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Root and shoot phenotypic traits and their expression in response to sowing density in spring barley (*Hordeum vulgare*)

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To all I love.

“There is more than you can see, obviously.”

PRELIMINARY WORDS

When I started my project finding phase for my PhD at IBG-2 at FZJ in July 2012, I did not know much about plants, especially roots, although I had studied Biology at the University of Bonn. Thus, most information in the literature I read was new to me and fascinating, especially the interaction between roots and their habitat (the soil or media they can grow in) and the roots' interaction with each other – within one plant and with a neighboring one. Further, the discrepancy between the "natural" habitat (i.e. everything outside the greenhouse, climate chamber, laboratory; with field soil) and the "artificial" growth conditions (i.e. everything inside a greenhouse, climate chamber, laboratory; mostly with substrate, on gels, in water) of plants and their roots captured me and the idea how to overcome that discrepancy seemed an interesting challenge.

These topics can fill one's life time, though, and I am very grateful to Fabio Fiorani (with whom I discussed a lot during the project finding phase and who became my group leader), Johannes Postma, Vicky Temperton, and Kerstin Nagel (who became my three official internal supervisors), and Dagmar van Dusschoten and Ulrich Schurr who helped me to reduce this broad field to a few research questions which to answer seemed to be feasible.

That performing the various experiments to address these questions rose only more questions, is another story that I will touch at the end of my thesis. ☺

SUMMARY

Much is known about plant performance under controlled conditions and much about performance under field conditions as well as on single plants and populations. The extent, however, to which the variability of traits, especially root traits, in single plants and individuals in a population differs from each other was still unclear.

To increase the comparability of results of experiments or studies in controlled conditions and in field trials it is crucial to investigate not only single plants but plants in populations, both in the lab and the field, since outside – both at an agronomic and a natural field-site – plants virtually never grow as one single individual but in a population or cluster (as plants of the same species growing in the same area or volume of substrate). Individuals growing in the same substrate will interact with each other within a defined volume, especially when they are larger in size and the amount of interaction will increase over time. The effects of plant-plant interactions on the plant phenotype and particular the root phenotype will depend on plant density. Therefore, I focused on sowing density as a critical aspect of the systematic comparison of plant root architecture.

In this thesis, I investigated the plant performance of spring barley (*Hordeum vulgare* L.), an important crop in Germany but also worldwide, since little was known about the barley root system. Hence, I studied spring barley grown as single plants and in clusters at various sowing densities both in rhizotrons and pots in the greenhouse, climate chamber, and outdoors at Forschungszentrum Jülich, Germany, and at a field site at Campus Klein-Altendorf, Germany, as presented in the following four chapters:

1) In the first chapter 3.1 (Hecht *et al.* 2016), I was interested in what a response curve to sowing density looks like. I focused on the results of the field trial and provided response curves of several traits to sowing density: Root length density (RLD), specific root length (SRL) especially in the 0-10cm topsoil layer, specific leaf area (SLA), the depth above which 50% of the fine roots are located (D50 of fine roots), stem mass fraction (SMF), and final grain yield increased with increasing sowing density. In contrast, shoot dry weight (SDW) per plant, tiller number per plant, and root mass fraction (RMF) decreased. SDW per tiller, leaf mass fraction (LMF), and D50 of major root axes were not affected by sowing density. Based on these findings, I concluded that observed responses may suggest that competition for light was greater than for nutrients.
2) In the second chapter 3.2 (Hecht *et al.* 2018), I addressed how the measured alterations of traits at the crop level (described in chapter 3.1) could be explained by changes of traits or trait components at the individual plant and organ level. I observed that, per plant, SDW, tiller number but also nodal root number decreased with increasing sowing density, while the branching angle, the lateral branching frequency, the number of seminal roots, and the ratio of nodal roots per tiller were not affected, however, the later increased over time. The older a plant was, the more tillers it produced (the maximum number depended on sowing density) and the tillers bore on average more nodal roots. Nonetheless, even in old plants at flowering, I found tillers with no nodal roots. Furthermore, the ratio of seminal (= smaller in diameter) to nodal roots (= thicker in diameter) increased with sowing density. In summary, I concluded that RLD increased because the number of roots increased (seminal and nodal roots) per area and the increased ratio of seminal to nodal roots may explain the greater SRL. In addition, I proposed a formula to estimate RLD from root counts, using the number of main axes, the lateral branching frequency, and the average length of a lateral root.
3) In the third chapter 3.3 (submitted to Annals in Botany, December 2018), I studied the differences in the response to sowing density of two lines with contrasting root systems. Here, I asked if a bigger root system selected in the greenhouse could be reproduced in the field and if so, if this bigger root system led to other changes in traits, like RLD or final grain yield. Further, I asked what factors might help in translation from lab to field. I observed that the two genotypes sometimes differed a lot and sometimes were very similar depending on the time point of sampling and the measured trait. Nonetheless, I could reproduce the bigger root system-phenotype partly but not as strong as described in literature. Despite the sometimes bigger root system, that line did not have greater final grain yield. For translation from lab to field, apart from temperature and light, the time point (i.e. the developmental stage of the plant or plant age) of the observation or measurement and the growth environment seem crucial.
4) The fourth chapter 3.4 (Burkart *et al.* 2018) is about using the green-red-vegetation-index (GRVI) of an image taken by a normal RGB-camera installed on a drone or un-manned aerial vehicle (UAV) to determine the developmental stage of a crop. I recorded the developmental stage using the BBCH scale and provided the data for correlating the BBCH stage to the measured GRVI. I concluded that it is indeed possible to use the GRVI to determine BBCH, however, the correlation needs to be set up individually for each crop and can then be used as a tool to determine BBCH. The development of this technique is thereby promising for future research, whereas in my study I still had to rely on manual measurements to determine the developmental stage of the plants.

In summary, in the course of this thesis, I gained deeper insight into plant and especially root plasticity in response to sowing density. For instance, nodal root formation was strongly associated with tiller formation and RLD with the number of roots. As sowing density affected plant growth earliest about four weeks after sowing, I recommend to take sowing density into account when experiments run longer than three weeks. Further, with respect to lab field translation, not only sowing density but also the developmental stage and plant age should be taken into account.

KURZFASSUNG

Das Wachstum von Pflanzen sowohl unter kontrollierten und unter Feldbedingungen als auch von Einzelpflanzen und von Pflanzenpopulationen ist gut untersucht. Inwieweit sich die Variabilität der einzelnen Merkmale, insbesondere der Wurzel, in Einzelpflanzen und Individuen innerhalb einer Population unterscheiden, war allerdings noch unklar. Um die Vergleichbarkeit der Daten aus verschiedenen Experimenten oder Studien zu erhöhen, ist es jedoch notwendig, nicht nur Einzelpflanzen sondern auch Individuen in einer Population, sowohl im Feld als auch im Gewächshaus, zu untersuchen. Pflanzen wachsen nämlich sowohl auf Agrarflächen als auch in der Natur nahezu niemals als Einzelpflanzen sondern in Populationen bzw. Beständen („cluster"). Individuen, die im gleichen Bodengrund wachsen, werden zwangsläufig miteinander interagieren, insbesondere je größer sie sind, sodass ihre Interaktion mit der Zeit zunehmen wird. Die Auswirkungen dieser Pflanz-Pflanz-Interaktion auf den Phänotyp, insbesondere der Wurzel, hängen von der Pflanzdichte ab. Daher konzentrierte ich mich auf Pflanzdichte als wichtigen Aspekt im systematischen Vergleich der Pflanzenwurzelarchitektur. In dieser Arbeit analysierte ich das Wachstum von Sommergerste (*Hordeum vulgare* L.), eine bedeutende Kulturpflanze in Deutschland aber auch weltweit, da bisher wenig ihr Wurzelsystem bekannt war. Ich untersuchte Einzelpflanzen und Bestände von Sommergerste bei verschiedenen Saatdichten sowohl in Rhizotronen und Töpfen im Gewächshaus, in der Klimakammer und draußen im Forschungszentrum Jülich als auch in der Feldstation Campus Klein-Altendorf. Die Ergebnisse stelle ich in den folgenden vier Kapiteln vor:

1) Im ersten Kapitel 3.1 (Hecht *et al.* 2016) interessierte mich, wie eine Saatdichten-Wirkungskurve („response curve") aussehen würde. Hier nutzte ich die Felddaten und erstellte Wirkungskurven für zahlreiche Merkmale: Wurzellängendichte (RLD), spezifische Wurzellänge (SRL) insbesondere des Oberbodens (0-10 cm), spezifische Blattfläche (SLA), die Bodentiefe, über welcher sich 50 % der Feinwurzeln befinden (D50), Massenanteil des Halmes (SMF) und Kornertrag stiegen mit zunehmender Saatdichte. Im Gegensatz dazu sanken das Sprosstrockengewicht (SDW) und die Anzahl der Bestockungstriebe pro Pflanze und der Massenanteil der Wurzeln (RMF). Das Sprosstrockengewicht pro Bestockungstrieb, der Massenanteil der Blattscheiden (LMF) und D50 der Hauptwurzelachsen wurden von der Saatdichte nicht beeinflusst. Aufgrund dieser Ergebnisse schlussfolgerte ich, dass die beobachteten Auswirkungen von Saatdichte auf das Pflanzenwachstum in meinen Experimenten eher durch Konkurrenz um Licht als um Nährstoffe ausgelöst wurden.
2) Im zweiten Kapitel 3.2 (Hecht *et al.* 2018), befasste ich mich damit, inwieweit die im ersten Kapitel beschriebenen Merkmalsänderungen im Bestand durch Änderungen von Merkmalen bzw. ihrer Komponenten auf Einzelpflanzen- bzw. auf Organebene erklärt werden könnten. Ich beobachtete, dass pro Pflanze das Sprosstrockengewicht, die Anzahl der Bestockungstriebe und die Anzahl der Nodalwurzeln mit steigender Saatdichte sanken. Der Wurzelwinkel, die Seitenwurzelfrequenz, die Anzahl der Seminalwurzeln und das Verhältnis von Nodalwurzeln zu Bestockungstrieben waren für alle Saatdichten gleich, wobei das letztere mit der Zeit zunahm. Je älter eine Pflanze war, umso mehr Bestockungstriebe wies sie auf, wobei das Maximum von der Saatdichte abhing. Die Bestockungstriebe älterer Pflanzen hatten im Durchschnitt mehr Nodalwurzeln. Jedoch hatten manche Bestockungstriebe während der Blüte keine Nodalwurzeln. Des Weiteren nahm das Verhältnis von Seminal- (dünner) zu Nodalwurzeln (dicker) mit der Saatdichte zu. Ich schlussfolgerte, dass der Grund für die Zunahme der Wurzellängendichte die steigende Anzahl von Seminal- und Nodalwurzeln pro Fläche war und ihr steigendes Verhältnis möglicherweise die spezifische Wurzellänge vergrößerte. Zusätzlich erstellte ich eine Formel, mit der die Wurzellängendichte anhand der Anzahl der Hauptwurzelachsen, der Seitenwurzelfrequenz und der mittleren Seitenwurzellänge geschätzt werden kann.
3) Im dritten Kapitel 3.3 (eingereicht bei Annals in Botany, Dez. 2018) untersuchte ich die Wirkung von Saatdichte auf zwei Genotypen mit unterschiedlichen Wurzelsystemen. Meine Fragen waren, ob ein größeres, im Gewächshaus selektiertes Wurzelsystem ebenfalls im Feld reproduziert werden könnte und wenn ja, ob dieses größere Wurzelsystem Merkmale, wie Wurzellängendichte oder Kornertrag, beeinflussen würde. Weiterhin wollte ich wissen, welche Faktoren bei der Übertragung von Gewächshausergebnissen aufs Feld („translation from lab to field") helfen könnten. Die beiden Genotypen unterschieden sich abhängig vom Zeitpunkt der Probennahme und dem Merkmal teils sehr stark, aber ähnelten sich teils auch sehr. Die Reproduktion des Wurzelphänotyps war teilweise erfolgreich im Feld, jedoch nicht so ausgeprägt wie in der Literatur beschrieben. Trotz des größeren Wurzelsystems hatte dieser Genotyp keinen erhöhten Kornertrag. Für die Übertragung von Gewächshausergebnissen aufs Feld spielen neben Temperatur und Licht der Zeitpunkt, d.h. das Entwicklungsstadium und das Alter der Pflanze, und das Wachstumsumfeld eine wichtige Rolle.
4) Das vierte Kapitel 3.4 (Burkart *et al.* 2018) handelt vom Grün-Rot-Vegetationsindex (GRVI) und seiner Korrelation mit dem Entwicklungsstadium der Pflanzen. Mit einer handelsüblichen RGB-Kamera, die an einem unbemannten Flugkörper (UAV) befestigt war, wurden Bildern von Feldpflanzen gemacht wurden. Das Entwicklungsstadium nahm ich mittels der sogenannten BBCH-Skala manuell auf und stellte diese Daten zur Korrelation mit dem zeitnah gemessenen GRVI zur Verfügung. Tatsächlich ist es möglich, mit Hilfe der GRVI-Kurve das Entwicklungsstadium zu bestimmen, jedoch muss die Korrelation für jede Spezies individuell vorab bestimmt werden. Die Entwicklung dieser Technik ist vielversprechend, bedeutet sie doch ein enormes Zeitersparnis im Vergleich zur manuellen Messung wie in meiner Arbeit.

Im Rahmen meiner Arbeit habe ich ein tieferes Verständnis für die Plastizität von Pflanzen und insbesondere ihrer Wurzeln in Abhängigkeit von Pflanzdichte entwickelt. So war zum Beispiel die Bildung der Nodalwurzeln stark mit der Bildung der Bestockungstriebe assoziiert. Da ein Einfluss der Saatdichte frühestens nach vier Wochen messbar war, empfehle ich Pflanzdichte in Experimenten, die länger als drei Wochen dauern, zu berücksichtigen. In Bezug auf die Übertragung von Gewächshausergebnissen aufs Feld sollte neben der Pflanzdichte auch das Entwicklungsstadium und das Alter der Pflanze berücksichtigt werden.

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1. Introduction

1.1 Spring barley (*Hordeum vulgare* L.)

Within this PhD thesis, I used spring barley (*Hordeum vulgare* L.) as a model plant for my experiments. As an important plant for agriculture (Tab. 1), spring barley has been cultivated in Eurasia (including Germany) for about 10,000 years and thus, spring barley is adapted to the European cool-summer humid continental climate conditions (Badr *et al.* 2000; Zohary *et al.* 2012).

According to the Food and Agricultural Organization of the United Nations (FAOSTAT 2012), cereals were the most produced crop plants in the world including maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum* ssp.), and barley (*Hordeum vulgare* L.) in 2010. All four cereals were ranked among the top 10 of the most produced crop plants, forming the top 4 of the most produced cereals worldwide (Tab. 1). Moreover, they have a long agricultural history with their origin in different regions of the world and nowadays cultivated and produced worldwide (Piperno and Flannery 2001; Matsuoka *et al.* 2002; Piperno *et al.* 2004, 2009; Ranere *et al.* 2009). In Germany, barley was on rank 4 of the most produced crop plant behind sugar beet, wheat, and potato in 2016 (FAOSTAT 2012), with a production of nearly 10.73 t (spring and winter barley together). Spring barley is mostly used for malting, whereas winter barley is mostly used for feeding (Proplanta 2006).

Tab. 1 World production of total production of cereals in 2010 and top 10 of most produced crop plants in the world in 2010 (FAOSTAT 2012). Data downloaded on 27 July 2012 and 01 October 2018. According to FAOSTAT (2012), numbers may include official, semi-official or estimated data.

Crop plant	World production [t]	
	2010	2016
Cereals, total	2,466,501,240	2,848,661,914
Maize	851,348,928	1,060,107,470
Rice, paddy	701,108,595	740,961,445
Wheat	640,327,135	749,460,077
Potatoes	332,513,046	376,826,967
Soybeans	264,942,943	334,894,085
Vegetables, fresh, not elsewhere specified	259,721,091	290,130,864
Cassava	240,698,482	277,102,564
Oil palm fruit	223,437,286	300,252,193
Tomatoes	153,240,438	177,042,359
Barley	123,303,344	141,277,993

Spring barley belongs to the *Triticeae* in *Poaceae* (von Bothmer and Jacobsen 1985) and its aboveground vegetative unit is, like in most other grasses, a tiller (Hodgson 1990). Within my studies, I used the German spring barley cultivar Barke that is often used in science (Gahoonia and Nielsen 2004; Schmalenbach and Pillen 2009; Auškalnienė *et al.* 2010; Dornbusch *et al.* 2011; Castillo *et al.* 2012; Füllner *et al.* 2012; Alqudah and Schnurbusch

2015), an introgression line, S42IL-176, and its German parent Scarlett, another spring barley cultivar. This introgression line is a cross of Scarlett and an Israeli wild accession (Schmalenbach and Pillen 2009; Schmalenbach *et al.* 2011). S42IL-176 exhibits 3 small introgressions on chromosome 1H, 2H, and 3H and one large introgression on chromosome 5H of Israeli genes into the German genome but bears a much greater root system (RDW, root volume, maximum rooting depth (MRD)), greater tiller number, and a more spreading growth habit (planophil) than its German parent (Schmalenbach *et al.* 2011; Naz *et al.* 2012, 2014).

1.2 Plant plasticity – responses to light, temperature, and nutrient availability

Since plant growth is dependent on the environmental conditions (Sultan 2000; Bingham and Bengough 2003; Hodge 2004; Bradshaw 2006; Walter *et al.* 2009; Lande 2009; Nicotra *et al.* 2010), it is important to be aware of this plasticity, if one wants to compare results of different experiments. Varying light, temperature, and nutrient availability, for instance, can affect plant growth enormously, as described in the following paragraphs.

The red and blue light of the incoming light are absorbed by the plants and used for photosynthesis, while the far-red light is reflected by or transmitted through the leaves (Woolley 1971; Holmes 1981). Thereby, the ratio of red (R) to far-red (FR) light is declining (Holmes 1981; Kasperbauer and Karlen 1986; Kasperbauer 1987). This altered light composition, i.e. reduced R/FR ratio, within a crop leads to a reduction in tiller production of the individual plants (Casal *et al.* 1986; Davis and Simmons 1994). Low light itself has also been shown to reduce tiller formation in grasses and cereal crops in comparison to normal lighted plants (Kamel 1959; Kays and Harper 1974; Casal *et al.* 1986). Belowground, reduced light decreased root weight per plant in barley (Kamel 1959) and increased SRL in the grass *Lolium perenne* (Evans 1983). Thus, both, low light and a reduced R/FR ratio, cause a decrease in tiller number of a plant regardless if the incoming radiation is altered by neighboring plants or artificially. As Kamel (1959) already argued roots are dependent on carbohydrates produced by the shoot and if shoot growth is limited by e.g. light intensity, root growth is probably also limited.

Temperature, similarly to light, has been shown to slow down tiller formation in some grasses and barley at very low and very high temperatures (Clark 1969; Bade *et al.* 1985; Füllner *et al.* 2012; Hossain *et al.* 2012). Further, increases in temperature below 15 °C accelerate flowering and thus development of the plant, while further increases in temperature above 15 °C had no effect (Karsai *et al.* 2008). Naturally, a temperature gradient occurs in the soil (Blume *et al.* 2010) but in lab experiments, soil temperature is often not controlled. In oilseed rape (*Brassica napus*), a vertical temperature gradient in the medium (nutrient solution in a hydroponic rhizotron) increased length of the total root system, number and density of lateral roots and rooting density in upper regions of the medium compared to a uniform temperature treatment (Nagel *et al.* 2009). Likewise, in barley, increasing soil temperatures led to greater root and shoot biomass and accelerated the developmental stages, whereas the greatest biomass gain was achieved when a temperature gradient instead of uniform temperature was applied (Füllner *et al.* 2012). Additionally, this gradient caused an accumulation of roots in the topsoil layer and a greater proportion of thicker roots. The accelerating effect of

temperature on plant development is also the reason why thermal time has been introduced to correct for the influence of temperature on the shoot development, when comparing data of different experiments, already about 30 years ago (McMaster and Smika 1988; McMaster and Wilhelm 1997, 2003; Miller *et al.* 2001). Thus, I transferred my data of the different experiments into growing degree days, although it was unclear if this strategy would work for root data as well as for shoot data, as roots experience soil temperatures much different from the air temperature (Carter 1928).

Under low nutrient concentration, Drew (1975) showed for example that root proliferation was enhanced in nutrient rich regions, in order to increase nutrient capture. While shallow, dense roots are more important for phosphate acquisition (Shane *et al.* 2003; Shane and Lambers 2005; Miguel *et al.* 2015), steep/deep roots are the most important trait for nitrogen (N) acquisition (Thorup-Kristensen 2001, 2006; Lynch 2013) and water uptake (Wasson *et al.* 2012; Lynch 2013). A reduced lateral branching frequency was even found to improve drought tolerance in maize (Zhan *et al.* 2015). Further, a change in branching angle towards stepper roots resulted in a much deeper rooting depth (Manschadi *et al.* 2008; Dathe *et al.* 2013) and thereby enhance nutrient uptake from deeper soil and hence plant productivity. Interestingly, a reduction of crown roots per plant in maize also led to an enhanced N acquisition in low N soils, as the rooting depth of these plants with less crown roots was simultaneously enlarged (Saengwilai *et al.* 2014). Deep growing roots have been shown to be important for N acquisition especially from deep soil (Thorup-Kristensen 2006; Kristensen and Thorup-Kristensen 2009). Thus, plants either grow more fine roots or deeper roots depending on the limiting nutrient. The link of some root architectural traits to a certain function was also recently reviewed by Paez- Garcia *et al*. (2015).

These studies show that root growth can be very plastic in response to both light and nutrient availability. Plant density affects both factors, and thereby I expected similar responses of roots to density.

1.3 Plant density and plant-to-plant-interaction

Apart from the very obvious differences of the climate conditions in greenhouse and field experiments described above, plant density is another prominent difference between lab and field experiments. Fig. 1 depicts a proposed approach that is needed to systematically compare lab and field results. Nowadays, most plant growth studies are performed on single plants grown in a pot or in an equivalent item in the greenhouse or lab, while farmers grow plants in a clusters (or populations, stands) in the field. This could complicate the translation of lab to field studies. Thus, I investigated plant performance, especially root growth and architecture, of single plants and clusters at different sowing densities. In the following paragraphs, I describe responses of shoot and the root growth to plant density and competition.

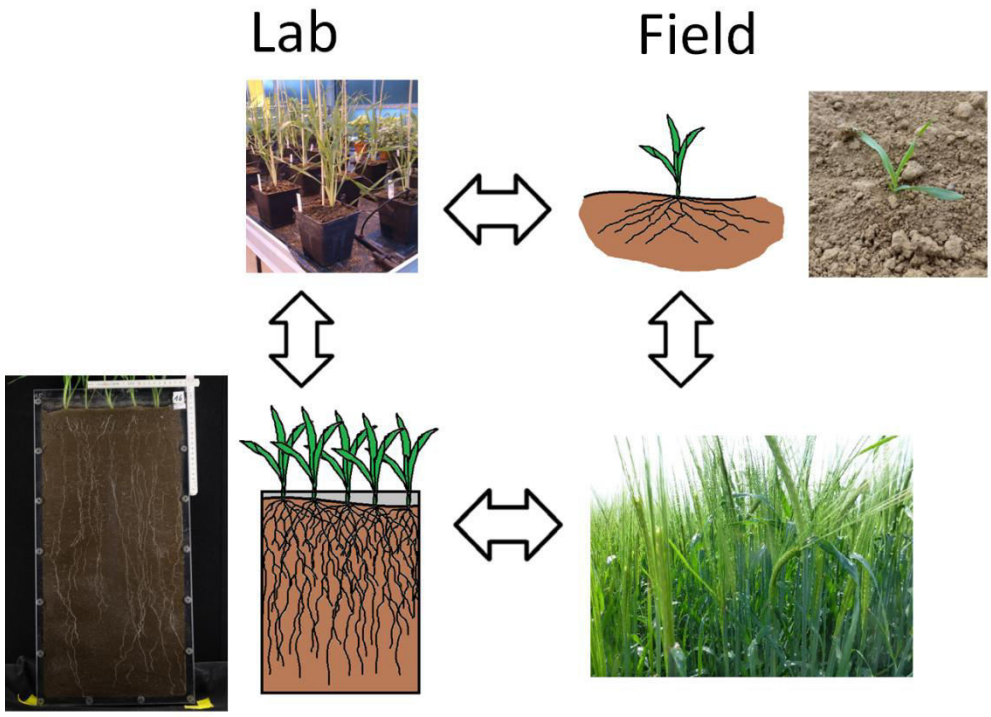

Fig. 1 Proposed approach needed to systematically compare variability of plant performance (by Vera Hecht). Additionally to single plants in the lab and stands or clusters in the field, plants in clusters (here shown in a rhizotrons) should be studied in the lab as well as single plants in the field.

Plant density has been shown to reduce the number of tillers per plant (Kamel 1959; Munir 2002; Turk *et al.* 2003; Soleymani *et al.* 2011), probably due to reduced light availability per plant (similar to low light response as described above), as well as shoot dry weight per plant (Harper 1977). At the same time, plant density increases the number of tillers per area (Kays and Harper 1974; Darwinkel 1978) and leaf number per area (Khalil *et al.* 2011; Moosavi *et al.* 2012). Additionally, leaf area per area (leaf area index: LAI) (Pospišil *et al.* 2000; Amanullah *et al.* 2007; Olsen and Weiner 2007; Moosavi *et al.* 2012) and specific leaf area (SLA) (Amanullah *et al.* 2007; Farshbaf-Jafari *et al.* 2014) increase with plant density, indicating a decreasing leaf thickness (Rodrigo *et al.* 1997; Amanullah *et al.* 2007; Abuzar *et al.* 2011; Khalil *et al.* 2011). Further, total biomass per area and yield per area increase with plant density (Kamel 1959; Singh and Singh 1981; Munir 2002; Farnia *et al.* 2014) and level-off at very high, supra-optimal densities (Singh and Singh 1981; Farnia *et al.* 2014). Weiner and Freckleton (2010) reviewed this response of biomass to increased plant density and concluded that total plant biomass per area was linearly proportional to plant density up to a critical plant density at which biomass per area saturated and not further increased, the final constant yield. Still, changes in biomass allocation may occur, as for example, plant height increases with density (Turk *et al.* 2003; Soleymani *et al.* 2011) and thus, stem mass fraction increases, as plants allocate more to stems than to leaves (Poorter *et al.* 2012).

Belowground, sowing density has been observed to decrease branching angle in grapevine and to increase RLD in the topsoil without affecting the ratio of primary, secondary and tertiary roots (Archer and Strauss 1985). In contrast, sowing density reduced the number of nodal roots per plant in maize (Demotes-Mainard and Pellerin 1992; Pellerin 1994). Kamel

(1959), who studied root growth of barley under different sowing densities, only examined root dry weights and root mass fraction and described both traits to be not affected by sowing density. However, he looked at rather medium to very high seeding rates (125-500 seeds m^{-2}) and it is unclear how the root samples were taken. The low root weights reported suggest that only a small part of the root system was collected. Furthermore, plant density has been shown to accelerate the uptake of soil water (Azam-Ali *et al.* 1984; Archer and Strauss 1989) and of nutrients, such as N, phosphate and potassium, (Gao *et al.* 2009; Ciampitti and Vyn 2011; Su *et al.* 2011; YS Li *et al.* 2014), simply because more plants take up more in a given time period.

In ecology, competition between plants rather than their density is studied. Nonetheless, the observations on root growth can be useful for understanding plant-to-plant interaction in a crop. In an ecological study, for instance, an accumulation of roots has been observed in mixtures of four grassland species (two grasses *Anthoxanthum odoratum* and *Festuca rubra* and two forbs *Leucanthemum vulgare* and *Plantago lanceolata*) which had increased total root biomass compared to the average of the monocultures. This additional root biomass – overyielding – was placed in the topsoil (Mommer *et al.* 2010). Thus, neighboring plants can induce the same response as a temperature gradient in the soil (described above). However, in the previously mentioned soil temperature studies (Nagel *et al.* 2009; Füllner *et al.* 2012), the density aspect was not taken into account and a direct comparison of single plants and plants grown in a population was not conducted. Other root growth responses to neighbors have been observed as well. For instance, Hodge *et al.* (1998, 1999) showed that the same species (*Poa pratensis*) grew differently within a nutrient patch depending on the presence or absence of a neighbor plant: single plants exhibit greater root length in the control treatment without any additional organic patch than in the treatment with an organic patch, but grown under competition, plants had increased root length in the organic patch.

Cahill *et al.* (2010) observed that the root systems of two neighboring plants (*Abutilon theoprasti*) tended to grow towards each other, regardless of the applied nutrient treatment (uniform and different positions of nutrient patches, namely at the edge of a pot or in the middle). However, pea (*Pisum sativum*) exhibited the opposite response to intraspecific neighbors (Gersani *et al.* 1998): in a split-root system a plant was grown with one half of the roots in one pot and the other half in an adjacent pot, making the plant a so-called fence-sitter. When competition was created in one of the two pots by planting other pea plants in that pot, the fence-sitter shifted its root system away from the pot with competition into the pot without any competition, while its total root biomass decreased only slightly compared to a fence-sitter without any competition. Indication for this mechanism of avoidance was also found by Archer and Strauss (1985) in grapevine as with increasing sowing density the branching angle became steeper.

In Fig. 2, growth performance of a species in a single plant (Fig. 2a) and a monocultural stand, i.e. a cluster (Fig. 2b, c), are depicted. Two strategies can be followed by the plants in the cluster: growth towards each other (Fig. 2b) or avoidance of the neighbor (Fig. 2c). Enhanced growth towards neighbor plants may result in accumulation of roots in the topsoil layer.

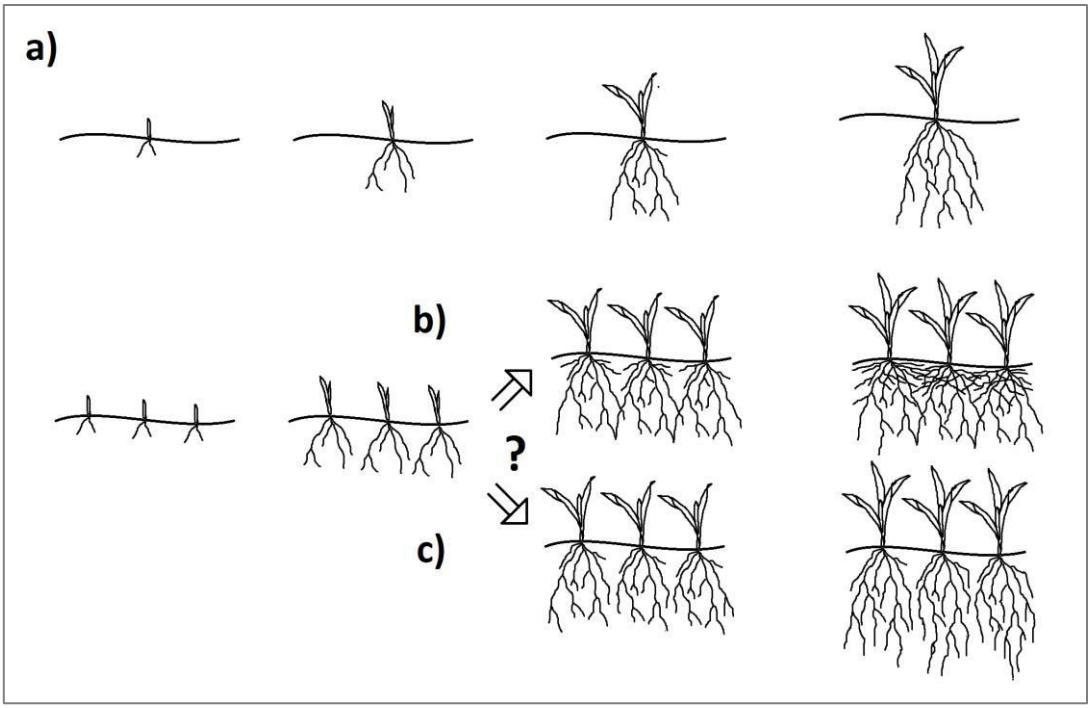

Fig. 2 Proposed growth performance of single plant (a) and monocultural population (b and c) with different alterations in root architecture (b: growth towards each other (Cahill *et al.* 2010) and accumulation of roots in topsoil layer, respectively; c: avoidance of the neighbor (Gersani *et al.* 1998) by steeper branching angle) (drawing by Vera Hecht).

1.4 Phenotyping methods

Currently, there are various phenotyping methods available and in use. In the last few years, non-invasive imaging techniques for plant phenotyping have been reviewed several times due to quickly new emerging methods and techniques (Fiorani and Schurr 2013; Araus and Cairns 2014; L Li *et al.* 2014; Humplík *et al.* 2015). Zhu *et al.* (2011) provided an overview including invasive techniques for root phenotyping. Here, the following descriptions focus on methods that I used within my experimental trials.

As non-invasive methods, I used rhizotrons (also called rhizoboxes, Fig. 3a-f), among others, in the so-called GrowScreenRhizo facility (Nagel *et al.* 2012). Rhizotrons are flat, deep boxes with a transparent plate or window at one side that allows non-invasive monitoring of root growth during the running experiment, however, only of roots that are visible at the transparent plate (Fig. 3d, e). I took images of the root system and determined the root length visible at the transparent window with RhizoPaint, a program in which roots are traced in the image and root length is determined both in pixel and cm. Moreover, I used nuclear magnetic resonance for visualizing the 3D structure of roots non-invasively during growth in a pot, the so-called magnetic resonance imaging (MRI) (Rascher *et al.* 2011; Metzner *et al.* 2014; van Dusschoten *et al.* 2016). It allows among others the determination of total root length and root counts at a certain depth. Additionally, I monitored the developmental stages of the plants in the field using the so-called BBCH scale (Lancashire *et al.* 1991) and provided the data for correlating to images of the plots in the field, in which I had measured BBCH, taken at the

same time by an unmanned aerial vehicle (UAV) controlled by Andreas Burkart. UAVs are commonly used for non-invasive phenotyping (Fiorani and Schurr 2013; Araus and Cairns 2014; L Li *et al.* 2014; Humplík *et al.* 2015). Here, it was equipped with a commonly used RGB-camera to photograph the plots and to obtain the greenness of the plants in a plot using a green-red-vegetation-index (GRVI) (Motohka *et al.* 2010).

$$GRVI = \frac{Green - Red}{Green + Red}$$

Equation 1 Formula of the green-red-vegetation-index (GRVI) which used the average green (Green) and the red (Red) values of an image as indicator of the greenness.

Fig. 3 Rhizotron experiments in climate chamber (a), in the GrowScreenRhizo facility (b), and outdoors at the research center Jülich (c). d) shows the root system visible at the transparent window of a single plant and e) of a cluster of five plants per rhizotron. At the end of the experiment, I cut the roots and the soil of the rhizotrons placed in the climate chamber into increments of 10 cm (f) before washing over a sieve using tap water (g). I scanned the washed roots, as shown for roots in h) of the outdoor placed rhizotron. Here, I washed the intact root systems of the middle plants and split it up into main root axes, i.e. seminal and nodal roots, just before scanning.

An invasive and destructive sampling method is the so-called shovelomics approach (Trachsel *et al.* 2010). I performed shovelomics in my field trials (Fig. 4a), in which I excavated plants with a shovel, washed the root system of the plant of interest, and determined several root traits of the root system, such as root counts, branching angle, and lateral branching frequency (Fig. 4b). Moreover, also in the field, I took soil cores with a cylinder of 9 cm in diameter that was mounted to a hydraulic, petrol-driven hammer (Fig. 4c, d). I washed the roots from the soil cores over a sieve, and scanned the washed and collected roots in a flatbed scanner and

determined root length and root diameter with WinRhizo^{TM}. At the end of all rhizotron and pot experiments, I performed a destructive harvest for collecting shoot and root samples (Fig. 3f-h).

Fig. 4 For shovelomics, basically only a shovel or spade and a bucket were required (a) for excavating the roots systems. I could phenotype the washed root system easily in the field (b). Taking soil cores was easier done with a cylinder mounted to a petrol-driven hammer (c), however, was still very labor-intensive. I also cut the soil cores into increments of 10 cm before washing of the roots for scanning in WinRhizo^{TM}.

1.5 Root system architecture

A root system architecture describes the 3D shape of a root system, including primary root, branch roots and root hairs (Osmont *et al.* 2007). In angiosperms, there are currently two main root systems accepted. One is the allorhizic root system (tap-root system), typically found in dicotyledons, having one primary root with lateral roots and rarely adventitious roots. Adventitious roots are roots that originate from non-root tissue, i.e. from the shoot, and are thus shoot-borne roots (Haissig 1973). The other one is the homorhizic root system (fibrous root system), typically found in monocotyledons like barley. It has many adventitious, shoot-borne roots that develop parallel to the primary root (Osmont *et al.* 2007). Moreover, the homorhizic root system can form additional embryonic roots (also emerging from the seed), the seminal roots (Jackson 1922; Hackett 1968; Osmont *et al.* 2007).

The barley root system (see also Fig. 5) consists of a seed-borne primary root (Hagemann 1957; Luxová 1986) and 4-7 seed-borne seminal roots (Jackson 1922; Krassovsky 1926; Hagemann 1957; Hackett 1969; Luxová 1986; Wahbi and Gregory 1995; Knipfer and Fricke

2011). The number of the seminal roots seems to be genetically determined (Robinson *et al.* 2016; Shorinola *et al.* 2018). Furthermore, the barley root system has typically adventitious roots. Barley usually grows adventitious roots from nodes of the stem or tillers (Jackson 1922; Krassovsky 1926; Hackett 1968), hence, I use the term nodal roots for roots emerging from a node and adventitious roots for all other shoot-borne roots which I found not very often and then in a low number of maximum three adventitious roots in my plants.

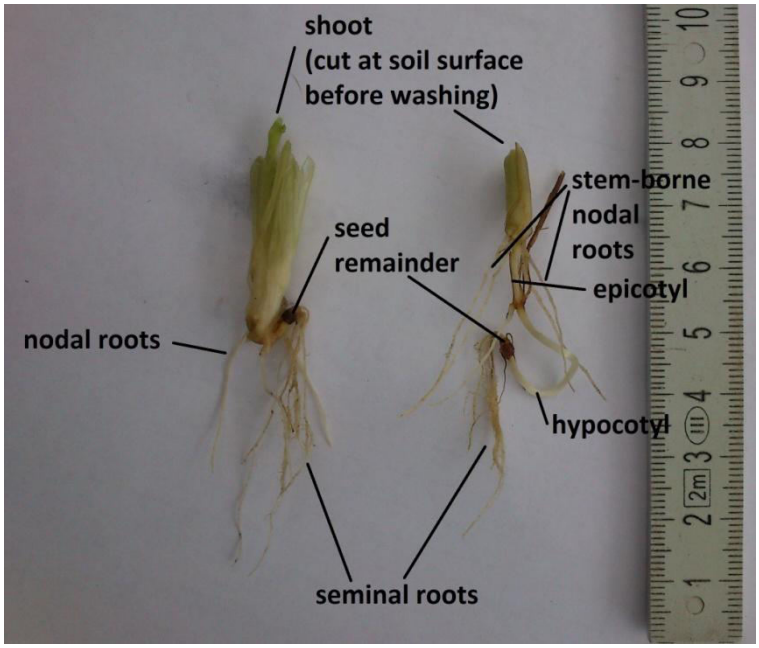

Fig. 5 The root systems and the shoot base of two spring barley plants washed from substrate. Before washing, I cut the shoots of the plants at the soil surface so that the plants were easier to handle. The seminal roots were thinner than the nodal roots. Note the stretched hypo- and epicotyls of the plant on the right side probably due to placing the seed with the so-called radicle from which the roots germinate upwards and with the so-called plumule from which the shoot will grow downwards.

Spring barley has a relatively small root and shoot system (in comparison to e.g. maize) and is thus, easy to handle in lab cultivations. Furthermore, its roots are sturdy and can therefore be washed from soil without too much damage (in comparison to e.g. soybean, personal observation). Jens Léon (University of Bonn) provided me seeds of two spring barley lines with contrasting root systems that are genetically very close (Schmalenbach *et al.* 2011; Naz *et al.* 2012, 2014), namely the introgression line S42IL-176 and one of its parents, Scarlett, a German spring barley cultivar. The introgression line S42IL-176 exhibits three small introgressions on the chromosomes 1H, 2H, and 3H and one large introgression on chromosome 5H. Further, it had a greater tiller number, a greater root biomass and volume, and greater root length in comparison to Scarlett (Naz *et al.* 2012, 2014).

2. Main research questions and hypothesis

Since not much was known about how sowing density would affect plant growth belowground in the field, I wanted to know to what extent and in what way biomass partitioning to roots was affected by sowing density in spring barley and what traits differed between single plants and clusters.

Further, I wanted to know what a response curve to sowing density looked like. Did roots and shoots respond in a similar way to sowing density? How would sowing density influence plant growth, crop production (final grain yield) and biomass allocation? To address these questions, I conducted two sowing density field experiments with spring barley over two consecutive years at the research facility of University of Bonn, at Campus Klein-Altendorf, Germany.

My hypotheses were, first, with increasing sowing density, root length density (RLD) and specific root length (SRL) would increase due to relatively greater investment into fine roots. Second, I expected an accumulation of roots in the topsoil, i.e. greater root length density in the topsoil in higher sowing densities, as an accumulation of roots in the topsoil in polycultures in comparison to monocultures have been observed.

The main findings of the field experiments (described in chapter 3.1 *Sowing density in the field* and in chapter 3.2 *Changes in RLD and SRL best explained by altered ratio of seminal to nodal roots*) revealed an increase in RLD and SRL, especially in the 0-10 cm topsoil layer, which led to the question what traits components at the individual plant level are behind the observed changes of RLD and SRL at crop-level. Thus, I performed rhizotron experiments with the same spring barley lines to monitor roots during plant growth followed by destructive harvests, when roots reached the bottom of the rhizotron. Additionally, I excavated root crowns of field grown spring barley plants to receive information on root system architecture.

I hypothesized that increased lateral branching frequency at higher sowing densities would cause an increased RLD and SRL and the accumulation of roots in the topsoil.

As the two investigated spring barley cultivars responded in a very similar way (described in chapter 3.1 *Sowing density in the field* and in chapter 3.2 *Changes in RLD and SRL best explained by altered ratio of seminal to nodal roots*), I was wondering if phenotypes with a contrasting root system at different sowing densities would help in translation of lab to field. Therefore, I used two spring barley lines with contrasting root systems – the first was a cross of a German high-yielding spring barley cultivar and an Israeli wild accession which had a much greater root system and as the second the German parent – in a range of various experiments (field, rhizotron in climate chamber, greenhouse, and outdoor, and pot) at two sowing densities. I asked if the selection for certain root traits under lab conditions worked and if the introgression line differed in the field with respect to important agronomic traits. What (other) factors may help in translation from lab to field?

Manual phenotyping of plants in the field can take a lot of time, especially, if at a great temporal frequency to monitor the complete growth phase. Therefore, while I was scoring the

developmental stage of the plants using the BBCH scale, which could take an entire day, Andreas Burkart (IBG-2, FZJ until 2015, now at JB Hyperspectral Devices UG) acquired images in about 20 min with a commonly used RGB-camera of the same plots with an unmanned aerial vehicle to calculate the GRVI as an estimator of greenness. The question was, if the developmental stage could be linked to the GRVI and if the GRVI then could be used to determine the BBCH stage.

3. Main results and discussion

In the following paragraphs, I discuss the main results of my publications forming this thesis. For a detailed description of material and methods, results, and discussion, I refer to the publications themselves in chapter 9 “Publications of this thesis”.

3.1 Sowing density in the field

In general, the two spring barley cultivars Barke and Scarlett responded similarly, so that I pooled genotypes for the analysis. Aboveground, tiller number per plant was constant during the first 4 weeks of plant growth across all 10 sowing densities. After that, tiller number per plant declined exponentially with sowing density, while tillers per area increased linearly with sowing density (Fig. 6a). This reduction in tiller number per plant with increasing plant density had been reported for barley previously (Kamel 1959; Munir 2002; Turk *et al.* 2003; Soleymani *et al.* 2011). As shoot dry weight per tiller was constant over sowing density (Fig. 6b), though increased over time, shoot dry weight per area increased also linearly (Fig. 6c). Gains in biomass by increasing plant density are a well-known response (Kamel 1959; Singh and Singh 1981; Munir 2002; Turk *et al.* 2003; Farnia *et al.* 2014). Final grain yield per area increased with sowing density, however, leveling-off with a maximum of about 7.5 t ha^{-1} at about 230 seeds m^{-2} (Fig. 6c), following the final constant yield concept (Weiner and Freckleton 2010), and slightly declining at even greater, supra-optimal sowing densities, similar to findings of other studies (Singh and Singh 1981; Farnia *et al.* 2014).

Belowground, root dry weight per core (data not shown) increased with sowing density but less than shoot dry weight so that root mass fraction (RMF) declined linearly with increasing sowing density (Fig. 7a). Further, RMF declined over time, which has been shown for several other non-woody species (Yin and Schapendonk 2004; Poorter and Sack 2012; Wang *et al.* 2015; and specifically for barley Kamel 1959), as plants grow larger. For the response of RMF to density, contrasting results have been found. For instance, Berendse and Möller (2009) found that in *Plantago lanceolata* RMF increased with increasing plant density, however, only under low N supply but not under high N supply, concluding that the increased RMF to increased plant density under low N supply was probably a plasticity response of the plant to low N availability. For barley, RMF was not affected by plant density under normal/high nutrient availability (Kamel 1959), however, as indicated in the introduction, it is unclear how the data were obtain and thus, how reliable they are. In another study, shoot mass fraction (leaf and stem mass fraction pooled together) increased with increasing N levels, so that, by implication, RMF must have decreased (Ågren and Franklin 2003). I grew the plants at normal fertilization as recommended for spring barley, so that the decrease in RMF in response to plant density probably is an adaptive response to light competition, as plants partitioned more biomass aboveground, namely, SMF.

SMF increased with sowing density (Fig. 7b), as also observed by Poorter *et al.* (2012). However, since plant height was not significantly affected by sowing density, increased SMF are likely due to increased number of tillers and thus stems. LMF was constant over sowing density (Fig. 7c), since, although leaf area per area (data not shown) increased similar to findings in other studies (Pospišil *et al.* 2000; Amanullah *et al.* 2007; Olsen and Weiner 2007;

Moosavi *et al.* 2012), specific leaf area also increased with sowing density (Fig. 8), as also reported for maize and amaranthus (Amanullah *et al.* 2007; Farshbaf-Jafari *et al.* 2014). Hence, plants had thinner but larger leaves at greater sowing densities, as found in previous studies (Rodrigo *et al.* 1997; Amanullah *et al.* 2007; Abuzar *et al.* 2011; Khalil *et al.* 2011).

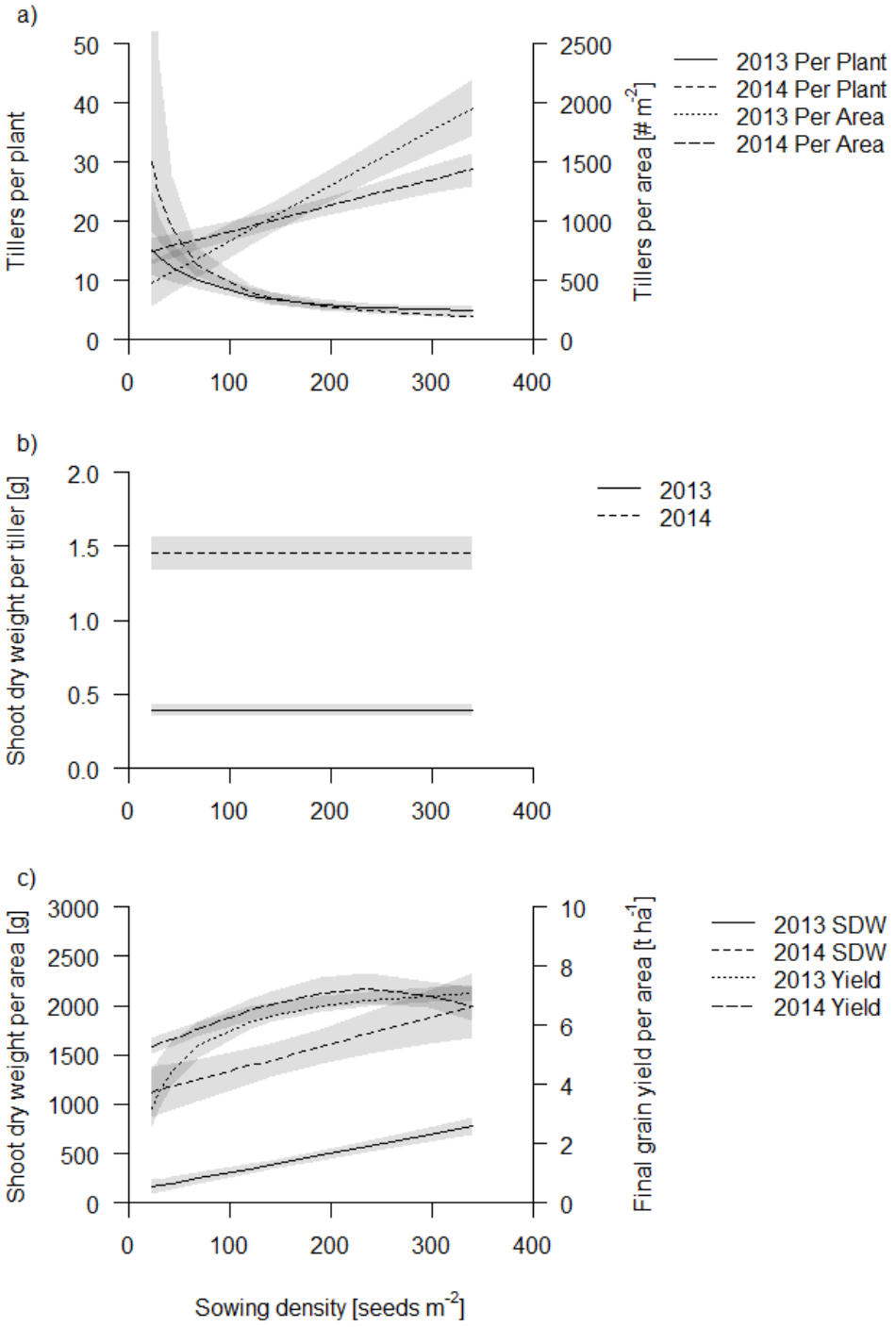

Fig. 6 Aboveground traits of the coring events of Scarlett and Barke in 2013 (solid line) and 2014 (dashed line) in the field at CKA. Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S1 in Hecht *et al.* (2016). (A) Tillers per plant and tillers per area; (B) Shoot dry weight per tiller; (C) Shoot dry weight per area and final grain yield. (Source: Fig. 2 in Hecht *et al.* (2016)).

3.1 Sowing density in the field

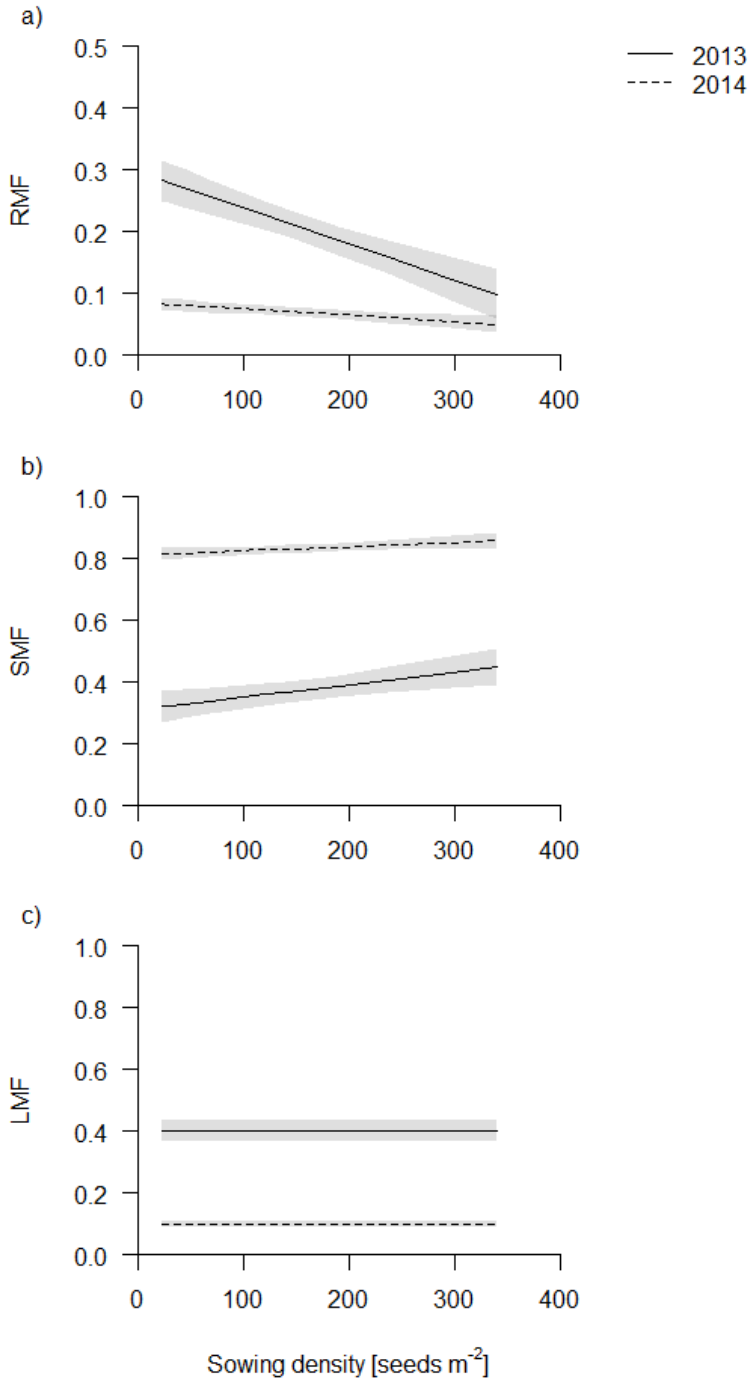

Fig. 7 Biomass partitioning of the coring in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S2 in Hecht *et al.* (2016). (A) Root mass fraction (RMF); (B) stem mass fraction (SMF); (C) leaf mass fraction (LMF). (Source: Fig. 3 in Hecht *et al.* (2016)).

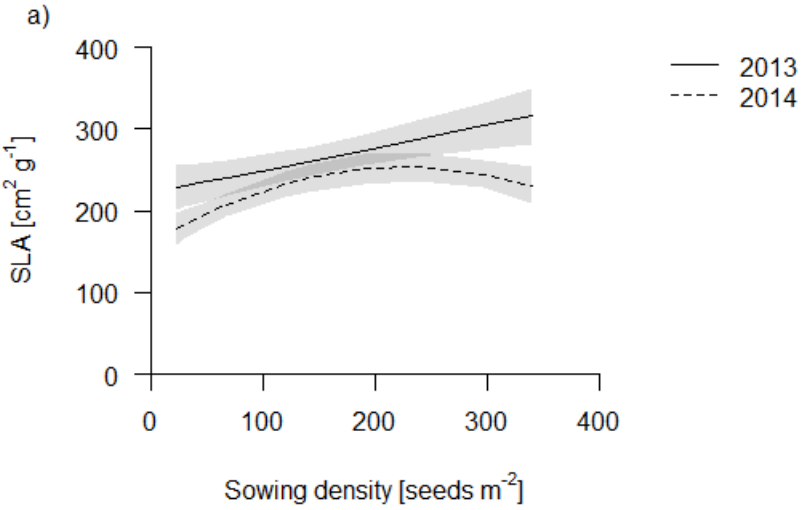

Fig. 8 Specific leaf area (SLA) of the coring in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S2 in Hecht *et al.* (2016). (Source: Fig. 5 in Hecht *et al.* (2016)).

Additionally, D50 of lateral roots increased with sowing density, except in 2014 within the plant row (Fig. 9a, b). This accumulation of roots in the topsoil has also been reported for several species (Tardieu 1988; Mommer *et al.* 2010; Kucbel *et al.* 2011; Chen *et al.* 2013; Ravenek *et al.* 2014). D50 of major root axes, however, was not affected, except in 2013 within the plant row, and was about 10 cm within the plant row in 2014 and increasing from about 18 cm to 10 cm in 2013 and deeper at about 22-23 cm between the plant rows (Fig. 9c, d). Thus, the difference in D50 of the major root axes between within the plant row and between the plant rows columns was 8 cm and 14 cm for 2013 and 2014, respectively. Using the half of the interrow distance (= 10 cm), the branching angle of the major root axes could be calculated as average angles of arctan(10/8) = 51° and arctan(10/14) = 36° degrees from vertical for both years (Fig. 10). The difference of the branching angles between the two years is caused by the small differences of the "in the row" D50 values, as D50 between the plant rows was approximately the same in both years.

SRL increased with sowing density strongest in the 0-10 cm soil layer (Fig. 9e, f). Here, within the plant row, SRL followed a saturating curve, slightly leveling off at very high sowing densities. Between the plant rows, SRL increased rather saturating with sowing density in 2013 and was constant in 2014. Greatest SRL were in between the plant rows in the 0-10 cm soil layer, whereas within the plant row greatest SRL were in the 10-20 cm soil layer, supposedly as in 0-10 cm the root crowns were located. SRL decreased with increasing depth (see also supplements in Hecht *et al.* (2016)). RLD was always greatest in 0-10 cm soil layer and greater within the plants row than between the plant rows, except for low sowing densities in 2013, which had between the plant rows greater RLD deeper down (10-20 cm and 20-30 cm soil layer) (Fig. 9g, h, supplements in Hecht *et al.* (2016)).

As RMF decreased with increasing sowing density and also over time, but plants increased RLD and SRL, the biomass was likely invested into more fine roots. To see what trait components were responsible for the changes in root distribution, I collected, among others,

data on lateral root branching frequency which is presented in the next chapter 3.2 Changes in RLD and SRL best explained by altered ratio of seminal to nodal roots and in Hecht *et al.* (2018) (chapter 9.2 *Plant density modifies root system architecture in spring barley (Hordeum vulgare L.) through a change in nodal root number*).

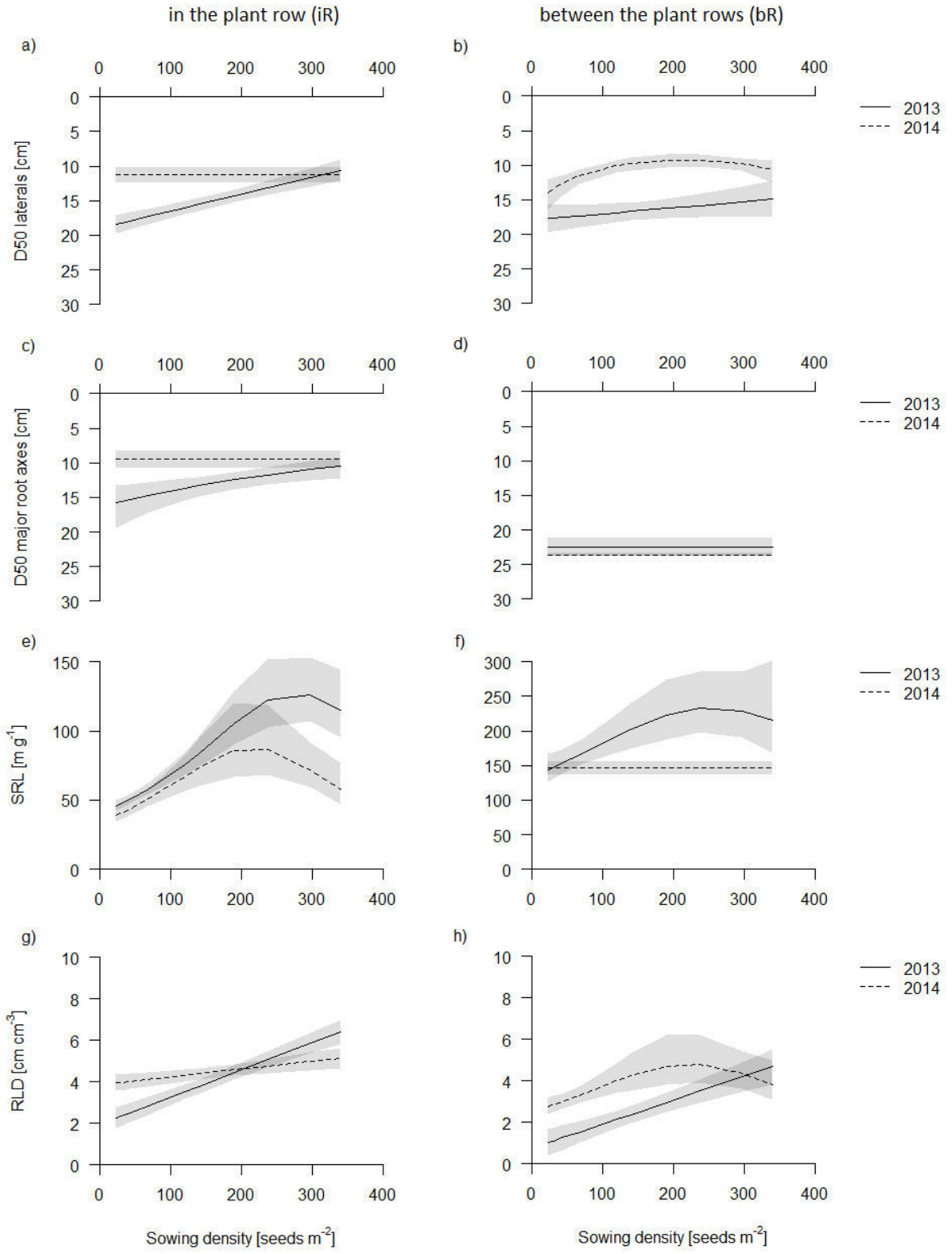

Fig. 9 Belowground traits of the coring in 2013 (solid line) and 2014 (dashed line) within the plant row (iR, left) and between the plant rows (bR, right). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2

and *p*-values see Table S3 in Hecht *et al.* (2016). D50 values of laterals iR (a) and bR (b); D50 values of major root axes iR (c) and bR (d); SRL in the topsoil iR (e) and bR (f); RLD in the topsoil iR (g) and bR (h). (Source Fig. 6 in Hecht *et al.* (2016)).

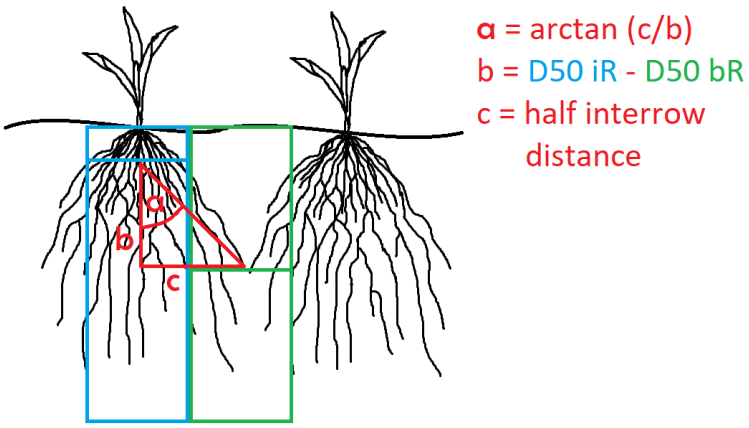

Fig. 10 Estimation of branching angle using D50 values of major root axes (Drawing by Vera Hecht).

3.2 Changes in RLD and SRL best explained by altered ratio of seminal to nodal roots

In the previous chapter 3.1 *Sowing density in the field* (see also Hecht *et al.* (2016) in chapter 9.1 *Sowing density: A neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (Hordeum vulgare L.)*), I describe the effect of sowing density on root distribution in the field and found that both, RLD and SRL, increased with increasing sowing density, while RMF decreased. In combination with the increasing D50 of fine roots, this data, i.e. accumulation of roots in the 0-10 cm topsoil layer, suggested that despite fewer root biomass more biomass was allocated to fine roots. Thus, I hypothesized that increased RLD and SRL and the accumulation of roots in the topsoil were caused by increased lateral branching frequency at higher sowing densities. As D50 of major roots were not affected, I expected branching angles to be not affected by sowing density, although in other studies sowing density was found to decrease branching angle (Archer and Strauss 1985).

At individual plant and organ level, seminal root count per plant was not affected by sowing density (data not shown but see Fig. A. 4 in supplementary information of Hecht *et al.* (2018)), probably since the number of seminal roots is genetically determined before germination (Robinson *et al.* 2016; Shorinola *et al.* 2018), and plants had on average 5 seminal roots per plant, similar to other findings in barley (4–7 seminal roots per plant (Hackett 1969; Wahbi and Gregory 1995; Knipfer and Fricke 2011)). Nodal roots per plant, however, declined with increasing sowing density, similar to findings in maize (Pellerin 1994; Liu *et al.* 2012), but increased over time (Fig. 11a). The number of nodal roots per tiller, though, was constant over sowing density and increased over time (Fig. 11b). Interestingly, although the number of tillers with a certain, high number of nodal roots increased over time, even old plants could have tillers with no nodal roots, even at high sowing density (Fig. 12).

3.2 Changes in RLD and SRL best explained by altered ratio of seminal to nodal roots

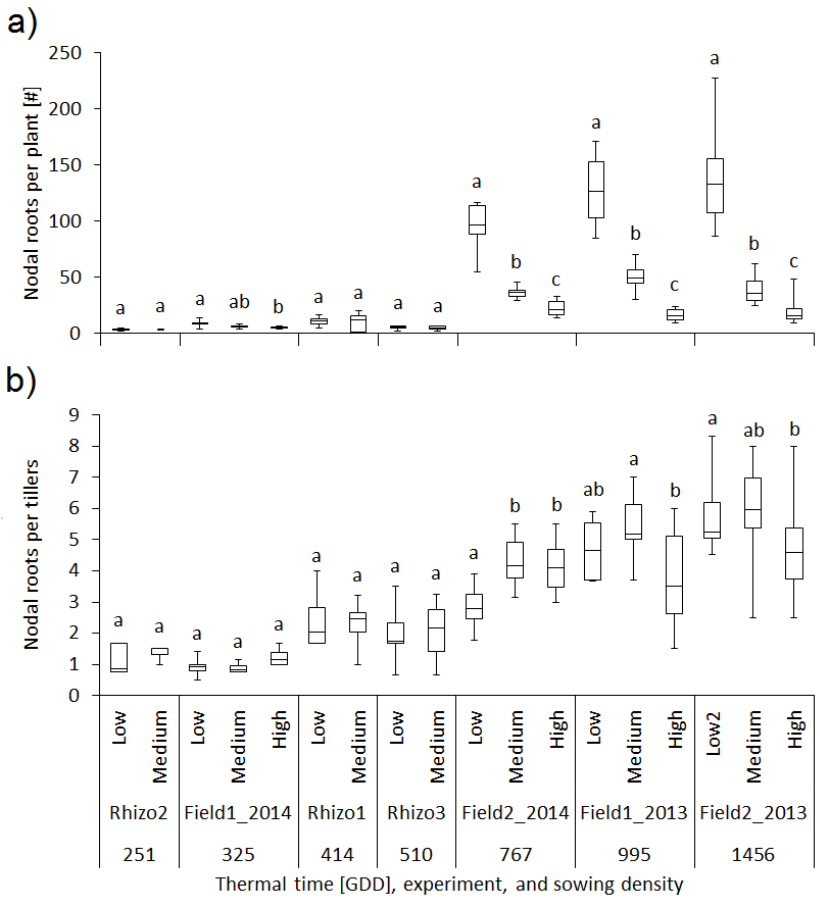

Fig. 11 Nodal root count (a) and ratio of nodal roots per tiller (b) for all different samplings of rhizotron (Rhizo1-3) and field experiments (Field1-2) over thermal time. a) Nodal root count increased over time, and decreased with sowing density, and b) nodal roots per tiller increased over time, but stayed more or less constant with sowing density. Data are presented as boxplots (median framed by the 50%-quantile, error bars indicate minimum and maximum values) and letters indicate significant difference between groups within one sampling (p<0.1). (Source: Fig. 5 in Hecht *et al.* (2018)).

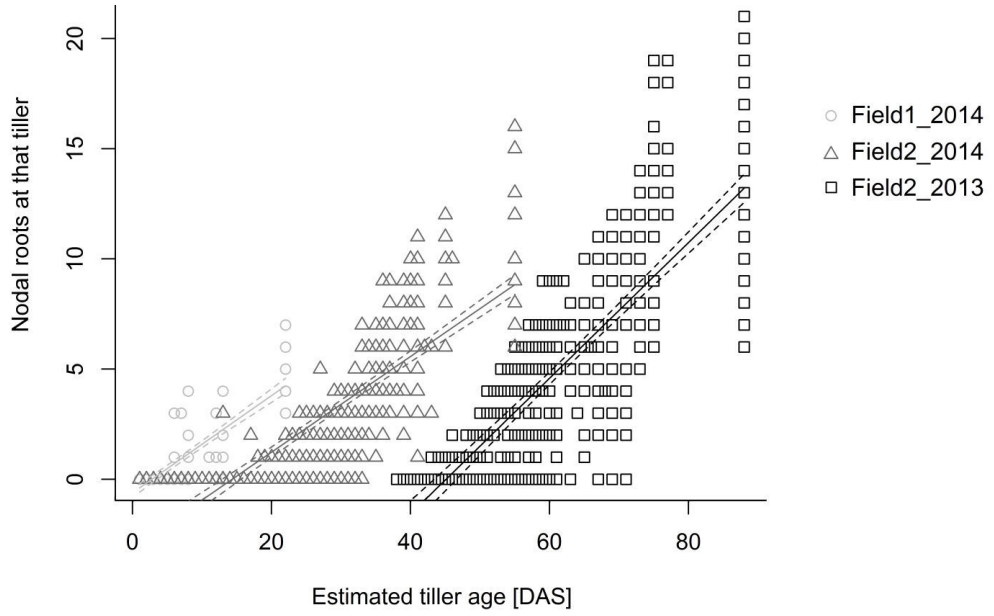

Fig. 12 Nodal roots at a certain tiller and tiller age in days after sowing (DAS) merged for all genotypes and densities of three different sampling time points in the field in the year 2013 and 2014. Data are raw data with linear regression (solid line) with 95% confidence interval (dashed lines). Dots are from Field1_2014 with its linear regression (y = 0.22056×-0.60999, adjusted R^2 = 0.77797, grey), triangles from Field2_2014 with its linear regression (y = 0.2171×-3.1053,

adjusted R^2 = 0.671, dark grey), squares from Field2_2013 with its linear regression (y = 0.3079×-13.8819, adjusted R^2 = 0.6117, black). (Source: Fig. 4 in Hecht *et al.* (2018)).

Still, root counts of both, seminal and nodal roots, increased per area, but nodal roots much stronger. Consequently, the ratio of seminal to nodal roots increased with increasing sowing density. Since seminal roots are smaller in diameter (Krassovsky 1926; personal observation), this changed ratio might explain the observed increases in SRL, especially, since lateral branching frequency was not affected by sowing density neither in young plants in rhizotrons nor in old plants in the field (Fig. 13).

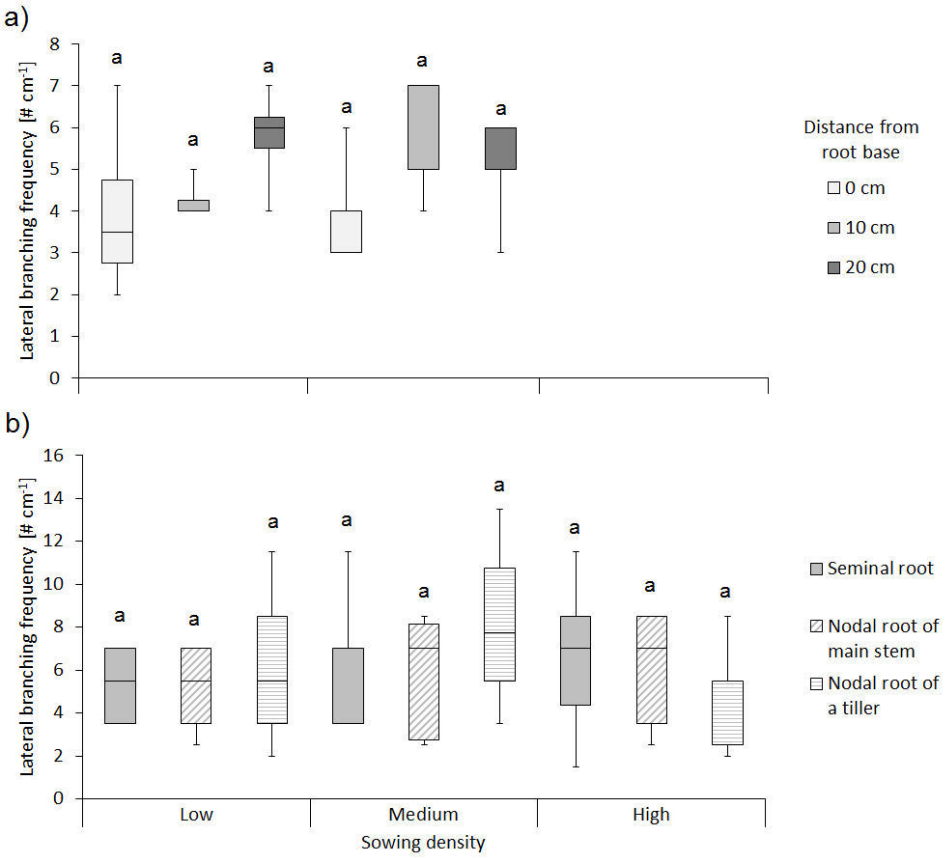

Fig. 13 Lateral branching frequency over sowing density of rhizotron (a) and field (b) experiments. a) Lateral branching frequency at 251 GDD measured along a seminal root at 0, 10, and 20 cm from root base (Rhizo2, 19 DAG). b) Lateral branching frequency of a seminal root, a nodal root from the main stem, and a nodal root of a tiller at 995 GDD (Field1_2013, 75 DAS). Data are presented as boxplots (median framed by the 50%-quantile, error bars indicate minimum and maximum values) and letters indicate significant difference between groups within one sampling (p<0.1). (Source: Fig. 5 in Hecht *et al.* (2018)).

Lateral branching frequency was on average 5 lateral roots per cm of main root, similar to the lateral branching frequency of 4 laterals per cm seminal root found in barley (Drew and Saker 1978). Since the average lateral root length was 2 cm (measured in rhizotrons as total lateral root length of washed roots scanned in WinRHIZO^{TM} divided by lateral root count, see also "Material and Methods" in Hecht *et al.* (2018)), I could estimate RLD from root counts using the following formula:

$$RLD = NMAA \times (1 + LRBD \times LL)$$

Equation 2 RLD = root length density in (cm cm^{-3}), NMAA = number of major axis per area (cm^{-2}), LRBD is the lateral root branching density (here, 5 cm^{-1}), LL = average lateral root length (here, 2 cm).

Using the formula, I obtained as RLD for low, medium and high sowing densities 2.6, 5.9 and 9.4 cm cm^{-3}, respectively. These values are quite close to the measured 2–6 cm cm^{-3} (Hecht *et al.* 2016). As lateral branching frequency and average lateral root length were the same at all sowing densities, the previously observed increase in RLD was most likely due to the increase in the number of main root axes per area, namely seminal and nodal roots.

As growth rates of main root axes (measured on roots visible on transparent window in rhizotrons) were accelerated, decelerated or not affected by sowing density, branching angles were not or slightly decreased by sowing density, as also found in grapevine (Archer and Strauss 1985), and since lateral branching frequency were neither affected, I did not find a direct explanation for the observed accumulation of roots in the topsoil (D50 of fine roots). However, I did not investigate higher order lateral branching or measured the length of the lateral roots in the field. These two factors might have caused the observed accumulation of roots in the 0-10 cm soil layer.

3.3 Translation from lab to field using contrasting root phenotypes at different sowing densities

Additionally, I was interested, if sowing density would help in translation from lab to field. Therefore, I grew two spring barley lines with contrasting root systems, the German cultivar Scarlett and the introgression line S42IL-176, at contrasting sowing densities or levels of competition, i.e. single plants and in stands or clusters, in two years in the field, in rhizotrons and in a pot experiment. The introgression line was selected in the greenhouse for its greater root system (root dry weight, root volume, maximum rooting depth) which was associated with greater tillering (Naz *et al.* 2012, 2014). To compare the two lines and their response in the various experiments, I used the ratio of S42IL-176 over Scarlett of a certain trait (see also "Material and Methods" in Hecht *et al.* (in preparation)).

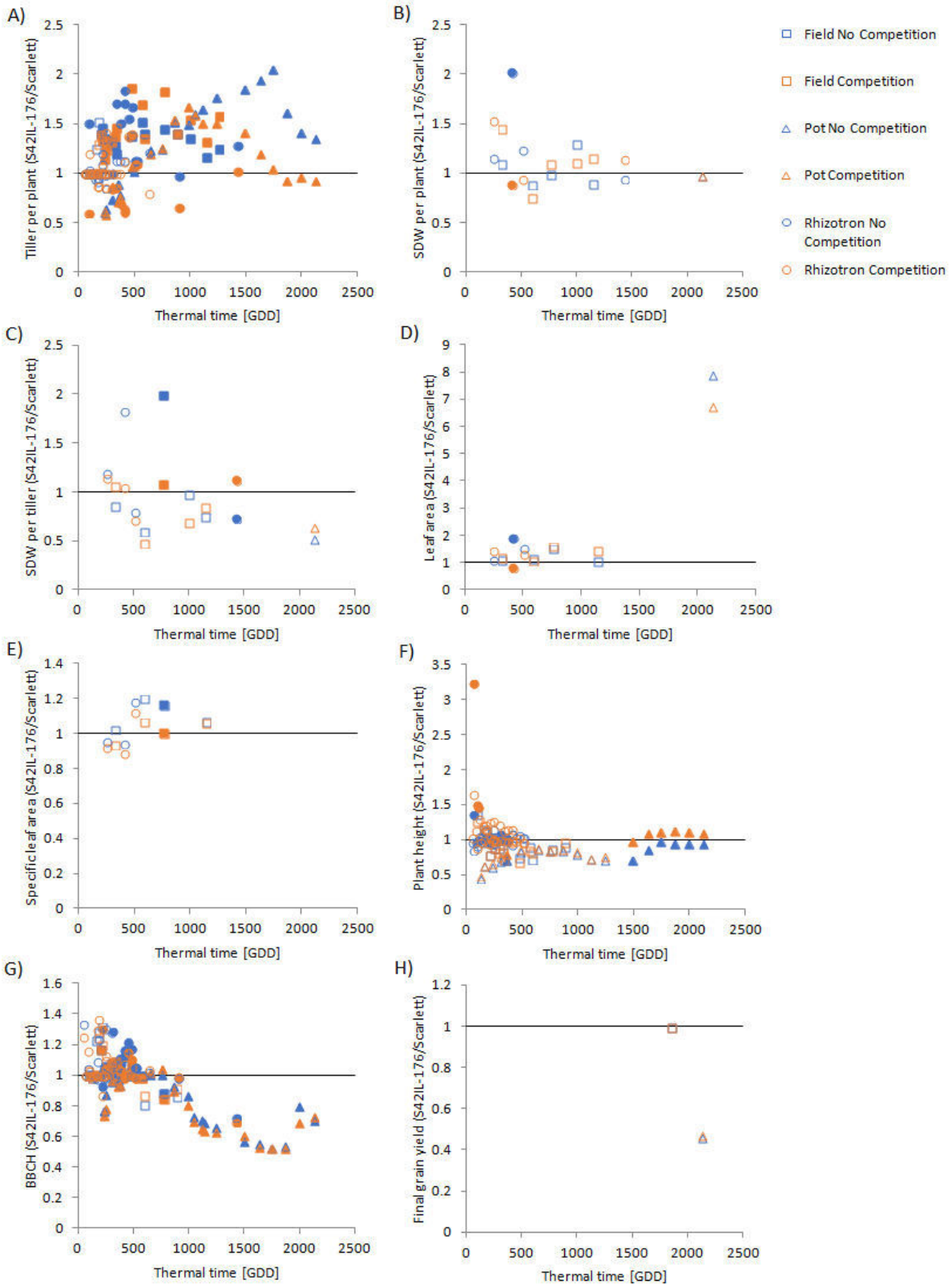

Fig. 14 Ratios of S42IL-176 over Scarlett for shoot traits in field, pot, and rhizotrons experiments. The ratios are of A) tiller number per plant, B) SDW per plant, C) SDW per tiller, D) leaf area per plant (note that the ratios at 2133 GDD were based on projected green leaf area), E) specific leaf area, F) plant height, G) BBCH, and H) final grain yield. Filled symbols represent a significant (p<0.1) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes. (Source: Fig. 1 in Hecht *et al.* (in preparation)).

Throughout all experiments, S42IL-176 bore more tillers than Scarlett at most sampling time points (Fig. 14a), as previously reported (Naz *et al.* 2012, 2014). However, especially young (<500 GDD) S42IL-176 plants and old S42IL-176 plants (>1600 GDD) grown under competition had similar tiller numbers as Scarlett. Sowing density had mostly a suppressing effect on the tiller phenotype of S42IL-176 in comparison to Scarlett. As shoot dry weight was about the same for S42IL-176 and Scarlett (Fig. 14b), contrary to the reported lower SDW for S42IL-176 (Naz *et al.* 2012, 2014), and only sometimes greater or less for S42IL-176 compared to Scarlett, SDW per tiller was mostly lower for S42IL-176 or the same as in Scarlett (Fig. 14c). Leaf area was mostly the same or greater for S42IL-176 (Fig. 14d) and SLA was lower for S42IL-176 in young plants and greater for S42IL-176 in older (Fig. 14e). Under competition, i.e. at greater sowing density, SLA was greater than under non-competitive conditions, i.e. at lower sowing density or in single plants (see supplemental material in Hecht *et al.* (in preparation), as the common response of SLA to plant density (Amanullah *et al.* 2007; Farshbaf-Jafari *et al.* 2014). Although the growth habit of S42IL-176 was described as planophil (= spreading over the ground), I only observed it in the field and in pot experiments but never in the rhizotrons. Despite the observed planophil growth habit in the field, plant height, measured at the stretched plants from ground to tip of the longest leaf, was only affected at booting, when Scarlett started to boot earlier and it took S42IL-176 until flowering to catch up (Fig. 14f). These differences in the development were reflected in BBCH: at tillering, S42IL-176 had greater BBCH but as soon as Scarlett started to boot, S42IL-176 had lower values (Fig. 14g). Final grain yield was the same in the field in 2014 and only half in the pot experiment (Fig. 14h).

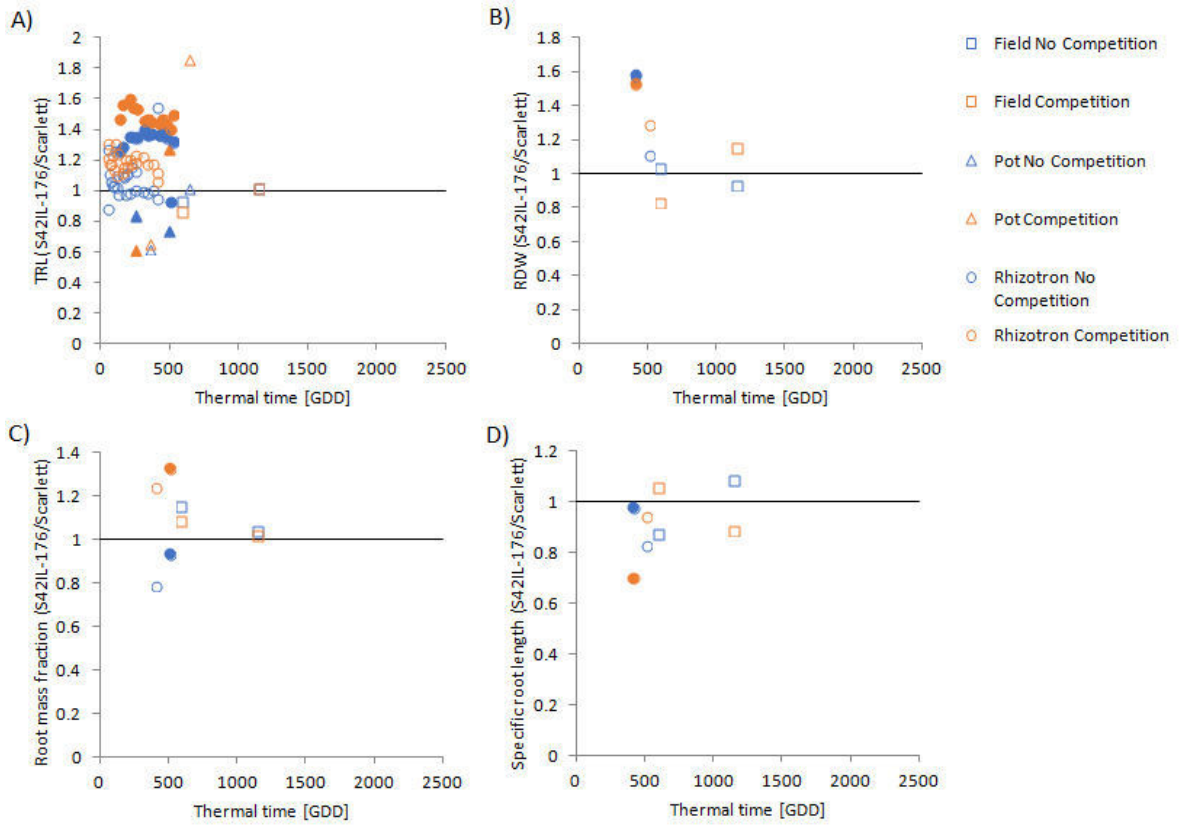

Fig. 15 Ratios of S42IL-176 over Scarlett for root traits in field, pot, and rhizotrons experiments. The ratios are of A) TRL, B) RDW, C) root mass fraction, and D) specific root length in total system within the plant row. Filled symbols represent a

significant (p<0.1) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes. (Source: Fig. 2 in Hecht *et al.* (in preparation)).

The root traits were more plastic and thus, the root phenotype of S42IL-176 in comparison to Scarlett was not expressed in all experiments. Total root length (TRL) was greater for S42IL-176 at almost all samplings in rhizotrons, however, in pot and field, young S42IL-176 plants had lower TRL (<500 GDD), while older plants had greater TRL in the pot experiment and the same in the field experiment (Fig. 15a). Also, the root dry weight-phenotype as described by Naz *et al.* (2012, 2014) was expressed in rhizotrons, but in the field, RDW depended on sampling time point and sowing density: young plants (<500 GDD) had less RDW under competitive conditions and the same RDW under non-competitive conditions, while in older plants it was the other way around (Fig. 15b). RMF was greater for S42IL-176 than for Scarlett under competitive conditions in rhizotrons, whereas RMF was lower under non-competitive conditions. In the field, young S42IL-176 (<600 GDD) had greater RMF than Scarlett, while older plants had about the same (Fig. 15c). SRL tended to be lower for S42IL-176 (Fig. 15d). Nodal root counts were greater only in young plants (<500 GDD) or old (>1000 GDD) plants (Fig. 16a), although greater tillering is associated with greater nodal root production, as I showed in the previous chapter. As S42IL-176 had more tillers per plant, nodal roots per tiller was actually lower for S42IL-176 (Fig. 16b). Lateral branching frequency in young plants was lower for S42IL-176 at seminal roots but greater in older plants at seminal and nodal roots (Fig. 17).

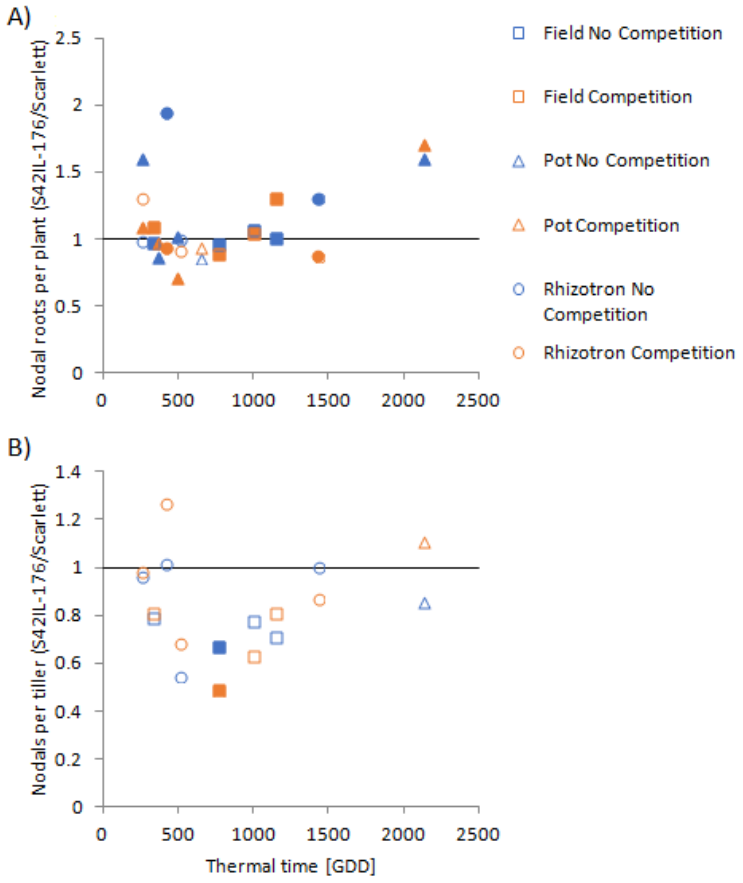

Fig. 16 Ratios of S42IL-176 over Scarlett for root counts in field, pot, and rhizotrons experiments. The ratios are of A) nodal roots per plant and B) nodal roots per tiller. Filled symbols represent a significant (p<0.1) GxE interaction (i.e.

genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes. (Source: Fig. 3 in Hecht *et al.* (in preparation)).

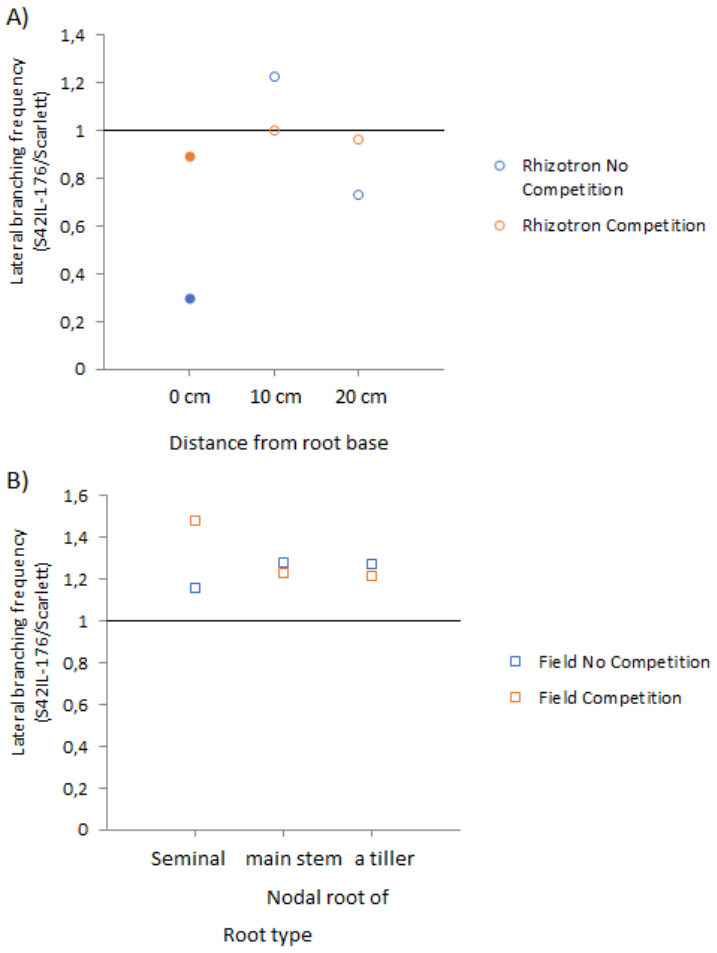

Fig. 17 Ratios of S42IL-176 over Scarlett for lateral branching frequency in field and rhizotron experiments. The ratios are of A) 0 cm, 10 cm, and 20 cm away from a seminal root base from one rhizotron experiment (251 GDD, Rhizo2) and B) seminal root, nodal root of a main stem, and nodal root of a tiller from field trial in 2013 (995 GDD, Field1_S). Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes. (Source: Fig. 4 in Hecht *et al.* (in preparation)).

For nutrient capture, rooting depth is an important trait (Thorup-Kristensen 2001; Kristensen and Thorup-Kristensen 2009; Thorup-Kristensen and Rasmussen 2015) and previously a greater maximum rooting depth (MRD) for S42IL-176 in comparison to Scarlett has been reported (Naz *et al.* 2012, 2014). Although S42IL-176 tended to have greater MRD, this difference was not significant (except in one rhizotron experiment) and GxE was never significant (Fig. 18a). The branching angle can influence rooting depth of the root system (Lynch 2013). Here, branching angles were the same for both genotypes, except once in young S42IL-176 field plants that had greater branching angles than Scarlett (Fig. 18b).

Despite the greater root system, S42IL-176 did not achieve greater yield. However, in a different environment, e.g. drought, this greater root system might be beneficial.

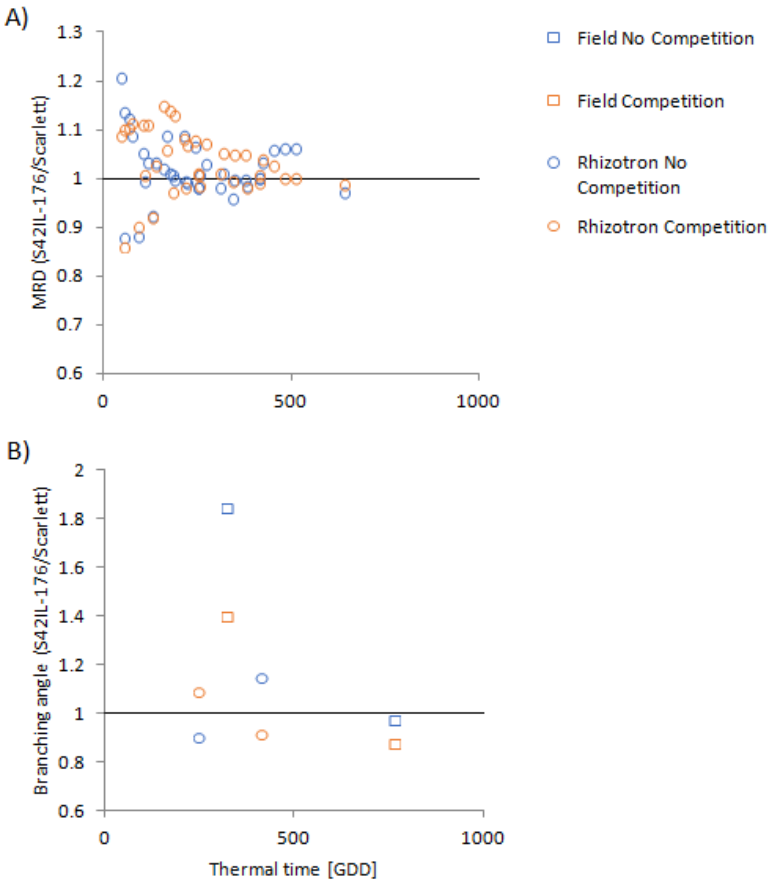

Fig. 18 Ratios of S42IL-176 over Scarlett for MRD (A) and branching angle (B) in field and rhizotron experiments. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes. (Source: Fig. 5 in Hecht *et al.* (in preparation)).

Further, the extend of the differences in traits between the two genotypes was time dependent (= developmental stage dependent), as some traits had a maximum value at which they saturated and it just took longer in lower sowing density to reach this maximum value (e.g. tillers per area, RLD).

Translation from lab to field remains challenging, however, sowing density only needs to be considered in experiments running longer than four weeks. For older plants, the developmental stage is very important, if plants of different experiments are compared directly. A direct comparison of plants at the same age, the same developmental stage, or the same GDD still revealed differences which are probably due to different climate conditions in greenhouse and field. In greenhouse, climate conditions are much more stable, in climate chamber they are consistently the same, and there is no wind or rain that could cause mechanical stress. In contrast, in field, everything is variable: light (quality, quantity, day length, fluctuating light), temperature (day-night fluctuation, temperature gradient in soil, different in air and soil), wind, rain, and soil (chemical composition/properties, soil structure).

Further, root traits seemed to be more plastic so that the root phenotype was less stable from lab to field. Regarding the stability of a certain phenotype in lab to field, root traits were more plastic than shoot traits. Since the translation from lab to field remains challenging, I wonder if a different approach, such as the ranking of many tested genotypes rather than the absolute

numbers of the measured traits, would be more useful in the translation. Would there be more stable phenotypes and more plastic phenotypes? What would the mechanism be behind this plasticity? And when could this plasticity be beneficial?

3.4 Developmental stage correlated to greenness of plot

Manual phenotyping usually is very time consuming, as I also experienced during my experiments. So, coming up with a faster solution, especially, when many plots have to be screened at a great frequency, will improve the phenotyping process. Here, the purpose was to take images of spring barley in a time series with a commonly used RGB-camera. Based on these images, the Green-Red-Vegetation-Index (GRVI) as an indicator of the greenness of a plot can be calculated which was then used for correlating to the developmental stage, i.e. the BBCH stage, of the plant.

While my manual scoring of the developmental stage of the plants could take the entire day depending on how much help I had, Andreas Burkart acquired the RGB-images of the entire field trial in about 20 min. In general, the GRVI increased with increasing sowing density except in 2014, where the second highest sowing density had greater GRVI values than the highest sowing density. Nevertheless, the GRVI showed a similar pattern in both years for both genotypes. Fig. 19 shows the GRVI and the BBCH for the cultivar Scarlett for the highest and lowest sowing density for the two years 2013 and 2014.

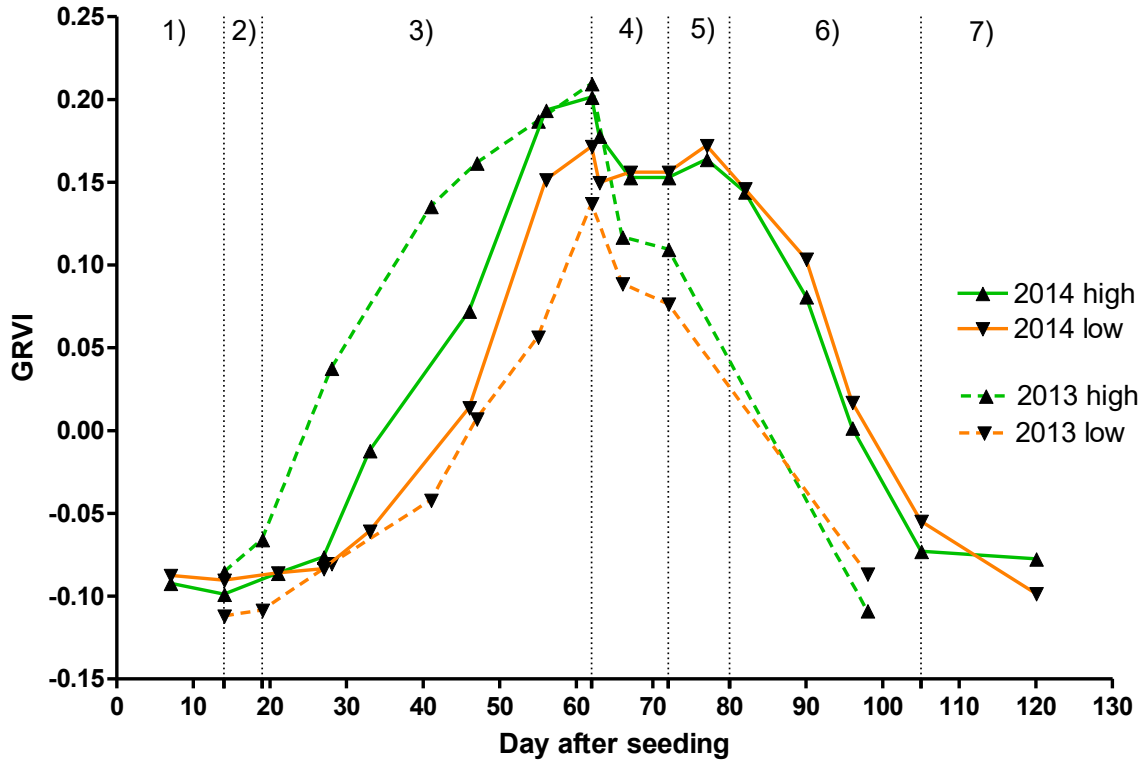

Fig. 19 GRVI development throughout the years 2013 (dotted) and 2014 (solid) for the cultivar Scarlett at the high (340 seeds m^{-2}, green) and low (31 seeds m^{-2}, red) sowing density. Growth stages are numbered on the top and refer as follows: 1) bare soil; 2) first leaves visible; 3) vegetative growth; 4) flowering and emergence; 5) laying down of the ears; 6) ripening; 7) fully mature. (Source: Fig. 7 in Burkart *et al.* (2018)).

In both years, the GRVI reached a maximum at 62 days after sowing (DAS), followed by a small decline and a short plateau, indicating flowering and ear emergence. At the laying down of the ears, between approximately 72 and 80 DAS, another little peak occurred (only observed in 2014, as in 2013 no images were acquired during that particular developmental stage). After that, the GRVI declined during ripening and grain filling to almost the same values as in the beginning of the season, which is accompanied by turning yellow of the plants until full maturity to the lowest GRVI at the end of the season (Fig. 19).

Thus, one can save a great amount of time, when using a camera to obtain the GRVI and correlate it to the developmental stage, especially, if many different lines have to be screened often during the growth cycle. Thus, the GRVI could be used in phenotyping as well as in precision farming to determine the developmental stage of a crop or in crop models to improve and verify the prediction of the crop model with UAV based remote sensing data. However, this GRVI-curve and correlation to BBCH has to be set up for each crop separately to identify the individual shape. Nonetheless, once established, this approach could facilitate decision making for crop cultivation in precision farming. In future, it would be interesting to know if the use of differently colored cultivars of the same species (more yellow, more bluish) has an effect on the GRVI. A blue green may cause greater GRVI, while a yellow green may show lower values at the same developmental stage. Would the threshold in the curve of the GRVI be the same (in absolute/relative terms) when a certain developmental stage (e.g. flowering) is reached? And more general, what would the GRVI-curve look like in other crops? These are open questions still to answer.

4. Conclusions

Sowing density is a modifier of root distribution and root system architecture with probable functional consequences. It affects root distribution and biomass allocation of the plants on crop-level and nodal root counts per plant on organ-level. Thus, as an important factor, sowing density should be considered in root studies but also in the development of root ideotypes in agriculture.

Further, the extend of differences in traits is both time dependent – and thus, developmental stage and plant age dependent – and sowing density dependent, as some traits have a maximum value at which they saturate and it just takes longer in lower sowing density to reach this maximum value (e.g. tillers per area and RLD).

Hence, translation from lab to field remains challenging, however, sowing density only needs to be considered in experiments running longer than four weeks. For older plants, the developmental stage is very important for the direct comparison of plants of different experiments. Direct comparison of plants at same age, developmental stage, or thermal time, though, still revealed differences which are probably caused by the different climate conditions in greenhouse and field, among others light (quality, quantity, day length, fluctuating light) and temperature (day-night fluctuation, temperature gradient in soil, different in air and soil).

Moreover, the stability of a certain phenotype in translation from lab to field is greater for shoot traits, as root traits are more plastic than shoot traits. Further, a greater root system did not necessarily result in greater yield but maybe would under different environmental conditions, for example, under drought stress.

A normal RGB-camera mounted to a UAV can be used in phenotyping for the determination of the developmental stage. Especially, if many different lines (>100) have to be screened often to determine e.g. flowering time or time until ripening, as it is much faster than recording the developmental stage manually and by eye. Moreover, this approach could be used in precision farming in decision making, as it depends on larger areas that can be covered more easily by an UAV than by a human.

Similarly to manual scoring of the developmental stages of the plant, not only the manual root sampling for phenotyping as in my experiments is very time-consuming and labor-intensive but also the further processing of these samples, i.e. root washing from the soil and scanning of the roots. Thus, automated high-throughput phenotyping methods for roots are required, as they would facilitate conducting root research experiments.

5. Outlook

With respect to climate change, not only temperature will increase but also the frequency of extreme weather events, namely unusually warm or cold day and nights, drought, and flooding, recently reviewed by Thornton *et al.* (2014). As climate will thus become less predictable, plant, and especially root, growth plasticity might be of great importance. My study shows that plasticity can be large, even when the experiments are conducted by the same researcher and under, in many aspects, comparable circumstances. Understanding this plasticity is very challenging, however, very important for the development of new ideotypes and thus, for translation from lab to field. In my thesis, I tried to gain more knowledge about root growth plasticity in response to plant density. Future work needs to be placed in the context of drought and nutrient uptake and plant plasticity. Thus, we need to find new ideotypes in agriculture that can deal with such extremes weather conditions. At the same time, the need for more sustainable, more nutrient efficient varieties grows, as managing of our nutrient and water resources sustainably is a requirement to ensure global food security (Drechsel *et al.* 2015). Therefore, for future ideotypes, not only increased nutrient efficiency but also the capability to cope with the extreme, varying environmental conditions will be crucial. A great plasticity as well as great trait stability can be beneficial, depending on the environment or the future scenario of that cultivar.

For example, early establishment and vigor of a plant in drought regions seem to be crucial (Rebolledo *et al.* 2013), and here, rooting depth is a very important factor in capture of deep water (Wasson *et al.* 2012; Lynch 2013). In combination with low N availability, rooting depth becomes an even more important trait (Thorup-Kristensen 2001; Kristensen and Thorup-Kristensen 2009), that the ideotype for this region should express regardless of the environmental conditions during early growth. Roots thicker in diameter will allow greater penetration rates and thus deeper rooting (Clark *et al.* 2002). Also lower total nodal root number will result in deeper roots (Saengwilai *et al.* 2014), as photoassimilates probably are used for growth of these roots and their laterals and not invested in forming new nodal roots. Regarding the formation of (higher order) lateral roots, the optimal lateral branching density depends on the limiting nutrient (Postma *et al.* 2014). In my study, I did not investigate higher order laterals or the length of lateral roots in the field; however, these two factors might be important in nutrient capture when nutrient deficiency occurs.

On the contrary, more frequent strong wind events may increase the risk of lodging in a crop (Drechsel *et al.* 2015). Greater nodal root number improves lodging in maize (Liu *et al.* 2012) which could be achieved by reduced plant density, as nodal root number increased with reduced plant density, but when drought is likely to occur, this greater root count may not be beneficial anymore, as it also may result in a lower number of deep roots. Thus, a combination of a few very deep roots plus an appropriate number of short to medium length nodal roots could ensure deep water capture and anchorage of the plant at the same time.

6. Abbreviations

BBCH scale for developmental stage, Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie

bR between the plant rows

CKA Campus Klein-Altendorf, research facility of University of Bonn

D50 depth at which 50% of the sampled roots are above located, cm

DAS days after sowing

Field1-2 Field experiments in 2013 and 2014 at CKA, for detailed description see Material and Methods in Hecht *et al.* (2016, 2018)

FZJ Forschungszentrum Jülich GmbH

GDD growing degree days, °C

GRVI green-red-vegetation-index

IBG-2 Institut für Bio- und Geowissenschaften: Pflanzenwissenschaften, am FZJ

iR within the plant row

LL average lateral root length, cm

LMF leaf mass fraction (= leaf dry weight/total plant dry weight)

LRBD lateral root branching density, cm^{-1}

MRD maximum rooting depth, cm

MRI magnetic resonance imaging

N nitrogen

NMAA number of major axis per area, cm^{-2}

RDW root dry weight, g

Rhizo1-4 Rhizotron experiments, for detailed description see Material and Methods in Hecht *et al.* (in preparation, 2018)

RLD root length density, cm cm-3

RMF root mass fraction (= RDW/total plant dry weight)

SDW shoot dry weight, g

SEM standard error of the mean, (= standard deviation/√(number or replicates))

SLA specific leaf area, cm2 g-1

SMF	stem mass fraction (= stem dry weight/total plant dry weight)
SRL	specific root length, m g-1
TRL	total root length
UAV	unmanned aerial vehicle

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9. Publications of this thesis

9.1 Sowing density: A neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (*Hordeum vulgare* L.)

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Sowing Density: A Neglected Factor Fundamentally Affecting Root Distribution and Biomass Allocation of Field Grown Spring Barley (*Hordeum Vulgare* L.)

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Studies on the function of root traits and the genetic variation in these traits are often conducted under controlled conditions using individual potted plants. Little is known about root growth under field conditions and how root traits are affected by agronomic practices in particular sowing density. We hypothesized that with increasing sowing density, root length density (root length per soil volume, cm cm^{-3}) increases in the topsoil as well as specific root length (root length per root dry weight, cm g^{-1}) due to greater investment in fine roots. Therefore, we studied two spring barley cultivars at ten different sowing densities (24–340 seeds m^{-2}) in 2 consecutive years in a clay loam field in Germany and established sowing density dose-response curves for several root and shoot traits. We took soil cores for measuring roots up to a depth of 60 cm in and between plant rows (inter-row distance 21 cm). Root length density increased with increasing sowing density and was greatest in the plant row in the topsoil (0–10 cm). Greater sowing density increased specific root length partly through greater production of fine roots in the topsoil. Rooting depth (D50) of the major root axes (root diameter class 0.4–1.0 mm) was not affected. Root mass fraction decreased, while stem mass fraction increased with sowing density and over time. Leaf mass fraction was constant over sowing density but greater leaf area was realized through increased specific leaf area. Considering fertilization, we assume that light competition caused plants to grow more shoot mass at the cost of investment into roots, which is partly compensated by increased specific root length and shallow rooting. Increased biomass per area with greater densities suggest that density increases the efficiency of the cropping system, however, declines in harvest index at densities over 230 plants m^{-2} suggest that this efficiency did not translate into greater yield. We conclude that plant density is a modifier of root architecture and that root traits and their utility in breeding for greater productivity have to be understood in the context of high sowing densities.

Keywords: biomass allocation, field, root architecture, root length density, root morphology

INTRODUCTION

Most of our knowledge of root system architecture and traits derives from controlled experiments in which solitary plants are grown in pots (Løes and Gahoonia, 2004; Lotfollahi, 2010), whereas data from field conditions are still relatively rare. This is partly because roots are difficult to access and evaluate in the field and relatively intensive sampling is needed to compensate for large variation caused by soil heterogeneity and other factors. Various root traits may enhance plant productivity by increasing drought tolerance and/or nutrient acquisition efficiency and may thereby be targeted by breeders (Postma and Lynch, 2011; Comas et al., 2013; Raza et al., 2014; Svačina et al., 2014; Heřmanská et al., 2015). The feasibility and relevance, however, of targeting root traits in breeding programs is still questioned as (a) high plasticity may cause traits to have low inheritance values, (b) phenotyping for root traits often has low throughput and low precision complicating the selection process, and (c) the function of the traits need to be understood under field conditions (Cobb et al., 2013; Fiorani and Schurr, 2013; Araus and Cairns, 2014; Kuijken et al., 2015; Paez-Garcia et al., 2015). Farmers, grow crops at relatively high target sowing densities in order to maximize yield. If the controlled environment studies are to have any relevance to breeding and agronomy, it is of great importance to know how sowing density influences root architecture, what traits are density-independent and to what extent density influences the root ideotype for nutrient and water acquisition. So far, little is known about the responses of roots to changing sowing densities, as most studies which deal with sowing density focus on the aboveground part of the plant. Further, sowing density can be easily changed by the farmer, so it is important to know how management influences root traits and thereby the agroecology of the crop.

Aboveground, increasing sowing density is known to decrease individual shoot biomass (Harper, 1977). In barley, increasing sowing density reduces tiller formation (Kamel, 1959; Munir, 2002; Turk et al., 2003; Soleymani et al., 2011). At very high densities, the smallest number of individual tillers was observed and in some cases plants may even not have survived, i.e., self-thinning occurs (Harper, 1977). Decreasing plant size may have direct consequences for the size-dependent root architectural traits, for example maximum rooting depth. However, due to growth regulatory mechanisms in response to plant density, plants may compensate by changing both their biomass allocation, architecture and morphology. Some of these changes are well-described for aboveground plant tissues. Etiolation responses to plant density, for example, can cause crop height to increase with increasing sowing density [for barley e.g., Turk et al. (2003), and Soleymani et al. (2011)], despite individual plants having reduced aboveground biomass. Similarly, maximum rooting depth might not simply be a function of plant size, and might become deeper, rather than shallower at higher densities. In either case, maximum rooting depth is known to be of critical importance for water acquisition and recovery of deep nitrate (Thorup-Kristensen, 2001, 2006; Lynch, 2013) which underlines the importance of knowing how sowing density may influence these traits.

While individual shoot biomass decreases with density, total biomass per area and grain yield increase with sowing density (for spring barley e.g., Kamel, 1959; Singh and Singh, 1981; Munir, 2002; Turk et al., 2003; Farnia et al., 2014), leveling-off at very high sowing densities (Singh and Singh, 1981; Farnia et al., 2014). Reviewing this response, Weiner and Freckleton (2010) concluded that total biomass on a given area was linearly proportional to plant density up to a critical plant or stand density beyond which total biomass per area does not increase (final constant yield). Changes in biomass allocation and individual plant morphology, however, may still occur. Plants become elongated (e.g., Turk et al., 2003; Soleymani et al., 2011) and allocate more to stems than to leaves (Poorter et al., 2012). Eventually, the biomass allocation to reproduction may be reduced as well, causing a lower harvest index at very high sowing densities (Weiner and Freckleton (2010), or *Trifolium incarnatum* Weiner (1980), for barley e.g., Farnia et al. (2014). These responses to density, however, have partly been bred out of the modern grain cultivars, as these cultivars (in contrast to older cultivars and land races) stay short, and maintain a high harvest index at high densities (Lee et al., 1989; Hammer et al., 2009; Soleymani et al., 2011; York et al., 2015). It is unclear how biomass partitioning to roots is affected by plant density, and whether breeding for short straw varieties and high harvest index has affected biomass partitioning to roots. Chloupek et al. (2006) observed that the root system size of semi-dwarf genotypes was significantly greater than of non-semi-dwarf controls, but Wojciechowski et al. (2009) found no effect of dwarfing genes on root elongation in either field or in soil-filled columns. Since much root phenotyping is done under non-competitive growth conditions, we asked to what extent and in what way biomass partitioning to roots of modern barley cultivars is affected by sowing density.

As we are not aware of any reports (except Kamel, 1959) in the literature of how plant density may alter barley root system architecture, we draw on a limited set of reports from other species that we found in the literature. Archer and Strauss (1985) observed steeper and greater root length densities (RLD) in denser stands of grapevine. Similarly, Azam-Ali et al. (1984) observed faster and deeper root growth at higher sowing densities of pearl millet. Manschadi et al. (1997) found an increase in RLD with increasing sowing density and over time for faba beans, although for the high sowing densities, RLD decreased after pod setting. Several studies of RLD in high density stands report high RLDs in the topsoil (for example Tardieu, 1988; Mommer et al., 2010; Kucbel et al., 2011; for example Chen et al., 2013; Ravenek et al., 2014). However, many of these studies do not contain low density controls, and most plants might forage the topsoil

Abbreviations: BBCH, crop developmental stages encoded in decimal numbers developed by BASF, Bayer, Ciba-Geigy and Hoechst after (Lancashire et al., 1991); D50, median of the interpolated root length distribution, i.e., the depth (in cm) which divides the root length in the core into equal parts (50% of root length above and 50% below that D50 value), calculated on root length up to 40 cm depth; DAS, days after sowing; LMF, leaf mass fraction; PAR, photosynthetic active radiation; RDW, root dry weight; RLD, root length density; RMF, root mass fraction; SLA, specific leaf area; SMF, stem mass fraction; SRL, specific root length; TPDW, total plant dry weight.

with greater intensity simply as the topsoil has generally greater nutrient availability (Jobbágy and Jackson, 2001; Kahle et al., 2010). Apparently, most stands forage the topsoil with a greater intensity and hence, we hypothesize that high sowing density will increase RLD in the topsoil. Whether topsoil foraging is a desirable trait in agriculture is still under discussion and probably depends strongly on the soil environment (Thorup-Kristensen, 2001; Dunbabin et al., 2003; Ho et al., 2005; Zhu et al., 2005; Lynch, 2013).

We ask if roots and shoots respond in a similar way to sowing density and how sowing density would influence plant growth, crop production (final grain yield) and biomass allocation. To address these questions, we set up two sowing density field experiments with spring barley over 2 consecutive years. We hypothesized that with increasing sowing density, root length density (root length per soil volume) will increase as well as specific root length (root length per root dry weight) due to relatively greater investment in fine roots. We expected these increases in root length density to be greater in the topsoil.

MATERIAL AND METHODS

To study the effect of sowing density on root and shoot growth and yield, we conducted sowing density experiments with spring barley in a field in Germany over 2 consecutive years. We took soil cores to investigate root length distribution around the time of flowering of two barley cultivars grown at ten different sowing densities.

Plant Material

We grew two German malting spring barley (*Hordeum vulgare* L.) cultivars "Scarlett" and "Barke." Scarlett grows shorter than Barke, however, Barke is more resistant to lodging. Scarlett ripens earlier than Barke (Lindemann et al., 2002). Barke is often used in scientific studies (Gahoonia and Nielsen, 2004; Schmalenbach and Pillen, 2009; Auškalnienė et al., 2010; Dornbusch et al., 2011; Castillo et al., 2012; Füllner et al., 2012; Alqudah and Schnurbusch, 2015).

Field Site

We conducted the experiments at Campus Klein-Altendorf (University of Bonn, Germany, 50°37′31.00″N, 6°59′20.54″E) in 2013 and 2014 on a loamy-clay silt soil (luvisol). Annual precipitation, average annual temperature and sun hours were 734.4 mm, 9.8°C and 1753 h in 2013 and 820.4 mm, 11.4°C, and 1934 h in 2014, respectively, and cumulative rainfall, thermal time (cumulative growing degree days), and cumulative photosynthetically active radiation (PAR) from sowing date until final harvest date were 285 mm, 1769.06°C, and 68.9 kWh m^{-2} in 2013 and 315 mm, 1864.45°C, and 74.9 kWh m^{-2} in 2014 (see Figure S1). Climate data were obtained from the service center of the rural area of Rhineland-Palatine (Dienstleistungszentrum Ländlicher Raum Rheinland-Pfalz)[1] and can be found on http://www.am.rlp.de.

[1]http://www.dlr.rlp.de/Internet/global/inetcntr.nsf/dlr_web_full.xsp?src=7647GJT68H&p1=V3802SO2OW&p2=M42BIW88I8&p3=9203R4M5VS (last accessed: January 12, 2016); http://www.am.rlp.de/Internet/AM/NotesAM.nsf/amweb/6d6fa012f043c619c1257171002e8a75?OpenDocument&TableRow=2.7 (last accessed: January 12, 2016).

Experimental Design

Sowing took place on 25 April 2013 and 20 March 2014 in 1.5 × 14.2 m plots in six rows (inter-row distance of 21 cm) in a randomized nested-block design with five replicates in ten different sowing densities (24, 31, 43, 68, 120, 140, 190, 238, 298, and 340 seeds m^{-2} as sowing density 1–10, respectively (recommended sowing density for spring barley in Germany between 250 and 300 seeds m^{-2}); **Figure 1**, Figure S2) using a Hege 95 single seed sowing machine (Hege, Waldenburg Germany). We took soil cores (9 cm in diameter, hammer: COBRA; cylinder: Eijkelkamp) in all sowing densities (in the plant row and between the plant rows, each $n = 1$) plus additional replicates in the lowest (24 seeds m^{-2}), medium (120 seeds m^{-2}), and highest (340 seeds m^{-2}) sowing densities (2013: in the plant row, $n = 3$; 2014: only Scarlett in the plant row and between the plant row, each $n = 4$). In 2013, we sampled 60 cm deep at 48–56 DAS (stem elongation phase, BBCH 30–49). We reduced the coring depth to 40 cm deep in 2014 at 88–97 DAS (around flowering, BBCH 69–87), as the samples below 40 cm contained relatively few roots and we did not observe any treatment effects below 40 cm. Soil coring was always complemented with shoot measurements, as described below. We harvested the grain for determining the final yield on 16 August 2013 (113 DAS) and 26 July 2014 (128 DAS), respectively.

Crop Husbandry

Plants were sprayed against pathogens and insects as recommended for barley cultivation (2013: insecticide Karate Zeon at BBCH 12, herbicides Azur and Hoestar, and fungicide Capalo at BBCH 29, and fungicide Adexar and insecticide Biscaya at BBCH 59; 2014: herbicides Azur and Hoester at BBCH 13, Capalo at BBCH 30, fungicides Input and Karate Zeon at BBCH 37, fungicide Adexar at BBCH 61). Fertilization was the same for all treatments and based on soil tests; in 2013: basis fertilization P_2O_5: 45 kg/ha, K_2O: 160 kg/ha, MgO: 24 kg/ha; N-application (total): 50 kg/ha; in 2014: basis fertilization P_2O_5: 30 kg/ha, K_2O: 60 kg/ha, MgO: 9 kg/ha; N-application (total): 45 kg/ha. In both years, N-application was somewhat lower than recommended for spring barley to avoid lodging (recommended N-application (total): 80–120 kg/ha).

Non-destructive Measurements during Growth

Approximately every 2 weeks we evaluated non-destructively three randomly chosen plants per plot. We stretched the plant to measure plant height from the plant base to the tip of the longest leaf (to the nearest 0.1 cm). We recorded the developmental stage of the plants according the so called BBCH stages (Lancashire et al., 1991). Further, we counted all tillers of an investigated plant (tiller count).

Shoot Sampling at Coring

To determine shoot traits, we collected five plants of each plot at each sampling time by cutting the plants at the base (ground level) in 2013 and for sowing density 5–10 in 2014, while only

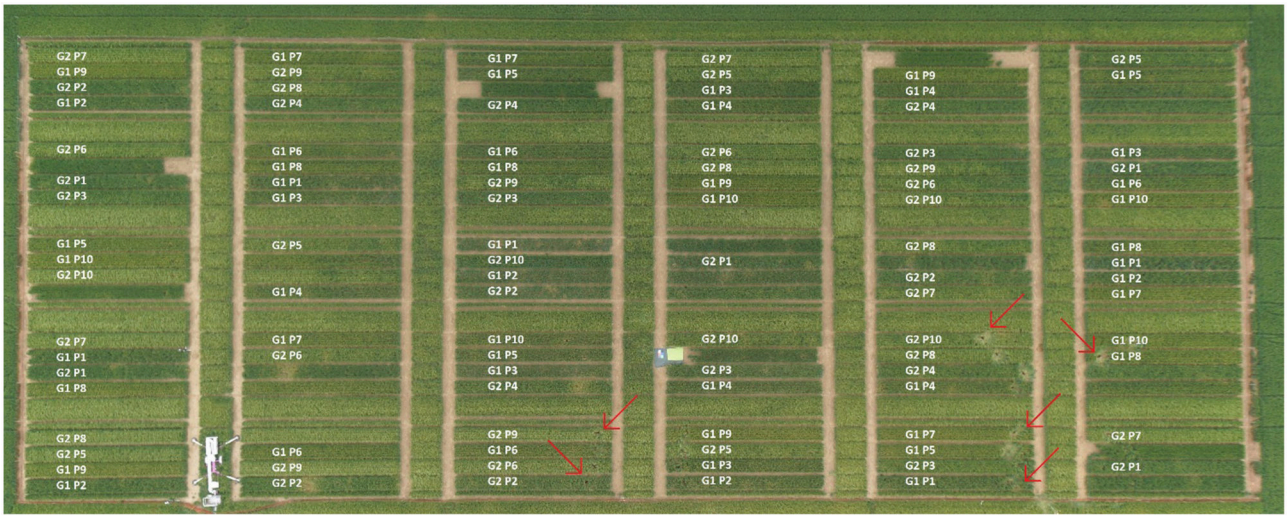

FIGURE 1 | Experimental design of 2014 at 90 DAS (1179.75°C GDD [growing degree days = average of daily maximum and minimum temperature minus base temperature (here, base temperature = 0°C), adapted according to McMaster and Wilhelm (1997)]. G1 and G2 refer to cultivar Scarlett and Barke. P1–P10 stand for the 10 different sowing densities: 24, 31, 43, 68, 120, 140, 190, 238, 298, and 340 seeds m^{-2}, respectively. Plots were 14.2 m long and 1.5 m wide. Data of the plots not used within this publication are left blank. Red arrows indicate some positions of coring. The design of 2013 can be found in Supplementary Figure S2. Picture with permission of A. Burkart.

three plants per plot were harvested for sowing density 1–5 in 2014 (88–97 DAS), in order to reduce the amount of sampled plant tissue. Of the harvested plants, we took three randomly chosen tillers as a sub sample, separated them into leaf sheaths and blades and photographed them to determine leaf area via segmentation based on green value. We oven dried the sub-samples and remaining shoot samples at 70° C for at least 2 days before determining their dry weight (to the nearest 0.01 g). We calculated specific leaf area (SLA) as leaf blade area over its corresponding dry weight.

Soil Coring, Root Washing, and Sample Processing

The soil cores, taken within 1 day after the harvesting of the shoots, were divided into 10 cm sections and individually packed into plastic bags and stored at 4°C until root washing. We manually washed the roots of each soil core section separately on a sieve (mesh size 500 µm) using tap water. After cleaning, we collected the roots from the sieve and stored them in 50% EtOH in 2013 and, since the handling of roots without EtOH was much easier and the time between washing and scanning was maximum 2 weeks, in tap water at 4°C in 2014, respectively, before scanning and analyzing with WinRHIZO™ (resolution 600 dpi, gray scale, manual threshold gray value 210, 20 diameter classes à 0.1 mm width). We oven-dried the scanned roots at 70°C until showing constant weight (to the nearest 0.00001 g). Similar to D95-values (depth of 95% of root length in a core) as in e.g., Lynch (2013) and Zhan et al. (2015), we calculated D50-values (median of the interpolated root length distribution, i.e., the depth in cm which divides the root length in the core into equal parts) on root length values down to 40 cm depth to be able to compare the data of the 2 years.

Root Measures

From the scans in WinRHIZO™, we obtained total root length (TRL) for each core section and calculated root length density (RLD) for each layer by dividing through the corresponding core volume. Furthermore, we calculated specific root length (SRL) for each layer separately as root dry weight (RDW) by its corresponding TRL.

Dry Weight Ratios

In order to calculate the biomass fractions, we converted shoot dry weight per plant and root dry weight per volume to dry weight per area using the here described formulas. Further, we only used the root dry weights of 0–40 cm depth in order to be able to compare the data of the 2 years. Hence, we calculated total plant dry weight per area (TPDW) as

$$TPDW = \left(\frac{shoot\ dry\ weight}{plant} * \frac{seeds}{area}\right) + RDW, \text{ in g m}^{-2}$$

with root dry weight per area of columns (RDW) as

$$RDW = \frac{\frac{root\ dry\ weight}{0-40\ cm\ soil\ column}}{area\ of\ column}, \text{ in g m}^{-2}$$

and used it to calculate root mass fraction (RMF), stem mass fraction (SMF) and leaf mass fraction (LMF) as

$$RMF = \frac{RDW}{TPDW},$$

$$SMF = \frac{\frac{stem\ dry\ weight\ of\ three\ tillers}{dry\ weight\ pf\ three\ tillers} * \frac{shoot\ dry\ weight}{plant} * sowing\ density}{TPDW},$$

and

$$LMF = \frac{\frac{leaf\ dry\ weight\ of\ three\ tillers}{dry\ weight\ pf\ three\ tillers} * \frac{shoot\ dry\ weight}{plant} * sowing\ density}{TPDW}.$$

Yield Determination

At final harvest, we determined the total weight of the seeds per plot (yield at harvest) and took a 100 g subsample for oven-drying at 105°C and determining seed dry matter. We corrected the seed weights for water content as follows:

$$yield = \frac{yield\ at\ harvest \times determined\ seed\ dry\ matter}{basic\ seed\ dry\ matter},$$

with basis seed dry matter = 86% (Richtlinien für die Durchführung von landwirtschaflichen Wertprüfungen und Sortenversuchen, 2014)[2].

Statistics

We used R version 3.2.3 (R Development Core Team, 2015) to analyze all data. As the two genotypes were not statistically different from each other in response to almost all of the measured parameters (exception: plant height with Barke taller than Scarlett, RMF not affected for Barke and declining for Scarlett in 2014), we pooled them for the analysis and consider our data as more generally true, and not cultivar dependent, although genotypic differences in plant responses to sowing density may exist among other genotypes. We fitted three different models: first, we fitted linear regressions (model 1) but if it was not appropriate ($R^2 < 0.3$), we applied linear regression on 1/y transformed data which is according to Willey and Heath (1969) for density-dependent data the most satisfactory equation (model 2), or non-linear saturating curves (Michealis-Menten kinetics) which is an inversion of model 2 and better describes saturating or asymptotic relationship between sowing density and e.g., yield (model 3; Willey and Heath, 1969). For the linear models, we used $\sim a + b * x + c * x^2$, but dropped terms if it would improve the AIC criteria (using the R function stepwise). The 1/y data transformation was fitted in the same way as the non-transformed data and used for data that seemed appropriate. For example, number of tillers per hectare was relatively linear with density, which means that the number of tillers per plant is mathematically a simple inversion with density. As responses to density are known to saturate at higher densities, linear fits do not always describe the density dose-response curves in a satisfactory way, and we fitted the nonlinear function $y = a + \frac{(b*x)}{(c+x)}$ (with y = measured trait, x = sowing density) to the data using *R*'s nls-function. Figures show model fits with 95% confidence intervals shaded in gray. Raw data can be found in the Supplementary Material.

[2]Richtlinien für die Durchführung von landwirtschaflichen Wertprüfungen und Sortenversuchen (2014). 2.8.1–2.8.29.

RESULTS

The results of the two cultivars were in almost all measured parameters the same. We therefore merged the data of the two cultivars for the analysis and here present the results of the combined analysis. If the cultivars differed in a measured trait, we point out the difference in the corresponding section below.

Sowing Density Affects Tiller Formation and Aboveground Biomass Production

Tiller count per plant was constant across all densities during the first 4 weeks of crop establishment (see Figure S3). At 4 weeks after sowing, plants had 2–5 tillers and this number did not further increase in the highest sowing densities ($\geq$140 seeds m^{-2}) over time. This tillering arrest was observed in both years (see Figure S3). For the lower sowing densities (24–120 seeds m^{-2}), tillering was arrested at later times, namely the earlier the higher the sowing density, and the rate of tiller formation was negatively correlated with sowing density. Consequently, the fit of tillers per plant at the coring event declined exponentially (**Figure 2A**, see Table S1). This decline from lowest to highest sowing density was about 5–6 times in 2013 (during stem elongation) and 6–7 times in 2014 (during grain filling). This relative decline in tiller number per plant was not as strong as the 14-fold increase in sowing density, so that the number of tillers per area increased with sowing density in both years, and the increase was approximately linear at the coring events. In 2013, the intercept of the linear fit was lower and the slope was steeper, possibly reflecting the earlier sampling time, however, yield was also more sensitive to sowing density in 2013 (see below). Surprisingly, at high densities, the number of tillers per m^2 was less in 2014 than in 2013. This might be simply year to year variation, but could also reflect senescence of smaller tillers or plants in the highest densities and thus a form of self-thinning. True self-thinning, that is mortality of the plant, could present an error in our estimation of shoot biomass and tiller counts per m^2, as we took our measures on individual plants. However, we took great care that we only sampled locations where all plants and neighboring plants were present.

The shoot dry weight per tiller stayed constant over sowing density and was 0.33 and 1.4 g per tiller in 2013 and 2014 respectively (**Figure 2B**, Table S1). Consequently, just as the tiller counts per area, shoot dry weight per area increased linearly in both years (**Figure 2C**, see also Table S1, Figure S4). Both fits of shoot dry weight per area had about the same slope, however, the intercept was significantly greater in 2014, in accordance with the later sampling time point (**Figure 2C**, Table S1). The increase of shoot dry weight per area from lowest to highest sowing density was five times in 2013 and two times in 2014. Biomass production is thought to be closely related to light capture, which at later stages, when the canopies at all densities have closed (full canopy closure at 62 DAS, data published elsewhere; Burkart et al., in preparation), is independent of sowing density. But absolute differences in biomass production, gained during earlier stages of development, are maintained.

In both years, greatest grain yield ($\sim$7.5 t ha^{-1}) was obtained at a sowing density of 230 seeds m^{-2} and declined slightly at

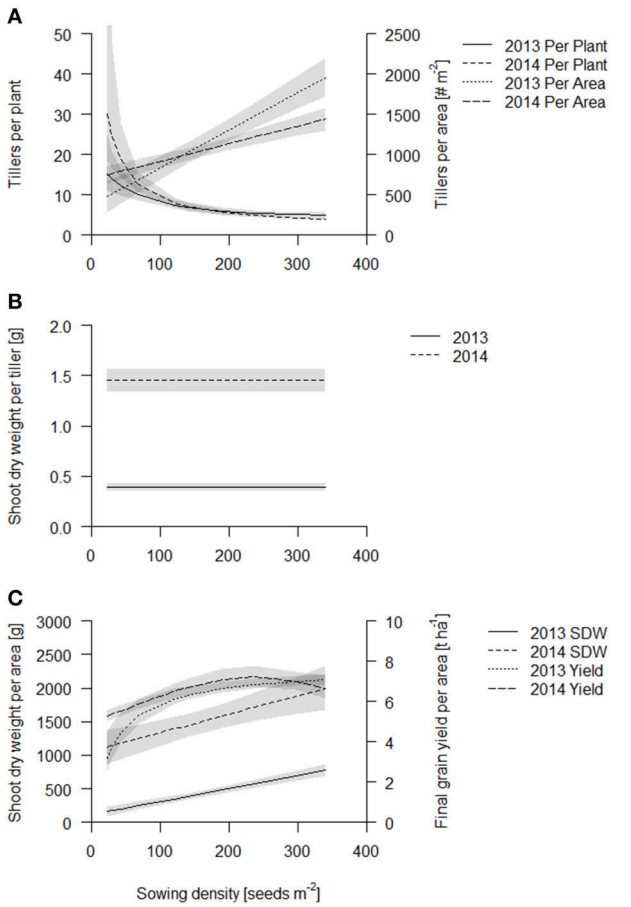

FIGURE 2 | Aboveground traits of the coring events of Scarlett and Barke in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S1. **(A)** Tillers per plant and tillers per area; **(B)** Shoot dry weight per tiller; **(C)** Shoot dry weight per area and final grain yield.

higher sowing densities (**Figure 2C**, Table S1). Grain yield was more sensitive to low sowing densities in 2013 than in 2014, so that the increase in yield from the lowest to the highest sowing density was about two times in 2013 and 1.5 times in 2014. Grain yield per area was the same for the two cultivars except in lower sowing densities (below 68 seeds per m^2), where Barke had somewhat lower values than Scarlett (see Figure S5). The grain yield suggests that early gains in biomass production at the high densities might not translate into grain yield.

In summary, during early crop development sowing density has little effect on individual plant development, and consequently the tiller count per area and the biomass production per area is greater at greater densities. However, as the crop develops, individual plants responded to the density treatment by reducing tiller formation but not the growth rate of the individual tillers. Eventually the crop canopy closes and biomass production per tiller and per area becomes less sensitive to sowing density. Early gains in biomass in the higher sowing densities also translate into increased yield, but at sowing densities greater than 230 plants m^{-2} yield stabilizes or declines slightly.

Sowing Density Affects Biomass Partitioning

The root dry weight in the cores increased with sowing density, but to a lesser degree compared to the shoot dry weight and consequently root mass fraction (=root dry weight per area up to 40 cm depth over total plant dry weight per area, RMF) declined linearly in both years with increasing sowing density (**Figure 3A**, Table S2) with the exception of cultivar Barke in 2014 which had a constant RMF across sowing densities (see Figure S6). Furthermore, RMF was higher in 2013 than in 2014. In 2013, the plants had not bolted yet, and during stem elongation and bolting relatively much biomass is presumably partitioned to the shoot.

In contrast to RMF, stem mass fraction (SMF) increased linearly in both years with increasing sowing density (**Figure 3B**, Table S2). Differences in absolute values again reflect the sampling time. In 2014, SMF included ears, which however does not introduce a bias, as density did not influence flowering time. Etiolation is a commonly observed response to competition, however, in our experiments plant height was not significantly affected by sowing density treatment (data not shown) and thus does not explain the increased SMF, rather the increase in SMF is caused by an increase in the number of stems, as reflected by the tiller count. Leaf mass fraction was not affected significantly (**Figure 3C**, Table S2).

We propose that leaf area per total root length may be a better indicator of a functional equilibrium than shoot to root ratios, as carbon fixation (aboveground) and nutrient uptake (belowground) are typically estimated on basis of geometry, not mass. In both years, leaf area per TRL increased with increasing sowing density (**Figure 4**, Table S2). This increase is partly explained by an increase in specific leaf area (SLA) with increasing density which, given a constant LMF, caused high density plots to have a greater leaf area (**Figure 5**, Table S2). Hence, plants had thinner but larger area leaves at higher densities. Specific root length (SRL) also increased, but not enough to compensate for the reductions in RMF.

Sowing Density Increases SRL in the Topsoil

Although in 2014 the average SRL was lower than in 2013, trends in both years were the same: sowing density increased SRL strongest in the topsoil layer (0–10 cm) (**Figures 6A,B**, Table S3, see also Figures S7, S8). SRL increased in the plant row in the topsoil layer (0-10 cm) with increasing sowing density (from 50 cm g^{-1} in 2013 and 40 cm g^{-1} in 2014 to 150 cm g^{-1} in 2013 and 70–80 cm g^{-1} in 2014, Figures S7, S8). SRL in the row, though, was much smaller in the topsoil than in all other soil layer (SRL in layers below 10 cm depth were between 100–250 cm g^{-1} in 2013 and 70–170 cm g^{-1} in 2014), supposedly because the root crowns, where all the nodal roots come together, are in those samples. SRL was in the row highest in 10-20 cm layer (∼150 cm g^{-1} in 2013 and 100–110 cm g^{-1} in 2014) and declined slightly with increasing depth (100–150 cm g^{-1} in 2013 and 90–100 cm g^{-1} in 2014) but was at these depths not affected by sowing density (see Figures S7, S8). In contrast to the in the row cores, between the plant rows cores had greatest SRL values in the

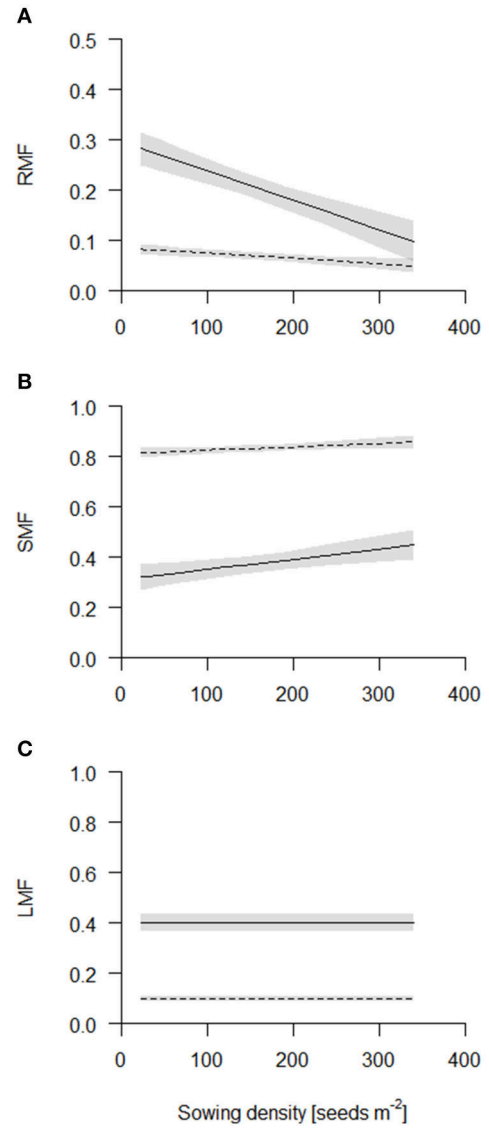

FIGURE 3 | Biomass partitioning of the coring in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S2. **(A)** Root mass fraction (RMF); **(B)** stem mass fraction (SMF); **(C)** leaf mass fraction (LMF).

topsoil (140–150 cm g^{-1} in 2014) and decreased with increasing depth. However, while in 2013 SRL increased with increasing sowing density in the topsoil, in 2014, SRL only increased in the lower sowing densities and was rather constant over sowing density from medium sowing densities on (**Figure 6A**). In the deeper layers, from 10 cm on, SRL was not affected by sowing density.

Root Length Density (RLD) in the Topsoil Increases with Increasing Sowing Density

In both years, root length density (RLD, cm root per cm^3 soil) was always highest in the plant row in the topsoil (0–10 cm), where the root crowns are (**Figure 6C**, see also Figures S9, S10). RLD increased in the row linearly with sowing density in 2013 from 2.5 cm cm^{-3}, to 4.5 cm cm^{-3}, to 6 cm cm^{-3} for the lowest, medium and highest sowing density, respectively (**Figure 6C**, Table S3). This increase in RLD was about 2–3-fold. In 2014, at the later harvest date, RLD however did not vary significantly among density treatments and were comparable to the RLD at the higher sowing densities in 2013 (**Figure 6C**). Between the plant rows, RLD was greatest in the top (0–10 cm) soil and declined with depth with the exception for the low densities in 2013, which had greater RLD deeper down (10–20 or 20–30 cm) (**Figure 6D**, Figures S9, S10). As for the in the row cores, RLD between the rows in the topsoil increased with increasing sowing density linearly in 2013 from about 1 cm cm^{-3} at the lowest to 3 cm cm^{-3} at the medium and 5 cm cm^{-3} at the highest sowing density (Table S3). In 2014, this effect was less strong and saturated above 200 plants m^{-2} (**Figure 6D**, Table S3).

Higher Densities had More Shallow Roots through Increases in Fine Root Production in the Topsoil

We estimated D50 values (depth of 50% of total root length in the top 40 cm of the core) in order to understand relative root placement. We calculated D50 values for thicker roots with diameters larger than 0.4 mm and thinner roots (diameters of 0.1–0.39 mm) separately, in order to approximately separate major axis from lateral roots. D50 values were always below 20 cm, indicating that plants always placed more roots in the topsoil, except for the thicker roots in between the row, which is supposedly explained by the downward angles at which these major axes grow (**Figures 6E,F**). The D50 values for the major axis were not influenced by sowing density, while the D50 of the fine roots tended to decrease, i.e., relatively more fine roots were placed in the topsoil, linearly from about 18 cm in lowest sowing density to about 10 cm in highest sowing density. Effects however were noisy, and only significant in 2013 in the row and in 2014 in between the row coring positions (Table S3, see also Figures S11, S12). In 2013, D50 of fine roots decreased in the row with increasing sowing density linearly from about 18 cm in lowest sowing density to about 10 cm in highest sowing density (**Figure 6E**).

DISCUSSION

We investigated how biomass partitioning to roots, root length density (RLD) and root distribution are influenced by sowing density by establishing dose-response curves to a wide range of sowing densities in the field. Earliest effects of sowing density on individual plants were visible around 30 DAS in the shoot and increased thereafter. Around anthesis (flowering), sowing density had strong effects on individual plants, affecting size, biomass partitioning and morphology of both roots and shoot. At the field level, canopy closure, leaf area index (data not shown), total shoot biomass, total root biomass, biomass partitioning, and relative rooting depth (as D50) were affected. Eventually, yield maximized at about 230 plants m^{-2}, which means that increased

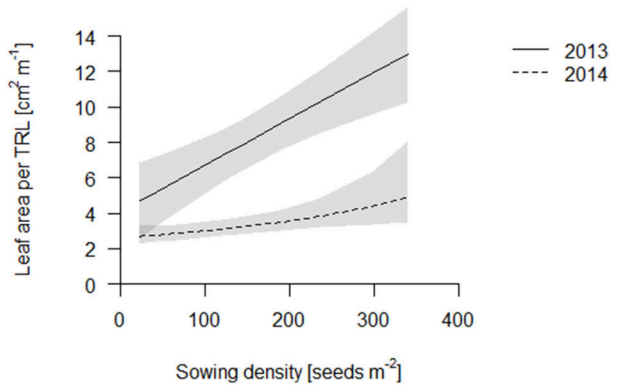

FIGURE 4 | Leaf area per total root length (TRL) of the coring in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S2.

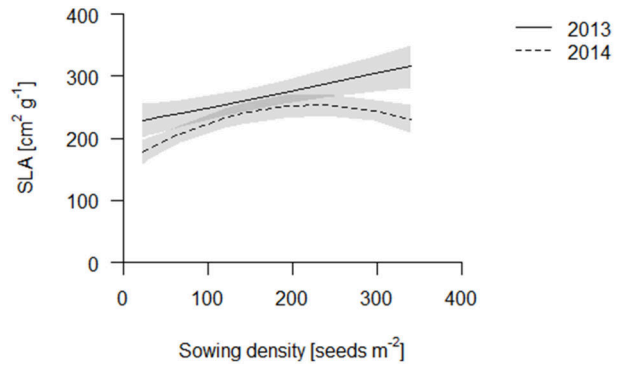

FIGURE 5 | SLA of the coring in 2013 (solid line) and 2014 (dashed line). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and *p*-values see Table S2.

biomass (g ha^{-1}) and RLD at 340 plants m^{-2} did not translate into further yield increases, rather harvest index declined. Our results may have consequences for extrapolating root function from the individual plant level to the field level and thus require careful (re)evaluation of the utility of root traits in the field, and the interpretation of genotypic contrasts observed in greenhouse-based phenotyping platforms, especially, since in greenhouse studies usually single plants are investigated.

Root Mass Fraction Decreases with Sowing Density, While Stem Mass Fraction Increases

Root mass fraction (RMF) of non-woody species typically reduces over time as plants grow larger (Poorter and Sack, 2012; Wang et al., 2015; Yin and Schapendonk, 2004; and specifically for barley Kamel, 1959). Ontogenetically, we may thus expect RMF to increase at high plant densities, as plants are smaller in high densities and smaller plants have greater RMF. So far, a variety of outcomes have been found for RMF response to plant density: Berendse and Möller (2009), for example, found such increased RMF with increasing density for *Plantago lanceolata* under low N supply, but not under high N supply, and concluded that increased RMF to plant density was better explained by plasticity responses to reduced nutrient availability in the denser populations. Under high/normal nutrient availability, Kamel (1959) did not find effects of plant density on the shoot to root ratios in barley, suggesting that plants exhibited neither plastic responses to reduced nutrient availability, nor ontogentic drift. Ågren and Franklin (2003), however, found shoot mass fraction to increase with increasing plant N concentration, suggesting that RMF actually declines at very high fertilization. Our results, also obtained under non-limiting fertilization levels, show a decrease in RMF in response to plant density and may be interpreted as an adaptive response to light competition.

Based on meta-analysis, Poorter et al. (2012) found that on average species tended to increase their SMF and specific stem length in response to density. Increased SMF and specific stem length are a possible reaction to competition for light assuming that plants elongate and increase their height in order to avoid shading by neighboring plants. Such etiolation responses have been nicely demonstrated by Nagashima and Hikosaka (2011) who placed pots with *Chenopodium album* at different heights and observed that the lowered plants simply stretched more such that they reached the same height as the higher plants. Similar to Poorter et al. (2012), we observed an increase in SMF. This increase in SMF did not correlate with an increase in crop height at anthesis, although stem elongation started slightly earlier in 2013 in the high densities (data not shown) giving rise to small differences in plant height during this earlier stage. Reports in the literature on plant height vary, with some finding increases (Munir, 2002), while others finding decreases (Turk et al., 2003; Soleymani et al., 2011) and yet others both increases and at very high densities decreases in plant height with increasing sowing density (Farnia et al., 2014). Conflicting reports may be caused by the fact that high competition for light might trigger an etiolation response, but that at the same time height is tempered for allometric reasons or reduced nutrient availability.

Crop species might also have lost the etiolation response as breeders have purposely targeted short straw varieties in order to reduce lodging risk and increase harvest index. Kiaer et al. (2013) concluded from a meta-analysis that crops are generally less competitive than wild species and that this is the result of selection under high-resource availability and weed-free conditions in which competitive ability was less important. If so, we may expect that crop height does not respond to sowing densities, which is what we observed in our two barley cultivars. The increase in SMF is thus not due to increased height but rather smaller plants had more tillers relative to their size, and thus more stems (about 80% of tillers carried ears, except in the three lowest sowing densities, where only 60% of the tillers carried ears, data not shown) and consequently the stem density per area was greatest at the highest sowing densities.

The Highest Densities had the Greatest Tiller Density and Biomass Production

The tiller count for the highest sowing densities (>140 seeds m^{-2}) reached a maximum tiller count of 2–5 tillers per plant

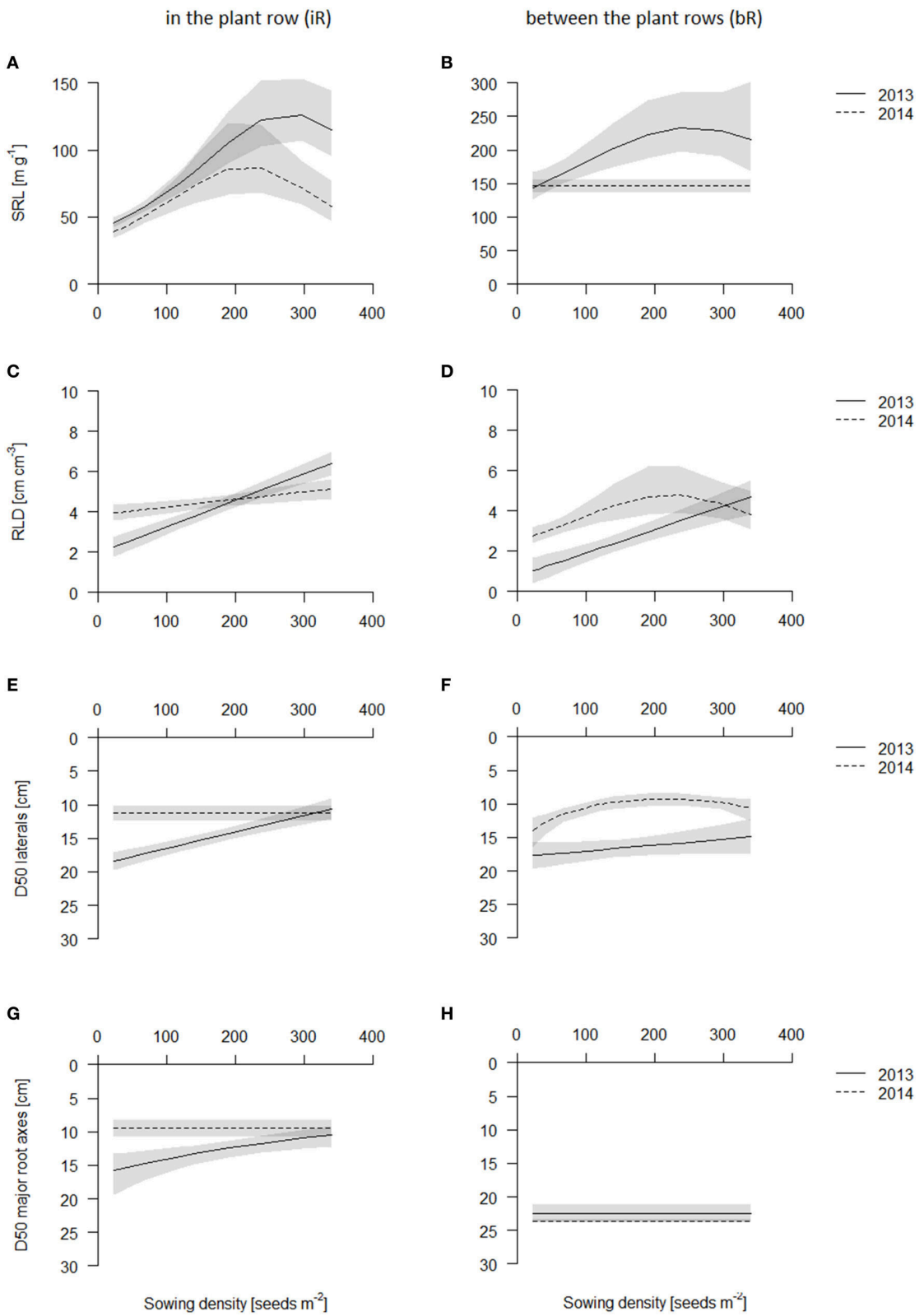

FIGURE 6 | Belowground traits of the coring in 2013 (solid line) and 2014 (dashed line) in the plant row (iR, left) and between the plant rows (bR, right). Data are presented as best fits with 95% confidence interval (gray). For equations, R^2 and p-values see Table S3. SRL in the topsoil iR **(A)** and bR **(B)**; RLD in the topsoil iR **(C)** and bR **(D)**; D50 values of laterals iR **(E)** and bR **(F)**; D50 values of major root axes iR **(G)** and bR **(H)**.

in both years at about 5 weeks after sowing, only 1 week after the earliest response of tiller counts to sowing density. Tillering in *Poaceae* is known to respond early to density, not due to direct competition but to changes in red to far-red ratios (Casal, 2013). Despite the early arrest in tillering, the highest sowing densities reached the highest tiller density of 2000 tillers per m^2 (1500 tillers per m^2 in 2013), which is two to three times greater than found in other studies (Finlay et al., 1971; Fukai et al., 1990; Munir, 2002; Soleymani et al., 2011). However, this difference probably reflects the contrast between the temperate climate of Germany and the arid climates of Jordan, Iran, and Australia. In Germany, guidelines recommend a spike density of 800–1150 spikes per m^2 (Landwirtschaftskammer Nordrhein-Westfalen) and with only 80% of the tillers carrying a spike we achieved 1000–1400 spikes per m^2. Surprisingly, this high tiller density did not compromise the dry weight per tiller in our study, such that not only the tiller density but also the shoot biomass per ha increased linearly with density which stand in contrast with the constant final yield concept (Weiner and Freckleton, 2010). Possibly constant yield is achieved later in time as observed by Fukai et al. (1990). Final constant yield does not usually distinguish between shoots and grain biomass. In our study, we found something similar to constant final yield in terms of grain yield, but not in terms of shoot biomass.

Grain yield was slightly reduced at the highest density, but even if grain yield was constant, the presumptive harvest index (final grain yield over total biomass at final harvest; in our study, we can only approximate harvest index by using biomass data from the coring events; data not shown) clearly declined with increasing density (assuming that stem and leaf mass did not increase drastically after anthesis). This decline in harvest index was also observed by Farnia et al. (2014) and may suggest that the higher biomass production was metabolically costly and reduced yield.

Root Length Density Increases with Sowing Density due to Greater Specific Root Length

The net effect of increasing total biomass but decreasing biomass partitioning to roots is that the total root biomass per ha stayed constant, or increased possibly slightly, but in general the plot to plot variation was large (data not shown). Thus, the increase in RLD in the topsoil is mostly caused by an increase in SRL, since root biomass itself stayed constant. Aerts (1999) lists several studies in which plants respond to interspecific competition by reducing RMF and increasing SRL and we thus may have observed a common response. Changes in SRL are, however, difficult to interpret as they may be caused by shifts in root anatomy, or in the relative production of fine roots verses major axis roots. In recent years, more attention has been drawn to root anatomical traits and their function (reviewed by Paez-Garcia et al., 2015), and for barley root cortical senescence may be of special importance (Schneider et al., in revision).

Determining changes in root anatomy of cored roots seems difficult, as we do not know the age or class of the root fragments that we collected and thereby have no indication if root anatomy, and in particular the rate of cortical senescence, was affected by sowing density.

Our data suggests that increases in SRL at least in part were caused by a greater portion of fine roots in the topsoil. Several studies have suggested that interspecific competition may lead to increased root proliferation (Mommer et al., 2010), and thereby increased production of lateral roots. Although these responses are not completely understood, one explanation may be that plants try to outcompete by depleting soil resources faster than their neighbors. Such responses would not be desirable in agriculture, as it would not increase the performance of the whole crop. Rather the crop should exhibit lateral root traits that maximize resource acquisition, as for example recently estimated using a root model by Postma et al. (2014a) who showed that high rooting density may increase phosphorus acquisition, but not nitrate acquisition unless nitrate concentrations are very high.

Greater Placement of Roots in the Topsoil with Increasing Density

In our study, plants accumulated root length in the topsoil supporting our hypothesis. Topsoil foraging is important for the acquisition of immobile nutrients (Dunbabin et al., 2003), and possibly reduces leaching of mobile nutrients (Thorup-Kristensen, 2001). Many crops and plant species explore the topsoil with greater intensity, for wheat see Schweiger et al. (2009) and Lotfollahi (2010) and for barley Lampurlanés et al. (2001) and Breuning Madsen (1985), who showed that the topsoil is foraged with greater intensity irrespective of soil or tillage type. Topsoil foraging has been strongly associated with changes in rooting angles of the major axis (Lynch, 2013). Our coring data suggests however that the depth of the major root axis did not change over sowing density for the two genotypes. Hence, the difference in D50 for the major axis between in the row and in between the row columns over the half row distance is 8/10 and 14/10 for 2013 and 2014, respectively. We think that these calculations may reflect average angles of $\arctan(10/8) = 51^\circ$ and $\arctan(10/14) = 36^\circ$ degrees from vertical for both years respectively, although the year difference is somewhat artificially caused by the "in the row" D50 values.

Changes in Biomass Allocation and Root Morphology and Architecture may be Adaptive Responses to Plant Competition

Increases in plant density cause the resource availability per plant to decline. Mathematically, we might assume that both light availability and nutrient availability scale linearly with the area per plant. Thus reductions in plant size could be simply seen as a decline in resource availability per plant. Plant growth, however, is not always a pure function of resource availability, neither does one resource alone usually determine production (law of the minimum), as plants can adapt their architecture and morphology in order to increase or balance resource capture (Ågren et al., 2012; Dathe et al., 2013; Postma et al., 2014b).

Our data shows that barley plants adapt their biomass allocation, root and shoot morphology, and architecture in response to plant density. Since biomass production increased

with density, our results suggest that these adaptations increased resource capture of the whole crop. Shoot:root ratios are thought to express a functional equilibrium between above- and belowground (Gleeson and Tilman, 1992). We computed leaf area per root length as a better expression of that functional equilibrium as it takes changes in SRL and SLA into account. Current models of nutrient uptake and photosynthesis typically integrate over area or length, not mass (Thornley, 1995; Boote et al., 2013; Dunbabin et al., 2013). In both years, leaf area per root length increased with increasing sowing density. This may suggest that increasing sowing density shifted plants into carbon limited growth. Through fertilization, farmers try to achieve yields that are not limited by nutrient capture, and consequently light capture is probably the limiting factor once a crop is established.

At high nutrition, Grime and Hodgson (1987) suggest that ideal competitive traits are fast shoot growth to avoid shading by neighbors, high relative growth rates and high morphological plasticity. Our measurements on shoot traits seem to at least partly confirm these traits, however, we also observed changes in the root traits. These suggest that the metabolic efficiency of the root system, that is the relative investment of biomass (carbon, N, P) into roots, is reduced while the nutrient uptake capacity of the root system is increased by increasing root length. These increases occurred mainly in the topsoil.

Simulation studies suggest that under agricultural conditions root competition for immobile nutrients is relatively low (Postma and Lynch, 2012; Postma et al., 2014a) and thus further increases in RLD probably do translate into greater uptake of nutrients in the topsoil. Acquisition of nitrogen may be improved through increased NH_4 uptake although NH_4 are typically low in well-aerated temperate soils. Effective nitrate uptake is thought to be associated with low RLD and exploration of large soil volumes, which is in the case of crops translates into deep rooting (Dathe et al., in press). We did not find differences in RLD deeper down, however, such that we cannot exclude that density may influence RLD below 60 cm. We conclude that at high sowing density, fertilized barley produces more leaf area through increased SLA, more stem biomass through increased allocation to stems, and more root length in the topsoil through increased SRL. Maximum yield was found around 230 plants m^{-2}, as in higher densities the harvest index declined, possibly due to over commitment to shoot biomass, while total light capture at the crop level was not increased.

The Strong Effects of Sowing Density on Individual Plant Traits Raises Questions about How to Scale up Research Results from the Lab to the Field

Currently, most root research is performed on individual plants growing in pots, relatively isolated from other plants. However, if root research is to have an impact on breeding strategies and agriculture as a whole (Kuijken et al., 2015), we need to understand how roots function in the context of high plant densities. Our research shows that high plant density can drastically change the root system, the relative rooting depth (D50), the biomass partitioning to roots and the root length distribution with depth. Changes in biomass partitioning and root morphology or architecture supposedly influence the functioning of the root system (Berendse and Möller, 2009; Lynch, 2013), and may partly compensate for increased competition at higher density, thus increasing biomass production and yield of the whole crop. In our research, both cultivars were very similar in nearly all aspects and responded similarly to sowing density. We suspect, however, that genotypic differences in response to plant density exist, since barley genotypes can differ quite dramatically in their responses to other factors like e.g., nutrient availability (Ayad et al., 2010; Karley et al., 2011). Plant performance of different genotypes and the utility of traits are often evaluated on the basis of early vigorous growth in plants growing at low densities. However, these genotypes may well lose their advantage in a high density stand. The highest yield in this study was obtained with relatively small individual plants, a high harvest index, and reduced biomass allocation belowground, while total crop nutrient uptake was guaranteed by high RLD and greater SRL, as, for example, maize showed to increase RLD under low N especially in the topsoil (Mu et al., 2015) and wheat plants with more roots (unpruned plants) had a greater N uptake under competition than plants with fewer roots (pruned plants) (Andrews and Newman (1970). Furthermore, early biomass production may simply result in early competition (Weiner and Freckleton, 2010), and not translate into yield. We suggest that variation in individual plant size during early growth stages may be compensated for by variation in plant density and thus has little meaning for agricultural production. We conclude that genotypes should not just be evaluated on absolute values, but rather in terms of efficiencies, such as root metabolic efficiency, harvest index, and nutrient uptake efficiency (uptake per unit root mass) as these characteristics are more important at the crop level than individual plant weight, which can be compensated for by sowing density.

CONCLUSIONS

Sowing density influenced individual plant size and relative biomass allocation to different plant organs. The changes in biomass allocation are opposite from what we may expect from general allometric rules; that is bigger plants have reduced RMF and increased SMF and LMF. Thereby, the changes in biomass allocation are not just related to size, but related to (adaptive) responses to competition. Our results indicate that plant density increased the SMF at the cost of the RMF. Increased SRL and increased overall biomass production allowed the high sowing density plants to maintain relatively high RLDs in soil, despite the reduced biomass allocation to roots. Sowing density increased RLD in the topsoil, especially in between the row, while not affecting the RLD further down. This may mean that deep rooting, at least in rather light- than nutrient-limited systems, is not sensitive to sowing density, as we initially hypothesized.

Plants reduced the investment into root biomass with increasing sowing densities and simultaneously enhanced the

investment of this root biomass into fine roots. Moreover, aboveground, biomass was invested into stems; however, although, plants did not raise the investment into leaf biomass fractions with increasing sowing density, they increased leaf area, by increasing SLA. The combination of these changes in allocation indicates that the plants in our study were generally more aboveground light-limited than belowground resource-limited.

Changes in root length distribution with depth, SRL and overall biomass allocation to roots suggest that architectural and morphological changes in the root system occurred, and possibly greater source limited tradeoffs for root growth had taken place. While these hypotheses require further investigation, they possibly have important consequences for root phenotyping of isolated plants, the functional interpretation of traits for nutrient and water acquisition, and the importance of traits that may increase the metabolic efficiency of the root system.

AUTHOR CONTRIBUTIONS

The conception and design of the work was performed by all authors. VH and JP acquired the data. VH processed the samples. Data analysis was done by VH and JP. Data interpretation was done by all authors. VH performed the drafting of the work with accompanying input from JP. All authors revised the manuscript critically for important intellectual content and approved the final version of the manuscript. Further, all authors agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2016.00944

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9.2 Plant density modifies root system architecture in spring barley (*Hordeum vulgare* L.) through a change in nodal root number

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REGULAR ARTICLE

Plant density modifies root system architecture in spring barley (*Hordeum vulgare* L.) through a change in nodal root number

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Abstract
Aim Previously, we showed that sowing density influences root length density (RLD), specific root length (SRL) especially in the topsoil, and shallowness of fine roots of field grown spring barley (*Hordeum vulagre* L.). Here, we ask which trait components may explain these observed changes.
Method We grew two spring barley cultivars at contrasting sowing densities in both field trials and rhizotrons, and excavated root crowns and imaged root growth.
Results In the field, tiller and nodal root numbers per plant decreased with increasing sowing density, however, nodal roots per tiller, seminal roots per plant, and lateral branching frequencies were not affected. Branching angle did not or only slightly declined with increasing sowing density. In rhizotrons, aboveground only tiller number was affected by sowing density. Root growth rates and counts were not (or only slightly) affected.
Conclusion Greater RLD at high sowing densities is largely explained by greater main root number per area. The altered seminal to nodal root ratio might explain observed increases in SRL. We conclude that sowing density is a modifier of root system architecture with probable functional consequences, and thereby an important factor to be considered in root studies or the development of root ideotypes for agriculture.

Keywords Nodal & seminal roots · Tiller counts · Lateral branching frequency · Branching angle · Lab to field · Plant competition

Abbreviations

C_2013	coring in field in 2013 for scanning of roots via NMR
CKA	Field Lab Campus Klein-Altendorf (field site, research location in Germany)
D50	depth at which one finds 50% of the total root length [cm]
DAG	days after germination
DAS	days after sowing
Field1_2013	first shovelomics sampling in the field in 2013
Field2_2013	second shovelomics sampling in the field in 2013

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Field1_2014	first shovelomics sampling in the field in 2014
Field2_2014	second shovelomics sampling in the field in 2014
GDD	growing degree days [°C]
NMR	nuclear magnetic resonance
MRD	maximum rooting depth (from soil surface to deepest root tip, cm)
RGR	relative growth rate
Rhizo1	first experiment in rhizotrons
Rhizo2	second experiment in rhizotrons
Rhizo3	third experiment in rhizotrons
RLD	root length density [root length per unit soil, cm cm^{-3}]
RSA	root system architecture
Sowing density	applied treatment, in seeds m^{-2}
SRL	specific root length [root dry mass per root length, g m^{-1}]
TRL	total root length (visible at window)

Introduction

Much research in plant science is conducted indoors, under controlled climatic conditions, but claims to have relevance to the field. Translation of results has often proven difficult. We consider that part of the differences found might be explained by environmental factors such as fluctuating light, varying temperature, rainfall and wind occurring. We propose, however, that plant competition, which is strongly affected by sowing density in a crop, may be an important factor in lab-to-field translation.

Plant density is known to have large effects on plant traits and growth above-ground; increasing sowing density reduces tiller number per plant (Kamel 1959; Munir 2002; Turk et al. 2003; Soleymani et al. 2011) and shoot dry weight per plant (Harper 1977), while increases tillers per area (Kays and Harper 1974; Darwinkel 1978), leaf number per area (Khalil et al. 2011; Moosavi et al. 2012), leaf area per area (=leaf area index) (Pospišil et al. 2000; Amanullah et al. 2007; Olsen and Weiner 2007; Moosavi et al. 2012) and specific leaf area (leaf area per mass of leaf) (Amanullah et al. 2007; Farshbaf-Jafari et al. 2014).

Despite knowledge about how sowing- and the resulting plant density affects plant traits aboveground, very little is known about how sowing density affects root traits and architecture belowground. Knowledge of effects of sowing density on roots could prove to be essential in the quest to find crop ecotypes and varieties that are better adapted to extreme weather events such as drought or low soil phosphorus levels, since sowing density may interact with a crop's ability to be resilient to drivers of global change. We are not aware of literature dealing with branching angle, root number, and lateral branching frequency and how these traits are affected by sowing density in barley. We found a few studies in other plant species on the topic. Sowing density has been found to decrease branching angle in grape vine and increase root length density (RLD) in the topsoil without affecting the ratio of primary, secondary and tertiary roots (Archer and Strauss 1985). Volis and Shani (2000) studied the desert annual plant *Eremobium aegyptiacum*, a Brassicacea, in its natural habitat and found the number of first order laterals and the lateral branching frequency at the main root to increase with plant density. Further, maize has been found to grow fewer roots at upper phytomers at higher plant densities (Demotes-Mainard and Pellerin 1992; Pellerin 1994). Kamel (1959) analyzed root dry weights and root mass fraction in barley and found that neither traits were affected by sowing density. His study used rather high seeding rates (125–500 seeds m^{-2}) and reported unusually low root mass fractions such that we do not know how generalizable their results are. We conclude that there is a knowledge gap with regard to how sowing density effects root architecture and that this knowledge gap may play an important role in understanding why experiments on individual plants in controlled conditions may not translate directly to experimentation in the field.

In a previous study, we reported that increasing sowing density affected a number traits in the field. Sowing density led to a reduction in tiller formation and shoot dry weight per plant and an increase in specific leaf area of field grown barley (Hecht et al. 2016) and suggested that the plants might respond more to light competition than nutrient competition. We observed that shoot dry weight per tiller was not affected by sowing density, however, it increased over time. Additionally, we found that increasing sowing density changed biomass allocation and fine root distribution. Specifically, stem mass fraction increased, while root mass fraction decreased with increasing sowing density, again pointing to a strong role of competition for light being a main driver

of such effects. In a meta-analysis, Poorter et al. (2012) showed that these changes in allocation have been observed for several other species. Within the root system, we observed that root mass was invested into fine root growth resulting in increased RLD as well as specific root length (SRL) (Hecht et al. 2016). This occurred especially in the topsoil, causing the depth at which one finds 50% of the total root length (D50) of fine roots to become shallower. In this study we focused on the individual plant phenotype and the organ level phenotypic traits such as root branching angles, nodal and seminal root counts and lateral root branching densities. We wanted to know which architectural root traits may explain the observed changes in specific root length, rooting depth and biomass allocation at the plot (field) level. We also asked if the effects of sowing density on root architectural traits could be reproduced under controlled conditions using rhizotron boxes placed along a gradient of environmental conditions going from highly controlled, but artificial, to less controlled but closer to reality, e.g. rhizotron boxes places in a growth chamber, a greenhouse, and outside.. We investigated branching angles, root counts, and branching frequencies of plants grown at high and low density in these three conditions and compared them to the same measurement taken on roots excavated in the field. We reason how plasticity in these root architectural traits may explain the earlier reported higher scale observations of SRL and RLD distribution with depth.

Given the lack of knowledge with regard to how sowing density effects root architecture, we may consider how sowing density influence the local environment of the plant, and how these environmental changes may cause phenotypic responses. Increasing sowing density does not only reduce the area available per plant but also the amount of intercepted light per plant (changing light quantity and quality) and water or nutrients (e.g. nitrogen) available to the individual plant (Weiner et al. 2001). These changes in the abiotic factors can alter plant architecture and such plasticity responses have been much studied (Kamel 1959; Casal et al. 1986; Thorup-Kristensen 2001; Manschadi et al. 2008; Wasson et al. 2012; Lynch 2013; Paez-Garcia et al. 2015) and might be a basis for interpreting root growth plasticity responses to increasing sowing density and will thereby be shortly discussed.

A decline in red (R) to far-red (FR) light – no matter, if by neighboring plants absorbing red and blue light (Woolley 1971; Holmes 1981; Kasperbauer and Karlen 1986; Kasperbauer 1987) or artificially –with increasing sowing density, leads to a reduction in tiller production of the individual plants (Casal et al. 1986; Davis and Simmons 1994). Similarly, low light has been shown to reduce tiller formation in grasses and cereal crops in comparison to plants growing under normal light conditions (Kamel 1959; Kays and Harper 1974; Casal et al. 1986). Belowground, reduced light decreased root weight per plant in barley (Kamel 1959) and SRL is increased in the grass *Lolium perenne* (Evans 1983). Thus, low light and a reduced R/FR ratio cause a decrease in tiller number of a plant regardless of whether the incoming radiation is altered by neighboring plants or artificially. In addition, as Kamel (1959) already argued, roots are dependent on carbohydrates produced by the shoot and if shoot growth is limited by e.g. light intensity, root growth might too be limited.

More plants at e.g. higher plant densities require and take up more water (Azam-Ali et al. 1984; Archer and Strauss 1989), but also nutrients, such as nitrogen, phosphate and potassium (Gao et al. 2009; Ciampitti and Vyn 2011; Su et al. 2011; Li et al. 2014). This does not necessarily occur proportionally due to competition, which can be asymmetric when plants of different sizes are competing with one another (Weiner and Thomas 1986). We hypothesize that competition might trigger similar root growth plasticity responses as under low resource availability, such as root proliferation (Marschner 2012), changes in rooting depth (Thorup-Kristensen 2001, 2006; Kristensen and Thorup-Kristensen 2009; Wasson et al. 2012; Lynch 2013), branching angle (Manschadi et al. 2008; Singh et al. 2010b; Dathe et al. 2013), axial root number (Saengwilai et al. 2014), and lateral roots (Postma et al. 2014; Zhan and Lynch 2015; Zhan et al. 2015). The linking of some root architectural traits to a specific function has also recently been reviewed by Paez-Garcia et al. (2015). However, as the literature is not clear on how plants respond to belowground resource deficiency, and as we do not know what resources high density plants compete for most, it is difficult to predict what responses in root system architecture (RSA) we might expect.

We hypothesized that, as the previously observed increase in RLD seemed to be caused mostly by greater investment into fine roots (expressed in the previously observed increased SRL), the lateral branching frequency, as a trait component of RLD, might be greater at higher sowing densities.

Furthermore, as in our previous study the D50 values of major root axes were not affected by sowing density (Hecht et al. 2016), we expect the branching angle to be the same for all sowing densities. In order to test these hypotheses, we measured many root architectural traits in a range of experiments in which we varied the sowing density. As one difference between controlled and field experiments is often plant density (in controlled experiments, often single plants are studied, while in a crop in the field plants usually grow in a stand), we designed these experiments along a gradient from highly controlled to greater realism in order to understand how plant density might play a role in the translation from lab to field. We thus grew our plants in rhizotrons and in the field. The rhizotron experiment was repeated under varying climatic conditions, in a growth chamber, in a green house, and outside, representing a gradient from highly controlled to near field conditions.

Material and methods

Within this study, we conducted three rhizotron experiments with spring barley as single plants and in clusters and two field experiments with a range of different sowing densities that corresponded to those used in the rhizotron experiments. Two of the rhizotron experiments were performed in 60x30cm-rhizotrons namely, Rhizo1 (rhizotron experiment 1) at 414 GDD (GDD = growing degree days, for calculation and explanation see below; 23 days after germination (DAG)) and Rhizo2 (rhizotron experiment 2) at 251 GDD (19 DAG), and the third rhizotron experiment was conducted in bigger 90x70cm-rhizotrons, namely Rhizo3, at 510 GDD (34 DAG) using GROWSCREEN-Rhizo (Nagel et al. 2012).

In total, there were five samplings in the field experiments at Campus Klein-Altendorf: in both years, 2013 and 2014, we excavated root crowns twice via the so called shovelomics approach (Trachsel et al. 2010), namely in Field1_2013 (75 days after sowing (DAS), 995 GDD) and in Field2_2013 (98 DAS, 1456 GDD) in 2013 and in Field1_2014 (35 DAS, 325 GDD) and in Field2_2014 (68 DAS, 767 GDD) in 2014. Additionally, we took soil cores for scanning via nuclear magnetic resonance (NMR) in 2013, namely C_2013. For a better comparison of rhizotron and field experiments, we first converted DAS of field trials into DAG by approximating the first DAG as 5 days before shoot emergence. Second, we converted DAG for all experiments into GDD, since temperatures can vary greatly among years and locations and plant development depends, among others, strongly on temperature (Kirby et al. 1982; Hunt and Thomas 1985; Miglietta 1989; McMaster and Wilhelm 1997, 2003; Füllner et al. 2012). Thermal time expressed in GDD is the cumulative heat based on daily mean temperature above a certain base temperature. Hence, thermal time is supposed to be a better way to compare developmental stages of plant growth than days of growth (Miller et al. 2001). We calculated GDD as

$$GDD = \sum_{i=1}^{n} \left\lceil \frac{(T_{max} + T_{min})}{2} \right\rceil - T_{base} \quad (1)$$

where T_{max} and T_{min} are daily maximum and minimum air temperature, respectively, and T_{base} is the base temperature (McMaster and Wilhelm 1997), for each day of growth from 1 DAG (i) until sampling (n). We set base temperature to 0 °C (e.g. McMaster and Smika (1988); McMaster and Wilhelm (2003)). In the following paragraphs, the experiments are described in more detail.

Plant material

We grew two German malting spring barley (*Hordeum vulgare* L.) cultivars 'Scarlett' and 'Barke'. Scarlett is shorter than Barke, however, Barke is more resistant to lodging. Scarlett ripens earlier than Barke (Landesanstalt für Pflanzenbau und Pflanzenschutz 2002). Barke is possibly one of the most researched barley cultivars of the last decade (Gahoonia and Nielsen 2004; Schmalenbach and Pillen 2009; Auškalnienė et al. 2010; Dornbusch et al. 2011; Castillo et al. 2012; Füllner et al. 2012; Alqudah and Schnurbusch 2015).

Rhizotron experiments

We conducted the experiments in greenhouse, climate chamber, and outdoor at the Forschungszentrum Jülich GmbH, Germany (N50° 54′ 35.96", E6° 24' 47.401"). We transferred pre-germinated seeds into rhizotrons (narrow boxes at 45° angle with a transparent plate on

the lower side through which one can track root growth visually) with a spacing representing low and medium sowing density of field experiments. More information on design, number of replicates, climate conditions are listed in Table 1. We harvested the plants, when the first roots reached the bottom of the rhizotrons.

Pre-germination and transfer to rhizotron

We stirred the seeds in a mixture of 1 ml Tween20 surfactant per 50 ml H_2O and 0.5%NaClO for surface sterilization in a beaker with a stirring rod for 1 min. We discarded the sterilization bath and rinsed the seeds with tap water for 5 min before vernalization in 0.5mMol $CaSO_4$ in a beaker covered by wrapping foil at 4 °C in the fridge overnight. The next day, we placed seeds on sterile petri dishes that contained filter papers soaked with 5 ml of 0.5mMol $CaSO_4$. We covered seeds with another wetted filter paper to prevent drying. We sealed petri dishes with Parafilm® and wrapped them in aluminum foil to keep the seeds in the dark. We kept them in a cupboard at room temperature. Seeds germinated after 2–3 days and seedlings with roots of 2–4 mm length were transferred into rhizotrons filled with substrate at 2–3 cm depth, and placed close to the transparent sheet.

Shoot measurements and sampling

During growth, every 2–3 days, we noted the growth stages of plants using the BBCH according to (Lancashire et al. 1991) and counted the number of tillers. At harvest, we cut the shoots at the base and dried the samples in the drying oven at 70 °C for minimum 5 days before determining dry weights.

Roots measurements and sampling

During growth, every 2–3 days, we photographed the visible roots at the transparent window, determined their length via RhizoPaint (Nagel et al. 2012), and determined maximum rooting depth (MRD) as distance from soil surface to deepest root tip. We calculated growth rates of MRD (GR_{MRD}) at measurement day j and k as:

$$GR_{MRD} = \frac{(MRDj_i - MRD_k)}{(GDD_j - GDD_k)} \quad (2)$$

Further, we calculated relative growth rates (RGR) of total root length (RGR_{TRL}) at measurement day j and k as:

$$RGR_{TRL} = \frac{(ln(TRL_j) - ln(TRL_k))}{(GDD_j - GDD_k)} \quad (3)$$

At harvest, we measured branching angle (BA) of the outermost seminal and outermost nodal roots separately and at 5 cm depth (Rhizo1). We present the average branching angle as the angle from vertical (see also Fig. 1 b):

$$BA = \frac{180 - (BA_{left} + BA_{right})}{2} \quad (4)$$

Moreover, we counted the number of seminal and nodal roots for each tiller separately (see Fig. A.1). All roots emerging close to the seminal roots but not from the seed tip, we counted as coleoptile nodal roots, and excluded from the here presented nodal root counts (compare to Singh et al. (2010a)). We washed the roots over a sieve using tap water. For Rhizo2, we measured lateral branching frequency at 1 cm, 10 cm, and 20 cm from the base of one seminal root as counts of lateral roots per cm root. We estimated the average lateral root length by 1) scanning the longest seminal root with all its laterals, 2) determining the total length in root diameter class 0.1–0.3 mm (WinRHIZO™, manual threshold 210, 600 dpi), and 3) dividing that length by the count of all lateral roots along the longest seminal root.

For Rhizo3, for a distribution histogram of the nodal roots per tiller, we grouped all tillers with a certain number of nodal roots per tiller for each plant into the following categories/bins: 0–2, 3–5, 6–8, and 9–11 nodal roots per tiller. For an example plant see also appendix Fig. A.1.

Field experiment

We sampled in the same field trials as described in Hecht et al. (2016), albeit at different time points, with different sampling methods and in different blocks. Here we provide a short summary of the experiment and the additional root sampling techniques used for this publication.

Table 1 Experimental design, treatment, and climate data of the rhizotron experiments

	Rhizo1	Rhizo2	Rhizo3
Location	Climate chamber at research center	Outdoor at research center	Greenhouse at research center
Rhizotron size (outer dimension), volume	60 cm × 30 cm × 2 cm, 3.4 l	60 cm × 30 cm × 2 cm, 3.4 l	90 cm × 70 cm × 5 cm, 18.3 l
Substrate	Kaldenkirchener field soil + sand (2:1 *v*/v)	Kaldenkirchener field soil	Klein-Altendorf field soil + substrate (1:1 v/v)
Watering (tap water)	To weight of rhizotron at field capacity (18% water per l soil)	To weight of rhizotron at field capacity (32% water per l soil)	Automatic irrigation (from 145 ml per day to 290 ml per day, 23%[a] water per l soil)
Fertilizer	same amount of fertilizer per plant (133.3 and 666.7 mg Hakaphos® blue in low and medium sowing density, corresponds to 60 kg N/ha applied usually at first N-application in field), mixed into substrate	same amount of fertilizer (666.7 mg Hakaphos® blue, corresponds to 60 kg N/ha) per rhizotron, mixed into substrate	Same amount per rhizotron, no additional fertilizer
Pesticides	Juwel® (fungicide) at BBCH 13	Juwel® (fungicide) at BBCH 13	Juwel® (fungicide) at BBCH 13
Cultivars	Scarlett	Scarlett	Scarlett, Barke
Plants per rhizotron (distance to neighbor plant[b]), representing low and medium sowing density of the field trials	1 and 5 plants (4 cm)	1 and 5 plants (4 cm)	1, 3 (21 cm) and 13 plants (4 cm)
Replicates per treatment (design)	5 (complete randomized design)	5 (randomized block design)	8 (randomized block design)
Final harvest date, days after germination, thermal time, cumulative PAR, developmental stage	5 November 2012, 23 DAG, 414 GDD, 38.65 kWh m^{-2}, BBCH 21–23, tiller formation	13 May 2013, 19 DAG, 250.62 GDD, 14.76 kWh m^{-2}, BBCH 19–21, tiller formation	3 February 2014, 34 DAG, 510 GDD, 6.44 kWh m^{-2}, BBCH 21–22, tiller formation
Air temperature (day/night)	20/16 °C (16 h/8 h)	Average 13.6/9.1 °C (15 h/9 h), with average maximum of 16.7 °C (total maximum 24.8 °C)	18/12 °C (16 h/8 h)
Light (Photosynthetically active radiation (PAR))	Ca. 960 μmol m^{-2} s^{-1} (16 h)	Average PAR 187.60 μmol m^{-2} s^{-1}, with average maximum of 296.25 μmol m^{-2} s^{-1} (overall max. 485.16 μmol m^{-2} s^{-1}) (15 h)	Average PAR 93.15 μmol m^{-2} s^{-1}, with average maximum of 198.09 μmol m^{-2} s^{-1} (overall max. 407.33 μmol m^{-2} s^{-1}) (9 h)
Air humidity	60%	NA	60%
Trial year	2012	2013	2014

[a] Minimum: 19%, maximum 31%, with average maximum of 25%

[b] corresponds to a sowing density in the field of about 300 seeds/m2, when inter-row distance 12 cm; in the within this study conducted field experiments, this neighbor-distance corresponds to medium sowing density, as inter-row distance is 21 cm

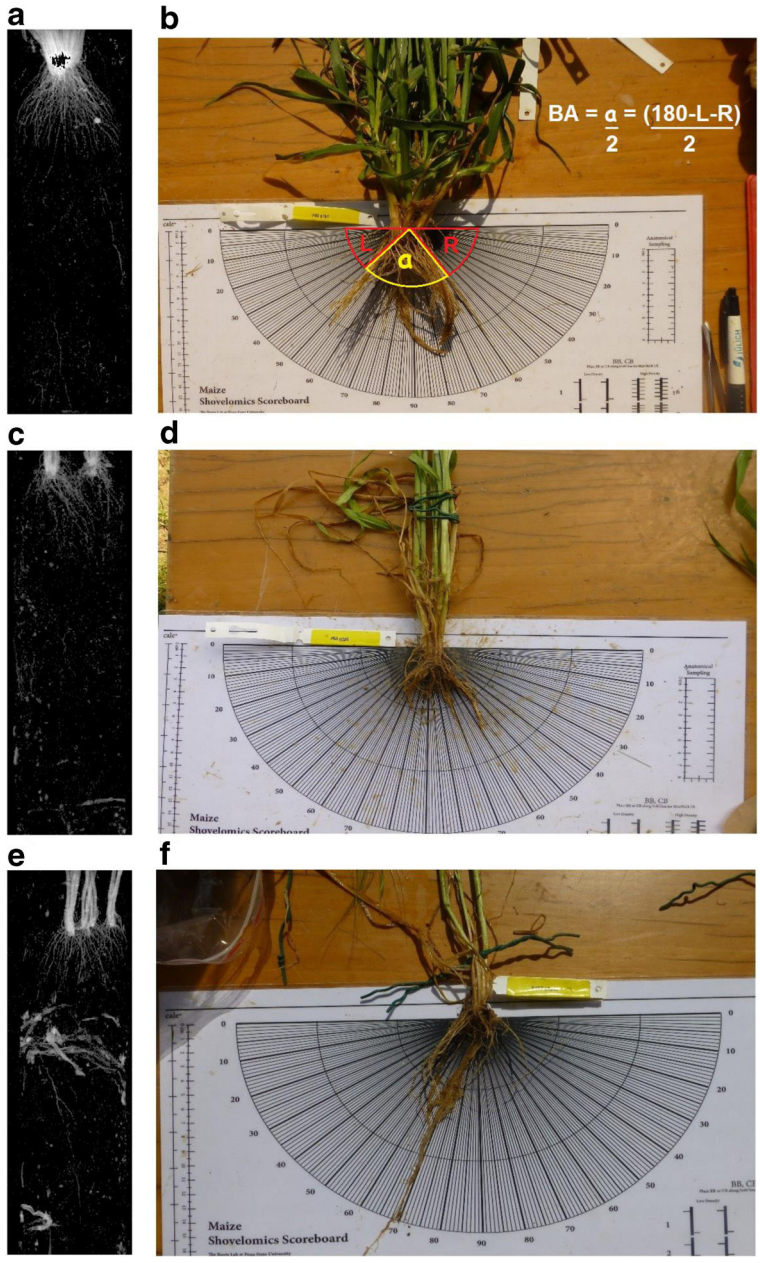

Fig. 1 NMR-images of cores taken 68 DAS (**a**, **c**, **e**) and photos of root systems of barley plants taken during shovelomics at 75–76 DAS at CKA in 2013 (**b**, **d**, **f**). a and b show Scarlett plants of low sowing density, c and d Barke plants of medium sowing density, and e and f Barke plants of high sowing density. Further, b shows the measuring of the branching angle (BA) at 5 cm depth of the freshly excavated and washed spring barley root system

Field site

We conducted two experiments at Campus Klein-Altendorf (CKA, University of Bonn, Germany, 50°37′31.00”N, 6°59′20.54″E) in 2013 and 2014 on a loamy-clay silt soil (luvisol). Annual precipitation, average annual temperature and sun hours were 734.4 mm, 9.8 °C and 1753 h in 2013 and 820.4 mm, 11.4 °C, and 1934 h in 2014, respectively, and cumulative rainfall, cumulative growing degree days (GDD in °C), and cumulative photosynthetically active radiation (PAR) from sowing date until final harvest date were 285 mm, 1769.06 GDD, and 68.9 kWh m^{-2} in 2013 and 315 mm, 1864.45 GDD, and 74.9 kWh m^{-2} in 2014 (see Fig. S 1 in Hecht et al. (2016)). Climate data were obtained from the service center of the rural area of Rhineland-Palatine (Dienstleistungszentrum Ländlicher Raum Rheinland-Pfalz) and can be found on http://www.am.rlp.de. For more [crop husbandry] details see Material and Methods in Hecht et al. (2016).

Experimental design

Sowing took place on 25 April 2013 and 20 March 2014 in 1.5 × 14.2 m^2 plots in six rows in a fully randomized block design with five replicates in ten different sowing densities as described in Hecht et al. (2016). Seedlings emerged on 5 May 2013 and 2 April 2014, respectively, so that – with an assumed period of 5 days from seed germination to seedling emergence – germination took place at 5 DAS in 2013 and 8 DAS in 2014, respectively, leading to a conversion of DAS to DAG of X-5 DAS = DAG in 2013 and Y-8 DAS = DAG in 2014, with X and Y the respective measurement day. Within this study, we only used data of the lowest, medium and highest sowing densities (24, 120, and 340 seeds m^{-2}; in 2013, second shovelomics-sampling 31 instead of 24 seeds m^{-2}, since too many plants died in the 24 seeds m^{-2} plots to find an appropriate slot to sample). For each genotype, we sampled root crowns of one plant per plot of each sowing density using the shovelomics-approach (Trachsel et al. 2010). For more details on sampling method, sampled sowing density, replicates, climate conditions at harvest see Table 2.

Shoot sampling and measurements

We cut the shoot at plant base, recorded BBCH (Lancashire et al. 1991) and counted the number of all tillers of each sampled plant. We oven-dried the shoot samples at 70 °C for minimum 2 days before determining dry weights (to the nearest 0.01 g).

Root sampling and measurements

After excavation, we washed the root crowns in soapy water and then determined the branching angle (BA) of the outermost roots at 5 cm below the plant base as described above in 2.2.3 but for nodal roots only. BA was determined as the angle from the horizontal on the left and right side from the center of the plant into the direction of neighbor plants within the same plant row and into the direction of the neighboring plant row. We verified that the nodal roots were sufficiently stiff as to not change angles during washing by visually comparing them to angles of unwashed nodal roots as imaged by NMR (See Fig. 1). Further, we counted the number of seminal roots and nodal roots for each tiller separately (see Fig. A.1) and all other (=adventitious) roots. Furthermore, for Field1_2013, we determined the lateral branching frequency of 1 cm of a randomly chosen seminal and nodal root at least 3 cm from the plant base, respectively. For the distribution histogram of the nodal roots per tiller see 2.2.3. For field data, we added an additional bin of 12 or more nodal roots per tiller. Apart from the absolute numbers, we also calculated the percentages of the different bins in these distribution histograms. Additionally, we estimated tiller age based on tiller counts at the several measurement days and plotted the number of nodal roots of a certain tiller over the age of that tiller. In 2013, we complimented the root crown excavation by imaging the roots crowns in intact soil cores (Fig. 1 a, c, e). Cores were 30 cm long, 9 cm in diameter, and taken directly over the plant (C_2013). After drilling, the soil cores were wrapped into wrapping foil and stored at 4 °C for later scanning with NMR (4.7 Tesla/300 mm Varian VNMRS vertical wide-bore MRI system (Varian Inc., Oxford, UK) (Jahnke et al. 2009)).

Statistics

We used R version 3.2.3 (R Development Core Team 2015) to analyze our data doing two-way ANOVA for each sampling time point separately. For that, we applied the following model:

$$y \sim Block + Sowing\ density + Genotype + Sowing\ density\ X\ Genotype,$$

with y = average of measured trait for a given rhizotron or plot. When block was not significant, we used a simplified model without block. Further, we averaged the data of the two genotype, since genotypes were statistically not significant from each other in most samplings (except Rhizo3 nodal roots per tiller Scarlett>Barke $p = 0.0458$; Field1_2014 nodal roots per plant Scarlett>Barke $p = 0.047$, Field2_2014 nodal roots per tiller Barke>Scarlett $p = 0.01116$, and Field2_2013 branching angle within the plant row (iR) Scarlett>Barke $p = 0.01323$).

Hence, we used the simplified model:

$$y \sim Sowing\ density.$$

As a posthoc-test, we performed TukeyHSD. Data are presented as boxplots as medians with 50% quantile and error bars indicating minimum and maximum values and significant differences among groups are indicated by letters with a significance level of $p < 0.1$.

Table 2 Details on climate data and sampling of the field experiments at CKA

	C_2013	Field1_2013	Field2_2013	Field1_2014	Field2_2014
Sampling method	Coring, 30 cm deep, 9 cm in diameter	Shovelomics	Shovelomics	Shovelomics	Shovelomics
Sampled sowing density (distance to neighbor plant)	24 (20.2 cm), 120 (4 cm), and 340 seeds m-2 (1.4 cm)	24 (20.2 cm), 120 (4 cm), and 340 seeds m-2 (1.4 cm)	31 (15.5 cm), 120 (4 cm), and 340 seeds m-2 (1.4 cm)	24 (20.2 cm), 120 (4 cm), and 340 seeds m-2 (1.4 cm)	24 (20.2 cm), 120 (4 cm), and 340 seeds m-2 (1.4 cm)
Replicates per treatment	3 (in 3 blocks)	3–4 (in 3–4 blocks)	6 (in 2 blocks)	3 (in 3 blocks)	4 (in 4 blocks)
Harvest (days after sowing, thermal time, cumulative PAR, developmental stage)	68 DAS, ca. 63 DAG, 861.46 GDD, 35.42 kWh m^{-2}, BBCH 41–49, booting	75 DAS, ca. 70 DAG, 995.11 GDD, 40.44 kWh m^{-2}, BBCH 65–75 anthesis/milk stage	98 DAS, ca. 93 DAG, 1455.65 GDD, 57.37 kWh m^{-2}, BBCH 89–92, ripening/senescence	35 DAS, ca. 27 DAG, 324.95 GDD, 14.32 kWh m^{-2}, BBCH 23–29, tillering	68 DAS, ca. 60 DAG, 766.55 GDD, 32.62 kWh m^{-2}, BBCH 38–48, stem elongation/booting
Trial year	2013	2013	2013	2014	2014

For counts (tiller and roots), we used the non-parametric Kruskal-Wallis-test appropriate for non-parametric data (McDonald 2014) and performed the Kruskal-Nemensyi-Test as a posthoc-test. Data are presented as boxplots as medians with 50% quantile and error bars indicating minimum and maximum values and significant differences among groups are indicated by letters at a significance level of $p < 0.1$.

Results

Here, we shortly summarize our results: we found sowing density effects on several plant traits in field trials but only few traits were affected in some rhizotron experiments, namely, tiller number and nodal root distribution at tillers at 510 GDD (Rhizo3, 34 DAG, BBCH21–22) and nodal roots per area at 251 GDD (Rhizo2, 19 DAG, BBCH19–21) and at 414 GDD (Rhizo1, 23 DAG, BBCH21–23). In the outdoor placed rhizotrons (Rhizo2) that experienced more field like weather conditions and had similar shoot development to field grown plants at that thermal time (tiller count and plant height (data not shown)) we did not find any further sowing density effects. In the field, we observed a strong effect of sowing density on tiller formation (in rhizotrons rather weak) and shoot dry weight (see section "Shoot tiller formation and dry weight"), causing high density plants to have fewer but on average older tillers (see section "Number of tillers with a certain number of nodal roots and tiller age"), with in total fewer nodal roots (see section "Seminal and nodal root counts"). Nodal root count per area increased with increasing sowing density, but not as much as seminal root counts per area (see section "Seminal and nodal root counts"). At high density stands, we had many more major axes per area, especially more fine seminal roots (see section "Seminal and nodal root counts"). Nonetheless, we found the following traits to be not (or only slightly) affected by sowing density: seminal roots per plant (see section "Seminal and nodal root counts"), nodal roots per tiller (see section "Nodal roots per tiller"), branching angle (see section "Branching angle"), lateral root emergence and lateral branching frequency (see section "Lateral roots: emergence, length, and branching frequency"), and growth rates of roots (see section "Root growth rates"). Thus, we did not find support for our first hypothesis that increased SRL is caused by greater lateral branching frequency.

However, our second hypothesis hold true that branching angles were not affected by sowing density.

Shoot tiller formation and dry weight

The number of tillers per plant decreased with increasing sowing density (Fig. 2, see Table A.1-A.6 for statistics, means and SEM). For rhizotron experiments, this trend was only significant after 510 GDD (Rhizo3, 34 DAG, BBCH21–22), while in the field, tiller counts were already significantly greater in low than in high sowing density at 325 GDD (Field1_2014, 35 DAS, BBCH23–29, 9.5 in low vs. 4.5 tillers in high sowing density, $p = 0.00069$). From 767 GDD on (Field2_2014, 68 DAS, BBCH38–48), tiller counts of low sowing density were significantly greater than in high and medium sowing density (low>high $p > 0.00001$, low>medium $p = 0.039$), while medium and high sowing density did not statistically differ from each other. Furthermore, at the highest and medium sowing density, tiller formation stopped after 767 GDD (Field2_2014, 68 DAS, BBCH38–48). In contrast, the number of tillers increased in the lowest sowing density until 995 GDD (Field1_2013, 75 DAS, BBCH65–75) and decreased slightly afterwards from 995 to 1456 GDD (Field2_2013, 98 DAS, BBCH89–92) as well as in the medium sowing density. For the highest sowing density, there was no change from 995 to 1456 GDD (Fig. 2).

Similarly to tiller counts per plant, shoot dry weight per plant decreased with increasing sowing density (see appendix Fig. A.2). This trend we could earliest measure after at 325 GDD (Field1_2014, BBCH23–29, 35 DAS): plants in low sowing density had 0.54 g per plant and had therefore significantly greater shoot dry weight per plant than in medium (0.30 g per plant) and in high sowing density (0.25 g per plant), while there was no statistical difference between medium and high sowing density (for p-values see Table A.7). In rhizotron experiments, however, shoot dry weight per plant was not significantly affected by sowing density even at 510 GDD (Rhizo3, 34 DAG, BBCH21–22) (see Table A.8). Until anthesis, plants gained 4.55 g, 12.36 g, and 34.37 g shoot dry weight per plant in high, medium, and low sowing density, respectively (see appendix Fig. A.2).

Number of tillers with a certain number of nodal roots and tiller age

In order to see, how the nodal roots are distributed at the tillers of a plant, we separated all tillers and counted the number of nodal roots for each tiller (Fig. 3). We grouped the tillers according to their number of nodal roots and compared the histograms of the different sowing densities. Young plants did not have so many tillers and therefore nodal roots yet. Hence, most tillers had 0–2 nodal roots (Fig. 3 a). This is also the bin in which a trend started to become visible: plants in low sowing density had more tillers with 0–2 nodal roots than plants in medium or high sowing density. At 767 GDD (Field2_2014), low sowing density plants still had most tillers in the 0–2 nodal roots per tiller-bin, while for medium and high sowing density the highest tiller count was in bin 3–5 nodal roots per tiller (Fig. 3 b).

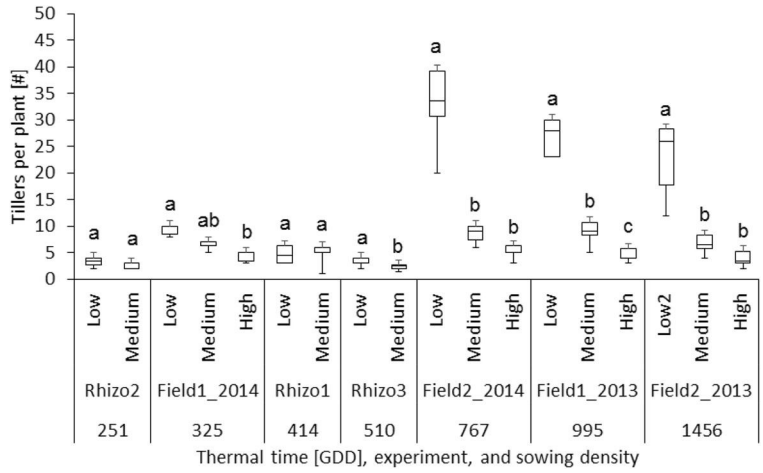

Fig. 2 Tiller number per plant over thermal time, experiment, and sowing density. Data are presented as boxplots (median framed by the 50%-quantile, error bars indicate minimum and maximum values) and letters indicate significant difference between groups within one sampling ($p < 0.1$)

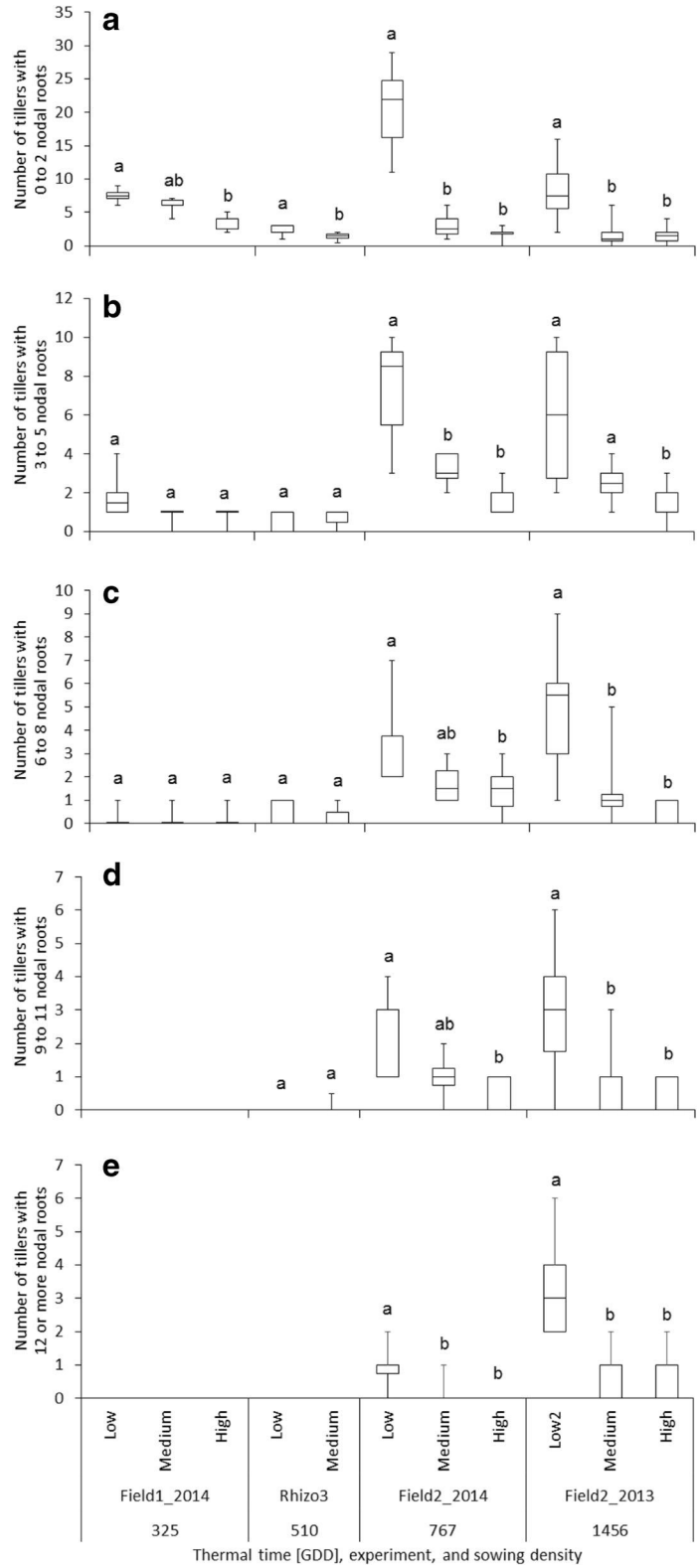

Fig. 3 Histogram of number of tiller that bear a certain number of nodal roots over thermal time: (**a**) 0 to 2 nodal roots per tiller, (**b**) 3 to 5 nodal roots per tiller, (**c**) 6 to 8 nodal roots per tiller, (**d**) 9 to 11 nodal roots per tiller, and (**e**) 12 or more nodal roots per tiller. The older the plants, the more tillers with higher nodal root counts they exhibited. Note the different y-axes. Letters indicate significant difference between groups within one sampling ($p < 0.1$). For description of x-axis and data presentation see Fig. 2. The percentages of the data presented as means with SEM can be found in the appendix in Table A. 13

Moreover, low sowing density had in almost all bins significantly more tillers than medium and high sowing density (see Table A.9, Table A.10, Table A.11, and Table A.12). At 1456 GDD (Field2_2013), all sowing densities had most tillers in bin 0–2 and 3–5 nodal roots per tiller. In general, there was only a significant difference in the total number of nodal roots per tiller between plants of low vs. medium and high sowing density. This was probably due to the fact that plants in low density had much more tillers and therefore in all bins they had more tillers with a certain number of nodal roots than in medium or high sowing density.

The overall distribution of nodal roots per tiller – relatively seen – was the same in a low-density, medium-density, and a high-density plant, as the percentages of the tillers with a certain number of nodal roots were not significantly different from each other at any sampling time point within one experiment (see Table A.13, Table A.14, and Table A.15) (except in Field2_2014 (767 GDD) for 0–2 nodal roots per tiller where low density plants had 59% in that bin, while medium and high sowing density had only up to 31% in that bin and were therefore significantly lower than low sowing density. Further, percentages were different for all experiments in bin 3–5 nodal roots per tiller, in which low sowing density almost always had significantly lower values than medium or high sowing density (Rhizo3 (510 GDD): medium>low $p = 0.0659$, Field2_2014 (767 GDD): medium>low $p = 0.04861$, high>low $p = 0.0807$, Field2_2013 (1456GDD): medium>low $p = 0.07086$)).

Assuming that tillers with more nodal roots were older, and based on the aboveground monitored appearance of tillers, we designated an age for each tiller and plotted tiller age against number of nodal roots (Fig. 4, for data separated for each genotype and sowing density see appendix Fig. A.3). Nodal roots per tiller increased significantly until the end of the season among samplings (overall means of Field1_2014 (325 GDD): 0.94 (± 0.15) nodal roots per tiller, Field2_2014 (767 GDD): 5.45 (± 0.22) nodal roots per tiller, Field2_2013 (1456 GDD): 3.19 (± 0.17) nodal roots per tiller were all highly significantly different (for all $p < 0.0001$) from each other). Moreover, it is noteworthy that there were tillers without any nodal roots even when plants were about 2-month-old or older, when plants were already elongating their stems and booting and no longer in the tiller formation phase.

Seminal and nodal root counts

The seminal root count was not affected by sowing density (except at 995 GDD (Field1_2013), where medium sowing density had significantly greater values than low ($p = 0.027$) and high ($p = 0.087$) sowing density). At all sampling time points in all experiments, seminal root count was about 5–7 (Fig. A.4).

For nodal roots, there was also no sowing density effect visible during the first five weeks after germination in rhizotron experiments (Rhizo2, 251 GDD, 19 DAG; Rhizo1, 414 GDD, 23 DAG; and Rhizo3, 510 GDD, 34 DAG), when plants were in the tiller formation phase and had about 3–11 nodal roots per plant (3–4, 10–11, and 4–6 nodal roots per plant at 251, 414, and 510 GDD, respectively, see also Table A.16). In the field, though, plants already showed a significant decline in nodal root count with increasing sowing density after four weeks after germination at 325 GDD (Field1_2014, 35 DAS) from 9 in the lowest to 6 in the medium and 5 nodal roots per plant in the highest sowing density (low>high sowing density, $p = 0.046$; Fig. 5 a, see also Table A.17 for statistics). At 767 GDD (Field2_2014, 68 DAS), at stem elongation and booting, low sowing density had about 95 nodal roots per plant and had thus significantly greater nodal root counts than medium and high sowing density which had about 37 and 22 nodal roots per plant, respectively, and which were also significantly different from each other (Fig. 5 a, for p-values see Table A.18). From here on, the number of nodal roots per plant increased further for low sowing density up to maximum 138 nodal roots per plant at grain filling at 1456 GDD (Field2_2013, 98 DAS), while the nodal root count for medium and highest sowing density stayed at about 39 and 20 nodal roots per plant, respectively (Fig. 5 a), so that the number of nodal roots per plant in low sowing density was significantly greater than high and medium sowing density, which were as well statistically significant from each other (Table A.19, Table A.20).

Although, the number of nodal roots per plant declined, the number of nodal roots per area (# m^{-2}) increased with increasing sowing density (see appendix Fig. A.5) and were up to almost 9 times greater in high (1813.3 nodal roots per area) than in low (212 nodal roots per area) sowing density at about four weeks after germination (325 GDD, Field1_2014) (high>low sowing density, $p = 0.00029$). At stem elongation (767 GDD, Field2_2014), though, nodal root counts per area

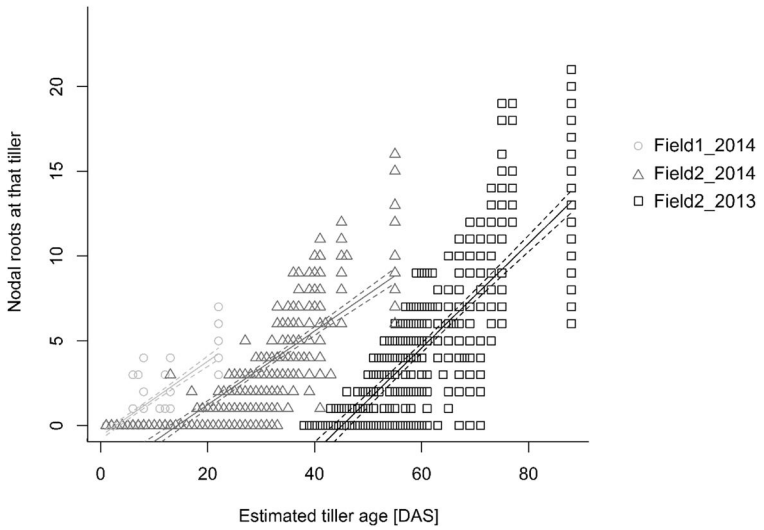

Fig. 4 Nodal roots at a certain tiller and tiller age in DAS merged for all genotypes and densities of three different sampling time points in the field in the year 2013 and 2014. Data are raw data with linear regression (solid line) with 95% confidence interval (dashed lines). Dots are from Field1_2014 with its linear regression (y = 0.22056×-0.60999, adjusted R^2 = 0.77797, grey), triangles from Field2_2014 with its linear regression (y = 0.2171×-3.1053, adjusted R^2 = 0.671, dark grey), squares from Field2_2013 with its linear regression (y = 0.3079×-13.8819, adjusted R^2 = 0.6117, black)

were about three times greater in high (7522.5 nodal roots per area) than in low (2277 nodal roots per area) sowing density (high>low sowing density, p = 0.000029). In addition, around the time of flowering and grain filling, nodal root counts per area were only about 1.8 times greater in high than in low sowing density but still significantly higher (995 GDD, Field1_2013: high: 5644, low: 3090.7, p = 0.0104) and at the end of grain filling/seed maturity, nodal root counts per area were only 1.6 times but still significantly greater in high than in low sowing density (1456 GDD, Field2_2013: high: 7083.3, low: 4285.8 nodal roots per area, p = 0.10). This was in contrast to the seminal root count which did not change over time and was not affected by sowing density or system/location and thereby the seminal root count was about 14 times greater per area in high compared to low sowing density (5–7 seminal roots per plant in all treatments, hence, about 5 × 24 = 120 in low and 5 × 340 = 1700 seminal roots per area in high sowing density).

Nodal roots per tiller

Like the nodal root count per plant, nodal roots per tiller increased over time from about 1–2 nodal roots per tiller during tiller formation (251 to 510 GDD, Rhizo1–3 and Field1_2014), over 3–4 nodal roots per tiller at stem elongation (767 GDD, Field2_2014), to 4–6 nodal roots per tiller at flowering/grain filling and maturing (995 (Field1_2013) to 1456 GDD (Field2_2013)) (Fig. 5 b). During the vegetative stages (251 to 510 GDD, Rhizo1–3 and Field1_2014), nodal roots per tiller was more or less constant in rhizotron experiments, however, nodal roots per tiller increased significantly with sowing density in the field trial at 325 GDD (Field1_2014, high > low sowing density, p = 0.0608; high > medium sowing density, p = 0.0361) during the vegetative stage. At the end of the vegetative stage, during stem elongation at 767 GDD (Field2_2014, 68 DAS), nodal roots per tiller increased with sowing density (Fig. 5 b, for p-values see Table A.21). During anthesis and grain filling (995 GDD, Field1_2013, 75 DAS), nodal roots per tiller declined with sowing density, though, significantly only between medium and high sowing density (Fig. 5 b, for p-values see Table A.22).

Branching angle

The branching angle (average angle of outer most roots from vertical, see Fig. 1 and material and methods) measured at 5 cm depth increased over time from 23 to 27° at tillering (251 GDD, Rhizo2, 19 DAG) to maximum 52.81° at stem elongation (767 GDD, Field2_2014, 68 DAS) and declined slightly at grain filling/maturing (1456 GDD, Field2_2013, 98 DAS) (Fig. 6). Sowing density significantly affected branching

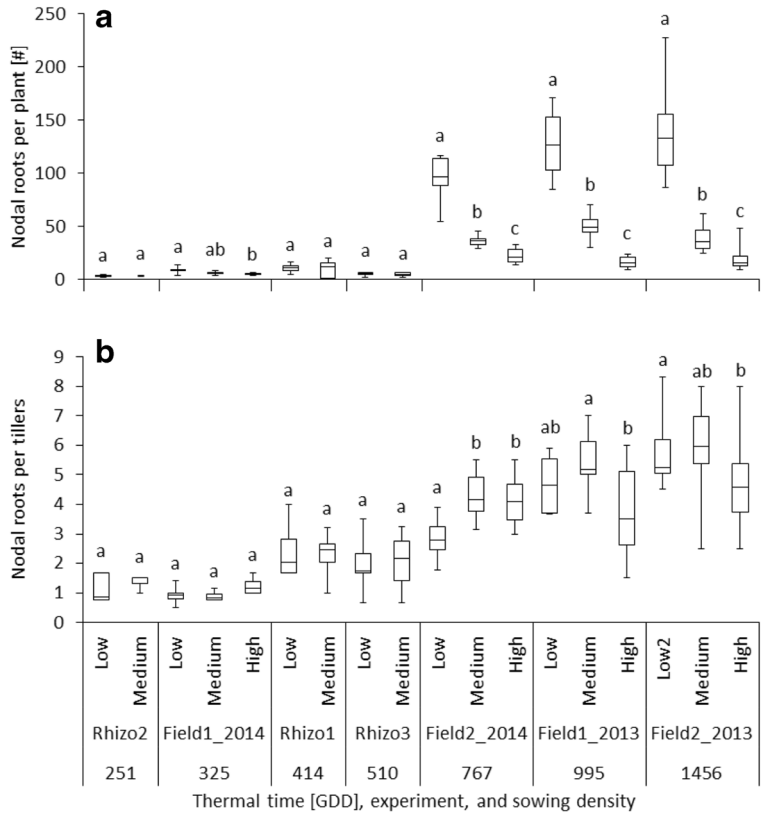

Fig. 5 Nodal root count (**a**) and ratio of nodal roots per tiller (**b**) for all different samplings over thermal time. **a** Nodal root count increased over time, and decreased with sowing density, and (**b**) nodal roots per tiller increased with time, but stayed more or less constant with sowing density. For description of x-axis and data presentation see Fig. 2

angle within the plant row (iR) only at 767 GDD (Field2_2014, 68 DAS): here, the branching angle declined with increasing sowing density from 52.83° in low to 41.62° in high sowing density (Fig. 6a, for p-values see Table A.23 and Table A.24). At the same time point, there was the same trend also for branching angle between the plant rows (bR), but this was not significant (Fig. 6a, b). About a month later, at 1456 GDD (Field2_2013, 98 DAS), at seed maturing, the branching angle also declined significantly with increasing sowing density, both iR and bR, however only statistically different between low and high sowing density (for p-values see Table A.25 and Table A.26).

Figure 1 shows images of excavated root systems via Shovelomics-approach and NMR-images of root systems taken via soil coring of plants in low, medium and high sowing density at about the same thermal time. In the appendix, there are more images of root systems in rhizotrons and of the earliest shovelomics sampling (Fig. A 1). The root systems of the Shovelomics-approach and NMR looked very much alike at the same sowing density. Furthermore, it seems as if the inclination angle of the nodal roots are actually the same and the differences in branching angle are caused by different widths of the plant base, i.e. the stool, as we measured from the center of the plant base and not from the point at the nodes of which the nodal roots emerged from.

Lateral roots: Emergence, length, and branching frequency

Lateral roots emerged earliest 5 DAG (90 GDD, Rhizo1) for plants in high sowing density but in most cases at 12 DAG (156–216 GDD, Rhizo1–2). They reached an average length of 2.1 cm averaged over all Scarlett plants (Rhizo2, 19 DAG, 251 °C; high sowing density: 2.5 cm (± 0.20 cm, SEM), low sowing density: 1.9 cm (± 0.08 cm, SEM)). Lateral branching frequencies of seminal roots were about 4–8 laterals per cm seminal root for both genotypes in all sowing densities at 251 GDD (rhizotron experiment, Rhizo2, 19 DAG) and 995 GDD (field experiment, Field1_2013, 75 DAS) (Fig. 7). Nodal roots had about the same lateral branching frequency of 4–9 laterals per cm of nodal root regardless of sowing density (Fig. 7b, Field1_2013, 75 DAS, 995 GDD). Both, lateral branching frequencies of seminal and nodal roots,

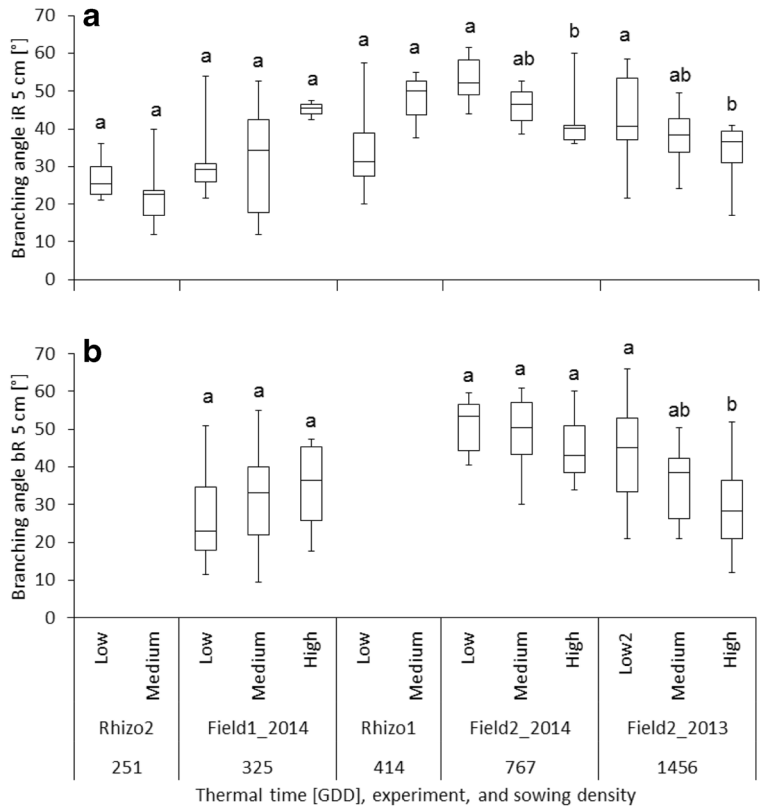

Fig. 6 Branching angle (BA) measured from vertical at 5 cm depth within the plant row (iR) (**a**) and between the plant rows (bR) (**b**) for all different samplings over thermal time. Branching angle was about the same iR and bR and increased somewhat with time. Significant differences became prominent only in old plants at or after flowering, where BA declined with increasing sowing density. For description of x-axis and data presentation see Fig. 2

were not significantly affected neither by sowing density nor by genotype (for *p*-values see Table A.27, Table A.28, Table A.29, and Table A.30).

Root growth rates

Growth rates of roots were only measured in rhizotron experiments. For Rhizo1 (414 GDD) most roots reached the bottom at 17 DAG and for Rhizo2 (251 GDD) at 16 DAG, while for Rhizo3 (510 GDD) roots reached the bottom at final harvest. Growth rates of maximum rooting depth (GR_{MRD}) were significantly different between sowing densities (data not shown) only during a few days (Rhizo2: 1 of 10 measurement days on which medium > low sowing density and 1 of 10 on which low > medium sowing density, Rhizo1: 3 of 9 measurement days on which medium > low sowing density (plus 3 days on which low > medium but roots had already hit the bottom in medium sowing density), Rhizo3: 2 of 11 measurement days on which Scarlett > Barke and 1 of 11 days on which Barke > Scarlett). The average GR_{MRD} until harvest or the day when roots hit the bottom were between 2.1 and 3.4 cm day^{-1} (0.14 and 0.26 cm GDD^{-1}) (see Table A. 31). For Rhizo3, there was no treatment effect, while for Rhizo1 GR_{MRD} was greater in medium sowing density ($p = 0.0136473$) and for Rhizo2 it was vice versa, low sowing density was significantly greater than medium sowing density ($p = 0.0538278$). The relative growth rates of total root length (RGR_{TRL}) did not show a sowing density effect for Rhizo2 and were on average 0.1968 ± 0.0188 cm cm^{-1} day^{-1} (0.0157 ± 0.0015 cm cm^{-1} GDD^{-1}) in low and 0.1902 ± 0.0143 cm cm^{-1} day^{-1} (0.0152 ± 0.0011 cm cm^{-1} GDD^{-1}) in medium sowing density. In Rhizo1, however, RGR_{TRL} was somewhat lower than in Rhizo2 and significantly greater for low (0.2346 ± 0.0146 cm cm^{-1} day^{-1}, 0.01303 ± 8.1026e-04 cm cm^{-1} GDD^{-1}) than medium (0.1845 ± 0.0039 cm cm^{-1} day^{-1}, 0.0102 ± 2.1437e-04 cm cm^{-1} GDD^{-1}) sowing density ($p = 0.0076282$).

Discussion

Our data suggests that in barley, and presumably other *Poaceae*, the root response to plant density is regulated

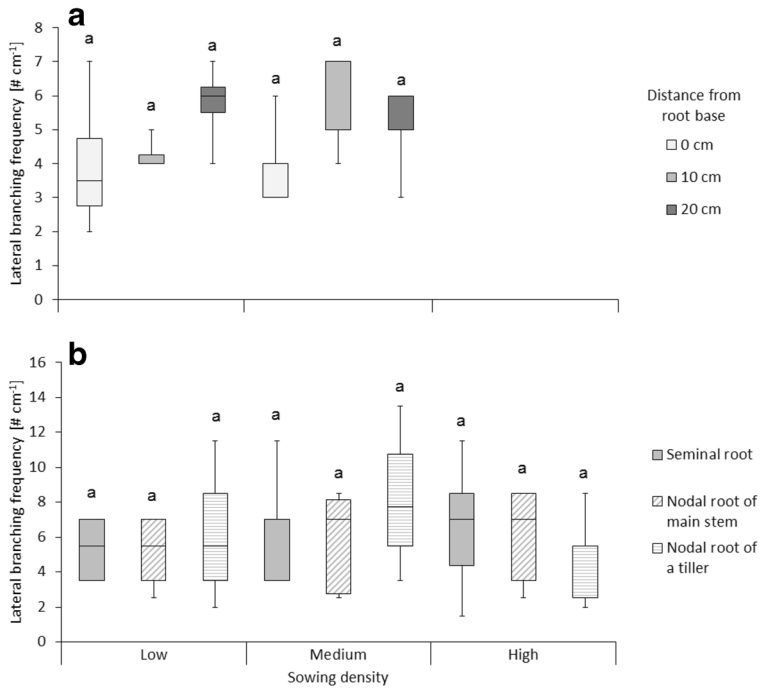

Fig. 7 Lateral branching frequency over sowing density of rhizotron (**a**) and field (**b**) experiments. **a** Lateral branching frequency at 251 GDD measured along a seminal root at 0, 10, and 20 cm from root base at 251 GDD (Rhizo2, 19 DAG). **b** Lateral branching frequency of a seminal, a nodal root from the main stem, and a nodal root of a tiller at 995 GDD (Field1_2013, 75 DAS). For explanation of data presentation see Fig. 2

over the tillering response, so by the aboveground part of the plant. We observed a strong reduction in the number of tillers in response to increasing sowing density. This response is common to many *Poaceae* (Kays and Harper 1974; Darwinkel 1978; Lafarge et al. 2002). We show, however, that this response has strong consequences for the RSA belowground. We discuss possible functional consequences of these architectural changes. Finally, we discuss the importance of the findings for root research and phenotyping. We show that it is challenging to simulate the sowing density effects under controlled conditions, whereas observed differences in root traits in the green house might be altered in the field, even when comparing plants at similar growing degree days.

Nodal root formation is associated to the tillering response to sowing density

A reduction in the number of tillers was one of the first responses to sowing density that we observed; a response well described in the literature. Already Kamel (1959) showed that tiller formation stops the latest at ear emergence, but in higher sowing densities up to two weeks earlier. Later studies have shown that this response is regulated over red to far-red ratios of the light, which decreases when neighboring plants absorb the red light (Woolley 1971; Holmes 1981; Casal et al. 1986; Kasperbauer and Karlen 1986; Davis and Simmons 1994). An early termination of the formation of tillers, causes high density plants to lack young tillers at flowering. Finally, sowing density also influences tiller mortality. Tiller death has been reported for cereals in many studies (e.g. (Kamel 1959; Darwinkel 1978; Anderson-Taylor and Marshall 1983)) and gets usually prominent around flowering, as we also found in this study in our field trials. After ear emergence, Kamel (1959) reports a decline in the number of tillers per plant, and this decline is stronger in the high sowing densities, similar to what we observed in our field data (except for Barke in 2014, data not shown). We conclude that not only the number, but also the age distribution of the tillers is altered by plant competition.

We consider the tillering response to competition of importance, as a large part of the root system is formed by the nodal roots coming from the tillers (Anderson-Taylor and Marshall 1983). Wahbi and Gregory (1995) showed for barley that the number of nodal roots increased linearly over thermal time and is linearly correlated with the number of leaves, and presumable tillers. Recently, this positive correlation of nodal roots (leaf node roots) and tiller number has been confirmed in *Brachypodium distachyon* (Chochois et al. 2015). At the same time, younger tillers carry fewer nodal roots

than older tillers (Anderson-Taylor and Marshall 1983). Thus, the increase in average tiller age with increasing sowing density discussed above may also affect the nodal root counts per tiller. It is thereby quite difficult to prove that sowing density had direct effects on nodal root formation, on top of the proposed regulation of the number of tillers. Such direct effects of plant density, for example, have been observed in the nodal root formation of maize, which mostly does not tiller (Liu et al. 2012).

Although in our study, there was a trend towards more nodal roots per tiller in higher sowing densities until flowering (and then to less nodal roots per tiller in higher sowing density), the number of nodal roots per tiller was relatively stable with sowing density. The observed fluctuation may be explained by the older age of the tillers in higher sowing density, where tiller formation stopped earlier and tiller death was less pronounced. Chochois et al. (2015) showed for *Brachipodium distachyon* that the number of leaf node axile roots is linearly correlated with the number of tillers, and we thereby conclude that the number of nodal roots per plant at different sowing densities is mostly a function of the number of tillers.

Estimating RLD and SRL from RSA traits

We asked what root traits might explain our previously published observations of increased RLD and SRL in high density plots. Even though the nodal root counts per plant declined with increasing sowing density, similar to findings in maize (Pellerin 1994; Liu et al. 2012), we still observed an increase in the total number of major root axis (i.e. seminal and nodal roots) per area. Similar to other findings in barley (4–7 seminal roots per plant (Hackett 1969; Wahbi and Gregory 1995; Knipfer and Fricke 2011)), both species had on average 5 seminal roots per plant, independent of the treatment applied. Consequently, we had 120, 600 and 1700 seminal roots per m^2 in the 24, 120 and 340 plants per m^2 plots, respectively. For nodal roots, these numbers were $24 \times 95 = 2280$, $120 \times 40 = 4800$, and $340 \times 20 = 6800$ nodal roots per m^2 at 767 GDD (Field2_2014) at somewhat higher GDD as the 60 cm-coring in Hecht et al. (2016). Given that we found no evidence of a sowing density effect on the lateral root branching density or the average lateral root length, we can estimate the average RLD by assuming the major roots are mostly growing vertically and, the laterals mostly horizontally.

$$\mathrm{RLD} = \mathrm{NMAA}^{*}\left(1 + \mathbf{LRBD}^{*}\mathrm{LL}\right) \quad (5)$$

Where RLD = root length density in (cm cm^{-3}), NMAA = number of major axis per area (cm^{-2}), LRBD is the lateral branching density (here, 5 cm^{-1}), LL = average lateral length (here, 2 cm). The resulting RLD for low, medium and high sowing densities would be 2.6, 5.9 and 9.4 cm cm^{-3}, for a rough computation fairly close to the measured 2–6 cm cm^{-3} (Hecht et al. 2016). Branching angles and the depth to which the different major axis grow influence the RLD in both horizontal and vertical directions, which we will discuss later. We conclude that increases in RLD may be explained by an increase in the number of major axes.

Our computations also show that the ratio of seminal to nodal roots increased. Given that seminal roots have smaller diameters and thereby greater SRL, we suggest that the observed increase in SRL in response to density might simply be a result of the greater portion of seminal over nodal roots in high sowing density.

We found no evidence that sowing density greatly affects steepness, or progression towards depth

In our previous publication, we show that high density plants place more fine roots in the topsoil relative to deeper soil layers (Hecht et al. 2016), which is similar to findings of other studies (e.g. (Tardieu 1988; Mommer et al. 2010; Kucbel et al. 2011; Chen et al. 2013; Ravenek et al. 2014)). Shallow rooting can be influenced by the angles at which the major axis grow, by the progression of the major axis towards depth or by varying lateral branching densities at depth.

Rather than measuring shallower growth angles in the high density plots, we measured steeper angles, although this trend was weak. Steeper angles in response to planting density have been observed by Archer and Strauss (1985) for grapevine. In maize, Liu et al. (2012) found a weak effect of planting density on the root branching angle at one internode in one cultivar, and, contrary to our findings, the branching angle became more shallow with increasing planting density. The weak trend towards steeper angles that we observed, might actually be caused by the reduction in tillers. Fewer tillers, cause the stool to be narrower and

thereby at 5 cm depth we might measure slightly steeper angles compared to the low density plants with many tillers, a wider stool. Going back to our NMR, shovelomics images, and rhizotron images, we suggest that the inclination angles of the roots are not affected by density supporting our hypothesis. These inclination angles, however, are more difficult to determine reliably as roots are not straight but curved. In general we observed a strong gravitropic response causing the major axes to grow quickly towards a vertical direction, similar as reported by Oyanagi et al. (1993). We conclude that in our studies branching angles do not explain observed differences in rooting depth.

Branching frequencies can differ strongly along one root (Drew et al. 1973; Drew and Saker 1978; Babe et al. 2012). We did not find any evidence for changes in lateral branching frequency, neither of seminal nor of nodal roots. Branching frequencies were relatively stable, and on average 5 roots per cm. So we have no evidence for our hypothesis that shallow rooting is caused by increased number of laterals in the topsoil layers. Possibly, the laterals grew longer, however, determining lateral root length is difficult, as laterals easily break during root washing. Similarly, we have no method for determining the length of the major axis in the field. If some of these major axis had slower progression towards depth, or reduced length, the whole root system would be shallower. In rhizotrons, we did not observe this, but once observed an accelerated and once a reduced rate for roots when plants were growing in competition. We conclude that we found no data directly explaining shallower rooting at high planting densities and that growth angles, lateral branching densities, and root elongation rates were relatively stable across treatments and genotypes.

Alteration in RSA could have functional consequences

We asked if these observed changes in RSA (RLD, SRL, D50 (published before in Hecht et al. (2016)), branching angle, root counts, lateral branching frequency (presented in this study)) might have functional consequences for the ability of the crop to access water and nutrients. Many papers in the literature discuss the effect of shallow and deep rooting on nutrient and water uptake (Thorup-Kristensen 2001, 2006; Manschadi et al. 2008; Kristensen and Thorup-Kristensen 2009; Singh et al. 2010b; Wasson et al. 2012; Lynch 2013). We regard the effects measured here however as probably too small to be of greater relevance. The strong effect on the number of major axes and the associated previously published increase in RLD could however have affected the ability of the crop to take up water and nutrients. Generally, greater RLD and SRL is associated with greater uptake capacity, especially for immobile nutrients such as phosphorus (Shane and Lambers 2005; Postma et al. 2014; Miguel et al. 2015). For nitrate and water uptake, the optimal RLD has been suggested to be low, as greater RLD quickly leads to increased competition, not increased uptake. For example Saengwilai et al. (2014) show that maize genotypes with fewer nodal roots grow better on low N soils, supposedly because they do not waste energy on competition. Similarly, Postma et al. (2014) predicted (based on modeling) that the optimal lateral branching density in maize for N uptake is few (2–5) branches per cm. A genotypic contrast study by Zhan and Lynch (2015) seems to confirm these results. Root classes are also thought to differ in function. Compared to seminal roots, nodal roots may have greater ability to penetrate deep soil, transport more water, but may have greater construction cost and reduced uptake per weight due to their generally lower SRL (Kuhlmann and Barraclough 1987; Araki and Iijima 1998). On the other hand, seminal roots, being older, might grow deeper when the soil permits but have more advanced cortical senescence and suberization which impacts uptake negatively (Schneider et al. 2017). Further research may be needed to understand what the root ideotype of barley is when growing at high sowing densities, especially with respect to the number of seminal and nodal roots.

Translating from lab to field

We performed our experiments along a gradient of highly controlled to close to practice. The translation of lab experiments to field practice has proven challenging. In a recent review, Poorter et al. (2016) suggest that light, temperature and planting density might be the three factors explaining much of the observed differences between lab and field grown plants. We investigated the effect of plant density along a range of temperature and light conditions. Our results suggests that it is difficult to trigger a realistic density effect under controlled conditions. No doubt this is in part due to the duration of the experiments in the greenhouse being relatively short. At relatively high indoor temperatures, we expected plants to develop faster and tiller earlier (Clark 1969; Bade et al. 1985; Füllner et al. 2012;

Hossain et al. 2012), but only at the same age, and not at the same growing degrees days, did we observe a reduction in tiller number similar to what we observed early on in the field, though, with only half as many tillers. Artificial light has a high red to far-red ratio that actually should stimulate tillering (Kasperbauer and Karlen 1986; Kasperbauer 1987) which was true for our climate chamber experiment when comparing tiller numbers of this rhizotron experiment to the other rhizotron experiments – it had more tillers than in the two other rhizotron experiments – but it was not the case when comparing to field data, as in field plants had more tillers at the same DAG and GDD (data not shown). Possibly low light conditions might have reduced the response in our greenhouse experiment (Kamel 1959; Kays and Harper 1974; Casal et al. 1986) so we could only find an effect on tiller formation but not on shoot dry weight or nodal roots. Interestingly, we also used rhizotrons that were placed outside in spring (April–May 2013) and found similar temperature and light profiles to the field experiments. Indeed, shoot growth (tiller number and shoot dry weights) was slowed down in these rhizotrons, but root growth much less so (GR_{MRD} were greater than in rhizotrons in greenhouse or climate chamber, the roots reached the bottom at 2–3 tillers, too early to cause a reduction in nodal roots due to a reduction in the formation of tillers). Possibly the root temperature in the rhizoboxes was higher than the air temperature or root growth is less temperature sensitive. In agreement with Poorter et al. (2016), we conclude that simulating density responses, similar to those found in the field, under controlled conditions probably requires high light, sufficiently low temperatures, sufficient far-red light, deep containers to accommodate root growth, and sufficient time.

Conclusions

Sowing density is a factor that significantly changes RSA and thereby probably root system functioning. Our data indicate that the effects of sowing density on RSA are in all likelihood regulated over the aboveground tillering response. A reduction in the number of tillers caused fewer nodal roots per plant to be formed. Never the less, the number of major axes per area increased explaining the in Hecht et al. (2016) observed higher RLD. As a larger proportion of that RLD was formed by seminal roots, and seminal roots have smaller diameters than nodal roots (data not shown) we may expect the SRL to increase, as was observed by Hecht et al. (2016). We found no definite explanation for the observed shallow placement of roots at higher density in the field experiments as neither root growth angles, nor branching frequencies or growth rates towards depth differed significantly among density treatments. Simulating the effect of sowing density under controlled conditions, where root growth is more easily observed, proved challenging. We suggest that light level, temperature, light quality, the depth of the container and the duration of the experiment are important factors to be considered when trying to simulate sowing density effects under controlled conditions. Given that sowing density might be an important factor in translating research to agronomic practice, especially in the development of root ideotypes, we suggest that further research is needed to understand the relative importance of the nodal versus seminal root system and its relation to the tillering response. In particular, our study makes clear, that field studies under realistic agronomic conditions can generally provide the most robust outcomes for such studies.

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9.3 Translation from lab to field: A case study of how greenhouse based selection for great root development may influence agronomic traits and performance in the field.

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Translation from lab to field: A case study of how greenhouse-based selection for larger root system may influence agronomic traits and performance in the field.

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Abstract

Background and aim Sowing density is an important modifier of root system architecture. This could pose a challenge for lab-based phenotyping of single plant root traits, and the selection of genotypes thereof, as the specific phenotype might not be expressed under competitive field conditions. We asked if introducing competition in the greenhouse by placing several plants in one pot or rhizotron would improve translation from lab to field.

Methods We did a meta-analysis of seven experiments, varying from highly controlled to field conditions, and compared the root and shoot phenotypes of the common spring barley cultivar Scarlett (*Hordeum vulgare* L.) and an introgression line thereof, S42IL-176, previously selected for having a bigger root system under greenhouse conditions.

Key results The previously reported tillering phenotype of S42IL-176 was reproducible across experiments, but the root phenotype was more plastic. S42IL-176 had clearly different agronomic aspects than Scarlett such as increased leaf area, but not larger root length density or yield in the field. Competition only influenced the phenotype in older plants, and the interaction of genotype and environment (GxE) was more often significant for older plants. The genetic contrasts were sometimes greater and sometimes smaller under competition, depending on the experiment and the trait of interest. Translation from younger to older plants proofed challenging, even when using relative numbers, or plotting against thermal time.

Conclusions Our case study demonstrates that selection for certain root traits based on non-competitive greenhouse conditions can result in observable differences in the field, although not all root traits were consistently expressed. Plant competition plays a role in lab to field translation mostly when plants are grown for longer than four weeks. Competition can both enhance and diminish phenotypic differences. Placing more plants into one pot might be a good strategy in lab-based plant phenotyping.

Keywords: Introgression line S42IL-176, *Hordeum vulgare* L., root plasticity, root traits, sowing density, greenhouse-based selection, lab to field translation, genotype x environment interaction (GxE)

Introduction

In plant breeding, trait-based selection is easier done under controlled conditions, where environmental noise is low in comparison to field conditions. Natural heterogeneity within field settings but also weather fluctuations increase error variance within phenotypic data (Araus and Cairns 2014). Furthermore, greenhouse-based studies are popular as they reduce overall costs: the short duration of the greenhouse experiments greatly facilitates throughput capacity and reduces costs in comparison to a full-season field experiment.

There is a currently much interest in improving root systems for improving plant performance such as plant production and yield. The leaf economics spectrum has been successful in predicting leaf traits in specific environment types, based on trade-offs between resource uptake efficiency and investment in defence compounds and ability to withstand stressful abiotic conditions (Wright *et al.* 2004). Far fewer root than leaf traits have been measured so far, making the question of whether such a trade-off exists in root tissues harder but not less important to answer. In addition, roots are inherently less modular and more plastic since they need to respond to and grow in a very different medium to the aboveground atmosphere (Fiorani and Schurr 2013). As root plasticity is often large (= large genotype x environment interactions, hereafter GxE), the heritability of traits may be low in roots and the translation of lab to field may be complicated. Clark et al. (2002) showed for rice that one cultivar increased its root penetration rate in response to flooding, both in a greenhouse-screen and under field conditions. However, for other high-performing cultivars (=high penetration rate) under greenhouse-conditions this reproducibility was not observed in the field. Furthermore, Clark *et al.* (2002) stated that certain traits (here, the diameter of the impeded longest nodal root) could serve as a predictor for root penetration rate, while others did not correlate (e.g. maximum rooting depth (MRD)). Trait selection based on young plants in the greenhouse can be reproducible in the field in young plants, however, not necessarily for older plants (Watt *et al.* 2013).

Plant density is an important aspect affecting root growth and thus, revealing root plasticity. We have shown that in barley the root response for density is mostly regulated over the tillering response in a light-limited rather than nutrient-limited system (Hecht *et al.* 2016). High plant density causes a reduction in the number of tillers (Kamel 1959; Munir 2002; Turk *et al.* 2003; Soleymani *et al.* 2011; Hecht *et al.* 2016) and, as the number of nodal roots per tiller remained constant (Hecht et al 2018), a reduction in number of nodal roots ensues (Demotes-Mainard and Pellerin 1992; Pellerin 1994; Hecht *et al.* 2018). The number of seminal roots, however, seems to be genetically controlled (Robinson *et al.* 2016; Shorinola *et al.* 2018) and is therefore independent from plant density. Consequently, plant density decreases the ratio of nodal roots to seminal roots. Since seminal roots are smaller in diameter (Krassovsky 1926), this reduction in the ratio of nodal to seminal roots is associated with a reduction in specific root length (Hecht *et al.* 2016, 2018).

The inclusion of plant density (competition) as a factor in greenhouse studies for trait-based breeding selection is typically low or non-existent compared to the field, as usually single plants are studied in pots. This could complicate the translation of trait-based selection in the greenhouse to the field, due to the aforementioned root plasticity.

Other aspects influencing translation from lab to field are temperature and timing of the experiment. Although for shoot research it is common to correct for temperature by comparing on the basis of similar thermal time (McMaster and Wilhelm 1997, 2003), it is not clear if this strategy works as well for roots, which experience soil temperatures that are quite different from the air temperature (Carter 1928). For instance, Füllner *et al.* (2012) could show that a soil temperature gradient close to that usually found in the field caused a much larger root growth in barley than constant temperatures.

To address these issues and to explore the potential for translating outcomes from greenhouse studies to the field, we compared the plasticity of S42IL-176 showing larger root systems under greenhouse conditions with its German parent Scarlett (Naz *et al.* 2012, 2014) across a

range of experiments conducted in the greenhouse and the field. The introgression line S42IL-176 was selected in the greenhouse for its larger root system (larger root dry weight (RDW), root volume, and MRD) which is associated with larger tiller number. In all experiments, we included two levels of sowing density (single plant or low density vs. high density).

We asked ourselves the following questions:

1) Is the phenotype of S42IL-176 reproducible and would this larger production of roots in S42IL-176 relative to Scarlett also be observed at high sowing density (i.e. under competition)?
2) Would a larger root production in S42IL-176 in the greenhouse also lead to other clearly improved agronomic traits in the field such as root length density distribution, shoot weight, or yield relative to Scarlett?

Hypothesis 1) related to Q 1): The root system size of S42IL-176 relative to Scarlett will be larger under low or non-competitive conditions compared to high competition., since overall root production per individual plant will be suppressed at higher densities (Hecht *et al.* 2016, 2018).

Hypothesis 2) related to Q 2): S42IL-176 has greater yield than Scarlett.

Moreover, many of the measured traits have an ontological correlation with plant development, with some traits being more pronounced in younger, others in older plants (*sensu* Watt *et al.* 2013). We therefore plotted root and shoot traits across experiments against thermal time (the cumulative temperature based on daily mean temperatures above a certain base temperature (McMaster and Wilhelm 1997, 2003)) and asked:

3) Were phenotypic differences between these genotypes stable over thermal time, i.e. can selection on young plants be translated to older plants?

To answer these three questions, we analysed data of four rhizotron experiments (in a climate chamber, greenhouse and outdoors; inter-plant distance under competitive conditions: 4 cm), one pot experiment (climate chamber, inter-plant distance under competitive conditions: ca. 4

cm), and two field experiments in which we grew both genotypes, Scarlett and S42IL-176, under competitive (high sowing density, 120 seeds m^{-2}, inter-plant distance: 4 cm) and non-competitive conditions (low sowing density, 24 seeds m^{-2}, inter-plant distance: 20.2 cm).

Material and Methods

Within this study, we present a meta-analysis of published and unpublished data across four rhizotron experiments (two in greenhouse, one in climate chamber, one outdoor, "Rhizo1-4", see also Hecht *et al.* (2018)), one pot experiment (climate chamber, "Pot") and two field studies with spring barley ("Field1-2", suffix "C" used for coring, suffix "S" for shovelomics, see also Hecht *et al.* (2016, 2018)). All studies included a density treatment (i.e. single plant (=no competition) vs. cluster (=competition) in rhizotrons and pot experiments and very low sowing density (24 seeds m^{-2}, no competition, as inter-plant distance 20.2 cm and inter-row distance 21 cm and if at all plant-plant-interaction happened presumably at very late growth stage only) vs. greater sowing density (120 seeds m^{-2}, competition) in field trials; in Germany, spring barley usually is sown between 250 and 300 seeds m^{-2} depending on the cultivar and region), and the two genotypes Scarlett and S42IL-176.

In all experiments, the following traits were measured: tiller number per plant, shoot dry weight (SDW) per plant at harvest, SDW per tiller, leaf area per plant (except Field1_S and Rhizo4), specific leaf area (except Field1_S and Rhizo4), plant height (except Field1_S and Rhizo4), BBCH, nodal root number per plant (except Field1_C and Field2_C), nodal roots per tiller (except Field1_C and Field2_C), root dry weight (RDW, except Pot, Rhizo4, Field1_S, Field2_S1, and Field2_S2), total root length (TRL, except Rhizo4, Field1_S, Field2_S1, and Field2_S2, for Pot only the first three measurement days), specific root length (except Pot, Rhizo4, Field1_S, Field2_S1, and Field2_S2). In rhizotron experiments, we additionally measured maximum rooting depth (MRD). Furthermore, we were only able to measure final grain yield in one field trial (2014, Field2) and in Pot and lateral branching frequency only in one field trial (2013, Field1_S) and in one rhizotron experiment (2013, outdoor, Rhizo2). All experiments and the samplings within these experiments are described in detail in Hecht et al. (2016, 2018), except for one rhizotron experiment ("Rhizo4") and the pot experiment ("Pot") that we describe in the following sections.

Plant material: We grew the German spring barley (*Hordeum vulgare* L.) cultivars 'Scarlett' and an introgression line of Scarlett with an Israeli wild accession. This introgression line S42IL-176 exhibits 3 small introgressions on chromosome 1H, 2H, and 3H and one large introgression on chromosome 5H but bears a much greater root system (RDW, root volume, MRD), greater tiller number, and a spreading growth habit (planophil) than its German parent (Schmalenbach *et al.* 2011; Naz *et al.* 2012, 2014). In Table 1, differences in traits between Scarlett and S42IL-146 are listed (Naz *et al.* 2012, 2014).

Experimental design: We conducted the rhizotron experiment (Rhizo4) in the greenhouse using the phenotyping platform *GrowScreen-Rhizo* (Nagel *et al.* 2012) and the pot experiment (Pot) in a climate chamber at Forschungszentrum Jülich GmbH, Germany (N50° 54' 35.96", E6° 24' 47.401"). We transferred pre-germinated seeds into rhizotrons (at 45° angle) as single plant (non-competitive condition/no competition) and seven plants per pot (competitive condition/competition) (further details see Table 2) and took measurements on shoot and roots and imaged the root systems at three time points during the experiment. We grew plants in round 12x40 cm pots (further details see Table 2) and thinned them to either single plant (non-competitive condition/no competition) or four plants per pot (competitive condition/competition), when the first leave emerged. We measured shoots by hand and photographed them in Pot. Further, we imaged roots via magnetic resonance imaging (MRI) (Rascher *et al.* 2011; Metzner *et al.* 2014; van Dusschoten *et al.* 2016) weekly from about two weeks after sowing until seed maturation in Pot. Plants were sprayed against pathogens as recommended for spring barley.

Shoot sampling and measurements: We measured plant height, counted tillers, and scored the developmental stage (BBCH (Lancashire *et al.* 1991)) weekly for Pot and counted tillers

and scored the developmental stage (BBCH (Lancashire *et al.* 1991)) at about 3, 5, and 8 weeks for Rhizo4 (dates corresponding to the harvests of rhizotron experiments and shovelomics sampling in field in 2014 (Hecht *et al.* 2018) and to harvest of plant in Naz *et al.* (2012, 2014)). For Pot, we also photographed the shoot to get projected leaf area (from green pixel counts). At harvest, we obtained biomass for SDW (oven-dried at 70° C until constant weight) and scanned leaf area for the pot experiment to calculate specific leaf area. Furthermore, we oven-dried ears separately from the remaining shoot to get final grain yield for Pot.

Roots measurements and sampling: For Rhizo4, we took images of the roots visible at the window at 23, 35, and 56 days after sowing (DAS) (639, 901, and 1430 growing degree days (GDD), respectively). Additionally, we measured MRD at the first imaging day where roots had reached the bottom only in some rhizotrons. At the second measurement day, roots had reached the bottom in all rhizotrons. At harvest, we counted the number of nodal roots of the middle plant (other adventitious roots, if present, were excluded). For Pot, we scanned the root systems via MRI in a 4.7 Tesla magnet (Rascher *et al.* 2011; Metzner *et al.* 2014; van Dusschoten *et al.* 2016) once per week to estimate root length and number of main root axes (i.e. seminal, nodal and adventitious roots). For estimating root counts, we used ImageJ to determine the number of objects (only objects > 1 pixel, equivalent to root number in that image) and their size (in pixels; black pixels that indicated root presence in that pixel) of 10 separate horizontal slices of the MRI-image in the first 3 cm from root base (Fig. S 1 [**Supplementary Information**]). As the soil moisture was too low during MRI measurements after the 4th measurement day, these measurements were excluded from the analysis because roots were only partly detectable with MRI. At harvest, we counted the number of nodal roots of one plant per pot (other adventitious roots, if present, were excluded).

Statistics: We used R version 3.2.3 and 3.5.0 (R Development Core Team 2015, stats and PMCMR packages) to analyse our data doing two-way ANOVA for each time point separately. For that, we applied the following model:

$y \sim Block + Sowing\ density + Genotype + Sowing\ density\ X\ Genotype$, with y = measured trait.

When block was not significant, or the design was not blocked, we used the simplified model

$y \sim Sowing\ density + Genotype + Sowing\ density\ X\ Genotype$,

As a posthoc-test we performed TukeyHSD.

For counts and ranks (tiller counts, BBCH, root counts), we used the non-parametric Kruskal-Wallis-test appropriate for non-parametric data (McDonald 2014) and performed the Kruskal-Nemensyi-test as a posthoc-test. For our meta-analysis, we calculated the ratio of S42IL-176 over Scarlett based on the means for each measurement (means with standard error of the mean (SEM) can be found in the appendix [**Supplementary Information**] and significant differences between groups are indicated by letters with a significance level of $p<0.1$) and present these ratios in the figures.

Results

We present results of a meta-analysis of eight shoot related and nine root related traits across seven experiments (Fig. 20-Fig. 24). These experiments ranged from highly controlled conditions in growth chambers to field plantings. All these experiments were conducted with the same two barley lines, Scarlett and S42IL-176. We present in our graphs (Fig. 20-Fig. 24) the ratio of the trait mean values between the two lines, i.e. large differences between the genotypes are shown as large differences from 1, where a ratio larger than 1 denotes a larger relative trait for S42IL-176. All experiments included a competitive (dense planting) and non-competitive (single plant, low density) treatment as indicated by different colours. Separation of the coloured symbols within a specific symbol type in the vertical direction is an indication of a possible GxE interaction. We indicated statistical significance of the GxE interaction by using closed instead of open symbols ($p<0.1$) within a given symbol type (e.g. circles for Rhizo1-4, triangles for Pot, squares for Field1-2). In order to make results of different experiments and harvests more comparable, we plotted against thermal time (in GGD) so that ontological effects could also be observed. We first describe the shoot and afterwards the root trait results.

Shoot traits

The introgression line S42IL-176 nearly always had more tillers than Scarlett (ratios > 1 in Fig. 20A), although significant variation existed (ranging for S42IL-176 from 0.5 to 2 times more tillers than Scarlett; Fig. 20A, Tab. S 2 [**Supplementary Information**]). This finding was found across all experiments and did not depend on whether the experiment was performed under controlled or field conditions. This result underlines that the tillering-phenotype as described by Naz *et al.* (2012, 2014) under controlled conditions was confirmed in our research. In very young plants (up to about 500 GDD) however, S42IL-176 had sometimes fewer tillers than Scarlett as Scarlett started to tiller about 2 days earlier than

S42IL-176 (Pot). After having grown for 1700 GDD under competitive conditions, S42IL-176 had about the same number of tillers as Scarlett, while under non-competitive conditions S42IL-176 had more tillers. Although we observed significant GxE-interaction, namely genotype and sowing density, most of the time, the effect was not consistent across experiments until plants got older. So, while for field and pot experiments, the GxE-interaction was always significant ($p<0.1$), except for very young plants (GDD<180), for rhizotron experiments, the GxE-interaction was never significant in the outdoor-placed rhizotrons (Rhizo2), and was not significant even until 270 GDD for the rhizotrons in climate chamber (except once at 90 GDD, Rhizo1), while for the rhizotrons in greenhouse (Rhizo3-4), GxE became significant only after 450 GDD.

Despite the large differences in number of tillers, the SDW of these lines was most of the time quite similar (Fig. 20B, Tab. S 3 [**Supplementary Information**]). Naz *et al.* (2012, 2014) reported that SDW of S42IL-176 was lower than SDW of Scarlett, despite greater number of tillers. However, during early harvests, S42IL-176 had more SDW than Scarlett. GxE for SDW was only significant for Rhizo1, where S42IL-176 had greater SDW than Scarlett under non-competitive, but not under competitive conditions.

Given the greater tiller production of S42IL-176 but similar SDW we may expect the SDW per tiller to be lower for S42IL-176, especially for older plants under non-competitive conditions. This trend can be somewhat observed in Fig. 20C (see also Tab. S 4 [**Supplementary Information**]), however, is not as strong as we expected and GxE was only significant in one rhizotron (Rhizo4, 1430 GDD, $p=0.0045477$) and in one field sampling (Field2_S2, 767 GDD, $p=0.0017349$).

For leaf area we have fewer observations, nevertheless, S42IL-176 had nearly always greater leaf area than Scarlett (Fig. 20D, Tab. S 5 [**Supplementary Information**]). There was one except at one sampling (414 GDD, Rhizo1), where S42IL-176 grown under competition had

less leaf area per plant. At this sampling point GxE was significant (414 GDD, Rhizo1). The green leaf area of S42IL-176 was 8 times greater at the end of the long term pot experiment as Scarlett senesced its leaves earlier (Pot, 2133 GDD).

Specific leaf area was lower for S42IL-176 than for Scarlett in young plants and greater for S42IL-176 in older plants (Fig. 20E, Tab. S 6 [**Supplementary Information**]). Plants under competitive conditions had greater specific leaf area than under non-competitive conditions (Tab. S 6 [**Supplementary Information**]), as is consistent with general responses of specific leaf area to light. Although this trend of a GxE was consistent across experiments, it was never significant for single experiments except once in the field (Field2_S2, 767 GDD). In young plants, the genotype effect was greater for plants under competitive conditions, whereas in older plants genotype effects were greater for plants under non-competitive conditions.

Naz et al. (2012, 2014) reported that S42IL-176 has a planophil growth habit in contrast to Scarlett which is erectophil (on a score of 1 being planophil to 5 being erectophil, Scarlett is scored as 5 and S42IL-176 as 2). In Field1-2 and Pot, S42IL-176 grew planophil, while in Rhizo1-4 S42IL-176 never grew planophil but erectophil, even in outdoor placed rhizotrons (Rhizo2) (see Fig. S 2-Fig. S 6 [**Supplementary Information**]). In indoor pots used for seed multiplication, S42IL-176 grew also erectophil but in pots used for seed multiplication placed outside it grew planophil.

Growth habit can affect the height of the crop. Here, plant height was measured by stretching the plant vertically (Fig. 20F, Tab. S 7 [**Supplementary Information**]). We did not observe consistent differences in plant height between the lines this way except during the booting phase (GGD ~1000-1500). As Scarlett started to boot some days earlier than S42IL-176, S42IL-176 was smaller and caught up at later stages (GDD>1500 °C). After booting, GxE was a significant. In non-competitive conditions, S42IL-176 stayed smaller than Scarlett, where as in competitive conditions it was somewhat taller.

Differences in plant development are perhaps best captured using the BBCH scale (Fig. 20G, Tab. S 8 [**Supplementary Information**]). At the vegetative stage, S42IL-176 had mostly greater BBCH than Scarlett due to greater tiller number. However, since Scarlett started to boot earlier, S42IL-176 then had lower BBCH and stayed behind Scarlett. GxE was significant during late tiller formation phase or when Scarlett had started to boot, but differences between competitive and non-competitive conditions seem small and not consistent across sampling points.

Final grain yield was only measured twice for S42IL-176: in one field (Field2) and in the pot experiment (Pot) (Fig. 20H, Tab. S 9 [**Supplementary Information**]). In Field2, it had the same final grain yield as Scarlett, namely 5.0 and 6.5 t ha^{-1} for non-competitive and competitive conditions, respectively. In Pot, however, it had only about half the final grain yield compared to Scarlett. GxE was never significant.

Root system traits

Although root length was sampled differently in the different experiments, we will assume that the ratio between the lines is a good estimate of the ratios for total root length (TRL, Fig. 21A, Tab. S 10 [**Supplementary Information**]). Total root length was always greater for S42IL-176 than for Scarlett in rhizotron experiments (Rhizo1-3), except for once (510 GDD, Rhizo3) under non-competitive conditions. The difference in TRL between the lines was consistently greater under competitive conditions. In Pot, S42IL-176 had initially lower TRL than Scarlett but later on greater TRL. In the field, young S42IL-176 had lower TRL (590 GDD, Field1_C) and older plants of both genotypes had similar TRL (1147 GDD, Field2_C). GxE was always significant in rhizotron experiments (except at 414 GDD, Rhizo1), sometimes in pot experiment (at 252 and at 504 GDD), but never significant for field experiments.

The root-phenotype regarding RDW of S42IL-176 in comparison to Scarlett as described by Naz *et al.* (2012, 2014) was consistently expressed in rhizotrons (Fig. 21B, Tab. S 11 [**Supplementary Information**]). In the field, a more complex picture emerged in which RDW ratios depended on sampling time, genotype and sowing density, i.e. competition (although GxE was not significant). Possibly the limited sampling in the field, using coring, affected the results here, but ontological or environmental effects cannot be excluded.
In rhizotron experiments, root mass fraction was greater for S42IL-176 plants than for Scarlett, when growing under competitive conditions, while under non-competitive conditions, root mass fraction was lower (Fig. 21C, Tab. S 12 [**Supplementary Information**]). In field, root mass fraction was greater for S42IL-176 than for Scarlett in younger plants, but similar for both genotypes in older plants. GxE was never significant, except in one rhizotron experiment (510 GDD, Rhizo3, $p=0.04$).
Specific root length tended to be lower for S42L-176 than for Scarlett (Fig. 21D, Tab. S 13 [**Supplementary Information**]). GxE interactions were mostly not significant, and were not consistent across experiments.

Root counts

We expected that the greater tillering and RDW-phenotype of S42IL-176 as reported by Naz *et al.* (2012,2014) under greenhouse conditions would be connected to a greater production of nodal roots, as we showed in a subsequent study happened after Naz *et al.* (2012,2014) that tiller production and nodal root production are highly correlated (Hecht et al., 2018). The number of nodal roots, however, was only greater for S42IL-176 in very young plants (GDD<300°C) and in older plants (GDD>1000°C) (Fig. 22A, Tab. S 14 [**Supplementary Information**]). It seemed thereby that rather than the expected consistently greater production of nodal roots, S42IL-176 differed in its ontology for nodal root formation. Indeed, S42IL-176 produced its first nodal roots about 1 day earlier than Scarlett (in Rhizo2, S42IL-176 at

17.2 DAG for non-competitive and competitive conditions, Scarlett at 17.8 DAG at competitive and at 18.25 DAG under non-competitive conditions). Sowing density, i.e. competition, nearly always had a significant interaction with genotype, but the interaction was not consistent across experiments or harvest time points. As S42IL-176 had greater tiller production, we observed that nodal root formation per tiller was lower in S42IL-176 (Fig. 22B, Tab. S 15 [**Supplementary Information**]).

In Rhizo2 (251 GDD), the lateral branching frequency (or lateral branching intensity) was lower for S42IL-176 than for Scarlett, except at 10 cm distance from seminal root base, where S42IL-176 had about 1.2 greater lateral branching frequency than Scarlett when growing under non-competitive conditions, and about the same lateral branching frequency, when growing under competitive conditions (Fig. 23A, Tab. S 16 [**Supplementary Information**]). At 0 cm and at 20 cm away from the seminal root base, S42IL-176 growing under non-competitive conditions had only 1/3 to 3/4 of the lateral branching frequency than Scarlett, when growing under competitive conditions. Further, GxE was statistically significant ($p=0.05099$) only at root base. In the field, we only determined the lateral branching frequency at the root base (Field1_S1). Here, the lateral branching density was consistently greater for S42IL-176 than for Scarlett, regardless of the root type or treatment (Fig. 23B, Tab. S 16 [**Supplementary Information**]).

Rooting Depth

Rooting depth is considered an important characteristic of nutrient foraging strategies (Thorup-Kristensen 2001; Kristensen and Thorup-Kristensen 2009; Thorup-Kristensen and Rasmussen 2015). We measured MRD in rhizotron experiments on roots visible at the window (Fig. 24A, Tab. S 17 [**Supplementary Information**]). Maximum rooting depth was named “root length” in Naz *et al.* (2012,2014), who observed a greater (deeper) MRD for S42IL-176 than for Scarlett. Although most of the dots are above 1, confirming that S42IL-

176 had somewhat greater MRD, in none of the observations we could establish a significant difference, neither did we observe significant effects of competition on MRD or a significant GxE.

Rooting depth in soil-based systems can be influenced by branching angle of the nodal and seminal roots (Lynch 2013). We did not observe significant effects of genotype or treatment on branching angle (Fig. 24B, Tab. S 18 [**Supplementary Information**]), except once in field for young plants (Field2_S1), where S42IL-176 had greater branching angle than Scarlett ($p=0.0263565$) and once in field in older plants (Field2_S2), where plants under non-competitive conditions had greater branching angle than under competitive conditions ($p=0.0174727$) (Tab. S 18 [**Supplementary Information**]). GxE was never significant.

Here, we summarize the main findings which are discussed in more detail further down: S42IL-176 produced more tillers than Scarlett and started to boot later than Scarlett so that it produced in total more tillers and thus nodal roots per plant. Furthermore, it had a larger TRL in the early vegetative phase (tiller formation phase) in rhizotrons but not in field, where S42IL-176 had less or the same TRL as Scarlett. Specific root length was lower especially in the vegetative phase probably due to the greater nodal root counts. Moreover, S42IL-176 had greater root biomass (namely RDW) in most cases, also, probably due to greater nodal root number per plant. Interestingly, the ratio of nodal roots per tiller was lower for S42IL-176 during the tiller formation phase, although it produced more nodal roots and more tillers, which means that it produced even more tillers than nodal roots.

Discussion

We asked if selection for phenotypic traits under artificial but controlled lab conditions can translate to the field into observable differences in agronomic traits and yield. There are many factors that might influence this translation, among others age-related ontology, abiotic (temperature, light) and biotic environment (competition). Poorter *et al.* (2016) reported that lab grown plants differ strongly from field grown plants with respect to several phenotypic traits. Here, we presented a case study in which we did a detailed lab to field comparison for one genotypic contrast and we focused especially on plant competition as a possibly important factor for improving lab to field translation.

Reproducibility of the larger root phenotype of the introgression line

The selection of S42IL-176 for comparison with Scarlett in our experiments was made based on its larger root system (greater RDW, root volume, MRD) which is associated with greater tiller number (Naz *et al.* 2012, 2014).

The tiller-phenotype of S42IL-176 as described by Naz *et al.* (2012,2014) was consistently observed across most samplings in all experiments. We hypothesized that translation of root traits may be more difficult as they are often more plastic than shoot traits. Indeed, the root-phenotype of S42IL-176 was not as strong expressed as described by Naz *et al.* (2012,2014) and sometimes completely missing. For example, we could observe an increase in RDW for S42IL-176 in rhizotrons but not as much as the 63 % reported by Naz *et al.* (2014) (and not even close to the 250 % increase reported by Naz *et al.* (2012)) but rather at the most 1.58 (corresponds to 58 % using calculation as in Naz *et al.* (2014)) times more in S42IL-176 than in Scarlett. Older S42IL-176 plants – similar to the plants in Naz *et al.* (2012,2014) with regard to age – had only similar RDW to Scarlett and in the field, S42IL-176 even had less RDW than Scarlett, such that the RDW-phenotype described by Naz *et al.* (2012, 2014) completely disappeared. For MRD ("root length" from base to tip of longest root in Naz *et al.*

(2012, 2014)), we could not reproduce the phenotype reported by Naz *et al.* (2014), as S42IL-176 was at the most 1.2 (corresponds to 20 % using calculation as in Naz *et al.* (2014)) times deeper but most of the time it had about the same or even less MRD and our S42IL-176 plants never reached the 42 % deeper roots reported by Naz *et al.* (2014). However, our S42IL-176 plants were close to the 10 % increase in MRD earlier reported by Naz *et al.* (2014). We stopped the experiments, when roots reached the bottom (at 19 and 23 DAG in Rhizo1-2, 35 DAS in Rhizo3) and thus, plants were younger by at least two weeks than in the studies of Naz *et al.* (2012, 2014). In the rhizotron experiment (Rhizo4) that had about the same duration as in Naz *et al.* (2012, 2014), we only sampled a part of the root system for root counts but not for MRD, as many plants had reached the bottom already by the 2nd measurement day (23 DAS, 21 DAG, 639 GDD, first imaging day). Here, S42IL-197 had the same MRD as Scarlett. The growth habit-phenotype (planophil for S42IL-176, erectophil for Scarlett) was reproducible for Scarlett but for S42IL-176 only reproducible in field or pots but never in rhizotrons. Competition suppressed the tiller-phenotype in rhizotrons and pot but not in field and competition rather enhanced the RDW-phenotype but did not affect the MRD-phenotype.

Moreover, we found S42IL-176 differed from Scarlett with respect to some traits that were not yet reported. For instance, lateral branching frequency in older plants was consistently greater for S42IL-176 than for Scarlett, regardless of root type. Overall, root traits were more variable across environments (here, rhizotrons, pots, fields, and sowing density) than shoot traits in our study. We conclude from our results that the selection of S42IL-176 did lead to a clearly different phenotype in both lab and field, and that this phenotype was relatively consistent aboveground, but much less so belowground.

This difficulty of reproducing greenhouse results has nicely been demonstrated by Anderson (1986) who observed within the same study for maize, that SDW, root mass fraction and N-

partitioning to roots of 7-week old greenhouse plants were similar to field grown plants in one year but not in the following year during which only SDW correlated with the greenhouse trial. Furthermore, field-grown maize plants always had lower specific root length and greater root diameter than plants grown in the greenhouse. Anderson's study is exemplary for the great variability in climate conditions plants experience in the field. Poorter *et al.* (2016) illustrates this variability by describing the r^2 of 53 studies in which biomass or yield was reported for a set of genotypes grown in subsequent years: The correlations ranged from -0.08 to 0.67 with a median r^2 of 0.08.

Interestingly the correlations for lab to field were significantly higher, although not great, with a median r^2 of 0.26 (Poorter *et al.* 2016). These correlations only focused on aboveground biomass. There are only a few studies that compared root traits measured in the greenhouse and the field. For example, Saengwilai *et al.* (2014) had consistent results for maize genotypes varying in crown root number grown in greenhouse and field trials. Lower crown root number was associated with greater rooting depths and greater low N-tolerance. Another example is given by Grumet *et al.* (1992) who observed that most cucumber genotypes that responded early to herbicide treatment placed in soil in the greenhouse also responded early in the field. Moreover, sometimes one trait observed in the greenhouse can serve as a predictor for the aimed trait in the field, as shown by Clark *et al.* (2002) for rice: the diameter of the impeded longest nodal root correlated better to root penetration rate than MRD.

In our studies, the tiller-phenotype was reproducible and to some extend the RDW-, SDW-, and growth habit-phenotype but not the MRD-phenotype. Hence, tillering seemed to be the most consistent trait that was less affected by GxE, whereas other traits were more variable.

Does S42IL-176 differ in the field with respect to important agronomic traits?

As the phenotype S42IL-176 was, at least in part, reproducible in the field, we ask if this led to changes of important agronomic traits. Indeed, as may be expected from a genotype with

more tillers, S42IL-176 had consistently greater leaf area than Scarlett in the field, though only once significantly so, but lower SDW per tiller. BBCH was greater during late tiller formation phase for S42IL-176, as S42IL-176 produced more tillers, and, since Scarlett booted and ripened earlier, and lower from booting onwards. At about the same plant age and developmental stage as in Naz *et al.* (2012, 2014), the genotype S42IL-176 expressed the tiller-phenotype and the RDW-phenotype, but neither the SDW-phenotype nor the MRD-phenotype. The growth habit-phenotype was only expressed in the field and in the pot experiment but never in rhizotrons. The longer an experiment took, the more stable the phenotype was expressed. Furthermore, despite quite consistent effects on RDW and fairly consistent effects on nodal root counts (both increased in S42IL-176), we did not observe consistent effects on specific root length and yield (S42IL-176 had the same yield as Scarlett in the field but only half of the yield of Scarlett in pot experiment).

When it comes to linking trait selection and yield increase, it has been shown in field trials with barley, that the selection for bigger root systems results in greater yield (Svačina *et al.* 2014). We did not observe an increase in yield, although root system size (RDW, TRL, nodal root number per plant) was increased in most samplings. We show thereby that, even though the root phenotype was not expressed strongly in the field, the selection of S42IL-176 did lead to different important agronomic root traits. We expect that, under different growth conditions, for example drought or water logging, these differences might translate into yield differences, however, to clearly show this would require further testing, possibly under more forms of edaphic stress.

Sowing density as an important factor in lab to field translation

We introduced sowing density into our rhizotron and pot experiments to get more similar results to a densely planted field. This only worked in experiments that ran long enough

(longer than 4 weeks) and better for shoot than for root traits. Moreover, competition sometimes reduced but also enhanced GxE effects, depending on the trait of interest. In our study, for example, competition reduced genotypic differences in tiller number in older plants. In contrast, competition enhanced genotypic differences in plant height or TRL. GxE-interactions are important reasons why phenotypes might not be reproducible.

Given that genotype by sowing density interactions were not consistent between lab and field, we ask if sowing density should be considered in phenotyping studies, for example growing several plants in one pot. As plant competition induced by sowing density played a role in plants older than approximately four weeks, a potential plant density effect only needs to be taken into account or taken care of in experiments running longer. Even when including more competition into the phenotyping studies, direct translation from lab to field remains challenging and other aspects such as light, temperature and developmental age need to be considered (Mishra *et al.* 2012; Poorter *et al.* 2016). However, before focusing on this, we first consider the use of relative versus absolute numbers.

Relative numbers translate better than absolute numbers

We presented our data as ratios of S42IL-176 over Scarlett, as these relative numbers were more stable over time. For instance, tiller number of S42IL-176 varied between 0.6 to 2 times that of Scarlett, while absolute means ranged from 1 to 56 tillers per plant. In general, except for leaf area, where S42IL-176 had about 8 times more green leaf area than Scarlett at maturity of Scarlett, most ratios ranged between 0.4 and 2, while absolute means could have up to 600-fold variation, as for example SDW that ranged 0.086 and 52.09 g per plant. Relating such a trait with a huge range like SDW to another trait, e.g. tiller number for SDW per tiller or leaf dry weight for specific leaf area, to get a size-independent trait, reduced the range of this proportion-trait (131- and 2.5-fold increases for SDW per tiller and specific leaf

area, respectively). However, this relation could still be very large as in the case of SDW per tiller and much greater than the genotype-ratios we used within this study.

In many cases, the biologist or agronomist will be more interested in the relative changes, as they are often assumed to be more comparable across ages and environments. A challenge thereby is that standard ANOVA analyses test absolute differences between means, which may cause GxE interactions to be significant, even when in relative terms there were no differences. We can conclude that relative differences were more stable across experiments in general but still were not always consistent. Finally, we will consider plant development, as influenced by temperature and light, as a translation factor.

Plant age and development as an explanatory factor in lab to field translation

The importance of the time point of sampling, when comparing results, can be seen in BBCH data describing the developmental stages of plants. The ratios of BBCH between the two genotypes were not stable over time and changed from above 1 (S42IL-176 > Scarlett) to below 1 (Scarlett > S42IL-176) and these differences in developmental stages were also reflected in other traits like tiller number and plant height. Also, GxE was more often significant in older plants. Running an experiment for a longer time improved similarity of lab and field results. This was backed up by the absolute values of the long-run rhizotron experiment Rhizo4 (1430 GDD, 56 DAS) being similar to the coring data in 2013 (Field1_C, plants are about 2 month old, 48-56 DAS, 590-741 GDD) for SDW, SDW per tiller and BBCH, when plants had about the same age, and were close to the values of shovelomics in 2013 (Field1_S, 995 GDD, 75 DAS) for tiller number per plant, nodal root number per plant, and nodal roots per tiller, when plants had grown for about the same thermal time period.

As discussed before, it is not surprising that trait measurements of younger plants do not relate to those of older plants, but we did expect relative numbers to be more stable. Using the ratios of the two genotypes helped in reducing the ontological effect, but not completely.

Thus, ontology remains an important factor to consider in lab to field translation, even when comparing ratios between genotypes.

Thermal time is often used to compare results of different experiments (McMaster and Wilhelm 1997; Miller *et al.* 2001; Alzueta *et al.* 2014) and a standard in many crop models (Ritchie *et al.* 1989; Keating *et al.* 2003; Eitzinger *et al.* 2004; Gaydon 2014). We expected that using thermal time would improve correlations for shoot traits of different experiments over (thermal) time, but not root traits, as soil temperature often strongly deviate from air temperatures (Carter 1928; Islam *et al.* 2015). Thermal time, however, did not improve fitting of BBCH across all experiments as compared to fitting against time in DAS, whereas, nodal root formation was better correlated to thermal time than to time in DAS (Fig. S 7 A-D [**Supplementary Information**]). Thus, we conclude that using GDD instead of DAS to compare experiments along developmental gradients can improve correlations (as in our case for nodal roots) but does not necessarily always do so (as in our case for BBCH). Correction for temperature (and light) by using thermal time (or photothermal time) only works in temperature ranges where the plant is responsive in a linear way. At very high temperatures or light, the response might be saturated or even negative. In barley, for example, temperatures greater than 15 °C did not result in earlier heading (as it is observed for temperature increases below 15 °C, Karsai *et al.* (2008)), meaning greater temperatures do not further accelerate growth. Possibly, the GGD model is oversimplified for translating experimental results and a different, non-linear, temperature model may be more appropriate for these traits (Yin *et al.* 1995). In addition, when focusing on root traits, much more uncertainty exists with respect to how temperature influences them, and further research is needed here, in order to know how lab results might be translated to field results, as for instance, Füllner *et al.* (2012) found that barley roots grown across a root temperature gradient grew much better than roots exposed to constant temperature, irrespective of the temperature.

Light is often low in lab conditions compared to the field (Poorter *et al.* 2016) and different light qualities in lab and field have been shown to affect shoot traits in *Arabidopsis* (Mishra *et al.* 2012). Our rhizotron and pot experiments had DLI (daily light integral) of 3 to 55 mol day^{-1} m^{-2}, with day lengths of 9-16 h, whereas in field trials we had in the first four weeks DLIs of 65 and 8 mol day^{-1} m^{-2} and average day length of 12.8 and 11.6 h in 2013 and 2014, respectively. In order to take differences in day length into account in the translation of experimental results, photothermal time has been proposed as an alternative to thermal time (Masle *et al.* 1989). Photothermal time is the average temperature during the light period as a proportion of the light period within a day, and correlated better to developmental stages in young wheat plants than thermal time (Masle *et al.* 1989). The fit of our data over photothermal time, however, was worse than for thermal time or time in DAS (Fig. S 7 E-F [**Supplementary Information**]). This can be partly due to the fact that light is intertwined with plant density, and we do not know how to correct for light levels in the translation between experiments, specifically lab to field, and only day length but not light intensity is taken into account in photothermal time. Hence, in our case, photothermal time did not help with our exploration of translation from lab to field.

Conclusions

Selection for traits in greenhouse can work (e.g. tiller number, nodal root number) despite a certain plasticity of the trait. In general, root traits are more plastic across different environmental conditions and thus less stable, less conserved than shoot traits. Furthermore, the age of the plant – and thus, developmental stage of the plant – is very important in two ways. One, a certain phenotype might change with age, as not all traits change to the same degree and two, when comparing results of different experiments, plants should have similar age. Therefore, running a controlled experiment for longer should help to get more comparable results to field trials. However, plant density has then to be taken into account,

especially, when traits that are sensitive to plant density or competition are studied. Moreover, ratios are more stable than absolute numbers and facilitate the comparison of genotypes but also of environments. Plant density, light and temperature have been identified as major translation factors but the translation of lab to field results remains challenging. Thus, more research is required to understand the plasticity of root traits and to identify the main drivers of this plasticity.

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Tables

Table 1 Phenotypic information of S42IL-176 from (Naz et al. 2012, 2014) on root length (corresponds to MRD), dry weight, volume, tiller number, growth habit and shoot dry weight. We calculated thermal time-based on the mentioned average temperature in the experiments in Naz et al. (2014) (18.2 °C in 2012; 14.2 °C in 2013).

Trait	**Relative trait performance of S42IL-176 compared to Scarlett from (Naz *et al.* 2014)** (=($LSM_{S42IL-176}$-$LSM_{Scarlett}$)/ $LSM_{Scarlett}$*100)	**Estimated values for least square means (LSM) (mean for shoot dry weight) from graphs of Naz *et al.* (2012)**		
		Scarlett	S42IL-176	Ratio S42IL-176/Scarlett (Relative trait performance as in Naz *et al.* (2014))
Root length [cm] (from stem base to root tip)	+ 41.8 %	39	43	1.1026 (+10.26 %)
RDW [g]	+ 63.3 %	1	3.5	3.5 (+ 250 %)
Root volume [cm3]	+ 42.2 %	11	36	3.2727 (+227.27 %)
Tiller number per plant (Naz *et al.* 2014) / change in tiller number (Naz *et al.* 2012)	+ 70.5 %	7.5	12.5	1.6667 (+66.67 %)
Growth habit (scored from 1 to 5 considering spreading growth type (1) to erect growth type (5), Scarlett is 5, ISR42-8 (Israeli parent) is 1, S42IL-176 is 2)	- 60.0 %	NA	NA	
SDW [g]	NA	Control: 20 Drought: 14	Control: 16 Drought: 12	Control: 0.8 (-20 %) Drought: 0.857 (-14.26 %)
Time point of stress application	30 DAS, BBCH 29-31	42 DAS, BBCH 30-31		

Plant age	56 DAS	70 DAS
Thermal time at harvest estimated from average temperature in greenhouse as mentioned in Naz *et al.* (2014)	795-1020 GDD	994-1274 GDD

Table 2 Experimental design, treatment, and climate data of rhizotron experiment (Rhizo4) and the pot experiment (Pot).

	Rhizo4	**Pot**
Location at research centre	Greenhouse	Climate chamber, greenhouse during NMR-measurements
Container type	rhizotron	pot
Container size (outer (inner) dimension/ height, outer (inner) diameter), volume	70 (60) cm x 5 (3.5) cm x 90 (80) cm, 18.3 l	40 cm x 12 (11.5) cm, 3.76 l
Substrate	Peat substrate	Sand : field soil (from Klein-Altendorf) 2:1
Watering	Automatic irrigation (from 145ml per day to 290 ml per day, 23 % water per l soil)	By hand and automated computer-driven irrigation
Fertilizer	Same amount per rhizotron (NH_4^+ 83 mg/l, NO_3^- 89 mg/l, P_2O_5 27 mg/l, K_2O 224 mg/l, pH in $CaCl_2$ 6.3), no additional fertilizer	540 mg of NPK (12-8-16 %)-fertilizer at sowing (corresponding to 65 kg N/ha), 400 ml of 10 % Hoagland solution at tillering (BBCH25-29, 31 DAS), 100 ml of 10 % Hakaphos green at tillering (BBCH 26-29, 38 DAS), 100 ml of 10% Hakaphos green at tillering and shooting (BBCH 28-32, 38 DAS)
Plants per container (distance to neighbour plant)	1 and 7 plants (4 cm)	1 and 4 plants (6.7 cm)

Replicates per treatment	9 (randomized block design)	4 (fully randomized design)
Final harvest	56 DAS (54 DAG)	118.5 DAS (Sca), 132.5 (IL)
Air temperature (day/night)	22/18 °C (16 h/8 h) (aimed), since experiment ran during an extremely hot summer, the average of the daily minimum and daily maximum temperatures were 19.4 °C and 33.5 °C, respectively, with an average daily temperature of 26 °C.	20/15 °C (16 h/8 h)
Light (PAR)	Ca. 218 µmol * m-2s-1 on daily average during light period, with maximum 1057 µmol * m-2 s-1 (17 h)	Ca. 960 µmol*$m^{-2}s^{-1}$ (16 h)
Air humidity	Daily average 47 %, with average daily minimum and maximum air humidity of 16 and 76 %	60 %
GDD at harvest	1430 °C	2133 °C (Scarlett)
Trial year	2018	2015

Supplemental tables

Tab. S 1 Pixel count (pixels of objects of 1 pixel are excluded) and object number for objects bigger than 1 pixel for the ten slices of the NMR image presented in Fig. S 1.

Tab. S 2 Tiller number per plant for all experiments at harvest, counts of during the experiments are not shown. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 3 Shoot dry weight (SDW) per plant for all experiments. Values are means (n=5-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 4 Shoot dry weight (SDW) per tiller for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 5 Leaf area per plant for all experiments. Values are means (n=5-8) with standard error of the mean (SEM). * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *). Note the different unit in Pot.**

Tab. S 6 Specific leaf area for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 7 Plant height for all experiments at harvest, measurements of during the experiments are not shown. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 8 BBCH for all experiments at harvest, measurements of during the experiments are not shown. Values are means (n=3-8), with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 9 Final grain yield for different experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 10 Total root length (TRL) per core or rhizotron for all experiments at harvest, measurements of during the experiments are not shown. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *). iR= within the plant row.**

Tab. S 11 Root dry weight (RDW) per plant for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 12 Root mass fraction for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 13 Specific root length (without roots with a diameter smaller than 0.1 mm) for entire system for all experiments. Values are means (n=3-8), with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 14 Nodal roots per plant for all experiments at harvest, measurements of during the experiments are not shown. Values are means (n=5-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 15 Nodal roots per tiller for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 16 Branching frequency at different locations along a seminal root (Rhizo2, 251 GDD) or at nodal or seminal root (Field1_S1, 995 GDD). Values are means with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 17 Maximum rooting depth for rhizotron experiments at harvest, measurements of during the experiments are not shown. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Tab. S 18 Branching angle at 5 cm within the plant row for all experiments. Values are means (n=3-8) with SEM. * behind experiment, genotype, or sowing density indicate significant GxE-, genotype- or sowing density-effect (p<0.1 *, p<0.05 **, p<0.01 *).**

Figure legends

Fig. 20 Ratios of S42IL-176 over Scarlett for shoot traits in field, pot, and rhizotrons experiments. The ratios are of A) tiller number per plant, B) shoot dry weight (SDW) per plant, C) shoot dry weight (SDW) per tiller, D) leaf area per plant, E) specific leaf area, F) plant height, G) BBCH, and H) final grain yield. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

Fig. 21 Ratios of S42IL-176 over Scarlett for root traits in field, pot, and rhizotrons experiments. The ratios are of A) total root length (TRL), B) root dry weight (RDW), C) root mass fraction, and D) specific root length in total system within the plant row. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

Fig. 22 Ratios of S42IL-176 over Scarlett for root counts in field, pot, and rhizotrons experiments. The ratios are of A) nodal roots per plant and B) nodal roots per tiller. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

Fig. 23 Ratios of S42IL-176 over Scarlett for lateral branching frequency in field and rhizotron experiments. The ratios are of A) 0 cm, 10 cm, and 20 cm away from a seminal root base from one rhizotron experiment (251 GDD, Rhizo2) and B) seminal root, nodal root of a main stem, and nodal root of a tiller from field trial in 2013 (995 GDD, Field1_S). Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

Fig. 24 Ratios of S42IL-176 over Scarlett for maximum rooting depth (MRD) (A) and branching angle (B) in field and rhizotron experiments. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes

Supplemental Figures

Fig. S 1 The ten different slices of the MRI image (within the first 3 cm below the root base). Black pixels indicate the presence of a root. The image is of S42IL-176 as a single plant in a pot on the first measurement day (14 DAG, 252 GDD). The pixel and root counts and their ratios going from upper left to lower right are presented in appendix Tab. S 1 [Supplementary information].

Fig. S 2 Images of Rhizo1 (climate chamber) and Rhizo2 (outdoor). A-D) show single plants of Scarlett at harvest (23 DAG, 414 GDD), E) five Scarlett plants in a rhizotron at harvest (23 DAG, 414 GDD), F-I) single plants of S42IL-176 at harvest (23 DAG, 414 GDD), J) five S42IL-176 plants in a rhizotron at harvest (23 DAG, 414 GDD), K) one box with several rhizotrons with single plants and clusters of Scarlett and S42IL-176 in

climate chamber (26.10.2012), note that all plants grow erectophil, and L) rhizotrons of Rhizo2 outdoor at 16 DAG.

Fig. S 3 Rhizotrons of Rhizo4 of shoot and root system of single Scarlett plants (A-F), single S42IL-176 plants (G-L), seven Scarlett plants per rhizotron (inter-plant distance 4 cm) (M-R), and seven S42IL-176 plants per rhizotron (inter-plant distance 4 cm) (S-X). Top images (A, B, G, H, M, N, S, and T) are at 639 GDD (23 DAS), middle images (C, D, I, J, O, P, U, and V) at 901 GDD (35 DAS), and bottom images (E, F, K, L, Q, R, W, and X) at 1430 GDD (56 DAS). Note that growth habit was very similar for both genotypes and roots reached the bottom at the first measurement already.

Fig. S 4 Excavated and washed plants (shovelomics, Field2_S1) in the field at 325 GDD (35 DAS) (A-E) and plants still in the plot photographed from the top (F-I). Plants of Scarlett are left (A, C, E, F, and H) and of S42IL-176 are right (B, D, C, and I). The respective sowing densities are 24 seeds m^{-2} (A, B, F, and G), 120 seeds m^{-2} (C, D, H, and I), and 340 seeds m^{-2} (E, only Scarlett, not enough seeds available for sowing S42IL-176 at 340 seeds m^{-2}).

Fig. S 5 Plots of hand sown S42IL-176 in the field in 2013 (Field1) at 338 GDD (29 DAS) and 476 GDD (41 DAS) at tiller formation phase. S42IL-176 is located within the red box surrounded by machine sown (outer rows) and hand sown (continued rows of S42IL-176) Scarlett.

Fig. S 6 S42IL-176 plants for seed multiplication placed outside in spring 2013 (A-D) and inside in climate chamber in winter 2012 (E-F). A) is at 7 DAS (day of transfer to outside), B) at 14 DAS, C) at 16 DAS, D) at 26 DAS, E) at 14 DAS, and F) 22 DAS (small plants in pots on righter side at 7 DAS). Note that plants outdoor changed growth habit from erectophil to planophil within 2 weeks, while in climate chamber plants stayed erectophil.

Fig. S 7 BBCH and nodal roots per plant (genotype and sowing density merged for each sampling time point) over thermal time (A, B), time in DAS (C, D), and photothermal time (E, F). The linear regression had greatest R^2 for BBCH over time in DAS, whereas for nodal roots per plant, the linear regression had greatest R^2 over thermal time. Photothermal time always showed worst R^2.

Figures

Translation from lab to field: A case study of how greenhouse based selection for great root development may influence agronomic traits and performance in the field.

Hecht VL[1], Nagel KA[1], Temperton VM[2], van Dusschoten D[1], Rascher U[1], Léon J[3], and Postma JA[1]*

Running title: translation from lab to field

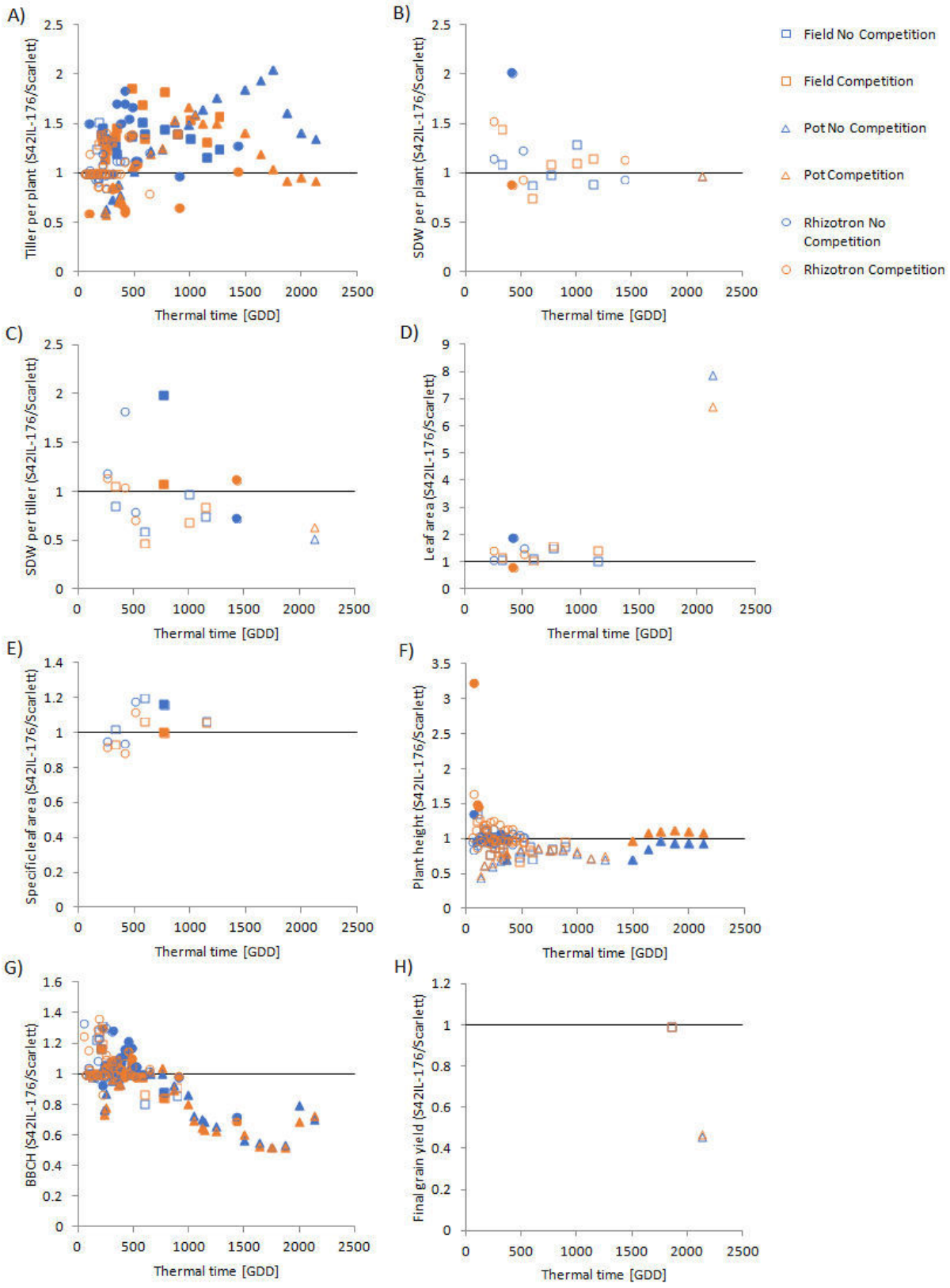

Fig. 25 Ratios of S42IL-176 over Scarlett for shoot traits in field, pot, and rhizotrons experiments. The ratios are of A) tiller number per plant, B) shoot dry weight (SDW) per plant, C) shoot dry weight (SDW) per tiller, D) leaf area per plant, E) specific leaf area, F) plant height, G) BBCH, and H) final grain yield. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

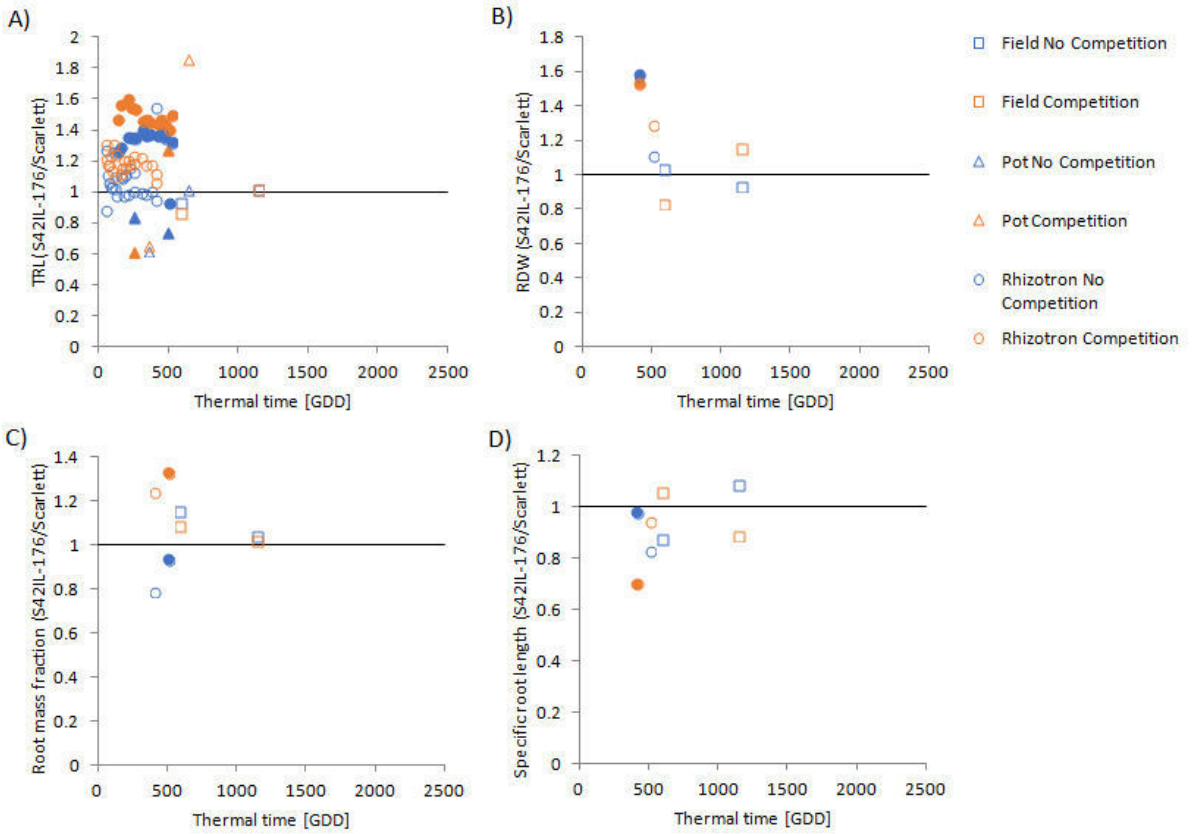

Fig. 26 Ratios of S42IL-176 over Scarlett for root traits in field, pot, and rhizotrons experiments. The ratios are of A) total root length (TRL), B) root dry weight (RDW), C) root mass fraction, and D) specific root length in total system within the plant row. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

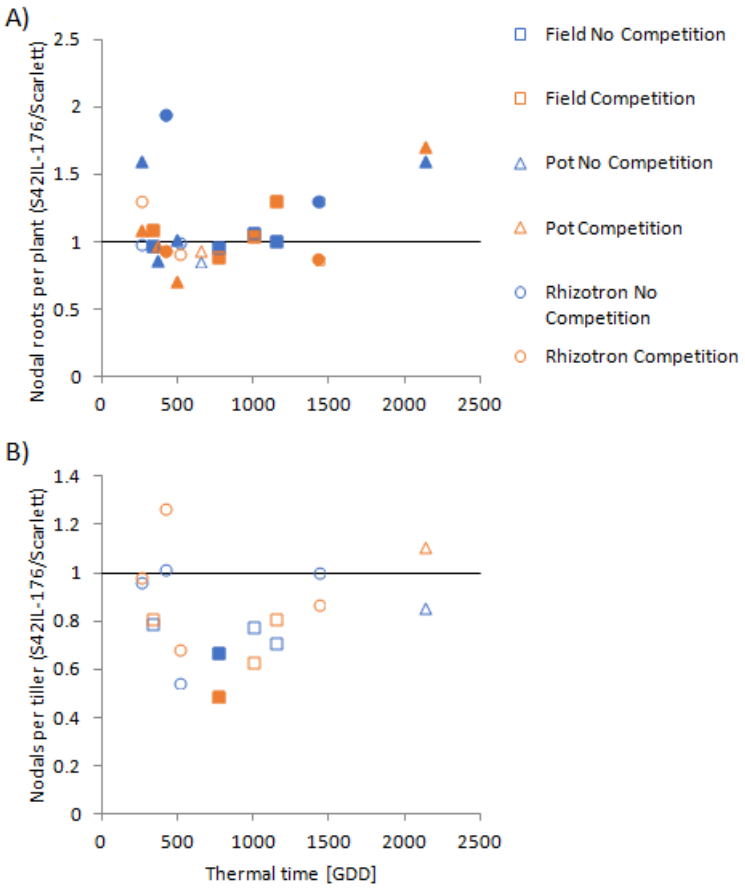

Fig. 27 Ratios of S42IL-176 over Scarlett for root counts in field, pot, and rhizotrons experiments. The ratios are of A) nodal roots per plant and B) nodal roots per tiller. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

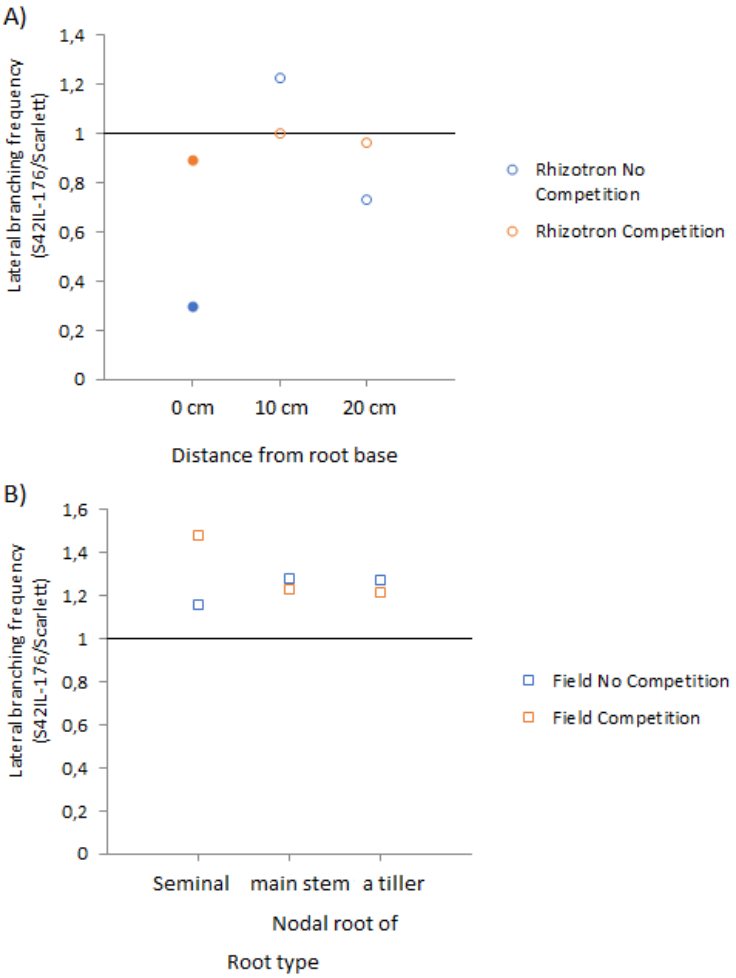

Fig. 28 Ratios of S42IL-176 over Scarlett for lateral branching frequency in field and rhizotron experiments. The ratios are of A) 0 cm, 10 cm, and 20 cm away from a seminal root base from one rhizotron experiment (251 GDD, Rhizo2) and B) seminal root, nodal root of a main stem, and nodal root of a tiller from field trial in 2013 (995 GDD, Field1_S). Filled symbols represent a significant (p<0.1) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes.

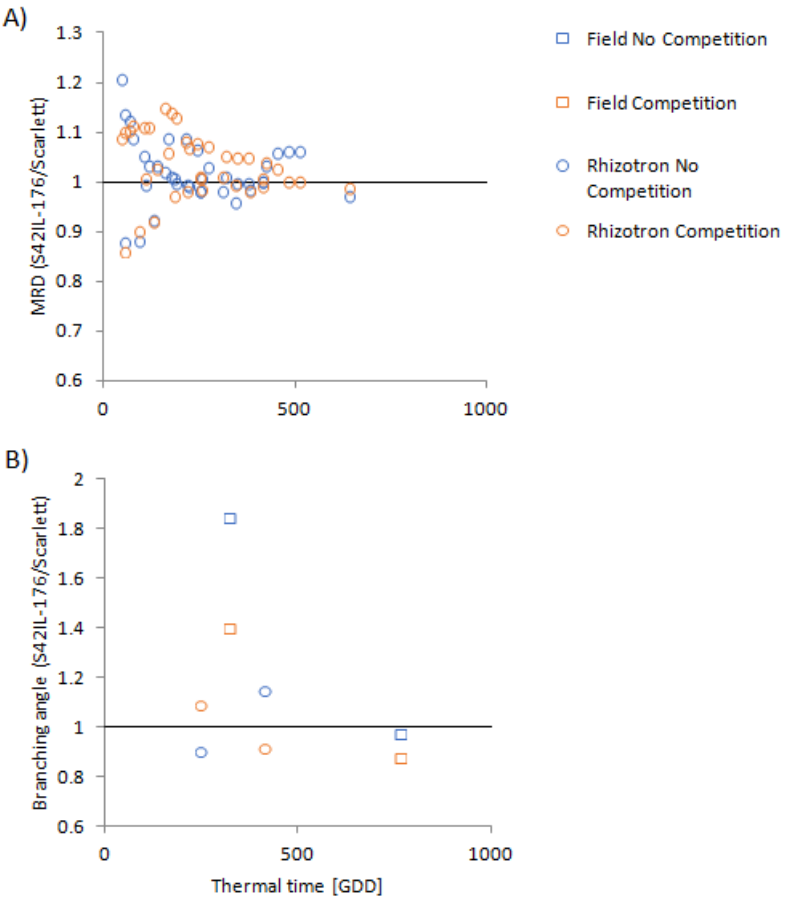

Fig. 29 Ratios of S42IL-176 over Scarlett for maximum rooting depth (MRD) (A) and branching angle (B) in field and rhizotron experiments. Filled symbols represent a significant ($p<0.1$) GxE interaction (i.e. genotype and sowing density) for the respective measurement day in the respective experiment and when symbols are open, GxE was not significant. Note the different y-axes

9.4 Phenological analysis of unmanned aerial vehicle based time series of barley imagery with high temporal resolution

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Phenological analysis of unmanned aerial vehicle based time series of barley imagery with high temporal resolution

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Abstract Emerging strategies and technologies in agriculture, such as precision farming and phenotyping depend on detailed data on all stages of crop development. Unmanned aerial vehicles promise to deliver such time series as they allow very frequent measurements. In this study, we analyse a field trial with two barley cultivars and two contrasting sowing densities in a random plot design over 2 consecutive years using the aerial images of 28 flight campaigns, providing a very high temporal resolution. From empirically corrected RGB images, we calculated the green-red-vegetation-index (GRVI) and evaluated the time-series for its potential to track the seasonal development of the crop. The time series shows a distinct pattern during crop development that reflected the different developmental stages from germination to harvest. The simultaneous comparison to ground based assessment of phenological stages, allowed us to relate features of the airborne time series to actual events in plant growth and development. The measured GRVI values range from −0.10 (bare soil) to 0.20 (fully developed crop) and show a clear drop at time of ear pushing and ripening. Lower sowing densities were identified by smaller GRVI values during the vegetative growth phase. Additionally, we could show that the time of corn filling was strongly fixed and happened around 62 days after seeding in both years and under both density treatments. This case study provides a proof-of-concept evaluation how RGB data can be utilized to provide quantitative data in crop management and precision agriculture.

Keywords UAV · Imaging · Agriculture · Phenotyping · Precision farming · Time series

Electronic supplementary material The online version of this article (doi:10.1007/s11119-017-9504-y) contains supplementary material, which is available to authorized users.

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Introduction

With human population growing up to 9 billion in the coming decades (Godfray et al. 2010), the need for food will increase too. Nonetheless the available agricultural area is reaching its limits. At the same time agriculture has to lower its ecological impact by the efficient use of resources such as water, fertilizer and pesticides. In current research, two promising techniques are addressing this problem. 1) Phenotyping (Fiorani & Schurr 2013) is an approach to support breeding efforts by the high-throughput characterization of the behaviour of a genotype under varying growth conditions. This way the best performing crop for specific landscapes and climates can be identified. 2) Precision farming (Bongiovanni & Lowenberg-DeBoer 2004) is using accurate and current knowledge about the crops state and adapts the farm management accordingly. By precision farming, the use of resources can be minimized while maintaining the maximum yield (McBratney et al. 2005).

Both approaches, phenotyping and precision farming require precise information with a high spatial resolution in a timely manner to assess what is going on at field level. Since the areas of interest are often too large to be covered by manual field work in a feasible amount of time, remote sensing techniques could be used to gather the necessary information. However, classical remote sensing based on satellites or airplanes often fails to deliver the data in the required spatial resolution and frequency in time. Satellites often have low revisit frequency or can be blinded by clouds, while plane based remote sensing requires highly skilled people and the expensive use of aircrafts. In this context, the current evolution of unmanned aerial vehicles (UAVs) has the potential to solve these problems (Anderson & Gaston 2013). Today's small UAVs are inexpensive, easy to deploy and allow to carry a specific sensor payload over agricultural fields on a daily basis. Various studies have shown the enormous potential of the use of UAVs for agricultural purpose (Zhang & Kovacs 2012). Consumer RGB-cameras allow outstanding insights about agricultural fields if mounted on a UAV just by changing the perspective of the viewer (Bendig et al. 2013, 2014). The recent study of Rasmussen et al. (2016) demonstrates the reliable derivation of plant indices from simple UAV based RGB cameras. Various other sensor types such as thermal-, multispectral- and hyperspectral- cameras can be deployed to investigate specific plant parameters such as water stress (Zarco-Tejada et al. 2011), chlorophyll content and canopy height (Aasen et al. 2015) or pasture quality (Capolupo et al. 2015; von Bueren et al. 2015). These preliminary works have proven the capability of tracking plant parameters using UAV based sensors with different working principles and complexity. But for the accurate retrieval of current information, a very high temporal frequency of data collection is necessary to fully cover the development of a crop. By having UAV observations of agricultural fields with a high temporal frequency, the decision making can be supported in a timely manner rather than missing the critical stages in plant development. For precision farming, high resolution maps of the current stage can be generated to support the farmer's management strategy. Further, by observing field phenotyping experiments with high temporal frequency, the full growth phase can be tracked and analysed. The extraction of plant parameters or growth state from imagery has been evolving since satellite data was available for this purpose and often relies on vegetation indices (Bannari et al. 1995). Vegetation indices make use of the differences in the spectral reflection of plants and are capable of retrieving information about overall conditions (Pettorelli 2013) or even can give insight into distinct stress events (Gamon et al. 1992; Rascher et al. 2015). Simple RGB cameras, however, lack the capability to decipher narrow spectral features but have only three wide spectral bands mimicking the colour

sensitivity of the human eye. But due to the low cost, the reliability and high spatial resolution of consumer RGB digital cameras and webcams, they appear in countless studies for the monitoring of environmental and plant phenology (Jacobs et al. 2009; Julitta et al. 2014; Nijland et al. 2014; Sakamoto et al. 2012). Multiple approaches are available to calibrate, normalize and analyse this image data including the calculation of vegetation indices from the three channels red, green and blue (Casadesús et al. 2007). Among those, the green-red-vegetation-index (GRVI) also known as normalised-green-red-difference-index (NGRDI) or normalized-differentiated-greenness-index (NDGI) serves as a reliable estimator for greenness (Motohka et al. 2010; Piekarski and Zwoliński 2014; Rasmussen et al. 2016).

In this study, we evaluate the potential of analysing simple RGB-imagery over an experimental spring barley field with high temporal frequency and spatial resolution, to correlate the outcome with the actual developmental stages of the crop.

Methods

Field site and experimental layout

In 2013 and 2014, the barley (*Hordeum vulgare*) experiment was sown at Campus Klein-Altendorf research station of the University of Bonn (GPS: 50.617285, 6.990364, WGS84). Two different cultivars (Scarlett, Barke) were sown at 10 different sowing densities (Fig. 1) and five repetitions, making up a total of 100 plots. Sowing density P10 = 340 seeds/m^2 reflects the regular sowing density which varies between 320 to 340 seeds/m^2. P1 represents a greatly reduced sowing density of 24 seeds/m^2. Each plot was 1.5 m wide and 15 m long. Because of the crop rotation the actual location of the experiment changed from 2013 to 2014 as well as details of the layout. Depending on weather conditions, the

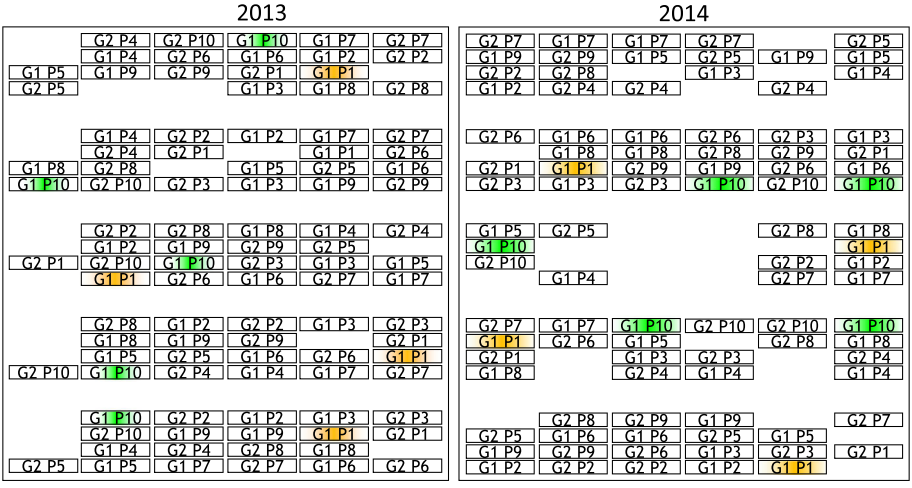

Fig. 1 Layout of the experimental field in the year 2013 and 2014. G1 = Genotype Scarlett, G2 = Genotype Barke. P1 to P10 refers to the different sowing densities with P1 = 24 seeds/m^2, and further up to P10 = 340 seeds/m^2. In the analysis a focus is set to G1P1 and G1P10 which are highlighted in *orange* and *green*. Plots that were not used in the analysis are *left* blank (Color figure online)

experiment was sown in 2013 at 25th April, in 2014 it was sown one month earlier at 20th of March. Crop cultivation followed good agricultural praxis. Plots that were harvested or damaged due to experimental practice were excluded from the analysis (Fig. 1). To avoid lodging, nitrogen fertilization was reduced (45 kg N/ha in 2013, 50 kg N/ha in 2014) and no plant growth regulator was applied. The weather station of the research station provided climate data covering both growing seasons.

UAV and camera

The UAV used during this study was a Falcon-8 (Asctec GmbH, Krailing, Germany), which proved to be a reliable flying platform throughout multiple scientific efforts (Burkart et al. 2014, 2015; von Bueren et al. 2015). The UAV was equipped with an RGB camera Sony NEX 5n (Sony Cooperation, Japan) offering a resolution of 4912 × 3264 pixels. A fixed lens with 16 mm focal length was mounted on the camera. Using this setup the whole experimental field could by covered within a single exposure from a flying altitude of 100 m above the ground (Fig. 2). The resulting ground resolution was about 20 × 20 mm per pixel. The true ground resolution however alters with aperture width, atmospheric density and ISO. The digital camera was set to shutter priority, leading to low integration times while other settings were automatically defined according to current conditions. Images were stored as JPG. The UAV was piloted manually with the camera pointing nadir. Using the live video transmission, the camera could be accurately pointed to cover the whole experiment with a single exposure. All flights were scheduled to happen around solar noon. After the flight or at consecutive days, a ground based assessment of the crop growth stage (BBCH-scale) including photography was conducted.(Lancashire et al. 1991; Meier et al. 2009). The UAV related efforts were conducted by one pilot in about 20 min per flight day, while the BBCH scoring required two investigators working for about 5 h. In 2014, prior to each flight, a set of colour references including colour sheets and green tarps was placed next to the centre of the experimental field.

Camera calibration

Other than in recent UAV based studies of agricultural surveys (Bendig et al. 2013; Berni et al. 2008) no orthomosaic was generated by stitching multiple images. By using a single image, various adverse effects that would appear in orthomosaics, such as changing ambient lights, stitching errors and averaging artefacts could be eliminated. However, the image will still suffer from a vignetting effect which introduces darker edges. This effect was characterized using the empirical method of the per pixel average of 1400 randomly chosen images that were taken by the camera-lens combination that was used in this study.

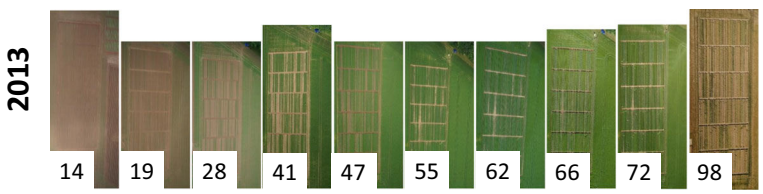

Fig. 2 Raw images as collected throughout the year 2013 over the barley experiment. The images were cut to show a similar region of interest. Numbers show the day after sowing when the image was taken. Due to slightly different flying heights and camera angles each image has different proportions and distortions

The resulting average shows the effects that are introduced by the camera and were transformed into a correction factor for each pixel of the camera (Burkart 2016). By applying this correction factor, the images of the study were corrected for the vignetting effect. To protect the camera from dirt and from degradation over the years, the lens was always covered when not in use, while the whole camera system was stored in a box.

Image processing

During each flight, 20 to 30 images of the experimental field were collected. Before image processing, the best image was chosen by evaluating consistent illumination, sharpness and visibility of the whole experiment. The raw imagery as used in this study is provided with the supplemental material. Even though it was tried to acquire all images at the same angle and position, it was not possible to achieve a perfect overlap. No artificial ground control points were placed in the field so we employed either manual or automatic feature detection in consecutive images to correctly rectify the images towards the base image. Different software suites were tested for the image correction. "Hugin" (http://hugin.sourceforge.net/) did require a high amount of manual efforts and no perfect overlap could be achieved in our tests. "ENVI" (Exelis Visual Information Solutions Inc., Boulder, US) was able to semi-automatically detect features, as soon a low amount of user set points (4–7 points) were defined. However the high error rate made manual assessment of each point necessary. If enough points (more than 25) were set with high accuracy, the polynomial rectification produced highly accurate overlaps. By using an automatic feature detection implemented in "Open Sift" (Hess 2010; Lowe 2004) pairs of following images of the time series were matched and rectified within single pixel accuracy by nonlinear distortion without altering the colour information (Fig. 3.). This approach performed almost automatic and produced highly accurate results. By applying this geometric correction step, all following image analysis could be applied to the whole stack of images of 1 year. As we compare just images, no geo-referencing nor geo-information-systems were used.

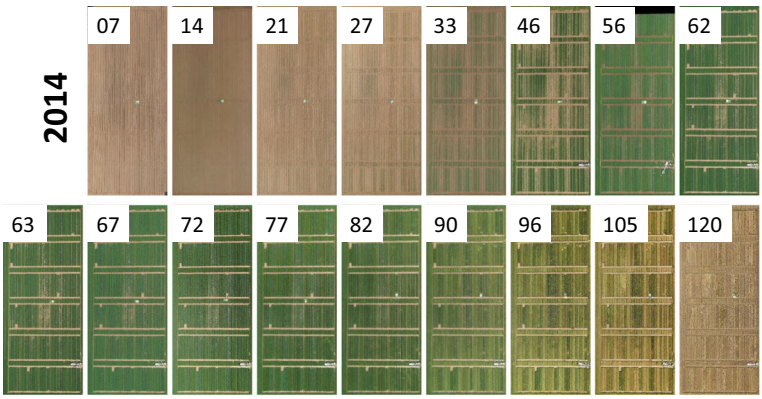

Fig. 3 Fully corrected images of the year 2014. Images were vignetting corrected, rectified and cut to show only the region of interest. Each image has the same dimensions in X and Y. In the centre of the experimental field, the reference targets are visible. The numbers show the day after sowing when the image was taken

Analysis

Each of the two image stacks from 2013 and 2014 were further analysed using the software ENVI. Using ENVI, the time series could be visualized and compared in form of data stacks with three spectral bands and multiple layers in time. Every treatment in its five repetitions was masked by a region of interest. Since other field work such as invasive sampling was done in the experiment, the regions of interest were chosen to not cover any disturbed sampling areas. Of these regions of interest, the average red and green values of the JPG imagery were extracted. In this study, we used the GRVI (Motohka et al. 2010) to compute an estimate for the greenness of each treatment using equation 1. For each flying date and treatment, we retrieved one data point that describes the current greenness. The use of this index reduces the influence from different light qualities and allows neglecting further camera calibration efforts. Using this dataset, we described the growth of the crop over the vegetation season and compared it to ground based observations.

$$GRVI = \frac{Green - Red}{Green + Red} \quad (1)$$

Results

The scoring of the barley experiment performed in 2013 and 2014 shows a normal plant development without unexpected events as shown after BBCH scale for both genotypes and all different sowing densities (Fig. 4). In 2013, the overall growth period was shifted to about 45 days later in the year, compared to 2014, which was accounted for by adapting the sowing date.

To ensure the analysis of the vegetation index is consistent over the year, despite changes in illumination or degradation of the sensor, the GRVI of the reference tarps was calculated. The GRVI of the tarps remained stable in general over the year 2014 and shows a standard deviation of SD = 0.00475 for the white reference, SD = 0.00976 for the green blanket and the highest of SD = 0.01025 was found for the green tarp (Fig. 5). The measured variation of GRVI of the plants canopy throughout the year 2014 is about 0.3 which leads to an uncertainty estimation of 3.3% of possible error in the present data. Since the references did show an almost stable retrieval of GRVI values in 2014, no further

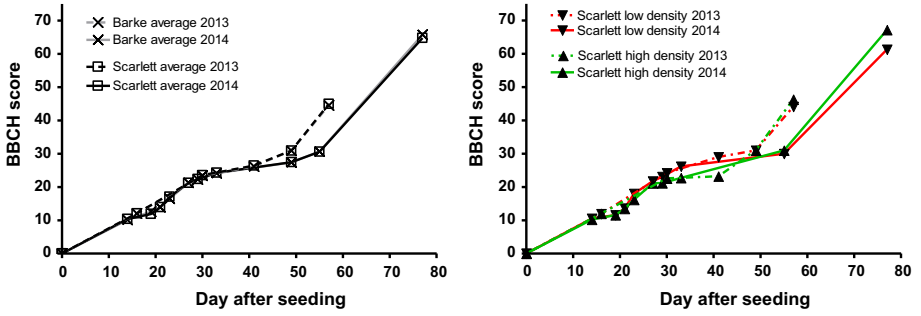

Fig. 4 *Left*: BBCH scoring of the two genotypes Scarlett (*black*) and Barke (*grey*). *Right*: BBCH scoring for the high (*green*) and low (*red*) sowing densities of the genotype Scarlett. Both figures are normalized to the day after sowing in the year 2013 (*dotted*) and 2014 (*solid*) (Color figure online)

normalization nor correction was employed. This way, the image data of 2013, not including a reference panel, could be used as well. Variations of the reference panels' results were found mostly due to their not favourable specular reflectance that changed with direct and indirect illumination. Correcting the data acquired in 2014 by the references introduces minor changes to the absolute values but does not alter the overall shape of the curves. While in 2013 levels of sowing densities corresponded well to the GRVI values between the highest and the lowest sowing densities, this does only partially apply to the year 2014. GRVI values of the sowing density G1P9 exceeded the values of G1P10 in the vegetative growth phase (Fig. 5).

Maps of GRVI (Fig. 6) show variability between plots as well as inside of one plot. Since in this analysis, we average over the total area of a plot the variation of the plot itself contributes to the GRVI value. Soil visibility and canopy colour are the main driver for the results and are discussed in the following. The development of GRVI or greenness of the experimental plots is shown in Fig. 7 for the low (P1) and high sowing densities (P10) of the cultivar Scarlett. The greenness values of the other sowing densities were found in between the highest and lowest with outliers in 2014 (Fig. 5). Further, the cultivar Scarlett does not significantly differ from Barke (Fig. 4), thus in the following, we focus only on the first cultivar. After germination, a steep increase of greenness happens until a maximum value of 0.2 is reached in both years at day 62 after sowing, when the canopy is fully closed and no soil is visible anymore. The low sowing density has a slightly smaller slope and does not reach the same maximum value of greenness. A sharp decrease of greenness to about 0.16 (0.11 for high and 0.09 for low sowing density in 2013) was measured in both cultivars and all sowing densities, within 10 days after the maximum peak. In the year 2013, after day 65, only two more data points were acquired. But in 2014, seven more data points were obtained describing a slight increase of greenness beginning at day 72, with a

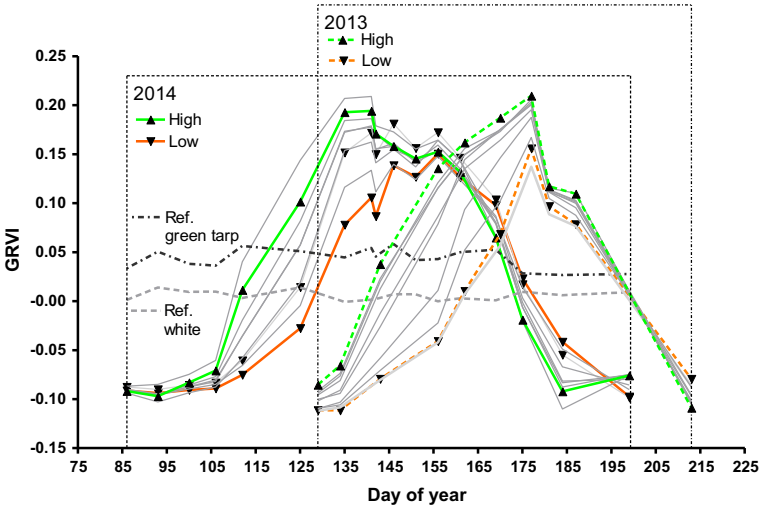

Fig. 5 Values of GRVI for all sowing densities of Scarlett shown for the years 2013 and 2014. High (G1P10—*green*) and low (G1P1—*red*) sowing densities are highlighted while the intermediate sowing densities are depicted in *grey*. The GRVI values of the *white* and *green* reference tarp that were used in 2014 are shown as *dotted line* (Color figure online)

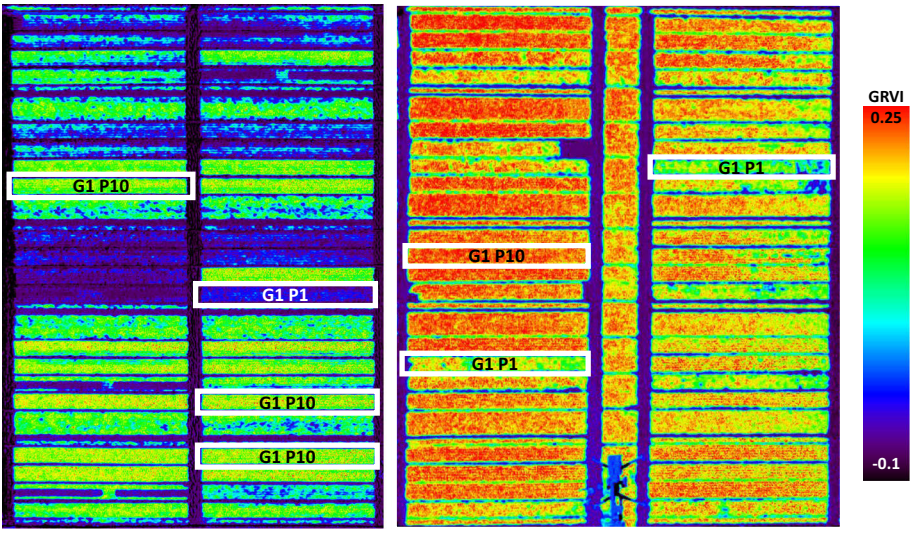

Fig. 6 Maps of GRVI of a subzone of the experimental field. The experimental plots of *Scarlett* with the sowing densities P1 and P10 are highlighted by *white* frames. *Left*: 41 days after sowing in 2013. *Right*: 62 days after sowing in 2014. The scale of GRVI is in both images between −0.1 and 0.25. For the coloured figure refer to the web version of the article

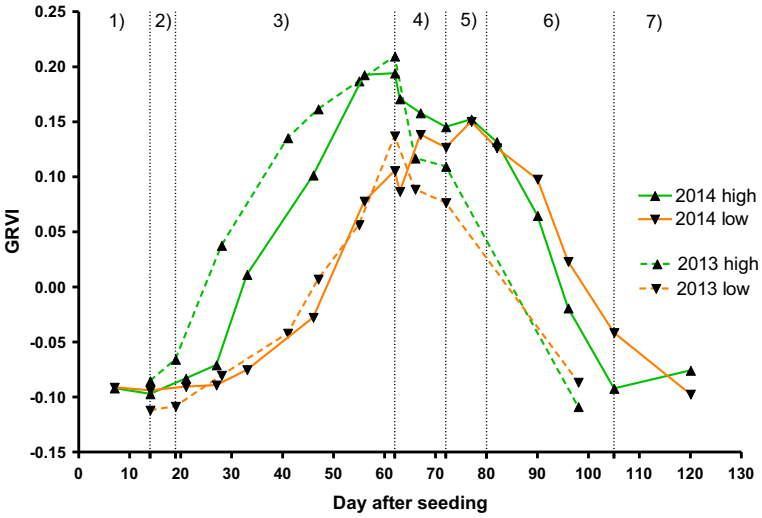

Fig. 7 GRVI development throughout the years 2013 (*dotted*) and 2014 (*solid*) for the cultivar Scarlett with the high (340 grains/m^2, *green*) and low (31 grains/m^2, *red*) sowing density. Stages of growth are numbered on the *top* and refer as follows: 1) bare soil; 2) first leaves visible; 3) vegetative growth; 4) flowering and emergence; 5) laying down of the ears; 6) ripening; 7) fully mature (Color figure online)

maximum of 0.152 at day 77. Until day 105, the greenness then decreases continuously down to a minimum of about −0.10. In the year 2014, the greenness then remains at the minimum value until harvest.

The high temporal resolution of this dataset allows the interpretation of the features seen in the graph that indicate growth stages of the actual plant development. Compared to the ground based observation of the crops by scoring and photography seven steps of crop growth could be determined which are: 1) bare soil, day 0–14; 2) first leaves visible, day 15–19; 3) vegetative growth, day 20–61; 4) flowering and ear emergence, day 62–71; 5) laying down of the ear, day 72–80; 6) ripening, day 81–104; 7) fully mature, for 2014 at day 105 while in 2013 this appears earlier from day 96 on.

First, even though the sowing dates were very different in the years 2013 and 2014, the development of greenness followed a similar pattern and shows the same features. Second, the pattern was highly similar in both cultivars and sowing densities. The treatment of different sowing densities changes the level of greenness values during the growth period of the crop until about day 65. The average difference between GRVI values of high to low sowing densities (Fig. 7) during the vegetative growth period is 0.108 in 2013 and 0.087 in 2014. After day 65, during the maturing period, the greenness levels of different sowing densities develop in a similar level. The overall pattern of the greenness development is conserved in all sowing densities, cultivars and both years.

Discussion

In this study, we have flown a UAV based camera system 28 times over experimental plots of barley, covering two vegetation seasons. By taking advantage of the high spatial resolution and unprecedented temporal frequency of UAV observations, we were able to detect distinct phenological events during the crop development. The analysis of plot greenness by GRVI is a basic approach, but already allows to identify key stages of development. The simple data processing chain is fast and appears to be robust against different illumination conditions, as shown by the evaluation of stable ground reference targets. Other than a vignetting correction no further camera calibration was employed. But the use of RAW imagery and a fully radiometrically calibrated camera could further enhance the accuracy of the data. In this study the design of the experimental field eliminated or lowered adverse optical effects, that otherwise would impact the results. By having five random repeats spread over the images, the bidirectional effects (Schaepman-Strub et al. 2006) are averaged out. In regular agricultural fields this angular effects might influence the retrieval of comparable data over the whole image, but on the other hand could be either corrected or used for further analysis of the canopy (Burkart et al. 2015; Grenzdörffer 2011). By scheduling the flights only during favourable weather conditions around solar noon, we could work around inhomogeneous illumination or varying sun angles.

The measured signal was processed to GRVI, to further enhance robustness of the uncalibrated RGB camera. Even though the GRVI is considered a rather basic indicator, its feasibility for continuous high resolution imagery is high (Motohka et al. 2010). Analysis of single or a few UAV flights as performed in various earlier studies (Aasen et al. 2015; Baluja et al. 2012; Peña Barragán et al. 2012) fail to track the vivid temporal pattern of the canopies colour change during the growth season. The high temporal resolution of our dataset is a prerequisite to observe the fast changes of canopy reflectance which cannot be detected by only a few UAV overpasses. It is further noticeable, that the pattern for both barley cultivars and both years are conserved. So it is likely that our approach can also be applied to other crops to track their seasonal development. Some differences in the

development of greenness are found in both of the years when looking at the gap between high and low sowing densities. In 2013 this gap in the vegetative phase is considerably larger while in 2014 the greenness pattern of the high sowing density is reduced (Fig. 7). Including the weather data we conclude this difference to be caused by the overall dryer and colder spring in 2014 compared to 2013. The accumulated rainfall during the first 50 days after sowing was 47.7 mm in 2014 and 142.2 mm in 2013 (Fig. 8). As consequence, the water availability could have been lower which mainly affects high sowing densities, since they have the larger water requirements. The flower and ear emergence happens, according to our data within both varieties and all sowing densities around day 62–71. Supporting our findings, genetic studies link the trait of ear emergence in vernalized spring varieties strongly to the number of photoperiods (Davidson et al. 1985). According to Wang et al. (2010) ear emergence in the variety Scarlett is observed around day 64 after sowing. In future experiments varieties with different flowering times will be tested.

In coming work, the GRVI or greenness of a crop canopy could be an output of agricultural crop models such as the "Agricultural Production Systems sIMulator" (APSIM) (Keating et al. 2003) or the "Informationssystem Integrierte Pflanzenproduktion" (ISIP) (www.isip.de) to improve and verify their prediction with UAV based remote sensing data. To foster more efforts in this direction we provide the raw data of this study as supplemental material and openly share additional material on request. Accompanying the colour information with 3D models of the canopy (Bendig et al. 2014) or hyperspectral data (Aasen et al. 2015; Burkart et al. 2014) would further enhance the quality of data that can be generated by UAVs surveying agricultural surfaces.

Crop phenotyping and precision farming follow two different approaches. While the aim of plant phenotyping is to cover the whole crop growth cycle, precision farming is interested in remote sensing data prior to a management decision. And while phenotyping happens on a relatively small spatial scale on limited experimental fields, precision farming depends on information about larger regions. So imagery could be provided for phenotyping by small flying platforms, that can be deployed on a daily basis such as copters used in this study. But even a tower or a balloon that is fixed at the experimental site and equipped with a digital camera could be feasible to constantly monitor the phenotyping fields. In precision farming, bigger areas have to be covered. Due to their inefficient way of

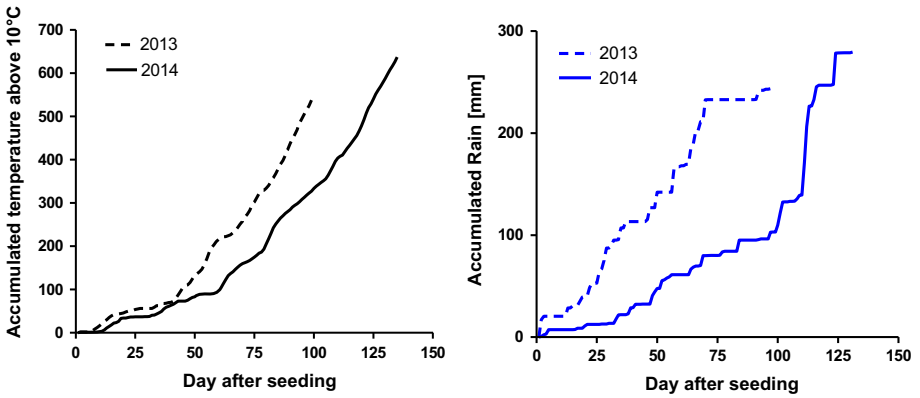

Fig. 8 *Left*: Accumulated air temperature above 10 °C. *Right*: Accumulated rainfall during the growth time. Both datasets were measured by the weather station nearby for the year 2013 (*dotted*) and 2014 (*solid*). The data is normalized in time to the day after sowing

flying, copters are outperformed by unmanned or even manned airplanes that can cover large areas in relatively short time. For the nearer future remote sensing airships (Dorrington 2007) with an endurance of several weeks could be the key technology to constantly gather information about agricultural regions, while flying below the clouds where no satellite can watch.

Integrating this remote sensing information in growth models to support decision making in precision agriculture could lead to a major benefit on optimising the use of resources while improving the ecological impact of agriculture.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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10. Talks and poster presentations

10.1 Talks

07/2015 "Sowing density: A neglected factor fundamentally affecting root distribution and biomass allocation of field grown spring barley (*Hordeum vulgare* L.)"
Hecht V.L.
University of Bonn

11/2014 „Wissenschaft an Wurzeln - von der Arbeit im Gewächshaus und im Feld"
Hecht V. L.
Talk at FZJ for members of Rotary Club

09/2014 "How planting density influences spring barley roots – results from experiments in lab and field."
Hecht V. L., Postma J. A., Temperton V., *et al.*
Talk at CSIRO Canberra, Australia

08/2014 "How planting density influences spring barley roots – results from experiments in lab and field."
Hecht V. L., Postma J. A., Temperton V., *et al.*
Talk at University of Western Australia in Perth, Australia

10.2 Poster presentations

07/2015 "Root architecture of spring barley changes in response to sowing density."
Hecht, V. L.; Postma, J. A.; Nagel, K.; *et al*
SEB conference 2015, Prague, Czech Republic, 30 Jun 2015 -3 Jul 2015

07/2014 "Do barley roots avoid neighbouring plant roots? Root growth responses of barley to planting density."
Hecht, V. L.; Postma, J. A.; Nagel, K.; *et al* SEB Conference 2014, Manchester, United Kingdom, 1 Apr 2014 - 4 Jul 2014

09/2013 "Root growth responses of barley to planting density"
Hecht, V. L.; Postma, J. A.; Temperton, V.
EUCAPRIA, NUE-CROPS, Breeding for Nutrient Efficiency, Göttingen, Germany, 24 Sep 2013 - 26 Sep 2013

03/2013 "A systematic comparison of root growth and architecture single plants and clusters"
Hecht, V. L.; Temperton, V.; Nagel, K.; *et al*
GfÖ, Arbeitskreis Experimentelle Ökologie - Workshop "Moving from the lab to the field: Putting ecology and ecophysiology in a new framework", Jülich, Germany, 18 Mar 2013 - 20 Mar 2013