Crops

Tábata A. Bublitz[1], Roman Kemper[2], Phillip Müller[3], Timo Kautz[4], Thomas F. Döring[2] and Miriam Athmann[5]

[1] Department of Soil Biology and Plant Nutrition, Faculty of Organic Agricultural Sciences, University of Kassel, Germany

[2] Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Germany

[3] Institute for Construction and Agricultural Machinery Techonology Cologne, Faculty of Plants, Energy and Machine Systems, Technical University of Cologne, Germany

[4] Department of Agronomy and Crop Science, Faculty of Life Sciences, Humboldt University of Berlin, Germany

[5] Department of Organic Farming and Cropping Systems, Faculty of Organic Agricultural Sciences, University of Kassel, Germany

---

This chapter has been published as Bublitz TA, Kemper R, Müller P, Kautz T, Döring TF, Athmann M (2022) Relating Profile Wall Root-Length Density Estimates to Monolith Root-Length Density Measurements of Cover Crops. Agronomy 12:48. doi:10.3390/agronomy12010048.

Abstract

Different methods have been proposed for in situ root length density (RLD) measurement. One widely employed is the time-consuming sampling of soil cores or monoliths (MO). The profile wall (PW) method is a less precise, but faster and less laborious alternative. However, depth-differentiated functions to convert PW RLD estimates to MO RLD measurements have not yet been reported. In this study, we perform a regression analysis to relate PW results to MO results and determine whether calibration is possible for distinct crop groups (grasses, brassicas and legumes) consisting of pure and mixed stands, and whether soil depth affects this calibration. The methods were applied over two years to all crop groups and their absolute and cumulative RLD were compared using a linear (LR) and multiple linear (MLR) regression. PW RLD was found to highly underestimate MO RLD in absolute values and in highly rooted areas. However, a close agreement between both methods was found for cumulative root length (RL) when applying MLR, highlighting the influence of soil depth. The level of agreement between methods varied strongly with depth. Therefore, the application of PW as main RLD estimation method can provide reliable estimates of cumulative root distribution traits of cover crops.

Keywords

Catch crops; cumulative root length; root distribution; calibration; regression analysis

6.1 1. Introduction

Root length density (RLD) of cover crops is frequently assessed in crop studies due to its high relevance for crop functions such as nitrogen uptake from deep soil layers [1–5], erosion control [6] and crop productivity [7]. Different quantitative methods have been employed to measure RLD in the field, of which soil core sampling [4,5,8–12], field installation of minirhizotrons [1–3,9,13–15] and soil monolith (MO) extraction [8,9,16,17] are the most frequently used. These methods are considered to be either very time consuming and/or expensive, but believed to be the most accurate in representing real values [8,18,19].

Böhm [8] suggested that a faster, less expensive and less laborious alternative [20–22] could be used for RLD estimation, named profile wall (PW) method. According to Böhm [8], relating PW estimations of RLD with MO measurements could help finding conversion factors to obtain similarly accurate root length. Difficulties related to the PW method, however, include its low RLD estimations compared to other techniques. In his studies to overcome such limitations, Lohmann [23] performed a comparative analysis of PW and MO, and determined a general conversion factor of 1 for faba beans (*Vicia faba* L.). Köpke [19] in turn, suggested to multiply the PW root lengths of oats (*Avena sativa* L. cv. Leanda) by 2.06 if PW RLU were counted within 8 weeks after sowing. The author reported a linear decrease of conversion factor at later plant development stages, because of stronger root development with e.g 12 and 13 weeks after sowing. Gäth and Meuser [24] later reported a conversion factor between both methods that varies with the vegetation periods of sugar beets (*Beta vulgaris* subsp. *vulgaris*). To have monoliths extracted at several plant stages requires high expenditure of time and labour, and the abovementioned studies demonstrate the unreliability of one mean conversion factor because they cannot do justice to the crops temporal dynamics.

When a temporal analysis is not possible, other factors such as the influence of soil depth on the comparison of PW and MO were already shown to be useful. In a novel attempt, Böhm [8] compared root length from PW and MO RLD for maize (*Zea mays* L.) at different depths and, although he did not develop any calibration tool, his study identified the necessity for finding useful predicting functions between both methods to overcome the difficulties involved in RLD quantification.

Regression analysis has been widely used in comparative studies [23,24] and in the calibration of root quantification methods [14,25–31]. Böhm performed a regression analysis between root counts from PW and RLD from MO as a function of soil bulk density, however, only within topsoil layers, and only for spring barley (*Hordeum vulgare* L.). No regression analysis between

PW and MO can be found applied to cover crops or that accounts for RLD changes with soil depth.

Cover crops are gaining importance as their use has numerous benefits including improved soil health, reduced soil erosion, and weed suppression [32]. Root data of cover crops are more and more needed to support the knowledge of the positive impacts that a high RLD of cover crops have on the level of shoot nitrogen uptake and nitrate content in the soil [7]. Therefore, the present study works on a novel dataset of RLD recorded for single and mixed cover crops using both PW and MO methods in the same experimental period.

This paper analyzes RLD estimations obtained with the PW method and RLD measurements performed with the MO method by means of a regression analysis of both absolute RLD values (cm/cm^3) and relative distribution of root length (RL) across the soil profile; in this paper referred as cumulative RL. The studied crops consist of pure and mixed stands grouped into three different crop families: Gramineae, Leguminosae and Brassicaceae.

The purpose of this study was to relate both methods to each other for each crop group using regression models, and to investigate the role of soil depth in data distribution, evaluating its contribution to the regression functions' accuracy using absolute and cumulative values. This study aims as well, at proposing an alternative to the development of a mean conversion factor, demonstrated to be a weak solution when PW and MO are not compared following crops temporal dynamics. Thus, because in the present experiment root length estimation in different plant stages was not possible to record, we hypothesize that a regression model is a good alternative in calibrating PW and MO RLD.

6.2 Materials and Methods

6.2.1 Experimental design and location

The field experiment was set up as a randomized complete block design with four blocks, of which three blocks (36 plots) were analyzed for root length density (RLD). The experiment was carried out in 2018/2019 with plot size of 1.5m × 12.5m, and in 2019/2020 with plot size 1.5m × 20m, inserted within the crop rotation of the farm in which the experiment was performed, as also described by Kemper et al. [33]. A plot seeder with row distance of 17cm was used on 12th August 2018 and 14th August 2019 for sowing. Separate plots were sown with single and mixed cover crops belonging to three different crop families, and for the present analysis, they were grouped accordingly: Gramineae (grasses group), Brassicaceae (brassicas group) and Leguminosae (legumes group). In mixed stands, crop group was determined on the basis of the dominant crop at the time of root sampling (Table 6.1). In mixtures, competition mechanisms were attributed to the resulting dominance of one or other mixture component, a process in which plant canopy and/or root system of one crop develops more significantly than the other [34–37], which for this study, was assessed by determining the partial land equivalent ratio (PLER) [38].

The experiment was performed at the research station for organic farming “Wiesengut”, located in the Sieg lowlands near Hennef (7° 17' East, 50° 48' North) about 65 m above sea level. The site is characterised by a flusivol with a silt-loam soil layer of 60cm to 120cm thickness over gravel, in a region with fluctuating groundwater level. The mean annual precipitation at the experimental site is 840 mm with a mean temperature of 10.3 °C over the experimental years. Precrop in both years was faba bean. After harvesting the precrop, the soil was ploughed to 23 cm depth and the seed bed was prepared with a rotary harrow. Hand and mechanical weeding were carried out during crop establishment, and field irrigation was carried out with a sprinkler, applying in 2018, 50 mm in one application before sowing, and 54 mm in three applications after sowing. In 2019, 30 mm was irrigated in one application before, and 12 mm in one application after sowing.

Table 6.1 Crops and mixtures used in the experiment. Crop group was determined according to crop family for single crops, and crop family of the dominant crop for mixtures. Grasses = Gramineae Family, Brassica = Brassicaceae Family, and Legumes = Leguminosae Family.

Crop/Mixture Species	Cultivar	Dominant Crop	Crop Group	Seed Quantity (kg/ha)
winter rye (*Secale cereale* L.)	Bonfire		Grasses	120
bristle oat (*Avena strigosa* Schreb.)	Pratex		Grasses	80
blue lupin (*Lupinus angustifolius* L.)	Boruta		Legumes	120
crimson clover (*Trifolium incarnatum* L.)	Linkarus		Legumes	30
oil radish (*Raphanus sativus* L. var. oleiformis Pers.)	Siletina		Brassica	25
tillage radish (*Raphanus sativus* L. var. *oleiformis* Pers.)	Deeptill		Brassica	12
winter turnip rape (*Brassica rapa* L. var. *silvestris* (Lam.) Briggs)	Jupiter		Brassica	15
Mixed Crops				
oil radish/green rye		oil radish	Brassica	12.5/60
oil radish/crimson clover		oil radish	Brassica	6.25/22.5
oil radish/crimson clover/winter rye		oil radish	Brassica	8.5/10/40
blue lupin/winter rye		winter rye	Grasses	60/60
blue lupin/winter rye		winter rye	Grasses	90/30

6.2.2 Profile wall method

The profile wall method was applied according to Böhm [8], as described by Kemper et al. [33], in which a trench of 1.2m depth and 1.0m width was opened along the plot's short end with an excavator. In every plot, the soil profile was manually smoothed with a sharp-edged spade and chisel. Subsequently, a toothed scraper was used to uniformly remove little amounts of soil by making a diagonal scrapping on both directions. Directly afterwards, a battery-operated water sprayer was used to complete the procedure of gently removing around 0.5cm of soil from the wall to expose its roots. After spraying, a 100cm × 100cm metal frame with 5 × 5cm grids was placed and fixed on the profile wall. The length of the exposed roots was estimated by counting the number of root length units (RLU) of 0.5cm inside each grid. Root length density per soil volume (cm/cm^3) was calculated by dividing the RLU sum of each grid by $12.5cm^3$ (5cm × 5cm × 0.5cm).

Root counting in the PW method took place from mid to end of October of both experimental years, and was performed by a different person each year. In 2018, the trench was refilled and re-opened between the first and second method repetitions, while in 2019, the trench remained open due to wet conditions. In March 2018, the trench was reopened 50cm further into the plot and in March 2019, plots within the still open trench had 50cm of soil removed from the profile wall to have an undisturbed counting surface. RLU were counted separately for large-sized vertical biopores and bulk soil, however, for this study they were combined for the whole plot.

6.2.3 Monolith method

The MO method was performed as described by Böhm [18]. Monoliths were extracted on a horizontal plane from each plot in both experimental years in 10 cm depth levels to maximum rooting depth: in October 2018, two monoliths with dimensions of 20 cm × 10 cm × 10 cm, and in October 2019, two monoliths in the topsoil layers (0-30 cm) and three monoliths in the sub-soil layers (below 30 cm) with dimensions of 17 cm × 10 cm × 10 cm, always next to each other. The monoliths were extracted until maximum soil depth of each plot before gravel started to appear, and then stored at 5°C until being washed out for soil removal. The washing process consisted in first soaking the monoliths in water in a 10-litre bucket, then the water was repeatedly poured through a sieve tower with different fine sieves, so straw and finer roots could be caught and separated. This process was repeated with the same bucket, using a hand shower to break soil crumbles and loosen all roots until it was empty. The sieve tower contained sieves of 4.0mm, 2.0mm, and 1.0mm mesh size in 2018, and additional sieves of 0.71mm, 0.63mm and 0.5mm in 2019. After that, the contents of the sieves was transferred to another bucket and poured through a funnel into a small sieve (mesh size 0.5mm) to facilitate root transfer to small plastic containers that were later (cold) stored. Non-root materials were removed manually with tweezers. To determine the root length density and the root diameter distribution, each sample was poured on a 297 x 420 mm glass dish and scanned with an Epson Expression 12000XL flatbed scanner, calibrated for determination of RLD with *WinRHIZO™* [39].

6.2.4 Statistical analysis

R version 4.0.4 [40] was used for data analysis. To determine the best method for estimating MO RLD on the basis of PW RLD, four regression models were applied for both absolute and cumulative RLD values, two of which were linear and two were non-linear models (Tables D1 and D2, supplementary material). The non-linear models were considered to present overfitting (see supplementary material) [41], and are therefore not further presented or discussed. The linear models were:

1) Simple linear regression (LR)

$$Y = a_0 + a_1(x) \tag{6.1}$$

2) Multiple linear regression (MLR)

$$Y = a_0 + a_1(x) + a_2(z) \tag{6.2}$$

where Y is root length density (RLD) in cm/cm^3 from MO method, x is root length density (RLD) in cm/cm^3 from PW method, and z is soil depth in cm, while a_0, a_1 and a_2 are coefficients to be estimated from the data.

Before the application of the regression models, cumulative RL was calculated by determining each absolute RLD proportion in relation to the total RL of the soil profile, and adding each value to the sum of previous depths. Absolute and cumulative data were then split into two groups, the first one consisting in 2/3 of the dataset, to be used as model training data, and the second, 1/3 of the dataset, to be used as testing data. The data split was performed using a random stratification within crop species and soil depth in order to represent each crop, experimental year and soil depth in both subsets.

The training dataset was used to fit the regression models and assess the best fitting equation using Akaike's Information Criterion (AIC) [42] as the evaluation metric in both absolute data (RLD) and cumulative data (RL). The regression equations were then applied to the testing data as a calibration tool to assess their ability in estimating MO RLD or RL using the equation parameters. Root mean squared error (RMSE) [43] and mean absolute error (MAE) were assessed between calculated and measured MO RLD or RL [23]. Additionally, the coefficient of determination (R^2) was computed in both training and testing datasets.

6.3 Results

MO and PW methods resulted in overall higher absolute RLD values, higher standard deviation (sd) and larger deviations between PW and MO methods in the first 30cm of soil compared to deeper layers. In the topsoil (0-30cm), grasses presented the highest mean absolute RLD and the greater difference between methods, while legumes had the lowest mean absolute RLD and the smaller difference between methods. In the subsoil (30-100cm), brassica crops presented higher differences between PW and MO than grasses and legumes. For legumes, methods where similarly close in the subsoil, and for grasses, the agreement tended to increase consistently, up to 100cm (Table 6.2). In cumulative data, grasses and legumes reached similar root distribution already at 30cm, but differed in their level of agreement between methods. Grasses, followed by brassicas, showed the highest agreement between methods at all depths, while legumes had the highest disagreements between methods. Differences in cumulative values between PW and MO, however, were very small for all crops, and values were mostly slightly negative, indicating a certain overestimation from PW (Table 6.2).

Table 6.2. Descriptive statistics of root length density (RLD) measurements from profile wall (PW) and monolith (MO) methods for different crop categories and soil depths. Mean RLD is the mean value in cm/cm^3 of all RLD absolute values, and Sd RLD is the standard deviation in cm/cm^3 of all RLD absolute values. Mean cum. RL is the mean cumulative root length distribution and Sd cum. RL is the standard deviation of the mean cumulative root length distribution. Mean Error is the mean difference between MO and PW. n is the number of observations for each depth.

Crop Family	Soil Depth (cm)	Mean RLD (cm/cm^3)		Sd RLD (cm/cm^3)		Mean cum. RL		Sd cum. RL		Mean Error (cm/cm^3) (Abs)	Mean Error (cm/cm^3) (Cum)	n
		MO	PW	MO	PW	MO	PW	MO	PW			
Brassicas	10	6.40	1.04	3.60	0.89	0.288	0.362	0.06	0.08	5.36	-0.07	27
	20	4.83	0.69	2.53	0.48	0.507	0.632	0.08	0.13	4.15	-0.13	27
	30	3.78	0.44	2.36	0.33	0.673	0.793	0.09	0.14	3.34	-0.12	27
	40	1.68	0.20	1.03	0.18	0.749	0.850	0.09	0.12	1.48	-0.10	27
	50	1.00	0.14	0.32	0.15	0.801	0.891	0.08	0.10	0.86	-0.09	27
	60	1.05	0.15	0.40	0.16	0.856	0.925	0.07	0.07	0.91	-0.07	27
	70	0.99	0.11	0.33	0.13	0.908	0.950	0.06	0.05	0.88	-0.04	27
	80	1.06	0.12	0.33	0.14	0.956	0.972	0.05	0.03	0.93	-0.02	25
	90	1.05	0.13	0.34	0.10	0.973	0.986	0.03	0.01	0.91	-0.01	15
	100	1.02	0.12	0.21	0.06	1.000	1.000	0.00	0.00	0.90	0.00	9
Grasses	10	11.56	1.72	6.49	1.40	0.442	0.476	0.11	0.07	9.84	-0.03	18
	20	5.68	0.98	1.95	0.63	0.687	0.773	0.09	0.06	4.70	-0.09	18
	30	4.33	0.49	1.93	0.31	0.866	0.923	0.06	0.05	3.84	-0.06	18
	40	1.54	0.16	0.84	0.14	0.931	0.965	0.04	0.03	1.38	-0.03	18
	50	0.43	0.06	0.34	0.07	0.949	0.979	0.03	0.02	0.37	-0.03	18
	60	0.37	0.04	0.30	0.05	0.964	0.988	0.03	0.01	0.32	-0.02	18
	70	0.29	0.03	0.21	0.03	0.976	0.994	0.02	0.01	0.26	-0.02	18
	80	0.25	0.02	0.17	0.02	0.986	0.997	0.02	0.00	0.23	-0.01	18
	90	0.25	0.02	0.14	0.02	0.992	0.999	0.01	0.00	0.23	-0.01	12
	100	0.20	0.01	0.11	0.01	1.000	1.000	0.00	0.00	0.19	0.00	9
Legumes	10	2.95	0.83	1.66	0.89	0.338	0.436	0.14	0.10	2.12	-0.10	12
	20	2.27	0.52	1.02	0.44	0.602	0.753	0.14	0.12	1.75	-0.15	12
	30	1.89	0.25	1.23	0.18	0.812	0.924	0.11	0.06	1.64	-0.11	12
	40	0.61	0.06	0.52	0.04	0.886	0.972	0.09	0.03	0.55	-0.09	12
	50	0.16	0.01	0.10	0.01	0.910	0.985	0.08	0.03	0.15	-0.08	12
	60	0.16	0.01	0.09	0.01	0.931	0.991	0.07	0.02	0.15	-0.06	12
	70	0.18	0.00	0.11	0.01	0.948	0.994	0.05	0.01	0.18	-0.05	11
	80	0.20	0.01	0.15	0.01	0.968	0.997	0.03	0.01	0.19	-0.03	10
	90	0.08	0.00	0.66	0.01	0.989	0.999	0.01	0.00	0.23	-0.01	8
	100	0.09	0.00	0.32	0.01	1.000	1.000	0.00	0.00	0.19	0.00	5

6.3.1 Model fitting

The results after data splitting show a significant correlation ($P < 0.05$) between MO and PW RLD in train data for both LR and MLR and both absolute and cumulative data (Table 6.3). In absolute data, MLR presented higher R^2 and lower AIC as well as higher visual data fitting (Figures 6.1 A, B and 6.2 A, B) than LR for grasses and brassica crops (Table 6.3). Legumes showed similar data fit for both models in absolute data (Table 6.3, Figures 6.1 C and 6.2 C).

In cumulative data, all crops showed higher R^2 and lower AIC (Table 6.3) when fitted with MLR, but only for brassica crops was there a pronounced difference compared to LR. Better graphical representation of subsoil RLD was achieved using MLR in all crops (Figures 6.1 D–F and 6.2 D–F) when compared to LR. Cumulative data in general presented better fit than absolute data, independently of regression function. This is visible in the very small or negative intercepts of both models (Table 6.3), indicating that PW RLD is more predictive of MO RLD for cumulative data than for absolute data.

Table 6.3. Coefficients and quality metrics of linear regression models in training dataset, by crop group and data type. RLD = root length density and cum. RL = cumulative root length. Models are: simple linear model ($Y = a_0 + a_1(x)$) and multiple linear model ($Y = a_0 + a_1(x) + a_2(z)$), where Y = monolith method RLD or cum. RL, x= profile wall RLD or cum. RL, z= soil depth (cm), a_0= intercept, a_1= coefficient to multiply by x, and a_2 = coefficient to multiply by z. AIC = Akaike's Information Criterion for model comparison. R^2 =coefficient of determination, and * indicates that the correlation between PW and MO was significant, with a p-value < 0.05.

Data Type	Crop Group	Model	AIC	R^2	a_0	a_1	a_2
Absolute Data (RLD)	Brassicas	Simple Linear	614.36	0.20*	1.32	3.28	-
		Multiple Linear	552.04	0.48*	4.41	1.08	-0.05
	Grasses	Simple Linear	414.51	0.68*	0.45	7.10	-
		Multiple Linear	400.59	0.72*	2.83	5.18	-0.04
	Legumes	Simple Linear	100.26	0.76*	0.25	5.99	-
		Multiple Linear	100.05	0.77*	0.53	5.46	-0.004
Cumulative Data (cum. RL)	Brassicas	Simple Linear	-236.34	0.72*	-0.01	1.10	-
		Multiple Linear	-325.69	0.85*	0.92	0.47	0.004
	Grasses	Simple Linear	-309.70	0.90*	-0.13	1.10	-
		Multiple Linear	-327.27	0.92*	-0.06	0.95	0.001
	Legumes	Simple Linear	-160.31	0.89*	-0.10	1.05	-
		Multiple Linear	-172.19	0.91*	-0.05	0.90	0.002

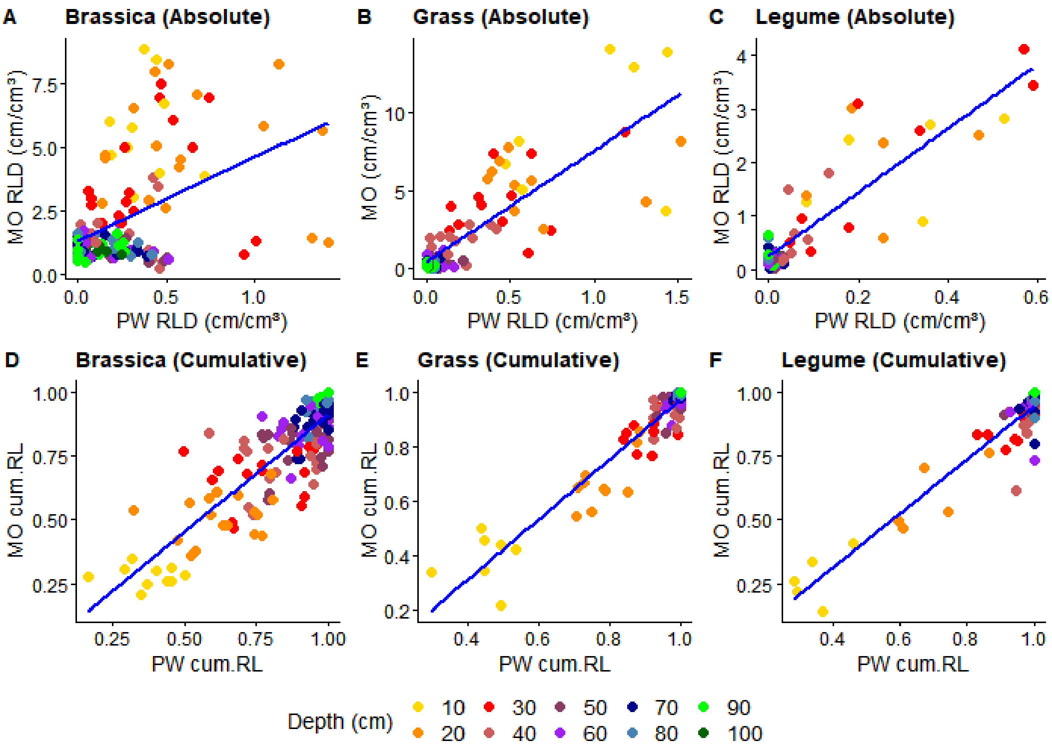

Figure 6.1. Linear regression fitting between monolith and profile wall root length density (RLD) in train data for absolute (A–C) and cumulative (cum.RL) data (D–F). Colors show soil depth (cm) of each value.

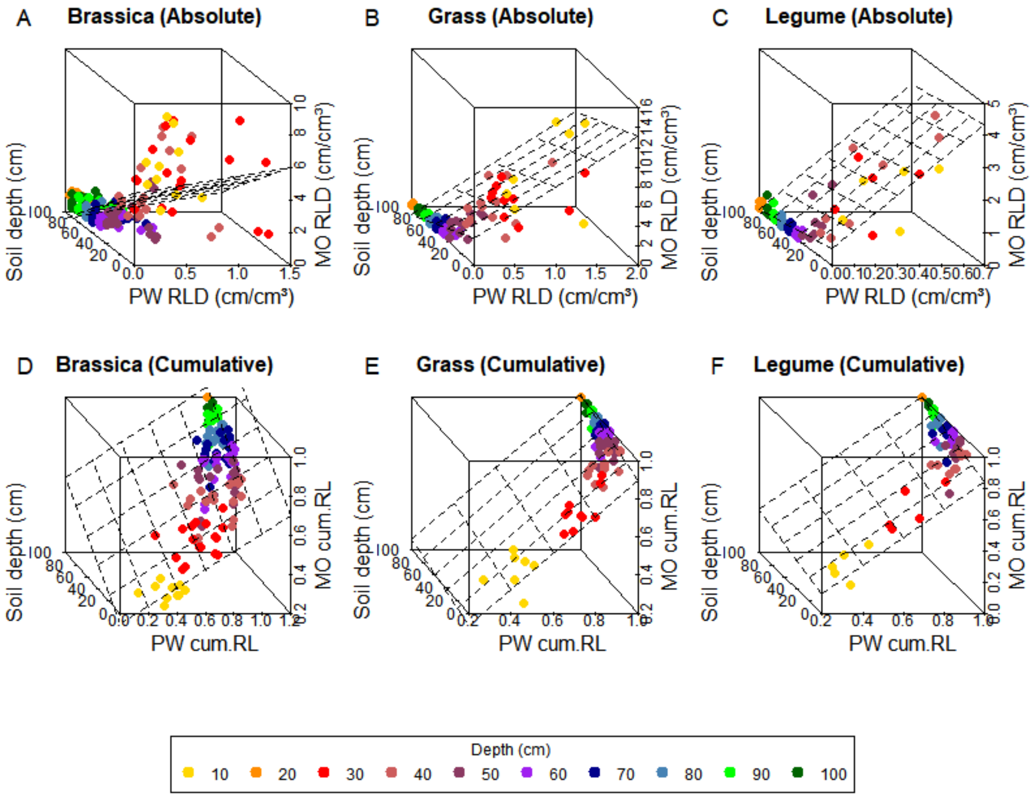

Figure 6.2. Multiple linear regression 3D fitting between monolith and profile wall root length density (RLD) with soil depth in train data for absolute (A–C) and cumulative (cum.LR) data (D–F). Colours show soil depth (cm) of each value.

6.3.2 Model testing

Model validation in test data showed a significant correlation ($P < 0.05$) between predicted and measured RLD from both models in all crop groups and both cumulative and absolute data (Table 6.4). In absolute data, calculated and measured RLD using MLR presented higher R^2, smaller MAE/RMSE, and greater accuracy than when using LR for grasses and brassica (Table 6.4, Figure 6.3 A, B, D, E), even though little agreement is still visible at 10-20cm (Figure 6.3 D, E).

For legumes, both regression models performed similarly (Table 6.4), with greater accuracy at subsoil (30-100cm) and lower accuracy at topsoil (0-30cm) (Figure 6.3 C, F). In cumulative data, MLR showed higher performance, with higher R^2 for all crops than LR, even though MAE and RMSE did not differ much. Calculated and measured cumulative RLD, contrary to the results for absolute data, seem to have a more homogeneous agreement throughout the soil profile for all crops except legumes (Figure 6.3 G-L as compared to A–F). When applying MLR in grasses and brassica, the homogeneity and agreement increased even more (Figure 6.3 J, K)

Table 6.4. Performance metrics of regression models in test dataset, by crop group and data type. RLD = root length density and cum. RL = cumulative root length. R^2= coefficient of determination, and * indicates that the correlation between predicted and measured RLD or cum. RL was significant, with a p-value < 0.05. MAE = Mean absolute error, and RMSE = Root mean absolute error.

Data Type	Crop Group	Model	R^2	MAE	RMSE
Absolute Data (RLD)	Brassicas	Simple Linear	0.23*	1.37	2.22
		Multiple Linear	0.42*	1.25	1.86
	Grasses	Simple Linear	0.63*	1.41	3.13
		Multiple Linear	0.68*	1.35	2.63
	Legumes	Simple Linear	0.73*	0.39	0.71
		Multiple Linear	0.73*	0.39	0.70
Cumulative Data (cum. RL)	Brassicas	Simple Linear	0.76*	0.09	0.11
		Multiple Linear	0.88*	0.07	0.08
	Grasses	Simple Linear	0.92*	0.04	0.05
		Multiple Linear	0.93*	0.04	0.05
	Legumes	Simple Linear	0.77*	0.07	0.11
		Multiple Linear	0.80*	0.06	0.10

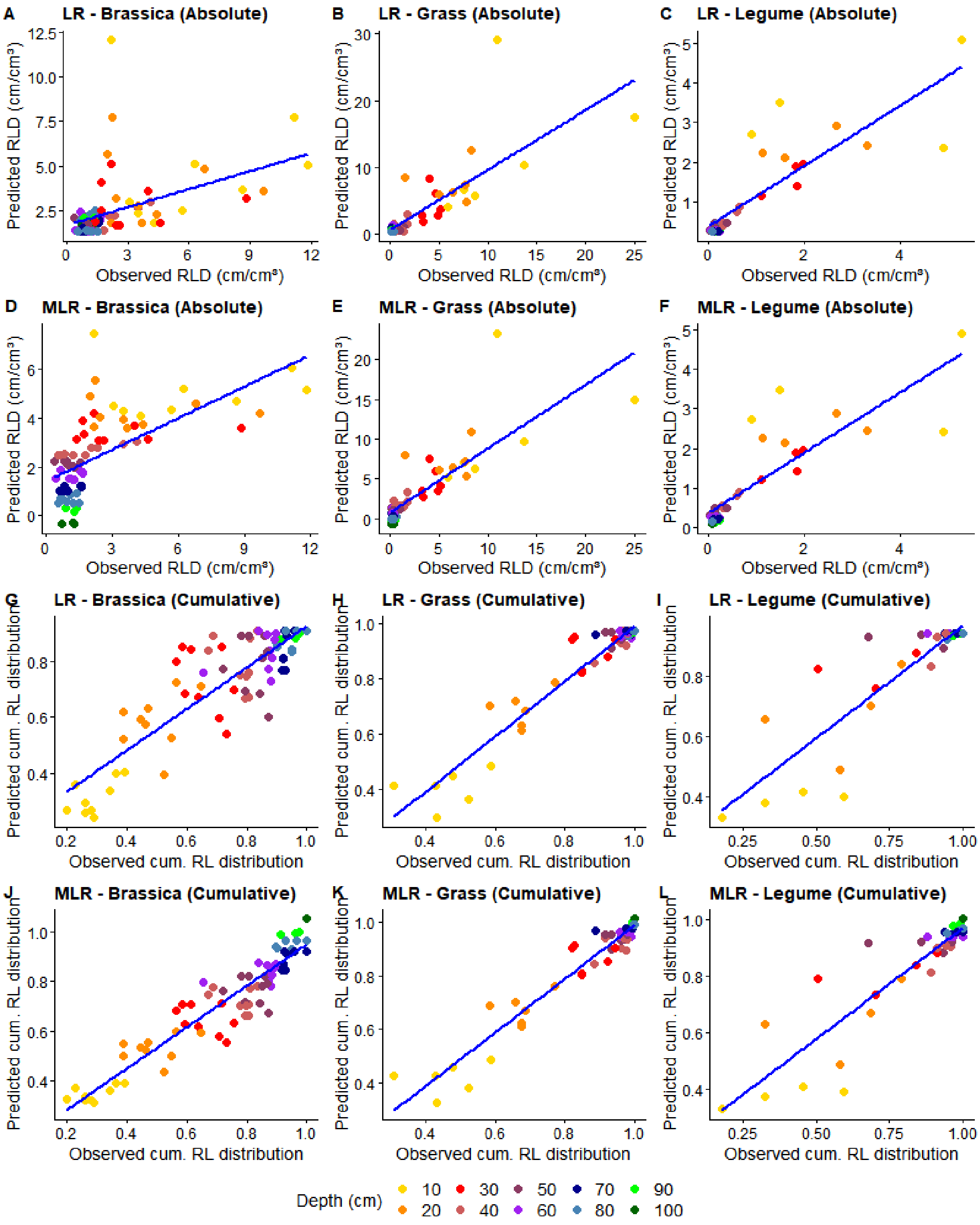

Figure 6.3. Relationships between observed and predicted root length density (RLD) using linear (LR) (A–C, G–I) and multiple linear regression (MLR) (D–F, J–L) for absolute (A–F) and cumulative (cum. RL) (G–L) data. Colours show soil depth (cm) of each value.

6.4 Discussion

PW RLD highly underestimated MO RLD and cum. RL in the topsoil, while in the subsoil, values of the two methods were closer. This indicates that highly rooted soil layers tend to have little agreement between methods (Table 6.2). The results disagree with findings of Böhm [8], of root length estimations from PW being close to half of MO measurements throughout the whole soil profile. Böhm, however, experimented with maize (*Zea mays* L.), and no studies comparing MO and PW results were found for the cover crops as used in the present study.

Crops within the same group (Table 6.1) are frequently reported to present similar rooting behavior [1,12,44], but in our study, the low accuracy of tested models in absolute data indicates that the number of datasets used, or replicates tested for each group, was not sufficient to capture the crop specific features. As it may be assumed that the high deviations between methods in the groups of brassica and grasses might be related to the amount of mixtures present, further tests related to this question were performed, and leaving the mixtures out did not improve the models, as well as comparisons across all crop species resulted in worse fitting (results not shown). Rooting behavior of dominant crops within mixtures might as well need further investigation for future calibration attempts.

Legumes showed high model accuracy and, while this may be related to the absence of mixtures (Table 6.1), the high accuracy is still surprising. Accuracy of calibration functions often depends on sample size, especially when dealing with mean observations [24], and legumes had the smallest sample size of the three groups analyzed. Also, lupin and crimson clover had contrasting root distributions throughout soil depth [33].

Further limitations in relating PW and MO methods involve human factors intrinsic to RLD estimation with PW. It is common for small roots to clump together in highly rooted regions, an obstacle to individual length estimation [8,26], especially when performed by two different people and at different sites or time points. Such factors mean different soil conditions for each person, affecting the real amount of soil removed by spraying, as well as different root status, influencing visual estimation of RLD.

Additionally, a lack of small sieves (0.71 mm, 0.63 mm, 0.5 mm) in 2018, for MO root washing step potentially induced MO RLD underestimation. Another potential source of error in our study was the sampling interval. Sinclair and Seligman [45] suggest that model evaluations should be done during the entire plant growth cycle such as performed by Kücke et al. [46]. In our study, however, MO RLD was recorded only once due to the method's labor-intensive nature. While our study attempts to compare methods in a generic sense for a wide scope of crops, it still fails to cover time and space variation, important parameters for real world representation [45,47].

The reproducibility of the calibration functions here presented for absolute values, therefore cannot be assured. Regression analysis, however, provided important insights about the influence of soil depth on the comparison between PW and MO. As mentioned before, the level of agreement between methods varies with depth, and can be better seen when visualizing data in a 3D plane (Figure 6.2). This explains why, for all crops, MLR was able to best describe the relationship between PW and MO in training data (Table 6.3, Figures 6.1 and 6.2) and calculate MO absolute RLD with higher accuracy than LR at testing phase (Table 6.4, Figure 6.3). The highly scattered data of brassicas is also a good indicator that LR might be, as well, less appropriate for deep rooting crops (Figure 6.1 A).

Comparisons between other root length estimation methods have already highlighted the importance of considering depth distribution [3,14,27,48], and future steps in the development of a calibration function between PW and MO techniques point to this direction. However, as MLR still presented certain limitations in describing depth-dependent variation (Figure 6.2 A–C, Figure 6.3 D–F), its utility for field experiments is limited. MLR fails to calibrate both methods in upper soil layers where RLD is higher, which is possibly why R^2 for absolute data in model fitting and testing (Tables 6.3 and 6.4) is low compared to other calibration studies [25,46,49,50].

In training data, high intercept values for MLR (Table 6.3) and high data scattering (Figure 6.2 A–C) already indicate a general lack of agreement between both methods, while in test data, the error (MAE/RMSE) relative to measured values is high (Table 6.4). Whether or not such errors are acceptable will depend on the research objectives. Studies focusing on the assessment of root growth differences between crop species might benefit from PW estimations of absolute RLD, while converting it into measurements from other methods using calibration functions can lead to errors. A higher accuracy of absolute value predictions still might be reached with more experimental replications and model validation on unseen data, as performed by Chopart et al. [51].

Relatively high agreement between methods was achieved when comparing cumulative RL values, for which generally high R^2 and low MAE/RMSE were found using MLR (Tables 6.3 and 6.4). Grasses and brassica crops showed higher predictive power while legumes had little improvement compared to absolute data, indicating that categories made of more than two crops are recommended for the development of a good conversion model, regarding not only absolute, but also cumulative data.

The good performance for grasses and brassica can be explained by the fact that calculating cumulative RLD through depth minimizes data variation, as it incurs in loss of information, resulting in more subtle changes from one soil layer to another. Cumulative root distribution can be used as a measure of crop performance [3] or as a tool for rooting type characterisation [48], demonstrating the utility of such data. In our study, the high depth-dependent RLD variations for grasses and brassica (Figure 6.3 G, H, J, K) seem to reduce in cumulative data when comparing measured and calculated RLD. This demonstrates the potential of root length estimation with PW method for studies focusing on crop-specific root-depth frequency.

Further studies on the relationship between PW and MO RLD estimations might benefit from more in depth studies about other potential factors involved in PW RLD estimations in highly-rooted areas, such as root orientation [49,51,52], crop management [29] and crop-specific root behavior inside mixtures. When working with absolute data, a more comprehensive approach where a different calibration function is developed for each specific soil layer might as well bring other perspectives to MO and PW comparisons.

6.5 Conclusions

Profile wall and monolith methods were found to have high disagreements in their root length density measurements, where values can be greatly underestimated if profile wall is chosen as main root length estimation technique. Underestimations were found for crop groups containing more mixtures and crops tested in only one year (Table 6.1), especially regarding absolute data. Our main findings from the regression analysis are that a simple linear regression is not able to describe the relationship between both employed methods in a sufficiently accurate manner, even though the relationship between PW and MO is significant. It is consequently inefficient in predicting root length density absolute values. It may still be used to compare cumulative values, but its performance is limited because it is unable to capture root length density covariation with depth.

Soil depth was found in this study to play a very important role in determining the degree of agreement between profile wall estimations and monolith measurements of root length density. In our study, the level of agreement between methods was inversely proportional to rooting intensity, making it harder to calibrate estimations with simple linear regressions for most crops tested here. All crop groups showed higher mean root length densities in the topsoil compared to the subsoil, independent on the estimation method used. Applying a multiple linear regression to that data allowed this depth-dependency to be better captured, especially in deep rooted crops. However, its low performance metrics indicated that it cannot be reliable in calibrating both techniques. The difficulties in relating both methods resulting from this study leads us into concluding that profile wall estimations of root length density from cover crops in absolute values tend to strongly deviate from monolith measurement values, and therefore, are difficult to convert.

On the other hand, when absolute root length density was converted into cumulative root distribution, all crops presented a very high agreement between methods. A multiple linear regression was able to best describe the relationship, with very small errors and high accuracy between predictions and measurements. These results indicate that for studies aiming at visualizing root distribution patterns of cover crops, the adoption of the profile wall as main method can in fact be recommended, especially regarding grasses, because its estimations were very close to that measured when extracting soil monoliths. We found as well that the profile wall method has potential for estimating accurately the cumulative root distribution of mixed cover crops, and of entire crop groups containing mixtures. Although this paper explored possibilities with regression models to compare methods and predict root length densities, many dataset improvements, and further validations in data acquired from a different experimental site (differing soil and climate conditions), timepoint and/or treatment would be necessary to present them as applicable tools in future crop root studies.

Supplementary Materials

The following are available online at https://www.mdpi.com/article/10.3390/agronomy12010048/s1, Table D1: Coefficients and quality metrics of non-linear regression models in train dataset, by crop group and data type; Table D2: Performance metrics of regression models in test dataset, by crop group and data type.

Author Contributions

M.A. and T.K. conceived the idea, planned the research and designed the experiments. R.K., P.M. and T.A.B. conducted the experiments in the field and collected the data. T.A.B. analysed the data and wrote the article. M.A., T.F.D., T.K and R.K. contributed to data interpretation, writing and editing of the article. All authors have read and agreed to the published version of the manuscript.

Funding

The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture.

Acknowledgments

We thank the technical staff of the ‘Wiesengut’ organic farming research station and our students for help in the field and laboratory.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Kristensen, H.L.; Thorup-Kristensen, K. Root growth and nitrate uptake of three different catch crops in deep soil layers. *Soil Sci. Soc. of Am. J.* 2004, *68*, 529–537.

[2] Thorup-Kristensen, K. Root development of nitrogen catch crops and of a succeeding crop of broccoli. *Acta Agric. Scand. - B Soil Plant Sci.* 1993, *43*, 58–64.

[3] Thorup-Kristensen, K. Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? *Plant Soil* 2001, *230*, 185–195.

[4] Barraclough, P.B. Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. *Plant Soil* 1989, *119*, 59–70.

[5] Wendling, M.; Büchi, L.; Amossé, C.; Sinaj, S.; Walter, A.; Charles, R. Influence of root and leaf traits on the uptake of nutrients in cover crops. *Plant Soil* 2016, *409*, 419–434.

[6] Baets, S. de; Poesen, J.; Gyssels, G.; Knapen, A. Effects of grass roots on the erodibility of topsoils during concentrated flow. *Geomorphology* 2006, *76*, 54–67.

[7] Sainju, U.M.; Singh, B.P.; Whitehead, W.F. Cover crop root distribution and its effects on soil nitrogen cycling. *J. Agron.* 1998, *90*, 511–518.

[8] Böhm, W. In situ estimation of root length at natural soil profiles. *J. of Agr. Sci.* 1976, *87*, 365–368.

[9] Böhm, W.; Maduakor, H.; Taylor, H.M. Comparison of five methods for characterizing soybean rooting density and development 1. *J. Agr.* 1977, *69*, 415–419.

[10] Gao, Y.; Duan, A.; Qiu, X.; Liu, Z.; Sun, J.; Zhang, J.; Wang, H. Distribution of roots and root length density in a maize/soybean strip intercropping system. *Agr. Water Manag.* 2010, *98*, 199–212.

[11] Gregory, P.J.; Reddy, M.S. Root growth in an intercrop of pearl millet/groundnut. *Field Crops Res.* 1982, *5*, 241–252.

[12] Yu, Y.; Loiskandl, W.; Kaul, H.-P.; Himmelbauer, M.; Wei, W.; Chen, L.; Bodner, G. Estimation of runoff mitigation by morphologically different cover crop root systems. *J. Hydrol* 2016, *538*, 667–676.

[13] Upchurch, D.R. Conversion of minirhizotron-root intersections to root length density. In *Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*; Taylor, H., Ed.; ASA CSSA SSSA: [S.l.], 1987; pp 51–65.

[14] Parker, C.J.; Carr, M.K.V.; Jarvis, N.J.; Puplampu, B.O.; Lee, V.H. An evaluation of the minirhizotron technique for estimating root distribution in potatoes. *J. Agric. Sci.* 1991, *116*, 341–350.

[15] Buckland, S.T.; Campbell, C.D.; Mackie-Dawson, L.A.; Horgan, G.W.; Duff, E.I. A method for counting roots observed in minirhizotrons and their theoretical conversion to root length density. *Plant and Soil* 1993, *153*, 1–9.

[16] Liao, R.; Bai, Y.; Liang, H.; An, S.; Ren, S.; Cao, Y.; Le, Z.; Lu, J.; Liu, J. Root growth of maize as studied with minirhizotrons and monolith methods. *Arch. Agr. Soil Sci.* 2015, *61*, 1343–1356.

[17] Li, L.; Sun, J.; Zhang, F.; Guo, T.; Bao, X.; Smith, F.A.; Smith, S.E. Root distribution and interactions between intercropped species. *Oecologia* 2006, *147*, 280–290.

[18] Böhm, W. *Methods of Studying Root Systems;* Springer Berlin Heidelberg: Berlin, Heidelberg, Germany, 1979.

[19] Köpke, U. Ein Vergleich von Feldmethoden zur Bestimmung des Wurzelwachstums Landwirtschaftlicher Kulturpflanzen. Ph.D thesis; University of Göttingen, Göttingen, Germany, 1979.

[20] Gregory, P.J. Plant roots: growth, activity and interaction with soils. *Ann. of Bot.* 2007, *100*, 151–152.

[21] Gaiser, T.; Perkons, U.; Küpper, P.M.; Puschmann, D.U.; Peth, S.; Kautz, T.; Pfeifer, J.; Ewert, F.; Horn, R.; Köpke, U. Evidence of improved water uptake from subsoil by spring wheat following lucerne in a temperate humid climate. *Field Crops Res.* 2012, *126*, 56–62.

[22] Han, E.; Kautz, T.; Perkons, U.; Uteau, D.; Peth, S.; Huang, N.; Horn, R.; Köpke, U. Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. *Biol. Fertil. Soils* 2015, *51*, 847–856.

[23] Yang, J.M.; Yang, J.Y.; Liu, S.; Hoogenboom, G. An evaluation of the statistical methods for testing the performance of crop models with observed data. *Agric. Syst.* 2014, *127*, 81–89.

[24] St-Pierre, N.R. Comparison of model predictions with measurements: A novel model-assessment method. *J. Dairy Sci.* 2016, *99*, 4907–4927.

[25] Delory, B.M.; Weidlich, E.W.A.; Meder, L.; Lütje, A.; Duijnen, R.; Weidlich, R.; Temperton, V.M. Accuracy and bias of methods used for root length measurements in functional root research. *Methods Ecol. Evol.* 2017, *8*, 1594–1606.

[26] Goubran, F.H.; Richards, D. The estimation of root length in samples and sub-samples: comparison of a visual and an automatic method. *Plant Soil* 1979, *52*, 77–83.

[27] Ruijter, F.J. de; Veen, B.W.; van Oijen, M. A comparison of soil core sampling and minirhizotrons to quantify root development of field-grown potatoes. *Plant Soil* 1996, *182*, 301–312.

[28] Ephrath, J.E.; Silberbush, M.; Berliner, P.R. Calibration of minirhizotron readings against root length density data obtained from soil cores. *Plant Soil* 1999, *209*, 201–208.

[29] Vepraskas, M.J.; Hoyt, G.D. Comparison of the trench-profile and core methods for evaluating root distributions in tillage studies. *J. Agr.* 1988, *80*, 166–172.

[30] Dusserre, J.; Audebert, A.; Radanielson, A.; Chopart, J.-L. Towards a simple generic model for upland rice root length density estimation from root intersections on soil profile. *Plant Soil* 2009, *325*, 277–288.

[31] Samson, B.K.; Sinclair, T.R. Soil core and minirhizotron comparison for the determination of root length density. *Plant Soil* 1994, *161*, 225–232.

[32] Osipitan, O.A.; Dille, J.A.; Assefa, Y.; Knezevic, S.Z. Cover Crop for Early Season Weed Suppression in Crops: Systematic Review and Meta-Analysis. *Agronomy Journal* 2018, *110*, 2211–2221, doi:10.2134/agronj2017.12.0752.

[33] Kemper, R.; Bublitz, T.A.; Müller, P.; Kautz, T.; Döring, T.F.; Athmann, M. Vertical root distribution of different cover crops determined with the profile wall method. *Agriculture* 2020, *10*, 503.

[34] Klimek-Kopyra, A.; Kulig, B.; Głąb, T.; Zając, T.; Skowera, B.; Kopcińska, J. Effect of plant intercropping and soil type on specific root length. *Rom. Agr. Res.* 2015, 221–230.

[35] Wit, C.T. de; Bergh, J. P. van den. Competition between herbage plants. *J. Agr. Sci.* 1965, *13*, 212–221.

[36] Grace, J.B.; Tilman, D. *Perspectives on Plant Competition;* Elsevier, 1990.

[37] Hauggaard-Nielsen, H.; Ambus, P.; Jensen, E.S. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. *Plant Soil* 2001, *236*, 63–74.

[38] Mead, R.; Willey, R.W. The concept of a 'Land Equivalent Ratio' and advantages in yields from intercropping. *Ex. Agric.* 1980, *16*, 217–228.

[39] Regent Instruments Inc. *WinRHIZO^{TM}*; Regent Instruments Inc.: Canada, 2017.

[40] Team, R.C. *R: A Language and Environment for Statistical Computing;* Vienna, Austria, 2021.

[41] Hawkins, D.M. The problem of overfitting. *J. Chem. Inf. Comput. Sci.* 2004, *44*, 1–12.

[42] Akaike, H. Information theory and an extension of the maximum likelihood principle. In *Proceedings of the 2nd international symposium on information theory*; Petrov, B.N., Csaki, F., Eds.; Akademiai Kiado: Budapest; pp 267–281.

[43] Loague, K.; Green, R.E. Statistical and graphical methods for evaluating solute transport models: overview and application. *J. Contam. Hydrol.* 1991, *7*, 51–73.

[44] Austin, E.E.; Wickings, K.; McDaniel, M.D.; Robertson, G.P.; Grandy, A.S. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. *GCB Bioenergy* 2017, *9*, 1252–1263.

[45] Sinclair, T.R.; Seligman, N. Criteria for publishing papers on crop modeling. *Field Crops Res.* 2000, *68*, 165–172.

[46] Kücke, M.; Schmid, H.; Spiess, A. A comparison of four methods for measuring roots of field crops in three contrasting soils. *Plant Soil* 1995, *172*, 63–71.

[47] Passioura, J.B. Simulation models: science, snake oil, education, or engineering? *J. Agron.* 1996, *88*, 690–694.

[48] Bodner, G.; Loiskandl, W.; Hartl, W.; Erhart, E.; Sobotik, M. Characterization of cover crop rooting types from integration of rhizobox imaging and root atlas information. *Plants (Basel)* 2019, *8*.

[49] Chopart, J.L.; Siband, P. Development and validation of a model to describe root length density of maize from root counts on soil profiles. *Plant Soil* 1999, *214*, 61–74.

[50] Heeraman, D.A.; Juma, N.G. A comparison of minirhizotron, core and monolith methods for quantifying barley (*Hordeum vulgare* L.) and fababean (*Vicia faba* L.) root distribution. *Plant Soil* 1993, *148*, 29–41.

[51] Chopart, J.-L.; Sine, B.; Dao, A.; Muller, B. Root orientation of four sorghum cultivars: application to estimate root length density from root counts in soil profiles. *Plant Root* 2008, *2*, 67–75.

[52] Lopez-Zamora, I.; Falcão, N.; Comerford, N.B.; Barros, N.F. Root isotropy and an evaluation of a method for measuring root distribution in soil trenches. *For. Ecol. Manag.* 2002, *166*, 303–310.

7 General Discussion

7.1 Methodology of root research: strengths, limitations and perspectives

In this thesis, the profile wall method and the monolith method, both using a soil trench, were used to study the root growth of cover crops on an organic experimental farm. FTIR spectroscopy was applied to discriminate root mass from different species in cover crop mixtures and photography was used to determine large-sized biopore density in the subsoil.

As previously discussed in chapter 2, the strengths of the profile wall method are the comparatively low technical and time requirements compared to methods based on root washing. This allows to sample with higher temporal resolution at a given sampling effort. Moreover, the profile wall method offers an easy separation of root length in bulk soil and large-sized biopores. Further advantages are that a high number of individual plants is included in the evaluation and that a high vertical resolution is possible. Besides, the profile wall method is a suitable method for comparing root distribution patterns (chapter 6). Limitations of the profile wall method are lower accuracy, less objectivity (chapter 2) and the fact that the profile wall method underestimates RLD compared to the monolith method, especially in densely rooted areas (chapter 6). Answering research question (6), the RLD of the profile wall method and of the monolith method do not agree. The problem of subjectivity due to the person counting the root length and the problem of underestimation of the RLD can be overcome by using software for analysing biological images, e.g., RootPainter (Han et al. 2021; Smith et al. 2022). Instead of manual counting, images of the profile wall are taken. Training a model for the segmentation can be easily conducted after field work by one person for several datasets. Han et al. (2021) showed that RLD is higher when profile wall images were segmented by RootPainter software compared to manual counting.

The monolith method is characterised by high precision in the analysis of root morphology; in addition, it allows the analysis of root mass and root chemical composition. However, these strengths come at a cost of high labour intensity, entailing high requirements of time and labour force. If only root morphology is of interest and not root mass or chemical composition, RootPainter (Smith et al. 2022) can help to differentiate roots and non-root material on the scanned images. Han et al. (2021) showed that RootPainter can clearly distinguish roots and non-root material. If dry mass, chemical analysis or FTIR spectroscopy of roots are of interest, there is still the need to separate non-root material and old roots from fresh roots. A possibility to overcome this problem might be the use of in-growth cores. These are mesh cylinders filled with sieved root-free soil which are inserted into a hole in a crop stand. The surrounding crops grow their roots into the cores and fine root biomass production can be estimated (Freschet et al. 2021; Vogt et al. 1998). Thus, this method enables the removal of particular organic matter and old roots in the soil before filling the in-growth cores and makes root washing easier. A further disadvantage, not of the monolith method itself, but due to its labour-intensive nature is, that it hardly allows repeated measurements during the crop growth cycle. With the profile wall method two investigations per season, one before and one after winter, were possible. Thus, the results revealed with the monolith method are only snapshots. The development of root formation was not displayed. Especially, the root growth dynamics in mixtures would have been interesting to investigate. Below-ground mixture effects might be limited in time or increase over time, as discussed in chapter 3. Information about root traits in sole and mixed cover crops was gathered just once, before the first frost event in late autumn. No information about the below-ground juvenile or after-winter development of cover crops could be obtained. If no root observation method like the profile wall method or minirhizotrons, but a root sampling method that allows repeated measurements is required, the in-growth core method might be considered.

In conclusion, both methods, profile wall and monolith method, are needed. The monolith method is the most precise method, and the absolute RLD values cannot be predicted from profile wall countings (chapter 6). However, for the comparison of root distribution patterns between different crops, the profile wall method is fast and well-suited. Consequently, the methods to determine the root growth of cover crops were chosen correctly in this thesis. The monolith method was used for the detailed investigation of root traits in sole and mixed cover crops, whereas the profile wall method was used to characterise root growth of different sole cover crops. The profile wall method thus answers the research question (1) of which root types of cover crops can be distinguished on the profile wall. It categorises winter rye and crimson clover as cover crops with a dense root system with many fine roots and high topsoil RLD; oil radish, winter turnip rape and phacelia as tap-rooted cover crops with high subsoil RLD and an intensive biopore use; bristle oat as fibrous rooted cover crops sharing some features with the tap-rooted cover crops; and blue lupin as a tap-rooted cover crop which has low RLD in topsoil as well as in subsoil (chapter 2).

FTIR spectroscopy is a well-suitable method for species-specific root mass discrimination (chapter 3 and 4). Statistical parameters showed well-fitting calibration models (Appendix B and C). However, FTIR spectroscopy relies on clean root samples obtained from monoliths or soil cores. qPCR-based methods (Haling et al. 2011) might be an alternative because root mass can be quantified directly from the soil without washing. However, these methods require special equipment and expertise. For a correct quantification of root biomass from DNA, a calibration with a traditional technique is needed (Haling et al. 2011). Since there are various influences such as plant age, species and root type on this indirect method (Haling et al. 2011), there is still a risk that root mass might be calculated incorrectly. As already discussed in chapter 3, FTIR spectroscopy only distinguishes root mass, but for a more detailed understanding of root growth in cover crop mixtures, there is a need for species-specific discrimination of root length. The DNA-based methods would not be helpful here either.

Biopore photography (chapter 5) is a simple method to determine the density of large-sized biopores. Image analysis with RootPainter (Smith et al. 2022) delivers objective and precise results. Identifying whether the recently cropped plants created biopores is not possible with this biopore photography as this method cannot distinguish between new biopores created by the last crop and older biopores. Thus, the photographed biopore density does not match the root pieces calculated from the monolith method. In the future, it would be desirable to develop a method to differentiate newly created from existing biopores.

7.2 Below-ground mixture effects, below-ground complementarity and vertical root niche differentiation in cover crop mixtures

In the field experiments with sole and mixed cover crops of this thesis, positive mixture effects in the shoot existed but were relatively low or non-significant (chapters 3 and 4). They tended to be higher for shoot nutrient uptake than for shoot biomass. The question of the below-ground mixture effects (research question 3) cannot be answered generalised. Positive mixture effects of RLD and RMD occurred in single layers. No clear pattern of positive mixture effects of RLD and RMD was found in lupin-rye mixtures (chapter 3). In the mixtures with oil radish, positive mixture effects for RLD and RMD were found in subsoil due to the strong proliferation of oil radish in deep layers (chapter 4) and their intensive biopore use (chapter 2). In all mixtures, positive mixture effects of SRL in both years and mixtures occurred throughout the whole profile except 0-10 cm in 2019 for the oil radish-containing mixtures (chapters 3 and 4). Higher SRL was also found in cover crop mixtures of rye and vetch by Bukovsky-Reyes et al. (2019). SRL is a root trait that is associated with resource acquisition and negatively correlates with root traits associated with resource conservation (Isaac et al. 2017). Moreover, SRL is understood as a trait representing below-ground economics as root length reflects the exploration potential for soil resources, and root mass reflects the cost for below-ground partitioning

(Freschet et al. 2021). In cover crop mixtures, plants thus might be forced to more economical root growth due to competition. SRL can be understood as a below-ground analogue of specific leaf area (Freschet et al. 2021). Accordingly, morphological changes due to competition, e.g., for light are known in shoot of intercrops. Higher specific leaf area was found in intercropped soybeans in mixtures to cope with shading (Li et al. 2020; Liu et al. 2017). SRL is inversely proportional to root tissue density (Ostonen et al. 2007). Low root tissue density is associated with high turnover and short longevity (Ryser 1996). Thus, rhizodeposition should be examined parallelly to SRL in future experiments, as discussed in chapter 4.

The results from the FTIR-spectroscopy helped to answer research question 2 (How does the root mass differ between the partners in the cover crop mixtures over the depth?). In the lupin-rye mixtures, homorhizous rye had the highest proportion of root biomass in topsoil, allorhizous lupin highest in the subsoil, whereas in the mixtures with oil radish, a clear pattern with homorhizous rye dominating the topsoil and allorhizous oil radish dominating the subsoil was not so pronounced in both years. This might be due to the dominance of oil radish (see chapter 4). Moreover, this might be influenced by the sampling size of the monolith and the chance to have a particularly vigorously developed taproot of oil radish in the sample.

The profiles of observed and expected RMD of the mixture partners did not differ considerably. Thus, vertical root niche differentiation in RMD between sole crops and mixtures was not clearly observed in any of the mixtures (chapters 3 and 4). In the case of mixtures containing oil radish, the weaker partners winter rye and crimson clover were suppressed in upper soil layers (chapter 4). Significant positive complementarity effects were not found for root mass; complementarity effects were predominantly negative (chapters 3 and 4). Thus, answering research question 4, below-ground complementarity or vertical root niche differentiation were not found in the studied cover crop mixtures.

The results raise the question of why no clear below-ground mixture effects of RLD and RMD (except in mixtures with oil radish in the subsoil), root mass complementarity or root vertical niche differentiation were found in the cover crop mixtures. Three possible approaches may answer this question.

First, the growing time might have been too short to build up below-ground mixture effects and complementarity, as discussed in chapters 3 and 4.

Second, more root mass or root length is not possible in the mixture, as competition in the shoot, e.g., for light, forces the mixture partners to invest in assimilates for elongation growth and restricts below-ground biomass partitioning. As weak above-ground and hardly any below-ground mixture effects were found, such a trade-off between shoot and root partitioning in mixtures is conceivable. Also, Bessler et al. (2009) discussed a trade-off between shoot and root partitioning in grassland species mixtures since they found above-ground overyielding in mixtures, whereas below-ground biomass remained constant in mixtures and the root-shoot-ratios were lower than expected in mixtures. In Figs. 3.2 and 4.1, for the majority of the mixtures, the root-to-shoot ratio tends to shift in advantage of the shoot biomass when comparing the observed with the expected biomass of the mixtures. Thus, if the net cost for tissue maintenance for roots is higher than for the shoot, less investment in roots is a benefit for the shoot, where competition for resources (e.g., light) is high (Bessler et al. 2009). The imbalance of mixtures and the dominance of oil radish, discussed in chapter 4, might reinforce the trade-off between shoot and root partitioning.

Third, more root mass or root length in the mixture may not be necessary. A reason for this might be that there is already sufficient root growth for soil exploitation to acquire mobile soil resources. At least in the topsoil, sufficient RLD might be more than reached. Moreover, higher SRL was observed as a morphological adaption in mixtures. A higher SRL enables sufficient nutrient and water uptake with less investment in root mass (Freschet et al. 2021). Roots with a higher SRL are thinner, and these “cheaper” roots result in a higher turnover (Ostonen et al. 2007; Ryser 1996). Thus, it is possible that the gross root mass in mixtures is higher than in sole crops after all. However, without quantifying net and gross root growth and its temporal dynamics, the question of below-ground mixture effects cannot fully be answered. Vertical root niche differentiation for root mass density was not found in these experiments. Here, it needs to be considered that vertical root niche differentiation was only analysed for root mass, not for root length, as it was methodologically not possible. Thus, possible effects based on root length dynamics, e.g., higher root length in biopores in mixtures with oil radish, might have been of no consequence for complementarity and niche differentiation of root mass.

Another aspect that needs to be highlighted for its impact on below-ground mixture effects is dominance, which may also be influenced by the environmental conditions and unbalanced mixture composition as discussed in chapters 3 and 4. For above-ground traits, Wendling et al. (2017) showed that cover crop mixture performance was higher when one species was not too dominant. In their study, complementary resource use was relevant for biomass production without N fertilisation, whereas with N fertilisation, resource partitioning was less important, and biomass production was more affected by the dominance of some species. Below-ground mixture effects may behave similarly and react to species dominance. Dominance in mixtures depends not only on species identity but also on seed density. However, little is known about the effect of seed density on RLD and RMD. As sole crops and also mixtures can compensate for low seed densities above-ground (Döring and Elsalahy 2022), it would be helpful to know about the relationship between seed density and root traits to better understand below-ground mixture effects.

Furthermore, it needs to be considered that the below-ground mixture effects constitute only overyielding and not transgressive overyielding. Significant transgressive overyielding was not found in the field experiments. Thus, mixtures did not perform better than the best sole crop. In their systematic review, Florence and McGuire (2020) analysed 243 comparisons of the best-performing sole crop and best-performing cover crop mixture for above-ground biomass, nitrogen retention and several other cover crop functions. They showed that in 88% of studies, the best sole crop and mixture were comparable, and in only 2% of the cases, the mixtures performed better than the best sole crop. This shows that the general potential of cover crop mixtures to perform better than all respective sole crops is relatively low, and the identity effect is very important in cover crop mixtures as discussed in chapter 4. Thus, from this perspective, the low below-ground mixture effects found in the field experiment of the thesis may not be surprising.

As no significant transgressive overyielding of root biomass was found, it was not possible to exploit the total soil profile more completely with mixtures through root mass. For the total root length of mixtures in the field experiments of this thesis, transgressive overyielding was neither found (Figure E.1). None of the mixture effects for total root length were significantly different from zero ($p \leq 0.05$, t-Test). Thus, also when root length is considered, it was not possible to exploit the total soil profile more completely with cover crop mixtures compared to sole cover crops. However, with the intensively rooting winter rye as sole crop, the bar was set very high.

7.3 Outlook on future research on cover crop mixtures concerning root traits

This thesis shows that root research is extremely laborious and time consuming. It contrasts with rather small mixing effects found for root traits. Thus, it arises the question of how to proceed in mixture research of traits like root mass, root length and specific root length. From a perspective on roots, methodological improvements, as discussed in chapter 7.1, offer new possibilities. From a mixture perspective, it is always problematic to calculate mixture effects for agroecosystems services of cover crops that are difficult to quantify. This is not only difficult for root traits but also for services like the reduction of nematodes, the promotion of invertebrates or other soil dwelling organisms. However, the major problem of mixture research is that there are endless possible combinations that would need to be tested. In the case of cover crops, this problem is particularly large, as there are more than 40 species that can be used as cover crops. For root traits, examined with the intensity and methods of this thesis, it is not even possible to test more than five different mixtures. Moreover, for mixtures of cover crops, it is shown in several studies (Freund et al. 2020; Wendling et al. 2019) that species identity is of very high importance, which makes it even more difficult to design mixtures with high mixture effects.

So, what are the opportunities to further explore root traits in mixtures and design mixtures based on the mixture effects of root traits? A possibility could be to refer from above-ground mixture effects to below-ground mixture effects. A potential relation between mixture indices of root and shoot mass could provide important information about mixture effects in the root zone without the need to carry out laborious root examinations. For maize-legume intercrops, significant correlations between the LER of RLD or RMD and the LER of grain yield, dry matter and N-uptake were found (Bilalis et al. 2005). The correlation between LER of shoot and root mass and the correlation between RME of shoot and root mass of cover crop mixtures of the field experiment in this thesis were both not significant (Fig. E.2). The correlation between pLER of shoot and pLER of root was only significant for winter rye (Fig. E.3). The analysis of both lupin-rye mixtures and mixtures containing oil radish, rye and crimson clover revealed big differences in mixture indices for shoot and root mass. Mixture effects of root mass were lower than those of shoot mass. Hence, it can be concluded that it was not possible to estimate root mass mixture effects from the shoot mixture effect in the cover crop mixtures of these field experiments. In contrast, Bilalis et al. (2005) achieved significant and very strong correlations between LER of root and shoot parameters for maize-legume intercrops. However, their experiment differed clearly from the present study as they used relay-strip-intercrops and examined roots only in the top 25 cm.

Another option is to combine mixture partners with functional complementarity. By combining functionally more divergent partners, mixture effects might be more pronounced (Amsili and Kaye 2021). However, to some degree, this was already done in this thesis by combining allorhizous and homorhizous cover crops. Still, the mixture effects were weak, which might be because the functional complementarity refers only to a few decimetres of root depth or growth period was short. Thus, even more extreme functional complementarity is needed to increase mixture effects. Cardinael et al. (2015) and Mulia and Dupraz (2006) demonstrated in agroforestry systems with winter annual crops and walnut trees that the tree roots were shifted downwards due to competition by the annual crops. Thus, to exploit mixture effects in the root zone to a greater extent, annual and perennial crops need to be combined, which can be done in agroforestry systems or with fodder crop strips between annual crops. However, this does not advance the composition of cover crop mixtures.

As vertical root niche differentiation was not detectable, it might be interesting to analyse cover crop mixtures for temporal root niche differentiation. This requires several samplings during the crop growth period. Methodological challenges regarding repeated samplings are discussed in chapter 7.1. Moreover, cover cropping systems could be developed to increase temporal root niche differentiation, like in a relay intercrop system, where the earlier sown species freely explores the soil volume and the later sown species recovers root growth after the early sown species is harvested (Yu et al. 2022). In the case of cover crops, the earlier sown species could be a frost-killed species with vigorous growth and nutrient uptake before winter and the later sown species could be a winter hardy species for soil coverage during winter and nitrogen transfer to the subsequent main crop. The later sown species may then recover after the other species is frost-killed.

Another way to find well-composed cover crop mixtures is to use participative approaches. They are popular in organic farming, e.g., in organic breeding (Colley et al. 2021). Cover crop mixtures could be tested in a decentralised way on several farms. Due to the different sites, information can be generated about the adaption of different mixtures to environmental conditions. For easily recordable parameters like soil coverage or coverage ratio of the mixture partners, decentralised on-farm trials are well suitable. For below-ground traits, testing mixtures on different sites would also be interesting due to adaption of the mixture on different soils. However, the huge effort for root analysis is not manageable in multi-site on-farm experiments. What might be possible is a spade diagnosis that could be extended for some deep rooting parameters with soil cores or a profile wall to quantify rooting depth or biopore use. The spade diagnosis (Beste 2002) is popular among organic farmers. However, this approach would not deliver enough data for scientific evaluation but may help farmers with their decision for the composition of cover crop mixtures.

As cover crops provide several ecosystem services (see chapter 1.1), trade-offs between these services may exist (Finney and Kaye 2017). In a mixture, different partners deliver different services, e.g., weed suppression and prevention of nitrate leaching by non-legumes and nitrogen fixation by legumes (Lavergne et al. 2021). Services of a specific partner may not be fully exploited if the mixture is not well designed and the specific partner is suppressed. Besides, shoot-based services, e.g., soil coverage and root-based services, such as nutrient uptake from deep layers can be conflicting, as assimilate partitioning between shoot and root is limited. Thus, objectives and priorities for the ecosystem services of the cover crop mixture need to be set before designing and testing different mixtures. For example, after vegetables with high residual nitrogen, deep-rooting and nutrient uptake are more important, whereas in a cropping system with reduced tillage soil coverage and weed suppression are more important.

7.4 Potential of cover crop roots and findings relevant for practical arable farming

The farmers' interest in cover crop roots might mainly be root intensity for aggregate stability and nutrient uptake, carbon storage, as well as biopore genesis potential. These functions can be represented by parameters examined in this thesis: RLD (Hudek et al. 2022; Sainju et al. 1998), root mass (Kätterer et al. 2011; Poeplau and Don 2015) and root diameter (Han et al. 2015a; Perkons 2018), respectively. But what is the scale of these functions of cover crops? In Table 7.1, the mean values from the field experiments of this thesis are shown for some root traits that may allow an assessment of the functions of cover crop roots. RLD in the top 30 cm represents the potential to improve topsoil structure and aggregate stability. RLD in the subsoil from 30 to 90 cm represents the potential for nutrient uptake from lower soil layers and prevention of nitrate leaching. Root dry mass per area represents the carbon storage potential. The number of root pieces (> 2 mm) per area in 40-50 cm soil depth represents the genesis potential for large-size biopores in the subsoil.

Table 7.1 Root traits of cover crops representing potential agroecosystem services of cover crop roots. Values are the means of two experimental years 2018 and 2019, * values from 2018 only, ** values from 2019 only.

agroecosystem service	"topsoil structure/ aggregate stability"	"subsoil nutrient uptake/ nitrate leaching prevention"	"carbon storage potential"	"biopore genesis potential"
root trait	RLD [cm cm^{-3}] in 0-30 cm soil depth	RLD [cm cm^{-3}] in 30-90 cm soil depth	root dry mass [t ha^{-1}]	root pieces m^{-2} (> 2 mm) in 40-50 cm soil depth
crimson clover	2.3	0.2	0.39	0
blue lupin	1.4	0.3	0.43	22
winter rye	7.5	0.6	1.02	9
bristle oat*	5.0	0.5	0.73	0
oil radish	3.8	1.1	0.79	5
winter turnip rape*	4.8	1.0	0.55	4
phacelia*	4.0	1.1	0.62	1
lupin - winter rye 50:50	5.1	0.5	0.74	6
lupin - winter rye 75:25**	3.5	0.5	0.37	0
oil radish - winter rye 50:50	4.4	1.1	0.65	1
oil radish - crimson clover - winter rye 33:33:33	4.6	1.1	0.76	0

Values of cover crops were 1.4-7.5 cm/cm³ for topsoil RLD, 0.2-1.1 cm/cm³ for subsoil RLD, 0.37-1.02 t/ha root mass, and 0-22 root pieces/m² (Table 7.1). High topsoil RLD was found for winter rye, the lupin-rye mixture (50:50), and bristle oat. Oil radish, the mixtures with oil radish, phacelia and winter turnip rape reached high subsoil RLD. Cover crop root mass was high for winter rye, oil radish, the three-species mixture, the lupin-rye mixture (50:50), and bristle oat. The highest number of root pieces was found for blue lupin.

In the study of Wendling et al. (2016), higher cover crop root mass values were found for oil radish (1.83 t ha^{-1}), bristle oat (1.46 t ha^{-1}) and phacelia (1.18 t ha^{-1}). Also, RLD found by Wendling et al. (2016) was higher. However, compared to fodder crops, which have a longer cropping duration, the values for the root traits of cover crops are generally low. RLD of chicory, lucerne and tall fescue in 45 to 55 cm soil depth was between 1 and 2 cm/cm³ for a cropping duration of one year (Han et al. 2015a). In the second cropping year, RLD of chicory and tall fescue between 45 and 105 cm soil depth reached values between 1.7 and 2.0 cm/cm³ for chicory and between 1.8 and 3.2 cm/cm³ for tall fescue (Han et al. 2015b). In the same field experiment, root pieces m^{-2} with a root diameter > 2 mm at 45-55 cm soil depth were calculated: 115

for lucerne, 53 for chicory, and 6 for tall fescue (Perkons 2018). With regard to research question 5, it can be said that the potential of cover crops to generate large-sized biopores is low compared to perennial fodder crops. In an own field experiment (Kemper 2023), after 1.5 years of growing time topsoil RLD of 22 cm/cm³ was found for a red clover-ryegrass mixture, subsoil RLD values between 0.6 and 0.9 cm/cm³ for various fodder crops, lucerne root dry matter of 6 t ha^{-1}and 195 root pieces m^{-2} (> 2 mm) in 40-50 cm soil depth for tall fescue.

Thus, potential of cover crops grown during autumn and winter to improve the topsoil structure, store carbon in the soil and generate large-size biopores is much lower compared to annually or perennially cultivated fodder crops. The subsoil RLD relevant for nutrient uptake is in a similar order of magnitude for short-grown deep-rooting cover and longer-grown fodder crops. In summary, deep-rooting cover crops provide similar catch crop service as perennial fodder crops, whereas the potential to improve soil structure and enhance soil organic matter is much higher in fodder cropping. For our temperate organic cropping systems, these findings mean that cover crops and perennial forages should be considered as complements rather than alternatives. This needs to be considered in practical farming for the planning of cropping systems and their crop rotations.

Perennial fodder crops have higher RLD, higher root mass and thicker roots than cover crops. To improve these traits of cover crops, an increase in the cropping duration might be important. Agronomic management options for this are sowing after early harvested crops, undersowing into the previous main crop, simultaneous sowing with combine harvest or direct drilling immediately after precrop harvest, and using winter hardy cover crops, which increase root intensity during winter (chapter 2). Future research needs to show whether these different agronomic management strategies can increase the performance of cover crop roots. A further agronomic management option that is discussed in practical farming is technical loosening of the lower topsoil before sowing cover crops (Hampl 2020). The effects of technical loosening on root intensity, root mass and rooting depth of cover crops also need to be investigated in future field experiments.

As shown above, cover crops have a lower potential to generate large-sized biopores compared to perennial fodder crops. However, there might be agronomic management strategies to increase the number of root pieces (>2 mm). A lower seed density may result in stronger individual plants with thicker roots, leading to more root pieces >2 mm and thus a higher biopore genesis potential. The relationship between root diameter and sowing density is shown by Hakl et al. (2021) for Lucerne where the taproot diameter decreases with plant density. Thus, a cover crop stand with a low density of a tap-rooted species as a sole crop or with a low competitive mixture partner might have an increased potential to generate large-sized biopores. However, reducing the cover crop density may be a trade-off with other functions such as weed suppression or soil coverage.

In the discussion about carbon sequestration by roots, it needs to be considered that there might be a difference between roots in subsoil and topsoil, as carbon losses from root material were higher in topsoil than in subsoils (Liang et al. 2018). Thus, subsoil root mass, which might be higher in perennial fodder crops than in cover crops, is of importance for long-term carbon sequestration. Besides root mass, which will be found with monolith sampling, also root exudates and dead roots are important. As discussed in chapter 4, the turnover of roots, as well as root exudation, might also be relevant for carbon storage. Austin et al. (2017) investigated that rhizodeposition of a winter rye cover crop accounted for over 30 % of the total below-ground carbon input of the cover crop.

Cover crops have more intensive rooting in the topsoil (chapters 2, 3 and 4) and do not reach larger diameters in subsoil (chapter 5) and thus may improve the structure in upper soil layers more effectively than lower soil layers. Their effect of soil structure improvement might be higher in no-till or minimal tillage systems compared to arable systems with regular ploughing. Abdollahi and Munkholm (2014) investigated the effect of cover crops on soil quality depending on the tillage system and found a significant interaction between cover crop and tillage system for the soil friability with a higher effect of the cover crop in the no-till system. The study of Abdollahi and Munkholm (2014) may support the above-mentioned assumption.

A basic question for farmers and seed companies is how to design proper cover crop mixtures. This question was not of fundamental interest to the field experiments of this thesis. However, their results delivered some suggestions about mixing cover crop species. Mixing deep rooting and intensively biopore using crops like oil radish, even in lower mixture proportions, increased the RLD and RMD in the subsoil compared to cover crops that were not intensively rooting into the subsoil (chapter 4). Moreover, balanced mixtures are needed to receive positive mixture effects (chapters 4 and 5). More information about competitiveness and maximal mixing proportions of the different cover crop species is thus necessary. A further open question that is relevant for practical agronomic management of cover crops is how mixtures perform under variable and unfavourable conditions, which will become more frequent due to climate change crisis.

In conclusion, the root traits of cover crops representing agroecosystem services differ between the various species, and mixtures are characterised by the dominant partner. Compared to roots of perennial fodder crops, the potential agroecosystem services of cover crop roots are likely to be lower. Agronomic management options for enhancing the potential of cover crop roots need further investigations.

8 References

Abdollahi L, Munkholm LJ (2014) Tillage System and Cover Crop Effects on Soil Quality: I. Chemical, Mechanical, and Biological Properties. Soil Science Society of America Journal 78:262–270. doi:10.2136/sssaj2013.07.0301

Akaike, H. (1998) Information theory and an extension of the maximum likelihood principle. In Proceedings of the 2nd International Symposium on Information Theory, Tsahkadsor, Armenia, 2–8 September 1971; Petrov, B.N., Csaki, F., Eds.; Akademiai Kiado: Budapest, Hungary, 267–281

Amsili JP, Kaye JP (2021) Root traits of cover crops and carbon inputs in an organic grain rotation. Renewable Agriculture and Food Systems 36:182–191. doi:10.1017/S1742170520000216

Andersen SN, Dresbøll DB, Thorup-Kristensen K (2014) Root interactions between intercropped legumes and non-legumes—a competition study of red clover and red beet at different nitrogen levels. Plant and Soil 378:59–72. doi:10.1007/s11104-013-2014-4

Athmann M, Kautz T, Pude R, Köpke U (2013) Root growth in biopores—evaluation with in situ endoscopy. Plant and Soil 371:179–190. doi:10.1007/s11104-013-1673-5

Athmann M, Sondermann J, Kautz T, Köpke U (2019) Comparing Macropore Exploration by Faba Bean, Wheat, Barley and Oilseed Rape Roots Using In Situ Endoscopy. Journal of Soil Science and Plant Nutrition 19:689–700. doi:10.1007/s42729-019-00069-0

Austin EE, Wickings K, McDaniel MD, Robertson GP, Grandy AS (2017) Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9:1252–1263. doi:10.1111/gcbb.12428

Baets S de, Poesen J, Gyssels G, Knapen A (2006) Effects of grass roots on the erodibility of topsoils during concentrated flow. Geomorphology 76:54–67. doi:10.1016/j.geomorph.2005.10.002

Baets S de, Poesen J, Meersmans J, Serlet L (2011) Cover crops and their erosion-reducing effects during concentrated flow erosion. CATENA 85:237–244. doi:10.1016/j.catena.2011.01.009

Bakker LM, Mommer L, van Ruijven J (2016) Can root trait diversity explain complementarity effects in a grassland biodiversity experiment? Journal of Plant Ecology 11:rtw111. doi:10.1093/jpe/rtw111

Bardgett RD, Mommer L, Vries FT de (2014) Going underground: root traits as drivers of ecosystem processes. Trends in ecology & evolution 29:692–699. doi:10.1016/j.tree.2014.10.006

Barraclough PB (1989) Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. Plant and Soil 119:59–70. doi:10.1007/BF02370269

Bedoussac L, Journet EP, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, Jensen ES, Prieur L, Justes E (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agronomy for Sustainable Development 35:911–935. doi:10.1007/s13593-014-0277-7

Bessler H, Temperton VM, Roscher C, Buchmann N, Schmid B, Schulze ED, Weisser WW, Engels C (2009) Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. Ecology 90:1520–1530. doi:10.1890/08-0867.1

Beste A (2002) Weiterentwicklung und Erprobung der Spatendiagnose als Feldmethode zur Bestimmung ökologisch wichtiger Gefügeeigenschaften landwirtschaftlich genutzter Böden. Dissertation. Justus-Liebig-Universität Gießen, Gießen

Bilalis DJ, Sidiras N, Kakampouki I, Efthimiadou A, Thomopoulos P (2005) Effects of organic fertilization on maize/legume intercrop in a clay loam soil and Mediterranean climate – Can the Land Equivalent Ratio (LER) index be used for root development? Journal of Food Agriculture and Environment 3:117–123. doi:10.1234/4.2005.670

Blanco-Canqui H, Shaver TM, Lindquist JL, Shapiro CA, Elmore RW, Francis CA, Hergert GW (2015) Cover Crops and Ecosystem Services: Insights from Studies in Temperate Soils. Agronomy Journal 107:2449–2474. doi:10.2134/agronj15.0086

Bodner G, Himmelbauer M, Loiskandl W, Kaul HP (2010) Improved evaluation of cover crop species by growth and root factors. Agronomy for Sustainable Development 30:455–464. doi:10.1051/agro/2009029

Bodner G, Leitner D, Kaul HP (2014) Coarse and fine root plants affect pore size distributions differently. Plant and Soil 380:133–151. doi:10.1007/s11104-014-2079-8

Bodner G, Leitner D, Nakhforoosh A, Sobotik M, Moder K, Kaul HP (2013) A statistical approach to root system classification. Frontiers in Plant Science 4:292. doi:10.3389/fpls.2013.00292

Bodner G, Loiskandl W, Hartl W, Erhart E, Sobotik M (2019) Characterization of Cover Crop Rooting Types from Integration of Rhizobox Imaging and Root Atlas Information. Plants 8. doi:10.3390/plants8110514

Böhm W (1976) In situ estimation of root length at natural soil profiles. The Journal of Agricultural Science 87:365–368. doi:10.1017/S0021859600027660

Böhm W (1979) Methods of Studying Root Systems, Vol 33. Springer, Berlin, Heidelberg

Böhm W, Maduakor H, Taylor HM (1977) Comparison of Five Methods for Characterizing Soybean Rooting Density and Development 1. Agronomy Journal 69:415–419. doi:10.2134/agronj1977.00021962006900030021x

Brust J, Claupein W, Gerhards R (2014) Growth and weed suppression ability of common and new cover crops in Germany. Crop Protection 63:1–8. doi:10.1016/j.cropro.2014.04.022

Brust J, Gerhards R (2012) Lopsided oat (Avena strigosa) as a new summer annual cover crop for weed suppression in Central Europe. Julius-Kühn-Archiv 1:257–264

Buckland ST, Campbell CD, Mackie-Dawson LA, Horgan GW, Duff EI (1993) A method for counting roots observed in minirhizotrons and their theoretical conversion to root length density. Plant and Soil 153:1–9. doi:10.1007/BF00010539

Bukovsky-Reyes S, Isaac ME, Blesh J (2019) Effects of intercropping and soil properties on root functional traits of cover crops. Agriculture, Ecosystems & Environment 285:106614. doi:10.1016/j.agee.2019.106614

Burr-Hersey JE, Mooney SJ, Bengough AG, Mairhofer S, Ritz K (2017) Developmental morphology of cover crop species exhibit contrasting behaviour to changes in soil bulk density, revealed by X-ray computed tomography. PloS one 12:e0181872. DOI: https://doi:10.1371/journal.pone.0181872

Bybee-Finley KA, Cordeau S, Yvoz S, Mirsky SB, Ryan MR (2022) Finding the right mix: a framework for selecting seeding rates for cover crop mixtures. Ecological applications: a publication of the Ecological Society of America 32:e02484. doi:10.1002/eap.2484

Cardinael R, Mao Z, Prieto I, Stokes A, Dupraz C, Kim JH, Jourdan C (2015) Competition with winter crops induces deeper rooting of walnut trees in a Mediterranean alley cropping agroforestry system. Plant and Soil 391:219–235. doi:10.1007/s11104-015-2422-8

Cardinale BJ, Wright JP, Cadotte MW, Carroll IT, Hector A, Srivastava DS, Loreau M, Weis JJ (2007) Impacts of plant diversity on biomass production increase through time because of species complementarity. Proceedings of the National Academy of Sciences 104:18123–18128. doi:10.1073/pnas.0709069104

Chopart JL, Siband P (1999) Development and validation of a model to describe root length density of maize from root counts on soil profiles. Plant and Soil 214:61–74. doi:10.1023/A:1004658918388

Chopart JL, Sine B, Dao A, Muller B (2008) Root orientation of four sorghum cultivars: application to estimate root length density from root counts in soil profiles. Plant Root 2:67–75. doi:10.3117/plantroot.2.67

Colley MR, Dawson JC, McCluskey C, Myers JR, Tracy WF, van Lammerts Bueren ET (2021) Exploring the emergence of participatory plant breeding in countries of the Global North – a review. The Journal of Agricultural Science 159:320–338. doi:10.1017/S0021859621000782

Corre-Hellou G, Fustec J, Crozat Y (2006) Interspecific Competition for Soil N and its Interaction with N2 Fixation, Leaf Expansion and Crop Growth in Pea–Barley Intercrops. Plant and Soil 282:195–208. doi:10.1007/s11104-005-5777-4

Couëdel A, Alletto L, Tribouillois H, Justes É (2018) Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. Agriculture, Ecosystems & Environment 254:50–59. doi:10.1016/j.agee.2017.11.017

Delory BM, Weidlich EWA, Meder L, Lütje A, Duijnen R, Weidlich R, Temperton VM (2017) Accuracy and bias of methods used for root length measurements in functional root research. Methods in Ecology and Evolution 8:1594–1606. doi:10.1111/2041-210X.12771

Diepenbrock W, Fischbeck G, Heyland KU, Knauer N (1999) Spezieller Pflanzenbau, 3rd edn. Verlag Eugen Ulmer, Stuttgart

Dirks I, Streit J, Meinen C (2021) Above and Belowground Relative Yield Total of Clover–Ryegrass Mixtures Exceed One in Wet and Dry Years. Agriculture 11:206. doi:10.3390/agriculture11030206

Döring TF, Elsalahy H (2022) Quantifying compensation in crop mixtures and monocultures. European Journal of Agronomy 132:126408. doi:10.1016/j.eja.2021.126408

Dusserre J, Audebert A, Radanielson A, Chopart JL (2009) Towards a simple generic model for upland rice root length density estimation from root intersections on soil profile. Plant and Soil 325:277–288. doi:10.1007/s11104-009-9978-0

Eissenstat DM (1992) Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition 15:763–782. doi:10.1080/01904169209364361

Elhakeem A, Bastiaans L, Houben S, Couwenberg T, Makowski D, van der Werf W (2021) Do cover crop mixtures give higher and more stable yields than pure stands? Field Crops Research 270:108217. doi:10.1016/j.fcr.2021.108217

Elhakeem A, van der Werf W, Ajal J, Lucà D, Claus S, Vico RA, Bastiaans L (2019) Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. Agriculture, Ecosystems & Environment 285:106627. doi:10.1016/j.agee.2019.106627

Elhakeem A, van der Werf W, Bastiaans L (2021) Radiation interception and radiation use efficiency in mixtures of winter cover crops. Field Crops Research 264:108034. doi:10.1016/j.fcr.2020.108034

Ephrath JE, Silberbush M, Berliner PR (1999) Calibration of minirhizotron readings against root length density data obtained from soil cores. Plant and Soil 209:201–208. doi:10.1023/A:1004556100253

Esnarriaga DN, Mariotti M, Cardelli R, Arduini I (2020) The Importance of Root Interactions in Field Bean/Triticale Intercrops. Plants 9. doi:10.3390/plants9111474

Fan J, McConkey B, Wang H, Janzen H (2016) Root distribution by depth for temperate agricultural crops. Field Crops Research 189:68–74. doi:10.1016/j.fcr.2016.02.013

Felten S von, Schmid B (2008) Complementarity among species in horizontal versus vertical rooting space. Journal of Plant Ecology 1:33–41. doi:10.1093/jpe/rtm006

Finney DM, Kaye JP (2017) Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. Journal of Applied Ecology 54:509–517. doi:10.1111/1365-2664.12765

Florence AM, McGuire AM (2020) Do diverse cover crop mixtures perform better than monocultures? A systematic review. Agronomy Journal 112:3513–3534. doi:10.1002/agj2.20340

Freschet GT, Pagès L, Iversen CM, Comas LH, Rewald B, Roumet C, Klimešová J, Zadworny M, Poorter H, Postma JA, Adams TS, Bagniewska-Zadworna A, Bengough AG, Blancaflor EB, Brunner I, Cornelissen JHC, Garnier E, Gessler A, Hobbie SE, Meier IC, Mommer L, Picon-Cochard C, Rose L, Ryser P, Scherer-Lorenzen M, Soudzilovskaia NA, Stokes A, Sun T, Valverde-Barrantes OJ, Weemstra M, Weigelt A, Wurzburger N, York LM, Batterman SA, Gomes de Moraes M, Janeček Š, Lambers H, Salmon V, Tharayil N, McCormack ML (2021) A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytologist 232:973–1122. doi:10.1111/nph.17572

Freund L, Mariotte P, Santonja M, Buttler A, Jeangros B (2020) Species identity, rather than species mixtures, drives cover crop effects on nutrient partitioning in unfertilized agricultural soil. Plant and Soil. doi:10.1007/s11104-020-04782-z

Gaiser T, Perkons U, Küpper PM, Puschmann DU, Peth S, Kautz T, Pfeifer J, Ewert F, Horn R, Köpke U (2012) Evidence of improved water uptake from subsoil by spring wheat following lucerne in a temperate humid climate. Field Crops Research 126:56–62. doi:10.1016/j.fcr.2011.09.019

Gao Y, Duan A, Qiu X, Liu Z, Sun J, Zhang J, Wang H (2010) Distribution of roots and root length density in a maize/soybean strip intercropping system. Agricultural Water Management 98:199–212. doi:10.1016/j.agwat.2010.08.021

Gentsch N, Boy J, Batalla JDK, Heuermann D, Wirén N von, Schweneker D, Feuerstein U, Groß J, Bauer B, Reinhold-Hurek B, Hurek T, Céspedes FC, Guggenberger G (2020) Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. Biology and Fertility of Soils 56:943–957. doi:10.1007/s00374-020-01475-8

Goubran FH, Richards D (1979) The estimation of root length in samples and sub-samples. Plant and Soil 52:77–83. doi:10.1007/BF02197734

Grace, JB, Tilman, D (1990) Perspectives on Plant Competition; Elsevier: Amsterdam, The Netherlands

Gregory PJ, Reddy MS (1982) Root growth in an intercrop of pearl millet/groundnut. Field Crops Research 5:241–252. doi:10.1016/0378-4290(82)90027-2

Gregory, PJ (2007) Plant roots: Growth, activity and interaction with soils. Ann. Bot., 100, 151–152

Griffiths M, Delory BM, Jawahir V, Wong KM, Bagnall GC, Dowd TG, Nusinow DA, Miller AJ, Topp CN (2022) Optimisation of root traits to provide enhanced ecosystem services in agricultural systems: A focus on cover crops. Plant, cell & environment 45:751–770. doi:10.1111/pce.14247

Grosse M, Heß J (2018) Sommerzwischenfrüchte für verbessertes Stickstoff- und Beikrautmanagement in ökologischen Anbausystemen mit reduzierter Bodenbearbeitung in den gemäßigten Breiten. Journal für Kulturpflanzen 70:173–183

Hakl J, Pisarčik M, Fuksa P, Šantrůček J (2021) Potential of lucerne sowing rate to influence root development and its implications for field stand productivity. Grass and Forage Science 76:378–389. doi:10.1111/gfs.12546

Halde C, Gulden RH, Entz MH (2014) Selecting Cover Crop Mulches for Organic Rotational No-Till Systems in Manitoba, Canada. Agronomy Journal 106:1193–1204. doi:10.2134/agronj13.0402

Haling RE, Simpson RJ, McKay AC, Hartley D, Lambers H, Ophel-Keller K, Wiebkin S, Herdina, Riley IT, Richardson AE (2011) Direct measurement of roots in soil for single and mixed species using a quantitative DNA-based method. Plant and Soil 348:123–137. doi:10.1007/s11104-011-0846-3

Hamblin AP, Tennant D (1987) Root length density and water uptake in cereals and grain legumes: how well are they correlated. Australian Journal of Agricultural Research 38:513. doi:10.1071/AR9870513

Hampl U (2020) Lockerung der Unterkrume im Ackerbau. Nichtwendende Bodenbearbeitung als Vorbereitung für den Gemengeanbau, https://www.bodenfruchtbarkeit.bio/bauerliches-praxiswissen/praxiswissen-lockerung-der-unterkrume, 07.02.2023

Han E, Kautz T, Perkons U, Lüsebrink M, Pude R, Köpke U (2015) Quantification of soil biopore density after perennial fodder cropping. Plant and Soil 394:73–85. doi:10.1007/s11104-015-2488-3

Han E, Kautz T, Perkons U, Uteau D, Peth S, Huang N, Horn R, Köpke U (2015) Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. Biology and Fertility of Soils 51:847–856. doi:10.1007/s00374-015-1032-1

Han E, Smith AG, Kemper R, White R, Kirkegaard JA, Thorup-Kristensen K, Athmann M (2021) Digging roots is easier with AI. Journal of experimental botany 72:4680–4690. doi:10.1093/jxb/erab174

Hansen DR, Spicer GS, Patterson R (2009) Phylogenetic Relationships Between and within Phacelia Sections Whitlavia and Gymnobythus (Boraginaceae). Systematic Botany 34:737–746. doi:10.1600/036364409790139637

Hartwig NL, Ammon HU (2002) Cover crops and living mulches. Weed Science 50:688–699

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Interspecific competition, N use and interference with weeds in pea–barley intercropping. Field Crops Research 70:101–109. doi:10.1016/S0378-4290(01)00126-5

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing 32P technique. Plant and Soil 236:63–74. doi:10.1023/A:1011909414400

Hauggaard-Nielsen H, Ambus P, Jensen ES (2003) The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. Nutrient Cycling in Agroecosystems 65:289–300. doi:10.1023/A:1022612528161

Hauggaard-Nielsen H, Jensen ES (2005) Facilitative root interactions in intercrops. In: Lambers H, Colmer TD (eds), Root Physiology: from Gene to Function, Vol 4. Springer, Dordrecht, pp 237–250

Hauggaard-Nielsen H, Jørnsgaard B, Kinane J, Jensen ES (2008) Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. Renewable Agriculture and Food Systems 23:3–12. doi:10.1017/S1742170507002025

Hawkins DM (2004) The problem of overfitting. Journal of Chemical Information and Computer Sciences 44:1–12. doi:10.1021/ci0342472

Heeraman DA, Juma NG (1993) A comparison of minirhizotron, core and monolith methods for quantifying barley (Hordeum vulgare L.) and fababean (Vicia faba L.) root distribution. Plant and Soil 148:29–41. doi:10.1007/BF02185382

Herrera JM, Feil B, Stamp P, Liedgens M (2010) Root growth and nitrate-nitrogen leaching of catch crops following spring wheat. Journal of environmental quality 39:845–854. doi:10.2134/jeq2009.0306

Heuermann D, Gentsch N, Boy J, Schweneker D, Feuerstein U, Groß J, Bauer B, Guggenberger G, Wirén N von (2019) Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils. Scientific reports 9:11531. doi:10.1038/s41598-019-48060-0

Hill JO, Simpson RJ, Moore AD, Chapman DF (2006) Morphology and response of roots of pasture species to phosphorus and nitrogen nutrition. Plant and Soil 286:7–19. doi:10.1007/s11104-006-0014-3

Homulle Z, George TS, Karley AJ (2022) Root traits with team benefits: understanding belowground interactions in intercropping systems. Plant and Soil 471:1–26. doi:10.1007/s11104-021-05165-8

Hudek C, Putinica C, Otten W, Baets S de (2022) Functional root trait-based classification of cover crops to improve soil physical properties. European Journal of Soil Science 73. doi:10.1111/ejss.13147

in‘t Zandt D, Fritz C, Wichern F (2018) In the land of plenty: catch crops trigger nitrogen uptake by soil microorganisms. Plant and Soil 423:549–562. doi:10.1007/s11104-017-3540-2

Isaac ME, Borden KA (2019) Nutrient acquisition strategies in agroforestry systems. Plant and Soil 444:1–19. doi:10.1007/s11104-019-04232-5

Isaac ME, Martin AR, Melo Virginio Filho E de, Rapidel B, Roupsard O, van den Meersche K (2017) Intraspecific Trait Variation and Coordination: Root and Leaf Economics Spectra in Coffee across Environmental Gradients. Frontiers in Plant Science 8:1196. doi:10.3389/fpls.2017.01196

Jensen ES (1996) Grain yield, symbiotic N2 fixation and interspecific competition for inorganic N in pea-barley intercrops. Plant and Soil 182:25–38. doi:10.1007/BF00010992

Jian J, Du X, Reiter MS, Stewart RD (2020) A meta-analysis of global cropland soil carbon changes due to cover cropping. Soil Biology and Biochemistry 143:107735. doi:10.1016/j.soilbio.2020.107735

Justes E, Bedoussac L, Dordas C, Frak E, Louarn G, Boudsocq S, Journet E-P, Lithourgidis A, Pankou C, Zhang C, Carlsson G, Jensen ES, Watson C, Li L (2021) The 4 C approach as a way to understand species interactions determining intercropping productivity. Frontiers of Agricultural Science and Engineering 0:0. doi:10.15302/J-FASE-2021414

Justes E, Constantin J, Dürr C, Hermon C, Le Bas C, Mignolet C, Savini I, Réchauchère O (2017) Study Context and Methodology. In: Justes E (ed), Cover Crops for Sustainable Farming. Springer, Dordrecht, pp 1–12

Kanders MJ, Berendonk C, Fritz C, Watson C, Wichern F (2017) Catch crops store more nitrogen below-ground when considering Rhizodeposits. Plant and Soil 417:287–299. doi:10.1007/s11104-017-3259-0

Kätterer T, Bolinder MA, Andrén O, Kirchmann H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agriculture, Ecosystems & Environment 141:184–192. doi:10.1016/j.agee.2011.02.029

Kautz T (2015) Research on subsoil biopores and their functions in organically managed soils: A review. Renewable Agriculture and Food Systems 30:318–327. doi:10.1017/S1742170513000549

Kautz T, Amelung W, Ewert F, Gaiser T, Horn R, Jahn R, Javaux M, Kemna A, Kuzyakov Y, Munch JC, Pätzold S, Peth S, Scherer HW, Schloter M, Schneider H, Vanderborght J, Vetterlein D, Walter A, Wiesenberg GL, Köpke U (2013) Nutrient acquisition from arable subsoils in temperate climates: A review. Soil Biology and Biochemistry 57:1003–1022. doi:10.1016/j.soilbio.2012.09.014

Kautz T, Perkons U, Athmann M, Pude R, Köpke U (2013) Barley roots are not constrained to large-sized biopores in the subsoil of a deep Haplic Luvisol. Biology and Fertility of Soils 49:959–963. doi:10.1007/s00374-013-0783-9

Kaye JP, Quemada M (2017) Using cover crops to mitigate and adapt to climate change. A review. Agronomy for Sustainable Development 37. doi:10.1007/s13593-016-0410-x

Kemper R (2023) Perspektive Boden - Wurzeleigenschaften von Zwischenfrüchten und Futterpflanzen. Vortrag am NutriNet-Feldtag: Luzernekleegras und Alternativen? 03.05.2023 Briesen (Spreewald)

Kemper R, Bublitz TA, Müller P, Kautz T, Döring TF, Athmann M (2020) Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method. Agriculture 10:503. doi:10.3390/agriculture10110503

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

Klimek-Kopyra A, Kulig B, Głąb T, Zając T, Skowera B, Kopcińska J (2015) Effect of plant intercropping and soil type on specific root length. Romanian Agricultural Research:221–230

Kolbe H, Schuster M, Hänsel M, Grünbeck A, Schließer I, Köhler A, Karalus W, Krellig B, Pommer R, Arp B (2004) Zwischenfrüchte im ökologischen Landbau. Fachmaterial der Sächsischen Landesanstalt für Landwirtschaft

Köpke, U (1979) Ein Vergleich von Feldmethoden zur Bestimmung des Wurzelwachstums Landwirtschaftlicher Kulturpflanzen. Ph.D. Thesis, University of Göttingen, Göttingen, Germany

Kozak M, Piepho HP (2018) What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. Journal of Agronomy and Crop Science 204:86–98. doi:10.1111/jac.12220

Kristensen HL, Thorup-Kristensen K (2004) Root Growth and Nitrate Uptake of Three Different Catch Crops in Deep Soil Layers. Soil Science Society of America Journal 68:529. doi:10.2136/sssaj2004.5290

Kücke M, Schmid H, Spiess A (1995) A comparison of four methods for measuring roots of field crops in three contrasting soils. Plant and Soil 172:63–71. doi:10.1007/BF00020860

Kutschera L, Lichtenegger E (1960) Wurzelatlas mitteleuropäischer Ackerunkräuter und Kulturpflanzen. DLG-Verlag, Frankfurt am Main, Germany

Kutschera L, Lichtenegger E, Sobotik M (2009) Wurzelatlas der Kulturpflanzen gemäßigter Gebiete mit Arten des Feldgemüsebaues. DLG-Verlag, Frankfurt am Main, Germany

Lavergne S, Vanasse A, Thivierge M-N, Halde C (2021) Using fall-seeded cover crop mixtures to enhance agroecosystem services: A review. Agrosystems, Geosciences & Environment 4. doi:10.1002/agg2.20161

Li C, Stomph TJ, Makowski D, Li H, Zhang C, Zhang F, van der Werf W (2023) The productive performance of intercropping. Proceedings of the National Academy of Sciences 120:e2201886120. doi:10.1073/pnas.2201886120

Li L, Sun J, Zhang F, Guo T, Bao X, Smith FA, Smith SE (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290. doi:10.1007/s00442-005-0256-4

Li L, Tilman D, Lambers H, Zhang FS (2014) Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. The New phytologist 203:63–69. doi:10.1111/nph.12778

Li L, Yang S, Li X, Zhang F, Christie P (1999) Interspecific complementary and competitive interactions between intercropped maize and faba bean. Plant and Soil 212:105–114. doi:10.1023/A:1004656205144

Li S, Evers JB, Werf W, Wang R, Xu Z, Guo Y, Li B, Ma Y (2020) Plant architectural responses in simultaneous maize/soybean strip intercropping do not lead to a yield advantage. Annals of Applied Biology 177:195–210. doi:10.1111/aab.12610

Li Y, Ghodrati M (1994) Preferential Transport of Nitrate through Soil Columns Containing Root Channels. Soil Science Society of America Journal 58:653–659. doi:10.2136/sssaj1994.03615995005800030003x

Liang Z, Elsgaard L, Nicolaisen MH, Lyhne-Kjærbye A, Olesen JE (2018) Carbon mineralization and microbial activity in agricultural topsoil and subsoil as regulated by root nitrogen and recalcitrant carbon concentrations. Plant and Soil 433:65–82. doi:10.1007/s11104-018-3826-z

Liao R, Bai Y, Liang H, An S, Ren S, Cao Y, Le Z, Lu J, Liu J (2015) Root growth of maize as studied with minirhizotrons and monolith methods. Archives of Agronomy and Soil Science 61:1343–1356. doi:10.1080/03650340.2014.1003812

Liu A, Ma BL, Bomke AA (2005) Effects of Cover Crops on Soil Aggregate Stability, Total Organic Carbon, and Polysaccharides. Soil Science Society of America Journal 69:2041–2048. doi:10.2136/sssaj2005.0032

Liu X, Rahman T, Song C, Su B, Yang F, Yong T, Wu Y, Zhang C, Yang W (2017) Changes in light environment, morphology, growth and yield of soybean in maize-soybean intercropping systems. Field Crops Research 200:38–46. doi:10.1016/j.fcr.2016.10.003

Livesley SJ, Stacey CL, Gregory PJ, Buresh RJ (1998) Sieve size effects on root length and biomass measurements of maize (Zea mays) and Grevillea robusta. Plant and Soil 207:183–193. doi:10.1023/A:1026461107110

Loague K, Green RE (1991) Statistical and graphical methods for evaluating solute transport models: Overview and application. Journal of Contaminant Hydrology 7:51–73. doi:10.1016/0169-7722(91)90038-3

Lopez-Zamora I, Falcão N, Comerford NB, Barros NF (2002) Root isotropy and an evaluation of a method for measuring root distribution in soil trenches. Forest Ecology and Management 166:303–310. doi:10.1016/S0378-1127(01)00679-X

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–76. doi:10.1038/35083573

Lütke Entrup E (1996) Feldfutterbau, Zwischenfruchtbau, Grünbrache. In: Heyland K-U (ed), Spezieller Pflanzenbau. Landwirtschaftliches Lehrbuch, 7th edn. Verlag Eugen Ulmer, Stuttgart

Mead R, Willey RW (1980) The Concept of a 'Land Equivalent Ratio' and Advantages in Yields from Intercropping. Experimental Agriculture 16:217–228. doi:10.1017/S0014479700010978

Meier IC, Tückmantel T, Heitkötter J, Müller K, Preusser S, Wrobel TJ, Kandeler E, Marschner B, Leuschner C (2020) Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. New Phytologist 226:583–594. doi:10.1111/nph.16389

Mendiburu F de (2019) Agricolae: Statistical Procedures for Agricultural Research. R Package Version 1.3-0. Available online at: https://CRAN.R-project.org/package=agricolae. (accessed January 26, 2021)

Mommer L, van Ruijven J, Caluwe H de, Smit-Tiekstra AE, Wagemaker CA, Joop Ouborg N, Bögemann GM, van der Weerden GM, Berendse F, Kroon H de (2010) Unveiling below-ground species abundance in a biodiversity experiment: a test of vertical niche differentiation among grassland species. Journal of Ecology 98:1117–1127. doi:10.1111/j.1365-2745.2010.01702.x

Mulder CP, Uliassi DD, Doak DF (2001) Physical stress and diversity-productivity relationships: the role of positive interactions. Proceedings of the National Academy of Sciences 98:6704–6708. doi:10.1073/pnas.111055298

Mulia R, Dupraz C (2006) Unusual Fine Root Distributions of Two Deciduous Tree Species in Southern France: What Consequences for Modelling of Tree Root Dynamics? Plant and Soil 281:71–85. doi:10.1007/s11104-005-3770-6

Munkholm LJ, Hansen EM (2012) Catch crop biomass production, nitrogen uptake and root development under different tillage systems. Soil Use and Management 28:517–529. doi:10.1111/sum.12001

Murrell EG, Schipanski ME, Finney DM, Hunter MC, Burgess M, LaChance JC, Baraibar B, White CM, Mortensen DA, Kaye JP (2017) Achieving Diverse Cover Crop Mixtures: Effects of Planting Date and Seeding Rate. Agronomy Journal 109:259–271. doi:10.2134/agronj2016.03.0174

Oram NJ, Ravenek JM, Barry KE, Weigelt A, Chen H, Gessler A, Gockele A, Kroon H de, van der Paauw JW, Scherer-Lorenzen M, Smit-Tiekstra A, van Ruijven J, Mommer L (2018) Below-ground complementarity effects in a grassland biodiversity experiment are related to deep-rooting species. Journal of Ecology 106:265–277. doi:10.1111/1365-2745.12877

Osipitan OA, Dille JA, Assefa Y, Knezevic SZ (2018) Cover Crop for Early Season Weed Suppression in Crops: Systematic Review and Meta-Analysis. Agronomy Journal 110:2211–2221. doi:10.2134/agronj2017.12.0752

Osipitan OA, Dille JA, Assefa Y, Radicetti E, Ayeni A, Knezevic SZ (2019) Impact of Cover Crop Management on Level of Weed Suppression: A Meta-Analysis. Crop Science 59:833–842. doi:10.2135/cropsci2018.09.0589

Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, Majdi H, Metcalfe D, Olsthoorn AFM, Pronk A, Vanguelova E, Weih M, Brunner I (2007) Specific root length as an indicator of environmental change. Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology 141:426–442. doi:10.1080/11263500701626069

Parker CJ, Carr MKV, Jarvis NJ, Puplampu BO, Lee VH (1991) An evaluation of the minirhizotron technique for estimating root distribution in potatoes. The Journal of Agricultural Science 116:341–350. doi:10.1017/S0021859600078151

Passioura JB (1996) Simulation Models: Science, Snake Oil, Education, or Engineering? Agronomy Journal 88:690–694. doi:10.2134/agronj1996.00021962008800050002x

Pausch J, Kuzyakov Y (2018) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. Global change biology 24:1–12. doi:10.1111/gcb.13850

Perkons U, Kautz T, Uteau D, Peth S, Geier V, Thomas K, Lütke Holz K, Athmann M, Pude R, Köpke U (2014) Root-length densities of various annual crops following crops with contrasting root systems. Soil and Tillage Research 137:50–57. doi:10.1016/j.still.2013.11.005

Perkons UK (2018) Bioporengenese durch homo- und allorhize Kulturpflanzen: Einfluss auf das Wurzelwachstum der Nachfrüchte. Dissertation. Universität Bonn, Bonn

Poeplau C, Don A (2015) Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agriculture, Ecosystems & Environment 200:33–41. doi:10.1016/J.AGEE.2014.10.024

Poeplau C, Don A, Schneider F (2021) Roots are key to increasing the mean residence time of organic carbon entering temperate agricultural soils. Global change biology 27:4921–4934. doi:10.1111/gcb.15787

Poirier V, Roumet C, Munson AD (2018) The root of the matter: Linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120:246–259. doi:10.1016/j.soilbio.2018.02.016

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramirez-Garcia J, Martens HJ, Quemada M, Thorup-Kristensen K (2015) Intercropping effect on root growth and nitrogen uptake at different nitrogen levels. Journal of Plant Ecology 8:380–389. doi:10.1093/jpe/rtu024

Ranaldo M, Carlesi S, Costanzo A, Bàrberi P (2020) Functional diversity of cover crop mixtures enhances biomass yield and weed suppression in a Mediterranean agroecosystem. Weed Research 60:96–108. doi:10.1111/wre.12388

Regent Instruments Inc. (2017) WinRHIZOTM.; Regent Instruments Inc.: Quebec City, QC, Canada

Roumet C, Lafont F, Sari M, Warembourg F, Garnier E (2008) Root traits and taxonomic affiliation of nine herbaceous species grown in glasshouse conditions. Plant and Soil 312:69–83. doi:10.1007/s11104-008-9635-z

Ruijter FJ de, Veen BW, van Oijen M (1996) A comparison of soil core sampling and minirhizotrons to quantify root development of field-grown potatoes. Plant and Soil 182:301–312. doi:10.1007/BF00029061

Ryser P (1996) The Importance of Tissue Density for Growth and Life Span of Leaves and Roots: A Comparison of Five Ecologically Contrasting Grasses. Functional Ecology 10:717. doi:10.2307/2390506

Ryser P (2006) The mysterious root length. Plant and Soil 286:1–6. doi:10.1007/s11104-006-9096-1

Sainju UM, Singh BP, Whitehead WF (1998) Cover Crop Root Distribution and Its Effects on Soil Nitrogen Cycling. Agronomy Journal 90:511–518. doi:10.2134/agronj1998.00021962009000040012x

Saleem M, Pervaiz ZH, Contreras J, Lindenberger JH, Hupp BM, Chen D, Zhang Q, Wang C, Iqbal J, Twigg P (2020) Cover crop diversity improves multiple soil properties via altering root architectural traits. Rhizosphere 16:100248. doi:10.1016/j.rhisph.2020.100248

Samson BK, Sinclair TR (1994) Soil core and minirhizotron comparison for the determination of root length density. Plant and Soil 161:225–232. doi:10.1007/BF00046393

Sapkota TB, Askegaard M, Lægdsmand M, Olesen JE (2012) Effects of catch crop type and root depth on nitrogen leaching and yield of spring barley. Field Crops Research 125:129–138. doi:10.1016/j.fcr.2011.09.009

Schenk HJ, Jackson RB (2002) The Global Biogeography of roots. Ecological Monographs 72:311–328. doi:10.1890/0012-9615(2002)072[0311:TGBOR]2.0.CO;2

Schmid B, Hector A, Saha P, Loreau M (2008) Biodiversity effects and transgressive overyielding. Journal of Plant Ecology 1:95–102. doi:10.1093/jpe/rtn011

Schröder D, Köpke U (2012) Faba bean (Vicia faba L.) intercropped with oil crops – a strategy to enhance rooting density and to optimize nitrogen use and grain production? Field Crops Research 135:74–81. doi:10.1016/j.fcr.2012.07.007

Schultz-Lupitz A (1895) Zwischenfruchtbau auf leichtem Boden, Berlin

Sieling K (2019) Improved N transfer by growing catch crops – a challenge. Journal für Kulturpflanzen 71:145–160

Sinclair TR, Seligman N (2000) Criteria for publishing papers on crop modeling. Field Crops Research 68:165–172. doi:10.1016/S0378-4290(00)00105-2

Smith AG, Han E, Petersen J, Faircloth Olsen NA, Giese C, Athmann M, Dresbøll DB, Thorup-Kristensen K (2020) RootPainter: Deep Learning Segmentation of Biological Images with Corrective Annotation. bioRxiv. doi:10.1101/2020.04.16.044461

Smith AG, Han E, Petersen J, Olsen NAF, Giese C, Athmann M, Dresbøll DB, Thorup-Kristensen K (2022) RootPainter: Deep Learning Segmentation of Biological Images with Corrective Annotation. New Phytologist. doi:10.1111/nph.18387

St-Pierre NR (2016) Comparison of model predictions with measurements: A novel model-assessment method. Journal of Dairy Science 99:4907–4927. doi:10.3168/jds.2015-10032

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

Thorup-Kristensen K (1993) Root Development of Nitrogen Catch Crops and of a Succeeding Crop of Broccoli. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 43:58–64. doi:10.1080/09064719309410233

Thorup-Kristensen K (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant and Soil 230:185–195. doi:10.1023/A:1010306425468

Thorup-Kristensen K, Magid J, Jensen LS (2003) Catch crops and green manures as biological tools in nitrogen management in temperate zones. Advances in Agronomy 79:227–302. doi:10.1016/S0065-2113(02)79005-6

Thorup-Kristensen K, Rasmussen CR (2015) Identifying new deep-rooted plant species suitable as undersown nitrogen catch crops. Journal of Soil and Water Conservation 70:399–409. doi:10.2489/jswc.70.6.399

Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science (New York, N.Y.) 294:843–845. doi:10.1126/science.1060391

Tosti G, Benincasa P, Farneselli M, Pace R, Tei F, Guiducci M, Thorup-Kristensen K (2012) Green manuring effect of pure and mixed barley – hairy vetch winter cover crops on maize and processing tomato N nutrition. European Journal of Agronomy 43:136–146. doi:10.1016/j.eja.2012.06.004

Tosti G, Benincasa P, Farneselli M, Tei F, Guiducci M (2014) Barley–hairy vetch mixture as cover crop for green manuring and the mitigation of N leaching risk. European Journal of Agronomy 54:34–39. doi:10.1016/j.eja.2013.11.012

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

Tribouillois H, Cohan JP, Justes E (2016) Cover crop mixtures including legume produce ecosystem services of nitrate capture and green manuring: assessment combining experimentation and modelling. Plant and Soil 401:347–364. doi:10.1007/s11104-015-2734-8

Tribouillois H, Fort F, Cruz P, Charles R, Flores O, Garnier E, Justes E (2015) A functional characterisation of a wide range of cover crop species: growth and nitrogen acquisition rates, leaf traits and ecological strategies. PloS one 10:e0122156. doi:10.1371/journal.pone.0122156

Upchurch (1987) Conversion of minirhizotron-root intersections to root length density:51

Vandermeer JH (1989) The Ecology of Intercropping. Cambridge University Press, Cambridge

Vepraskas MJ, Hoyt GD (1988) Comparison of the Trench-Profile and Core Methods for Evaluating Root Distributions in Tillage Studies. Agronomy Journal 80:166–172. doi:10.2134/agronj1988.00021962008000020006x

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional! Oikos 116:882–892. doi:10.1111/j.0030-1299.2007.15559.x

Vogt KA, Vogt DJ, Bloomfield J (1998) Analysis of some direct and indirect methods for estimating root biomass and production of forests at an ecosystem level. Plant and Soil 200:71–89. doi:10.1007/978-94-011-5270-9_61

Vos J, van der Putten P, Hassan Hussein M, van Dam AM, Leffelaar PA (1998) Field observations on nitrogen catch crops - II. Root length and root length distribution in relation to species and nitrogen supply. Plant and Soil 201:149–155. doi:10.1023/A:1004367530320

Wendling M, Büchi L, Amossé C, Jeangros B, Walter A, Charles R (2017) Specific interactions leading to transgressive overyielding in cover crop mixtures. Agriculture, Ecosystems & Environment 241:88–99. doi:10.1016/j.agee.2017.03.003

Wendling M, Büchi L, Amossé C, Sinaj S, Walter A, Charles R (2016) Influence of root and leaf traits on the uptake of nutrients in cover crops. Plant and Soil 409:419–434. doi:10.1007/s11104-016-2974-2

Wendling M, Charles R, Herrera J, Amossé C, Jeangros B, Walter A, Büchi L (2019) Effect of species identity and diversity on biomass production and its stability in cover crop mixtures. Agriculture, Ecosystems & Environment 281:81–91. doi:10.1016/j.agee.2019.04.032

Wezel A, Casagrande M, Celette F, Vian JF, Ferrer A, Peigné J (2014) Agroecological practices for sustainable agriculture. A review. Agronomy for Sustainable Development 34:1–20. doi:10.1007/s13593-013-0180-7

White CM, DuPont ST, Hautau M, Hartman D, Finney DM, Bradley B, LaChance JC, Kaye JP (2017) Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture, Ecosystems & Environment 237:121–133. doi:10.1016/j.agee.2016.12.016

Willey RW (1979) Intercropping-Its Importance and Research Needs: Part 1. Competition and Yield Advantages. Field Crop Abstracts 32:1–10

Williams PC, Sobering DC (1993) Comparison of Commercial near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds. Journal of Near Infrared Spectroscopy 1:25–32. doi:10.1255/jnirs.3

Williams PC, Sobering DC (1996) How do we do it: a brief summary of the methods we use in developing near infrared calibration, In: Near Infrared Spectroscopy: The Future Waves, NIR Publications, Chicester UK, pp 185–188

Williams SM, Weil RR (2004) Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop. Soil Science Society of America Journal 68:1403–1409. doi:10.2136/sssaj2004.1403

Wit CT de, Bergh J (1965) Competition between herbage plants. J. Agr. Sci. 13:212–221

Wittwer RA, Dorn B, Jossi W, van der Heijden MGA (2017) Cover crops support ecological intensification of arable cropping systems. Scientific reports 7:41911. doi:10.1038/srep41911

Xiong P, Zhang Z, Peng X (2022) Root and root-derived biopore interactions in soils: A review. Journal of Plant Nutrition and Soil Science. DOI: https://doi:10.1002/jpln.202200003

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proceedings of the National Academy of Sciences 96:1463–1468. doi:10.1073/pnas.96.4.1463

Yang JM, Yang JY, Liu S, Hoogenboom G (2014) An evaluation of the statistical methods for testing the performance of crop models with observed data. Agricultural Systems 127:81–89. doi:10.1016/j.agsy.2014.01.008

Yin S, Wang C, Lv C, Zhou Z (2023) Short-term responses of root traits and carbon exudation to drought in a Larix gmelinii plantation. Plant and Soil 484:393–405. doi:10.1007/s11104-022-05800-y

Yu RP, Yang H, Xing Y, Zhang WP, Lambers H, Li L (2022) Belowground processes and sustainability in agroecosystems with intercropping. Plant and Soil 476:263–288. doi:10.1007/s11104-022-05487-1

Yu Y, Loiskandl W, Kaul HP, Himmelbauer M, Wei W, Chen L, Bodner G (2016) Estimation of runoff mitigation by morphologically different cover crop root systems. Journal of Hydrology 538:667–676. doi:10.1016/j.jhydrol.2016.04.060

Yu Y, Stomph TJ, Makowski D, Zhang L, van der Werf W (2016) A meta-analysis of relative crop yields in cereal/legume mixtures suggests options for management. Field Crops Research 198:269–279. doi:10.1016/j.fcr.2016.08.001

Zhang C, Postma JA, York LM, Lynch JP (2014) Root foraging elicits niche complementarity-dependent yield advantage in the ancient ‘three sisters’ (maize/bean/squash) polyculture. Annals of botany 114:1719–1733. doi:10.1093/aob/mcu191

Zhang Z, Peng X (2021) Bio-tillage: A new perspective for sustainable agriculture. Soil and Tillage Research 206:104844. DOI: https://doi:10.1016/j.still.2020.104844

Appendix A: Supplementary material from article “Vertical Root Distribution of Different Cover Crops Determined with the Profile Wall Method”

Table A1 Estimated parameters for a three-parameter logistic regression of root length density depending on soil depth for different cover crops before (October) and after winter (March) in two seasons (2018/2019 and 2019/2020).

year	date	cover crop	a	b	c
2018/2019	October	crimson clover	0.83138	0.00173	0.30753
		winter rye	0.99876	0.01335	0.18859
		bristle oat	0.53122	0.00028	0.29419
		blue lupin	0.30839	0.03469	0.13749
		oil radish	0.42701	0.07936	0.12263
		winter turnip rape	0.43448	0.02378	0.14787
		phacelia	0.40328	0.15073	0.07797
	March	crimson clover	2.47656	0.68408	0.08258
		winter rye	1.53348	0.19271	0.09375
		bristle oat	0.39113	0.00512	0.19105
		blue lupin	0.32555	0.07996	0.11815
		oil radish	0.45722	0.46920	0.10225
		winter turnip rape	0.54409	0.12904	0.10934
		phacelia	0.18929	0.00396	0.21934
2019/2020	October	crimson clover	6.49826	1.22644	0.09067
		winter rye	7.77116	0.61248	0.08983
		bristle oat	2.09426	0.24000	0.08827
		blue lupin	0.33569	0.00138	0.20173
		oil radish	-0.54847	-1.18386	0.01471
		winter turnip rape	-0.57801	-1.18181	0.01434
		phacelia	0.83521	0.68140	0.03017
	March	crimson clover	5.39990	0.23258	0.09977
		winter rye	17.12518	1.01202	0.06898
		bristle oat	2.05598	0.07650	0.09648
		blue lupin	2.11465	1.34926	0.03691
		oil radish	3.04881	0.06645	0.10068
		winter turnip rape	5.99178	0.87936	0.05116
		phacelia	-1.81859	-2.47120	0.02932

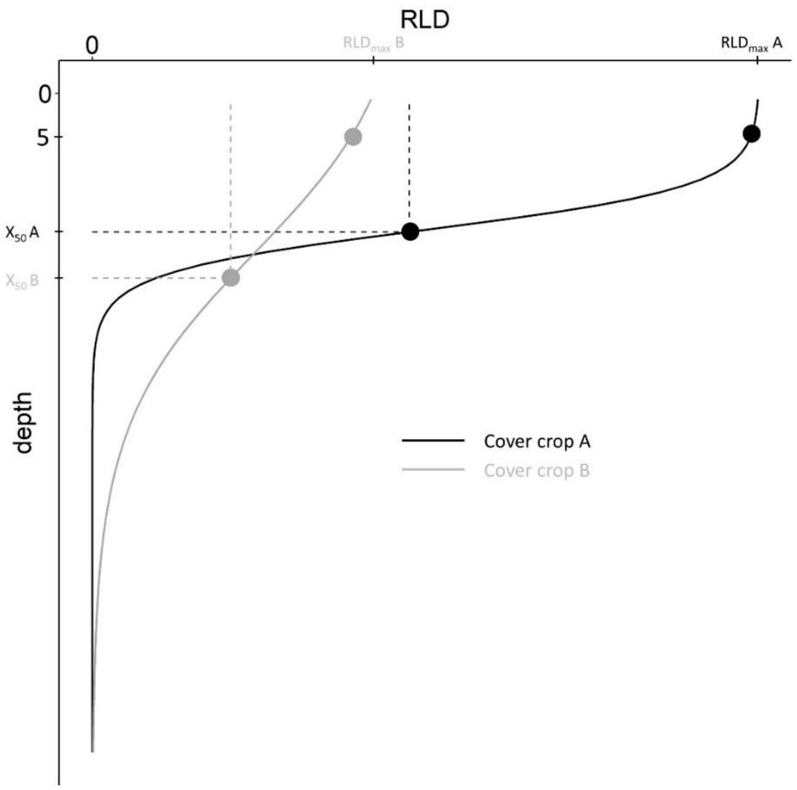

Figure A1. Example of distribution of root length density depending on soil depth for two different crops. Two points on the curve show the RLD at a depth of 5 cm and the depth at half maximal RLD.

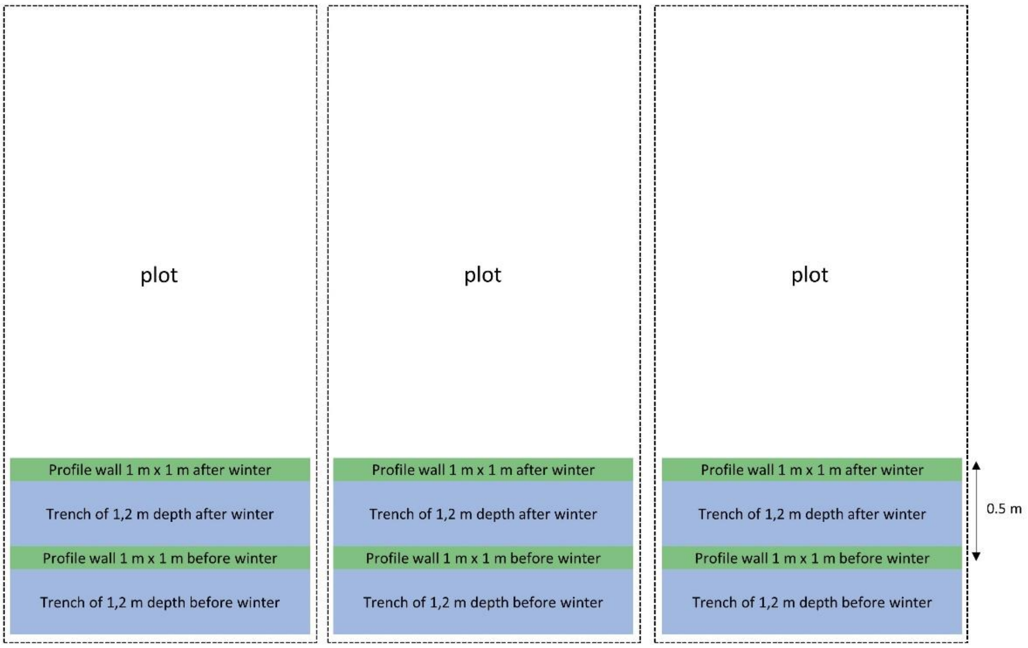

Figure A2 Position of the plots and the trench relative to the plots

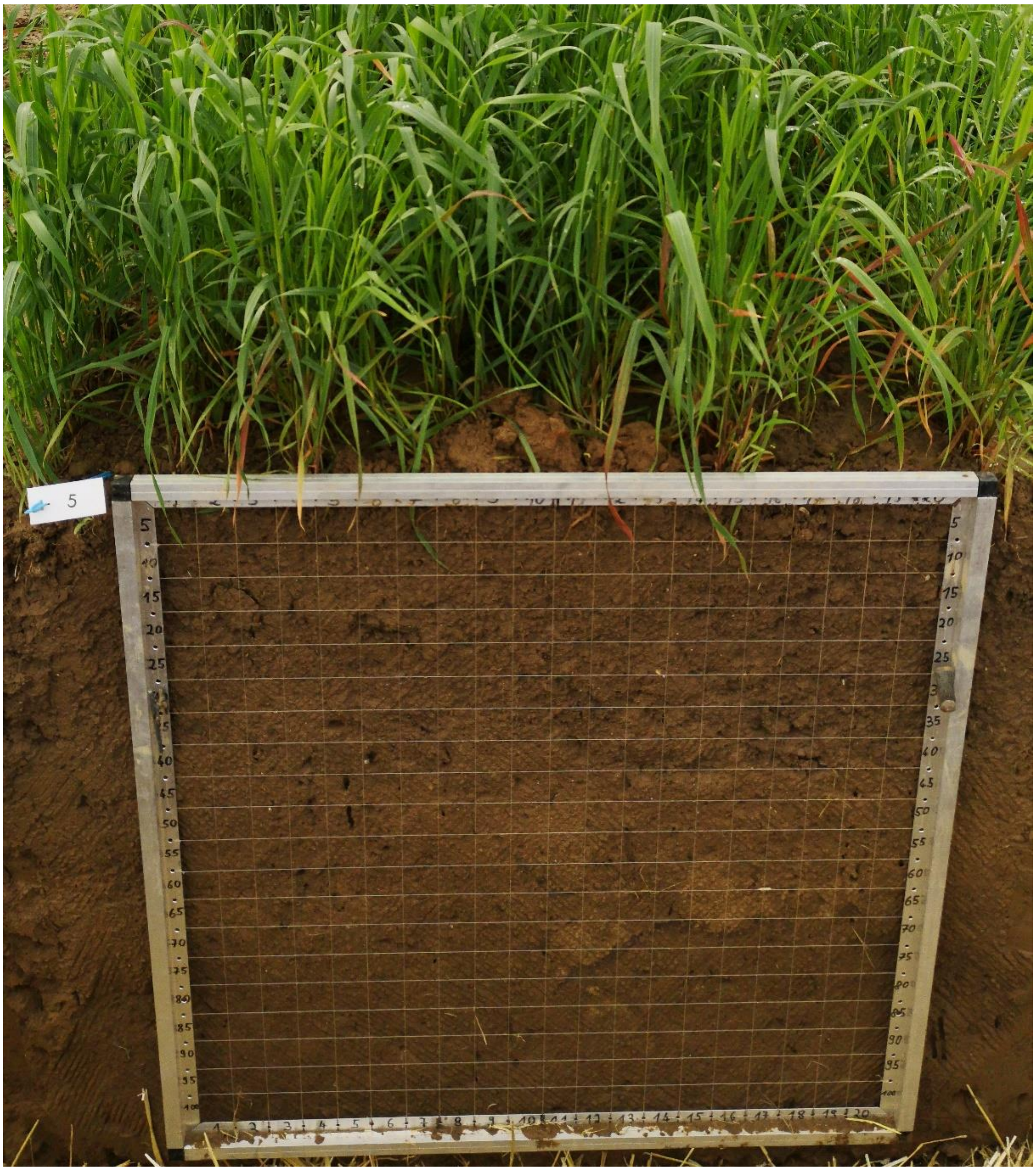

Figure A3 Photo of metal frame with a grid of 5 cm × 5 cm for manual root counting.

Appendix B Supplementary material from article “Root traits in cover crop mixtures of blue lupin and winter rye”

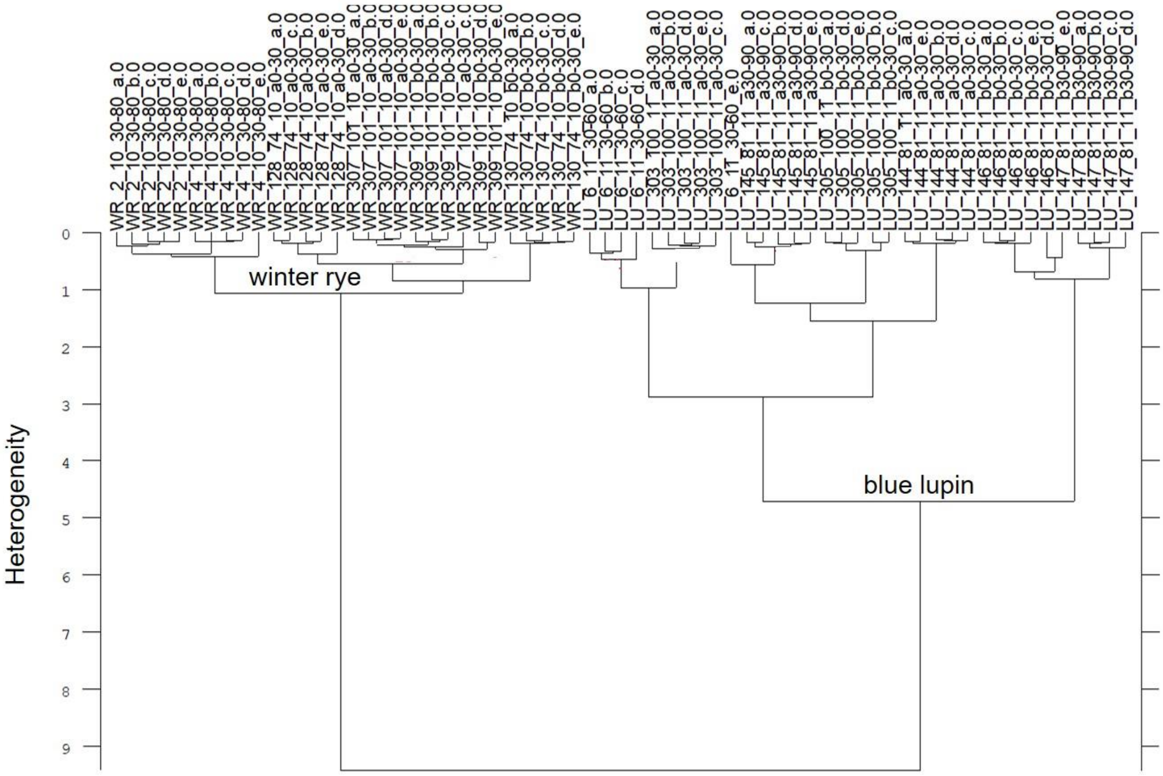

Fig. B1 Cluster analysis of FTIR spectra of single species sample of dried and ground roots of winter rye (WR) and blue lupin (LU) of 2018. File names consist of species name, two (or three) numbers which indicate the plot numbers in the field, the depth of which the samples were taken und replicated measurements (a-e). Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (1800 – 850 cm^{-1}), Ward’s algorithm and Euclidian distance.

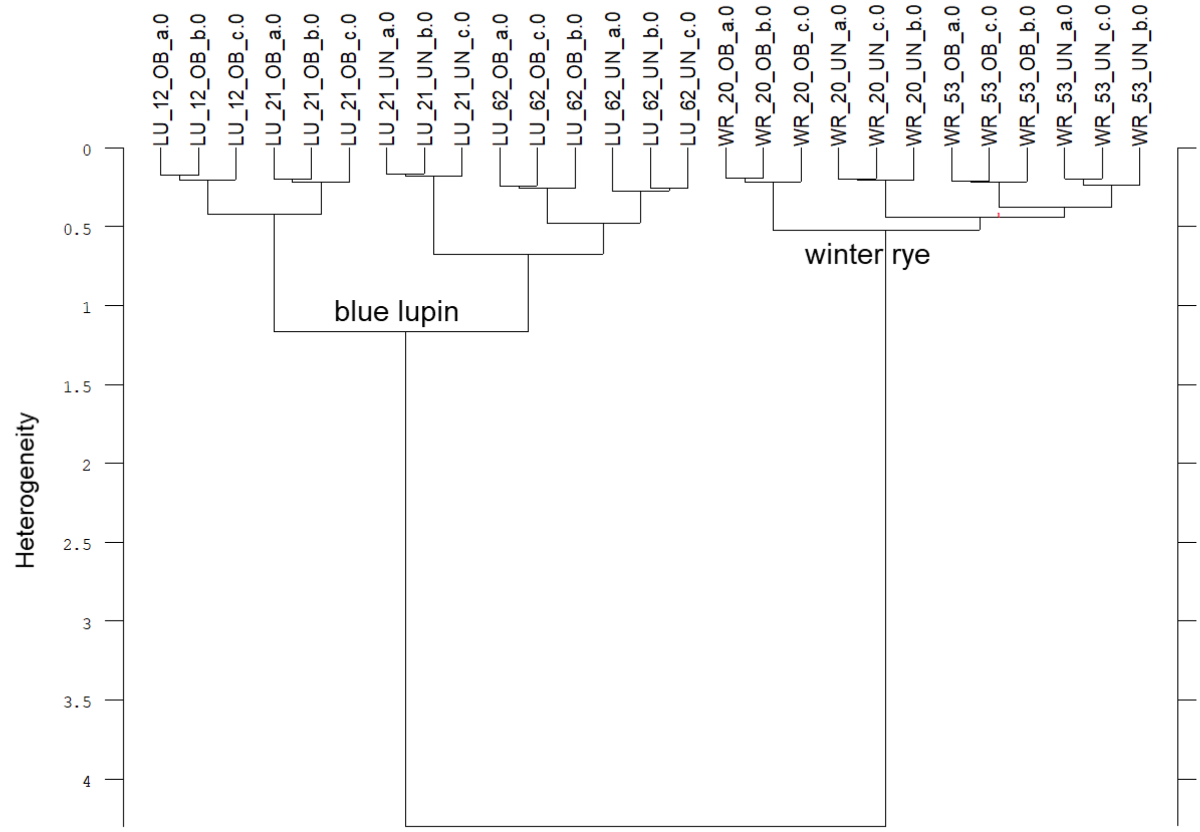

Fig. B2 Cluster analysis of FTIR spectra of single species sample of winter rye (WR) and blue lupin (LU) of 2019. File names consist of species name, the number indicates the plot number in the field, the depth of which the samples were taken (OB means 0-30 cm and UN means 30-90 cm depth) und replicated measurement (a-c). Cluster analysis was evaluated with the second derivative and vector normalization of the reduced frequency range (1800 – 850 cm^{-1}), Ward's algorithm and Euclidian distance.

Details for the model establishing

For establishing a 2-species model, a calibration set of 35 "artificial mixtures" was generated in 3 % steps from 0 % to 100 % for blue lupin and winter rye, respectively. These mixtures covered the complete calibration range thoroughly. 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) regressions 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 detects the best datapreparation 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 (for this purpose the 20 additional “artificial mixtures” were used). Both internal validation and external calibration were compared with the calculated statistical parameters of each calibration. For each year a separate model was generated.

The finally chosen model for 2018 as well as for 2019 (Table B1) showed for the calibration/internal validation and external calibration the following different statistical parameters (Table B1): The R^2, the coefficient of determination, was > 97 % in both years. The RPD, the residual predication deviation, is calculated by the ratio of standard deviation and the standard error of prediction. The RPD should be as high as possible but at least three (Williams and Sobering 1996). Values > 2.5 are satisfactory for screening and values of 5 – 10 are adequate for quality control and > 10 are excellent and equivalent than to reference chemicals (Williams and Sobering 1993). The RPD values of the calibrations are > 6 (Table B1). The RMSECV is the root mean square error of cross validation and accordingly, the RMSEP is the root mean square error of prediction and both values can be to taken to assess the quality of the model. These values should be as low as possible. Due to the optimization process, the models with the lowest RMSECV was chosen. For the chosen models, the values were < 5 (Table B1). The regression line of the calibration illustrates the differences between the true and known values and the predicted values. The intercept and the slope of the regression line can be used to assess the quality of the model. In both years, the intercepts of the calibration were very low with values under 2 (Table B1). The slope for the regression line should be close to 1. Here, regression lines showed values > 0.93 (Table B1, displayed in Fig. B3).

The software is also marking values outside the calibration range. The outside values are values below 0 % or above 100 % which are arithmetically possible but not logical. If one species had 104 %, the corresponding species amounted to -4 %. The values were corrected to 100 % and 0 %.

Table B1 Model details and statistical values for the models of blue lupin (LU) and winter rye (WR) of the years 2018 and 2019. Abbreviation: 1st der. – first derivative, SNV – vector normalization, MSC – multiplicative scattering correction, R² - 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 (LU and WR), slope– slope of the regression line "predicted concentration values vs. true concentration values" (see also Fig. 3), RMSEP – root mean square error of prediction.

		LU-WR 2018	**LU-WR 2019**
Data pre-processing		1st der. + SNV	1st der. + MSC
Frequency range [cm^{-1}]		4000 – 3280 2560 – 1120	4000 – 3280 1840 – 1120
Calibration/ internal validation	R²	97.58	97.82
	RPD	6.43	6.77
	RMSECV	4.66	4.42
	Offset LU	1.728	1.344
	Offset WR	1.463	1.478
	Slope	0.968	0.972
External validation	RPD	5.16	5.97
	RMSEP	4.93	4.45
	Offset LU	0.518	2.498
	Offset WR	0.725	4.200
	Slope	0.988	0.933

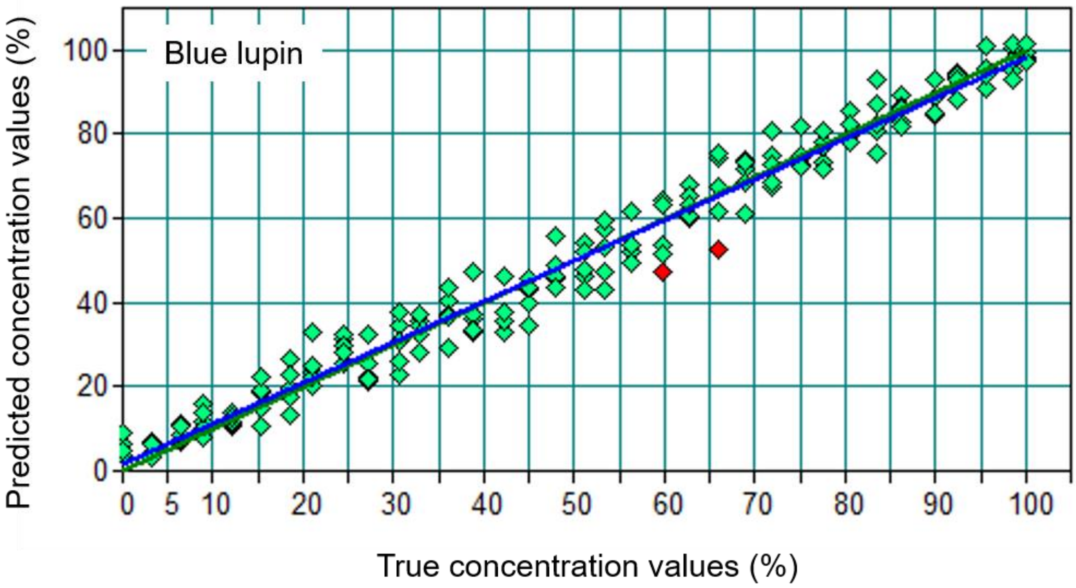

Fig. B3 Predicted concentration values of blue lupin (blue regression line) in comparison to the true (known) concentration values (green dots and green regression line) of the "artificial mixtures" of the model of 2018. The red dots are outlier calculated by the model. The corresponding figure of winter rye of 2018 and for both species for 2019 are very similar and are not shown.

Table B2 Pearson's correlation between relative mixture effect (RME) of root traits and root traits: root length density (RLD), specific root length (SRL) and root mass density (RMD). Significant correlations ($p \leq 0.05$) are given in bold.

RME of root trait	(RME of) root trait	correlation coefficient
RME of RLD	RLD	**0.25**
RME of RLD	SRL	-0.14
RME of RLD	RMD	**0.31**
RME of SRL	RLD	**0.30**
RME of SRL	SRL	**0.58**
RME of SRL	RMD	0.055
RME of RMD	RLD	0.1
RME of RMD	SRL	**-0.44**
RME of RMD	RMD	**0.31**
RME of RLD	RME of SRL	-0.049
RME of RLD	RME of RMD	**0.71**
RME of SRL	RME of RMD	**-0.38**

Williams PC, Sobering DC (1993) Comparison of Commercial near Infrared Transmittance and Reflectance Instruments for Analysis of Whole Grains and Seeds. Journal of Near Infrared Spectroscopy 1:25–32. doi:10.1255/jnirs.3

Williams PC, Sobering DC (1996) How do we do it: a brief summary of the methods we use in developing near infrared calibration, In: Near Infrared Spectroscopy: The Future Waves, NIR Publications, Chicester UK, pp 185–188

Appendix C Supplementary material from article “Oil radish, winter rye and crimson clover: root and shoot performance in cover crop mixtures“

Fig. C1 Trench in the experimental field in 2019 at experimental station ‘Wiesengut’, transversely to the short end of the plots (on the right), for investigation of cover crop root traits with the monolith method.

Table C1 Mean values ± standard deviation (n=4 for shoot, n=3 for root) of partial land equivalent ratio (pLER), land equivalent ratio (LER), and relative mixture effect [%] (RME) in 2018 and 2019 for a 50:50 mixture of oil radish (OR) and winter rye (WR). Significant differences for pLER from 0.5, for LER from 1 or for RME from 0 (p ≤ 0.05, t-test) are given in bold.

		2018					2019				
		root dry mass	shoot dry mass	N shoot uptake	P shoot uptake	K shoot uptake	root dry mass	shoot dry mass	N shoot uptake	P shoot uptake	K shoot uptake
pLER	OR	0.38 ± 0.22	0.48 ± 0.15	0.57 ± 0.17	0.52 ± 0.21	0.47 ± 0.16	0.79 ± 0.15	0.83 ± 0.21	0.74 ± 0.19	0.70 ± 0.16	0.66 ± 0.20
	WR	0.19 ± 0.14	**0.62 ± 0.05**	**0.68 ± 0.10**	0.67 ± 0.13	**0.73 ± 0.14**	**0.17 ± 0.01**	**0.15 ± 0.08**	**0.17 ± 0.10**	**0.20 ± 0.11**	**0.19 ± 0.11**
LER		0.56 ± 0.28	1.10 ± 0.16	1.26 ± 0.17	1.19 ± 0.20	1.20 ± 0.22	0.97 ± 0.13	0.97 ± 0.15	0.92 ± 0.10	0.90 ± 0.13	0.85 ± 0.12
RME [%]		-46.33 ± 24.88	8.03 ± 16.66	24.65 ± 15.87	18.73 ± 19.46	11.85 ± 20.91	-15.98 ± 17.34	11.20 ± 18.98	-3.75 ± 12.03	-1.10 ± 14.28	5.40 ± 24.24

Table C2 Mean values ± standard deviation (n=4 for shoot, n=3 for root) of partial land equivalent ratio (pLER), land equivalent ratio (LER), and relative mixture effect [%] (RME) in 2018 and 2019 for a 33:33:33 mixture of oil radish (OR), winter rye (WR) and crimson clover (CC). Significant differences for pLER from 0.33, for LER from 1 or for RME from 0 (p ≤ 0.05, t-test) are given in bold.

		2018					2019				
		root dry mass	shoot dry mass	N shoot uptake	P shoot uptake	K shoot uptake	root dry mass	shoot dry mass	N shoot uptake	P shoot uptake	K shoot uptake
pLER	OR	0.54 ± 0.55	0.53 ± 0.14	**0.57 ± 0.11**	0.48 ± 0.11	0.44 ± 0.07	1.12 ± 0.86	**0.93 ± 0.30**	**0.83 ± 0.162**	**0.80 ± 0.21**	**0.71 ± 0.18**
	WR	0.31 ± 0.21	**0.48 ± 0.07**	**0.57 ± 0.09**	**0.61 ± 0.07**	**0.59 ± 0.12**	**0.10 ± 0.05**	**0.11 ± 0.03**	**0.13 ± 0.028**	**0.15 ± 0.04**	**0.14 ± 0.04**
	CC	0.13 ± 0.05	**0.03 ± 0.02**	**0.02 ± 0.01**	**0.03 ± 0.02**	**0.03 ± 0.02**	0.13 ± 0.09	**0.02 ± 0.02**	**0.02 ± 0.014**	**0.02 ± 0.02**	**0.03 ± 0.02**
LER		0.97 ± 0.62	1.04 ± 0.20	1.16 ± 0.13	1.12 ± 0.17	1.07 ± 0.16	1.35 ± 0.99	1.05 ± 0.29	0.98 ± 0.174	0.97 ± 0.24	0.88 ± 0.22
RME [%]		-1.71 ± 60.51	9.85 ± 22.71	-0.95 ± 11.81	23.13 ± 17.68	7.88 ± 17.28	22.67 ± 57.31	30.63 ± 25.98	-0.78 ± 16.583	26.48 ± 30.03	30.03 ± 32.29

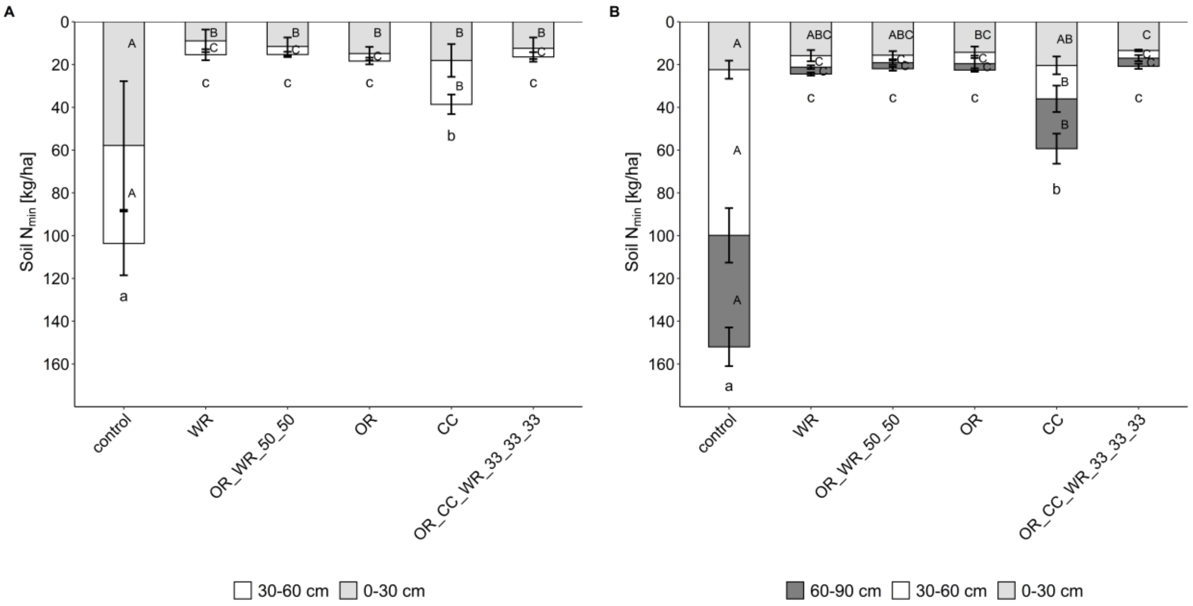

Fig. C2 Mean values ± standard deviation (n = 4) of soil mineral nitrogen [kg/ha] (N_{min}) under a weed-free control, sole cover crops oil radish (OR), winter rye (WR), crimson clover (CC), 50:50 cover crop mixtures of radish and rye (OR_WR_50_50) , 33:33:33 cover crop mixtures of radish, clover and rye (OR_WR_50_50) in 2018 (A) and 2019 (B). Bar colours refer to the soil layer, with light grey for 0-30 cm, white for 30-60 cm, and dark grey for 60-90 cm. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different treatments for total N_{min} within one year. Different uppercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different treatments for N_{min} within each soil layer within one year.

Table C3 Mean values ± standard deviation of net biodiversity effect (NE), complementarity effect (CE) and selection effect (SE) of a 50:50 mixture of oil radish and winter rye for shoot nutrient uptake [g/m²] (n = 4). Effects significantly different from 0 ($p \leq 0.05$, t-test) are given in bold.

Year	parameter	NE	CE	SE
2018	N uptake	**1.9 ± 1.1**	**1.9 ± 1.2**	-0.1 ± 0.1
2018	P uptake	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.1
2018	K uptake	1.7 ± 3.3	3.2 ± 3.5	-1.5 ± 1.3
2019	N uptake	-0.3 ± 1.0	-0.6 ± 0.8	**0.4 ± 0.2**
2019	P uptake	0.0 ± 0.3	-0.1 ± 0.2	**0.2 ± 0.1**
2019	K uptake	0.3 ± 3.3	-3.0 ± 3.0	3.3 ± 2.2

Table C4 Mean values ± standard deviation of net biodiversity effect (NE), complementarity effect (CE) and selection effect (SE) of a 33:33:33 mixture of oil radish, crimson clover and winter rye for shoot nutrient uptake [g/m²] (n = 4). Effects significantly different from 0 ($p \leq 0.05$, t-test) are given in bold.

year	parameter	NE	CE	SE
2018	N uptake	-0.1 ± 1.1	1.4 ± 1.2	**-1.5 ± 0.6**
2018	P uptake	0.4 ± 0.3	0.2 ± 0.3	**0.2 ± 0.1**
2018	K uptake	1.2 ± 2.6	1.1 ± 2.5	0.1 ± 0.4
2019	N uptake	-0.2 ± 1.5	-0.4 ± 1.7	0.2 ± 0.5
2019	P uptake	0.3 ± 0.4	-0.1 ± 0.4	0.4 ± 0.1
2019	K uptake	3.4 ± 4.2	-2.5 ± 4.7	**5.9 ± 1.8**

The cluster analysis (Figs. C3-C6) confirmed that species separation is possible. Details of the cluster analysis calculation are listed in the figure description.

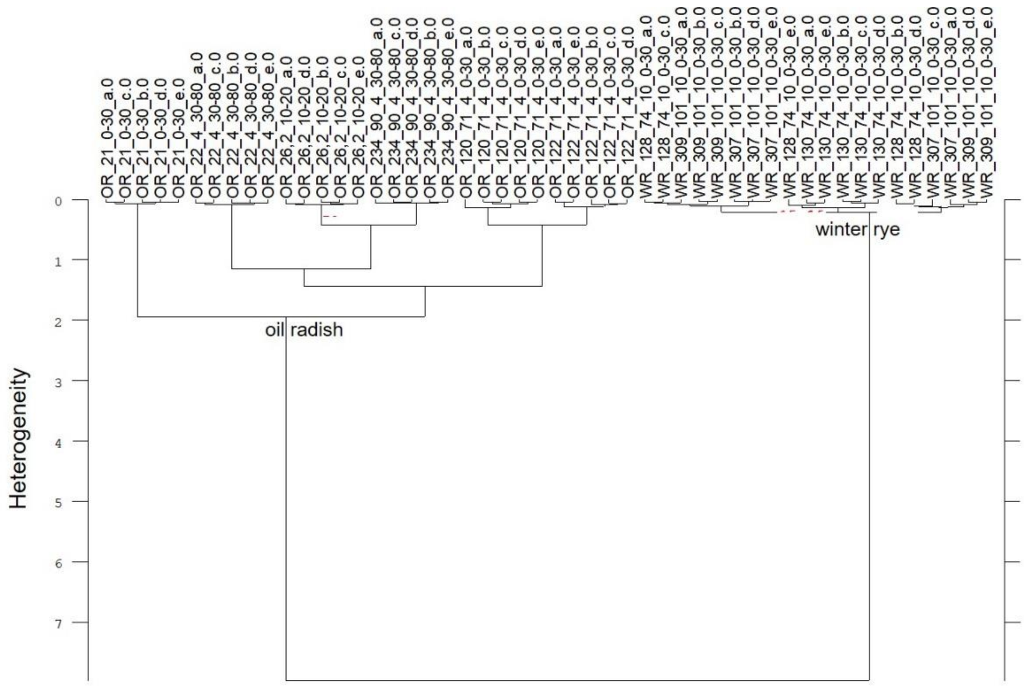

Fig. C3 Cluster analysis of oil radish (OR) and winter rye (WR) of 2018. FTIR Spectra were pre-processed by the first derivative and vector normalization, the frequency range was reduced (3640-3316, 1788-1538 and 1292-1017 cm^{-1}) and the Euclidian's distance and Ward's algorithm were applied. Interspecific heterogeneity was 7.98 and intraspecific heterogeneity was 1.95 for oil radish and 0.22 for winter rye.

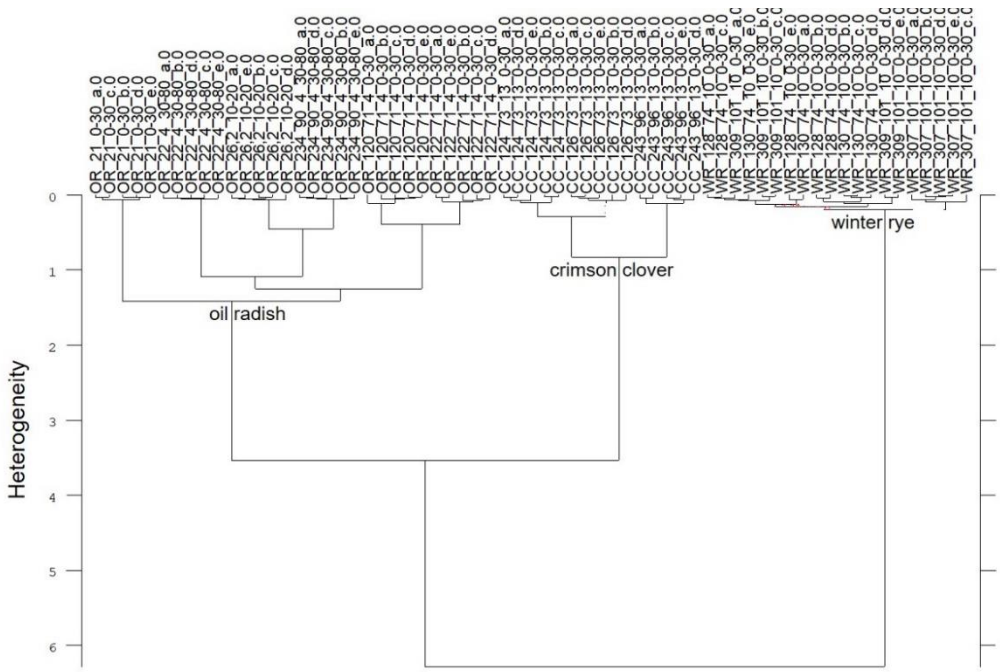

Fig. C4 Cluster analysis of oil radish (OR), crimson clover (CC) and winter rye (WR) of 2018. Spectra were pre-processed by the first derivative and vector normalisation, the frequency range was reduced (3281-2558 and 1842-760 cm^{-1}) and the Euclidian's distance and Ward's algorithm were applied. Interspecific heterogeneity was 6.29 (overall) and 3.54 (OR-CC) and intraspecific heterogeneity was 1.42 for oil radish, 0.83 for crimson clover and 0.20 for winter rye.

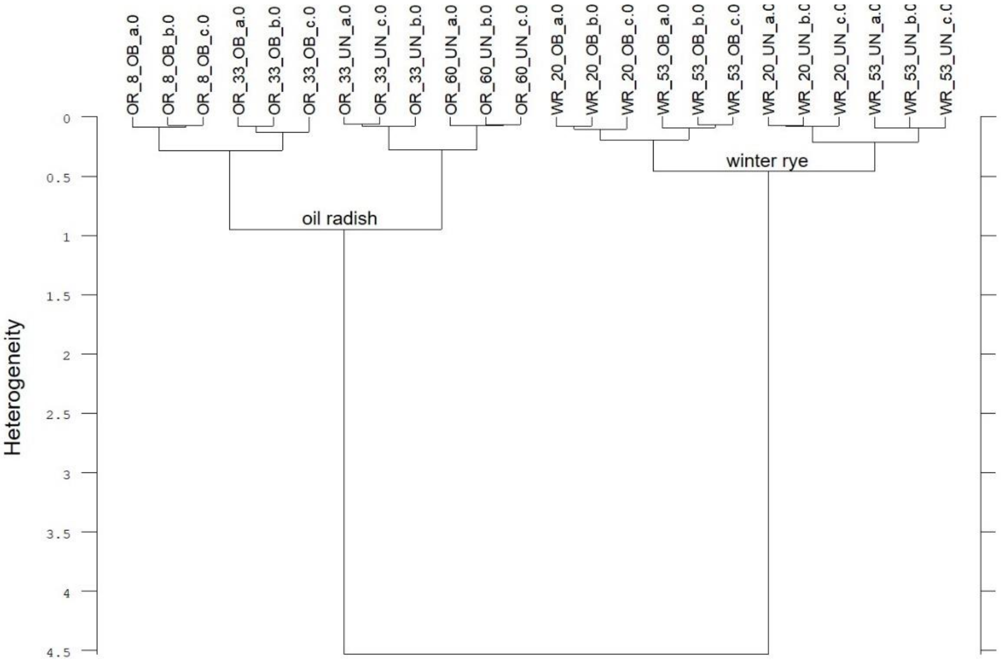

Fig. C5 Cluster analysis of oil radish (OR) and winter rye (WR) of 2019. FTIR Spectra were pre-processed by the first derivative and vector normalization, the frequency range was reduced (3641-3278 and 1479-1117 cm-1) and the Euclidian's distance and Ward's algorithm were applied. Interspecific heterogeneity was 4.53 and intraspecific heterogeneity was 0.95 for oil radish and 0.46 for winter rye.

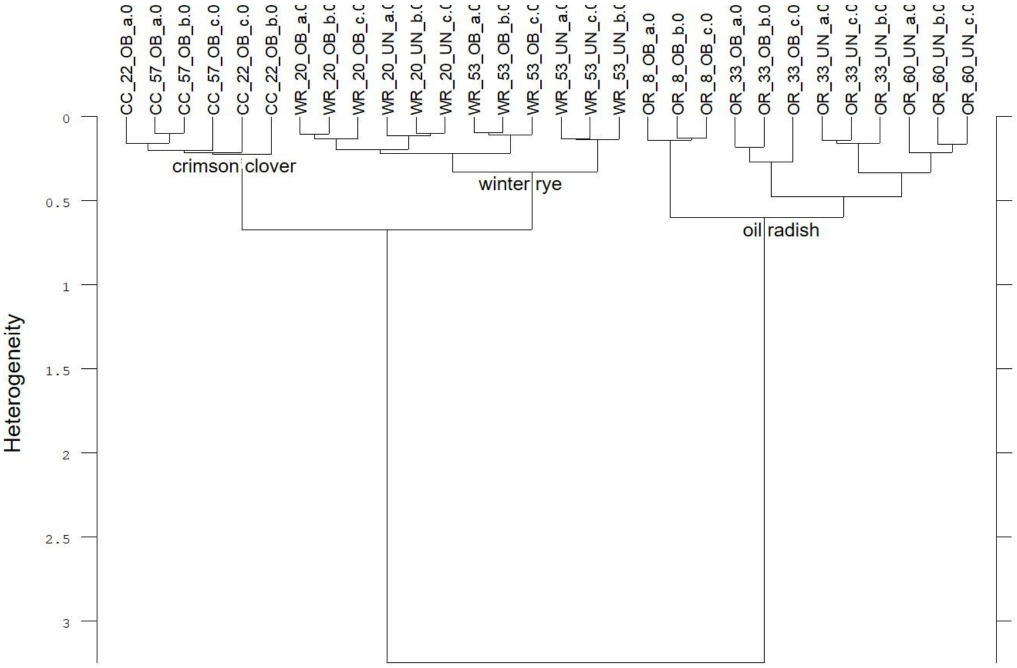

Fig. C6 Cluster analysis of oil radish (OR), crimson clover (CC) and winter rye (WR) of 2019. Spectra were pre-processed by the first derivative and vector normalization, the frequency range was reduced (3281-2558 and 1842-760 cm-1) and the Euclidian's distance and Ward's algorithm were applied. Interspecific heterogeneity was 3.50 (overall) and 0.67 (WR-CC) and intraspecific heterogeneity was 0.60 for oil radish, 0.23 for crimson clover and 0.33 for winter rye.

Details for the model establishing

The details for establishing a 2-species model are in accordance with Kemper et a. (2022). For a 3-species model of oil radish (OR), crimson clover (CC) and winter rye (WR) of 2018, a calibration set of 57 artificial mixtures was generated. In accordance with the described 2-species model, the artificial mixtures completed the calibration range (0-100 %) for all three species thoroughly. Thirty-two additional artificial mixtures with known species composition were generated to be used for external calibration of the model. As described for the 2-species model, a 3-species model was calculated on the basis of multivariate calibration with the method of partial least square regressions using the software Quant 2 (Opus, version 7.2, Bruker Optics, Ettlingen, Germany). The established model was evaluated by an internal validation (cross validation) and was subsequently optimized by the Quant 2 software. Six of the proposed optimized models were verified by an external calibration (therefore the 32 additional artificial mixtures were used). Both internal validation and external calibration were compared with the calculated statistical parameters of each calibration. The finally chosen model of 2018 (Table C6) was determined on the basis of the parameters explained in Kemper et al. (2022).

With the chosen model, the species proportion of the mixed samples could be calculated in %. Outside values (above 100 and below 0) were arithmetically possible but not logical and were therefore corrected as follows. First, an additive shift was conducted such that all values got positive values (example see Table C7). Then, a weighted directed compression (WDC) was executed. With this, the values ranged between 0 and 100 %. Additionally, the sum of the values for the three species amounted to 100% (example in Table C7). If the calculated values are within the 0-100% range, but the sum is more or less than 100%, a WDC without shift was used to correct the data.

After calculating the 3-species model in 2019, the results were not considered to be satisfactory. As seen in Fig. C6, the species did not match their systematic background (comparing Figs. C4 and C6). Hence, the 3-species models of 2019 could not be used for model calibration. We decided to use the 3-species model of 2018 (Table C6) for the mixed samples of 2019. As a verification, we calculated the 2-species mixture samples of 2019 with the 2-species model of 2018. The percentage aberration amounted to 9.31% and was considered to be acceptable.

Table C5: Model and statistical details for the models of oil radish (OR) and winter rye (WR) for the years 2018 and 2019. Abbreviations: 1st – first derivative, SNV – vector normalization, R² - 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”, RMSEP – root mean square error of prediction.

		OR-WR 2018	OR-WR 2019
data pre-processing		1st + SNV	1st + SNV
frequency range [cm^{-1}]		3640 - 3316 1787 - 1538 1292 - 1018	3640 - 3278 1479 - 1118
calibration/ internal validation	R²	98.29	98.89
	RPD	7.65	9.49
	RMSECV	3.9	3.18
	Offset OR	1.206	0.938
	Offset WR	0.985	0.965
	Slope	0.98	0.981
external validation	RPD	5.33	6.44
	RMSEP	4.25	3.84
	Offset OR	-0.0624	0.538
	Offset WR	-0.009	-0.043
	Slope	1.006	0.995

Table C6: Model and statistical details for the models of oil radish (OR), crimson clover (CC) and winter rye (WR) for the year 2018. Abbreviations: 1st – first derivative, SNV – vector normalization, R² - 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", RMSEP – root mean square error of prediction.

		OR-CC-WR 2018		
		OR	CC	WR
data pre-processing		1st + SNV		
frequency range [cm^{-1}]		3279 - 2558		
		1841 - 760		
calibration/ internal validation	R²	98.18	98.25	98.41
	RPD	7.42	7.57	7.93
	RMSECV	4.07	3.91	3.69
	Offset	0.913	0.653	0.802
	Slope	0.973	0.977	0.976
external validation	RPD	2.78	4.03	3.64
	RMSEP	5.38	5.03	4.8
	Offset	3.266	2.23	0.344
	Slope	0.907	0.911	1.004

Table C7: Example of the calculated result of a 3-species model. First, an additive shift and secondly, a weighted directed compression (WDC) was conducted to get results between 0 and 100%. All values are in %.

	Predicted values	Additive shift	WDC
GR	-40	0	0
OR	10	50	22.73
CC	130	170	77.27
Sum	100	220	100

Kemper R, Döring TF, Legner N, Meinen C, Athmann M (2022) Root traits in cover crop mixtures of blue lupin and winter rye. Plant and Soil. doi:10.1007/s11104-022-05366-9

Appendix D Supplementary material from article "Relating Profile Wall Root-Length Density Estimates to Monolith Root-Length Density Measurements of Cover Crops"

Table D1. Coefficients and quality metrics of non-linear regression models in train dataset, by crop group and data type. RLD = root length density and cum. RL = cumulative root length. Models are: quadratic model ($Y = a_0 + a_1(x) + a_2(x^2)$) and cubic model ($Y = a_0 + a_1(x) + a_2(x^2) + a_3(x^3)$), where Y = monolith RLD, x = profile wall RLD, a_0 = intercept, a_1 and a_2 = coefficients to multiply by x. AIC = Akaike's Information Criterion for model comparison. R^2 = coefficient of determination, and * indicates that the correlation between PW and MO was significant, with a P-value < 0.05.

Data Type	Crop Group	Model	AIC	R^2	a_0	a_1	a_2	a_3
Absolute Data (RLD)	Brassicas	Quadratic	604.49	0.26*	0.88	7.17	-3.77	-
		Cubic	604.08	0.28*	1.04	3.74	5.44	-5.18
	Grasses	Quadratic	404.33	0.71*	0.10	11.54	-3.73	-
		Cubic	405.23	0.72*	0.19	8.81	3.06	-3.53
	Legumes	Quadratic	97.38	0.77*	0.18	8.77	-5.83	-
		Cubic	92.92	0.80*	0.10	15.02	-42.19	46.19
Cumulative Data (cum. RL)	Brassicas	Quadratic	-237.62	0.73*	0.17	0.36	0.40	-
		Cubic	-236.70	0.73*	0.11	0.68	-0.14	0.27
	Grasses	Quadratic	-328.24	0.92*	0.30	-0.20	0.88	-
		Cubic	-328.92	0.92*	0.75	-2.38	4.14	-1.53
	Legumes	Quadratic	-160.34	0.89*	0.05	0.50	0.39	-
		Cubic	-158.38	0.89*	-0.02	0.89	-0.23	0.31

Table D2. Performance metrics of regression models in test dataset, by crop group and data type. RLD = root length density and cum. RL = cumulative root length. R^2 = coefficient of determination, and * indicates that the correlation between predicted and measured RLD or cum.RL was significant, with a P-value < 0.05. MAE = Mean absolute error, and RMSE = Root mean absolute error.

Data Type	Crop Family	Model	R^2	MAE	RMSE
Absolute Data (RLD)	Brassicas	Quadratic	0.00*	1.59	2.61
		Cubic	0.00	2.94	13.30
	Grasses	Quadratic	0.17*	1.62	4.51
		Cubic	0.05	4.43	22.01
	Legumes	Quadratic	0.70*	1.06	1.32
		Cubic	0.69*	0.50	0.95
Cumulative Data (cum. RL)	Brassicas	Quadratic	0.75*	0.09	0.11
		Cubic	0.75*	0.09	0.11
	Grasses	Quadratic	0.92*	0.04	0.05
		Cubic	0.93*	0.04	0.05
	Legumes	Quadratic	0.78*	0.07	0.10
		Cubic	0.79*	0.07	0.10

Appendix E: Further results

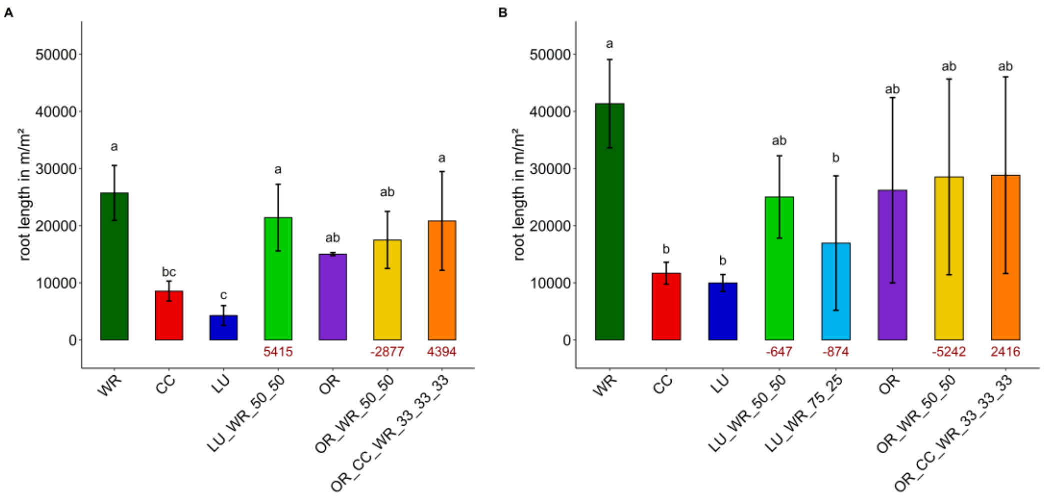

Fig E.1 Mean values ± standard deviation (n = 3) of total root length [m] down to 1 m soil depth of cover crops under an area of 1 m² in 2018 (A) and 2019 (B) at Wiesengut experimental farm in Hennef. Root length refers to the RLD shown in the Fig. 3.5 and 4.2. Different lowercase letters indicate significant differences ($p \leq 0.05$, HSD Tukey-test) between different cover crops. The absolute mixture effects (AME) of total root length in cover crop mixtures are represented in red numbers below the bars.

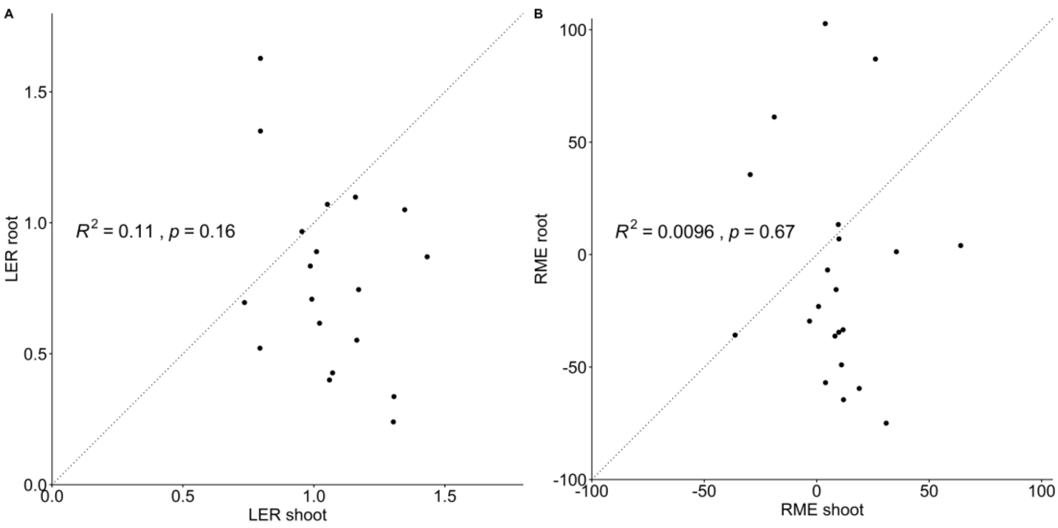

Fig. E.2 Relationship between land equivalent ratio (LER) of shoot and root mass (A) and relative mixture effect (RME) of shoot and root mass (B) for cover crop mixtures of winter rye and lupin (50:50 and 75:25), oil radish and winter rye (50:50) and oil radish, crimson clover and winter rye (33:33:33) grown at Wiesengut experimental farm in Hennef in 2018 and 2019. LER and RME values are from Fig. 3.3 and Tables C1 and C2.

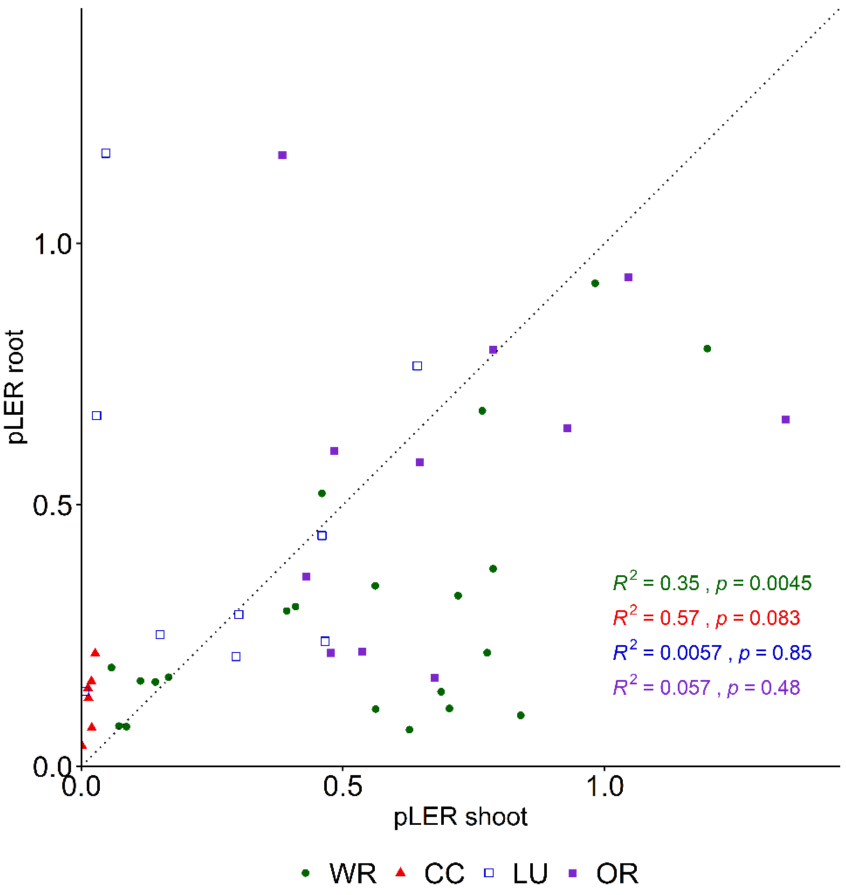

Fig. E.3 Relationship between partial land equivalent ratio (pLER) of shoot mass and partial land equivalent ratio (pLER) of root mass for the cover crop mixture partners winter rye (WR, n=21), crimson clover (CC, n=6), blue lupin (LU, n=9) and oil radish (OR, n=12) grown at Wiesengut experimental farm in Hennef in 2018 and 2019. pLER values are from Fig. 3.3 and Tables C1 and C2. Coloured symbols refer to species: green circle for rye, red triangle for clover, open blue square for lupin and closed violet square for radish.

Appendix F: List of further publications

Conference presentations

Roman Kemper, Thomas F. Döring, Nicole Legner, Catharina Meinen, Miriam Athmann (2021) Root growth of rye and lupin in cover crop mixtures, 11th Symposium of the International Society of Root Research, 24-28 May 2021, Missouri, USA.

R Kemper, P Müller, TF Döring, M Athmann (2021) Exploring The Total Soil Volume: Root Length Densities And Rooting Depth Of Different Cover Crops Determined With The Profile Wall Method, Organic World Congress 2021, September 8-10, 2021, Rennes, France.

Publications for agricultural practice

Kemper R, Döring T, Athmann M (2022) Ein Blick auf die Wurzeln – Wurzelforschung: Untersuchungen bei Zwischenfruchtmischungen. Lumbrico, 12: 23-27.

Posters and presentations for knowledge transfer on field days

Roman Kemper, Phillip Müller, Thomas Döring, Miriam Athmann. Wurzelwachstum und N-Aufnahme von Zwischenfrüchten in Reinsaaten und Mischungen. Ökofeldtage, Frankenhausen, 3. und 4. Juli 2019.

Roman Kemper. Optimierung der Bodennutzung durch Mischanbau von Fein- und Pfahlwurzlern. Feldtag Zwischenfruchtanbau und Klimaanpassung auf dem Versuchsbetrieb Wiesengut, Hennef, 7. November 2019.

Roman Kemper. Optimierung der Bodennutzung durch Mischanbau von Fein- und Pfahlwurzlern. Naturland-Feldtag Zwischenfruchtmanagement im Ökolandbau. Naturland Hof Bredtmann. Velbert, 29. Oktober 2020.

Roman Kemper. Wurzeleigenschaften in Zwischenfruchtmischungen. Nachwuchswissenschaftliches Kolloquium der Gesellschaft für Pflanzenbauwissenschaften e. V. (GPW). 30.04.2021

Roman Kemper, Miriam Athmann. Dürre oder Land unter auf dem Acker: Die ökologische Antwort auf Wetterextreme. Was können wir aus der Wurzelforschung im Projekt

MIKODU lernen? DLG-Feldtage. Versuchsgut Kirschgartshausen, Mannheim. 14. bis 16. Juni 2022.

Roman Kemper, Muhammad Arslan, Thomas Döring. Wurzel- und Bioporenforschung. Ökofeldtage, Gladbacher Hof 28. bis 30. Juni 2022.

Roman Kemper. MIKODU: Fruchtfolgen für optimierte Nutzung der Bodenressourcen: Mischanbau allorhizer und homorhizer Arten zur komplementären Durchwurzelung. BÖL-Veranstaltung Nährstoffmanagement im ökologischen Landbau. Fulda. 24. und 25. November 2022.

Roman Kemper. Zwischenfruchtmischungen: Ein Blick auf die Wurzeln. NutriNet Online-Abend des Regionetzwerks Bayern: Zwischenfrüchte und deren Leistung. 14. Februar 2023.

Roman Kemper. Durchwurzelung von Zwischenfruchtbeständen. Vortrags- und Praxistag Bodenstruktur: Erkennen – Beurteilen – Fördern. Fachhochschule Kiel, Osterrönfeld. 20. April 2023.

Kemper R (2023) Perspektive Boden - Wurzeleigenschaften von Zwischenfrüchten und Futterpflanzen. NutriNet-Feldtag des Regionetzwerks Brandenburg: Luzernekleegras und Alternativen? Briesen (Spreewald). 03. Mai 2023.