



Linking wheat nitrogen use to root traits: Shallow and thin embryonic roots enhance uptake but reduce conversion efficiency of nitrogen

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ABSTRACT

Nitrogen (N) is considered quantitatively most important for crop growth and productivity. The improvement of crop N use efficiency is economically beneficial to farmers and reduces the negative environmental impact of agriculture. Root traits are promising, yet underexploited breeding targets to improve N use efficiency. We aimed to evaluate (1) the effects of genotype and environment on N use efficiency and various root architectural and anatomical traits; and (2) the relationships between root traits, N uptake and conversion efficiency. Nine spring wheat genotypes were grown on compacted and non-compacted soil during two years with contrasting weather conditions in Central Sweden. Wheat genotype and year caused considerable variation in several root and N use efficiency traits. Negative correlations were found between N uptake efficiency and N conversion efficiency; root number and diameter; root number and angle; and metaxylem number and diameter. The N uptake efficiency increased with shallower root angle, higher root number, smaller root diameter, higher metaxylem number and smaller metaxylem diameter; whilst N conversion efficiency showed the opposite pattern. We conclude that a negative relationship observed between N uptake efficiency and N conversion efficiency can be linked to trade-offs between embryonic root traits.

1. Introduction

Nitrogen (N) is considered quantitatively most important for increasing crop productivity, although other nutrients can significantly (co-)limit crop growth and productivity (Reich et al., 2014; Weih et al., 2021). Nitrogen fertilization is widely used to enhance crop yield and quality across many regions of the world. However, N fertilization has environmental impacts through nitrate leaching to surface water and groundwater, and nitrate conversion into nitrous oxide by denitrifying soil bacteria (Xu et al., 2012; Huang et al., 2017). The improvement of crop N use efficiency will therefore help to reduce the environmental impact of agriculture (Foulkes et al., 2009; Xu et al., 2012).

Nitrogen use efficiency can be assessed with various approaches. A discussion of different methodologies for assessing N use efficiency and its components is found elsewhere (Weih et al., 2018). In this study we use the N accumulation efficiency concept (Weih et al., 2011, 2018), which divides the overall N accumulation efficiency into N uptake efficiency (the ratio between the mean N amount in plant during the entire growth period and the N amount in the initial seed), grain-specific N

efficiency (also called N conversion efficiency; the ratio between the grain yield and the mean plant N amount during the entire growth period) and grain N concentration. A negative relationship (possibly indicating a trade-off) between N uptake efficiency and N conversion efficiency has been reported previously and seems to be common, but the underlying mechanisms are often unclear (Maire et al., 2009; Weih et al., 2018).

Root traits are promising yet underexploited breeding targets to improve N use efficiency (Lynch, 2019). Root architectural traits such as a steeper main root angle, which indicates a deeper root system, are advantageous for accessing subsoil N pools especially under N-limiting conditions (Lynch, 2013, 2019; Saengwilai et al., 2014b; Gao and Lynch, 2016). Root anatomical traits such as cortical aerenchyma formation are linked to the metabolic costs of root construction and maintenance, and greater cortical aerenchyma is associated with enhanced N acquisition (Saengwilai et al., 2014a; Klein et al., 2020). The investigation of multiple root traits that affect soil resource acquisition and transport may reveal synergisms (i.e., greater than the additive effects of individual traits) and trade-offs among them (Lynch, 2019). For example, the

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combination of large cortical cell sizes and few cortical cell files was reported as a synergistic strategy to decrease the metabolic cost of root exploration (Klein et al., 2020). However, due to resource limitation of growth processes and cost-benefit considerations, certain trait combinations are less likely to occur, leading to the frequent observation of negative associations between two or more traits (Stearns, 1989). For example, trade-offs can be expected to occur between fewer thick and numerous thin roots, that is between root number and diameter (Kadam et al., 2015; Wen et al., 2019; Klein et al., 2020).

Plant performance is controlled by its genotype (G), the growing conditions (environment, E) and the interactions between them (G x E). Individual genotypes may respond differently to growing conditions, and interactions between genotype and environment may occur. Knowledge about the variation in traits related to N use efficiency among genotypes, and differences in performance in different environments (G x E) is highly desired for breeding.

With spring wheat as a model plant, we aimed to evaluate (1) the effects of genotype and environment on N use efficiency and various root architectural and anatomical traits; and (2) the relationships between root traits, N uptake and N conversion efficiency. To address the aims, nine spring wheat genotypes were grown on compacted and non-compacted soils during two growing seasons with contrasting weather conditions in Central Sweden. We hypothesized that a negative relationship between N uptake efficiency and N conversion efficiency is correlated with trade-offs, or negative relationships, between root traits; e.g., between root number and diameter.

2. Materials and methods

2.1. Site description

A field trial was carried out during the 2018 and 2019 growing seasons in Uppsala, Sweden (59° 45' N, 17° 42' E) with a boreal-temperate climate. The months May, June and July (i.e., from the germination to the grain-filling of spring wheat) in 2018 were warmer and drier than the corresponding long-term means; whilst May, June and July in 2019 were cooler and wetter than in 2018 (Weih et al., 2021). Artificial irrigation of about 10 mm of water was applied at 28 and 34 days after sowing (i.e., during stem elongation) due to an extended dry period in 2018.

2.2. Plant material and experimental design

Nine spring wheat genotypes were used in this study: the cultivars 'KWS Alderon' ('Alderon', Germany, KWS W185), 'Bjarne' (Sweden, NK 97520), 'Boett' (Sweden, SW 71034), 'Dacke' (Sweden, W 26267), 'Diskett' (Sweden, SW 45456), 'Happy' (Sweden, SW 91003), 'Quarna' (Switzerland, CH 21112283), 'Rohan' (Sweden, SW 01198), and a landrace originating in Dalecarlia ('Dala', Sweden). The cultivars are commonly grown in northern Europe, and have been shown previously to considerably vary in root architectural and anatomical traits at early growth stage (Colombi et al., 2019; Liu et al., 2021).

The field trial had a split-plot design with four replicates. Main plots were assigned to two treatments, soil compaction and non-compaction; and subplots (2 m x 12 m) were assigned to the nine wheat cultivars. The soil compaction treatment was conducted by double track-by-track passing using a front loader with four wheels and an average wheel load of 42 kN in April 2018. Afterwards, soil surface was loosened to a depth of approximately 50 mm with a tine harrow before sowing. The details of the compaction procedure and the effects of the compaction treatment on soil parameters are presented elsewhere (Liu et al., 2022). Seeds with a rate of 550 seeds m⁻² were sown on 10th May 2018. At sowing, 140 kg ha⁻¹ of N, 24 kg ha⁻¹ of P and 46 kg ha⁻¹ of K were applied. In 2019, the same site was used, and the soil was re-compacted using the same method whilst the position of the genotype plots was re-randomized. Seeds were sown and fertilization was applied on 23rd

April 2019.

2.3. Soil sampling and analyses

Fourteen days after sowing, soils (0–0.3 m depth) from each subplot along a transect across the eight main plots were sampled using an auger. The soils from each main plot were then mixed together, and sub-samples were taken for the measurement of pH, total N and organic carbon (Table 2). Soil pH was determined using a pH meter (SS-ISO10390). Total N and organic carbon were analyzed by dry combustion with a LECO CNS/2000 analyzer using a standard method (SS-ISO13878 and SS-ISO10694). The soil data are presented and discussed in detail by Liu et al. (2022).

At crop flowering (BBCH 65), soil cylinders of 72 mm diameter and 50 mm height were sampled at 0.1 and 0.3 m depths from four randomly-selected subplots in each main plot. Soil cylinder samples were oven dried at 105 °C for 72 h to measure soil bulk density. The soil compaction treatment performed in this study increased soil bulk density and had similar effects in the two years (Table 2). Volumetric soil water content was continuously recorded using soil moisture probes (5TM, Decagon Devices, Pullman, USA) from crop germination until maturity. The moisture probes were placed at 0.1 and 0.3 m depths in four out of eight main plots, and within each main plot in the plots of one representative genotype ('Diskett'), because we had no reason to expect significant genotype-specific effects on soil moisture. Data were recorded in 30-minutes intervals using data loggers (Em50, Decagon Devices, Pullman, USA). The dynamics of soil moisture, and the impacts of soil compaction and weather condition on it is described in detail by Liu et al. (2022).

2.4. Assessment of nitrogen use efficiency

The BBCH-scale (Lancashire et al., 1991) was used for defining the developmental stages of the wheat plants. Shoots within two 0.5 m x 0.5 m areas in each plot were sampled at the beginning of stem elongation (BBCH 30), flowering (BBCH 65) and maturity (BBCH 89). The shoots were harvested at approximately 15 mm above the soil surface, oven-dried at 65 °C for 72 h. Plant samples were ground in a stainless steel grinder to pass a 1-mm mesh, then the N concentrations of sown seeds, shoots and grains of five representative plants sampled in each of the 0.5 m x 0.5 m plots were analyzed on a LECO CNS/2000 analyzer using a standard method (SS-ISO13878). Seed and shoot N contents were obtained by multiplying the N concentrations with the corresponding biomasses. The mean plant N content in the biomass during the entire growth period (N') was calculated based on the seed N contents (assuming 550 seeds m⁻²) and the above ground plant N contents at different developmental stages (BBCH 30, 65, 89), by taking into account the different lengths of the corresponding periods of time. Thus, N' was calculated based on the periods between sown seed and stem elongation, stem elongation and flowering, and flowering and maturity. To assess grain yield, the central plot area (2 m x 6 m) was harvested at fully ripe (BBCH 89) with a combine harvester on 17th August 2018 (i.e., 99 days after sowing) and 23rd August 2019 (i.e., 122 days after sowing), respectively. The large difference in harvesting time between years was due to the large difference in weather conditions between the two study years (Table 1).

The N use efficiency was assessed in terms of the N accumulation efficiency (NAE) concept, where NAE is the product of N uptake efficiency, N conversion efficiency (also called grain specific N efficiency in the original publication) and grain N concentration (Weih et al., 2011). The N uptake efficiency and N conversion efficiency were calculated according to the following equations (Weih et al., 2018):

$$\text{N uptake efficiency} = \text{N}' / \text{N amount in initial seed} \quad (1)$$

$$\text{N conversion efficiency} = \text{grain yield} / \text{N}' \quad (2)$$

Table 1

Weather conditions during the experimental periods of the years 2018 and 2019, and the corresponding long-term (1896–2019) means. All data were collected at the Ultuna climate station near Uppsala, located 3 km south-west from the experimental site.

	Temperature (°C)			Precipitation (mm)			Days with precipitation of at least 1 mm (d)	
	2018	2019	1896–2019	2018	2019	1896–2019	2018	2019
April	6.0	6.6	3.9	36.3	3.6	30.7	8	1
May	15.3	10.2	9.8	6.7	59.8	35.7	2	12
June	16.3	17.6	14.4	20.7	25.7	50.2	3	7
July	21.6	16.6	16.8	81.7	60.3	66.2	2	8
August	17.8	17.1	15.2	68.7	65.8	71.0	11	9

Table 2

Soil properties at the experimental site in the years 2018 and 2019.

Soil properties	2018	2019
pH (H ₂ O) in 0–0.3 m depth	5.8	5.7
Total nitrogen (g kg ⁻¹) in 0–0.3 m depth	2.4	2.3
Total organic carbon (g kg ⁻¹) in 0–0.3 m depth	25.9	24.7
Bulk density at 0.1 m depth under non-compacted treatment (g cm ⁻³)	1.35	1.31
Bulk density at 0.1 m depth under compacted treatment (g cm ⁻³)	1.49	1.53
Bulk density at 0.3 m depth under non-compacted treatment (g cm ⁻³)	1.51	1.51
Bulk density at 0.3 m depth under compacted treatment (g cm ⁻³)	1.58	1.63

2.5. Root sampling

Root crowns were sampled at the beginning of stem elongation (BBCH 30) to characterize various architectural and anatomical traits. Four representative plants were selected from 0.5 m x 0.5 m areas at both ends of each plot. Following the shovelomics approach (Trachsel et al., 2011), the topsoil root system of these plants were excavated with a shovel to a depth of approximately 0.2 m and washed. All four root crowns were included for architectural measurements and two of them were selected for anatomical analyses. The numbers of embryonic roots were counted. The angle between the outermost embryonic roots and the soil surface were measured along an arc with a 50 mm radius (Colombi and Walter, 2016). Hence a low root angle depicted a shallow embryonic root system, while a high root angle referred to a steep embryonic root system. To assess the anatomical traits, 3 cm-long samples of embryonic roots were taken 3 cm from the root bases. For each plot, samples from two embryonic roots from two different plants were studied for the quantification of anatomical traits. These samples were preserved in 50% ethanol and stored at 4 °C in darkness until further measurement. Root cross sections of around 150 µm thickness were manually cut from each individual root sample with a razor blade and stained with Toluidine Blue (0.1% in distilled water) for 1 min. Cross sections were imaged at a resolution of 8 megapixel at 100 × magnification using a digital microscope camera (Mirazoom MZ808, Owl Tech Limited, Hong Kong, China), which was connected to a bright field microscope (Kern Optics OBF 122, Kern & Sohnb GmbH, Balingen, Germany; Objective: 10 × magnification, 0.25 numerical aperture). The cross-sectional areas of the root and the cortex, the aerenchyma, the radial diameter of metaxylem vessel were assessed manually in ImageJ version v 1.52 t (National Institute of Health, Bethesda, MD, USA). Moreover, the diameter of 15 cortical cells across cortical cell files (excluding epi- and endodermal cells) was measured to determine average cortical cell diameter.

2.6. Statistical analyses

All statistical analyses were performed using R version 4.0.0 (R Core Team, 2020). Linear mixed-effects models were used to study the effects of genotype, soil compaction treatment, year and their interactions on the variation in N use efficiency traits and root traits with the 'lme4'

package (Bates et al., 2015). Year, soil compaction treatment, genotype and their interactions were set as fixed effects, and block, main-plot and plot as random effects. Effect sizes (η^2) were calculated with the 'sjstats' package (Lüdtke and Lüdtke, 2017). Correlation analysis and linear regression analyses were used to assess the relationships among N use efficiency traits and root traits.

3. Results

3.1. Variation in nitrogen use efficiency traits

Nitrogen uptake efficiency, N conversion efficiency and grain N concentration were significantly affected by genotype, year, and their interaction (Table 3, S1). No significant main effects of soil compaction on any of the N use efficiency traits were found, but the N uptake efficiency was significantly affected by the interaction between soil compaction and year (i.e., the compaction treatment increased N uptake efficiency in 2018 but decreased it in 2019, Table 3). The effect sizes of genotype were large ($\eta^2 \geq 0.49$) for all the three N use efficiency traits; whilst the effect sizes of the interactions between genotype and environment (here represented by the compaction treatments and year) were mostly low ($\eta^2 < 0.08$), except for the genotype by year interaction for N uptake efficiency ($\eta^2 = 0.17$). The year-to-year variation in weather generated large effect sizes for grain N concentration ($\eta^2 = 0.34$). More specifically, N uptake efficiency and grain N concentration were lower, whilst N conversion efficiency was higher in the dry year 2018 than the relatively wet year 2019 (Table 3).

3.2. Variation in root traits

Root angle, root number and metaxylem diameter varied significantly among the genotypes, but were not significantly affected by the interactions between genotype and environment (Table 3, S1). The other root traits (i.e., root diameter, cortical cell diameter and cell file number, aerenchyma percent of cortex and metaxylem number) were not significantly affected by neither genotype nor the interactions between genotype and environment. The effect sizes of genotype were large ($0.14 \leq \eta^2 \leq 0.46$) for root angle, root number and metaxylem diameter. The year-to-year variation in weather generated large effect sizes for root angle ($\eta^2 = 0.28$), and medium effect sizes for root number and diameter, metaxylem number and diameter ($0.09 \leq \eta^2 \leq 0.13$). The compaction treatments had a significant effect on two root traits, i.e., root diameter and cortical cell diameter, both with an effect size of 0.13. More specifically, embryonic roots were steeper, fewer and thicker in the dry year 2018 than the relatively wet year 2019, and metaxylem vessels were wider and fewer in 2018 than 2019 (Table 3). Compared to the non-compacted treatment, the compacted treatment increased root diameter and cortical cell diameter in both years (Table 3).

3.3. Relationships among nitrogen use efficiency and root traits

When data were analyzed across all genotypes, treatments and years, significantly negative relationships were found between N uptake efficiency and N conversion efficiency, root number and diameter, root

Table 3

Results from ANOVA and corresponding effect sizes (η^2) for year (df = 1), soil compaction treatment (df = 1), genotype (df = 8) and their interactions on root growth and nitrogen (N) use efficiency of nine spring wheat genotypes field-grown in Central Sweden during two years under two treatments; and the mean values across the nine wheat genotypes for each treatment (compacted and non-compacted) and year (2018 and 2019). ***, ** and * denote significant effects at $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively, n.s. denotes non-significant effects ($n = 4$).

Trait (unit)	ANOVA								Treatment mean			
		Genotype (G)	Year (Y)	Treatment (T)	Y \times T	G \times Y	G \times T	G \times Y \times T	2018 compacted	2018 non-compacted	2019 compacted	2019 non-compacted
N uptake efficiency (g g ⁻¹)	P	***	***	n.s.	***	***	n.s.	n.s.	7.71	6.77	11.88	12.30
	η^2	0.493	0.075	0.004	0.056	0.170	0.012	0.022				
N conversion efficiency (g g ⁻¹)	P	***	**	n.s.	n.s.	***	n.s.	n.s.	56.34	57.10	47.17	46.24
	η^2	0.578	0.112	0.000	0.002	0.073	0.019	0.020				
Grain N concentration (g g ⁻¹)	P	***	***	n.s.	n.s.	***	n.s.	n.s.	0.025	0.025	0.031	0.031
	η^2	0.502	0.337	0.006	0.001	0.061	0.005	0.007				
Embryonic root angle (°)	P	***	***	n.s.	n.s.	n.s.	n.s.	n.s.	31.92	34.53	23.72	26.35
	η^2	0.141	0.279	0.029	0.000	0.035	0.026	0.021				
Embryonic root number (-)	P	***	**	n.s.	n.s.	n.s.	n.s.	n.s.	4.89	4.63	5.52	5.13
	η^2	0.460	0.120	0.038	0.001	0.014	0.010	0.025				
Embryonic root diameter (μm)	P	n.s.	*	**	*	n.s.	n.s.	n.s.	471.3	413.3	421.6	397.7
	η^2	0.054	0.088	0.129	0.024	0.031	0.027	0.021				
Metaxylem diameter (μm)	P	***	*	n.s.	n.s.	n.s.	n.s.	n.s.	46.73	44.19	42.22	40.87
	η^2	0.191	0.103	0.017	0.003	0.037	0.051	0.021				
Metaxylem number (-)	P	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	1.26	1.35	1.50	1.75
	η^2	0.032	0.127	0.037	0.003	0.039	0.048	0.059				
Cortical cell diameter (μm)	P	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	31.45	26.78	28.04	24.48
	η^2	0.084	0.032	0.126	0.002	0.042	0.029	0.030				
Cortical cell file number (-)	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	3.58	3.43	3.47	3.42
	η^2	0.032	0.002	0.008	0.002	0.025	0.041	0.059				
Aerenchyma percent of cortex (%)	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	1.91	1.67	1.68	1.82
	η^2	0.000	0.000	0.037	0.027	0.056	0.030	0.051				

number and root angle, as well as metaxylem number and diameter (Fig. 1). Thus, N uptake efficiency and grain N concentration increased with shallower root angle, higher root number and smaller root diameter; whilst N conversion efficiency showed the opposite pattern (Fig. 2). Nitrogen uptake efficiency and grain N concentration increased with higher metaxylem number and smaller metaxylem diameter; whilst N

conversion efficiency showed the opposite pattern (Fig. 3). Nitrogen uptake efficiency, N conversion efficiency and grain N concentration were not significantly correlated with the other anatomical traits investigated (i.e., aerenchyma area, cortical cell diameter and cell file number; Supplementary Fig. S1).

When data were assessed across the nine genotypes within each

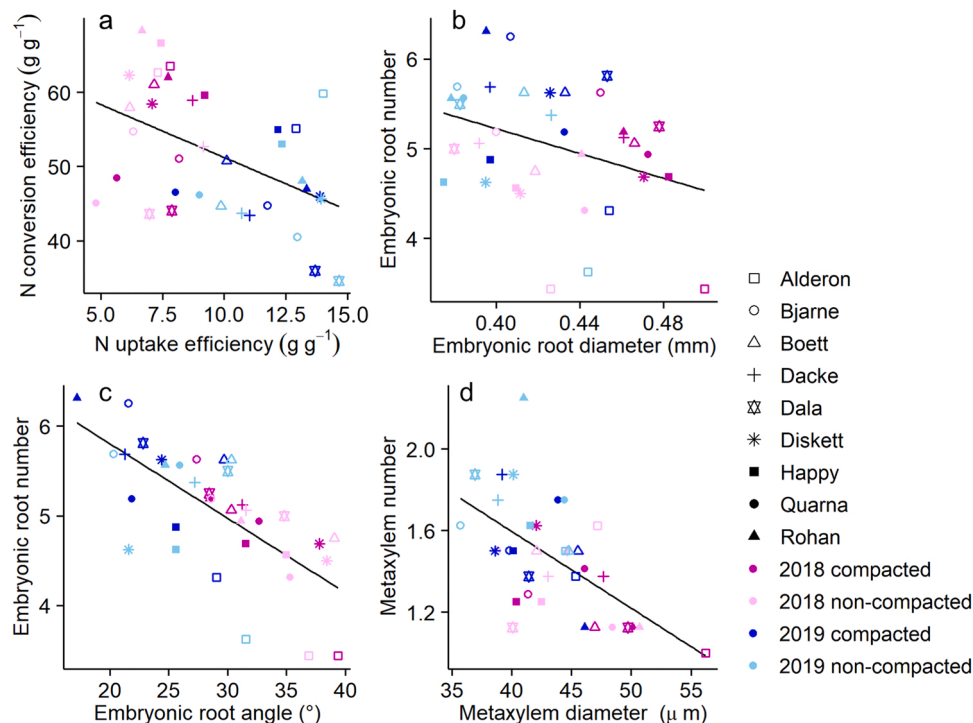


Fig. 1. Linear regressions between root traits at the beginning of stem elongation, and nitrogen (N) use efficiency of nine wheat genotypes grown under two soil compaction treatments during the years 2018 and 2019 in Central Sweden. Individuals are the mean values of four blocks for each genotype under each soil compaction treatment in each year. Statistics: (a) $r^2 = 0.23$, $p = 0.003$; (b) $r^2 = 0.12$, $p = 0.037$; (c) $r^2 = 0.48$, $p < 0.001$; (d) $r^2 = 0.35$, $p < 0.001$.

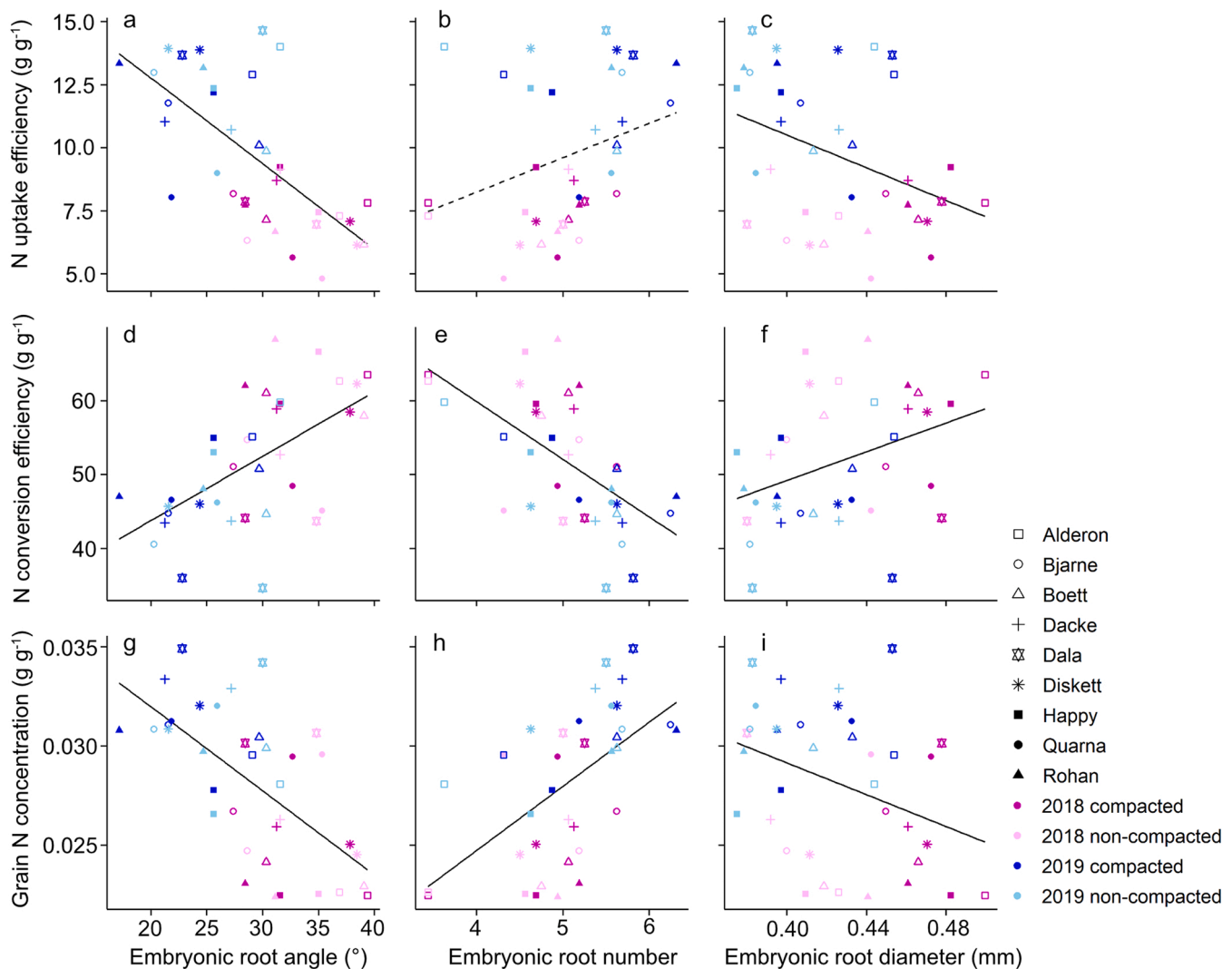


Fig. 2. Linear regressions between root architectural traits at the beginning of stem elongation, and nitrogen (N) use efficiency for nine wheat genotypes grown under two soil compaction treatments during the years 2018 and 2019 in Central Sweden. Individuals are the mean values of four blocks for each genotype under each soil compaction treatment in each year. Statistics: (a) $r^2 = 0.45$, $p < 0.001$; (b) $r^2 = 0.10$, $p = 0.057$; (c) $r^2 = 0.15$, $p = 0.021$; (d) $r^2 = 0.34$, $p < 0.001$; (e) $r^2 = 0.38$, $p < 0.001$; (f) $r^2 = 0.15$, $p = 0.019$; (g) $r^2 = 0.41$, $p < 0.001$; (h) $r^2 = 0.34$, $p < 0.001$; (i) $r^2 = 0.13$, $p = 0.029$. Dash line indicates non-significant relationship.

treatment and year, N conversion efficiency significantly increased with decreasing root number and greater metaxylem diameter under the non-compacted treatment in 2019 (Supplementary Fig. S2). The genotypic variation in the other root traits was not significantly correlated with any of the N use efficiency traits.

4. Discussion

This study mainly focused on early root growth and its influence on the main aspects of N use efficiency (i.e., N uptake efficiency and N conversion efficiency) of spring wheat field-grown during two growing seasons with contrasting weather. By studying nine wheat genotypes exposed to various environmental conditions, we found considerable variation in root architectural and anatomical traits, and identified several of them to be likely linked to the negative relationship between N uptake efficiency and N conversion efficiency.

4.1. Nitrogen uptake efficiency was influenced by root architectural traits

It has been shown previously for post-embryonic roots that steep angle and low number of roots can be used as indicators for a deep root

system, which is beneficial for accessing mobile nutrients that quickly move through the soil profiles and become more concentrated at greater depth, such as N (Lynch, 2013, 2019; Gao and Lynch, 2016; Schneider et al., 2021). Contrary to the above studies, shallower root angle and higher quantity of embryonic roots (i.e., shallower embryonic root systems) were associated with greater N uptake efficiency in our study. Fertilization was applied in our study at sowing and concentrated in the topsoil early in the growing season. This could have facilitated large N uptake by a great number of shallow-angled embryonic roots early in the growing season as proposed by Lynch (2013). The effect size of genotype for root number was relatively high in comparison to the effect sizes of the environmental variables (compaction treatment and year) in our study (Table 3). This suggests that the genetic difference explains more of the phenotypic variation in this trait than the difference in the growing conditions. The effect size of year was higher than the effect size of genotype for root angle in our study (Table 3), suggesting that the angle of the embryonic root system is more dependent on the environmental conditions than genotype. Other studies have shown moderate to high heritability for root number and angle (Sanguineti et al., 2007; Christopher et al., 2013; Richard et al., 2015; Colombi and Walter, 2017).

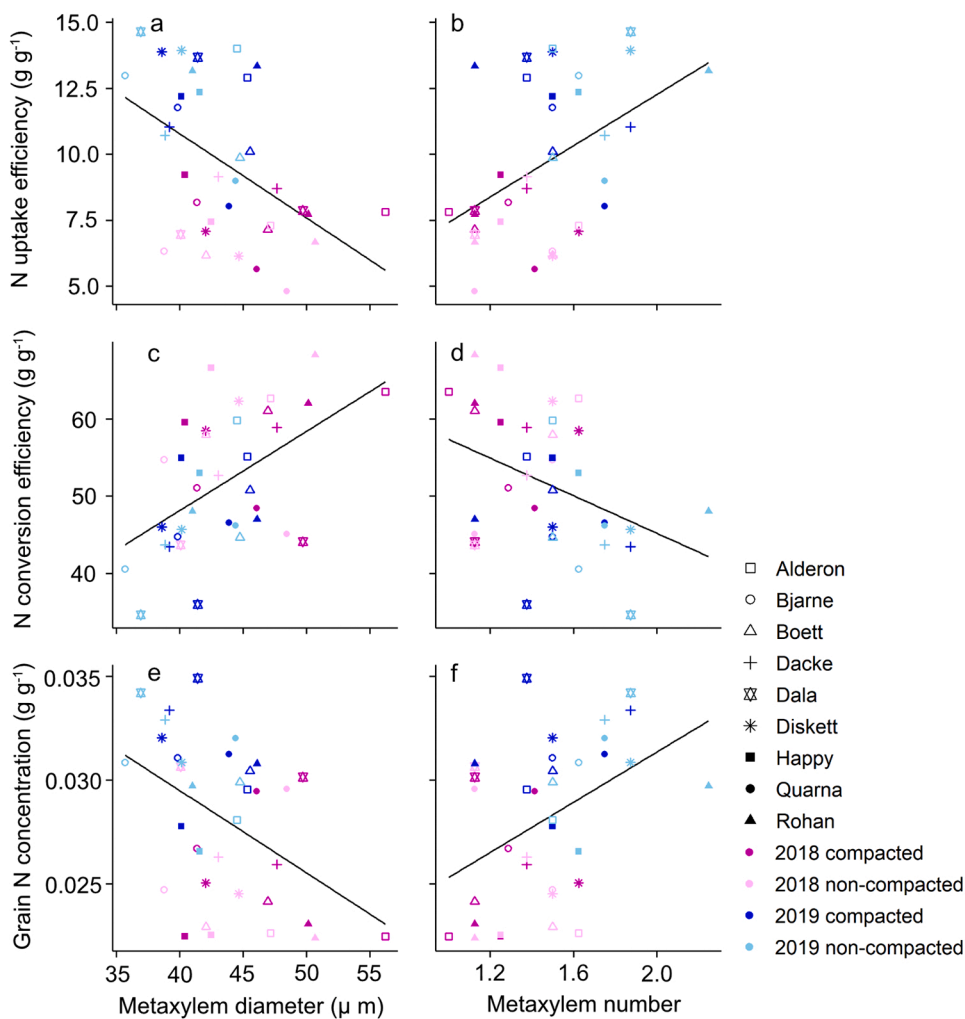


Fig. 3. Linear regressions between root xylem vessel traits at the beginning of stem elongation, and nitrogen (N) use efficiency of nine wheat genotypes grown under two soil compaction treatments during the years 2018 and 2019 in Central Sweden. Individuals are the mean values of four blocks for each genotype under each soil compaction treatment in each year. Statistics: (a) $r^2 = 0.23$, $p = 0.003$; (b) $r^2 = 0.21$, $p = 0.005$; (c) $r^2 = 0.27$, $p = 0.001$; (d) $r^2 = 0.15$, $p = 0.019$; (e) $r^2 = 0.21$, $p = 0.005$; (f) $r^2 = 0.19$, $p = 0.008$.

Soil compaction has been shown previously to result in reduced root number (Grzesiak et al., 2002; Colombi and Walter, 2017) and steeper root angle (Correa et al., 2019). In the field where our study was conducted, soil compaction increased soil moisture and decreased soil penetration resistance in the drier year 2018; whilst the same treatment decreased soil moisture and increased soil penetration resistance in the wetter year 2019 (Liu et al., 2022). The lack of clear effects of soil compaction on root number and angle in this study is presumably due to the interaction effects of weather and soil physical conditions described by Liu et al. (2022). We also expected soil compaction to negatively affect N uptake efficiency, but the results documented by Liu et al. (2022) suggest a more complex pattern due to the interaction between the compaction treatment and the contrasting weather conditions in the two years studied, which also may have affected the pattern in N uptake efficiency. Thus, soil compaction increased N uptake efficiency in 2018 but decreased it in 2019 (see the significant Year x Treatment effect in ANOVA, Table 3), along with a similar pattern seen in soil moisture. Soil moisture was therefore apparently a strong driver of N uptake efficiency in our study.

4.2. Nitrogen uptake efficiency was associated with root xylem traits but not cortex traits

The capacity for axial transport of water and nutrients through a root system is largely determined by the size and number of xylem vessels; and decreased, constant or increased size and number of xylem vessels in response to drought have been reported previously for wheat, barley,

rice and maize (Kadam et al., 2015; Klein et al., 2020; Oyiga et al., 2020). In our study, the decreasing soil moisture (Liu et al., 2022) indicates that the availabilities of water and N to plants were decreasing from the beginning towards the end of each growing season. Under such conditions, thinner and more numerous metaxylem vessels are expected to improve N uptake efficiency, as was seen in our study (Fig. 3), by restricting net N uptake early in the growing season and thereby increasing N uptake during later growth. Similarly, thinner and more numerous metaxylem vessels have been reported previously to improve water use efficiency under terminal drought (Zaman-Allah et al., 2011; Feng et al., 2016). The effect sizes of both genotype and year on metaxylem diameter were relatively high in our study (Table 3), supporting the elsewhere reported finding of moderate heritability for this trait (Oyiga et al., 2020).

Root cortical aerenchyma formation has often been found to be increased by edaphic stress, including hypoxia, drought and suboptimal availability of N (Jackson et al., 1985; Saengwilai et al., 2014a; Klein et al., 2020). However, aerenchyma formation was not responsive to soil compaction or drought in this study, probably because the effects of soil compaction and drought on soil properties were still relatively mild at the beginning of the growing season (Liu et al., 2022). In maize, a more intense aerenchyma formation, larger cortical cell sizes and fewer cortical cell file numbers have been regarded as promising breeding targets, because they could reduce the metabolic costs of soil exploration by decreasing root respiration, and improve water and N capture under drought and N stress (Lynch et al., 2021). In contrast to Lynch et al. (2021), root aerenchyma, cortical cell sizes and cell file numbers were

not associated with any of the N use efficiency traits in our study using spring wheat. In addition, no significant difference was found among the studied genotypes for the above mentioned root traits (Table 3), whilst other studies have shown moderate to high heritability for them in maize and durum wheat (Nazemi et al., 2016; Schneider et al., 2020). The potential of these root traits to be used as candidate traits in wheat breeding therefore needs to be further investigated.

4.3. Is the negative relationship between nitrogen uptake efficiency and nitrogen conversion efficiency linked to trade-off(s) between root traits?

A negative relationship between N uptake efficiency and N conversion efficiency has often been found (Lambers and Oliveira, 2008; Maire et al., 2009; Weih et al., 2018), whilst the mechanistic basis behind is unclear. With increasing N uptake by roots, leaf N concentration increases but the photosynthetic rate per unit N decreases (Lambers and Oliveira, 2008). Moreover, with increasing N availability, plant growth and productivity are often increasingly co-limited by nutrient elements other than N (Reich et al., 2014; Weih et al., 2021). In our study, the inverse relationship between N uptake efficiency and N conversion efficiency closely reflected the negative relationships between some of the root traits investigated (i.e., root number and diameter, root number and angle, as well as metaxylem number and diameter; Fig. 1). It is therefore possible that the negative association between N uptake efficiency and N conversion efficiency, as observed in this study and by others (Lambers and Oliveira, 2008; Maire et al., 2009; Weih et al., 2018), is partly caused by the trade-offs between root traits (i.e., root number and diameter, root number and angle, metaxylem number and diameter) observed already at an early growth stage in our study. Physiological trade-offs are invoked by physiological links between two or more traits, which means that the benefit obtained through one physiological process entails a restraint for another process (Stearns, 1989); examples for physiological trade-offs are probably the negative associations between some of the root traits (e.g., root number and diameter) observed in our study. Genetic trade-offs are based on antagonistic pleiotropy among genes controlling different traits or gene linkage (Roff, 1993), and the genetic links between traits could possibly be eliminated by modern plant breeding technology (Weih, 2003). On the one hand, if the negative association between N uptake efficiency and N conversion efficiency is mechanistically linked to the physiological trade-offs between the above root traits, it is unlikely to be overcome by breeding, because physiological trade-offs cannot be eliminated even with modern plant breeding technology (Weih, 2003). On the other hand, if the negative association between N use efficiency aspects is linked to the trade-offs between root traits, the link provides an opportunity for breeding, because the root traits investigated here could then be used in the breeding to modify N use efficiency aspects. Further investigations are needed to verify the generality of our findings regarding the links between the N use efficiency and root traits, applying different environmental conditions and using different germplasm. For example, it will be interesting to see whether germplasm could be identified combining high N uptake efficiency and high N conversion efficiency, along with a large quantity of thick embryonic roots.

5. Conclusion

A high number of thin embryonic roots was associated with a high N capture. Our results support our hypothesis that trade-offs between embryonic root traits (e.g., root number and diameter, root number and angle, metaxylem number and diameter) are linked to an overall negative relationship between N uptake efficiency and N conversion efficiency seen in wheat and many other plants. Root architectural and anatomical traits are likely to be inherited independently of carbon allocation traits, which offers an opportunity for using root number and angle, or metaxylem number and diameter as selection criteria in the breeding towards improved N uptake efficiency. Our results suggest that

any selection or breeding targeting root architectural traits in conjunction with N use efficiency traits needs to consider the temporal dynamics of soil N and water conditions, which can vary greatly between different locations and years.

CRedit authorship contribution statement

Hui Liu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Tino Colombi:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **Ortrud Jäck:** Conceptualization, Investigation, Methodology, Supervision, writing – review & editing. **Anna Westerbergh:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Martin Weih:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data supporting the finding of this manuscript are available on reasonable request to the corresponding author.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108603.

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