Biomass, root distribution and overyielding potential of faba bean/wheat and white clover/ryegrass mixtures

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Juliane Streit
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1. Referent: Prof. Dr. Rolf Rauber

2. Korreferent: Prof. Dr. Johannes Isselstein

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Contents

Abbreviations

asl	Above sea level
ATR	Attenuated total reflection
Av	Average
β	Regression coefficient for vertical root distribution
DWD	Deutscher Wetterdienst
FTIR	Fourier transform infrared
IMPAC^3	Novel genotypes for mixed cropping allow for improved sustainable land use across arable land, grassland and woodland
IR	Infrared
Lp	*Lolium perenne*
N, N_2	Nitrogen
R^2	Coefficient of determination
RBa	Accumulated root biomass from 0 to 60 cm soil depth
RMSECV	Root mean square error of cross validation
RMSEE	Root mean square error of estimation
RMSEP	Root mean square error of prediction
RPD	Residual predictive deviation
R:S	Root:Shoot
RY	Relative Yield
RYT	Relative yield total
SE	Standard error
Ta	*Triticum aestivum*
Tr	*Trifolium repens*
Vf	*Vicia faba*

Chapter 1

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General Introduction

1.1. Mixed cropping

Mixed cropping systems have the potential to simultaneously enhance yields and improve resource use (Hauggaard-Nielsen et al. 2008; Hauggaard-Nielsen et al. 2011). They are defined as the simultaneous growing of two or more field crops at the same field (Andrews and Kassam 1976). Terminology differences exist among the different land use systems. In the following study, the term intercropping will be simultaneously used with mixed cropping or mixture. Similarly, the cultivation of a single crop species will be defined as either pure stand or sole stand (Vandermeer 1992). Even though mixed cropping systems exist for the majority of the farming history, their practice declined drastically due to agricultural intensification and sole stand breeding progress in the 20th century (Borlaug 2000; Geno and Geno 2001). In Germany, agricultural farming practice has also undergone a change towards less diverse cropping systems with few main crops and short rotation systems in the last decades (Stein and Steinmann 2018). With the increasing world population, demand for food is constantly rising. Furthermore, climate change and a stagnating increase in cereal yields enhance the demand for more sustainable cropping systems. In fact, research on mixed cropping has increased since the 1970s (Geno and Geno 2001).

Mixed cropping comprises several advantages: One of the most cited benefit is dry matter or grain overyielding compared to the sole stand equivalents (Nyfeler et al. 2009; Rauber et al. 2001). Other benefits include a more efficient resource use and increased yield stability (Ergon et al. 2016; Lithourgidis et al. 2006). Carbon sequestration and soil conservation are enhanced in mixed cropping systems (Anil et al. 1998; Halty et al. 2017; Picasso et al. 2008).

Furthermore, numerous studies demonstrated a better suppression of weeds for mixed cropping compared to pure stands (e.g. Picasso et al. 2008). A major advantage of integrating legumes into mixtures is the increment in yield potential, since legumes improve the soil nitrogen (N) pool via symbiotic N_2 fixation (Jensen 1996; Xiao et al. 2004). The integration of legumes in crop rotations, such as for instance faba bean, enhances the diversification and provides habitat for insects (Crist et al. 2006; Hauggaard-Nielsen et al. 2008; Jensen et al. 2010).

1.2. Mixed cropping including faba bean or white clover

The majority of the global faba bean (*Vicia faba* L.) production is located in China, Ethiopia and Australia (FAOSTAT 2018). The cultivation area for faba bean in Germany was 46,000 ha in 2017 (Bundessortenamt 2018). The crop serves as protein rich human food, as feed for animals and is grown for bioenergy and green manure purposes. An increase in cultivation area of faba bean in Europe has the potential to reduce the import dependency on soybean (Köpke and Nemecek 2010). Numerous studies showed that faba bean/cereal intercrops have significant higher yields than their sole stand equivalents (e.g. Pristeri et al. 2006). Furthermore, yield stability of faba bean is enhanced when intercropped (Hauggaard-Nielsen et al. 2008). Winter forms of faba bean are characterized by higher yields and an increased weed suppression compared to the summer types (Haymes and Lee 1999; Link and Arbaoui 2005). For German cultivation purposes, winter hard cultivars are required. Therefore, the major focus of winter faba bean breeding programs in Germany are resistance and tolerance to frost stress (Link et al. 2010). To this date, there is only one winter faba bean cultivar (cf. Hiverna) available on the German market (Bundessortenamt 2018).

White clover (*Trifolium repens* L.)/perennial ryegrass (*Lolium perenne* L.) mixtures are of common practice in temperate grassland systems (Frame et al. 1998). Clover/grass mixtures demonstrate many advantages over their pure stands such as: a higher yield production, a higher yield stability and an improved forage quality (Ergon et al. 2016; Nyfeler et al. 2009; Sleugh et al. 2000). Due to a permanent ground cover, soil erosion is reduced and carbon sequestration is enhanced (Halty et al. 2017). Similar to bean/wheat intercrops, clover/grass mixtures use nitrogen (N) more efficiently than sole stands through N transfer from legumes to non-legumes (Rasmussen et al. 2012). Cultivars, which were bred for pure stand purposes are often also used in mixed cropping systems (Davis and Woolley 1993; Nelson and Robichaux 1997). Unlike France and United Kingdom, official testing trials in Germany do not test the white clover cultivar performance in mixtures.

The performance of mixtures depends on genotypic properties as well as on environmental conditions (Carton et al. 2018; Neugschwandtner et al. 2015). Trait expressions can vary between pure stands and mixed stands; well performing cultivars in pure stands don't necessarily perform well in mixed stands. In order to intensify mixed cropping systems sustainably, cultivar development has to be targeted for these systems considering the interspecific interactions (Carr et al. 1998; Hauggaard-Nielsen and Jensen 2001). It is still unclear in which way the genetic variation within legume cultivars/genotypes affects the above- and belowground biomass of faba bean/wheat and white clover/ryegrass mixtures.

1.3. The effect of mixed cropping on root growth

Above- and belowground competition, facilitation processes and complementarity in temporal and spatial growth patterns are the main mechanisms behind the widely demonstrated grain overyielding of legume/cereal mixtures (Hauggaard-Nielsen and Jensen 2001; Li et al. 2006; Rauber et al. 2001). These mechanisms lead to a better resource utilization of the mixtures compared to their sole stands. Most intercrop studies focused on aboveground processes. However, numerous studies showed that the yield advantage of mixtures is connected to root interactions and belowground competition (Connolly et al. 2001; Hauggaard-Nielsen and Jensen 2005; Tofinga et al. 1993). Roots are essential for a plant's anchorage, carbon capture, water and nutrient uptake (McElrone et al. 2013). Several studies demonstrated that mixtures produce significantly higher root biomass as opposed to their pure stand equivalents (Ma and Chen 2016). Root overyielding was observed in the case of faba bean/maize mixtures (Li et al. 2006; Xia et al. 2013) and clover/ryegrass mixtures (Davidson and Robson 1990). However, there is also evidence that a higher plant species richness leads to decreased root biomass (Bessler et al. 2009). Besides root biomass, horizontal and vertical root distribution is also altered by the presence of a mixing partner (Li et al. 2006). Nevertheless, there are only a few studies which investigated differences in horizontal and vertical root distribution between sole stands and mixtures (Gao et al. 2010). The modification of spatiotemporal root distribution and enhanced root biomasses in mixtures might lead to an increased nutrient uptake and eventually to higher yields (Hauggaard-Nielsen et al. 2001; Nachi and Le Guen 1996). Brooker et al. (2015) emphasize that the main challenge for research on mixed cropping systems is to understand its underlying processes. More efforts have to be put into the research of roots and their role in the yield formation process (Koevoets et al. 2016; Lynch 2007). Research of belowground interspecific interactions in mixtures is still limited by the laborious task to identify roots on a species level.

1.4. Root species differentiation in mixed stands via FTIR spectroscopy

To determine roots of different species in mixtures, a diverse set of approaches have been employed. The most common method is to separate roots of the mixture species by their differing gross root morphology, such as root colour or texture (Li et al. 2006). Other determination approaches are based on ^{13}C discrimination, fluorescence or DNA (Gealy and Fischer 2010; Faget et al. 2009; Riley et al. 2010). Some of these methods, however, are associated with high costs, high training requirement and high handling times (Rewald et al. 2012). In particular, distinguishing between closely related species and within young roots might be difficult.

The determination of unknown biological substances via infrared (IR) spectroscopy started with the identification of microorganisms (Thomas and Greenstreet 1954). IR spectroscopy records the absorption of different IR frequencies by an irradiated sample in the path of an IR beam. The most frequently used IR region is the mid infrared region between the wavenumbers 400 and 4000 cm^{-1} (Sherman Hsu 1997). The chemical composition of the sample determines the absorption at certain wavenumbers and thereby creates a spectral fingerprint (Sherman Hsu 1997). Spectral peaks can be attributed to the presence of certain chemical compounds and functional groups (Naumann 2000; Sherman Hsu 1997). Fourier transform infrared (FTIR) spectroscopy is a subtype of IR spectroscopy and was first used for bacteria identification (Naumann et al. 1988). For this method, a broadband light source is guided through a Michelson Interferometer (Sherman Hsu 1997). The resulting interferogram is then converted with the Fourier transform algorithm into light absorption for each wavelength (spectrum). As a further development, attenuated total reflection (ATR) devices were established. Samples are placed on an ATR crystal where the infrared beam is totally reflected at the interface between the sample and the ATR crystal (Naumann et al. 2010). FTIR-ATR spectroscopy is suitable for thick or highly absorbing solid and liquid materials. Furthermore, FTIR-ATR offers many advantages such as low sample preparation, highly characteristic finger print regions and short measuring times (Meinen and Rauber 2015; Sherman Hsu 1997). FTIR-ATR spectroscopy can be used to discriminate roots from different species, e.g.: pea/oat (Naumann et al. 2010), oak/spruce (Lei and Bauhus 2010) and wheat/rapeseed (Rewald and Meinen 2013). Recently, Legner et al. (2018) demonstrated that the separation of pea, oat, maize and barnyard grass via FTIR-ATR spectroscopy was possible regardless of cultivar and provenience. Furthermore, FTIR-ATR spectroscopy has been proven to be a successful tool for the quantification of root masses in diverse plant mixtures. Species quantification was possible for root mixtures of maize/barnyard, grass/wild oat (Meinen and Rauber 2015) and faba bean/chamomile (Rewald and Meinen 2013). The authors emphasize that FTIR-ATR spectroscopy is a promising tool for the quantification of root proportions in mixed cropping systems.

1.5. Objectives and structure of this thesis

In Germany, breeding and official testing trials traditionally focus on improving the performance of pure stand cultivars. However, cultivars which were bred for pure stand purposes might not be suitably adapted to mixed cropping systems. The present study was conducted within the interdisciplinary project IMPAC³ (Novel genotypes for mixed cropping allow for improved sustainable land use across arable land, grassland and woodland). The main goal of IMPAC³ was to understand the causality of positive mixing effects by comparing mixed cropping systems across arable land, grassland and woodland. Altogether 12 research groups carried out systematic analyses on the interspecific interactions, beneficial traits and trait expression of novel genotypes within legume/non-legume mixtures. Hereby, the project fosters the knowledge about cultivars suitable for mixed cropping systems for future breeding.

The present study focused on the comparison of pure stands and mixtures in the land use systems arable land and grassland. In a field experiment, various novel winter faba bean as well as white clover genotypes were established in pure stands and mixed stands with non-legume species (i.e. winter wheat and perennial ryegrass). The novel genotypes of winter faba bean and white clover used in the present study were available to the project but not on the market. The genotypes were phenotyped beforehand and varied in traits such as plant height, maturity, flowering, leaf size or mixture yield. The present study investigated interspecific differences (winter faba bean/winter wheat; white clover/perennial ryegrass) as well as intraspecific differences between eight winter faba bean genotypes and between eight white clover genotypes.

In the present study the following parameters were investigated (A=arable land, G=grassland):

- Aboveground biomass (A)
- Aboveground overyielding potential (A)
- Root biomass (A, G)
- Horizontal and vertical root distribution (A)

- Belowground overyielding potential (A, G)
- Root:Shoot ratio (A)

For this purpose, sampling was conducted in May 2015 and May 2016 in arable land and in June 2016 in grassland.

The main objectives of this study were (a) to test the capacity of FTIR spectroscopy for root mass quantification in bean/wheat mixtures and clover/grass mixtures, (b) to quantify biomass in sole stands and mixtures of bean/wheat and clover/grass systems, (c) to examine intraspecific differences between bean genotypes and between clover genotypes and (d) to compare root growth of mixtures in arable land and grassland and investigate if they are characterized by similar patterns.

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Chapter 2

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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

Juliane Streit, Catharina Meinen, William Christopher Dougal Nelson, Daniel Johannes Siebrecht-Schöll, Rolf Rauber

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2.1. Abstract

Background and aims Legume-cereal mixtures are often characterized by higher biomass and grain yields compared to their sole crop equivalents due to complementary resource use. Little is known about the contribution of the root system to this overyielding potential and the related cultivar differences. This study investigated pure stands and mixtures of eight winter faba bean (*Vicia faba* L.) genotypes and one winter wheat cultivar (*Triticum aestivum* L., cv. Genius) with regard to their intra- and interspecific variation of shoot and root biomass and overyielding potential at full flowering of the bean.

Methods Shoot biomass of 1 m² was harvested and roots were sampled with a root auger down to 0.6 m soil depth in two sampling years. Fourier transform infrared (FTIR) spectroscopy was successfully used to determine species specific root biomasses in mixtures. Statistics were performed using linear mixed effects models.

Results Mixtures of winter faba bean and winter wheat overyielded more below- than aboveground. Bean genotypes grown in mixtures with wheat differed significantly in their root biomass, root:shoot ratio and overyielding potential but not in their shoot biomass.

Conclusions Genotype differences in root biomass and overyielding indicate breeding potential of winter faba bean cultivars for mixed cropping.

Keywords: *Vicia faba*, *Triticum aestivum*, Legume-Cereal Intercropping, FTIR Spectra, Root Shoot Ratio, Overyielding

2.2. Introduction

Mixed cropping is known to improve temporal and spatial resource use and has the potential to increase plant production and reduce resource consumption at the same time (Hauggaard-Nielsen et al. 2008; Hauggaard-Nielsen et al. 2011). Other benefits of mixed cropping besides increased aboveground biomasses (Bulson et al. 1997; Knudsen et al. 2004; Pristeri et al. 2006; Rauber et al. 2001) include improved yield stability (Lithourgidis et al. 2006), improved soil conservation (Anil et al. 1998) and an improved defense against weeds, plant pathogens and pests (Banik et al. 2006; Gronle and Böhm 2012). Combining N_2-fixing legumes with cereals positively influences nutrient availability and uptake and therefore reduces the need for synthetic mineral N fertilizer (Jensen 1996; Xiao et al. 2004).

It is still unclear in which way the genetic variation within species affects the complementarity among species in a mixture. In the past, plant breeding programs mainly focused on the development of cultivars for pure stands. These pure stands cultivars were also used in mixed cropping systems (Davis and Woolley 1993; Nelson and Robichaux 1997). Studies by Carr et al. (1998) and Hauggaard-Nielsen and Jensen (2001) however, emphasized the importance of cultivar development for mixed cropping systems to sustainably intensify such systems. Moreover, previous breeding efforts have focused on aboveground phenotyping and yield improvement. Research on architecture and the root contribution to stress resistance may sustain future yield improvements (Den Herder et al. 2010; Koevoets et al. 2016; Lynch 2007).

The main challenge for mixed cropping research is to understand its underlying processes (Brooker et al. 2015). Above- as well as belowground traits and processes have to be considered throughout the year. Complementary resource use due to temporal and/or spatial niche partitioning and a more efficient exploitation of these resources were suggested to be the main reasons for yield increment in mixtures (Hauggaard-Nielsen et al. 2001a; Li et al. 2006; Tsubo and Walker 2004). The widely demonstrated grain overyielding of legume-cereal mixtures compared to their pure stands cannot occur without earlier below- and aboveground competition and facilitation processes. Those processes tend to shift during the vegetation period (Li et al. 2014; Li et al. 2016; Zhang et al. 2015). Even though agronomists have demonstrated the aboveground overyielding potential of legume-cereal mixtures for decades, few experimental studies have focused on species specific root biomasses and their impact on mixture performance. Higher yields in intercrops can however depend on root interactions and belowground competition (Connolly et al. 2001; Hauggaard-Nielsen and Jensen 2005; Tofinga et al. 1993). Vertical belowground niche complementarity and facilitation processes between legumes and cereals could lead to the improved utilization of resources. Stimulation of root growth or modification of spatiotemporal root distribution patterns of the species involved, may account for higher yields due to an increased total nutrient uptake in the mixture (Hauggaard-Nielsen et al. 2001b). Some studies have already demonstrated increased root biomasses from pure to mixed stands (Corre-Hellou and Crozat 2005; Li et al. 2006; Ma and Chen 2016). Belowground biomass overyielding during vegetative stages could lead to higher resource availability as well as to aboveground biomass overyielding and subsequent grain overyielding at harvest time (Nachi and Le Guen 1996).

Differences in root biomass of genetically different species and subsequently the root distribution of intercrops remains unclear. Research on belowground interspecific competition in mixtures has been limited so far by the difficulty to identify roots on a species level. Moreover, little is known about whether within species genetic variation affects root biomass. Methods for root species identification which are based on DNA, ^{13}C or root morphology are often associated with high handling times and require extensive training (Rewald et al. 2012). Infrared spectroscopy has been proven to be a successful and fast tool to distinguish between the roots of different species: sunflower-maize (Dokken and Davis 2007), corn-soybean (White et al. 2011), pea-oat (Naumann et al. 2010), oak-spruce (Lei and Bauhus 2010) and wheat-loose silky bentgrass (Rewald and Meinen 2013). Fourier transform infrared (FTIR) spectroscopy can not only separate roots according to species but it also estimates species specific proportions within a root sample (Meinen and Rauber 2015). In this field experiment, FTIR spectroscopy was used to analyze species specific root proportions in a mixed cropping system of winter faba bean (*Vicia faba* L.) and winter wheat (*Triticum aestivum* L.). Eight winter faba bean genotypes in pure stands and substitutive mixtures with one winter wheat cultivar were grown to test the following hypotheses:

(1) Mixtures of winter faba bean and winter wheat show above- and belowground overyielding at the full flowering stage of bean.

(2) Winter faba bean genotypes differ significantly in their shoot and root biomass at the full flowering stage of bean.

(3) Winter faba bean genotypes in mixtures with winter wheat show significant differences in their overyielding potential.

2.3. Materials and Methods

2.3.1. Experimental site and design

The field experiment was carried out in 2014/2015 and 2015/2016 at the experimental station “Reinshof” close to Goettingen, Germany (51°29´N, 9°55´E, 160 m above sea level). The mean annual temperature was 9.2°C and mean annual precipitation 651 mm (1981-2010, DWD 2018). The soil type was Gleyic Fluvisol (WRB) with a bulk density of 1.56 g cm^{-3} and had a particle size distribution of 21 % clay, 68 % silt and 11 % sand (top soil, April 2016). Soil pH was 7.0 (0.01 M $CaCl_2$). Penetration resistance was on average 1.18 MPa, with higher soil density found below 0.3 m soil depth. Previous landuse was cropland (rye). Eight winter faba bean (*Vicia faba* L.) genotypes and one winter wheat (*Triticum aestivum* L.) cultivar were grown in pure stands and in alternate row mixtures with a substitutive design (0.225 m row distance). Winter faba bean genotypes were chosen from the breeding program of NPZ (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany) and from the breeding research program at the department for Crop Sciences, University of Goettingen (Table A1) (Link and Arbaoui 2005). The wheat cultivar Genius was chosen as a mixture partner because of its medium height, high N-uptake capacity, low susceptibility to mildew and stable yields (NORDSAAT Saatzucht GmbH, Langenstein, Germany, Bundessortenamt 2017). Within the mixtures, each species was sown at 50 % of its pure stand seed density in a 0.5:0.5 replacement design. Plant densities were: 40 seeds m^{-2} in bean pure stands, 320 seeds m^{-2} in wheat pure stands, 20 bean seeds m^{-2} and 160 wheat seeds m^{-2} in mixtures. The experiment was arranged in a fully randomized split-plot design with 4 replications. Total plot size for final harvest was 10.5 m^2, and the central subplot size for biomass and root sampling was 1 m^2. Crops were sown on 28 October 2014 and 05-06 October 2015 (Table 1). Crops were grown without any fertilizer and were not irrigated. Pests and diseases were controlled with appropriate pesticides, weeds were manually controlled.

Table 1 Monthly precipitation (mm), monthly mean temperature (°C) (October till May) and sowing date, sampling date, sampling time (DAS, days after sowing), total precipitation and accumulated thermal time from sowing till sampling (degree days, base temperature 5°C) for the growing seasons 2014/2015 and 2015/2016 (DWD 2018).

	Growing season	
	2014/2015	2015/2016
Oct	49.6 mm (12.23 °C)	37.4 mm (8.57 °C)
Nov	11.3 mm (7.15 °C)	94.5 mm (8.25 °C)
Dec	41.5 mm (3.25 °C)	21.0 mm (7.47 °C)
Jan	43.2 mm (2.78 °C)	41.8 mm (1.49 °C)
Feb	22.0 mm (1.76 °C)	46.9 mm (3.70 °C)
Mar	58.2 mm (5.30 °C)	31.6 mm (4.42 °C)
Apr	46.5 mm (8.40 °C)	28.4 mm (8.06 °C)
May	30.0 mm (12.16 °C)	41.4 mm (13.75 °C)
Sowing date	28 October 2014	05-06 October 2015
Sampling date	27-28 May 2015	23-26 May 2016
Sampling time	210 DAS	230 DAS
Precipitation	245.8 mm	304.7 mm
Accumulated thermal time	466.0 °C d	635.3 °C d

2.3.2. Plant and root sample collection

To attain the maximum root biomass of bean, root samples and shoot biomass were collected at the full flowering stage of bean (Ehlers and Goss 2016) on 27-28 May 2015 and 23-26 May 2016. Shoot biomass was determined by sampling 1 m^2 aboveground biomass at the ground level of the central subplot. Fresh weights of bean and wheat were measured separately. Subsamples of each species were dried at 60°C for 24 h and again at 105°C for 24 h and weighed. Total sample dry matter was calculated from dry and fresh subsamples weights. Aboveground data was expressed as bean shoot biomass, wheat shoot biomass and total shoot biomass (g m^{-2}). After aboveground harvest, soil cores were taken from the same subplot. Root cores were taken with a root auger (0.0874 m diameter) down to 0.6 m soil depth. Two cores were sampled in pure stands, three in mixed stands: on the bean row, on the wheat row and between rows, respectively. Soil samples were air-dried for 72 h and transferred to plastic bags. In order to extract roots, air-dried soil samples were washed with a root-washing machine and cleaned of soil residues and other organic matter (custom made, mesh size 1 mm). Live and dead roots were distinguished based on root turgor and elasticity, only live roots were further investigated. Lateral roots of faba bean were cut from the tap root. Due to randomized sampling on the bean row, not all samples were taken directly on bean plants. Tap roots were therefore excluded from further analysis. Roots were dried at 55°C for 72 h until a constant weight was reached, ground in a centrifugal mill (Retsch, ZM 200, 0.12 mm) and stored in glass vials.

2.3.3. FTIR spectroscopy and cluster analysis

FTIR spectra of dried and ground roots were recorded with an Alpha-P FTIR spectrometer combined with a platinum ATR unit (Bruker Optics, Ettlingen, Germany). Spectra were recorded with a 4 cm^{-1} resolution (32 Scans) and at wavenumbers of 400-4500 cm^{-1}. Background measurements for CO_2 compensation were applied every 10 minutes. Dried and ground bean and wheat roots of pure stands showed species specific FTIR spectra (Fig. 1). Similar absorption was found between wavenumber 860-950, 1070-1200, 1750-2400 and 3400-4000 cm^{-1}. Roots of both species demonstrated similar peaks at 1050, 1250, 1620, 2900 and 3300 cm^{-1}, however mean absorbance of bean roots (0.017) was significantly higher than the absorbance of wheat roots (0.015) ($n=2540$, $p \leq 0.01$). The average difference between the absorbance of bean and wheat was 4.92%. The highest absorbance peak 0.088, was found at 1029 cm^{-1}. Differences in spectral amplitude between species were found at 400-860, 950-1070, 1200-1750 and 2400-3400 cm^{-1}.

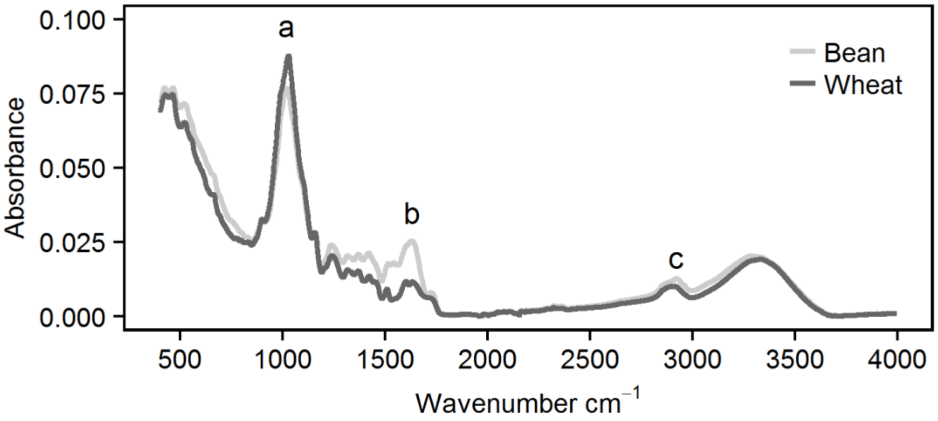

Figure 1 Mean FTIR spectra of dried and ground roots from bean (light grey) and wheat (dark grey) pure stands. Spectra were vector-normalized and offset-corrected. Mean spectra of 112 measurements of wheat and 148 measurements of bean. Letters indicate different functional groups: cellulose and hemicellulose (a), proteins (b) and lipids (c).

Species discrimination was tested with a cluster analysis of the pure stand samples. Cluster analysis and dendrogram development were performed with the software package OPUS IDENT (Version 7.0, Bruker, 2011, Fig. 2).

Figure 2 Cluster analysis of FTIR spectra recorded from dried and ground roots of pure stands of bean (Vf) and wheat (Ta). Analysis included eight bean genotypes (Vf1-8) and one wheat genotype with 3 replicated measurements (a, b, c) for the experimental years 2015 and 2016. Numbers next to wheat pure stands represent wheat plots adjacent to bean plots. Please note the break on the y-axis. Samples were cumulated from 4 blocks and all sample positions and depths. Spectra were pre-processed by first derivative and vector normalization. Ward's algorithm was applied in the frequency range of 3997.3 to 2750 and 1800 to 401 cm^{-1}.

For the cluster analysis, a composite pure stand sample of different positions within the plot (on bean row, on wheat row, between rows) and of all blocks was prepared for each genotype and year. Bean pure stand samples were prepared from the eight bean genotypes for 2015 and 2016, respectively (n=16). Wheat pure stand samples consisted of the cultivar Genius and were prepared identically to pure bean samples (n=16). All 32 samples were measured spectrometrically three times (total n=96). Frequency ranges of the cluster analysis were 3997 to 2750 and 1800 to 401 cm^{-1} due to continuous peak differences in these regions. Clusters were developed by using pretreated spectra (first derivative plus vector normalization), Ward's algorithm and Euclidean distance. Cluster analysis revealed a 100% separation of both species (Fig. 2). FTIR spectra of wheat and bean roots of pure stands formed two distinct clusters. Heterogeneity between species was very high with 10.03. Intra-specific heterogeneity was much smaller with 1.48 for bean and 1.74 for wheat.

In order to calculate the species proportions within mixtures, bean and wheat roots of pure stands were used to prepare artificial samples to calibrate and validate chemometric FTIR models. FTIR model type was a PLS1 algorithm. Calibrations as well as model developments were conducted for each genotype within a mixture and for each year. The total number of FTIR models was 16. For this process, the software package OPUS QUANT 2 (Version 7.0, Bruker, 2011) was used. FTIR spectra of 35 calibration samples with known species composition were used to prepare a two-species calibration (3% steps from 0 to 100% of bean and wheat, respectively). All samples were measured spectrometrically with the same setting as the cluster measurements five times. Three internal test samples were chosen for cross validation in order to evaluate chemometric models and test for their prediction reliability. The internal cross validation of the 16 FTIR models showed R^2 values above 90.94, root mean square error of cross validation (RMSECV) below 8.96% and residual prediction deviation (RPD) higher than 3.35 (Table A2). Chemometric models were improved automatically with the "Optimize" option from the QUANT software (standard method Opus 7.0). This procedure checks common frequency regions in combination with several data preprocessing methods like first derivative, vector normalization and multiplicative scatter correction. The optimal model was chosen by using the lowest RMSECV, the highest R^2 and highest RPD. Species proportions derived from the FTIR models were then multiplied with the weight of the total root sample. Total root biomass as well as bean and wheat root biomass of 0-0.6 m soil depth was averaged over sampling positions (on bean row, on wheat row, between rows) per plot and expressed as total root biomass, bean root biomass and wheat root biomass (g m^{-2}).

In order to test the predictive quality of the FTIR models, an external validation was carried out. For this, 20 independent samples with known species proportions were prepared for each genotype and both years. External validation samples were measured spectrometrically with the same setting as the calibration samples. FTIR models predicted the species proportion of the external sample set. The predictive error of determination (RMSEP) did not exceed 15 % and RPD values were greater than 3 for most of the models. Therefore, the external validation revealed a satisfactory predictive quality for most of the 16 FTIR models (Diller 2002; Meinen and Rauber 2015). Additionally, the predictive character of FTIR spectroscopy was compared with the results of a morphological species determination approach. For this, bean and wheat roots of 288 mixed stand soil samples from 2015 were identified by their differences in gross root morphology (root colour and texture). The difference in average bean proportion between the approaches was only 4 % (morphology 44 %, FTIR 48 %), confirming the high accuracy of the FTIR models.

2.3.4. Calculation of relative yield total of above- and belowground biomass, comparative wheat and bean biomasses and root:shoot ratio

The relative yield total (RYT) for mixtures of winter faba bean and winter wheat, was calculated for above- (RYT_{shoot}) and belowground (RYT_{root}) biomass according to de Wit (1960) and de Wit and van den Bergh (1965). The RYT for a mixture of bean and wheat is the sum of the relative yield values for bean (RY bean) and wheat (RY wheat):

$$RYT_{shoot|root} = RY\ bean + RY\ wheat \quad (1)$$

$$RY\ bean = Y\ bean_{mix}\ /\ Y\ bean_{pure} \quad (2)$$

$$RY\ wheat = Y\ wheat_{mix}\ /\ Y\ wheat_{pure} \quad (3)$$

where Y $bean_{mix}$ and Y $wheat_{mix}$ are the yields (shoot biomass, root biomass) of bean and wheat, respectively in the mixture and Y $bean_{pure}$ and Y $wheat_{pure}$ are the yields of bean and wheat in pure stands. A RYT > 1 indicates a yield advantage for mixed cropping compared to the pure stands.

The eight faba bean genotypes were assessed for their total overyielding potential and overyielding consistency. Genotypes were first ranked according to their RYT_{shoot} and RYT_{root} values separately for each sampling year and given a rank number from 1 (lowest) to 8 (highest). This rank number was divided into the groups “low performance” (1-4) and “high performance” (5-8). Performance consistency for RYT_{shoot} or RYT_{root} was given when a genotype was in the same group in both sampling years. RYT_{shoot} and RYT_{root} had the same weighting and total overyielding potential for each genotype was derived from the average rank number of RYT_{shoot} and RYT_{root} of both years (n=4).

As the species specific plant density of pure stands (bean: 40 seeds m^{-2}, 6 rows, 1 m^2; wheat: 320 seeds m^{-2}, 6 rows, 1 m^2) was double than that of the mixtures (bean: 20 seeds m^{-2}, 3 rows, 1 m^2; wheat: 160 seeds m^{-2}, 3 rows, 1 m^2), we divided the pure stands bean or wheat biomass by two to have comparative values for the mixtures („comparative shoot biomass”). The same calculation was applied for the root biomasses. Data was expressed as comparative bean or wheat shoot biomasses and comparative bean or wheat root biomasses (g m^{-2}).

Root:shoot (R:S) ratios of wheat, bean and their total (bean+wheat) were calculated per plot by dividing wheat, bean or total root biomass (0-0.6 m, averaged over sample positions, g m^{-2}) by wheat, bean or total shoot biomass of 1 m^2 (g m^{-2}).

2.3.5. Statistical analysis

For the analysis of root FTIR absorbance differences between bean and wheat grown in pure stands, a linear model was applied. All other traits were analyzed using Linear Mixed-Effect Modelling (LME, “nlme” package, Pinheiro et al. 2017). For each model that focused on the comparison between bean pure stands and mixtures, the response variable was modeled with the fixed factors year (2015, 2016), bean genotype (Vf1-8) and treatment (pure stand, mixture). Blocks (1 to 4) and the main plots (1 to 8) were treated as random factors and main plots were nested within blocks. In order to compare the single wheat genotype in pure stand with each of the eight bean genotypes in both treatments (pure stand or mixture), a new variable called “WBGT” was created. This variable combines the wheat pure stand (W: Ta) with the bean genotype (BG: 1-8) and the treatment (T: pure stand, mixed stand). Fixed factors in this case were year and WBGT. For the comparison of total shoot or root biomass, WBGT consisted of wheat pure stands, the eight mixtures and the eight bean pure stands. For the comparison of comparative wheat shoot or root biomasses and the comparison of wheat R:S ratio, WBGT consisted of wheat pure stands and the eight mixtures. The homogeneity of variance and normal distribution of the residuals of each model were checked visually. Additionally, models were compared with the second-order Akaike Information Criterion (AICc). When normality and homoscedasticity of residual variance was not given, models were corrected with an appropriate variance structure. Bean shoot biomass, wheat R:S ratio and RY root bean residuals showed a heteroscedastic pattern and were log or square root transformed. Differences between factor levels were tested for significance by obtaining post-hoc pairwise least-square means and contrasts using the LSD test, as implemented in the “lsmeans” package (Lenth 2016). $p \leq 0.05$ was used as a significance level. All statistical analyses and graph creation were carried out using the statistics software R 3.3.2 (R Core Team 2016).

2.4. Results

2.4.1. Relative shoot and root yields of bean and wheat and relative yield total of mixtures

The relative shoot yield of bean in mixtures was affected by the bean genotype (Table 2). Year had a significant effect on the performance of bean genotype. Relative yield differences between genotypes were more pronounced in 2016 than in 2015 (Table 3a). Relative shoot yield of wheat did not significantly differ between mixtures and years. Mixtures of bean and wheat were characterized by a higher relative shoot yield total in 2016 (1.11) than in 2015 (0.94), bean genotype however did not affect the aboveground RYT (Table 2). The relative root yields of bean and wheat were highly dependent on the bean genotype and its interaction with year. In 2015, RY wheat exceeded RY bean on average by a difference of 0.4 (Table 3b). In 2016, RY bean exceeded RY wheat by an average difference of 0.6. The relative root yield total of the eight bean/wheat mixtures was significantly affected by bean genotype, year and its interaction. Relative yield and relative yield total were generally higher for root compared to shoot biomass (year average RYT_{root} 1.37, RYT_{shoot} 1.03).

The analysis of genotypic differences revealed that relative shoot and root yields of bean were the highest for mixture Vf7-Ta in 2015 and Vf6-Ta in 2016. Relative shoot yields of wheat were the highest for mixture Vf1-Ta in 2015 and Vf2-Ta in 2016. The highest relative wheat root yields were found for mixture Vf4-Ta in 2015 and for Vf5-Ta in 2016. Relative root yield totals were the highest for mixtures Vf4-Ta in 2015 and Vf3-Ta in 2016.

According to their overyielding potential, genotypes in mixture could be ranked as follows Vf5-Ta > Vf3-Ta > Vf1-Ta/Vf2-Ta > Vf6-Ta/Vf7-Ta > Vf4-Ta > Vf8-Ta (Table 4). The highest total overyielding potential was found in mixture Vf5-Ta (6), followed by Vf3-Ta (5.5) and Vf1-Ta and Vf2-Ta (5). Low total overyielding potential was found in mixture Vf6-Ta and Vf7-Ta (4.75), Vf4-Ta (3.5) and Vf8-Ta (1.5). RYT_{shoot} overyielding consistency was given for mixtures Vf3-Ta, Vf4-Ta, Vf5-ta and Vf8-Ta. Mixtures Vf5-Ta and Vf8-Ta demonstrated RYT_{root} overyielding consistency.

Table 2 P values for the factors year, bean genotype, treatment and WBGT and their interactions explaining shoot and root biomass, comparative biomasses, relative yield (RY), relative yield total (RYT), proportion and root:shoot ratio (R:S) of bean, wheat and the summed up species total using linear mixed effect models.

	Trait	Species		Year	Bean Genotype	Treatment	WBGT	Year x Bean Genotype	Year x Treatment	Year x WBGT	Bean Genotype x Treatment	Year x Bean Genotype x Treatment
Shoot	Biomass	Total *[1]	¥ x #	**0.0045**	0.1298	< **0.0001**		**0.0413**	0.5564		**0.0097**	**0.0450**
		Total *[2]	¥ x §	**0.0057**			< **0.0001**			**0.0001**		
	Comparative Biomasses	Bean	log, # x ^	< **0.0001**	**0.0300**	0.6057		< **0.0001**	**0.0491**		0.1376	0.0720
		Wheat	§	**0.0478**			0.7464			0.4043		
	RY	Bean	#	0.3087	**0.0176**			**0.0040**				
		Wheat	#	0.0911	0.4334			0.4436				
	RYT	Total	#	**0.0035**	0.5592			0.7169				
	Proportion	Bean	#	0.1808	**0.0310**			0.4597				
Root	Biomass	Total *[1]	#	**0.0109**	< **0.0001**	< **0.0001**		**0.0117**	0.6389		**0.0110**	**0.0115**
		Total *[2]	§	< **0.0001**			< **0.0001**			**0.0005**		
	Comparative Biomasses	Bean	# x ^	**0.0025**	< **0.0001**	< **0.0001**		0.0866	**0.0002**		**0.0015**	**0.0070**
		Wheat	P x §	0.5939			< **0.0001**			**0.0273**		
	RY	Bean	sqrt, #	0.2020	**0.0007**			**0.0264**				
		Wheat	#	0.3412	< **0.0001**			**0.0051**				
	RYT	Total	#	**0.0196**	< **0.0001**			**0.0011**				
	Proportion	Bean	#	**0.0013**	**0.0072**			**0.0186**				
Root:Shoot	R:S Ratio	Bean	# x ^	0.1905	**0.0004**	< **0.0001**		0.1881	0.8929		**0.0177**	**0.0174**
		Wheat	sqrt	0.1906			0.1387			0.1956		
		Total	log, #	0.9110	**0.0208**			0.0695				

Factor WBGT comprised wheat pure stands and all eight bean genotypes in pure stands and/or mixtures. Log and sqrt models were log or square root transformed; variance structure varPower was implemented in the model (P); variance structure varIdent was implemented in the model, allowing for differing variances for each year (¥), bean genotype (#), treatment (^) or species genotype (§) where x indicates an varIdent interaction of two factors. Bold p values indicate significant factors and interactions at $p \leq 0.05$ (LSD post-hoc test).

*[1] Total biomass was compared between bean genotypes in pure- and mixed stands.
*[2] Total biomass was compared between pure stand wheat and bean genotypes in pure- and mixed stands.

Table 3 Relative (a) shoot and (b) root yields of bean (RY bean, n=4), wheat (RY wheat, n=4) and relative yield total (RYT, n=4) of the eight bean genotypes in mixture with wheat (Vf1-8-Ta) for 2015 and 2016. Given are means. Different lowercase letters indicate significant differences between genotypes within one year and trait. Capital letters indicate significant differences between years within one genotype and trait. Asterisks indicate significant different values from 0.5 for RY and 1 for RYT (p≤0.05, LSD post-hoc test).

a

	2015			2016		
	RY bean	RY wheat	RYT	RY bean	RY wheat	RYT
Vf1-Ta	0.47 aA	0.54	1.01	0.47 bA	0.65	1.12
Vf2-Ta	0.55 aA	0.40	0.95	0.51 bA	0.69	1.20*
Vf3-Ta	0.56 aA	0.42	0.99	0.59*abA	0.53	1.12
Vf4-Ta	0.39 aA	0.51	0.91	0.49 abA	0.53	1.01
Vf5-Ta	0.48 aA	0.49	0.96	0.57 abA	0.61	1.18
Vf6-Ta	0.38*aA	0.47	0.85*	0.72*aB	0.45	1.18
Vf7-Ta	0.56 aA	0.42	0.98	0.64 abA	0.43	1.07
Vf8-Ta	0.47 aA	0.42	0.89	0.43 bA	0.60	1.02
Av.	0.48	0.46	0.94*A	0.55*	0.56	1.11*B

b

	2015			2016		
	RY bean	RY wheat	RYT	RY bean	RY wheat	RYT
Vf1-Ta	0.49 aA	0.87 abcA	1.36 abA	0.61 bcA	0.80 aA	1.41*abcA
Vf2-Ta	0.50 aA	0.51 bcA	1.01 abA	0.74*abcA	0.78*aA	1.52*abcA
Vf3-Ta	0.52 aA	0.78*abA	1.30 abA	0.94*abB	0.63*aA	1.57*abA
Vf4-Ta	0.85 aA	0.99*aA	1.84*aA	0.54 bcA	0.56 aB	1.10 bcB
Vf5-Ta	0.69*aA	0.77 abA	1.46 abA	0.66 bcA	0.81*aA	1.46*abcA
Vf6-Ta	0.78*aA	0.54 abcA	1.31*aA	1.17*aA	0.55 aA	1.71*aB
Vf7-Ta	0.82 aA	0.55 bcA	1.37 abA	0.75 abcA	0.66 aA	1.42 abcA
Vf8-Ta	0.51 aA	0.49 cA	1.00 bA	0.40 cA	0.60*aA	1.00 cA
Av.	0.65*	0.69*	1.33*	0.73*	0.67*	1.40*

Table 4 Ranking of the eight bean/wheat mixtures (Vf1-8-Ta) according to their aboveground (RYT_{shoot}) and belowground (RYT_{root}) relative yield total for 2015 and 2016. Rank numbers ranged from 1 (lowest rank) to 8 (highest rank). Consistency of one trait was given when a genotype had the same performance (1-4) or (5-8) in both years (*). Overyielding potential was the mean of RYT_{shoot} and RYT_{root} of both years. Consistency of overyielding potential was given when one (*) or both traits (**) demonstrated consistency.

	RYT_{shoot}		RYT_{root}		Overyielding potential
	2015	2016	2015	2016	
Vf1-Ta	8	4	5	3	5
Vf2-Ta	4	8	2	6	5
Vf3-Ta	7*	5*	3	7	5.5*
Vf4-Ta	3*	1*	8	2	3.5*
Vf5-Ta	5*	7*	7*	5*	6**
Vf6-Ta	1	6	4	8	4.75
Vf7-Ta	6	3	6	4	4.75
Vf8-Ta	2*	2*	1*	1*	1.5**

2.4.2. Total shoot and root biomass

Total shoot biomass was significantly affected by year and treatment (Table 2 *1). Additionally, year influenced the effect of bean genotype on total shoot biomass. The differences in shoot biomass between pure stands of bean and mixtures were dependent on the bean genotype. Shoot biomass of bean pure stands and mixtures was generally higher in 2016 than in 2015 (Fig. 3, Table 5a). Bean pure stands produced on average more shoot biomass (492.0 g m^{-2}) than wheat pure stands (384.7 g m^{-2}) and mixtures (448.2 g m^{-2}). In 2015, only one bean genotype grown as a pure stand yielded significantly more than wheat pure stands (Vf4) (Table 2 *2, Fig. 3, Table 5a). In 2016, this occurred in six out of eight instances. The best performing bean genotypes in pure stands regarding shoot biomass were Vf4 in 2015 and Vf8 in 2016. Highest total shoot mixture biomass was found for mixture Vf4-Ta in 2015 (399.5 g m^{-2}) and Vf7-Ta in 2016 (581.7 g m^{-2}). Total root biomass from 0 to 0.6 m soil depth was significantly affected by the year, treatment and bean genotype (Table 2 *1). In pure stands and mixtures, total root biomass was higher in 2016 than in 2015. Mixtures exceeded bean pure stands in their total root biomass on average with 93.7 g m^{-2}. Mixture Vf4-Ta showed the highest total root biomass in 2015 (103.0 g m^{-2}) and mixture Vf3-Ta in 2016 (137.1 g m^{-2}) (Fig. 3, Table 5b).

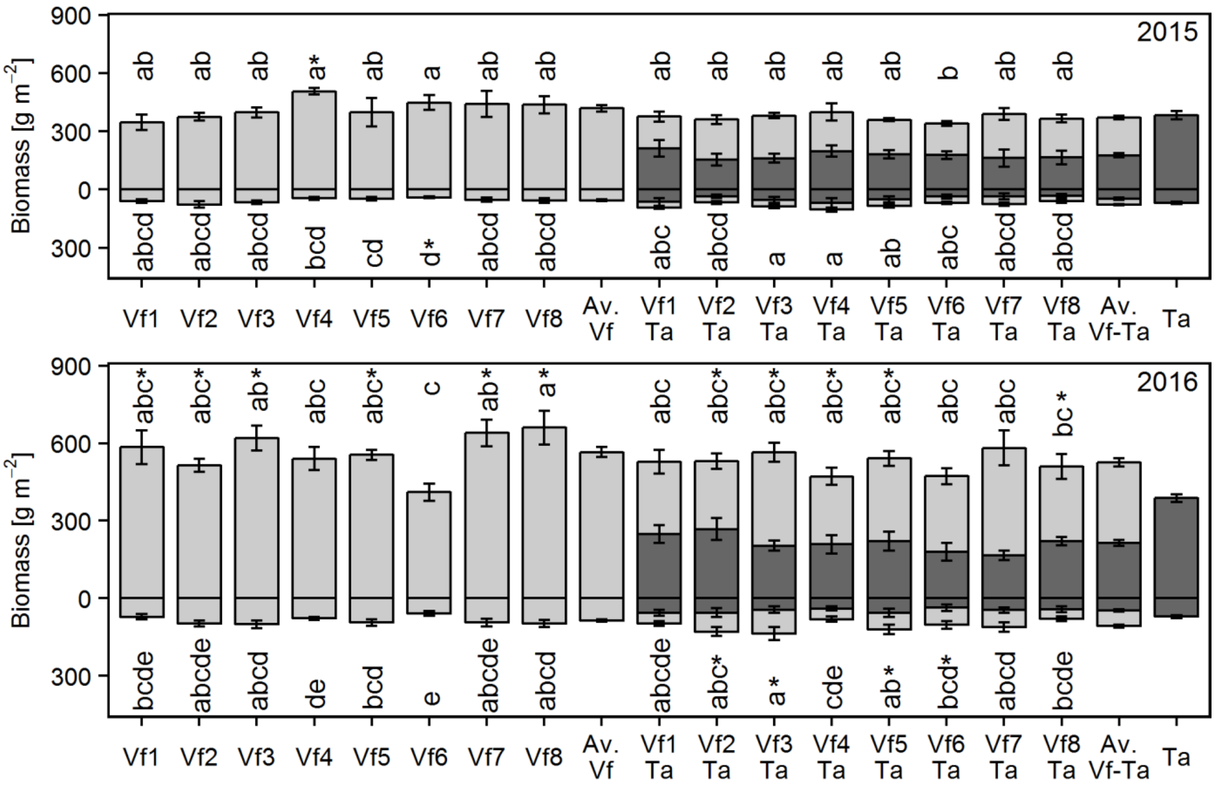

Figure 3 Mean total shoot and root biomass per species, treatment, genotype and treatment average (Av.) in 2015 and 2016. Treatments were Vf (bean pure stand, n=4), Ta (wheat pure stand, n=8) and Vf-Ta (mixture, n=4). Numbers adjacent to treatments represent bean genotypes (1-8). Bars below 0 represent root biomass, bars above 0 shoot biomass. Colors of bars refer to species: light grey for bean, dark grey for wheat. Given are means ± SE. Different lowercase letters indicate significant differences between genotypes and treatments per shoot or root biomass within one year. Asterisks indicate significant differences between wheat pure stand (Ta) and any other given bar ($p \leq 0.05$, LSD post-hoc test).

2.4.3. Comparative shoot and root biomasses of bean and wheat

Comparative shoot biomasses of bean were significantly higher in 2016 compared to 2015 (Table 2, Fig. 4a,b). Mixtures did not demonstrate higher comparative bean shoot biomasses than bean pure stands (Fig. 4b). The factor year had a stronger effect on the comparative bean biomasses (F-value 547.893) than bean genotype (F-value=2.839) and post-hoc test results did not demonstrate differences between genotypes (Fig. 4a). Comparative shoot biomasses of wheat were significantly higher in 2016 compared to 2015 and did not depend on the treatment or accompanied bean genotype in mixtures (Table 2, Fig. 4c).

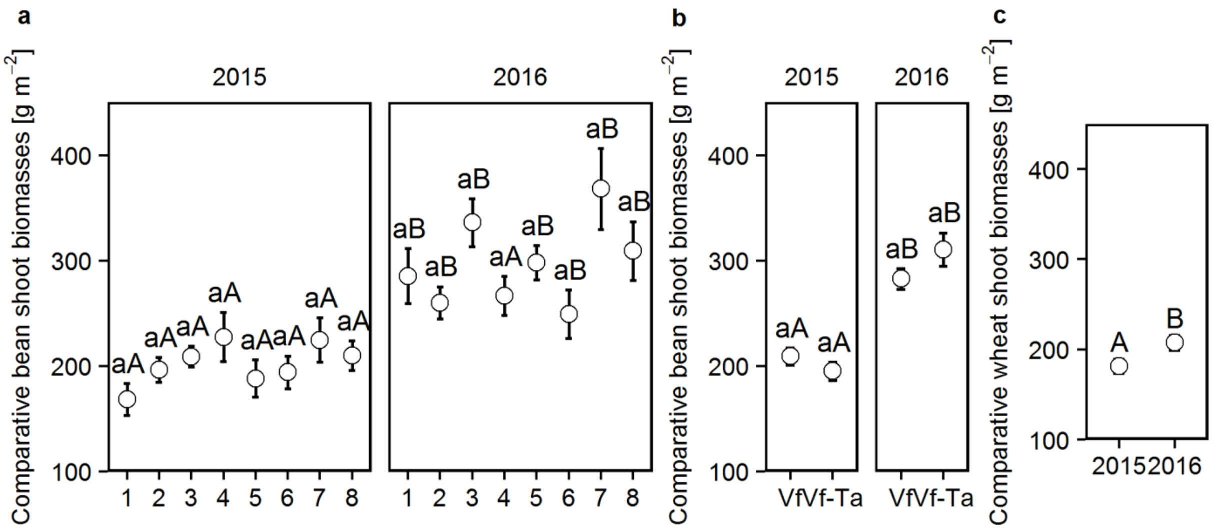

Figure 4 Mean comparative bean and wheat shoot biomasses per bean genotype and treatment for the years 2015 and 2016: (a) Comparative bean shoot biomasses for the eight bean genotypes (1-8) in both years averaged over treatments (n=8), (b) Comparative bean shoot biomasses for bean pure stand (Vf) and mixture (Vf-Ta) averaged over bean genotypes (n=32), (c) Comparative wheat shoot biomasses in both years averaged over treatment and genotype (n=48). In order to compare species specific biomasses of pure stands with mixtures, shoot biomasses of pure stands were divided by 2 to calculate comparative shoot masses. Given are means ± SE. Different lowercase letters indicate significant differences within one year (a) between genotypes and (b) between treatments. Different capital letters indicate significant differences between years (a) within the genotype (b) within treatment ($p \leq 0.05$, LSD post-hoc test).

Mixtures showed similar species specific root biomasses (bean 46.3 g m^{-2}, wheat 47.3 g m^{-2}). Comparative bean root biomasses were significantly affected by year, treatment, bean genotype and their interactions (Table 2, Fig. 5a). Comparative root biomasses of bean in pure stands and mixtures were higher in 2016 than in 2015. When averaged over both years, all eight mixtures exceeded the comparative bean root biomasses of pure stands. Comparative wheat root biomasses were affected by the factor WBGT and the interaction of year and WBGT (Table 2, Fig. 5b). The best performing, but not significant bean genotypes in pure stands with regard to comparative root biomasses were Vf2 in 2015 and Vf3 in 2016. Mixture Vf7-Ta showed the highest comparative bean root biomasses in 2015, V3-Ta in 2016. Comparative wheat root biomasses were the highest in mixture Vf4-Ta for 2015 and in Vf5-Ta for 2016 (Fig. 5b).

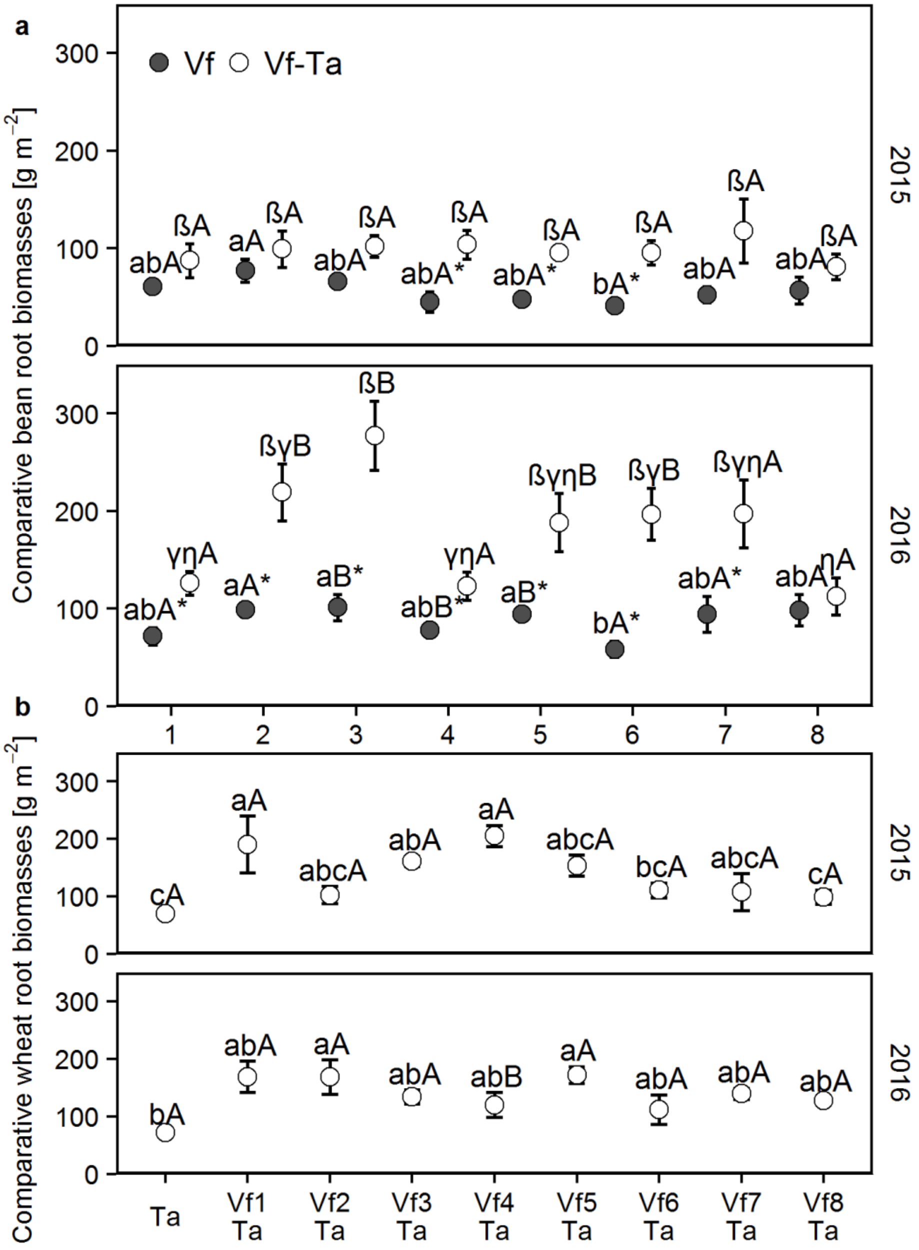

Figure 5 Mean comparative bean and wheat root biomasses per bean genotype and treatment for the years 2015 and 2016: (a) Comparative bean root biomasses of the eight bean genotypes (1-8) for the treatments pure stand (Vf, black) and mixed stands (Vf-Ta, white), (b) Comparative wheat root biomasses for pure stand of wheat (Ta, n=16) and the 8 mixtures (Vf1-8-Ta, n=4). In order to compare species specific biomasses of pure stands with mixtures, root biomasses of pure stands were divided by 2 to calculate comparative root masses. Given are means ± SE. Different lowercase letters indicate significant differences within one year (a) between genotypes in comparative bean root biomasses of pure stands and (b) between wheat pure stands and mixtures in comparative wheat root biomasses. (a) Different greek letters indicate significant differences within one year between genotypes in comparative bean root biomasses of mixed stands. Different capital letters indicate significant differences between years (a) within treatment and genotype and (b) within wheat pure stands and mixtures. Asterisks indicate significant treatment differences ($p \leq 0.05$, LSD post-hoc test).

2.4.4. Bean and wheat proportion in shoot and root biomass

The bean shoot proportion was only affected by bean genotype (Table 2). Bean had higher shoot proportions in both years than wheat (Table 5a). Mixture Vf7-Ta had the highest bean shoot proportion in 2015 and 2016. The mixture with the highest wheat shoot proportion averaged over both years was Vf1-Ta. The bean root proportion of bean/wheat mixtures was significantly affected by year, bean genotype and its interaction (Table 2, Table 5b). In 2015, wheat had higher root proportions than bean, whereas in 2016 bean exceeded wheat. Mixture Vf7-Ta had the highest bean root proportion in 2015, Vf3-Ta in 2016. The mixture with the highest wheat root proportion was Vf4-Ta in 2015 and Vf1-Ta in 2016.

Table 5 Species proportions (%) for (a) shoot biomass and (b) root biomass of bean and wheat in the eight mixtures (Vf1-8-Ta) and the average (Av.) over mixtures for 2015 and 2016 and the total biomass of wheat+bean (g m^{-2}). Given are means (n=4). Different lowercase letters indicate significant differences in species proportion between bean genotypes within one year. Capital letters indicate significant differences in species proportion between years within one genotype ($p \leq 0.05$, LSD post-hoc test).

a

	2015			2016		
	Bean	Wheat	Total Biomass	Bean	Wheat	Total Biomass
Vf1-Ta	44.34	55.66	375.4	52.18	47.82	528.0
Vf2-Ta	57.57	42.43	360.0	49.96	50.04	530.4
Vf3-Ta	58.12	41.88	380.8	63.99	36.01	565.5
Vf4-Ta	48.99	51.01	399.5	55.89	44.11	471.5
Vf5-Ta	50.04	49.96	358.9	59.31	40.69	540.7
Vf6-Ta	48.49	51.51	341.2	62.55	37.45	471.9
Vf7-Ta	60.07	39.93	390.1	70.61	29.39	581.7
Vf8-Ta	56.09	43.91	366.1	55.42	44.58	509.8
Av.	52.96	47.04	371.5	58.74	41.26	524.9

b

	2015			2016		
	Bean	Wheat	Total Biomass	Bean	Wheat	Total Biomass
Vf1-Ta	35.30 aA	64.70	92.6	43.83 abA	56.17	98.3
Vf2-Ta	48.64 aA	51.36	67.3	56.94 aA	43.06	129.3
Vf3-Ta	38.63 aA	61.37	87.8	66.69 abB	33.31	137.1
Vf4-Ta	33.64 aA	66.36	103.0	51.34 abB	48.66	81.1
Vf5-Ta	38.87 aA	61.13	82.9	51.24 abA	48.76	120.2
Vf6-Ta	46.34 aA	53.66	68.8	64.36 abA	35.64	102.9
Vf7-Ta	53.13 aA	46.87	75.1	57.49 abA	42.51	112.1
Vf8-Ta	44.68 aA	55.32	60.0	45.88 bA	54.12	80.1
Av.	42.40	57.60	79.7	54.72	45.28	107.7

2.4.5. Root:shoot ratio

Bean had, when averaged over sampling year and treatment, lower root:shoot (R:S) ratios (0.17) than wheat (0.22). According to LME, bean R:S ratio was affected by bean genotype, treatment and its interaction with year (Table 2). Mixed stands had on average 25 % higher bean R:S ratios than pure stands, ratios were slightly higher in 2016 than in 2015 (Table 6). Mixtures also showed higher wheat R:S ratios (0.26) than wheat pure stands (0.19), however within mixed stands wheat ratios were lower in 2016 than in 2015. Total R:S ratios of mixed stands (0.22) were only dependent on the accompanied bean genotype. Bean genotype Vf2 had the highest bean R:S ratio in pure stands in both sampling years (Av. 0.20). This genotype also had the highest mixture R:S ratio in 2016 (0.29). In 2015, the highest wheat R:S ratios were in mixture Vf4-Ta (0.38) and in 2016 in mixture Vf5-Ta and Vf7-Ta (0.29). Total R:S ratio was found to be the highest in mixture Vf4-Ta in 2015 (0.27) and Vf3-Ta in 2016 (0.25).

Table 6 Bean, wheat and total root:shoot ratio of bean pure stands (Vf, pure), wheat pure stands (Ta, pure) and mixed bean and wheat stands (mixed) of the eight bean genotypes (Vf1-8) and averaged over genotypes (Av.) of 2015 and 2016. Given are means (n=4). Different lowercase letters indicate significant differences between genotypes within one treatment and year. Different capital letters indicate significant differences between years within one treatment and genotype (p≤0.05, LSD post-hoc test)

	Bean				Wheat				Total	
	2015		2016		2015		2016		2015	2016
	pure	mixed	pure	mixed	pure	mixed	pure	mixed	mixed	mixed
Ta					0.19		0.19			
Vf1	0.18 abA	0.18 aA	0.13 aA	0.16 abA		0.30		0.23	0.25	0.19
Vf2	0.21 aA	0.16 aA	0.19 aA*	0.29 aA		0.23		0.23	0.18	0.24
Vf3	0.17 abcA	0.15 aA	0.17 aA*	0.26 aAB		0.35		0.22	0.23	0.25
Vf4	0.09 cA*	0.20 aA	0.15 aA	0.16 abA		0.38		0.20	0.27	0.17
Vf5	0.13 abcA*	0.18 aA	0.17 aA	0.20 abA		0.28		0.29	0.23	0.22
Vf6	0.10 bcA*	0.20 aA	0.15 aA*	0.23 aA		0.21		0.21	0.20	0.22
Vf7	0.13 abcA	0.19 aA	0.15 aA	0.19 abA		0.23		0.29	0.20	0.21
Vf8	0.14 abcA	0.14 aA	0.15 aA	0.13 bA		0.23		0.20	0.17	0.16
Av.	0.14	0.17	0.16	0.20	0.19	0.28	0.19	0.23	0.22	0.21

2.5. Discussion

2.5.1. FTIR spectroscopy for root species differentiation

The interspecific variation in the chemical composition of roots is the basis for chemometric infrared spectroscopy model development and the subsequent analysis of plant root composition (Naumann et al. 2010; Rewald and Meinen 2013; White et al. 2011). Several spectral peaks from our study were attributed to the presence of certain functional groups and chemical compounds: cellulose and hemicellulose at 1020 cm^{-1}, proteins (Amid I+II) at 1630 cm^{-1} and lipids at 2919 cm^{-1} (Naumann 2000). Cluster analysis showed a 100 % differentiation success between bean and wheat roots, which indicates that the chemical root composition of bean and wheat is species specific. This is in accordance to the findings of Naumann et al. (2010) and Rewald and Meinen (2013). FTIR spectroscopy was thus used to predict the species proportions in root mixtures of bean and wheat. Internal cross validation of the 16 bean/wheat FTIR mixture models revealed a high model quality (Diller 2002). There was no indication for an effect of genotype or year on model success. The quality of prediction was similar to FTIR models of maize/wild oat (Meinen and Rauber 2015) and faba bean/chamomile (Rewald and Meinen 2013). The short measuring time of less than 30 seconds per sample makes FTIR spectroscopy a promising high throughput tool for species determination.

2.5.2. Higher interspecific variation in shoot biomass than root biomass

Interspecific variation between bean and wheat in pure stands was more evident for shoot biomass than for root biomass. On average, wheat pure stands produced 22 % less shoot biomass than bean pure stands at the time of sampling. This might be due to a smaller biomass potential, the absence of nitrogen fertilization, or the shorter phenological development of wheat (BBCH 49-59, end of booting to end of heading) in comparison to faba bean (BBCH 65, full flowering). The faba bean shoot biomasses of 492 g m^{-2} at flowering are in line with studies from Germany (Hof and Schmidtke 2006; Menke 2011) and Italy (Pampana et al. 2016). The average shoot biomasses of wheat from booting to heading in our study was 384 g m^{-2}, which are smaller than reported values for unfertilized wheat treatments by Nielsen and Halvorson (1991).

Both species developed similar root biomasses in our study. Root growth of wheat normally exceeds that of grain legumes like faba bean (Gregory et al. 1995; Turpin et al. 2002). Our results can to some extent be explained by the species differences in root systems: Winter wheat forms a homorhizous, fibrous root system with a high number of thin lateral roots. Faba beans have an allorhizous, tap root system with secondary roots (Kutschera et al. 2009). These differences conclude that wheat likely produced a higher root number and area than faba bean but similar biomasses. Wheat root biomasses increase until flowering and significantly decrease towards maturity. Smaller root biomasses of wheat could therefore be attributed to a sampling time before full root development was achieved (Ehlers and Goss 2016; Muñoz-Romero et al. 2010; Schroetter et al. 2006). Menke (2011) found smaller root biomasses of winter cereals like rye and barley compared to winter faba bean. Roots are more variable in their biomass production than the shoot and they are strongly linked to phenology, soil properties, climatic conditions, sampling techniques and sampling depths. The faba bean root biomasses in our study of 71 g m^{-2} at bean flowering are smaller compared to studies from Menke (2011) and Rengasamy and Reid (1993), who reported average root biomasses over years and treatments of approximately 200 g m^{-2} and 140 g m^{-2}, respectively. These differences are due to the exclusion of taproots in our study. Literature revealed high variability for wheat root biomasses ranging from 39.4 g m^{-2} to 150 g m^{-2} at anthesis (Ehlers and Goss 2016; Zhang et al. 2004b).

Cereals are generally seen as strong competitors compared with legumes due to a bigger root system and deeper root distribution (Hauggaard-Nielsen et al. 2001b; Gregory et al. 1995; Gregory and Eastham 1996). Our root and shoot biomass results do not confirm this theory. Wheat showed no clear advantage over faba bean with regard to relative shoot or root yields. Furthermore, the proportion of wheat in the total shoot or root biomass was not higher than the proportion of bean. This indicates that there is no higher competition ability of wheat in comparison to faba bean when unfertilized.

2.5.3. Overyielding potential and change in biomass allocation

Our results indicate a significant but small increase in shoot biomass of wheat and faba bean when grown in mixtures at the time of bean full flowering in the year 2016 but not in 2015. Our first hypothesis was therefore partly confirmed. In 2016, bean produced on average 5 % and wheat on average 6 % more shoot biomass in mixtures than in pure stands. Previous studies demonstrated the aboveground mixture overyielding potential in diverse ecosystems (Cardinale et al. 2007) and a number of studies reported grain overyielding for bean/cereal mixtures (Knudsen et al. 2004; Li et al. 2001; Pristeri et al. 2006). Li et al. (2011) reported shoot biomass overyielding at earlier stages than at final harvest. A study by Hof and Schmidtke (2006) demonstrated a two percent average shoot biomass overyielding of winter faba bean/winter wheat mixtures during wheat anthesis. Furthermore, our results showed that roots of winter faba bean and winter wheat mixtures significantly overyielded in in both years in contrast to shoot biomass. Bean and wheat roots produced on average 38 % and 36 % more biomass in mixtures than in pure stands. A meta-analysis by Ma and Chen (2016) reported a positive effect of species diversity on the belowground productivity across terrestrial ecosystems like forests, planted grassland and croplands. Our findings of root overyielding in mixtures are well in line with several studies on root biomass in cropland mixtures: faba bean/maize (Li et al. 2006), faba bean/chickpea (Xia et al. 2013) and pea/barley (Corre-Hellou and Crozat 2005). The belowground species proportions in mixtures were balanced in this study. Wheat produced on average 49 % and bean 51 % of root biomass. This and observations during the washing process indicate intermingled root growth of both species with the potential of interspecific interaction. Researchers emphasize the importance of belowground interactions as a major driver for overyielding (Li et al. 2007; Wilson 1988; Zhang et al. 2004a). In a microplot experiment Li et al. (1999) reported a positive yield effect on maize when roots from maize/faba bean mixtures freely intermingled and were exposed to interspecific interactions but no effect when roots were separated by plastic sheets. Our observed RYT root values indicate that belowground facilitation in bean/wheat mixtures is more prominent than competition at the time of bean flowering. The aboveground RYT values indicate a change from a less complementary resource use and competition tendencies in 2015 to competition avoidance and facilitation in 2016. The study site was characterized by an above-average precipitation in May 2016 but not in 2015. A study by De Costa et al. (1997) showed that well-irrigated winter faba beans produced high aboveground biomasses. Higher biomasses and overyielding potential in 2016 than in 2015 are therefore likely caused by wetter conditions in May. Furthermore, it was shown that the sowing date has an impact on above- and belowground biomass accumulation of winter wheat (Barraclough and Leigh 1984; Gregory and Eastham 1996). The earlier sowing in growing season 2015/2016 (23 days) compared to growing season 2014/2015 and the resulting differences in accumulated thermal time (635.3 °C days, 466.0 °C days) may also have contributed to the higher biomasses in the second year.

A higher allocation of biomass towards roots in mixtures during vegetative stages could lead to increased water and nutrient uptake. This could enhance the development of leaf biomass and eventually increase the translocation of photosynthate assimilation products into the grain. Aboveground biomass overyielding and subsequent grain overyielding may therefore become visible at a later date, during the vegetation period. Nachi and Le Guen (1996) demonstrated a positive correlation between grain yield and total biomass accumulation for spring-sown faba bean during their growth period. The belowground overyielding potential of winter faba bean/winter wheat mixtures at the full flowering of bean could be a prerequisite for yield advantage. This assumption was confirmed by yield analyses in August 2015 and 2016 from the same project (subproject Plant breeding). It showed that winter faba bean/winter wheat had an average grain RYT of 1.1 (data not shown) and therefore demonstrated a yield advantage.

Wheat exceeded bean in its root:shoot ratio (R:S). R:S ratios of legumes normally exceed those of cereals when taproots are included (Gregory and Eastham 1996; Li et al. 2014). Studies by Crawford et al. (1997), Li et al. (2014) and Muñoz-Romero et al. (2011) reported higher ratios for faba bean than in our study (0.28 to 0.60). Smaller R:S ratios of bean than wheat found in our experiment may therefore be due to taproot exclusion. Mixtures showed higher R:S ratios compared to pure stands in our study. Root overyielding in both sampling years, together with the greater R:S ratios in mixtures, indicates a change in biomass allocation from above to belowground plant structures. This finding is in line with Li et al. (2014) and Yang et al. (2010), who demonstrated increased R:S ratios for wheat in mixtures compared to pure stands. In contrast, Mariotti et al. (2009) showed a R:S ratio decrease from pure stands towards mixtures for cereals. Faba bean had higher R:S ratios in 2016 than in 2015, which might be due to the earlier sowing in 2015/2016 and the interannual differences in weather conditions. The accumulated precipitation in March and April of 2016 was much lower than the long-term average for that area indicating drought stress during the phase of intense root growth. An increased R:S ratio with mild or moderate drought stress might improve the plant water status due to enhanced root tip production and water acquisition (Comas et al. 2013). The observed genotypic differences of winter faba bean in regard to R:S ratio supports our second hypothesis which predicted shoot and root differences between genotypes.

2.5.4. Effect of bean genotype on shoot and root biomass and overyielding potential

The eight novel winter faba bean genotypes in our study had a high pretested aboveground variability in winter hardiness, plant height, start of flowering, maturity and yielding potential in pure stands. At time of flowering, bean genotypes in pure stands had significantly different shoot biomasses. Comparative shoot biomass analysis showed that there was no significant difference in bean shoot biomass between pure stands and mixtures and that genotype had a smaller effect on the shoot biomass than year. The higher intraspecific variation in bean root biomass compared to bean shoot biomass, as observed in our study, is likely due to the development peak of roots at full flowering. Aboveground biomass peaks and aboveground differences among genotypes occur during later development stages. Nachi and Le Guen (1996) showed that the factor year had a stronger effect on the dry matter accumulation of spring faba beans at the start of flowering as opposed to the factor genotype. This finding is consistent with our observations, as differences between the two years in shoot biomass were greater than those due to the bean genotype. This suggests that aboveground genotype comparisons need to account for seasonal differences. The significant effect of the bean genotype x treatment interaction on total shoot and root biomass, comparative bean root biomasses and bean R:S ratio shows that the eight bean genotypes performed differently in pure stands and in mixtures. This concurs with Hauggaard-Nielsen and Jensen (2001), Francis et al. (1982) and Watiki et al. (1993) who reported an interaction between cultivar and cropping system. We found that certain winter faba bean genotypes had a high overyielding potential at bean full flowering. This trait might be advantageous for multi-species systems and could lead to a higher productivity. However, it might be worthwhile to investigate other yield forming factors, such as leaf area index, as well as genotypic yield differences in winter faba bean/winter wheat mixtures in order to understand the complex processes of these mixtures.

Our study found a high degree of plasticity among the bean genotypes in terms of belowground biomass and overyielding potential. Our second hypothesis was therefore partly confirmed. Studies by Khan et al. (2010) and Wasson et al. (2014) also demonstrated genotypic variation in root traits, such as rooting depth, total lateral root length, mass, number and root penetration rate. They emphasize the importance of root phenotyping for genotype selection within breeding programs.

When averaged over two sampling years there were a few genotypes with clear trait performances. Vf7 and Vf8 had the highest pure stand shoot biomass and Vf2 and Vf3 the highest pure stand root biomass. The ranking of the eight genotypes by their total overyielding potential and its consistency over both sampling years revealed the highest mixture suitability for Vf5 and the lowest for Vf8. Our third hypothesis, that the genetic variation of faba bean affects mixture productivity was therefore confirmed. The overyielding consistency of both genotypes could indicate a continuous above- and belowground advantage (Vf5) or disadvantage (Vf8) despite differing weather conditions. Genotype Vf5 might have the highest complementarity potential and therefore the best mixture relevance. The assessment of overyielding potential during vegetative stages might serve as a tentative estimate for a later grain overyielding. We assume that genotypes which show an enhanced root biomass in mixture might have an advantage in resource capture. Yield analyses in August 2015 and 2016 from the same project (sub project Plant breeding) showed that the superiority of mixture Vf5-Ta was reflected in a high and consistent grain overyielding (RYT, data not shown). Mixture Vf8-Ta demonstrated the lowest grain overyielding in both years. These results give indication that above- and belowground overyielding at vegetative stages indeed has an impact on the later yield advantage in bean/wheat mixtures.

2.6. Conclusions

Overall, the results of this research demonstrate the potential of Fourier transform infrared spectroscopy for the characterization of plant root proportions in mixed cropping systems. It can be used to gain a better understanding of the underlying belowground processes in plant mixtures. We observed genotypic differences in overyielding potential and consistency in the mixtures with more pronounced below- than aboveground overyielding. Bean genotypes with belowground overyielding potential could be advantageous for intercropping at nutrient poor and drought prone sites. Different intraspecific variation of winter faba bean in pure stands and mixtures indicate the need of specific winter faba bean breeding and cultivar selection for mixed cropping systems.

2.7. Acknowledgements

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Chapter 3

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Intercropping effects on root distribution of eight novel winter faba bean genotypes mixed with winter wheat

Juliane Streit, Catharina Meinen, Rolf Rauber

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3.1. Abstract

The spatial root distribution of plant species is generally altered by intra- and interspecific competition. The assessment of species specific root distribution in intercrops was limited so far because of the difficulties to identify roots on a species level. We investigated horizontal and vertical root distribution of eight winter faba bean genotypes (*Vicia faba* L.) and one winter wheat cultivar (*Triticum aestivum* L.) grown in sole stands and in 50/50 substitutive row intercrops.

Root samples were taken on and between rows with a root auger down to 60 cm soil depth in May 2015 and May 2016 at a field site in central Germany. We used Fourier transform infrared (FTIR) spectroscopy for root species identification. Vertical root distribution was described by the equation $y = 1 - \beta^d$ according to Gale and Grigal (1987).

Horizontal root distribution did not differ between bean and wheat and between sole stands and intercrops averaged across the eight bean genotypes: Bean and wheat root biomass was on average 65% lower between rows than on rows in sole stands and in intercrops. Both species proliferated into the soil space between the rows and into the intercropping partner's row to a similar extent. Bean developed 36% of its root biomass in 0-10 cm soil depth, while wheat had 51%. Bean and wheat had shallower roots on their own row in intercrops (β_{bean}=0.93; β_{wheat}=0.86) compared to their own row in sole stands (β_{bean}=0.94; β_{wheat}=0.90). In the intercrops both species occupied deeper soil layers on their partner's row (β_{bean}=0.95; β_{wheat}=0.96) compared to their own row (β_{bean}=0.93; β_{wheat}=0.86). This change in vertical root distribution was more evident for wheat than for bean. Bean genotypes grown in sole stands did not differ in their horizontal and vertical root distribution. However, there were significant differences between bean genotypes on-wheat rows in the intercrops: bean genotype Vf6 had the largest horizontal spread but the most shallow root growth on the wheat row, while Vf5 showed the lowest horizontal spread and the highest root fractions in deep soil layers on the wheat row. The alteration of the vertical root distribution of both species in intercrops, compared to the sole crops, might possibly lead to a better resource utilization and an intercrop advantage.

Keywords: *Vicia faba, Triticum aestivum,* Intercropping, FTIR, Vertical root distribution

3.2. Introduction

Faba bean serves as protein rich human food, as feed for animals and is grown for bioenergy and green manure purposes. In 2016, the highest faba bean yield production was in China, Ethiopia and Australia (FAOSTAT 2018) while Germany only used the area of 38,800 ha. Reasons for that are the low demand for faba bean as a basic food but also the susceptibility to fungal diseases and instable grain yields (FAOSTAT 2018; Jensen et al. 2010; Stoddard et al. 2010). Winter faba beans have the potential of higher yields and an increased weed suppression as opposed to their spring types (Haymes and Lee 1999; Link and Arbaoui 2005). Intercropping is defined as a type of agricultural system which cultivates two or more crop species or genotypes at the same time (Li et al. 2013; Vandermeer 1992). Its benefits include i.a. a more effective resources use, protection from diseases or weeds and increased yields compared to their sole stands (Ghosh et al. 2007; Rauber et al. 2001; Zhu et al. 2000). These positive effects of intercropping have already been demonstrated in faba bean/cereal intercrops (Bulson et al. 1997; Haymes and Lee 1999). The mentioned yield increment is known to be caused by above- and belowground interactions between intercropped species.

In the past, most intercrop studies focused on aboveground processes. Belowground traits are generally less studied, even though there are examples where root competition is more pronounced than shoot competition (Casper and Robert 1997; Wilson 1988). Roots are important for a plant’s anchorage, play an essential role in its water and nutrient uptake and therefore maintain carbon capture and transpiration (McElrone et al. 2013). Shoot biomass is generally correlated with root dry weight (Carton et al. 2018). Furthermore, interspecific root interactions were reported to enhance shoot biomasses (Li et al. 1999; Zhang et al. 2004a). In the last recent years, authors such as Koevoets et al. (2016) and Lynch (2007) emphasized the need for an intensified research of roots and their role as a yield forming factor.

Plant roots are influenced by various abiotic factors such as soil structure, water availability, nutrient gradients within the soil and biotic factors like intra- and interspecific competition. These factors are known to alter i.a. root distribution (Li et al. 2006; Zhang et al. 2004b). Nevertheless, there are only a few studies which compare the vertical and horizontal root distribution of different plant species of sole stands with those of intercrops (Gao et al. 2010; Li et al. 2006; Yang et al. 2010). This is mainly due to technical difficulties in root sampling and root discrimination of competing species. Most intercrop studies separate roots of the component species by their differing gross root morphology like root colour or texture (e.g. Li et al. 2006). This distinguishing process however, might be ambiguous and time consuming, especially for closely related species (e.g. grasses) and young roots. Infrared spectroscopy (IR) has long been used to identify and quantitatively determine unknown substances (Thomas and Greenstreet 1954). In the last decade, roots of different species in mixtures have been successfully distinguished by FTIR spectroscopy: pea-oat (Naumann et al. 2010), maize-barnyard grass (Meinen and Rauber 2015) and maize-sunflower (Dokken and Davis 2007). Studies on spatial root distribution gain insights in the complex belowground interaction processes. Intra- and interspecific competition and facilitation are important processes in intercropping systems especially belowground (Hauggaard-Nielsen and Jensen 2005). A plastic response to interspecific competition, like a change in root distribution, enables plants to use the soil volume more optimal. Subsequently, water and nutrient uptake as well as shoot performance are increased (Lynch 1995; Robinson et al. 1999; Shi et al. 2018). It was demonstrated in intercrop studies that a higher water uptake and shoot overyielding were accompanied by the lateral proliferation of maize roots into the row of the component species (Adiku et al. 2001; Li et al. 2006).

Root properties as target traits for breeding of drought-tolerant crops received increasing attention (Schoppach et al. 2014). The effect of genetic variation within one species (lines, cultivars) on root biomass has been reported for maize (Cai et al. 2012) and lupin (Carton et al. 2018). These studies however only focused on single plants or sole crop stands. The research question, whether the presence of interspecific competition by intercropping partners alters the genotype specific root response, has not been fully answered. Intercropping needs to be improved through selection of suitable cultivars or plant breeding programs. Therefore, it is important to know whether a significant variation of root growth exists between the genotypes. This information can only be obtained by a high-throughput quantification of root species in mixtures.

In the present study, we investigated the horizontal and vertical root distribution of eight winter faba bean genotypes (*Vicia faba* L.) and one winter wheat (*Triticum aestivum* L.) cultivar in sole stands and in intercrops. The hypotheses of the present study are:

(H1) the horizontal root distribution differs between faba bean and wheat and

(H2) the vertical root distribution differs between faba bean and wheat.

(H3) the horizontal root distribution differs between sole stands and intercrops and

(H4) the vertical root distribution differs between sole stands and intercrops.

(H5) the eight bean genotypes differ in horizontal root distribution in sole stands and intercrops and

(H6) the eight bean genotypes differ in vertical root distribution in sole stands and intercrops.

3.3. Materials and Methods

3.3.1. Study site and experimental design

The field trial was conducted at the experimental station Reinshof, Goettingen, Germany (51°29´N, 9°55´E, 160 m above sea level) in 2014-2015 and 2015-2016 as part of the IMPAC³ project (https://www.uni-goettingen.de/de/528191.html). The climate of the region is temperate with four distinct seasons. The mean annual temperature is 9.2 °C and the mean annual precipitation is 651 mm (1981-2010, DWD). Soil analyses in April 2016 classified the soil type as a Gleyic Fluvisol (WRB). Top soil contained 11% sand, 21% clay and 68% silt. Soil pH was 7.0 and bulk density was 1.56 g/cm³. The experimental field was cropped previously with rye. Eight winter faba bean genotypes (Vf1-Vf8; *Vicia faba* L.) and the winter wheat cultivar Genius (*Triticum aestivum* L.) were grown in three cropping systems: sole bean, sole wheat and intercropped bean and wheat. The eight winter faba bean genotypes were selected from the breeding program of the Department of Crop Sciences, University of Goettingen and NPZ (Norddeutsche Pflanzenzucht Hans-Georg-Lembke KG, Hohenlieth, Germany) because of their variability in plant height, maturity time or winter hardiness (Table 1). Genius was chosen as intercrop partner because of its medium height, high N-uptake capacity, low susceptibility to mildew and stable yields (Bundessortenamt 2017). The experiment was set up as a split-plot design with 4 replicates (blocks). Blocks were divided in eight mainplots where each mainplot consisted of 3 plots: one bean genotype in sole stand, in intercrop with wheat and one wheat sole stand. Intercrops were sown in a substitutive row intercropping design, where each species was sown at 50% of its sole stand density. Sowing densities were: 40 seeds/m² in sole bean, 320 seeds/m² in sole wheat, 20 bean seeds/m² and 160 wheat seeds/m² in intercrops. Row spacing was 22.5 cm in all treatments. An area of 1 m² at the northern end of the plot was chosen for root sampling (total plot size 10.5 m²). Sowing was conducted in October 2014 and 2015. Weeds were controlled manually and pests and diseases were treated with appropriate pesticides. Plots were neither irrigated nor fertilized.

Table 1 Winter faba bean genotypes included in the experiment with some known characteristics.

Code	Genotype	Characteristics
Vf1	S_004-1-6	Medium tall, Low tillering, Late flowering, Medium maturing, High yielding
Vf2	S_062-2-2	Very short, High tillering, Medium early flowering, Medium maturing
Vf3	S_069-1-1	Very tall, Medium tillering, Medium late flowering, Medium maturing, High yielding
Vf4	S_265-1-1	Very tall, Very high tillering, Medium early flowering, Medium maturing
Vf5	Hiverna/2-5-1	Medium tall, Low tillering, Medium early flowering, Low yielding, Pure line developed from Hiverna (German cv.), Superior winter hardiness
Vf6	Côte d'Or/1-1-3	Very tall, High tillering, Late flowering, Late maturing, Source of superior winter hardiness
Vf7	WAB-Fam157-1-2	Medium tall, Low tillering, Early flowering, Early maturing, High yielding
Vf8	WAB-EP98-267-11	Medium tall, Medium tillering, Late flowering, Late maturing, High yielding, Sibling of cv. Nordica

3.3.2. Root sampling

To ensure maximum root development of bean, root sampling was conducted at full flowering stage of bean (BBCH 65; Ehlers and Goss 2016) on 27-28/05/2015 and 23-26/05/2016. BBCH of wheat was 49 to 59, at the end of booting to end of heading. Soil coring was conducted using a cylindrical root auger (8.74 cm diameter, Eijkelkamp, Giesebeek, Netherlands) with integrated caulking hammer (Makita, type 1400 HM, Ratingen, Germany) down to 60 cm soil depth. Two cores were taken in sole stands (on rows, between rows) and three were taken in intercrops (on bean row, on wheat row, between rows). Soil cores were divided into 0-10, 10-20, 20-30, 30-40, 40-50 and 50-60 cm soil samples, air-dried for 72 h and stored in plastic bags. The area of each sample obtained by the root auger was 59.99 cm², the volume was 599.95 cm³. Samples of each soil layer were cleaned from soil residues and other organic matter by using a semi-automated root-washing machine (mesh size 1 mm). Tap roots and dead roots were excluded from further analysis. Root samples were ground in a centrifugal mill (0.12 mm, ZM 200, Retsch) and stored in glass vials.

3.3.3. FTIR analysis

In order to calculate root species proportions in intercrops, dried and ground root samples were further processed with Fourier transform infrared (FTIR) spectroscopy. Sole wheat and sole bean root samples were used to prepare a calibration curve consistent of 35 artificial calibration samples with known species proportions. The calibration curve ranged in bean and wheat proportions from 0 to 100%, with 3% steps, respectively. This calibration process was done for all eight bean genotypes and both years. Calibration samples were measured spectrometrically with a FTIR-ATR spectrometer (Alpha P, Bruker Optics, Ettlingen, Germany) with a resolution of 4 cm^{-1} (32 Scans) in the spectral range of 400-4500 cm^{-1} (5 replicates). Subsequently, FTIR models for each calibration were created using the software package OPUS QUANT 2 (Version 7.0, Bruker, 2011). FTIR models used a PLS1 algorithm. Models were improved automatically with the "Optimize" function of the software which tested several wavenumber ranges and mathematical pretreatment on the spectra (e.g. first derivative, vector normalization). Models with the highest residual prediction deviation (RPD), highest R^2 and lowest root mean square error of cross validation (RMSECV) were chosen (Table 2). An internal cross validation of three calibration samples and an external validation of 20 independent mixture samples were applied to evaluate model prediction reliability. FTIR spectra of intercrop samples were then recorded with the same settings as calibration samples.

Subsequently, FTIR models predicted the root species proportion of each intercrop sample. Bean and wheat proportion were multiplied with the total root biomass of each sample to obtain wheat and bean root biomass per area and soil layer (g/cm^2). Root biomass was summed up from 0 to 60 cm for each species (bean, wheat) and for each sampling position (on-bean, between, on-wheat) on plot level and expressed as accumulated bean or wheat root biomass per area (RBa, g/m^2).

Table 2 Statistical parameters of the Fourier transform infrared models in terms of calibration and internal cross validation. In order to predict the root species proportions in intercrops, 56 or 36 spectra (n) from composite ground root samples with known species proportions were recorded for each of the eight bean genotypes (Vf1-8) and both years 2015 and 2016 separately (_15,_16). Coefficient of determination (R²), root mean square error of estimation (RMSEE), root mean square error of internal cross validation (RMSECV) and residual predictive deviation (RPD) describe the model quality. Models with a high prediction quality are characterized by high RPD and low RMSECV values. See Streit et al. (2019) for detailed model characteristics.

		Calibration			Internal cross validation		
Model	n	R²	RMSEE	RPD	R²	RMSECV	RPD
Vf1_15	56	0.97	5.60	5.41	0.96	6.26	4.77
Vf2_15	35	0.97	5.45	5.69	0.96	6.39	4.73
Vf3_15	35	0.97	5.33	5.79	0.96	6.17	4.90
Vf4_15	35	0.99	3.07	9.96	0.99	3.52	8.51
Vf5_15	35	0.99	3.39	8.99	0.98	4.05	7.43
Vf6_15	35	0.97	5.12	5.94	0.96	6.15	4.84
Vf7_15	35	0.99	2.29	13.40	0.98	3.74	8.01
Vf8_15	35	0.99	3.07	9.94	0.99	3.56	8.40
Vf1_16	35	0.99	2.13	14.50	0.99	2.45	12.40
Vf2_16	35	0.97	5.18	5.85	0.96	6.01	4.99
Vf3_16	35	0.99	3.71	8.29	0.98	4.21	7.17
Vf4_16	35	1.00	2.10	14.50	0.99	2.49	12.00
Vf5_16	35	0.98	4.37	6.95	0.98	4.50	6.70
Vf6_16	35	0.98	4.65	6.58	0.91	8.95	3.36
Vf7_16	35	0.98	3.97	7.80	0.98	4.11	7.46
Vf8_16	35	0.98	4.06	7.50	0.97	4.77	6.32

3.3.4. Analysis of vertical root distribution

The vertical root distribution was described using the nonlinear function according to Gale and Grigal (1987):

$$y = 1 - \beta^{d}$$

where y is the cumulative root fraction (0-1) from the soil surface to the depth d of 60 cm, calculated on the basis of the root biomass (g/m²). Cumulative root fraction was multiplied by 100 and expressed in 0-100%. β is the estimated curve parameter which describes the shape of the vertical root distribution. High β values (e.g. 0.98) correspond to a large proportion of roots at deeper soil layers and low β values (e.g. 0.90) to large root proportions near the soil surface. According to Jackson et al. (1996), β values range between 0.91 and 0.98. Therefore, even small differences in this value can be linked to marked changes in vertical root distribution. The β values were calculated separately for wheat and bean and for each bean genotype, cropping system and position. Calculation of the β values and associated figure development was carried out by the software Xact (version 8.05f, SciLab, Hamburg, Germany).

3.3.5. Statistical analyses

Statistical analyses were performed using the open source software R (version 3.3.2., R Core Team 2016). The influence of fixed factors on target variables was examined using linear mixed effect models (LME, “nlme” package, Pinheiro et al. 2017). The target variables were accumulated root biomass for bean and wheat (RBa wheat, bean) and β values for bean and wheat. Replicate blocks with nested mainplots and nested plots were used as random factors. Depending on the model, fixed factors (factor levels) were year (2015, 2016), bean genotype (none, Vf1-8), cropping system (sole, intercrop), species (bean, wheat), and position (on-bean, between, on-wheat). In order to ensure orthogonal data sets, subsets were created (Table 3). For example, for the analysis of bean β values, sole stands of wheat had to be excluded. Target variables with a heavy skewed data distribution were treated with a Box-Cox power transformation (Box and Cox 1964). Appropriate variance structures (e.g. varIdent, varPower) were added to the model when model residuals did not show normal distribution and/or homogeneity of variance. Models with the best residual plot and the lowest second-order Akaike Information Criterion (AICc) were chosen. Afterwards, a pair-wise mean value post-hoc comparison of significant fixed effects was performed using the LSD test with a significance level of $p \leq 0.05$ (“lsmeans” package, Lenth 2016). Due to the high number of factors, not all significant interactions are shown. See Table 3 for detailed lme model information on F and p values for the different factors and interactions.

Table 3 Results of linear mixed effects models analyzing the traits accumulated root biomass (RBa) and vertical root distribution (ß values). In order to analyze orthogonal data sets, subsets were created for cropping system (sole bean, intercrop, sole wheat), sampling position (on-bean row, between rows, on-wheat row) and species (bean, wheat). Given are F and P values for the factors year, bean genotype, cropping system, position, species and their interactions. All traits were pretreated with a box-cox transformation. The variance structure varIdent was used in the models allowing for differing variances for each (*) bean genotype and (#) sampling position. Bold p values indicate significant factors and interactions at p≤0.05 (LSD post-hoc test).

Parameter	**Accumulated root biomass (Rba)**						**Vertical root distribution (β)**					
Cropping System	**Sole Bean Intercrop**		**Sole Wheat Intercrop**		**Intercrop**		**Sole Bean Intercrop**		**Sole Wheat Intercrop**		**Intercrop**	
Position	**on-bean between**		**on-wheat between**		**on-bean between on-wheat**		**on-bean between**		**on-wheat between**		**on-bean between on-wheat**	
Species	**Bean**		**Wheat**		**Bean, Wheat**		**Bean**		**Wheat**		**Bean, Wheat**	
	*****		*****		*****				*** #**		*****	
Factor	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Year (Y)	19.242	**0.0046**	0.909	0.3772	10.075	**0.0192**	0.460	0.5229	2.565	0.1604	0.127	0.7341
Bean Genotype (G)	4.247	**0.0013**	5.133	**0.0027**	3.455	**0.0052**	2.032	0.0734	1.399	0.2694	1.775	0.1180
Cropping system (CS)	34.372	**< 0.0001**					1.808	0.1851				
Position (P)	881.273	**< 0.0001**	831.561	**< 0.0001**	120.181	**< 0.0001**	73.665	**< 0.0001**	744.191	**< 0.0001**	165.069	**< 0.0001**
Species (S)					0.359	0.5496					38.970	**< 0.0001**
Y x G	0.840	0.5607	1.728	0.1674	2.168	0.0569	0.607	0.7470	2.557	0.0524	2.387	**0.0377**
Y x CS	0.001	0.9768					6.667	**0.0129**				
Y x P	0.072	0.7896	1.380	0.2437	1.344	0.2628	0.007	0.9356	5.452	**0.0221**	2.073	0.1276
Y x S					35.032	**< 0.0001**					0.203	0.6525
G x CS	1.894	0.0912					0.915	0.5033				
G x P	2.930	**0.0080**	7.665	**< 0.0001**	0.785	0.6849	0.683	0.6859	4.711	**0.0001**	1.744	**0.0465**
G x S					6.029	**< 0.0001**					1.856	0.0765
CS x P	49.119	**< 0.0001**					5.875	**0.0172**				
P x S					635.204	**< 0.0001**					238.937	**< 0.0001**
Y x G x CS	1.475	0.1989					0.764	0.6201				
Y x G x P	1.455	0.1927	2.225	**0.0341**	1.417	0.1455	1.045	0.4053	1.174	0.3253	4.089	**< 0.0001**
Y x G x S					2.587	**0.0137**					1.757	0.0955
Y x CS x P	4.733	**0.0320**					0.546	0.4616				
Y x P x S					0.938	0.3930					0.836	0.4345
G x CS x P	1.575	0.1520					0.771	0.6126				
G x P x S					2.795	**0.0007**						
Y x G x CS x P	1.099	0.3699					1.138	0.3464				
Y x G x P x S					1.655	0.0659					1.848	**0.0316**

3.4. Results

3.4.1. Horizontal distribution of accumulated root biomass

The accumulated root biomass from 0 to 60 cm soil depth (RBa, g/m²) of bean and wheat was affected by the factor position (Table 3). Both species showed a significant decrease of their RBa from on-rows to between rows in all cropping systems (av. -65%, Fig. 1). Sole stands of bean and wheat had similar RBa on their own rows (bean on-bean: 99 g/m², wheat on-wheat: 103 g/m²) and between the rows (bean between: 40 g/m², wheat between: 40 g/m²). In intercrops, roots of bean and wheat spread into the rows of their intercrop partner. The species proportions of bean and wheat between the rows in the intercrops were: 53% bean, 47% wheat. On their intercropping partner's row, both species showed similar proportions (on-bean row: 16% wheat, on-wheat row: 15% bean).

Bean RBa in sole stands and intercrops was furthermore affected by the main factor bean genotype and its interaction with position and the interaction of cropping system x position (Table 3). There was no significant interaction of bean genotype with cropping system and position (Fig. 1, Table 3). Root biomasses on-bean rows varied between 86.5 g/m² and 140.5 g/m² in sole stands (Fig. 1) and between 64 g/m² and 107 g/m² in intercrops. Bean RBa between rows ranged from 31 g/m² to 50 g/m² in sole stands and from 11 g/m² to 32.5 g/m² in intercrops. Bean RBa on-wheat rows in intercrops varied between 7 g/m² and 34 g/m². On average, bean RBa was a little higher in bean sole stands (98 g/m²) than in intercrops (82 g/m²).

Wheat RBa was significantly affected by bean genotype, position and the interaction of bean genotype x position (Table 3). There were no significant differences in wheat RBa between the intercrops on-bean rows (Fig. 1). The wheat RBa showed significant differences between sole stand of wheat and the eight intercrops between rows: The wheat RBa in wheat sole stands was significantly higher (40 g/m²) than in most of the intercrops (intercrop average 20 g/m²). However, between rows, wheat RBa of Vf1-Ta and Vf3-Ta was similar to wheat sole stand. Wheat RBa on-wheat rows was similar in sole stands (101 g/m²) and intercrops (average of 103 g/m²).

The RBa values of intercrops were affected by the factors position and bean genotype (Table 3). Bean and wheat roots dominated on their own row (Fig. 1). Bean RBa (23 g/m²) and wheat

RBa (20 g/m²) in intercrops were fairly similar between rows. On-wheat rows in intercrops, Vf5-Ta had the lowest (7 g/m²) and Vf6-Ta the highest (34 g/m²) bean RBa.

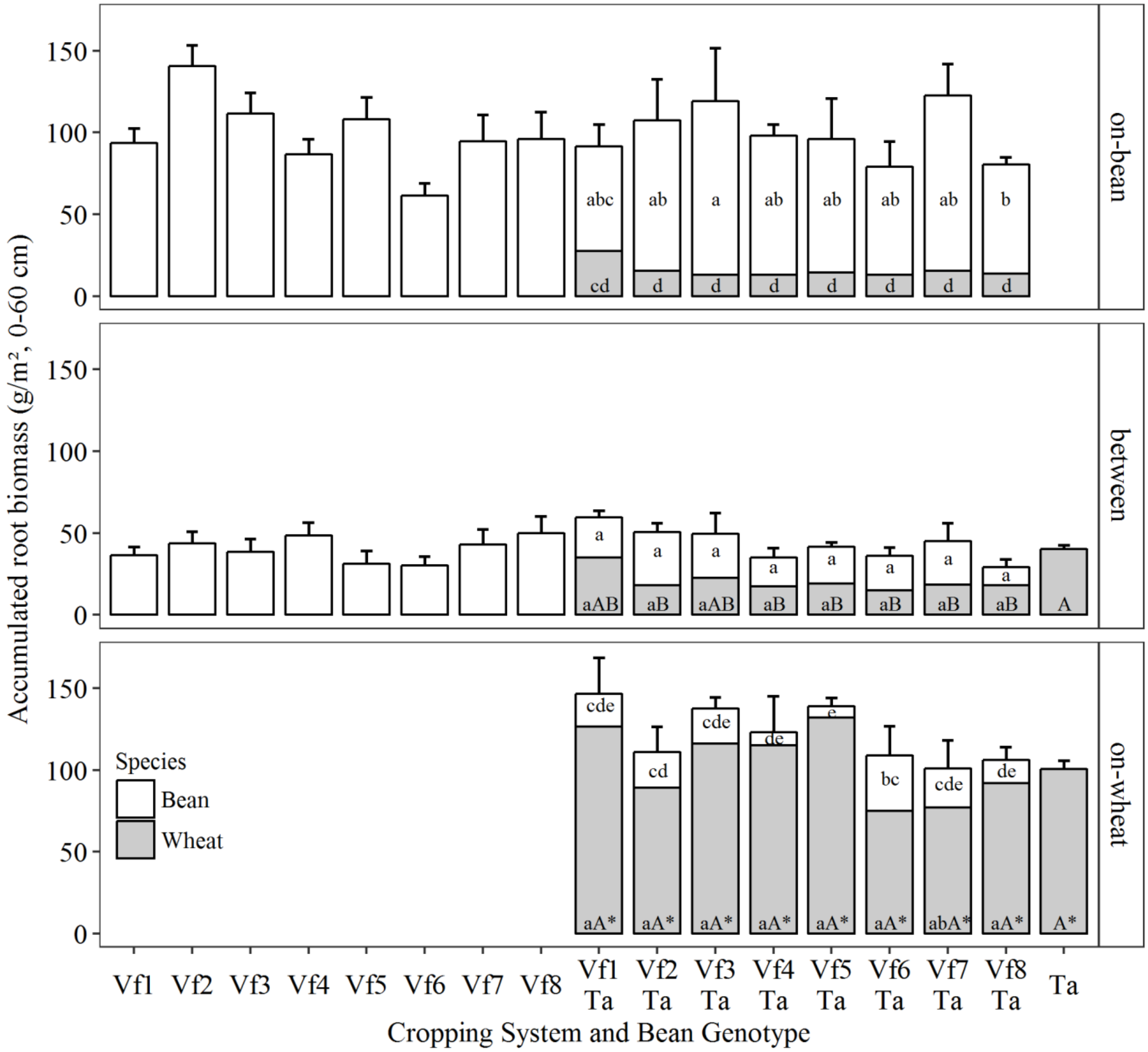


Figure 1 Accumulated root biomass from 0 to 60 cm soil depth (RBa, g/m²) for the species wheat (grey) and bean (white) of the eight bean genotypes in sole stands (Vf1-8), the eight intercrops (Vf1-8 Ta) and wheat sole stand (Ta) for the three sampling positions on-bean, between and on-wheat pooled over sampling years. Given are means + SE of the species total. Different lowercase letters indicate significant differences between species and intercrops within one position. Different uppercase letters indicate significant differences in wheat RBa between intercrops and wheat sole stand within one position. Asterisks indicate significant differences in wheat RBa in intercrops between the positions on-wheat and between ($p \leq 0.05$, LSD post hoc test). For statistical differences between the genotypes in species total accumulated root biomass pooled over sampling positions see Streit et al. 2019.

3.4.2. Vertical distribution of root biomass

The vertical decrease of root biomass, as indicated by β values, differed between species and cropping system and was affected by position (Table 3). The root biomass of bean and wheat decreased exponentially with increasing soil depth in sole stands and in intercrops (Fig. 2). When pooled over positions, sole stands of bean had lower root fractions (75%) in the topsoil (0-30 cm) than sole stands of wheat (81%). In 0-10 cm soil depth, sole stands of bean had on average 36% of their total root fraction, while wheat sole stands had on average 51% (data not shown). In particular, on-wheat rows of wheat sole stands about 68% of the total root biomass was found in the upper 10 cm of the soil, whereas it was only 33% between the rows. In bean sole stands, roots were distributed more equally: on the bean row 42% and between the rows 31% of total root biomass was found in 0-10 cm soil depth. In the upper 10 cm of intercrops, wheat and bean on their own row had higher root fractions (wheat 80%, bean 48%) compared to their sole stands (wheat 68%, bean 42%) (Fig. 2).

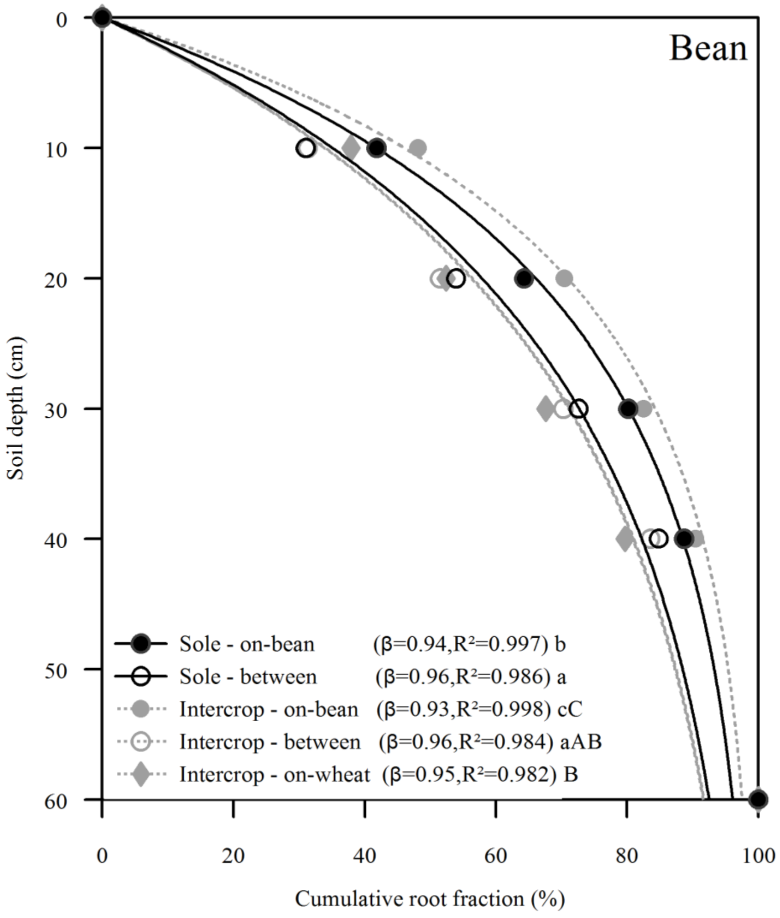
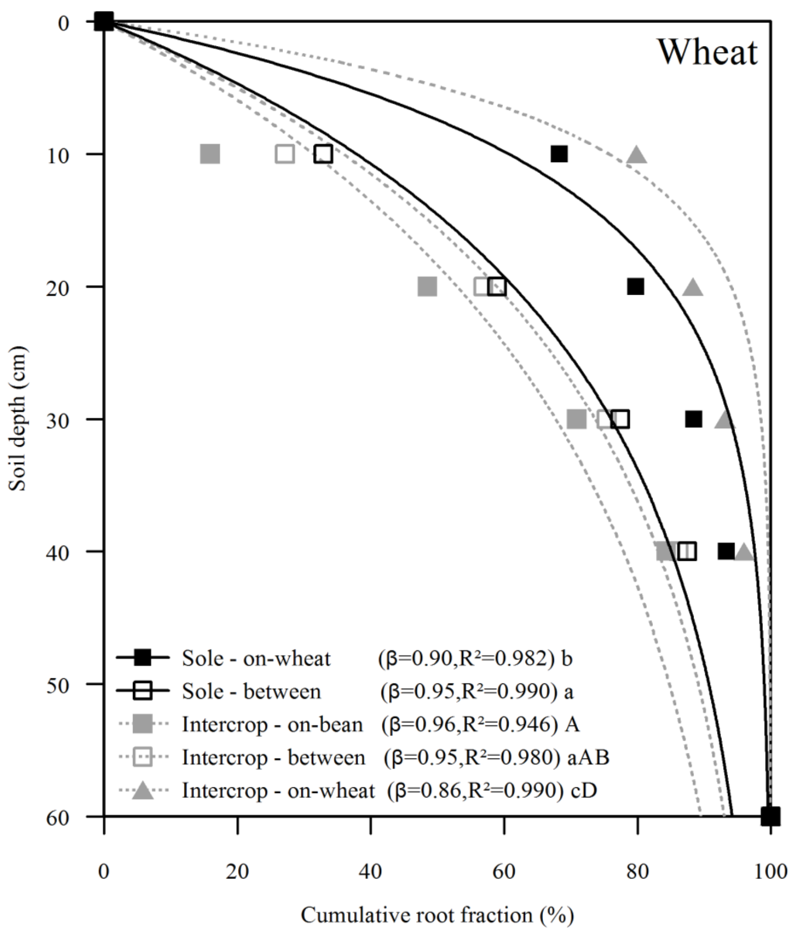


Figure 2 Change of cumulative bean and wheat root fraction (%) with soil depth (cm) per cropping system (sole stand, intercrop) and sampling position (on-bean, between, on-wheat). The curve parameter β was used as a measure of vertical root distribution ($y = 1 - \beta^{d}$ describes the change of cumulative root fraction *y* with soil depth *d* after Gale and Grigal 1987, R^2 is the regression coefficient). Given are means per cropping system, position and species pooled over bean genotypes and years (sole wheat n=32, sole bean n=64, intercrop n=64). Different lowercase letters indicate significant differences between cropping system and position for one species. Different uppercase letters indicate significant differences between bean and wheat and positions within the cropping system intercrop ($p \le 0.05$, LSD post hoc test).



Bean had on average higher β values than wheat and therefore higher root fractions in deeper soil layers than wheat (Fig. 2). The β value of bean on-bean row in intercrops (0.93) was significantly lower than the β value of bean on-bean row in sole stands (0.94) (Fig. 2). Wheat demonstrated a similar pattern: Wheat β values on-wheat row in intercrops (0.86) were significantly lower than wheat β values on-wheat row in sole stands (0.90). The change in β values was more pronounced for wheat (Δ 0.04) than for bean (Δ 0.01). Therefore, both species produced higher root fractions in shallower soil layers on their own row in intercrops compared to their own row in sole stands.

In intercrops on-wheat rows, bean developed slightly higher root fractions in deeper soil layers (β=0.95) than beans on-bean rows (β=0.94) in sole stands (Fig. 2). Sole stand wheat on-wheat row produced the majority of root biomass in shallower soil layers (β=0.90), but in intercrops wheat roots on-bean rows occupied deeper soil layers (β=0.96). The change in β value was more pronounced for wheat (Δ 0.06) than for bean (Δ 0.01). Therefore, on their intercropping partner's rows both species produced higher root fractions in deeper soil layers compared to their own row in sole stands. There was no significant difference between bean β values between rows in sole stands (0.96) and intercrops (0.96). The same applies to wheat between rows, where the β value was 0.95 in sole stands and in intercrops.

There were no significant differences of bean β values between the bean genotypes in sole stands (Table 3, Table 4). This was true for on-bean rows and between rows. Furthermore, there were no significant differences between bean genotypes in bean β values in intercrops on-bean rows and between rows. The vertical distribution of bean roots on-wheat rows in intercrops, however, differed between bean genotypes: On-wheat row in intercrops, genotype Vf5 had the highest bean β value (0.97) and therefore more roots in deeper soil layers than genotype Vf6 which had the lowest β value (0.92) (Table 3). Furthermore, there were significant differences in wheat β value between wheat sole stand and some of the eight intercrops on the wheat rows (Table 3): Wheat intercropped with bean genotype Vf4, Vf5 and Vf8 had significantly lower wheat β values (β=0.84, 0.85, 0.84) than sole wheat (β=0.90) on-wheat rows.

Table 4 β values of eight bean genotypes (Vf1-8) and wheat in the cropping systems sole bean, sole wheat and intercrop for the sampling positions on-bean, between and on-wheat pooled over sampling years. β describes the change of cumulative root fraction with depth, higher β values indicate that proportionally more roots are in deeper soil layers. Given are means. Different lowercase letters indicate significant differences between genotypes and sole stand of wheat (Ta) within one position ($p \leq 0.05$, LSD post-hoc test).

	Sole Bean		Intercrop						Sole Wheat	
	on-bean	between	on-bean		between		on-wheat		on-wheat	between
Genotype	Bean	Bean	Bean	Wheat	Bean	Wheat	Bean	Wheat	Wheat	Wheat
Ta	-	-	-	-	-	-	-	-	0.90 a	0.95 a
Vf1	0.94	0.94	0.93 a	0.95 a	0.96 a	0.96 a	0.93 cd	0.87 ab	-	-
Vf2	0.94	0.96	0.94 a	0.96 a	0.96 a	0.96 a	0.96 abc	0.87 ab	-	-
Vf3	0.94	0.96	0.95 a	0.96 a	0.95 a	0.95 a	0.94 bcd	0.86 ab	-	-
Vf4	0.95	0.96	0.93 a	0.96 a	0.96 a	0.95 a	0.96 abc	0.84 b	-	-
Vf5	0.95	0.96	0.92 a	0.96 a	0.96 a	0.95 a	0.97 a	0.85 b	-	-
Vf6	0.95	0.96	0.94 a	0.96 a	0.96 a	0.95 a	0.92 d	0.86 ab	-	-
Vf7	0.94	0.95	0.93 a	0.96 a	0.95 a	0.95 a	0.95 abcd	0.87 ab	-	-
Vf8	0.95	0.96	0.93 a	0.97 a	0.95 a	0.96 a	0.96 ab	0.84 b	-	-

3.5. Discussion

This study showed that FTIR spectroscopy can be used for the detailed analysis of horizontal and vertical root distribution of different species in intercrops. When using this method we were able to gain valuable knowledge about the root distribution of bean and wheat e.g. on the row of their intercropping partner. According to Diller (2002), all FTIR models of this study had a good prediction performance (RPD>3).

3.5.1. Spatial root distribution of bean and wheat

Our results show that roots of bean (genotype average) and wheat in intercrops and sole stands spread laterally from their own row into the space between the rows and into the intercrop partner row. The accumulated root biomass (RBa) decreased significantly from on-rows to between rows. Bean and wheat showed a similar rooting pattern. The decrease was present for both species for sole stands and intercrops. Our first and third hypothesis that horizontal root distribution differs between species (H1) as well as between sole stands and intercrops (H3) cannot be confirmed. Our results are in agreement with a previous finding of Manschadi et al. (1997) who reported an average decrease of 28% of faba bean root length density (RLD) from center cores to adjacent cores. Amato and Pardo (1994) also showed a marked decline of wheat and faba bean roots from on-row to between rows. According to Casper and Robert (1997), the competitive ability of species depends on their occupation of soil space by root growth, its morphological and physiological plasticity and its spatiotemporal soil partitioning. Studies by Corre-Hellou et al. (2007) and Li et al. (2006) found cereals like maize and barley to be dominating in root competition. Contrasting to their results, we observed that both, wheat and bean, had a similar root proliferation into the row of the intercropped partner. This indicates that winter faba bean and winter wheat had a comparable belowground competitive ability.

The root biomass of both faba bean and wheat decreased exponentially with soil depth and their change of cumulative root fraction with depth was described with β values according to Gale and Grigal (1987). The calculated curves were a good fit for the change of cumulative root fraction with depth as the regression coefficients were high for all treatments ($R^2>0.945$). Plant roots generally have an exponential decrease of their biomass with soil depth and the vertical root distributions found in our experiment reflect the results of several studies, e.g. the global meta-analysis by Jackson et al. (1996). There were interspecific differences in the vertical root distribution of faba bean and wheat grown in sole stands, which confirms our second hypothesis (H2): Wheat produced more roots near the soil surface as around 51% of its root biomass was found in 0-10 cm soil depth. Bean on the other hand had only around 36% of its cumulative root biomass in 0-10 cm. Together with the higher β values of bean compared to wheat, the conclusion arises that wheat has a shallower root system than faba bean. This, however, is contradictory to several studies which reported shallower root distribution of faba bean compared to cereals (Li et al. 2006; Manschadi et al. 1998; Xia et al. 2013). Differing results to our study probably originate from the inclusion of the tap roots in the analysis of the mentioned articles. Wheat produced around 81% and bean around 75% of its root biomass in 0-30 cm soil depth. In accordance to our study is a meta-analysis by Jackson et al. (1996), who reported average β values of 0.96 for field crops (e.g. pea, soy beans, wheat). In the particular study, 70% of the field crop root biomass was located in the upper 30 cm. Temperate grasslands had lower average β values (0.94) than field crops and had 83% of their total biomass in the upper 30 cm.

The present study showed that the vertical root distribution of bean and wheat differs between sole stands and intercrops. Our fourth hypothesis (H4) was therefore confirmed. The overlap of neighbouring root systems and intermingled root growth of intercropped faba bean and wheat in this study has necessarily led to interspecific belowground interactions. Root habitats of the intercrop partners overlap and competition and/or facilitation processes take place. Geno and Geno (2001) and Vandermeer (1992) concluded that in intercropping systems both competition and facilitation occur and are sometimes present at the same time. Competition for nutrients and water in intercrops happen when plant species absorb resources from the same soil layers.

Both species on their own row produced more roots near the soil surface in intercrops than in sole stands, as indicated by β values. When roots of wheat proliferate the soil space under the bean row in intercrops, bean increased root biomass in the upper soil layers. The same pattern was also found for wheat. These results suggest that interspecific competition might decrease in top soil. Simultaneously, intraspecific competition might increase in the upper soil layers. Shallower roots on their own row in intercrops possibly might be advantageous for a better nutrient and water acquisition from the topsoil (Khan et al. 2010). Similar changes in root distribution like in our results have been reported by Li et al. (2006), who found higher faba bean root proportions present in the upper soil layers when intercropped with maize. In addition, Tosti and Thorup-Kristensen (2010) observed a shallower rooting depth of faba bean in interspecific mixture with beet root compared to single plant stands.

This study furthermore showed that bean and wheat had higher root fractions in deeper soil layers on their intercrop partner's row compared to their own row in sole stands. Bean and wheat naturally dominate the upper soil layers on their own row. When bean or wheat proliferate the soil space under the intercropping partner's row, they occupy deeper soil layers. Our result is consistent with a study by Hauggaard-Nielsen et al. (2001), who demonstrated higher root fractions of pea and barley below a soil depth of 12.5 cm in intercrops compared to their sole stands. The authors argue that deeper roots in intercrops lead to a more complete exploitation of the soil profile and an improved nutrient acquisition. We assume that the increased root growth in deeper soil layers on the intercropping partner's row might mitigate interspecific competition. Facilitative processes, such as a transfer of N from bean to the wheat plants, may also have occurred in the present experiment (Xiao et al. 2004). This transfer could lead to an increased root growth of wheat compared to the sole stands.

In total, the comparison of sole stands and intercrops showed a simultaneous relocation of roots to shallower soil layers on the own row and to deeper soil layers on the partner's row. However, the changes in vertical root distribution were more pronounced for wheat than for bean, in terms of β values, which indicates a stronger plastic response of wheat to interspecific competition. Ho et al. (2005) observed, that common bean genotypes which allocate roots to shallower soil depths are better adapted to low phosphorus environments. At the same time, the authors showed that genotypes that have higher root fractions in deeper soil layers are better adapted to drought. We assume that intercrops in the present study might be better equipped to absorb topsoil nutrients and rainwater and at the same time acquire more water from deeper soil layers. These patterns might contribute to an intercrop advantage. Streit et al. (2019) showed that both faba bean and wheat developed significant higher root biomasses in intercrops compared to their sole crops. The possible yield advantage of an intercrop can be analyzed by calculating the relative yield total (RYT) from the relative yields of each species (de Wit 1960; de Wit and van den Bergh 1965). A RYT value higher than 1 indicates overyielding in intercrops. Streit et al. (2019) observed that the relative root yields of bean (0.69) and wheat (0.68) were almost identical. Both species therefore contributed equally to the belowground intercropping advantage (RYT>1). Together with the RYT>1, our results suggest that winter faba bean/winter wheat intercrops could possibly have a better resource utilization than sole stands. Interspecific competition might have been mitigated through the ‘avoidance strategy’ of both species. Zhang and Li (2003) stated that RYT values greater than 1 either result from strong facilitation or from low competition between the species. A symmetric belowground facilitation was shown to produce an overyielding in both intercropping partners (Li et al. 2006). A more efficient resource utilization might lead to an increased shoot performance of the intercrops compared to their sole stands (Lynch 1995; Robinson et al. 1999; Shi et al. 2018). For the present study, detailed analyses of plant-soil interactions are needed in order to draw conclusions about possible occurring belowground facilitation processes. With our method we were not able to detect from which row roots originated. The β values of e.g. wheat on-wheat rows in sole stands reflect not only the vertical root distribution of the sampling row. They rather comprise the combination of roots from the sampling row and roots from adjacent rows which extended into the sampling row.

3.5.2. Intraspecific variation in root distribution

In the present study we detected intraspecific differences in horizontal and vertical root distribution between intercrops but not between bean sole stands. This indicates that the investigated eight genotypes of this study have a similar spatial root distribution when growing in sole stands.

The detected intraspecific differences in bean RBa on-wheat rows in intercrops indicate differences in horizontal spread and competitive ability between the eight investigated bean genotypes. In particular, bean genotype Vf6 had the largest horizontal spread into wheat rows in intercrops, genotype Vf5 the lowest. Furthermore, there were differences between the eight intercrops in the vertical root distribution of bean on-wheat rows. For instance, genotype Vf6 had the shallowest bean root distribution on-wheat rows in intercrops, genotype Vf5 the deepest. Interestingly, Vf6 produced higher root fractions in deeper soil layers on-bean rows in intercrops than Vf5. Competition for soil space and nutrients in intercrops is presumptively much higher on-rows than between the rows. Nevertheless, the vertical distribution of bean roots on-wheat rows depends on the horizontal spread of the bean roots from their own row. Some genotypes produced more RBa on-wheat rows than others. The differences in vertical root distribution between genotype Vf5 and Vf6 support and complete our observations of the horizontal distribution of these two species. Genotype Vf6 seems to have a stronger competitive ability than Vf5, as Vf6 had the largest horizontal spread on-wheat rows and produced more roots near the soil surface than Vf5.

Our results indicate differences in competitive ability between the bean genotypes in intercrops. However, we were not able to detect intraspecific differences in horizontal and vertical root distribution between the bean genotypes in sole stands. Therefore, we conclude that hypotheses H5 and H6 were only confirmed for the intercrops. Even though authors such as Belachew et al. (2018) and Khan et al. (2010) found an extensive variation in rooting depth between faba bean accessions, there are no studies on the genotypic variation in horizontal and vertical root distribution between faba beans on the field level. Furthermore, none of the mentioned studies did investigate genotype differences in intercrops. Studies addressing root distribution for other legumes in sole stands are however present: e.g. Armstrong et al. (1994) found differences in the vertical root distribution between six field pea genotypes.

It was shown that the improvement of plant performance under unfavourable conditions such as water shortage or nutrient deficiencies can be caused by alterations of the root system architecture (Gruber et al. 2013; Khan et al. 2010; Uga et al. 2013). Furthermore, Belachew et al. (2018) found large differences in the root response of faba bean genotypes to water deficit. It was already shown that root growth is closely connected to shoot traits and yield (Belachew et al. 2018; Xia et al. 2013). In intercrops, bean genotypes Vf5 and Vf6 showed the most promising root distribution on their own row and on-wheat rows. Intercrops with Vf5 and Vf6 might have an advantage in water and nutrient acquisition during periods of drought or nutrient deficiencies. Therefore, we consider genotypes like Vf5 and Vf6 as candidates for an improved bean-wheat intercropping system.

3.6. Conclusions

The use of FTIR spectroscopy in this study allowed a detailed analysis of the horizontal and vertical root distribution of intermingled roots of two plant species. Compared to sole stands we saw that in the presence of an intercropping partner (1) both winter faba bean and winter wheat increased their root biomass in shallower soil layers on their own row and that (2) both species had higher root fractions in deeper soil layers on their partner’s row. We conclude that this plastic response to interspecific competition might lead to an enhanced water and nutrient utilization in both the shallower and the deeper parts of the soil. We further conclude, that this change in vertical root distribution might allow for an intercropping advantage, especially under conditions of drought and nutrient deficiency. Finally, we detected differences in vertical root distribution between the bean genotypes in intercrops but not in bean sole stands. We therefore conclude that possible advantages in bean/wheat intercrops depend on the genotype of the legume.

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Chapter 4

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Root biomass and belowground overyielding of *Trifolium repens* L. and *Lolium perenne* L. in pure stands and mixtures

Juliane Streit, Catharina Meinen, Rolf Rauber

Manuscript Draft

4.1. Abstract

Clover/grass mixtures are of common practice in central Europe as their herbage yields usually exceed that of their pure stands. Aboveground overyielding of multi-species mixtures might be mirrored belowground. The analysis of root species proportions in mixtures has been limited so far by the lack of a simple and time efficient method. Furthermore, little is known of the genotypic variation of white clover in root biomass and the white clover root proportion in clover/grass mixtures.

A substitutive field experiment with eight novel white clover genotypes (*Trifolium repens* L.) and one perennial ryegrass genotype (*Lolium perenne* L.) was established as (i) unfertilized clover pure stand of each clover genotype, (ii) unfertilized ryegrass pure stand, (iii) N-fertilized ryegrass pure stand and (iv) unfertilized mixture of each clover genotype with ryegrass. Relative seeding densities were 0.4 for clover and 0.6 for ryegrass. The study assessed the root biomass from 0 to 60 cm soil depth in June 2015. Clover and ryegrass root proportion in mixtures was determined via Fourier transform infrared (FTIR) spectroscopy. Belowground relative yield total (RYT) was calculated for each mixture.

In pure stands as well as in mixtures, clover produced significantly lower root biomasses than ryegrass. Clover root biomass in pure stands differed significantly between genotypes. In pure stands clover root biomass ranged between 67-179 g m^{-2}, in mixtures between 37-88 g m^{-2}. Fertilization had no effect on the root biomass of ryegrass. All eight clover/grass mixtures reached RYT values higher than 1 indicating a relative root overyielding. This was mainly caused by the high relative root yields of ryegrass (0.9) compared to clover (0.6).

We assume that the belowground overyielding in the present study has resulted from niche complementarity and improved resource utilization. Clover genotype differences in root overyielding in mixtures might affect nutrient acquisition and aboveground productivity. We suggest that white clover breeding should include analyses on root traits such as belowground overyielding potential or rooting depth.

4.2. Introduction

White clover (*Trifolium repens* L.) is one of the most important legume grassland species in temperate regions (Frame et al. 1998). It is commonly grown together with non-legume species such as perennial ryegrass (*Lolium perenne* L.) due to mixture benefits. The inclusion of legumes increases the productivity of unfertilized grassland systems, enhances the fodders digestibility and protein content (Nyfeler et al. 2009; Sleugh et al. 2000). Besides higher dry-matter yields, legume/grass mixtures improve the yield stability of grasslands (Ergon et al. 2016). Other mixture benefits include increased carbon sequestration, reduced soil erosion and weed suppression (Halty et al. 2017; Picasso et al. 2008).

Overyielding of legume/grass mixtures can be attributed to facilitative processes such as the increase of soil fertility by legume atmospheric nitrogen (N_2) fixation (Rasmussen et al. 2012). However, one of the most stated underlying mechanisms behind mixture overyielding is the complementarity between species in resource capture. Competition is reduced by complementarity which leads to a better exploitation of resources. Vertical complementarity in soil water uptake and root distribution enhances resource acquisition resulting in a higher mixture productivity (Hernandez and Picon-Cochard 2016; Wardle and Peltzer 2003). It is unknown to which extent root traits contribute to the mixing performance of clover/ryegrass mixtures. Aboveground overyielding might be mirrored belowground.

Belowground complementarity in grasslands usually arises from differences in vertical root distribution and the inclusion of deep rooted species. White clover and perennial ryegrass differ in their root system architecture and root biomass. White clover has a less extensive rooting system than ryegrass (Lucero et al. 1999). Due to the presence of a tap root, clover has a higher extraction ability of water from deeper soil layers than ryegrass (Grieu et al. 2001). Studies on the effect of plant diversity on root biomass in grassland systems show contradictory results. Some studies show a clear increase of belowground biomass with higher species richness (Ma and Chen 2016; Mommer et al. 2010), while others show no effect (Gastine et al. 2003) or a negative effect of diversity on root biomass (Bessler et al. 2009). The question, if clover/grass mixtures overyield belowground and therefore have an increased nutrient acquisition is of great agronomic importance.

Nitrogen (N) fertilization is known to enhance aboveground biomass, including the herbage yield of grassland systems (Nyfeler et al. 2009; Nevens and Rehuel 2003). Even though N fertilization has a higher impact on aboveground parts of the plant than belowground, various studies have demonstrated the positive effect of N fertilization on root growth (Fageria and Moreira 2011). Nyfeler et al. (2009) showed that the herbage production of legume/grass mixtures is similar to pure stands with high fertilization treatments. This pattern might be also found belowground. We want to investigate, if the inclusion of clover has the same effect on root biomass as a fertilizer input.

Even though most of the biomass in grasslands is allocated belowground rather than aboveground, diversity related studies on belowground productivity are scarce (Poorter et al. 2012). One of the reasons for the lack of studies is the difficulty to investigate roots in the field and to determine roots according to species. Determination methods which are based on manual sorting (Wardle and Peltzer 2003), ^{13}C discrimination (Gealy and Fischer 2010) or DNA (Riley et al. 2010) are often difficult and time consuming. Root discrimination and root mass quantification via Fourier Transform Infrared (FTIR) Spectroscopy offers a new way to study root systems of diverse plant mixtures (Meinen and Rauber 2015; Naumann et al. 2010). Spectroscopy for the analysis of the botanical composition of grassland root mixtures has been applied in the form of near-infrared reflectance spectroscopy (NIRS) (Roumet et al. 2006; Rumbaugh et al. 1988). To our knowledge, FTIR spectroscopy has not been used so far for root mass quantification of grassland mixtures.

The effect of cultivar on root systems has been studied for several crop species, e.g. wheat (Hodgkinson et al. 2017) and rice (Uga et al. 2013). Even though researchers still investigate plant traits and processes that are responsible for mixture benefits, testing and breeding for new cultivar is generally performed in pure stands. Prieto et al. (2015) emphasized that in grassland breeding programs taxonomic as well as the genetic diversity have to be considered. Differences in root morphology, architecture and depth between genotypes generally play an important role in the acquisition of water and nutrients. Positive relationships exist between aboveground biomass, deep-root biomass (Mueller et al. 2013) and root number/root volume (Troughton 1963). The few studies which exist on differences in root biomass between white clover genotypes are often not field based (Caradus 1981).

In the present study we investigated the cumulative root biomass from 0 to 60 cm soil depth of eight novel white clover genotypes (*Trifolium repens* L.) and one perennial ryegrass genotype (*Lolium perenne* L.) as pure stands and mixtures. Root biomass of clover, ryegrass, clover/grass mixtures and one fertilized ryegrass treatment was assessed in June 2015 in a man-made grassland field experiment in central Germany (Goettingen). The hypotheses of the following study were:

(H1) N-fertilization has a positive effect on the root biomass of ryegrass,

(H2) Clover has a positive effect on the root growth of ryegrass in mixtures,

(H3) Clover/ryegrass mixtures overyield in regard to root biomass,

(H4) Clover genotypes differ in their root biomass and root overyielding.

4.3. Materials and Methods

4.3.1. Experimental site and design

The field experiment was conducted at the experimental station ‘Reinshof’ of the University of Goettingen, Germany (51°29´N, 9°55´E, 160 m above sea level) and was established in May 2014. The experiment was situated on a Gleyic Fluvisol, which contained 21% clay, 68% silt and 11% sand (0-30 cm soil depth). The pH was neutral with 7.0 (0.01 M $CaCl_2$). The long-term average annual temperature and precipitation of the site were 9.2°C and 651 mm (Deutscher Wetterdienst, 1981-2010). The preceding crop was rye. Eight novel white clover genotypes (*Trifolium repens* L.) and one perennial ryegrass genotype (*Lolium perenne* L.) were selected from the portfolio of the Deutsche Saatveredelung (DSV, Lippstadt, Germany, Table 1). The following four crop stands were established: (i) unfertilized white clover pure stands (Tr1-8), (ii) unfertilized ryegrass pure stand (Lp-N0), (iii) N-fertilized ryegrass pure stand (Lp-N1) and (iv) binary substitutive mixtures of white clover (40%) and unfertilized ryegrass (60%) (Tr1-8/Lp-N0). See Table 1 for detailed crop stand description and seeding densities. The fertilizer treatment included a total of 240 kg ha^{-1} calcium ammonium nitrate (NH_4NO_3 + $CaCO_3$ * $MgCO_3$) per year which was applied in March and right after aboveground dry matter harvests in May, July and August 2015 with differing amounts (Table 1). No fertilizer was applied in 2014 in all four crop stands. The experiment was conducted as a split plot design with four replicates/blocks. Each block comprised eight mainplots. Mainplots consisted of randomized plots of one clover genotype in pure stand and in mixture. The other crop stands were arranged randomized within the block. Total plot size was 5 x 1.5 m. Each plot comprised eight seeding rows with 18.6 cm row distance. Plot borders were treated with herbicides (a.i. glyphosate) to prevent the spread of clover into adjacent plots. Weeds in the plots were controlled manually. Poisoned wheat against field mice and slug pellets have been applied.

Table 1 Crop stands, dates and amounts of fertilizer application (kg N ha^{-1}) and genotype codes of the species white clover (Tr) and perennial ryegrass (Lp) of the present study. White clover was present with 8 genotypes and perennial ryegrass with one genotype. Perennial ryegrass was either fertilized (N1, 240 kg N ha^{-1} a^{-1}) or unfertilized (N0). White clover in pure stands and mixtures was not fertilized. White clover and perennial ryegrass seed material was provided by the Deutsche Saatveredelung (DSV, Lippstadt, Germany).

Crop stand code	**Crop stand description**	**Seeding density (seeds m^{-2})**	**Number of plots per block**
Tr1-8	Pure stand clover 1 to 8 unfertilized	1000	8
Lp-N0	Pure stand ryegrass unfertilized	1000	2
Lp-N1	Pure stand ryegrass fertilized	1000	2
Tr1-8/Lp-N0	Mixture clover 1 to 8/ryegrass unfertilized	Clover: 400 Ryegrass: 600	8

Fertilizer application dates and amounts (kg N ha^{-1}) for Lp-N1

12/03/2015 (80); 15/05/2015 (60); 10/07/2015 (60); 26/08/2015 (40)

White clover		**Perennial ryegrass**	
Tr1	EGB PX 90305	Lp	Elp 060687
Tr2	EGB PX 90312		
Tr3	EGB PX 90702		
Tr4	EGB PX 90710		
Tr5	EGB PX 90913		
Tr6	EGB PX 90914		
Tr7	EGB PX 90915		
Tr8	EGB PX 90909		

4.3.2. Root sampling

Roots were sampled from 29/06/2015 to 02/07/2015. Two root samples per plot were taken with a root auger down to 60 cm soil depth (8.9 cm diameter, Eijkelkamp, Giesebeek, Netherlands). Samples were taken on the seeding row and between the rows. Soil samples were air dried for 72 hours and stored in plastic bags until root washing. The roots were cleaned by removing the soil and other organic residues by using a semi-automated root washing machine (mesh size 1 mm) and tweezers. Tap roots and lateral roots were not separated for the analysis. The extracted roots were dried for 72 h at 55°C and weighed for dry matter determination. Roots were then ground in a centrifugal mill (0.12 mm, ZM 200, Retsch, Haan, Germany) and stored in glass vials.

4.3.3. FTIR quantification models

Spectral analysis was done using an Alpha-P Fourier Transform Infrared Spectrometer (FTIR) with an integrated platinum attenuated total reflection (ATR) diamond (Bruker Optics, Ettlingen, Germany). Quantification models were developed to determine the species specific root proportions in binary mixtures of clover1-8/ryegrass (Tr1-8/Lp). Eight two-species models for each clover genotype in mixture with ryegrass were developed (Table 2). Each two-species model was calibrated with 35 artificial root mixtures in 3.5% steps from 0% to 100% of clover and ryegrass, respectively. Each calibration sample had a total weight of 10 mg. Five small root powder subsamples of each calibration sample were placed on top of the ATR diamond and recorded with the FTIR device. Spectra were recorded with the wavenumber range of 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1} and 32 scans. Model development was conducted with the software OPUS QUANT 2 (Version 7.0 Bruker, 2011). An internal cross-validation of three calibration samples was carried out to describe the predictive power of the model. A standard ‘Optimize’ function of the software was used to find the best wavenumber range and mathematical pretreatments of the spectra for each model. Models were chosen based on a high residual predictive deviation (RPD) and a low root mean square error of internal cross validation (RMSECV). Additionally, an independent external validation with known species proportions was carried out. External validation comprised 20 samples for the two-species models (Table 2). Five subsamples of each mixture samples were measured with the same settings as the calibration samples. Models were then applied on each mixture spectra to determine the species-specific root proportions. Root proportions of each species were averaged over the five subsamples and the sample positions. Subsequently, root proportions were multiplied with the total root biomass of the sample. Data was expressed as clover and ryegrass root biomass (0-60 cm, g m^{-2}).

Table 2 Statistical parameters of Fourier transform infrared models for the analysis of root species proportions in the white clover/perennial ryegrass mixtures (Tr/Lp) of the eight different clover genotypes (Tr1-8). Spectra of 35 calibration samples and 20 external validation samples of dried and ground roots with known species proportion were recorded five times between 400 and 4500 cm^{-1}. Coefficient of determination (R^2), root mean square error of estimation (RMSEE), root mean square error of internal cross validation (RMSECV) and residual predictive deviation (RPD) are estimates for model quality. Models with a RPD > 3 and low RMSECV values are considered to have a satisfactory predictive quality.

	Calibration			**Internal cross validation**			**External validation**	
Model	R^2	RMSEE	RPD	R^2	RMSECV	RPD	RMSEP	RPD
Tr1/Lp	0.993	2.62	11.90	0.988	3.29	9.17	5.61	4.94
Tr2/Lp	0.991	2.95	10.30	0.985	3.67	8.13	5.16	5.27
Tr3/Lp	0.991	3.02	10.30	0.984	3.82	7.91	6.84	4.19
Tr4/Lp	0.989	3.32	9.35	0.984	3.87	7.91	4.84	5.83
Tr5/Lp	0.985	3.70	8.28	0.982	4.07	7.36	5.60	4.55
Tr6/Lp	0.985	3.64	8.26	0.980	4.19	7.09	5.68	4.81
Tr7/Lp	0.991	2.80	10.80	0.988	3.22	9.21	5.56	4.82
Tr8/Lp	0.986	3.68	8.39	0.974	4.92	6.15	6.52	3.97

4.3.4. Relative yield total

The relative yield total (RYT) is an index to evaluate yield advantage of a mixture compared with pure stands. If the RYT is greater than 1.0 the mixture has a yield advantage over the pure stand. RYT is often calculated for grain yields or shoot biomass data (Davidson and Robson 1990; Rauber et al. 2001) but can also be applied to belowground root biomass (Mommer et al. 2010). In our experiment RYT was calculated for root biomass from 0 to 60 cm soil depth. According to de Wit and van den Bergh (1965), RYT is defined as the sum of the relative yields of the mixture components (RY_{clover}, $RY_{ryegrass}$) and expressed as follows:

$$RYT = RY_{clover} + RY_{ryegrass} = M_{clover}/P_{clover} + M_{ryegrass}/P_{ryegrass}$$

where RY_{clover} and $RY_{ryegrass}$ are the yields per area in mixture (M_{clover}, $M_{ryegrass}$) divided by their yield per area in pure stand (P_{clover}, $P_{ryegrass}$) for clover and ryegrass, respectively. RY and RYT were calculated for each for the eight clover/ryegrass mixtures. For this, the root biomasses of the two unfertilized ryegrass pure stands plots were averaged. If the RY of clover or ryegrass is higher than their particular relative seeding density (clover 0.4, ryegrass 0.6, Table 1), overyielding occurred. For this, we use the term „relative root overyielding".

4.3.5. Statistical analyses

Statistical analyses were performed with the software R 3.3.2. (R Core Team 2016). Seven models were set up in order to analyze the effect of (M1) species on the mean FTIR spectra absorbance, (M2) crop stand on the species total root biomass (clover genotype average), (M3) crop stand on the clover root biomass (clover genotype average), (M4) crop stand on the ryegrass root biomass, (M5) crop stand and clover genotype on the clover root biomass, (M6) clover genotype and species on the relative yield and (M7) clover genotype on the relative yield total. For M1 the absorbance differences between clover and ryegrass of mean FTIR curves (n=40, Fig. 1) were analyzed with a linear model and subsequent anova ($p \leq 0.05$).

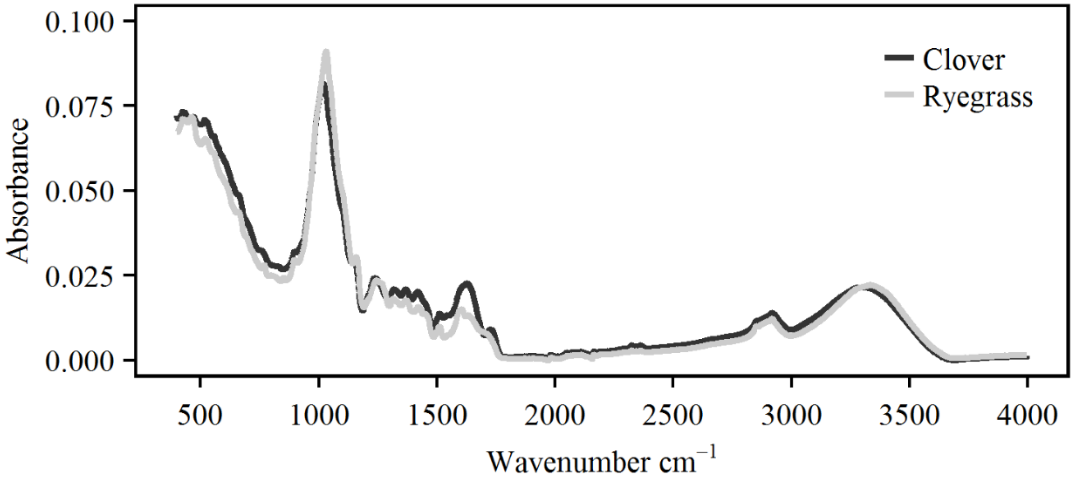

Figure 1 Mean Fourier transform infrared spectra of dried and ground roots of the species white clover (dark grey, n=40) and perennial ryegrass (light grey, n=40) grown in pure stands. Mean spectra were vector-normalized and offset-corrected.

The other models were linear mixed effect models (lme, ‘nlme’ package, Pinheiro et al. 2017) with subsequent anovas ($p \leq 0.05$). For M2 and M3 we first averaged the eight clover genotypes and summed up the species specific root biomasses. For M2, M3, M4 and block and mainplot were added as random factor, where mainplot was nested in block. For M5, M6 and M7 block was added as random factor. The variance structure varIdent was added to the models to account for variance heterogeneity between the crop stands (M2, M4) (Table 3). A pairwise LSD test was used to discriminate differences between the means of the factor levels at $p \leq 0.05$ except for M7 and M5 where we applied $p \leq 0.1$ (LSD test, ‘lsmeans’ package, Lenth 2016). Additionally, we tested the significant differences of the RYT values against the value 1.0 for each mixture and the mixture average with linear models and a subsequent anova ($p \leq 0.05$).

4.4. Results

4.4.1. Spectral root patterns

The spectra of dried and ground roots of clover and ryegrass in pure stands showed a similar peak distribution between 800 and 1200 cm^{-1} and between 1800 and 2800 cm^{-1} (Fig. 1). The mean absorbance significantly differed between the species ($p \leq 0.05$, Table 3). The absorbance of species differed most markedly at a wavenumber of 1620 cm^{-1}. White clover had a significantly higher mean absorbance (0.017) than ryegrass (0.016). The highest absorbance value of the two species was found in perennial ryegrass with 0.91 at 1032 cm^{-1}.

4.4.2. Crop stand differences in root biomass

The root biomass of the species total was significantly affected by the crop stand (Table 3, Fig. 2). Clover pure stand (Tr) had the lowest root biomass with on average 121 g m^{-2} (Fig. 2). The crop stands mixture (Tr/Lp-N0) and ryegrass unfertilized (Lp-N0) showed roughly the same total root biomasses with 348 g m^{-2}. Even though there was no significant effect of fertilization on the root biomass of ryegrass, fertilized ryegrass (Lp-N1) demonstrated a slightly higher root biomass (394 g m^{-2}) than Tr/Lp-N0 and Lp-N0. In Tr/Lp-N0, clover represented 16% (54 g m^{-2}) and ryegrass 84% (294 g m^{-2}) of the total root biomass. Clover root biomass was significantly higher in Tr than in Tr/Lp-N0. Ryegrass biomass was slightly higher in Lp-N0 than in Tr/Lp-N0. Lp-N1 had significantly higher root biomasses than ryegrass in Tr/Lp-N0.

Table 3 Summary analysis of variance with results of a linear model analyzing the effect of species on mean Fourier transform infrared absorbance (M1) and mixed effects models (M2-7) analyzing the effect of crop stand, clover genotype, species and their interactions on total, clover and ryegrass root biomass, on relative yield and relative yield total. The variance structure varIdent was implemented in the model, allowing for differing variances for crop stands (M2, M4). Bold p-values indicate significant factors and interactions at $p \leq 0.05$.

	Mean Absorbance M1		Root biomass total M2		Root biomass clover (Genotype Average) M3		Root biomass ryegrass M4		Root biomass clover M5		Relative yield M6		Relative yield total M7	
Factor	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Crop Stand (C)			88.818	**< 0.001**	32.645	**< 0.001**	6.924	**0.0081**	39.941	**< 0.001**				
Clover genotype (G)									1.772	0.1166	1.861	0.0988	2.371	0.0594
Species (S)	4.961	**0.0260**									7.293	**0.0097**		
C x G									2.113	0.0614				
G x S											1.028	0.4252		

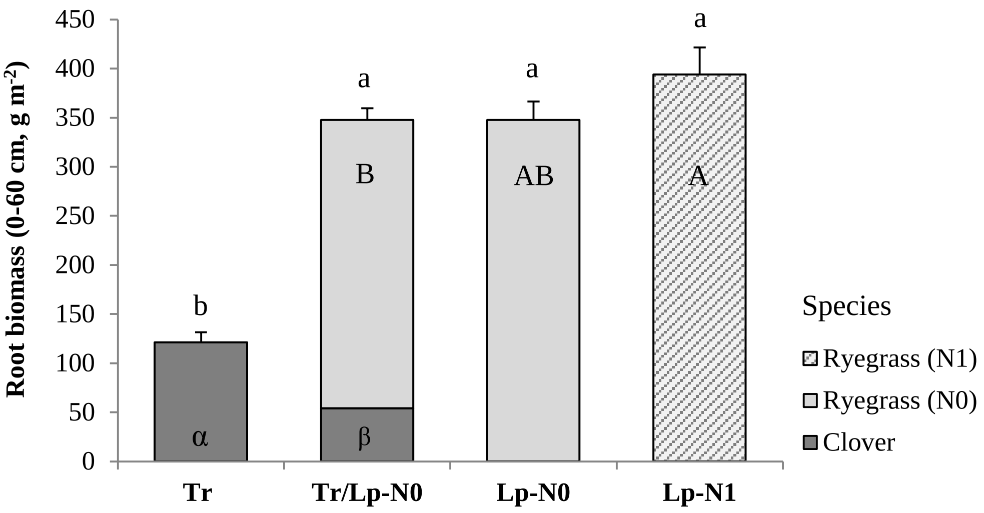

Figure 2 Root biomass from 0 to 60 cm soil depth (g/m²) of the species white clover (Tr, dark grey) and perennial ryegrass (Lp, light grey) for the crop stands white clover pure stand (Tr, n=32), white clover/perennial ryegrass mixture (Tr/Lp-N0, n=32) and perennial ryegrass pure stand without fertilizer (Lp-N0, n=8) and with fertilizer (Lp-N1, n=8). The eight white clover genotypes were pooled. Given are means + SE total. Differences between the crop stands total in root biomass are marked by different lowercase letters, differences between the crop stands in ryegrass root biomass by capital letters and differences between the crop stands in clover root biomass by greek letters ($p \leq 0.05$, LSD post-hoc test).

4.4.3. Effect of genotype on white clover root biomass

Crop stand had a significant effect on the root biomass of clover (Table 3, Fig. 3). Additionally, there was a significant crop stand x genotype interaction ($p \leq 0.1$). Clover genotypes Tr1, Tr2, Tr4, Tr5, Tr7 and Tr8 had significantly lower root biomasses in mixture than in pure stands (Fig. 3). Root biomass significantly differed between the clover genotypes in pure stands but not in mixture. The range of clover root biomass in pure stands was between 67 g m^{-2} (genotype Tr6) and 179 g m^{-2} (genotype Tr7). In mixtures, clover root biomass ranged between 37 g m^{-2} (Tr1/Lp-N0) and 89 g m^{-2} (Tr6/Lp-N0). All clover genotypes, except for Tr6, produced lower root biomasses in mixtures as compared to their pure stands (Fig. 3). Genotypes Tr3, Tr4, Tr5 and Tr7 had a similar performance, in terms of root biomass, in pure stands and in mixtures. For instance, Tr3 had the second lowest root biomass in pure stand as well as in mixture. In contrast, Tr1 demonstrated the second highest root biomass in pure stand, but the second lowest in mixture. Furthermore, Tr6 had the lowest root biomass in pure stand and the highest in mixture.

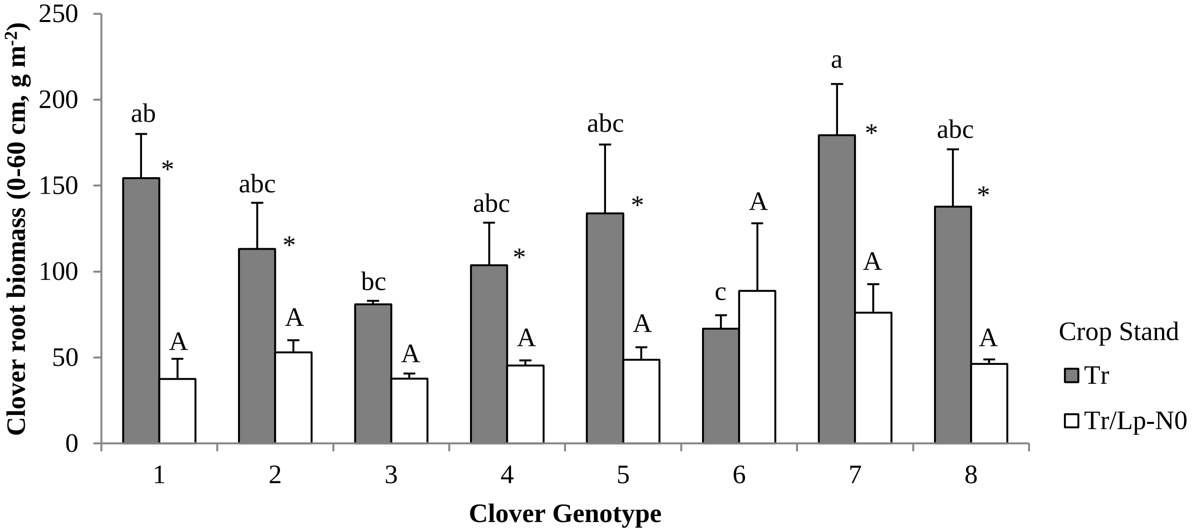

Figure 3 Differences in root biomass from 0 to 60 cm soil depth (g/m²) among eight white clover genotypes (1-8) grown as white clover pure stand (Tr, dark gray) and two species mixture with perennial ryegrass (Tr/Lp-N0, white). Given are means + SE (n=4). Significant differences between the genotypes within the crop stand clover pure stand are indicated by lowercase letters and differences within the crop stand mixture by uppercase letters. Asterisks indicate significant differences between the crop stands within a genotype ($p \leq 0.1$, LSD post-hoc test).

4.4.4. Relative yield and Relative yield total

The relative yield (RY) was significantly affected by the main factor species (Table 3). The main effect of the factor species showed that the average relative yield of clover (0.6) was significantly lower than of ryegrass (0.9) (Fig. 4). The variation within the RY was higher within clover than ryegrass. All mixtures exceeded the RYT value of 1.0. Five out of eight mixtures and the average, were significantly higher than 1.0 (Fig. 4). The average RYT value of all the mixtures amounted to 1.4 (Fig. 4). There was a significant effect of clover genotype on RYT ($p \leq 0.1$) (Table 3). The highest RYT value was found in Tr6/Lp-N0 (2.38) (Fig. 4). The lowest RYT values could be observed in mixtures with Tr1, Tr3 and Tr8 (RYT = 1.0, 1.2, 1.2, respectively).

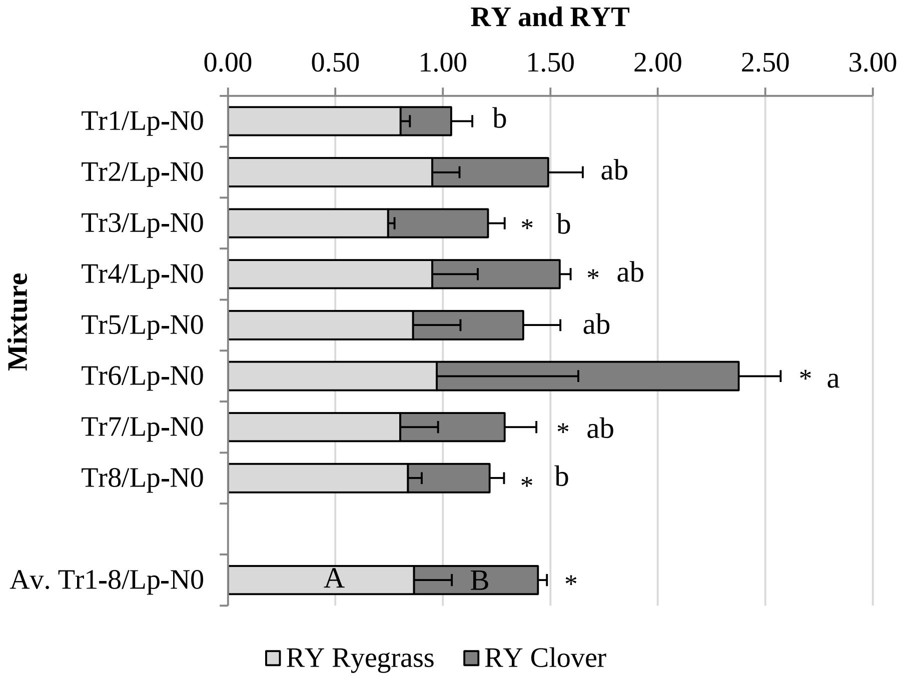

Figure 4 Belowground relative yield (RY) and belowground relative yield total (RYT) of the eight white clover/perennial ryegrass (Tr1-8/Lp-N0) mixtures and the average (Av.). The belowground RYT is the sum of the RY of ryegrass (light grey) and clover (dark grey). RYT higher than 1.0 indicate a relative root biomass increment in the mixtures. Given are means + species SE (n=4). Asterisks indicate a RYT significantly >1.0 ($p \leq 0.05$, LSD post-hoc test). Different lowercase letters indicate significant differences in RYT among the mixtures ($p \leq 0.1$, LSD post-hoc test). Different uppercase letters indicate significant differences between ryegrass and clover in the average RY ($p \leq 0.05$, LSD post-hoc test).

4.5. Discussion

4.5.1. FTIR spectroscopy for root species quantification in grassland mixtures

In the present study, clover and ryegrass roots showed clear FTIR absorbance differences. The external validation of the FTIR models presented a satisfactory predictive quality according to Diller (2002) and Meinen and Rauber (2015): the predictive error of determination (RMSEP) did not exceed 6.9% and the residual predictive deviation was higher than 3.96. As far as we know, the present experiment is the first study which verified the suitability of FTIR spectroscopy for root species quantification in clover/grass mixtures.

4.5.2. Differences in root biomass between clover and ryegrass

Our study showed clear differences in root biomass between white clover and unfertilized perennial ryegrass. This was true for pure stands as well as for mixtures. Averaged over the eight genotypes, clover root biomass was significantly lower than ryegrass root biomass in pure stands. These results are consistent with several studies, which observed smaller root biomasses of white clover pure stands than ryegrass pure stands (Lucero et al. 1999; Vinther 2006).

Root biomass values of unfertilized ryegrass (348 g m^{-2}, 0-60 cm) and fertilized ryegrass (394 g m^{-2}, 0-60 cm) in the present study are considerably lower than in an experiment of Menke (2011). The mentioned study was conducted at the same experimental site as ours and reported 100-130 g m^{-2} root biomass of ryegrass in December and 450-650 g m^{-2} in May (30 kg N ha^{-1}, 0-60 cm). Higher root biomasses compared to our values in the study of Menke (2011) might be related to Menke‘s two times higher seed density.

In the present study, the average root biomass of clover pure stand amounted to 121 g m^{-2} (0-60 cm). Vinther (2006) observed white clover root biomasses (0-20 cm) of 20-50 g m^{-2}. A study by Louarn et al. (2015) reported white clover root biomass of 13 g m^{-2} (0-20 cm). Root biomasses of white clover reached 62 g m^{-2} in 0-10 cm in an experiment of van Eekeren et al. (2009). The comparison of the present results with literature shows that research on root properties should be more standardized. Root biomass does not decline linearly with soil depth, an extrapolation of root biomass of the latter studies to 0-60 cm is therefore not possible.

4.5.3. Effect of N fertilization on root biomass of ryegrass

The present study shows that N fertilization had no significant effect on the root biomass of ryegrass pure stands, our first hypothesis (H1) was therefore rejected. According to the functional equilibrium theory, plants tend to allocate more biomass to the roots when nutrients are limited in order to enhance nutrient uptake (Brouwer 1962). Our results and several other studies do not support this theory (Davidson and Robson 1986; Sainju et al. 2017; van Eekeren et al. 2009). Some experiments, in contrast, demonstrated a positive correlation between N fertilization and ryegrass root biomass (Fageria and Moreira 2011; Murphy and Zaurov 1994). An application of higher N fertilizer amounts in the present study might result in an increase of root biomass. Furthermore, our results showed that the root biomass of the fertilized ryegrass was similar to the total root biomass of mixtures. Nyfeler et al. (2009) showed that legume/grass mixtures can achieve similar aboveground dry matter yields as pure stands of grass with high fertilizer application. This pattern was confirmed by Davidson and Robson (1986) for root biomasses. The authors observed higher total root biomasses in clover/grass mixtures compared to fertilized ryegrass treatments. This was not confirmed in our study. Nevertheless, ryegrass in mixed stands produced similar root biomasses compared to unfertilized pure stands despite a relative seeding density of only 0.6. This shows the positive effect of clover on the root biomass of ryegrass and therefore confirms the second hypothesis (H2).

4.5.4. Root biomass in clover/ryegrass mixtures

The presence of a mixture partner resulted in a decrease in absolute clover root biomass. Even though there was a slight decrease in ryegrass root biomass from pure stand to mixture, ryegrass root biomass was not significantly affected. In mixtures, ryegrass roots dominated and contributed 84% to the total root biomass, while clover contributed only 16%. The total mixture root biomass of 348 g m^{-2} in the present study at 0-60 cm soil depth is presumably lower than values observed for 0-10 cm soil depth by van Eekeren et al. (2009), who found a root biomass of a white clover/ryegrass mixture of 193 g m^{-2}. Skinner et al. (2006) reported total root biomass for a white clover/orchardgrass mixture of 254 g m^{-2} (0-60 cm).

In a pot experiment, Ren et al. (2017) stated that interspecific root competition led to an increase in the relative root yield of ryegrass but a decrease in clover relative root yield. The authors concluded that the competitive ability of ryegrass and clover in mixtures strongly depends on interspecific root interactions. In the present study, relative seeding densities were 0.4 for clover and 0.6 for ryegrass. The average relative yield (RY) of clover in our study was 0.6 and ryegrass RY was 0.9. Therefore, both species overyielded relatively but ryegrass contributed much more to the root biomass advantage of mixtures than clover. Davidson and Robson (1986) also observed higher relative root yields of ryegrass than of clover in mixtures. By analyzing RYT, the present study clearly showed a relative root overyielding of the clover/ryegrass mixtures. Our third hypothesis (H3) is therefore confirmed. Several other studies found evidence of relative root overyielding in grassland systems: A relative root overyielding of clover/grass mixtures compared to pure stands were demonstrated by Davidson and Robson (1986). Van Eekeren et al. (2009) observed RY values of 0.3 for clover and 0.8 for ryegrass, resulting in a RYT of 1.1. A four-species-mixture with white clover had RYT values higher than 3.0 (Chen et al. 2008). Mommer et al. (2010) reported an absolute root overyielding with 40% higher root biomasses of grass/forb mixtures compared to pure stands. Furthermore, the root biomass of grasslands is positively related to the number of species or functional groups (Ma and Chen 2016; Reich et al. 2004). Bessler et al. (2009) observed an increased investment in shoot biomass with a simultaneous reduction in biomass partitioning to roots in mixtures. The authors argue that the reduced biomass partitioning to roots is caused by the improved nitrogen (N) nutrition of non-legumes in mixtures.

Facilitative processes like an increase in soil N availability via symbiotic N_2 fixation by legumes are proposed to be one of the reasons for higher dry matter yields in grassland mixtures compared to pure stands (Lambers et al. 2004). Non-legume species such as ryegrass benefit from the improved soil N. Transfer of fixed N_2 from legumes to non-legumes can occur via direct N transfer, rhizodeposition or nutrient cycling mediated by root herbivory (Høgh-Jensen and Schjoerring 2001; Murray and Clements 1998; Ta and Faris 1988). Up to 50% of ryegrass N can be derived from N transfer by clover (Ledgard 1991). In a field experiment, McNeill and Wood (1990) reported an N benefit of 42.7 kg N ha^{-1} for grass derived from clover. This positive effect of N_2 fixation on aboveground compartments might be mirrored belowground: Hernandez and Picon-Cochard (2016) demonstrated the facilitative effect of N_2 fixating clover on the root biomass of ryegrass. An increase in N further enhances the competitive ability of ryegrass but reduces that of clover (Mouat et al. 1987). Even though N transfer was not investigated in our study, the much higher RY of ryegrass than of clover suggests that ryegrass roots might have benefited from N transfer by clover. Therefore, clover/grass mixtures of the present study might have a better resource utilization compared to pure stands.

The positive diversity-productivity relationship can also be attributed to niche complementarity and root partitioning between species (Cardinale et al. 2007). Clover and ryegrass are both considered to be shallow rooting (Caradus 1981; Keith Syers et al. 1984). Nonetheless, they show differences in their root system and belowground competitive ability (Haynes 1980). In our study, relative root overyielding in clover/grass mixtures was mainly attributed to the increased root biomass of ryegrass in relation to its seeding density. According to Ravenek et al. (2016) and Rajaniemi (2007), the competitive success of a plant depends on fast root growth, high root densities and high root biomass. Higher RYs of ryegrass than of clover in our study result from the higher competitive ability of ryegrass compared to clover (Haynes 1980; Ren et al. 2017). Species differences in rooting depth could have led to niche differentiation in our clover/grass mixtures (Berendse 1981). In accordance to the results of Mommer et al. (2010) and Skinner et al. (2006), the relative root overyielding in the present study might be linked to changes in rooting depth from pure to mixed stands.

Root overyielding of grassland mixtures has the potential to increase the soil organic matter and N availability which ultimately leads to a higher aboveground productivity (Mommer et al. 2010). Shoot and root biomass was also positively correlated in studies by Cahill Jr (2003). The relative root overyielding of the present study might increase the water and nutrient utilization of mixtures and therefore aboveground biomasses. This pattern is supported by dry matter analyses of the same project (subproject Grassland Science), which revealed that binary mixtures of clover and ryegrass are characterized by transgressive aboveground overyielding (Heshmati et al. 2018). It is important to mention that our investigation of the root biomass at one sampling time is only a rough estimate of the actual belowground biomass production and seasonal patterns were not recorded.

4.5.5. Differences in root biomass between white clover genotypes in pure stands and mixtures

The eight white clover genotypes used in our study originated from a breeding program which made a selection based on maximum winter hardiness, mixture performance and persistence in mixed swards. Genotypes varied in their expression levels of certain plant traits, which are important for mixtures (flowering time, leaf size, flowering intensity, mixture yield). White clover breeding, however, does not only aim for yield maximization but rather for a high persistence, reliability and contribution in clover-grass swards (Abberton and Marshall 2005). These traits rely on niche complementarity and root partitioning (Cardinale et al. 2007).

The results of the present study showed that the eight investigated white clover genotypes in pure stands differed in their root biomass. White clover has a high variation in root morphological traits (Caradus and Woodfield 1998). Differences between clover genotypes in root dry weight were also reported by Caradus (1977; 1981) and Frankow-Lindberg (1997). Furthermore, the present study showed that the clover genotypes did not differ significantly in their root biomass in mixtures. However, there were significant differences between the genotypes in mixtures in terms of root overyielding. Our fourth hypothesis (H4), that clover genotypes differ in their root biomass and root overyielding, was therefore partly confirmed.

Genotype Tr6 demonstrated the highest relative root overyielding while Tr1 the lowest. To our knowledge, there are no field studies available on the interspecific variation in root traits between white clover genotypes in mixtures. We were able to show that the performance of four clover genotypes (Tr2, Tr4, Tr5, Tr7) was similar in pure stands and in mixtures. Contrasting to this, Tr1 had a high root biomass in pure stands and a low root biomass in mixtures, while genotype 6 had the highest root biomass in mixtures and the lowest in pure stands. Based on the available data, we suggest to further investigate Tr6, in particular the belowground overyielding potential across several growing seasons and differing environment.

More research is needed on rooting depth and tap root properties of clover genotypes in pure stands as well as in mixtures as targeted breeding on these root traits might enhance drought tolerance (Marshall et al. 2016; Woodfield and Caradus 1987), soil C sequestration (Kell 2012) or water drainage (Holtham et al. 2007). The results of the present study suggest that the selection of white clover genotypes should not be entirely based on root biomass but also other beneficial root traits for resource acquisition. The belowground overyielding potential of clover genotypes might be one of those traits. Increased root biomasses in mixtures compared to pure stands might enhance C inputs to soil and aboveground productivity (Caradus and Woodfield 1998; Mommer et al. 2010; Ojeda et al. 2018). We therefore recommend that clover breeding programs should combine the selection for aboveground biomass with the selection for root architectural traits.

4.6. Conclusions

The present study demonstrated a significant relative root overyielding for most of the eight clover/grass mixtures. We assume that belowground overyielding could be linked to root partitioning, niche complementarity and optimized resource utilization in mixtures. We detected differences in root biomass between the clover genotypes in pure stands. Furthermore, the present study detected differences in belowground relative overyielding between the clover genotypes. We assume that the relative belowground overyielding of mixtures in the present study might increase nutrient and water acquisition. Hence, aboveground productivity could be positively affected by root overyielding. We suggest that white clover breeding programs should include root traits, such as relative root overyielding, in their selection process.

4.7. Acknowledgements

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Chapter 5

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General Discussion

The present study evaluated the biomass, root distribution and the overyielding potential of arable and grassland crops in legume-based pure stands and mixtures. Inter- and intraspecific differences between the eight novel winter faba bean genotypes and winter wheat, as well as between eight novel white clover genotypes and perennial ryegrass were investigated. In the following chapters, the observations from the contrasting land use systems arable land and grassland will be compared. In particular, it will be discussed if FTIR spectroscopy is a suitable method for the root quantification in these systems, if arable and grassland show similarities in root traits, and whether there are novel genotypes in both systems which are promising for future research.

5.1. Root mass quantification in bean/wheat and clover/grass mixtures via FTIR spectroscopy

The possibility to identify taxa belowground via FTIR spectroscopy is based on absorbance differences at multiple wavenumbers. These absorbance differences are caused by chemical composition differences between roots of different species (Rewald et al. 2012). The present study is the first experiment which successfully differentiated between winter faba bean and winter wheat as well as between white clover and perennial ryegrass roots (Chapter 2, Chapter 4). So far, FTIR spectroscopy has been used to determine root species of e.g. maize and barnyardgrass (Legner et al. 2018) or sugar beet and common lambsquarters (Meinen and Rauber 2015). The mean FTIR spectra of dried and ground roots of pure stands of bean, wheat, clover and ryegrass had similar absorbance peaks but at the same time absorbance differences in certain wavenumber regions: All species demonstrated absorbance peaks at 1050, 1620, 2960 and 3300 cm^{-1}. However, when considering the whole spectral range, roots of bean and clover had a similar average absorbance (0.017). Ryegrass roots had the second lowest absorbance with 0.016 and wheat the lowest (0.015). Absorbance differences between the species were most prominent in the wavenumber regions: 400-860, 950-1070, 1200-1750 and 2400-3400 cm^{-1}. According to Naumann (2000), Naumann et al. (2010) and Wilson et al. (2000), the absorbance at several wavenumber regions of the present study can be attributed to the presence of functional groups: 900-1185 cm^{-1} to cellulose and hemicellulose, 1185-1800 cm^{-1} to protein and lipids and 2700-3000 cm^{-1} to lipids. Interestingly, both legumes as well as both non-legumes had similar absorbance tendencies: At the cellulose/hemicellulose peak, mean absorbance of bean and clover were lower than of wheat and ryegrass. Similarly, at the protein and lipid peak, the legumes bean and clover demonstrated a higher absorbance than the non-legumes wheat and ryegrass. Root C/N analyses within the present study showed that bean (C/N ratio of 14) and clover (21) had smaller C/N ratios than wheat (50) and ryegrass (56) (data not shown). Our results indicate that the chemical composition of roots is similar within legumes (bean, clover) as well as within non-legumes (wheat, ryegrass). This result is in line with aboveground dry matter analyses by Sleugh et al. (2000), who reported that the crude protein content of legumes is considerably higher than of non-legumes, while the neutral-detergent fiber content is higher in non-legumes. Furthermore, cluster analysis of bean and wheat roots of the present study demonstrated that the interspecific heterogeneity was higher (10.03) than of the latter studies (6.1, 4.9), indicating a more accurate species differentiation.

Besides for species determination, FTIR can be used to determine biomass proportions in soil samples (Rewald and Meinen 2013). The present study demonstrated that FTIR spectroscopy is a promising tool for the quantification of root species proportions in bean/wheat and clover/grass mixtures. According to Diller (2002), the predictive quality of a model is excellent with a RPD>10, good to very good with a RPD 5-10 and sufficient with a RPD 3-5. Additionally, low root mean square error of calibration (RMSEE), cross validation (RMSECV) and prediction (RMSEP) values suggest a high model quality. The means of the statistical parameters of the 16 bean/wheat and the eight clover/ryegrass FTIR models demonstrate a high model quality (Table 1). However, the clover/ryegrass models reached on average a higher precision and accuracy in terms of RPD and root mean square errors than the bean/wheat models (Table 1). This finding is underlined by the lower standard error values of grassland models compared to arable models.

Table 1 Mean and standard error of several statistical parameters from FTIR models of arable land (n=16) and grassland (n=8) mixtures in terms of calibration, internal validation and external validation. Model quality is described by residual predictive deviation (RPD), root mean square error of calibration (RMSEE), root mean square error of cross validation (RMSECV) and root mean square error of prediction (RMSEP). See Chapter 2 and 4 for detailed model characteristics.

		Calibration		Internal validation		External validation	
		RMSEE	RPD	RMSECV	RPD	RMSEP	RPD
Arable land	Av.	3.97	8.57	4.83	7.00	7.63	4.03
	SE	0.29	0.76	0.41	0.62	0.69	0.45
Grassland	Av.	3.22	9.70	3.88	7.87	5.73	4.80
	SE	0.14	0.45	0.18	0.34	0.22	0.19

Model quality is described by residual predictive deviation (RPD), root mean square error of calibration (RMSEE), root mean square error of cross validation (RMSECV) and root mean square error of prediction (RMSEP). See Chapter 2 and 4 for detailed model characteristics.

So far, FTIR spectroscopy has been used to determine root species proportions in maize/barnyard grass mixtures (Meinen and Rauber 2015) and faba bean/chamomile mixtures (Rewald and Meinen 2013). The arable land and grassland models of the present study had on average lower external test set validation RPDs (Table 1) than the latter studies (6.19, 8.56). Crops of the latter studies were grown in greenhouses with controllable growth conditions (e.g. substrate, fertilization). The slightly lower quality of FTIR models in the present study might be therefore due to a higher heterogeneity within samples.

One disadvantage of FTIR spectroscopy for species quantification is the requirement of exact calibration series. The chemical composition of roots might change during ontogeny and under different abiotic and biotic environments (Rewald et al. 2012). Therefore, individual calibrations are needed for different species, locations, years or seasons. In case of the present study, calibrations were prepared separately for each mixture (bean/wheat or clover/grass), year and genotype. This is why FTIR model prediction properties, such as RMSEE or RPD, also differed between mixtures, years and genotypes. In case of year, the cluster analysis of Chapter 2 revealed no distinct year clusters. We assume that this is caused by the consistent cultivation at the same site and a similar managing practice in both growing seasons (tillage, no fertilization, same proceeding crop). When FTIR calibrations are prepared for arable mixtures on the same site with similar managing practice, a merging of years could be considered.

FTIR spectroscopy offers several advantages: In comparison to dispersive spectrometers, FTIR spectrometers have a high speed and high sensitivity (Sherman Hsu 1997). Measuring times per sample were less than 30 seconds in the present study. FTIR spectroscopy has a high signal-to-noise ratio and needs low sample preparation: e.g. solid samples only require to be dried and ground (Rewald et al. 2012). Moreover, this method has low maintenance costs and produces no chemical waste (Meinen and Rauber 2015). Additionally, only small amounts of root material is needed for a single measurement (Meinen and Rauber 2015): In the present study, sample weights ranged between 0.8 mg and 1.35 g. For the preparation of a single calibration approximately 300 mg of dry and ground root material was needed from each species. As a consequence, FTIR spectroscopy is a promising tool for the high throughput belowground species determination of mixtures with large sample numbers. With the present study we were able to demonstrate the high potential of FTIR spectroscopy for root mass quantification of different species in legume and non-legume mixtures. Moreover, FTIR spectroscopy can be utilized for a detailed analysis of the horizontal and vertical root distribution.

5.2. Root overyielding in arable and grassland mixtures

The present study showed that root biomasses in 0-60 cm soil depth of grassland species were on average higher than of arable species. When averaged over genotypes in pure stands, clover (121 g/m²) and ryegrass (348 g/m²) exceeded the root biomass of bean (71 g/m²) and wheat (70 g/m²). However, when taproots are included, bean (194 g/m²) would have a higher root biomass than clover. Because the grassland experiment was permanently established in May 2014 while crops of arable land rotated every year, crops in grassland accumulate more root biomass. Root biomass in permanently established grassland studies generally increase with experimental age (Ma and Chen 2016).

The study furthermore showed that both land use systems did not overyield absolutely belowground, meaning that mixtures had no higher absolute root biomasses than the pure stands. However, arable and grassland mixtures were characterized by relative root overyielding. Both mixtures were of a substitutive design which enables the comparison of the relative yield total (RYT) for belowground biomass. The relative seeding densities in mixtures were 0.5 (bean)/0.5 (wheat) in arable land and 0.4 (clover)/0.6 (ryegrass) in grassland. The average root RYT was both 1.4 in arable land and grassland. In arable land, relative yields of bean (0.7) and wheat (0.7) were more balanced than the relative yields of clover (0.6) and ryegrass (0.9).

In contrast to our results, there are a few studies which do not report a positive effect of an increased plant-species richness on the root biomass: Gastine et al. (2003) could not find significantly higher absolute root biomasses of legume/grass mixtures compared to the pure stands. Bessler et al. (2009) even reported lower root biomasses of multispecies mixtures than of pure stands. However, the observed relative root overyielding in both land use systems in the present study is in accordance with numerous studies (Table 2). Ma and Chen (2016) reported an average fine root overyielding of 28.4% across several land use systems. The results of the present study and the literature review strongly suggest that the relative root overyielding of legume/non-legume mixtures could be an overall pattern across land use systems such as arable land and grassland.

Table 2 Literature review on root overyielding in the land use systems arable land (A) and grassland (G) with the corresponding plant species. Absolute root overyielding: mixtures are characterized by higher root biomass values than pure stands. Relative root overyielding: the sum of the relatives root yields of the species is higher than 1.

Land use system and publication	**Species**
Absolute root overyielding	
(A) Xia et al. (2013)	faba bean/chickpea/maize
(A) Li et al. (2006)	faba bean/maize
(G) Mommer et al. (2010)	sweet vernal grass/red fescue/oxeye daisy/ribwort plantain
(G) Ma and Chen (2016)	review
Relative root overyielding	
(A) Xu et al. (2010)	milk vetch/switchgrass
(A) Xu et al. (2008)	sainfoin/switchgrass
(A) Corre-Hellou (2005)	fertilized pea/barley
(G) Ren et al. (2017)	white clover/ryegrass
(G) Davidson and Robson (1986)	white clover /ryegrass
(G) Van Eekeren et al. (2009)	white clover/ryegrass
(G) Chen et al. (2008)	white clover/alfalfa/sudan grass/tall fescue

The positive impact of plant diversity on biomass production is generally caused by species complementarity of mixtures. Complementarity between plant species was therefore suggested to be the main reason for aboveground (Cardinale et al. 2007) and belowground overyielding (Ma and Chen 2016). In mixed cropping systems, both competition and facilitation take place (Geno and Geno 2001). Root overyielding in the arable and grassland mixtures of this study, in particular from the non-legumes, might be due to the facilitative process of N transfer from legumes to non-legumes (Lambers et al. 2004; Xiao et al. 2004). This synergetic effect leads to a better resource utilization. By analyzing the relative root yields of the mixture components (Chapter 4), the present study showed that ryegrass had a higher competitive ability than clover but did not totally suppress clover. In contrast to this, relative root yield of faba bean and wheat were more balanced. According to Li et al. (2006), the results indicate an asymmetric interspecific facilitation in the clover/ryegrass mixture and a symmetric interspecific facilitation in faba bean/wheat mixtures.

By analyzing the root distribution of bean/wheat mixtures, the present study furthermore showed that bean and wheat roots intermingled in mixtures (Chapter 3). Therefore, interspecific belowground interactions such as competition were likely to be present in the investigated mixture. Several authors stated that belowground interactions are the major driver for above- and belowground overyielding (e.g. Li et al. 1999; Ren et al. 2017; Zhang et al. 2004). In chapter 3 it was demonstrated that in mixtures, faba bean and wheat on their own row produced higher root fractions in shallower soil layers than in pure stands, while simultaneously, both species had more roots in deeper soil layers on the partner's row than on their own row. A plastic response of root distribution to interspecific competition like in the present study is in accordance with several other studies (Hauggaard-Nielsen et al. 2001; Li et al. 2006; Tosti and Thorup-Kristensen 2010). The alteration of root distribution in presence of a mixture partner mitigates interspecific competition. Therefore, complementarity between species could be enhanced in terms of occupied soil space. Changes in root distribution could lead to a more complete exploitation and a better utilization of resources (Hauggaard-Nielsen et al. 2001). This, in turn, could cause an increased root biomass production. Even though, root distribution in clover/grass mixtures was not investigated in the present study, we assume that niche complementarity in terms of root distribution might have caused root overyielding in grassland mixtures (Cardinale et al. 2007; Skinner et al. 2006). An enhanced resource utilization in mixtures, caused by alteration in root distribution, can ultimately have a positive effect on the aboveground biomass.

There are numerous studies which observed a positive correlation between root biomass and aboveground biomass (Belachew et al. 2018; Cahill Jr 2003; Carton et al. 2018; Xia et al. 2013). Higher root biomasses in mixtures compared to pure stands could lead to an increased water and nutrient acquisition. Furthermore, soil organic matter and nitrogen availability might increase with higher root biomasses (Mommer et al. 2010). Subsequently, aboveground biomass and thus photosynthetic assimilation is enhanced (Lynch 2007; Shi et al. 2018). In the present study on arable crops, root dry matter of faba bean and wheat was positively correlated with aboveground dry matter at bean full flowering (Fig. 1, $R^2=0.35$, $p<0.001$).

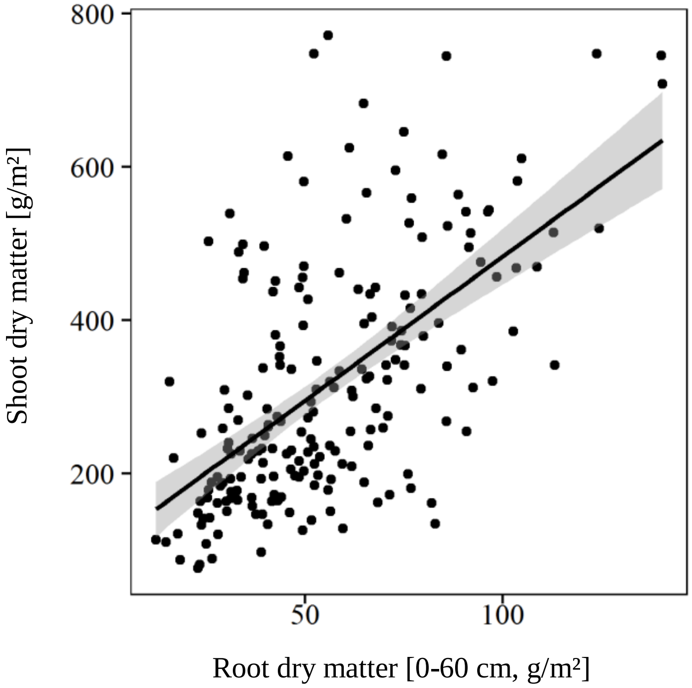

Figure 1 Correlation between root dry matter and shoot dry matter of bean and wheat in 2015 and 2016. Root biomass comprises the accumulated root biomass from 0 to 60 cm soil depth, averaged over sampling positions. Solid line represents the regression curve with 0.95 confidence interval. Pearson's correlation was based on n=200.

Nachi and Le Guen (1996) showed that biomass accumulation during the vegetative growth period is positively correlated to grain yield of faba bean. The grain yield analyses of Siebrecht-Schöll et al. (IMPAC³ project, Plant breeding, University of Goettingen) reported highest grain yield for bean/wheat mixture Vf5-Ta and lowest for the mixture Vf8-Ta (Fig. 2). The overyielding potential in terms of root and shoot biomass was also the highest for Vf5-Ta and the lowest for Vf8-Ta. The results of the present study therefore indicate that root overyielding leads to shoot overyielding and is thus one of the prerequisites for grain yield advantages of faba bean/wheat mixtures. Nevertheless, aboveground interspecific interactions and complementarity in shoot architecture in mixtures also occur e.g. in terms of differences in light acquisition (Cardinale et al. 2007; Gross et al. 2007). These aboveground processes also contribute to an optimized complementarity between species in mixtures and a subsequent increased plant production.

5.3. Utilizing novel winter faba bean and white clover genotypes in arable and grassland mixtures

In Germany, there is only one winter faba bean cultivar available on the market (cv. Hiverna). White clover in contrast, has 18 cultivars in the official German testing trials (Bundessortenamt 2018). However, these white clover cultivars are not tested in mixture but only in pure stands. In order to investigate whether the cultivar has an effect on the mixture performance, a wide range of genotypes is required. Therefore, seed material of the present study originated from breeding initiatives from the Georg-August-University of Goettingen, Norddeutsche Pflanzenzucht (NPZ, winter faba bean) and the Deutsche Saatveredelung (DSV, white clover). A major advantage of the available seed material of the present study is that it is pretested and phenotyped. Clover seed material was already pretested for its mixture performance by the DSV.

Faba bean cultivars demonstrate a large genetic variability in aboveground properties such as shoot biomass and grain yield (Link et al. 2010; Neugschwandtner et al. 2015); knowledge about genetic variability in root traits is however limited. Differences in root traits between faba bean accessions were observed by Belachew et al. (2018), Grzesiak et al. (1997), Khan et al. (2010) and Zhao et al. (2017). Even though the authors found differences in rooting depth, total root length, tap root length or lateral root length, they cannot provide information about these accessions in mixtures or under field conditions.

By using FTIR spectroscopy, we could show in the present study that the eight faba bean genotypes differed in their horizontal and vertical root distribution in mixtures with wheat (Chapter 3). Faba bean genotypes Vf5 and Vf6 in mixture had a high horizontal spread into the wheat row and higher root fractions in deep soil layers, respectively (Fig. 2). If the other genotypes are taken into account, a slight tradeoff between these two traits is noticeable: Genotypes with higher root fractions in deeper soil layers tend to have a lower spread into the wheat row and lower root biomasses on that sampling position. The relation between a high horizontal spread into the wheat row and deeper vertical root distribution was characterized by a weak negative correlation (R^2=-0.28). Genotypes Vf5 and Vf6 most likely differ in their belowground competitive ability. The downward directed avoidance strategy of Vf5 under the wheat row reduces interspecific competition. Simultaneously, the water and nutrient acquisition from deeper soil layers might be enhanced for this particular genotype in mixtures. Greater rooting depths and root lengths might contribute to an advantage in drought tolerance (Grzesiak et al. 1997; Khan et al. 2010). The number of lateral roots and the overall proportion of lateral roots within the root system are important for deep soil foraging of faba bean (Zhao et al. 2017).

Moreover, the results indicated that the change in vertical root distribution of wheat in presence of faba bean depends on the bean genotype (Chapter 3): we could observe that wheat on its own row reacted to the presence of faba bean genotypes 4, 5 and 8 with a significant increase of root fractions in shallower soil layers compared to wheat pure stand. Contrary, wheat in mixture on its own row with faba bean genotypes 1, 2, 3, 6 and 7 had a similar vertical root distribution as in pure stand. Simultaneously, bean genotypes 4, 5 and 8 were characterized by the highest root fraction in deep soil layers (Chapter 3, Fig. 2). This pattern indicates a spatial niche partitioning for soil space on the wheat row for some particular faba bean/wheat mixtures. Therefore, species complementarity between faba bean and wheat is notably high on the wheat rows for these mixtures.

The present study furthermore demonstrated that the eight faba bean genotypes differed in their above- and belowground overyielding potential at full flowering of faba bean (Chapter 2). In the evaluation of the eight faba bean genotypes according to their overyielding potential, genotype Vf5 displayed the highest and Vf8 the lowest advantage in the mixture (Fig. 2). Surprisingly, genotypes Vf5 and Vf8 had a contrasting overyielding potential but a similar root distribution on the wheat row in mixtures. We therefore suggest investigating the effect of spatial root distribution on the overyielding potential of bean/wheat mixtures. As already mentioned, grain yield analyses within the same project (Plant breeding, data not shown) reported consistently (years 2015 and 2016) the highest grain yield advantage of mixtures with genotypes Vf3, Vf2 and Vf5 (Fig. 2). Genotypes Vf8 and Vf6 had the lowest grain RYT.

Based on the results of root distribution and biomass as well as on overyielding potential and grain overyielding potential we consider genotype Vf5 to be the most promising candidate for a improved winter faba bean/winter wheat mixtures.

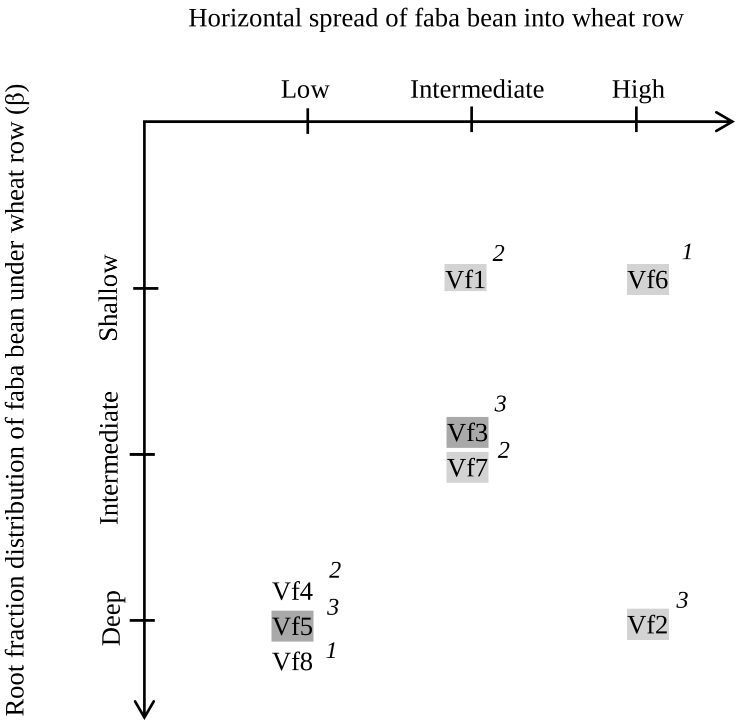

Figure 2 Evaluation of the spatial root distribution and overyielding potential of eight winter faba bean (Vf1-8) genotypes in mixture. Shading indicates above- and belowground overyielding potential and consistency of the genotypes at full flowering of bean; dark grey: high, light grey: intermediate, white: low. See Chapter 2 and 3 for details. Italic numbers next to the genotypes indicate their low (*1*), intermediate (*2*) or high (*3*) grain overyielding potential in 2015 and 2016 (data from project Plant Breeding).

The present study furthermore showed that the eight investigated white clover genotypes differed in their root biomass in pure stands but not in mixtures (Chapter 4). Furthermore there were differences in RYT between the clover/ryegrass mixtures ($p \leq 0.1$). Similar to the present study, differences in root biomass between white clover genotypes in pure stands were also observed by Caradus (1981), Caradus and Woodfield (1998) and Frankow-Lindberg (1997). However, similar to the above described faba bean genotypes, the majority of the latter studies were not field based experiments and neither tested the cultivars in mixtures.

Genotype Tr6 had the highest root biomass in mixture and the highest root overyielding (Chapter 4, Table 3). Based on these results we suggest to further research clover genotype Tr6 for its mixture potential. It should be tested the root overyielding of Tr6 in mixture with ryegrass is also evident under several growing seasons and differing sites. Nonetheless, the most important research question is, whether the high root overyielding of Tr6 in mixtures is translated into an aboveground overyielding. Similar to faba bean/wheat mixtures, the high root overyielding of clover genotype Tr6 could possibly lead to a higher resource acquisition and thus to an increased dry matter yield.

Table 3 Evaluation of the root overyielding potential of the eight white clover genotypes (Tr1-8) in mixture with unfertilized perennial ryegrass (Lp-N0).

Low	Intermediate	High
Tr1/Lp-N0	Tr2/Lp-N0	Tr6/Lp-N0
Tr3/Lp-N0	Tr4/Lp-N0	
Tr8/Lp-N0	Tr5/Lp-N0	
	Tr7/Lp-N0	

Plant breeding programs mainly focus on the development of cultivars for pure stands as they are grown more frequently than mixtures. Mixed cropping systems usually utilize the same cultivars, even though cultivar performance can vary between pure and mixed stands (Carton et al. 2018; Nelson and Robichaux 1997; Neugschwandtner et al. 2015). A significant cultivar x cropping system interaction for pea cultivars in terms of grain yield was observed by Hauggaard-Nielsen and Jensen (2001). This pattern was clearly confirmed by the present study: novel genotypes of bean and clover performed differently in pure stands and in mixtures. In arable land, bean genotype x cropping system interactions were visible for the traits total shoot and root biomass, comparative bean root biomasses and bean root:shoot ratio (Chapter 2). Furthermore, the vertical root distribution of bean differed between pure and mixed stands (Chapter 3). In grassland, the clover genotype x cropping system interaction was also evident for root biomass (Chapter 4). To our knowledge there are no studies available on performance differences between clover genotypes between pure and mixed stands in terms of dry matter yield or root traits. In order to improve mixed cropping systems, the breeding of new legume cultivars should be targeted for these systems. Furthermore, testing of new cultivars should be conducted as field studies to ensure the performance of plants in agronomical environments.

With the present study we were able to show that certain novel bean and clover genotypes possess traits that are advantageous for mixtures with non-legume species. A high root overyielding of certain bean genotypes in bean/wheat mixtures at full flowering of bean was a good indicator for grain overyielding. The superiority of clover genotype Tr6 in root overyielding might possibly be reflected in dry matter mixture advantage. In order to improve the crops water and nutrient efficiency, researchers such as Lynch (2007) and Zhao et al.

(2017) emphasize that root traits should be included in breeding efforts. Future yield improvements might be dependent upon the progress of research on root system architecture and stress resistance (Den Herder et al. 2010; Koevoets et al. 2016; Lynch 2007). In accordance to the latter studies, we recommend to include research on genotypic differences in root biomass and distribution in the evaluation process of mixed cropping systems.

5.4. Concluding remarks

The present study showed that FTIR spectroscopy has a high potential to quantify root species proportions and root distribution in legume/non-legume mixtures. The tested winter faba bean/winter wheat and white clover/perennial ryegrass mixtures were both characterized by root overyielding. This pattern, together with simultaneous changes in root distribution might lead to an enhanced resource utilization. The results of this study suggest that the aboveground yield advantage of legume/non-legume mixtures is clearly linked to belowground processes. The present study furthermore showed that there were genotype differences within each of the two legumes. Additionally, legume genotypes performed differently in pure and in mixed stands. We therefore recommend that breeding and selection of new cultivars for mixture purposes should be performed in mixed cropping systems. Investigations on root properties, such as root distribution and root overyielding, should be included in the breeding process. Furthermore, the integration of root properties in the selection process of legume genotypes could promote the development of drought adapted varieties, as already demonstrated for other species (Bucksch et al. 2014). Even though mixed cropping systems have the potential to increase the diversity of agricultural landscapes and to sustainably enhance yields, they are not of common practice in Germany. For the successful integration of mixtures into the German crop rotation, it is necessary to increase the attractiveness of these systems for the farmers (Lemken et al. 2017). With the present study we were able to foster the knowledge of underlying processes of mixture advantage. Legume/non-legume mixtures are complex agricultural systems, where below- as well as aboveground processes have to be considered. Interdisciplinary research on mixed cropping systems, as applied by the IMPAC^3 project, can contribute to a better understanding of these complex interactions.

5.5. References

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Summary

Mixtures of legumes and non-legumes are often characterized by higher grain and biomass yields compared to their pure stands. Complementarity between plant species is assumed to be the major driver behind this aboveground overyielding. Cultivar characteristics can affect mixture performance. Nevertheless, novel legume cultivars/genotypes are primarily bred and tested for pure stand purposes. However, well-performing genotypes in pure stands do not necessarily perform similarly well in mixtures. To fully understand mixed cropping systems, it is necessary to investigate their underlying spatiotemporal above- and belowground processes. Roots are of particular importance for the plant, as they acquire water and nutrients. Nonetheless, little is known about differences in root biomass and distribution between pure stands and mixtures. So far, the lack of a simple and time-efficient method has hampered the analysis of root species proportions in mixtures.

In the present study, novel legume genotypes of arable land and grassland were sown as pure stands and mixtures with non-legumes. Two different field experiments were conducted at the experimental station ‘Reinshof’ of the Georg-August-University of Goettingen (Germany) to investigate the biomass, root distribution and overyielding potential of these pure and mixed stands.

In the arable land experiment, eight genotypes of winter faba bean (*Vicia faba* L.) and one cultivar of winter wheat (*Triticum aestivum* L., cv. Genius) were sown in pure stands and in substitutive 50/50 mixtures. The intra- and interspecific variation of shoot and root biomass, the horizontal and vertical root distribution and the overyielding potential were investigated in all crop stands at full flowering of faba bean. Aboveground biomass of 1 m² was harvested and roots were sampled in May 2015 and May 2016. Root samples were taken on and between rows with a root auger down to 60 cm soil depth. Fourier transform infrared (FTIR) spectroscopy was used to quantify species-specific root biomasses in mixtures. The vertical root distribution was evaluated using the equation $y = 1 - \beta^d$ (Gale and Grigal 1987). To assess above- and belowground mixture overyielding, the relative yield total (RYT) was calculated for shoot and root biomass. The results showed that all FTIR quantification models performed well in the prediction of root species proportions. Roots of both species proliferated into the soil space between the rows and under the mixture partner’s row to a similar extent. In mixtures, faba bean and wheat on their own row produced higher root fractions in shallower

soil layers than in pure stands, while simultaneously both species had more roots in deeper soil layers under the partner's row than on their own row. Overyielding of faba bean/wheat mixtures was more pronounced for belowground biomass than for aboveground biomass. In mixtures, faba bean genotypes differed significantly in root biomass, root:shoot ratio, overyielding potential and vertical root distribution on wheat rows but not in shoot biomass.

In the grassland experiment, the root biomass of eight genotypes of white clover (*Trifolium repens* L.) and one perennial ryegrass genotype (*Lolium perenne* L., Elp 060687) were investigated. Four different crop stands were established in May 2014: (i) unfertilized clover pure stand of each clover genotype, (ii) unfertilized ryegrass pure stand, (iii) N-fertilized ryegrass pure stand and (iv) unfertilized mixture of each clover genotype with ryegrass. Similar to the first experiment, root sampling was conducted from 0 to 60 cm soil depth in June 2015. Clover and ryegrass root proportion in mixtures was determined via FTIR spectroscopy. Belowground RYT was calculated for each mixture. The results showed that FTIR models demonstrated a satisfactory residual predictive deviation. In pure stands as well as in mixtures, clover produced significantly lower root biomasses than ryegrass. Nitrogen fertilization did not affect the root biomass of ryegrass. In pure stands, clover root biomass differed significantly between genotypes. Furthermore, root RYT was higher than one in all the eight clover/ryegrass mixtures but differed between the genotypes. This belowground overyielding was mainly caused by the high relative root biomass of ryegrass.

The present study showed that FTIR spectroscopy is a suitable tool for the identification and quantification of root species in legume/non-legume mixtures. From the two experiments, it can be concluded that both faba bean/wheat and clover/ryegrass mixtures overyield with regard to root biomass. Root overyielding in legume/non-legume mixtures compared to the pure stand equivalents might lead to better resource utilization and enhanced aboveground yields of these systems. The fact that genotypes performed differently in pure and mixed stands shows the potential of legume breeding for mixture purposes. In both arable land (Vf5) and grassland (Tr6), one legume genotype was identified for further breeding in mixed cropping systems. The results of the present study suggest that investigations of root properties should be included in mixture breeding processes.

Appendix

Table A1 Description of the genotypes used in the present study: Eight winter faba bean inbred lines (Vf1-8) and winter wheat cultivar Genius (Ta). Winter faba bean genotypes from NPZ (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany) and the department for Crop Sciences, University of Goettingen, Germany. Description from the department for Crop Sciences, University of Goettingen, Germany. Wheat cultivar Genius from NORDSAAT Saatzucht GmbH, Langenstein, Germany, description from Bundessortenamt (2017).

Species	Code	Entry	Traits and specific features	Maximum winter hardiness	Highest Yield
Winter faba bean	Vf1	S_004-1-6	Medium tall, Low tillering, Late flowering, Medium maturing, High yielding	x	
	Vf2	S_062-2-2	Very short, High tillering, Medium early flowering, Medium maturing	x	
	Vf3	S_069-1-1	Very tall, Medium tillering, Medium late flowering, Medium maturing, High yielding		x
	Vf4	S_265-1-1	Very tall, Very high tillering, Medium early flowering, Medium maturing	x	
	Vf5	Hiverna/2-5-1	Medium tall, Low tillering, Medium early flowering, Low yielding, Pure line developed from Hiverna (German cv.), Superior winter hardiness	x	
	Vf6	Côte d'Or/1-1-3	Very tall, High tillering, Late flowering, Late maturing, Source of superior winter hardiness	x	
	Vf7	WAB-Fam157-1-2	Medium tall, Low tillering, Early flowering, Early maturing, High yielding		x
	Vf8	WAB-EP98-267-11	Medium tall, Medium tillering, Late flowering, Late maturing, High yielding, Sibling of cv. Nordica		x
Winter wheat	Ta	cv. Genius	Medium tall (BSA-score 5). Seed protein content: high (E). Stable yield. Resistance to mildew, leaf rust and fusarium. N-uptake capacity: high.		

Table A2 Statistical parameters of FTIR models in terms of calibration, internal cross validation and external validation. Models were prepared for each bean genotype in mixture and year separately (Vf1-8-Ta). Calibration set consisted of 35 or 56 spectra with 3 or 5 replicates of each model (n). Spectra were mathematically pretreated with first derivative (1), vector normalization (2), multiplicative scatter correction (3) or 17 smoothing points (4) at the different wavenumber ranges. Lowest root mean square error of cross validation (RMSECV) was chosen during the optimization procedure of internal cross validation. Model quality is described by coefficient of determination (R²), root mean square error of estimation (RMSEE) and residual predictive deviation (RPD). All models were validated externally by using 20 spectra with 3 or 5 replicates. External validation is described by standard error of prediction (SEP), root mean square error of prediction (RMSEP) and RPD.

			Calibration						**Internal cross validation**					**External validation**					
		Wavenumber ranges [cm^{-1}]	n	Rank	R²	RMSEE	RPD	Math. pretreatment	R²	RMSECV	RPD	MD limit	Bias	n	Slope	SEP	RMSEP	RPD	Offset
2015	Vf1-Ta	1825.1 - 736.8	280	6	96.58	5.60	5.41	1; 2	95.61	6.26	4.77	0.15	-0.0433 (0.0433)	175	0.946	7.02	7.00	3.64	2.744 (2.69)
	Vf2-Ta	3635.9 - 2909.0 1823.6 - 1460.9	105	4	96.91	5.45	5.69	2	95.53	6.39	4.73	0.29	0.0192 (-0.0192)	60	0.878	13.70	15.00	1.69	12.058 (0.159)
	Vf3-Ta	1462.3 - 1099.6	175	6	97.02	5.33	5.79	1; 2; 4	95.83	6.17	4.90	0.27	-0.054 (0.054)	100	0.969	6.69	7.28	3.76	3.726 (-0.606)
	Vf4-Ta	2549.2 - 1098.2	175	6	98.99	3.07	9.96	2	98.62	3.52	8.51	0.27	-0.131 (0.131)	100	0.981	4.39	4.53	6.76	2.213 (-0.273)
	Vf5-Ta	3273.3 - 2547.8 2186.5 - 1098.2	175	3	98.76	3.39	8.99	1; 2; 4	98.19	4.05	7.43	0.15	0.127 (-0.127)	100	0.944	4.87	5.06	5.40	1.091 (4.488)
	Vf6-Ta	3997.3 - 3634.5 3273.2 - 2910.4 2549.1 - 2186.4 1825.1 - 1460.9 1101.0 - 736.8	175	6	97.17	5.12	5.94	3	95.74	6.15	4.84	0.25	-0.12 (0.12)	100	0.833	7.95	8.07	3.23	10.018 (6.715)
	Vf7-Ta	3997.4 - 3271.9 2910.6 - 2547.8 2186.5 - 1461.0	175	7	99.44	2.29	13.40	1; 3; 4	98.44	3.74	8.01	0.31	-0.0607 (0.0607)	100	0.823	8.10	8.23	3.50	6.358 (10.845)
	Vf8-Ta	3997.4 - 2909.2 2186.5 - 1098.2	175	6	98.99	3.07	9.94	2	98.58	3.56	8.40	0.28	-0.0992 (0.0992)	100	0.936	5.91	6.10	4.42	1.14 (5.254)
2016	Vf1-Ta	3638.9 - 2917.7 1839.0 - 758.1	175	3	99.35	2.13	14.5	1; 2; 4	99.35	2.45	12.40	0.14	-0.0122 (0.0122)	100	0.995	2.97	2.98	9.46	-0.132 (0.591)
	Vf2-Ta	3997.4 - 3637.5 3279.0 - 2557.7 1839.3 - 1478.0 1119.5 - 758.1	175	3	97.08	5.18	5.85	1; 3; 4	95.98	6.01	4.99	0.17	- 0.128 (0.128)	100	0.91	7.11	7.07	3.30	4.068 (4.982)
	Vf3-Ta	1839.3 - 1478.0 1119.5 - 758.1	175	5	98.55	3.71	8.29	1, 3; 4	98.05	4.21	7.17	0.36	-0.0452 (0.0452)	100	0.969	6.90	6.86	3.75	1.591 (1.526)
	Vf4-Ta	3997.4 - 2917.7 1893.3 - 1118.0	175	6	99.52	2.10	14.5	2	99.30	2.49	12.00	0.29	-0.138 (0.138)	100	1.016	10.70	10.70	2.47	-1.602 (-0.025)
	Vf5-Ta	3997.4 - 3277.6 2199.2 - 1118.0 759.5 - 399.6	175	1	97.93	4.37	6.95	1; 3; 4	97.78	4.50	6.70	0.047	0.0772 (-0.0772)	100	0.933	8.21	8.19	2.97	3.207 (3.522)
	Vf6-Ta	3997.4 - 3637.5 2919.1 - 2557.7 2199.2 - 1118.0 759.5 - 399.6	175	8	97.69	4.65	6.58	2	90.95	8.95	3.36	0.36	-1.35 (1.35)	100	0.756	11.10	11.20	2.41	10.274 (14.152)
	Vf7-Ta	1839.3 - 758.1	175	2	98.36	3.97	7.80	1; 3; 4	98.20	4.11	7.46	0.11	-0.0171 (0.0171)	100	0.999	5.98	5.95	4.25	-0.077 (0.15)
	Vf8-Ta	3997.4 - 3637.5 1839.3 - 758.1	175	3	98.22	4.06	7.5	1; 2	97.49	4.77	6.32	0.14	0.0156 (-0.0156)	100	0.997	7.97	7.93	3.39	0.249 (0.013)

Publications

Journals

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. DOI:10.1007/s11104-018-03904-y.

Talks

Streit J, Meinen C, Rauber R (2017) Oberirdische Biomasse und Wurzelverteilung verschiedener Weißklee-Genotypen im Gemenge mit Deutschem Weidelgras und Zichorie. Mitt. Ges. Pflanzenbauwiss. 29, 112-113, Witzenhausen, Germany.

Streit J, Meinen C, Rauber R (2016) Quantitative Analyse der Wurzelverteilung in einem Winterackerbohnen-Winterweizen Gemenge mittels Fourier Transform Infrarot (FTIR) Spektroskopie. Mitt. Ges. Pflanzenbauwiss. 28, 54-55, Gießen, Germany.

Streit J, Meinen C, Rauber R (2016) Quantitative analysis of the root distribution in a faba bean-wheat intercropping system by Fourier transform infrared (FTIR) spectroscopy. Second International Legume Society Conference ILS2, p. 274, Tróia, Portugal.

Posters

Meinen C, **Streit J**, Legner N, Naumann A, Rauber R (2018) FTIR-Spectroscopy for root discrimination in multi-species mixtures. 10th Symposium of the International Society of Root Research. Israel.

Streit J, Meinen C, Rauber R (2018) Root distribution of poplar and robinia in a short rotation coppice mixture. PLANT 2030 Status Seminar. Potsdam, Germany.

Streit J, Meinen C, Rauber R (2017) FTIR spectroscopy analysis on root distribution in white clover-ryegrass mixtures. PLANT 2030 Status Seminar. Potsdam, Germany.

Streit J, Meinen C, Rauber R (2016) Quantitative analysis of the root distribution in a faba bea-wheat intercropping system by FTIR spectroscopy. PLANT 2030 Status Seminar. Potsdam, Germany.

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Eidesstattliche Erklärung

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

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2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

   Göttingen, den ....

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   (Unterschrift)