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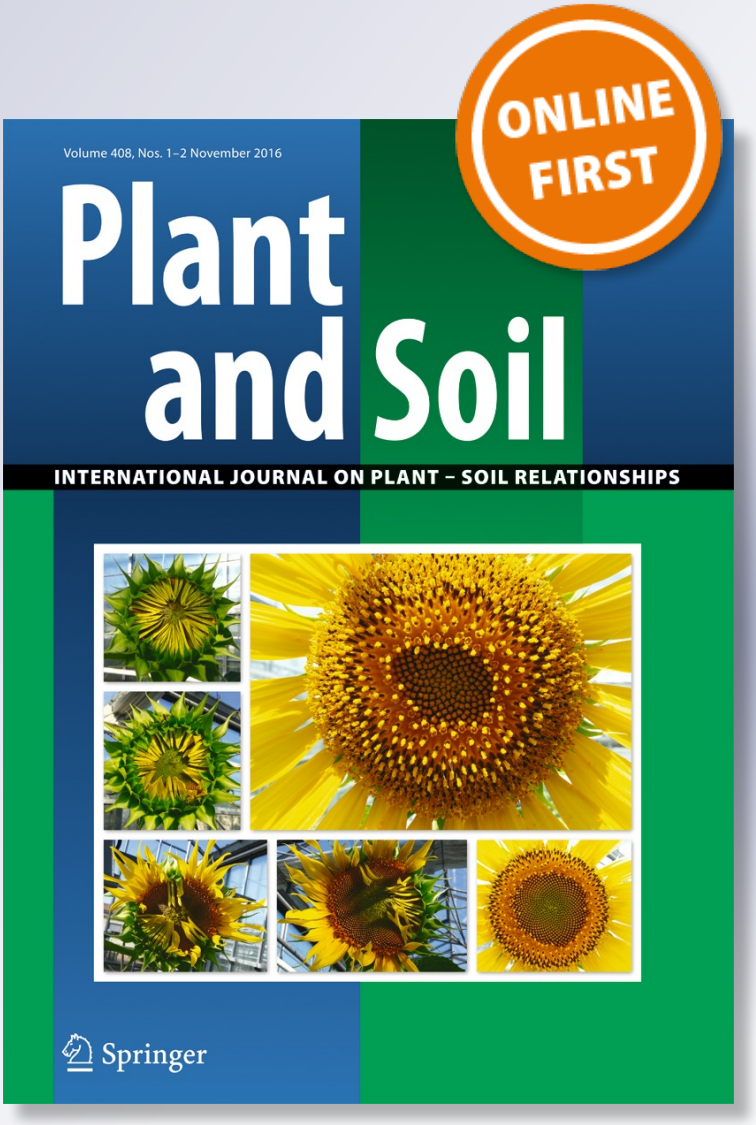

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REGULAR ARTICLE

Five decades of selection for yield reduced root length density and increased nitrogen uptake per unit root length in Australian wheat varieties

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Abstract
Background and aims Given the worldwide effort to improve the nitrogen (N) economy of crops, it is critical to understand the mechanisms of improved N uptake which have resulted from selection pressure for grain yield in Australian wheat (*Triticum aestivum* L.). Changes in root system traits and N uptake were examined in nine Australian wheat varieties released between 1958 and 2007.
Methods Wheat varieties were grown in rhizo-boxes in a glasshouse. We measured nitrogen uptake and mapped root growth and proliferation to quantify root length density (RLD), root length per plant, root biomass, specific root length, and plant nitrogen uptake per unit root length.
Results Selection for yield reduced total RLD and total root length, and increased N uptake per unit root length that overrode the reduction in root system size, effectively explaining the increase in N uptake. Importantly, N uptake in our experiment under controlled conditions matched field measurements, reinforcing the agronomic significance of the present study.
Conclusions Wheat varieties released in Australia between 1958 and 2007 increased their N uptake, not because of increasing their root length and RLD, but for progressively increasing the efficiency of their root system in capturing N. Our collection of varieties is therefore an interesting model to probe for variation in the affinity of the root system for nitrate.

Keywords Australian wheats · Breeding · N capture · Root system · Rhizo-boxes

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Introduction

Austin et al. (1980) compared the phenotypes of wheat varieties released in the UK between 1908 and 1978. Following this influential paper, similar studies have been performed for all major crops (Slafer 1994). The rationale is that the understanding of changes in phenotype resulting from selection for yield and agronomic adaptation can give some indication of pathways for future progress. For example, as harvest index is reaching its biological and agronomic limit in some

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wheat breeding programs, enhanced biomass and maintenance of harvest index are required for further yield gain (Foulkes and Reynolds 2015; Parry et al. 2011).

Changes in the phenotype of Australian wheat varieties have been characterised from 1860 to 1984 (Loss et al. 1989; Siddique et al. 1989, 1990a, 1990b) and from 1958 to 2007 (Sadras and Lawson 2011, 2013; Sadras et al. 2012). Collectively, these studies showed that yield improvement was associated with shortened time to flowering in the early breeding period, increased harvest index for the whole period 1860 to 2007 and increased biomass in the last five decades. The increase in biomass was associated with increased radiation use efficiency between stem elongation and flowering, and the increase in radiation use efficiency is related to both higher nitrogen uptake and improved distribution of light and nitrogen in the canopy. Further, newer varieties have a higher nitrogen nutrition index which highlights the improved nitrogen status of crops irrespective of changes in biomass. Given the worldwide effort to improve the nitrogen economy of crops, an investigation into the mechanisms of improved nitrogen uptake which have resulted from selective pressure for grain yield in Australian wheats is of interest.

Intra-specific variation in root morphological and physiological traits have been reported for Australian and European wheat (Loes and Gahoonia 2004; White et al. 2015; Manschadi et al. 2008; Richards and Passioura 1989). Owing to the combination of scarce rainfall and unfertile soil, research on roots in Australia has focused on water (Passioura 1983; Richards and Passioura 1989; Manschadi et al. 2008) and nitrogen uptake (Liao et al. 2004, 2006; Palta et al. 2011; Palta and Yang 2014; Pang et al. 2014, 2015). Passioura (1983) highlighted the superficial view "that more, and more vigorous, roots gives more drought resistance" and advanced the notion of redundant root systems in relation to water uptake. Large root systems, however, favour nitrogen uptake of wheat in deep sandy soils in Western Australia (Liao et al. 2004, 2006; Palta et al. 2011; Palta and Yang 2014; Pang et al. 2014, 2015). Enhanced nitrogen uptake may also result from higher root length density (Shortemeyer et al. 1993; Palta et al. 2011; Manschandi et al. 2013) or nitrogen uptake per unit root length (Liao et al. 2006; Pang et al. 2015), changes in root distribution in the soil profile or a combination of these traits (Nakamura et al. 2002; McMurtrie et al. 2012).

Here, we report a study aimed to determine changes in the root system and nitrogen uptake in a collection of Australian wheats released between 1958 and 2007. It was hypothesised that increased N uptake in response to selection for yield is associated with increased root length and root length density. To test this hypothesis, plants were grown in rhizo-boxes to map the growth, appearance, branching and distribution pattern of the root system (Liao et al. 2006; Palta et al. 2007; Nagel et al. 2015; Judd et al. 2015).

Materials and methods

Plant material and growth conditions

Nine of the thirteen Australian wheat (*Triticum aestivum* L.) varieties released between 1958 and 2007, previously used by Sadras et al. (2012) to probe for time trends in N and water use efficiency were chosen in this study. The varieties were Heron (released in 1958), Halberd (released in 1969), Condor (released in 1973), Spear (released in 1984), Janz (released in 1989), Frame (released in 1994), Krichauff (released in 1997), Wyalkatchem (released in 2001) and Gladius (released in 2007). These varieties were selected using two criteria: wide adoption in the winter-rainfall environments of Australia and narrow phenological range (Sadras and Lawson 2011).

Plants were grown in 24-L glass-walled rhizo-boxes, 0.24-m long, 0.10-m wide and 1.0-m deep filled with soil to a depth of 1.0 m. The rhizo-boxes were from polyvinyl chloride (PVC), filled with one glass side and have been described elsewhere (Liao et al. 2006; Palta et al. 2007; Benlloch-Gonzalez et al. 2014a, b). Briefly, the acrylic glass side was covered with a removable, black PVC sheet to avoid light exposure to the roots. The rhizo-boxes were placed on steel stands positioned at an angle of 30° to force the roots to grow along the acrylic glass side as described elsewhere (Dieffenbach et al. 1997; Dessureault-Rompré et al. 2006; Liao et al. 2006; Palta et al. 2007; Benlloch-Gonzalez et al. 2014a, b). Visibility of the root system is not different in rhizo-boxes under the 30° and 45° angle when seeds are sown in contact with the acrylic glass side (Nagel et al. 2012; Pfeifer 2013). The 30° angle inclination facilitates handling, mapping and scanning of the root system when using large rhizo-boxes (Adu et al. 2013). Each steel stand held two rhizo-boxes and each steel stand was 0.03 m apart. The soil was

a reddish-brown sandy clay loam; Red Calcic Dermosal (Isbell 1993), collected from the top 0–15 cm of soil from a field site at Cunderdin (31°64′ S, 117°24′ E), Western Australia. It consisted of 63.5 % brown sand, 8.3 % silt and 28.3 % clay with a pH, measured in a 1:5 suspension of soil in 0.01 M $CaCl_2$, of 6.0. The soil contained 6 μg g^{-1} of nitrate-N, 4 μg g^{-1} of ammonium-N, 46 μg g^{-1} of Colwell P, 691 μg g − 1 of Colwell K. Air-dried soil was sieved to 2 mm and mixed with coarse yellow sand (200–2000 μm particle size) in a 4:1 ratio using a cement mixer for uniformity. The soil was packed in each rhizo-box from the top, slowly in 0.2-m sections. The soil in each section was packed to a bulk density of approximately 1.55 g cm^{-3}. Four seeds were sown in a row in contact with the acrylic glass wall in each rhizo-box, corresponding to a field sowing density of 160 plants m^{-2} (Lemerle et al. 2004). At sowing, on 20 August 2014, the equivalent of 60 kg N ha^{-1} as urea; 72 kg P ha^{-1} as amended superphosphate (with Cu, Zn, Mo, S) and 70 kg K ha^{-1} as potash was mixed into the top 0.1 m of soil in each rhizo-box. These rates correspond to the optimal nutrient supply for wheat grown on soils of the Cunderdin district (Flower et al. 2012).

Plants were grown in an evaporatively-cooled glasshouse at The University of Western Australia, Perth, Australia (31°93′ S, 115°83′ E) with an average maximum air temperature of 26°, a minimum temperature of 13 °C, and mean relative humidity of 59 %. During the experiment, the glasshouse received 11 to 12.5 h of natural day light (photoperiod), with an average maximum photosynthetic photon flux density (PPFD) of 952 ± 21 μmol m^{-2} s^{-1} measured at the plant level at 13:00 h. The nine varieties were grown in a completely randomised block design with three replicates and the rhizo-boxes were rotated weekly to minimise spatial variability.

The plants were hand watered twice per week with tap water to maintain the soil water content close to 90 % field capacity and to avoid drainage of excess water. Plant phenology was monitored regularly using the scale of Zadoks et al. (1974). The experiment finished 56 days after sowing (DAS) when three seminal roots of each plant had reached the bottom of the rhizo-boxes, which coincided with plants reaching booting stage (Z41).

Shoot and root traits

When the experiment finished at 56 DAS and the plants were reaching booting stage (Z41), destructively above- and below-ground measurements were made. Above-ground measurements included shoot biomass, leaf area (LA), leaf dry weight, specific leaf area (leaf area per unit leaf weight, SLA), tiller number, and shoot nitrogen. Destructively below-ground measurements included total root length, total root biomass, total root length density (root length per unit of soil volume; RLD) and specific root length (SRL), which is an indirect measurement of root thickness (root length per unit of biomass). All plants in each rhizo-box were harvested as a replicate by cutting the shoots from the roots at the crown and the number of tillers recorded before being dried at 70 °C and weighed. LA was measured using a portable leaf area meter (LI-3000, LI-COR Biosciences, Nebraska, USA). Immediately after the shoots were harvested, each rhizo-box was opened by removing the glass wall and the soil profile was sampled in 0.2-m sections from the top to the bottom. The roots in each section were recovered from the soil by washing and repeated sieving through 1.4-mm sieve to produce a clean sample as described by Palta and Fillery (1993). After the roots were recovered from a section of the soil profile, they were placed in plastic bags and stored at 4 °C until they were measured often after 2 days. The root length in each root sample was measured as described by Liao et al. (2002). Briefly, roots were stained for 30 min with 0.1 % (*w*/*v*) methyl blue just prior to analysis, placed in a 0.2 × 0.3 m glass tray in about 3 mm of water and roots untangled to avoid any overlapping. The glass try was placed on the scanner, and the roots were scanned at 600 pixels per mm using a ScanJet Lic, Hewlett Parkard scanner connected to a computer. The root material was then dried at 70 °C for 48 h and weighed. The images were analysed for root length using WinRHIZO 2008 (model Pro, second version, Regent Instruments, Québec, Canada). Total root length density was calculated as the total root length to 1.0 m of the soil profile divided by the soil volume in the rhizo-box (0.0108 m^3). The distribution of RLD down the soil profile was calculated as the root length in 0.2-m sections from the top to the bottom of each rhizo-box divided by the soil volume of the corresponding section (0.00228 m^3).

At weekly intervals after the one-leaf stage (7 DAS), root growth was monitored through the glass wall of each rhizo-box. On each occasion, the black PVC cover-sheet was removed and replaced with a transparent plastic film and all of the visible new roots were traced using a waterproof permanent pen. Immediately

afterwards all visible new growth was marked on the glass wall of the rhizo-box to identify new root growth at the next measurement. The glass wall was then re-covered with the black PVC cover-sheet. The transparent film for each mapping day was scanned at 600 pixels per mm using a portable scanner (Jenkins PS4100; East Bentleigh, Vic, Australia) and the JPG images were analysed for root length using WinRHIZO 2008 (model Pro, second version, Regent Instruments, Québec, Canada).

Shoot N was determined using a VG-Micromass Sira 10 (V-G Isogas Ltd., Middlewich, England) connected to a Europa Roboprep C-N Analyzer (Europa Scientific Ltd., Crewe, England) after plant tissues were ground to a fine powder using a ball mill.

By combining the destructively measured shoot and root traits, we calculated nitrogen uptake per unit root length and root: shoot biomass ratio.

Statistical analysis

To probe for cultivar effect (year of release) on plant traits, data were first checked for normality and then analysed with one-way ANOVA using Genstat (15th edition; VSN International, UK). Where significant treatment effects were identified, LSD ($P = 0.05$) are given in figures and tables. Chronological trends of phenotypic traits were tested using least-square regressions of trait deviation vs year of release.

Results

Phenology and shoot traits

Wheat varieties released between 1958 and 2007 emerged within 1–2 days of each other at 7–9 DAS. In all varieties booting, flag leaf sheath extending (Z41) coincided with at least 50 % of the seminal roots reaching the bottom of the rhizo-boxes at 1.0 m depth, at which time the experiment was terminated. Time to booting stage (Z41) decreased gradually with the year of release, so that varieties released from 1989 to 2007 reached booting 5–6 days earlier than varieties released from 1958 to 1984 (Table 1). Leaf area at booting ranged from 785 cm^2 $plant^{-1}$ in Heron to 356 cm^2 $plant^{-1}$ in Krichauff ($P < 0.05$; Table 1) but showed no trend with year of release. Likewise, there was no trend in leaf dry weight, specific leaf area, tiller number or shoot biomass with year of release (Table 1).

Root traits and growth

RLD, total root length, total root biomass and specific root length in the soil profile at the end of the experiment are shown in Fig. 1. RLD decreased non-linearly with year of release (Fig. 1a). RLD in varieties released from 1997 to 2007 was about 38 % of Heron, the earliest variety in the series (Fig. 1a). Root length per plant followed a similar pattern of non-linear decline with year of release (Fig. 1b). Total root length declined from 16.2 m $plant^{-1}$ in Heron to 10.1 m $plant^{-1}$ in the most recent variety, Gladius. Total root biomass declined linearly with year of release at a rate of 0.058 g $plant^{-1}$ $year^{-1}$ (Fig. 1c). Specific root length showed no trend with year of release (Fig. 1d).

RLD in the 0–20 cm and 20–40 cm layers of the soil profile was lower in varieties released between 1997 and 2007 than those released between 1958 and 1994 ($P < 0.001$; Fig. 2a). In the 40–60 cm and 60–80 cm layers RLD gradually decreased with year of release ($P < 0.001$). In these soil layers, RLD in Gladius (released in 2007) was 46–50 % lower than in Heron (released in 1958). In the 80–100 cm layer, root length did not differ between varieties (Fig. 2a). The distribution of root length down the 1.0 m soil profile followed the same pattern of distribution as RLD in the 40–60 and 60–80 cm layers of the soil profile, decreasing with year of release, with significant differences between early (1958–1969) and later varieties (1973–2007) ($P < 0.001$; Fig. 2b). Root biomass in the 0 to 20, 20 to 40, 40 to 60 and 60 to 80 cm layers was higher in the early group of varieties than in those released after 1997 ($P < 0.001$; Fig. 2c). Root biomass in the 80 to 100 cm layer was not different with the year of release. There were not differences among varieties in specific root length except for the 20–40 cm layer where varieties released between 1997 and 2007 had higher specific root length than those released between 1958 and 1973 ($P < 0.001$) (Fig. 2d).

The root: shoot ratio at booting ranged from 0.59 to 1.65, but showed no trend with the year of release (Table 1). Likewise, not noticeable trend was observed in the proportion of root-to-total plant biomass (proportion of dry matter allocated to the root system) with year of release (Table 1). 231.

Table 1 Leaf area (LA), leaf dry weight (LDW), specific leaf area (SLA), number of tillers, shoot biomass, root:shoot biomass ratio and the proportion of total plant biomass (root plus shoots) allocated to the roots at booting (Z41), of wheat cultivars released from 1958 and 2007 grown in rhizo-boxes until booting (Z41)

Year of release	Cultivars	Time to booting (DAS)	LA (cm^2 $plant^{-1}$)	LDW (g $plant^{-1}$)	SLA (cm^2 g^{-1})	Tillers $plant^{-1}$	Shoot biomass (g $plant^{-1}$)	Root:shoot ratio	Root-to-total biomass (%)
1958	Heron	54	785.0	2.89	272.4	11.9	4.99	1.50	60.2
1969	Halberd	54	501.9	2.07	249.2	8.5	3.65	1.52	60.3
1973	Condor	54	763.9	3.12	245.4	11.8	5.49	1.42	58.8
1984	Spear	52	494.6	2.57	192.3	8.4	5.73	0.92	47.6
1989	Janz	47	493.8	1.72	290.2	8.0	2.80	1.65	61.3
1994	Frame	47	452.2	2.54	178.4	10.4	4.76	1.34	56.6
1997	Krichauff	49	356.1	2.07	171.6	8.0	7.74	0.59	37.0
2001	Wyalkatchem	46	551.3	1.95	282.8	10.25	3.88	1.18	60.0
2007	Gladius	48	515.8	2.21	233.8	7.38	5.08	0.92	48.0
	$LSD_{0.05}$	4.0	111.2	0.54	27.4	1.54	1.55	0.25	4.4

The non-invasive measured total root growth down the soil profile accumulated from the 1-leaf stage to booting was gradually less with the year of released (Fig. 3). Varieties released from 1958 to 1979 had faster and more root growth than varieties released later, particularly those released from 1997 to 2007. The differences in root growth

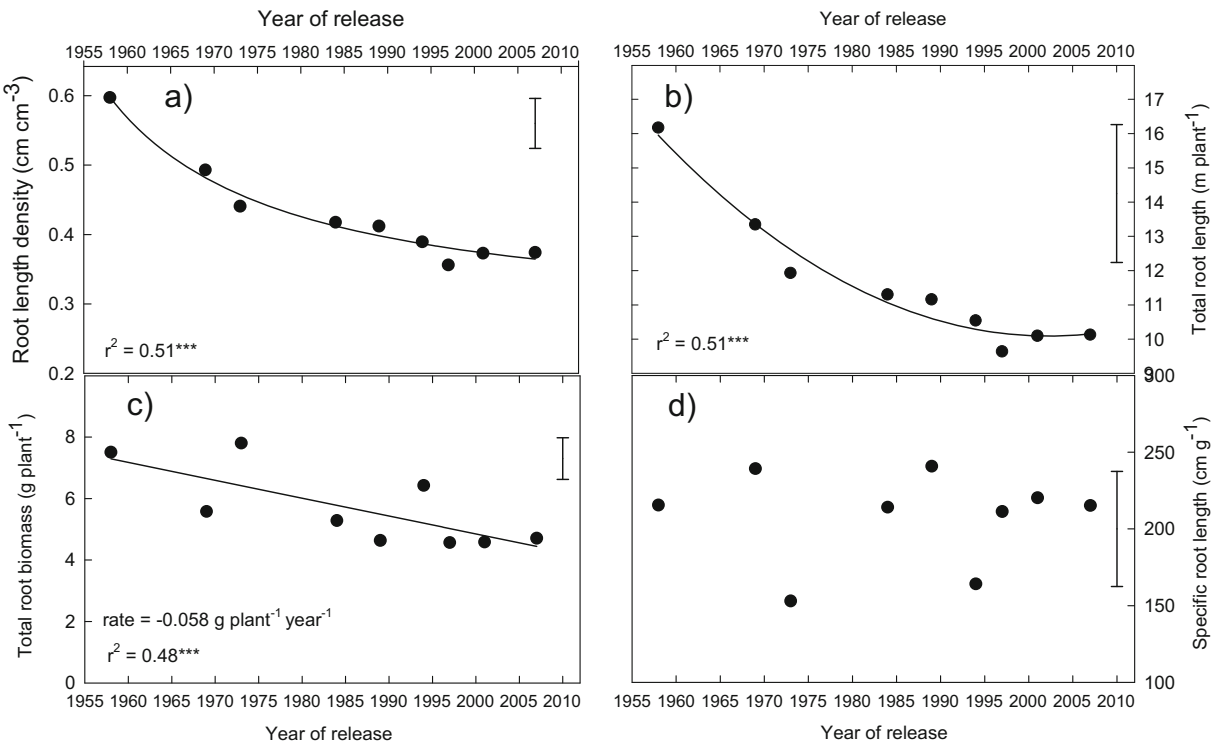

Fig. 1 **a** Destructively measured total root length density (RLD), **b** total root length per plant, and **c** total root biomass and **d** specific root length at the end of the experiment in wheat varieties released between 1958 and 2007 grown in rhizo-boxes. Traits were measured at booting (Z41) in 1.0-m soil profiles. Values are the mean of three replicate rhizo-boxes (four plants per box) per variety. Vertical bars represent LSD at $P < 0.05$ for comparison between year of release

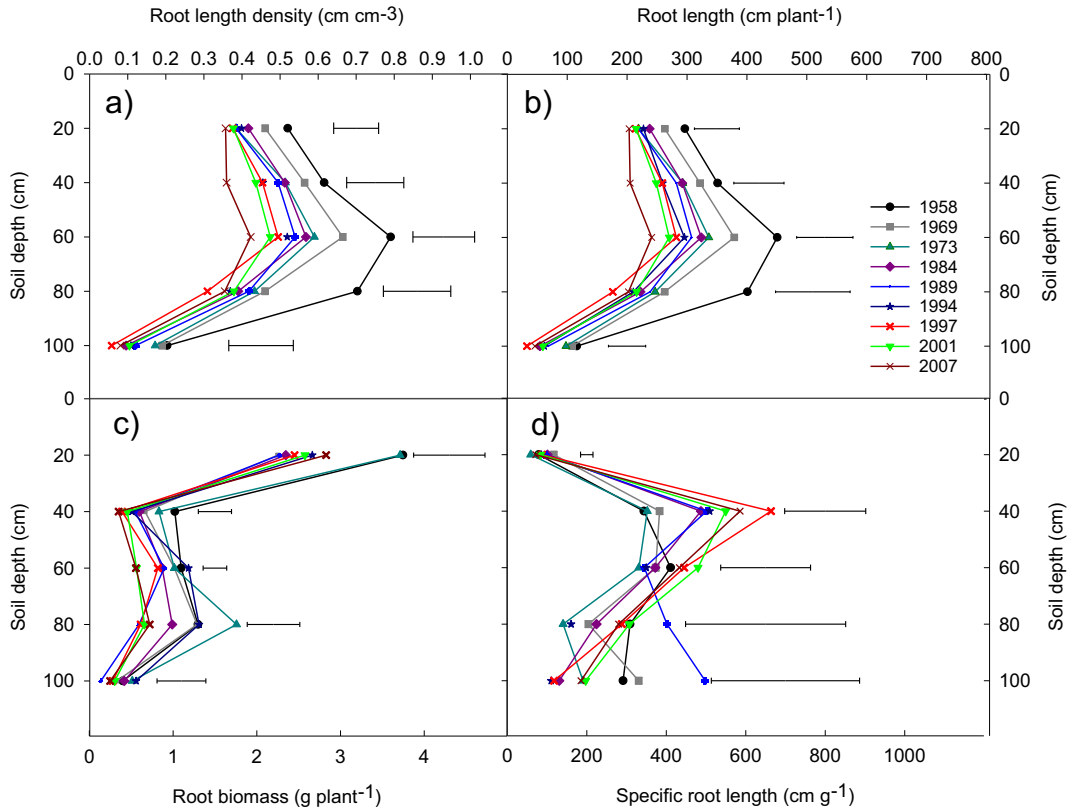

Fig. 2 Destructively measured vertical profiles (0.2-m sections from the top to the bottom of each rhizo-box) of **a** root length density, **b** root length, (**c**) root biomass and (**d**) specific root length at booting (Z41) at the end of the experiment in wheat varieties released between 1958 and 2007 grown in rhizo-boxes. Values are the mean of three replicate rhizo-boxes (four plants per box) per variety per year of release. Horizontal bars represent LSD at $P < 0.05$ for comparison between years of release at each depth of the soil profile

were apparent after 21 DAS and maximum root growth rates were 36.8–47.5 cm plant^{-1} day^{-1} for varieties released from 1958 to 1979 and 27.5–34.1 plant^{-1} day^{-1} for varieties released from 1997 to 2007.

Figure 4 shows the rooting patterns at booting (Z41) of Heron, the oldest variety in the series and Gladius the latest one Branching in Heron down the 1.0 m soil profile was substantially greater than in Gladius, with a total of 1487 root tips per plant, at the transparent plate of the rhizo-boxes, compared with 1103 in Gladius ($P < 0.001$; data not shown).

Nitrogen traits

Total plant N uptake at booting (Z41; leaf sheath extending) increased with year of release at a rate of 1.25 g plant^{-1} year^{-1} (Fig. 5a). More recent varieties had 89 % more plant N uptake than Heron. The time trends in N uptake per unit root length and N uptake per unit root biomass were parallel to that for total plant N uptake (Fig. 5 bc). Varieties released between 1997 and 2007 had twice as much N uptake per unit root length than that of Heron. There was a strong correlation between the mean N uptake measured at anthesis in field plots in two sites in South Australia under low and high available N (Sadras and Lawson 2013) and N uptake measured at booting in the plants grown in rhizo-boxes in this study (Fig. 5d).

Discussion

Selection for yield reduced wheat root system and increased nitrogen uptake

Breeders select primarily for yield, grain quality and disease resistance (Richards et al. 2014), but this selective pressure can lead to extraordinary changes in

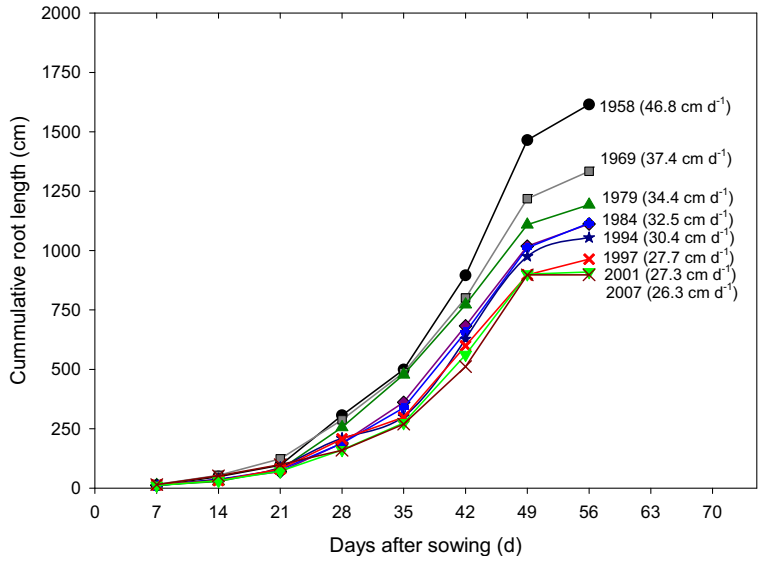

Fig. 3 Non-invasive measured total root growth down the soil profile accumulated from the 1-leaf stage to booting (Z41) for wheat varieties released between 1958 and 2007 grown in rhizo-boxes. Values are the mean of three replicate rhizo-boxes (four plants per box) per variety per year of release. Values in parentheses are the maximum growth rate

phenotype. Wheat breeding for grain yield in Australia between 1958 and 2007 improved N status at anthesis, quantified as NNI in association with an increased capacity for N uptake (Sadras and Lawson 2013). This change in the Australian wheat phenotype is unique, as selection for yield in bread wheat in UK and Argentina, and durum wheat in Italy has had little or no effect on nitrogen uptake (Austin et al. 1980; Slafer and Andrade 1993 Giunta et al. 2007; Barraclough et al. 2010; see review by Sadras et al. 2016).

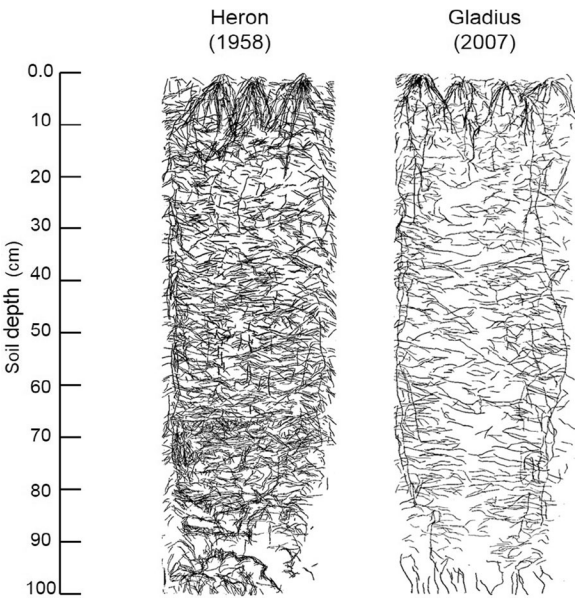

Fig. 4 Comparison of the non-invasive measured root distribution patterns of Heron released in 1958 and Gladius released in 2007 on the glass-wall of rhizo-boxes grown to booting stage (Z41)

Independent studies of wheat in deep sandy soils in Western Australia have found that high N uptake is associated with a large root system (Liao et al. 2004, 2006; Palta et al. 2011; Palta and Yang 2014; Pang et al. 2014, 2015). We thus hypothesised that selection for yield increased the size of the root system, thus accounting for enhanced N uptake.

Our study did not support this hypothesis. Instead, we found an overall reduction in root system size (RLD, root length, root biomass) and an increase in N uptake per unit root length which counteracted the smaller root system and effectively explained the increase in N uptake. Importantly, N uptake in our experiment under controlled conditions matched field measurements, reinforcing the agronomic significance of the present study.

Root tips dynamics, an indicator of root proliferation (Zoon and Tienderen 1990; Demchencko and Demchencko 2001) were not measured in this study, but presumably the reduction in root length and RLD, mainly in the 40–60 and 60–80 cm layers of the soil profile, can be attributed to a reduction in root proliferation in these layers, since the number of root tips at booting in Gladius, the most recent variety in the series, substantially decreased (Robinson et al. 1999; Robinson 2001). These changes were not reflected in the SRL, indicating that root thickness did not change with year of release. Similarly, selection for yield of winter wheat

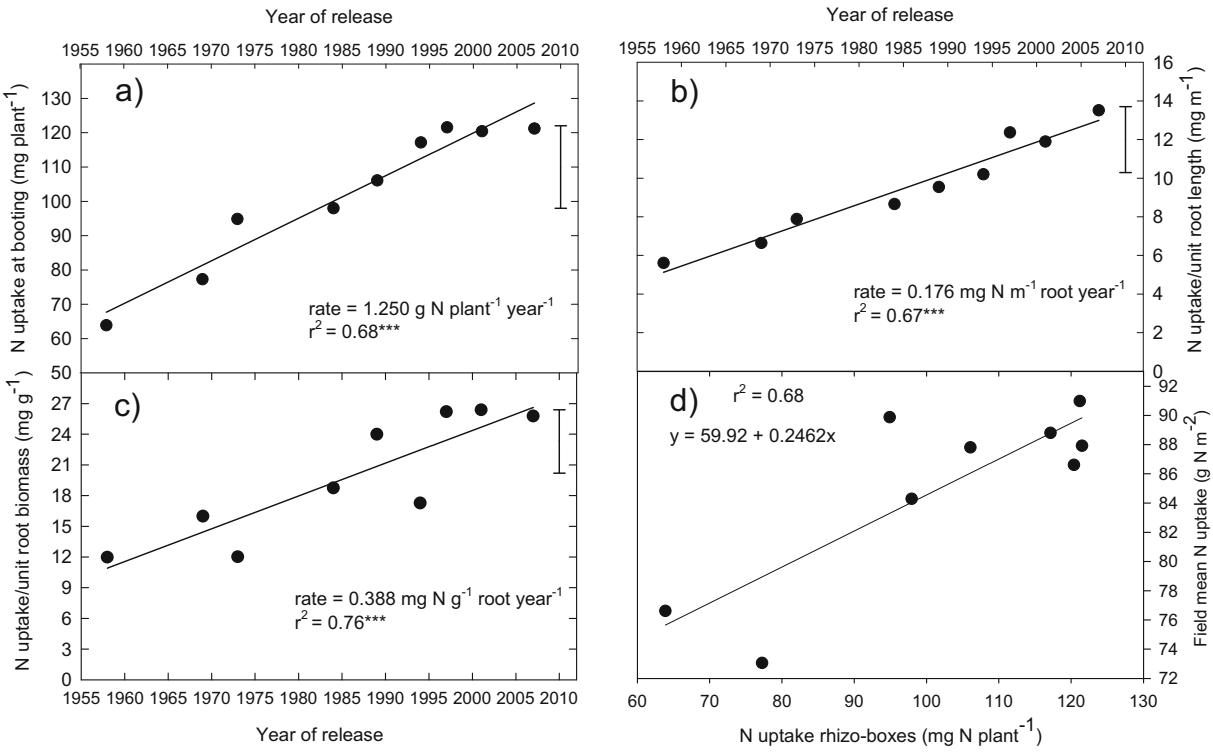

Fig. 5 Destructively measured total N uptake, (**b**) N uptake per unit root length, and (**c**) N uptake per unit root biomass for wheat varieties released between 1958 and 2007 grown in rhizo-boxes to booting stage (Z41). Values are the mean of three replicate rhizo-boxes (four plants per box) per variety per year of release. Vertical bars represent LSD at $P < 0.05$ for comparison between years of release. (**d**) Comparison of mean N uptake at anthesis in wheat varieties released between 1958 and 2007 grown in the field in South Australia (Sadras and Lawson 2013), and mean N uptake at booting in 1.0 m deep rhizo-boxes in the glasshouse (this study)

in the UK between 1970 and the early 2000s has apparently reduced RLD (White et al. 2015).

Early varieties (1958–1973) had more root biomass to 0.8 m than the most recent varieties (1997–2007) and was independent of differences in SRL, except in the 20–40 cm layer of the soil profile, where more recent varieties had significantly thinner (higher SRL) roots than those of early varieties. Since thinner roots favour water and N uptake (Barraclough et al. 1989; Melino et al. 2015; Corneo et al. 2016), it is possible that these roots in the 20–40 cm layer are associated with improved uptake of N. Vigorous root systems in wheat are characterized by early, prolific branching of thin roots in the 0.2–0.7 m layer of the soil profile and increased total root length and RLD (Liao et al. 2004, 2006; Palta et al. 2011). These characteristics have been associated with improved N uptake by anthesis in deep sandy soils (Liao et al. 2004, 2006; Palta et al. 2011; Palta and Yang 2014; Pang et al. 2014, 2015) although some varieties with non-vigorous root systems can uptake as much N by anthesis (Pang et al. 2014, 2015). As a result, the gradual increase in N uptake with year of release, paralleled with the gradual reduction in total root length and RLD, is likely explained by an improved affinity for NO_3^- with selection for grain yield. The strong decline in RLD in the 20–40 and 40–60 cm soil layers with year of release ($P < 0.001$) and the negative correlation between N uptake with RLD at booting ($r^2 = 0.55$; $P < 0.001$) rejects the hypothesis of this study and indicates that selection for wheat grain yield in Australia has inadvertently reduced root growth while increasing total N uptake. Whether these roots were redundant for the capture of N or their function overcompensated for by the activity of other roots (Wiedenroth and Erdmann 1985; Scholes et al. 2013; Ros et al. 2003) is an interesting physiological question. Root growth and proliferation has a carbon cost (Gregory et al. 1997; Rogers et al. 1996; Palta and Watt 2009). Consistently with the early view of Passioura (1983), the reduction in root biomass in response to selection for yield suggests that, for the same photosynthetic rate, surplus carbon may be allocated to

alternative sinks, including structural shoot biomass (Palta et al. 2011) and labile reserve carbohydrates (Sadras and Lawson 2011), and contribute to floret survival or grain growth (Kirby 1988; Ferrante et al. 2013; Dias de Oliveira et al. 2015). Indeed, the root:shoot ratio—which depend on the partitioning of photosynthate carbon— and the proportion of total biomass allocated to the roots were higher in varieties released early (between 1958 and 1989 (except for that released in 1984) than those released between 1994 and 2007. It is likely that the lower root:shoot ratio of the late released varieties may be influenced by their short time to reach booting since allocation of photosynthate carbon to the roots changes dramatically with floral initiation, at double ridge stage (Z24; Palta and Gregory 1997).

N uptake in this study was measured in rhizo-boxes in a glasshouse. Although these growing conditions may have resulted in a stronger growth response to N than for field crops (Limpens et al. 2012), the mean N uptake from varieties grown in rhizo-boxes positively correlated with that of the same varieties in the field at two sites in South Australia (Sadras and Lawson 2013). Despite the different growing conditions, the correlation confirms an improved N uptake in wheat varieties released between 1958 and 2007 in Australia (Sadras and Lawson 2013) even with the progressive reduction in root length. It is not clear that the increase in N uptake with the year of release in this experiment was driven by the N supply. This is because N in the soil at sowing and supply during sowing was the same in each rhizo-box to ensure that each variety had the same availability of N. It is not neither clear that the increase in N uptake with the year of release was driven by the N demand as leaf area, tiller number and shoot biomass showed no trend with year of release. N availability in each rhizo-box at sowing was high (16 kg N/ha as soil N plus 60 k N/ha as N supply) and this is consistent with the findings of Kamiji et al. (2014) that show biomass in wheat is the driver for N uptake under low N availability, but not under high N availability.

Implications for water use

In most of the Australian grain belt, rainfed crops are chronically short of water, hence the potential implications of the reduced size of root system in response to selection for yield found in this study. Selection for yield of wheat in Australia from early breeding until the mid1980s reduced seasonal evapotranspiration in parallel with shorter cycles; mean daily evapotranspiration did not vary among varieties and soil evaporation accounted for by 40 % of the seasonal evapotranspiration irrespective of cultivar (Siddique et al. 1990b). Seasonal evapotranspiration remained unchanged for the varieties released between 1958 and 2007 (Sadras and Lawson 2013). As a result of the increase in yield and largely unchanged water use after accounting for phenology, yield per unit water use of Australian wheats increased linearly over a century to 2007 (Sadras et al. 2016). Hence, the reduction in root size is congruent with the early view of root redundancy in relation to water uptake (Passioura 1983). It has been speculated that the apparent reduction in root length density of winter wheat between 1970 and the early 2000s, coupled with reduced rainfall, might be one of the factors underlying yield stagnation in the UK since the 1990s (White et al. 2015). The question remains, to what extent further reduction in root system are feasible before water uptake is compromised in wheat adapted to the winter-rainfall environments of Australia?

Conclusions

Wheat varieties released in Australia between 1958 and 2007 increased their N uptake by progressively increasing the efficiency of their root system in capturing N, and not by increasing root length and RLD. Total root length, RLD, root length and root biomass at booting progressively decreased with the year of release, while N uptake per unit root length and per unit biomass gradually increased. The reduction in root biomass may have resulted in less carbon being released from the roots through other root functions. The improved efficiency of the root system in capturing N likely resulted from an increased affinity for NO_3^- (Pang et al. 2015) or from increased activity of the root system compensating for the loss in root length and RLD in the 40–60 and 60–80 cm layers of the soil profile. The collection of wheats in this study is an interesting model to further investigate the physiology of N uptake and its genetic basis.

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