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RESEARCH ARTICLE

# Combining spring wheat genotypes with contrasting root traits for a better use of water resources in soil? Evidence from column-scale water stable isotopic experiments

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*Background and Aims* The advantages of genotype mixtures on soil water balance are still poorly understood. We aim to determine the impact of soil water conditions (well-watered or chronic water deficit) on the root water uptake (RWU) of two contrasting root genotypes (“shallow root system” SRS and “deep root system” DRS) and their mixture at the booting stage.
We conducted a controlled plant-soil column experiment and quantified daily vertical profiles of the fraction of RWU (fRWU, % $\text{cm}^{-1}$), i.e. the relative contribution of RWU normalized by the thickness of each layer. This calculation was achieved by applying Bayesian modelling on non-destructive soil and transpiration water stable isotopic measurements after pulse labelling. We compared these results to the monitored plant soil water status, plant physiology and root traits.
The SRS genotype had a higher fRWU than the DRS genotype in the topsoil and vice versa in the subsoil. In the mixture, both genotypes maintained complementary fRWU distributions and, in response to the water deficit, increased their contribution in subsoil

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by 0.5% $cm^{-1}$ and decreased their contribution in topsoil by -1.2% $cm^{-1}$ compared to the monoculture.
This study introduces novel observations of root water uptake plasticity, which is determined by genotype root traits, soil water availability, and interactions with neighboring plant root traits. This study highlights the potential of contrasting root traits mixtures to improve their water and nutrient access facing water deficit.

**Keywords** Wheat mixture · Water stable isotopes · Water deficit · Root water uptake plasticity

## Introduction

One potential strategy for agriculture to maintain high yields and ensure the soil health in the context of climate change involves the (re)introduction of greater biodiversity to agroecosystems (Altieri et al. 2017; Messéan et al. 2021). At the landscape scale, this can be achieved through the implementation of hedges, patchwork cultivation, or grass strips (Vanneste et al. 2020). At the field scale, biodiversity enhancement can be achieved through the implementation of diverse crop rotations. These rotations may include various cultivation cycles of different durations. Examples include perennial grains (Duchene et al. 2020) and pluriannual forages, which are more commonly used (Martani et al. 2022). Between cycles of the crop of interest, the introduction of catch crop or cover crop can be a complementary practice (Moreau et al. 2012; Vincent-Caboud et al., 2019). Biodiversity can also be cultivated within the same time and space, for example through the implementation of agroforestry systems, which combine large perennial and small annual plants (Mettauer et al. 2023; Nerlich et al. 2013). Alternatively, different annual crops can be grown in the same field, a practice known as intercropping, blending or maslins (Blanc et al. 2024; Brooker et al. 2015; Duchene et al. 2017; Hawes et al. 2021). It has been demonstrated that these practices have beneficial effects linked to the mixing of genetically different individuals interacting in the same environment. On the one hand, the combined effects of physiological diversity and plastic adjustment of genotypic traits ultimately lead to improved yield stability and pest resistance (Gaba et al. 2015; Angeletti et al. 2022; McAlvay et al. 2022; Schöb et al. 2023). Genetic mixture cross-pollination has been demonstrated to enhance the number of fertilised flowers and the quality of their fruits, a phenomenon that has been observed in the context of large-scale cereal crops (Chabert et al. 2024). Interactions between patterns of resource extraction, such as water, nutrients, or light, can be spatially or temporally complementary due to morphological or physiological differences between genotypes (deep or shallow roots, shifted phenology, etc.). This allows niche complementarity for a given resource. Furthermore, plants interact with each other through various chemical communication channels (soluble exudates in the soil solution or VOCs diffusing into the soil gas phase) (Stirling et al. 2024; Mathieu et al. 2025). It has been demonstrated that this communication can also be mediated by microbial modulations (under the effect of rhizodepositions), which influence the growth, nutrient mineralization activity, or microbial recruitment of neighboring plants (Zhou et al. 2024; Ghatak et al. 2025). These interactions have the capacity to amplify the positive effects of mixtures, for example by modifying soil microbiome dynamics or pathogen tolerance (Feng et al. 2024; Mathieu et al. 2025).

However, the necessity for specific technical itineraries, inputs (fertilizer, phytosanitary, etc.) and adapted machinery to account for differences in physiological development of the associated species are significant obstacles to the implementation of co-cropping, especially in Europe (Brannan et al. 2023; Bybee-Finley and Ryan 2018; Wezel et al. 2014). A promising yet less well-studied alternative to adding more inter-specific plant diversity to agroecosystems is to take advantage of the existing intra-specific crop biodiversity (Albert et al. 2011; Bruelheide et al. 2018; Jung et al. 2010; Litrico and Violle 2015).

Wheat occupies a central place in global food production and its yield is sensitive to climate change (Ortiz et al. 2008; Wilcox and Makowski 2014). Consequently, wheat genotype mixtures have gained interest over the last decades due to higher epidemiologic resistance, leading to more stable yields or even higher production. Thus, most current studies on wheat genotype mixtures focus on disease suppression and yield improvement (Kiær et al. 2009; Borg et al. 2018; Kristoffersen et al. 2020; Vestergaard & Jørgensen, 2024; von Cossel et al. 2025). Some studies have also reported a systematic improvement in water use efficiency in mixtures, but no study described the mechanisms at the individual level that drove this emerging effect in wheat mixture populations (Wang et al. 2016; Giunta et al. 2020; Huang et al. 2024).

The combination of these advantages, as well as the relative ease with which they can be adapted to different agricultural techniques, compared to interspecific mixtures (also known as co-cultures), has sparked growing interest among farmers, who are increasingly resorting to this practice, often in random mixtures of genotypic diversity (Perrone et al. 2017). In order to propose a clear overview of the possibilities and outcomes for farmers, the beneficial effects of this type of "random mixture" could be enhanced by studying the designing selection criteria for mixed varieties for specific environmental conditions (Lamichhane et al. 2018; Barot et al. 2017). While a significant proportion of plant-plant interactions occurs beneath the soil, these interactions have often been overlooked in research (Homulle et al. 2022). This is particularly true given that wheat selection has traditionally focused on aboveground traits, which has led to difficult or even impossible access to descriptions of root traits for commercial genotypes (Baca Cabrera et al. 2025; Huang and Zhang 2025). The extant literature highlights the necessity of (i) a comprehensive understanding of the underlying mechanisms of the ecosystem services observed and (ii) the identification of the trait configurations that lead to both high performance and stability of the mixture over time (Demie et al. 2022; Hughes et al. 2008; Lecarpentier et al. 2019).

Within these mechanisms, the strategies and patterns of root water uptake, in addition to the nutrients contained therein, are of critical importance to the plant's adaptation to water deficit. Water uptake is determined by the hydraulic soil–plant-atmosphere gradient and is further regulated by the root traits, such as architecture, rooting depth and density distribution, as well as root and microbial exudation (Doussan et al. 2024; Freschet et al. 2021; Giraud et al. 2023). Research has demonstrated that deep-rooting wheat genotypes enhance grain yield under water deficit conditions (El Hassouni et al. 2018; Nakhforoosh et al. 2014; Wasson et al. 2012) and have associated these outcomes with enhanced water use efficiency (Feng et al. 2017; Hafeez et al. 2024). Root plasticity also enables plants to adapt their root traits and their water uptake to optimize water uptake under water-deficit conditions, shifting it either more toward the subsoil (Wang et al. 2024; Xu et al. 2016) or the topsoil (Shazadi et al. 2024). However, the interaction between wheat genotypes with contrasting root traits in a cropped mixture and their divergent root water uptake strategies remains to be fully understood.

The objective of this study was to evaluate whether combining wheat genotypes with contrasting root traits could improve water supply to the plant population. We focused on the booting stage because it is crucial for yield determination, involving the stabilization of the number of fertile spikes and flower formation, as well as the development of grain filling potential (Fischer 1985; Wu et al. 2022; Xu et al. 2022). First, we hypothesized that the two genotypes, which were selected for their contrasting root traits—shallow vs. deep root systems—would exhibit significantly different root water uptake (RWU) profiles at this key stage of development. Secondly, water deficit should result in the RWU shifting to the subsoil and wetter soil layers, affecting all the genotypes. Thirdly, when the two genotypes are cultivated in mixture, it is hypothesized that each genotype will keep its specific water extraction niche, thereby enabling both plants to cope better with water deficit conditions.

Our study uses a controlled plant-soil column experiment to analyze the water use of two wheat genotypes with similar root biomass and contrasting root angle of insertion in the early stages of development (Rambla et al. 2022). Root traits of these genotypes also exhibit contrasting traits in later stages of development (Lattacher et al. 2025a). The experimental system enabled the quantification of the daily vertical profiles of RWU for wheat genotypes cultivated under adequate irrigation (WW) and water deficit (WD) conditions in monoculture and mixture, based on stable isotope measurements of soil water and transpiration flux after isotopic labeling. For this, a set of non-destructive methods and Bayesian modeling were employed, building upon the earlier work of Deseano Diaz et al. (2023). This approach enabled us to estimate for the first time to the best of our knowledge, the individual RWU profile of each genotype growing in mixture in the same soil column, at the same time of day, non-destructively, with a spatial resolution more detailed than what is currently done in the literature. These results were then compared with continuous non-destructive monitoring of soil water status, plant physiology (leaf area, tillering, biomass, stomatal density, etc.) and root system (3D MRI scans), verified a posteriori by destructive observation (root scans and biomass). Based on these experimental evidences, we then discuss the inter-genotype plasticity of root water uptake patterns and potential benefits of cropping wheat genotype mixtures to cope with water deficit.

## Materials and methods

### Experimental setup

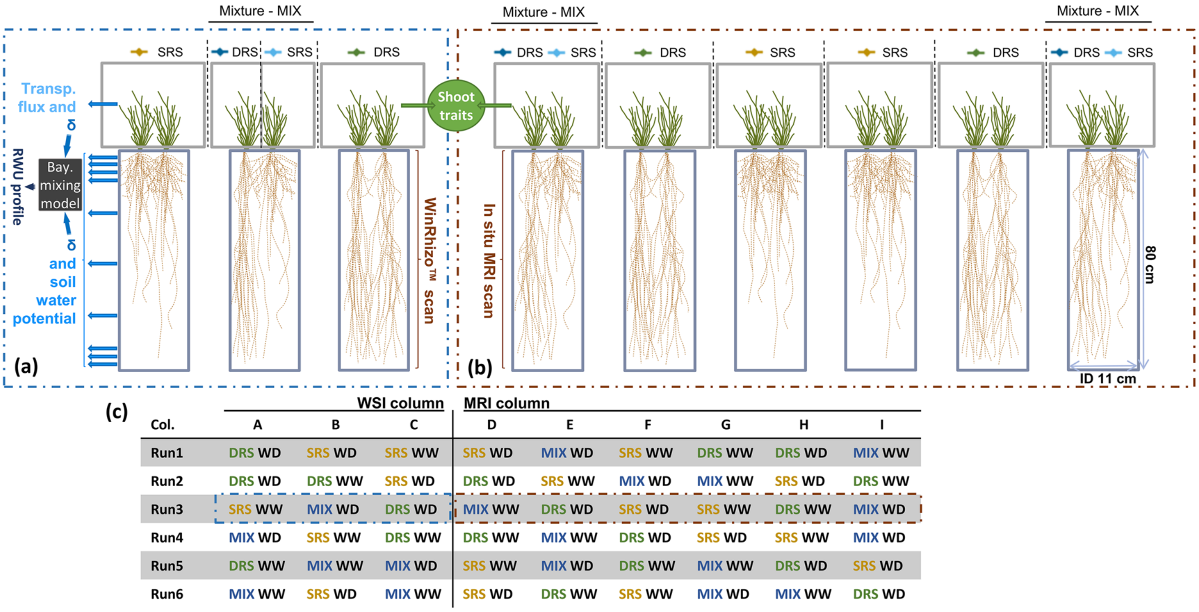
For the study, soil columns with an internal diameter of 11 cm and a height of 80 cm were used. These columns contained air-dried soil with a silt loam texture containing 22% clay, 66% silt, and 12% sand (Weihermüller et al. 2007) that had been sieved to a mesh size of 2 mm, collected from the upper 30 cm an agricultural field (Haplic luvisol) in Selhausen, Germany (50°52′07.8" N, 6°26′59.7" E), with temperate oceanic climate. The soil within the columns was compacted using a vibrating plate (Haver EML 450 Digital Plus N, Haver & Boecker, Oelde, Germany) to attain a homogeneous dry bulk density of 1.4 g $cm^{-3}$ across the entire soil column. This density is representative of field conditions measured on average in the origin agricultural field. Subsequently, the soil of all columns was saturated with water from below via a porous plate. Then the soil columns were covered for a period of two weeks to prevent evaporation and to establish a stable level of homogeneous hydraulic status and microbial activity within the entire column. Thereafter, four pre-germinated sprouts were placed within each column and some days later, the two less vigorous were removed.

Two experimental spring wheat (*Triticum aestivum* L.) lines, UQR012 (shallow-rooted system: SRS) and UQR015 (deep-rooted system: DRS) with strongly contrasting seminal root angles, determined at early growing stage, were used (Rambla et al. 2022). The genotypes were developed by backcrossing a donor source for narrow root angle to the high-yielding spring wheat genotype Borlaug100 (Rambla et al. 2022).

We tested six ‘plant modality x water treatment’ combination composed of three plant modalities—monoculture of SRS, of DRS and the mixture (MIX) of both – and two water treatments—well-watered (WW) vs. water deficit (WD) conditions during four successive days at the booting stage. This resulted in the following plant modalities and water treatments combinations: WW-SRS, WW-DRS, WW-MIX, WD-SRS, WD-DRS and WD-MIX. Each combination was replicated over nine soil columns replicates, which were generated in six separate runs due to the spatial limitation of the climate chamber (Fig. 1 c). Each of the nine replicates include three soil columns for the water stable isotopic measurements (WSI columns) and six soil columns for magnetic resonance imaging measurements (MIR columns) (Fig. 1 a, b).

We conducted this experiment in a climate chamber under controlled conditions, with air temperature set to 20 ± 0.22 °C, relative humidity was set to 50.0 ± 2%. Light was provided with homemade LED panels (LEDs: CXA2520-0000-000N0YN430H, Lighting Solutions, Ludenscheid, Germany) cooled with a water bath (RP1845, LAUDA, Germany). The light intensity followed a sinusoidal-like 24-h cycle from 0 µmol $m^{-2}$ $s^{-1}$ at "night" (from 8 pm to 6 am) to 1200 µmol $m^{-2}$ $s^{-1}$ at "midday" (1 pm).

From the sowing time on, water was applied to the topsoil using two different watering treatments. Watering regimes were adjusted to varying intensities, targeting for the 6th week of growth a matric potential corresponding to a pF value of 2.0–3.0 for the WW treatment and a pF value of 3.5–4.5 for the WD treatment. The pF value corresponds to the $\log_{10}$ of the absolute soil water matric potential (in kPa), and reflects soil water availability. The WW treatment therefore corresponds to wet soil condition, close to field capacity (pF ~ 2.0–2.5), where water is easily available to plants. The WD treatment corresponds to drier soil conditions where water is highly retained, requiring the roots to exert strong suction to extract water (pF 4.2 corresponds generally to the permanent wilting point). The watering for the WW treatment represented a total quantity of about 1300 mL of water over 6 weeks of growth, equivalent to 137 mm of rainfall, approaching the 10-year average of rainfall accumulated over 6 weeks between March and May in Selhausen (Zoomash). For the WD treatment, only 200 ml of water was applied over the same growth period, equivalent to 21 mm of rainfall, similar to two of the dry spring months in 2021 or 2022, to achieve chronic and progressive water deficit (Lynch, 2018).



|  | WSI column |  |  | MRI column |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|
| Col. | A | B | C | D | E | F | G | H | I |
| Run1 | DRS WD | SRS WD | SRS WW | SRS WD | MIX WD | SRS WW | DRS WW | DRS WD | MIX WW |
| Run2 | DRS WD | DRS WW | SRS WD | DRS WD | SRS WW | MIX WD | MIX WW | SRS WD | DRS WW |
| Run3 | SRS WW | MIX WD | DRS WD | MIX WW | DRS WD | SRS WD | SRS WW | DRS WW | MIX WD |
| Run4 | MIX WD | SRS WW | DRS WW | DRS WW | MIX WW | DRS WD | SRS WD | SRS WW | MIX WD |
| Run5 | DRS WW | MIX WW | MIX WD | SRS WW | MIX WD | DRS WW | MIX WW | DRS WD | SRS WD |
| Run6 | MIX WW | SRS WD | MIX WW | SRS WD | DRS WW | SRS WW | MIX WD | MIX WW | DRS WD |

**Fig. 1** Experimental design: example of Run3 consisting of its columns (stable water isotopes (WSI), panel a) vs MRI columns, panel b)) and their specific underground and aerial measurements. In the lower panel c), randomly distributed composition of each experimental series (Run1-Run6) in terms of plant modality (two individuals with "shallow root systems ‘ – SRS or with ’deep root systems’ – DRS grown in monoculture, and their mixture – MIX) and water treatment (well-watered conditions – WW and water deficit – WD). The WSI columns (left side, A-C) included soil water potential sensors and gas-permeable membranes for online analysis of the stable isotopic composition of soil water (δ) and gas exchange chambers for non-destructive determination of transpiration flux and isotopic composition. The MRI columns (right side, D-I) did not include soil sensors, and the gas exchange chambers were used only to simulate the same atmospheric conditions that prevailed for the WSI columns. Each run of experiments included three WSI columns and six MRI columns

Evaporation from the topsoil was minimized by covering it with a plastic film during the experiment.

The WSI columns were specifically designed to include gas probes (KM-PPMF-055–00-000-0000-0, 0.155 cm wall thickness, 0.55 cm i.d., 0.86 cm o.d., 0.2 µm pore size; Katmaj Filtration®, Poland) for the online and non-destructive determination of the soil water vapor $\delta^2$H and $\delta^{18}$O (Rothfuss et al. 2013). We also installed soil matric potential (SMP, MPa) and temperature (T, °C) sensors (Teros 21, Meter, München, Germany) (Fig. S1). The WSI columns were complemented by six polyvinyl chloride (PVC)-made columns of the exact same dimensions for magnetic resonance imaging (MRI) measurements. These MRI columns did not include any metal parts or metal-built sensors, which allowed for the visualization of the 3D root architecture with (MRI) at the 6th week of plant growth. Each WSI column was equipped with a gas exchange chamber containing the two plants from their shoot collars from 39 DaS. In the context of MIX conditions, the WSI columns were divided into two impermeable half-chambers (see Sect. "Labeling strategy, water stable isotopic monitoring, and RWU determination"). The MRI columns were also equipped with gas exchange chambers from 39 DaS to maintain consistent humidity and temperature conditions during the final four days of the experiment. However, the plants in the MRI columns were grouped into sets of three columns in a single gas exchange chamber (79 L in volume, 33 cm in width, 40 cm in height and 60 cm in length).

The thermal insulation of the soil column sides allowed the establishment of a soil temperature gradient as would be observed in field conditions, with 2 °C higher values in the topsoil layers than lower values in the subsoil layers. In the transpiration chambers, no significant differences were observed between the cultivation modalities and the water treatment in terms of air humidity (74 ± 9%), air temperature (24.0 ± 0.8 °C) and vapor pressure deficit (VPD) (0.78 ± 0.27) (Table 1).

A mineral fertilization, equivalent to 60 kg N ha$^{-1}$, was applied in each soil column at the three leaves-stage. The growth period was restricted to 6 weeks (until 43 days after seeding – DaS) when the plants reached ear emergence from boot, equivalent to Z51-59 on the Zadoks scale (Zadoks et al. 1974).

## Labeling strategy, water stable isotopic monitoring, and RWU determination

Labeling was conducted to increase the precision of the RWU estimates of the WSI columns. The labeling approach was designed to lead to 1) diverging vertical profiles in soil water $\delta^{2}$H and $\delta^{18}$O; 2) the increase in soil water $\delta^{2}$H and $\delta^{18}$O gradients across depth and 3) to a non-significant rise in soil water content value. This was done by adding small amounts (10 ml) of isotopically labeled water ($–149.0‰ < \delta^{2}H < 1026.0‰$, $–302.0‰ < \delta^{18}O < 96.0‰$) at 0, 20, 40, 60, and 80 cm depth via perforated tubing (Synflex "1300" 1/4", Eaton, Dublin, Ireland) at 28 and 34 DaS, in order to achieve contrasting isotopic composition profiles later between 39 and 42 DaS for estimating the fRWU. We targeted a monotonic $\delta^{18}$O profile on the one hand, and a non-monotonic $\delta^{2}$H profile in the shape of a V with a maximum in the middle of the column on the other. We aimed the following labelling $\delta^{18}$O and $\delta^{2}$H values: 0 cm: + 70‰/–70‰; −20 cm: + 30‰/+ 90‰; −40 cm: + 5‰/+ 170‰; −60 cm: −15‰/+ 4‰; −80 cm: −20‰/−70‰.

The weight loss in the WSI columns was recorded (IFS60K0.5D, Kern, Balingen, Germany) throughout the experiment to calculate the transpiration rate gravimetrically. In the sixth and final week of each experimental series, plant transpiration rate (Tr, mL/min) and transpiration isotopic composition values $\delta^{2}H_{Tr}$ and $\delta^{18}O_{Tr}$ ($\delta_{Tr}$) were measured on the WSI column plants, following the method described in Deseano Diaz et al. (2023). For this we used a gas exchange chamber (29 cm diameter, 40 cm length, 27 L volume) that was connected to a laser spectrometer (L2130-i, Picarro Inc. Santa Clara, USA) (Fig. S2) for 20 min per plant during the afternoon between 12 and 3 pm to measure maximum water flux (due to maximum light radiation at 1 pm).

The gas exchange chamber was equipped with an air inlet and outlet, a fan and temperature and humidity sensor humidity and air temperature sensors (RFT-2, precision for RHchamber and Tchamber = 2% and 0.1 °C, respectively; METER Group, Munich, Germany) and fans (500F, DC-Axiallüfter, ebm-papst, Mulfingen, Germany). Under mixture (MIX), each gas exchange chamber was divided into two air-tight chambers, each of which was equipped with the features described above. The WSI column plant chambers were constantly supplied with 6 to 12 l min$^{-1}$ of air taken from the climatic chamber ($T_{cc} = 20 \pm 0.22$ °C, $RH_{cc} = 50.0 \pm 2\%$) (Fig. 1). Measurements of relative air humidity ($RH_{chamber}$, %) and air temperature

**Table 1** Mean and standard deviation (sd) resulting of environmental parameters of the experiment in the afternoon between 1 and 3 pm at the booting stage (between 39 and 43 DaS) (air temperature in °C; air humidity in %, vapor pressure deficit (VPD) in kPa, pF matric potential and soil temperature in °C) for each culture type, genotype and water treatment (n = 3)

| | | | Air temperature (°C | | ) Air humidity (%) | | VPD (kPa) | | pF | | Soil temperature (OC) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Culturetype | Genotype | Water treatment | mean | sd | mean | sd | mean | sd | mean | sd | mean | sd |
| Monoculture | DRS | WD | 24.3 | 0.9 | 68 | 10 | 0.92 | 0.31 | 3.6 | 0.3 | 20.9 | 0.7 |
| Mixture | DRS | WD | 24.2 | 0.4 | 71 | 12 | 0.88 | 0.33 | 3.7 | 0.3 | 21.1 | 0.6 |
| | SRS | WD | 24.4 | 0.2 | 76 | 11 | 0.72 | 0.32 | | | | |
| Monoculture | SRS | WD | 24.3 | 0.7 | 69 | 8 | 0.91 | 0.27 | 3.6 | 0.5 | 21.1 | 0.7 |
| Monoculture | DRS | WW | 23.4 | 0.6 | 79 | 4 | 0.65 | 0.13 | 2.8 | 0.4 | 21.1 | 0.9 |
| Mixture | DRS | WW | 24.4 | 0.8 | 73 | 7 | 0.82 | 0.23 | 2.9 | 0.8 | 21.4 | 0.5 |
| | SRS | WW | 24.5 | 0.7 | 76 | 5 | 0.73 | 0.15 | | | | |
| Monoculture | SRS | WW | 23.8 | 0.6 | 78 | 5 | 0.68 | 0.14 | 2.6 | 0.6 | 21.0 | 0.7 |

($T_{chamber}$, °C) in the gas exchange chambers were used to calculate the vapor pressure deficit (VPD, kPa).

We sampled soil water vapor (swv) for 13 min at 13 depths (1, 3, 5, 8, 18, 20, 40, 42, 62, 64, 74, 77 and 79 cm depth) in the WSI columns with gas-permeable probes (Fig. 1). The probes were flushed, further diluted with dry synthetic air (at 70 and 30 ml $min^{-1}$, respectively; GF40 mass flow controller, Brooks Instruments, Hatfield, USA), and analyzed online with a laser spectrometer (L2130-i, Picarro Inc., Santa Clara, USA) for isotopic analysis ($\delta^2H_{swv}$ and $\delta^{18}O_{swv}$). $\delta^2H_{soil}$ and $\delta^{18}O_{soil}$ ($\delta_{soil}$) were assessed from $\delta^2H_{swv}$ and $\delta^{18}O_{swv}$ readings considering thermodynamic equilibrium between soil water vapor and liquid phases according to (Rothfuss et al. 2013) (Fig. S2). Data acquisition at a 30-s time resolution as well as the flushing and dilution of the soil gas probes and gas-exchange chambers were automatized in LabView® (NI™, Austin, USA). The raw isotopic data was calibrated following Deseano Diaz et al. (2023) and Kühnhammer et al. (2020) against two soil water vapor standards ($\delta^2H_{st1} = 102.4 \pm 1.4‰$ and $\delta^{18}O\ _{st1} = 30 \pm 0.3‰$, $\delta^2H_{st2} = -78.4 \pm 0.6‰$ and $\delta^{18}O_{st2} = -18.8 \pm 0.1‰$).

## Aboveground physiological measurement and root traits

The number of leaves and tillers, the dimensions of the blades (length and width) and sheaths (diameter and length) as well as leaf blade chlorophyll content (SPAD 502Plus, Konica Minolta, Munich, Germany) were manually and non-destructively measured twice a week during growth. We related the transpiration to the total leaf area by adding the area of both sides of the leaves' blades and sheaths, although the latter is close to negligible compared to the former. The leaf blade surface area was calculated considering the measured length of each leaf blade, a constant increasing leaf width over times inferred from regular measurements points, and a coefficient of 0.75 to take into account the oblong wheat leaf shape (Chanda and Singh 2002). The leaf sheath surface was calculated considering it as a cylinder with an average diameter of 5 mm and a length equal to the maximum leaf sheath-distance of each tiller. The stomata density was obtained by destructively sampling the flag leaf, applying a nail polish to the bottom part and counting the number of stomata on the nail polish impression with a stereo microscope (Axiophot 2, Zeiss, Germany) (Pathoumthong et al. 2023).

During the 4th and 6th week of plant growth, the root traits of the plants in the six MRI columns were monitored non-destructively using a 4.7 T MRI magnet (Magnex, Oxford, UK) and a MR Solutions console (Guildford, UK). We constructed a linear axis on top of the magnet to handle these long and heavy soil columns (16 kg) and integrated this axis into the scanning software. 3D root scans at different soil column depths were realized to fully measure the root traits, which were concatenated afterwards. Signal to noise ratio allowed the detection of roots with a diameter > ~ 350 µm, which was sufficient to visualize the crown, the seminal and part of the lateral wheat root structure. Digital root fresh weight (DRFW, a.u) was obtained at 7 mm-vertical resolution by integrating over each depth interval the intensity of the horizontal MRI signal, following Deseano Diaz et al. (2023) and Ceolin et al. (2024) working with contrasted soil water conditions as well. The MRI signal intensity, which is proportional to the water content of the roots, was used as proxy of the root biomass. Comparison with the destructive analysis showed (WinRhizo™, see next paragraph), that these roots greater than 350 µm for our wheat lines at the booting stage represented 20% of the total root surface area on average across soil layers. Root angles and tips were also automatically extracted by processing the 3D MRI scans at the start-up stage using NMRooting software, and manually verified according to van Dusschoten et al. (2016). Then, the number of root tips was aggregated by layers of 2 cm soil depths.

At the end of the experiment (43 DaS), the roots in the WSI columns were destructively sampled from the soil layers 0–6, 6–17, 17–28, 28–39, 39–50, 50–61, 61–73, 73–80 cm, weighted and scanned (Expression 10000XL Model J181A; EPSON, Japan). The images were analyzed with WinRhizo™ (Regent Instruments Inc., Quebec, Canada) for determination of the root surface per root diameter classes in each of the aforementioned soil layers. The number of basal roots was assessed by counting them manually on the topsoil root scans and the total root biomass after scanning by drying and weighting them. Aboveground biomass was assessed by sampling the leaves, stems and ears, and drying and weighing them. The water use efficiency (WUE, g/L) was calculated as the ratio of whole plant (above- and below-ground) weight to the amount of cumulated transpired water between the sowing and the booting stage.

### Statistics and data representation

Statistical analyses were performed in R (Version 4.2.0; R Core Team 2020). Assumptions of normality and homoscedasticity of model residuals were evaluated using qqplots and Levene tests (Fox et al. 2001). When residuals showed substantial deviations from these assumptions, logarithmic or square-root transformations were applied to stabilize variance and improve residual distributions. The significance of differences was assessed with a linear mixed-effects model using the lme function from the nlme package (Pinheiro and Bates 2000). Genotype, depth, and water regime were considered as fixed effects, while columns and runs were treated as random effects. We used the following symbols to indicate statistical significance: “”: $p > 0.05$; “*”: $p < = 0.05$; “**”: $p < = 0.01$; “***”: $p < = 0.001$; “****”: $p < = 0.0001$. The comparison among treatments were carried out via the emmeans test adjusted with the bonferroni method with the package stat_pwc {ggpubr}. Differences in root tips count depth-related slopes among plant modalities were assessed using estimated marginal trends (emtrends) with post hoc pairwise comparisons adjusted using the Bonferroni correction. To interpolate the plant aboveground parameter evolution (e.g., leaf number) over the time, the local polynomial regression fitting function “loess” was used.

Daily-resolved depth profiles of the relative contribution of RWU to plant transpiration, normalized by the thickness of each layer (fraction of Root Water Uptake; fRWU), were computed from 39 to 42 DaS using the multi-source mixing model Stable Isotope Analysis in R (SIAR) (Parnell et al. 2010) following the method of Couvreur et al. (2020) and Deseano Diaz et al. (2023). The parameters (prior, number of iterations, and values for burn-in and thinning) of the function siarmcmcdirichletv4 of the R package “siar” used are the same as those described in Deseano Diaz et al. (2023). The function uses a Markov chain Monte Carlo algorithm to produce estimated source proportions (water at different soil layers) in the observed product (the plant transpiration). These estimates on soil liquid water ($\delta_{soil}$) and transpired water vapor ($\delta_{Tr}$) isotopic compositions, assumed steady state transpiration yielding to $\delta_{Tr} = \delta_{RWU}$. For each ‘plant modality x water treatment’ combination, we had three repetitions of columns measured over four successive days, giving a maximum of twelve fRWU profiles per combination, except when a technical problem during the measurement hindered us from obtaining either the soil profile or the plant transpiration data. The fRWU contribution from 18 and 20; 40 and 42, 62 and 64 cm depth were grouped together to better represent respectively the soil layers 14–30, 30–52, 52–69 cm. The Sink Term (mL $\text{min}^{-1}$ $\text{cm}^{-1}$) was calculated as the product of the fRWU ($\text{cm}^{-1}$) and the transpiration rate (mL $\text{min}^{-1}$) calculated from the water vapor mixing ratio measured with the Picarro laser spectrometer, according to the plant transpiration rate formula in Deseano Diaz et al. (2023).

## Results

### Root and shoot traits

Across plant modalities and water treatments, the total dry biomass (1.7 ± 0.4 g) and root surface area (32 ± 6 $\text{dm}^2$) was very similar (Table S1). Although the differences were not significant, we note that the MIX led to higher biomass than both DRS and SRS monocultures in WW (respectively 56% and 26% more) but was intermediate under WD (resp. 2% more and 7% less). The root crown number was significantly higher under WW treatment than under WD (water: $p < 0.001$). Under both WD and WW treatment, the mixture MIX showed on average higher root crown numbers than both monocultures (12% and 15% less respectively under WW; 5% and 15% less under WD; non-significant (NS)) (Table S1). The average root crown angles were similar among the plant modality and the water treatments (Fig. S3).

The digital root fresh weight (DRFW), root tips density and the root surface densities calculated from scanned roots were significantly higher in the topsoil than in the other soil layers (depth: $p < 0.001$) (Fig. 3, S4, S8). Thus, the depth of 70% of the cumulative DRFW (D70, cm) deepened with WD from an average depth of 30 cm to a depth of 48 cm (water: $p < 0.01$, Table S1). Root distribution was greater in the topsoil under the WW treatment than under the WD treatment (10% difference at this soil layer) and the opposite was observed in subsoil layers (depth x water: $p < 0.001$), i.e. from 6 to 12% in average at 70–80 cm (Fig. 2). The root surface area of SRS was greater than that of DRS for root diameters between 150 and 300 µm. The opposite was observed in the subsoil (Fig. S4).

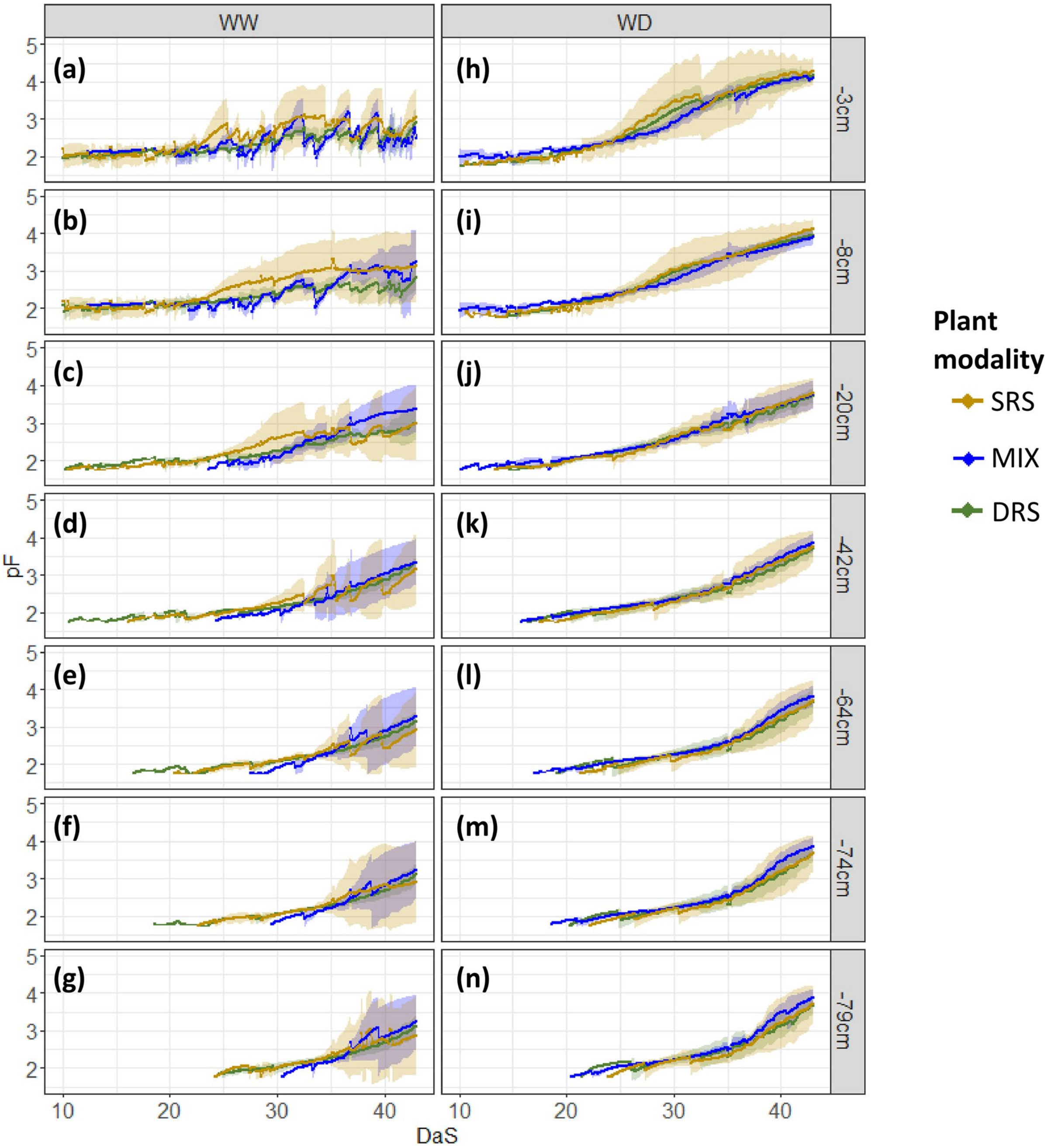


**Fig. 2** Evolution of soil water potential, expressed in pF, average (solid line) and standard deviation (transparent envelope) measured using Teros21 sensors, for well-watered treatments ("WW," panels a to g) and water deficit treatments ("WD," panels h to n) among seven soil depths displayed on the right-hand side (3, 8, 20, 42, 64, 74, 79 cm). The colors represent the three crop types: DRS monoculture, dark green; mixture – MIX, blue; and SRS monoculture, orange

The DRFW was greater for SRS than for DRS in the topsoil from 0 to 10 cm, whereas DRS showed higher DRFW at soil depths below 40 cm (Fig. 3) and SRS D70 was shallower than DRS whatever the water treatment (Table S1, NS). The root surface densities generally showed the same patterns in some

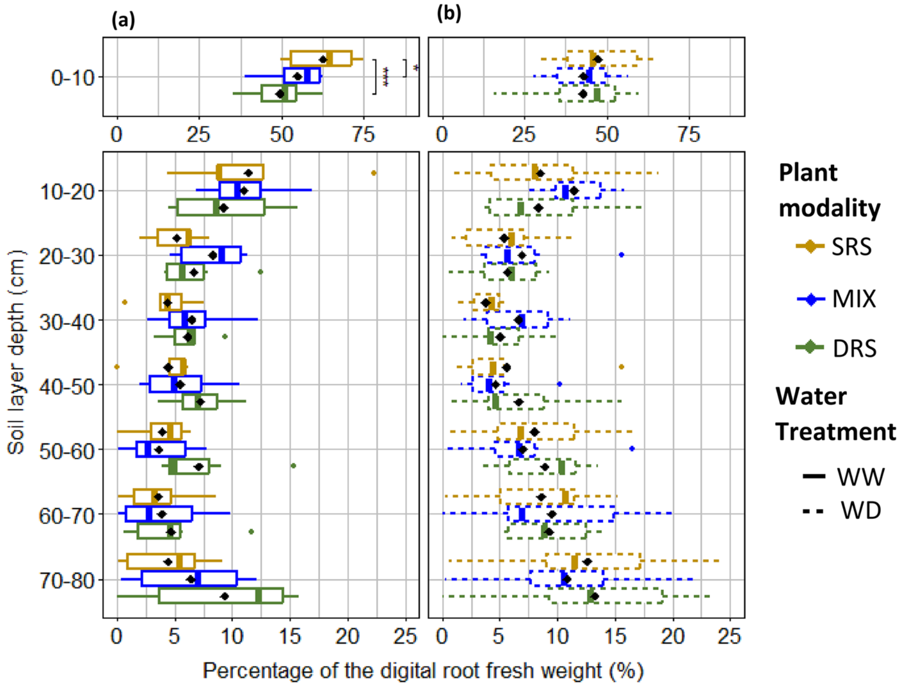


**Fig. 3** Distributions of the percentage of digital root fresh weight (DRFW) per 10 cm soil layer, measured in situ by MRI at 43 DaS for the well-watered (WW, lines, panel a) and water deficit (WD, dots, panel b) treatments among the plant modalities (DRS in monoculture, dark green; Mixture – MIX, blue; and SRS in monoculture, orange) (n = 6)

root diameter classes (Fig. S4). Root distribution across the profile in the MIX treatment was generally between SRS and DRS or on the same level (Fig. 3). SRS showed a steeper reduction in root tip density with depth, whereas DRS plants maintained higher root tip numbers in subsoil layers (plant × depth: $p < 0.001$). The MIX displayed an intermediate response (Fig S8).

The plant shoot dry biomass at the elongation stage was not significantly different among the treatments and modalities (2.6 ± 1.1 g). Differences between water treatments began to appear on the surface at 35 DaS (stem elongation) and were significant at the booting stage (43 DaS). At this stage, the water deficit treatment significantly decreased the plant shoot surface able to transpire (water: $p < 0.05$) (Fig. 4 a-d) and the tiller number per plant became significantly lower for DRS as compared to SRS (plant: $p < 0.05$), which was pronounced for the WD treatment (water x plant: $p < 0.01$) (Fig. 4 e–h). However, we noted no significant differences for the leaf stomatal density between the two genotypes (Table S1).

The tiller/crown root ratio, however, was significantly higher under WD than WW (water: $p < 0.01$), and SRS showed higher values than MIX and DRS (plant: $p < 0.05$, Table S1). The biomass and surface area ratios of aboveground and belowground organs remained stable regardless of water treatment and plant modality (resp. 2.92 ± 0.72 and 0.75 ± 0.29).

### Soil water status

With regard to soil water status, a gradual increase in average pF was observed in the different soil horizons as the plants developed during the 43 days after sowing (Fig. 2). The average value in pF was lower than 3 in WW due to the regular addition of water to the top of the column, but exceeded this value in WD for the topsoil from 30 DaS onwards. During the early stages of plant development, soil water availability was fairly similar across the three treatments, and even higher in the mixture than in the monoculture (which could reflect less intense water uptake). From 35 days after sowing, which corresponds to the transition between the elongation and tillering stages, the trend in the subsoil (−42, −64, −74, and −79 cm depth) changed in both WD and WW, with MIX decreasing soil water availability quicker than the two monocultures (Fig. 2).

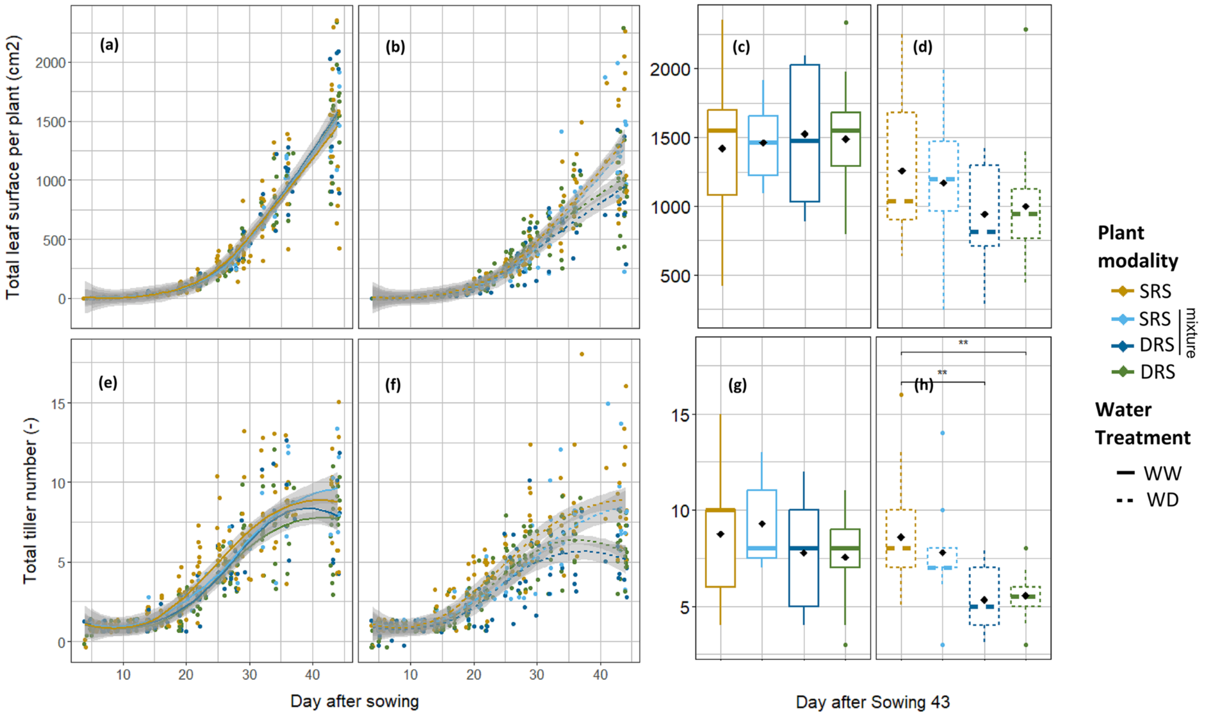


**Fig. 4** Total leaf area per plant (in cm$^2$, panels a, b, c, and d), and number of tiller per plant (panels e, f, g, and h) for the four plant modalities (DRS monoculture, dark green; DRS mixture, dark blue; SRS mixture, light blue; SRS monoculture, orange) under well-watered conditions (WW, lines, panels a, c, e and g) and water deficit (WD, dots, panels b, d, f and h), throughout growth, modelled by LOESS regression (Locally Estimated Scatterplot Smoothing, 95% confidence interval) (a, b, e and f) and at 43 DaS (c, d, g and h) (n = 18 for SRS and DRS in monoculture, n = 9 for SRS and DRS in mixture)

More precisely at the booting stage (between 39 and 42 DaS), the matric potential was significantly higher in water-deficit (WD) conditions (pF 3.7 ± 0.4 on average) than in well-watered (WW) conditions (pF 2.8 ± 0.6) (water: $p < 0.01$, Fig. 5 and Table 1). The total transpiration throughout the entire experiment (water: $p < 0.05$, Table S1) as well as the hourly transpiration rate between 39 and 43 DaS (water: $p < 0.001$, Fig. S5) were significantly lower under WD. However, the WUE was similar across water treatments and plant modalities (2.38 ± 0.52 mL g$^{-1}$, Table S1).

The SMP for the different plant modalities showed significant differences at each depth (plant x depth: $p < 0.0001$). In the interactions with water treatment, we observed under WD that SMP profiles all decreased from the topsoil to the subsoil layers for all three plant modalities. However, under optimal WW conditions, the SMP profiles of SRS and DRS were contrasting (Fig. 5). For SRS, SMP profile decreases from dry topsoil (2.62 ± 0.42) to moister subsoil (2.43 ± 0.73). For DRS, the SMP increased from the moist topsoil (2.42 ± 0.35) to the drier subsoil (2.98 ± 0.39), with a local maximum in the middle at 42 cm (3.16 ± 0.34). MIX behaved intermediately, similar to DRS (from moist topsoil to drier subsoil) but with more variability and a shallower local maximum than the DRS one, at 20 cm (3.28 ± 0.49) (Fig. 5).

Thus, this effect of water treatment differed between plant modalities and between profiles, with SRS showing an almost constant difference at all depths, while DRS showed pronounced differences in the upper soil layers and the MIX treatment showed the smallest difference at a depth of 20 cm (plant x water x depth: $p < 0.001$, Fig. 5). When we compared the matric potential “WW-WD” in MPa and not on a logarithmic scale, we found that the largest absolute difference in SMP was in the topsoil and was the most pronounced for SRS (Fig. S6).

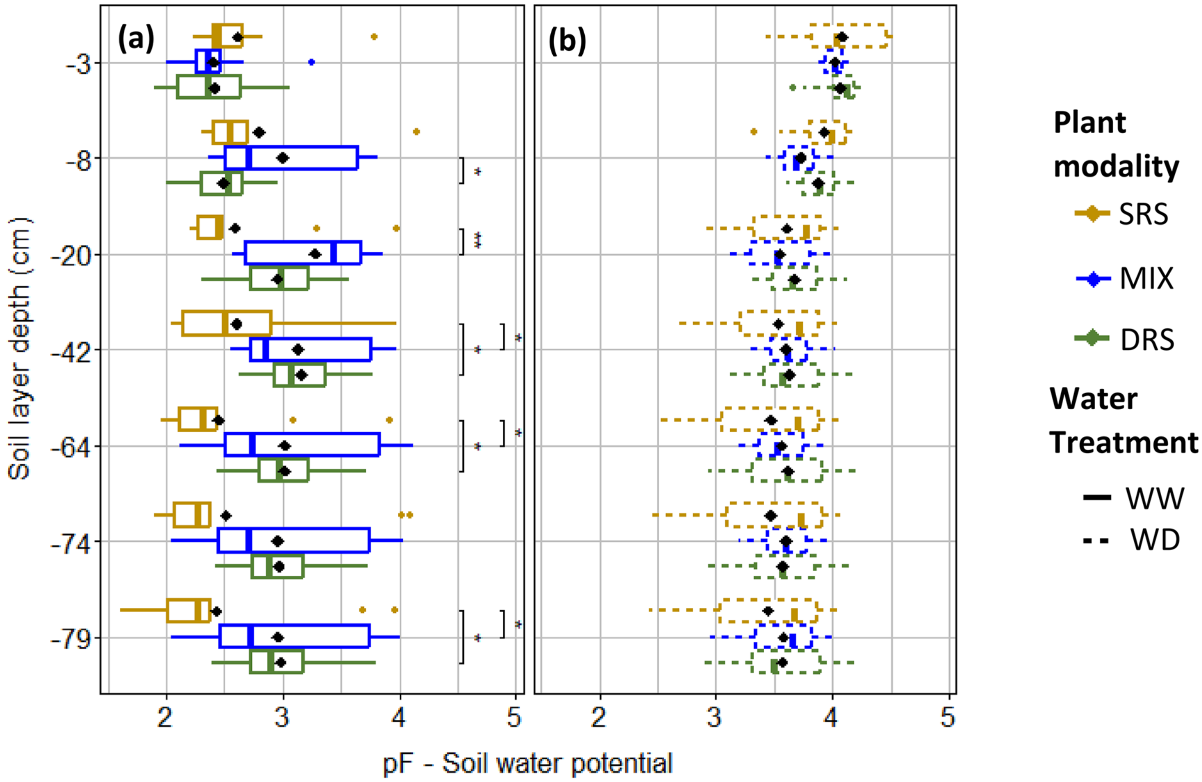


**Fig. 5** Soil depth profile distribution of water potential between 39 and 42 DaS in the afternoon during the plant transpiration measurements, expressed as pF, for the well-watered (WW, lines, panel a)) and water deficit (WD, dots, panel b) treatments among the plant modalities (DRS in monoculture, dark green, panel a; Mixture – MIX, blue, panel b; and SRS in monoculture, orange, panel c) ($n = 3$)

## Root water uptake

At the booting stage between 39 and 42 DaS, the wheat roots extracted water more intensively from the topsoil layer (the top 7 cm; 4.04 ± 0.75% cm$^{-1}$ in WW; 4.33 ± 1.4% cm$^{-1}$ in WD), less intensively from subsoil (69 to 80 cm; 1.46 ± 0.65% cm$^{-1}$ in WW; 1.96 ± 1.03% cm$^{-1}$ in WD). The least of the fRWU contributions came from intermediate soil layers (0.67 ± 0.34% cm$^{-1}$ WW; 0.57 ± 0.30% cm$^{-1}$ WD) (Fig. 6). However, this pattern changed with the reduction in water availability with the root water uptake intensifying mainly in the subsoil (also to a lesser extent in the topsoil) and decreasing in the intermediate soil layers (depth x water: $p < 0.001$).

The fRWU profiles were also affected by the genotypes with SRS having extracted more water from the topsoil layer than DRS while the opposite was observed in the subsoil layer (depth x plant: $p < 0.001$) (Fig. 6). These differences between DRS and SRS were more prominent for WD compared to WW (water x depth x plant: $p < 0.001$). We observed this same trend within the mixtures under WD, where SRS in mixture (4.23 ± 1.54% cm$^{-1}$) extracted more water than DRS in mixture in the first 7 cm soil layer (3.19 ± 0.87 cm$^{-1}$) and less in the 76–80 cm subsoil layer (2.34 ± 0.35% cm$^{-1}$ SRS in mixture; 3.50 ± 0.54% cm$^{-1}$ DRS in mixture). Thus, under WD we noted a constant absolute difference between DRS and SRS (both in monoculture and mixture) of 1.09 ± 0.46% cm$^{-1}$ from 0 to 7 cm and from 76 to 80 cm (Fig. 6b).

In a similar vein, it was observed that in mixtures, the absolute root water uptake (Sink Term; mL min$^{-1}$ cm$^{-1}$) of SRS in the topsoil were higher than those of DRS under WW ($p < 0.001$) (Fig. 7a). Under WD, the SRS also absorbed more water than the DRS between 0 and 2 cm ($p < 0.05$), but in the subsoil

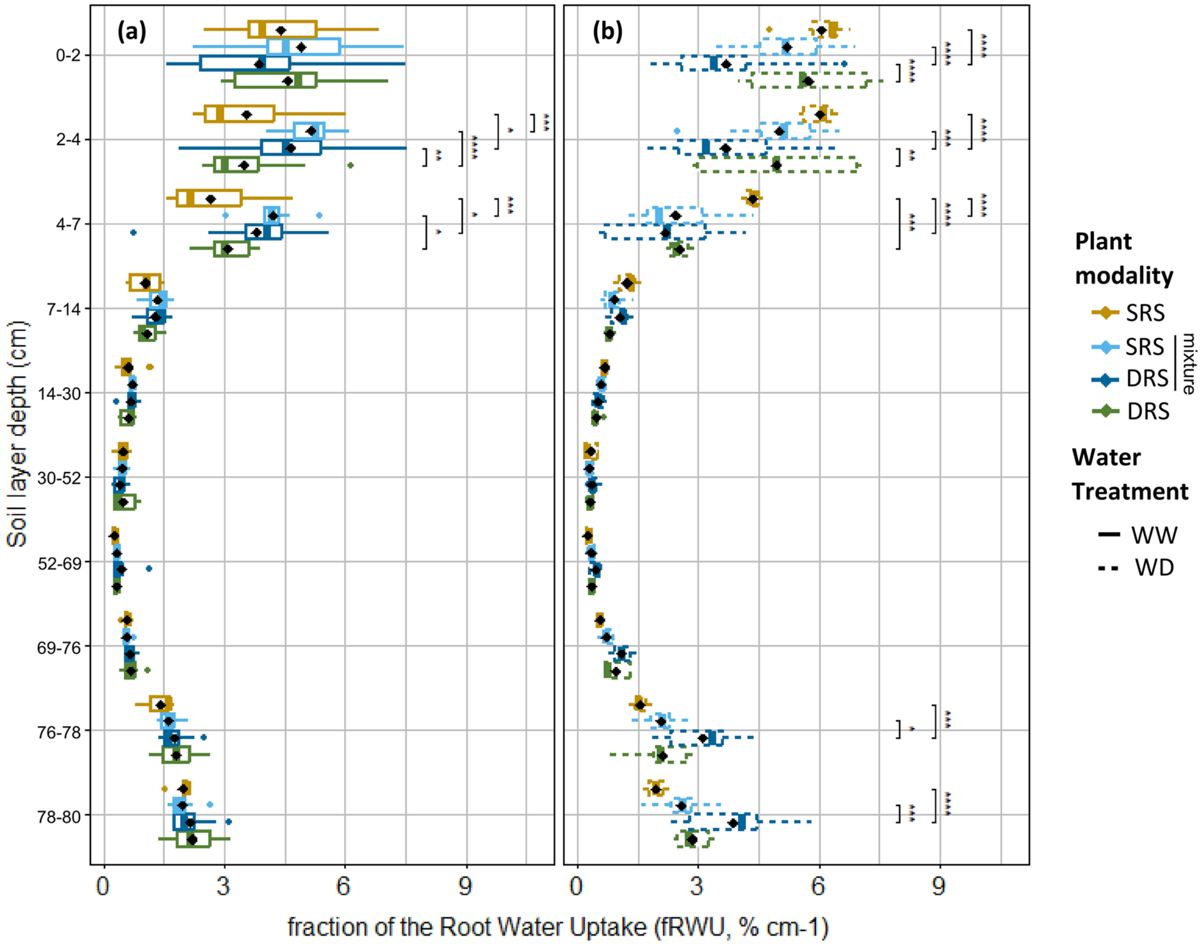


**Fig. 6** Fraction of the Root Water Uptake (fRWU, % cm$^{-1}$) distributions (panels a and b) and mean values (panels c and d) estimated between 39 and 42 DaS, for the four plant modalities (DRS in monoculture, dark green; DRS in mixture, dark blue; SRS in mixture, light blue; SRS in monoculture, orange), across ten soil layers (0–2; 2–4; 4–7; 7–14; 14–30; 30–52; 52–69; 69–76; 76–78; 78–80 cm) under well-watered (WW, lines, panels a) and water deficit (WD, dots, panels b) conditions (n = 7–12)

DRS absorbed much more water than SRS ($p < 0.001$) (Fig. 7b). However, in monoculture under WW, DRS transpired significantly more than SRS (Fig. S5) and differences appeared in the topsoil ($p < 0.05$) (Fig. 7c) while under WD, with more equivalent transpiration, DRS absorbed more water than SRS in the subsoil ($p < 0.05$) (Fig. 7d). In addition to this relative difference between SRS and DRS, it was found that both genotypes in mixture under WD reduced their water extraction from the topsoil compared to monoculture (−1.2% cm$^{-1}$ on average for layers 0 to 7 cm; at these depths, water x plant: $p < 0.001$). In contrast, both increased their fRWU from the subsoil layers in mixture (+0.5% cm$^{-1}$ for layers 69 to 80 cm; at these depths, water x plant: $p < 0.001$) (Fig. 6b). However, this was reversed under WW, with both SRS and DRS in mixture extracting more water from the surface than the same genotypes in monoculture (+2.0% cm$^{-1}$ from 2 to 7 cm depth) (Fig. 6a).

The amplitude of root water uptake plasticity in response to water deficit differed for each plant modality (Fig. S7). By calculating the difference between the fRWU profiles under WD and those under WW, it can be observed that SRS exhibited a significantly higher level of contribution in the initial 30 cm of soil in order to cope with water deficiency. This result was less pronounced for DRS in subsoil, but it greatly increased the amplitude of

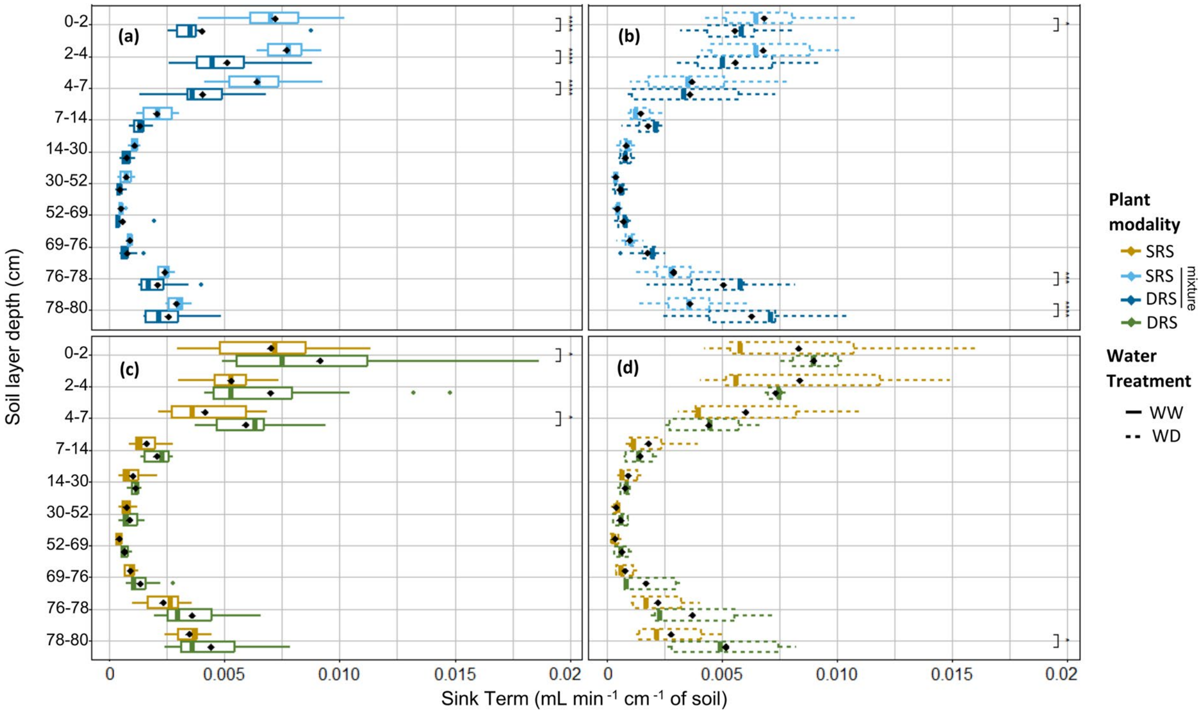


**Fig. 7** Distributions of the Sink Term (mL min$^{-1}$ cm$^{-1}$) estimated between 39 and 42 DaS, for the four plant modalities (DRS in monoculture, dark green; DRS in mixture, dark blue; SRS in mixture, light blue; SRS in monoculture, orange), across ten soil layers (0–2; 2–4; 4–7; 7–14; 14–30; 30–52; 52–69; 69–76; 76–78; 78–80 cm) under well-watered conditions (WW, lines, panels a and c) and water deficit (WD, dots, panels b and d) (n = 7–12)

the fRWU's shift toward subsoil (i.e., its "natural niche") in response to water deficit. In the context of mixture, both DRS and SRS exhibited comparable WW-WD plasticity profiles for fRWU, which resulted in a decrease in their contribution in the topsoil and an increase in the subsoil. However, this effect was more pronounced for DRS than for SRS (Fig. S7).

When the vertical distribution of fRWU (% cm$^{-1}$) and root biomass density (dDRFW, % cm$^{-1}$) were compared layer by layer, it was observed that most points were proportional (Fig. 8). The mixed linear regression of dDRFW explaining fRWU, taking into account the random effect of depths, shows a conditional R$^2$ of 0.67. Two main areas were identified where biomass and water uptake values were found to be non-proportional. Firstly, in the topsoil (0–4 cm), root biomass densities were found to be almost twice as high as their respective water uptake fractions. Secondly, in the subsoil, between 76 and 78 cm deep, the fRWUs were much higher than their corresponding root biomasses. Furthermore, it was observed that for the SRS under WD at 4–7 cm, the fRWUs exhibited higher values in comparison to the dDRFWs (Fig. 8).

## Discussion

### Root traits and water uptake

We corroborated the first hypothesis, namely that the SRS genotype in monoculture extracts more water in the topsoil layers than the DRS genotype in monoculture, and the converse is observed in the subsoil layers, especially under chronical water deficit conditions (Fig. 6, 7). The soil pF profiles at this booting stage are consistent with this, showing significant differences between genotypes, reflecting contrasting histories of root water (Fig. 5). Previous modeling studies have shown that root ideotype water uptake profiles can be partially explained by root biomass

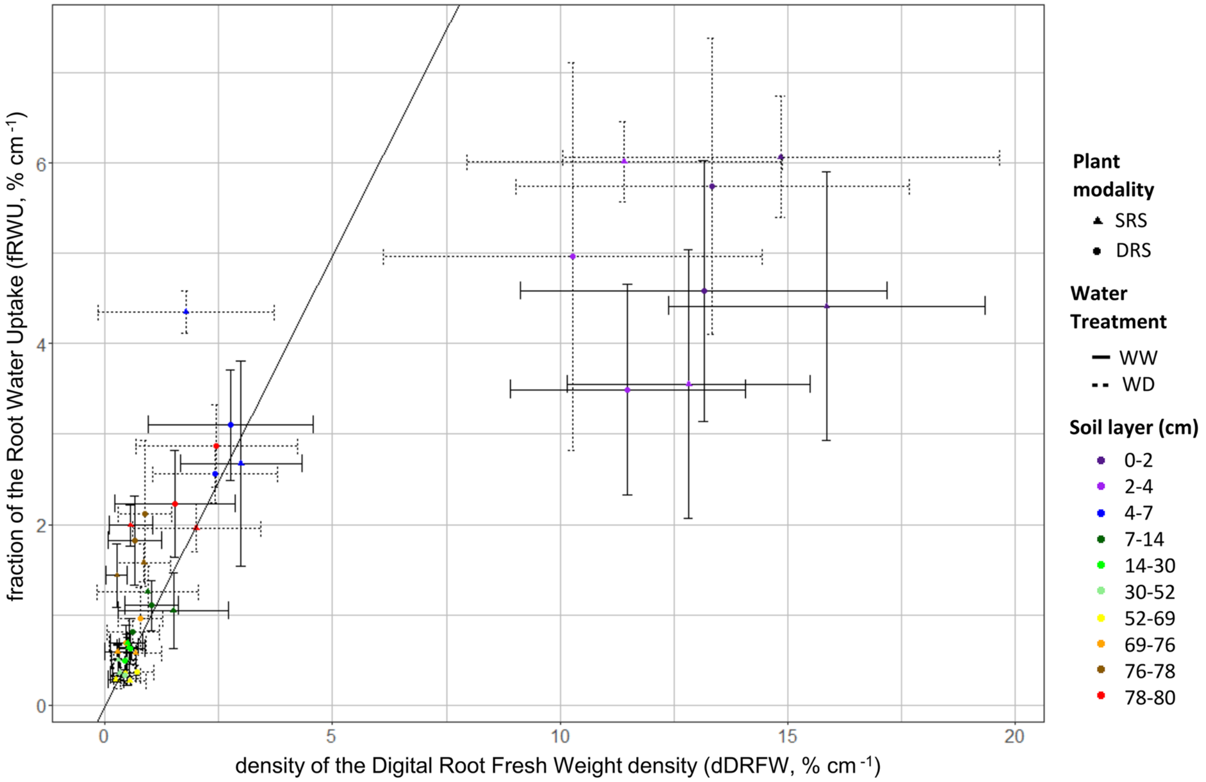


**Fig. 8** Density of the Digital Root Fresh Weight (dDRFW, % cm$^{-1}$, measured at 43 DaS) over the fraction of Root Water Uptake (fRWU, % cm$^{-1}$, estimated between 39 and 42 DaS). The points represent the average value of two plant modalities (DRS in monoculture, circle shape; SRS in monoculture, triangle shape), across ten soil layers (0–2; 2–4; 4–7; 7–14; 14–30; 30–52; 52–69; 69–76; 76–78; 78–80 cm) under well-watered conditions (WW, lines) and water deficit (WD, dots). Black bars indicate standard deviations

and morphology distributions (Fort et al. 2017; McMurtrie et al. 2012; Bramley et al. 2009). This was observed in our experimental study as the distribution of root biomass, fine root surface and root tip density exhibited higher values in the topsoil for SRS and in the subsoil for DRS (Fig. 3; S4; S8). Lattacher et al. (2025b) also observed in the same experiment, following a $^{13}$C-CO$_2$ labelling, a trend towards an increased δ$^{13}$C signal in the subsoil roots for MIX and DRS compared to SRS in both water treatments, thus confirming the contrasting depth-related root activities exhibited by these genotypes. While the root biomass density profile (dDRFW, % cm$^{-1}$) can explain most (67%) of the variability in fRWU distribution (% cm$^{-1}$) for both genotypes in monoculture, this relation is not constant at all depths—water uptake by root biomass units is lower in the topsoil and higher in the subsoil (Fig. 8). Physiological processes such as suberization, cambial growth and root hair loss may affect the water uptake properties of the oldest roots in the topsoil, as such alterations have been reported in wheat and other Poaceae during earlier stages of development (Kreszies et al. 2019; Schneider et al. 2020; Terletskaya et al. 2020; Guhr et al. 2025).

It is noteworthy that, while the disparity in basal root angles between the two genotypes – the initial selection criterion – proved significant in the early stages of development (Rambla et al. 2022), in our experiment at booting stage these differences were only marginal and no longer significant (Fig. S3). This highlights the fact that the early characterization of root traits is not necessarily a good indicator for later stages. Therefore, it is imperative to validate these root traits results at the different studied stages of development (Comas et al. 2013).

We acknowledge that our experimental system standardized soil and climate conditions, which are more heterogeneous in agricultural fields. However, we believe this simplification was necessary in order to observe specific plant responses to water treatments and a first step to prove the concept. Our conditions were based on a typical temperate climate in Western Europe, comparing average (WW) or dry (WD) spring conditions. We used soil from the same pedoclimatic ensemble, compressed to an average bulk density observed in the field (Weihermüller et al. 2007). We simulated rainfall using surface irrigation and modulated light intensity sinusoidally to reproduce average daily sunlight variations. Additionally, we induced a temperature gradient between the light-exposed topsoil and the insulated subsoil, similar to what is found in field conditions (Wang et al. 2025). These efforts enabled us to obtain root distribution patterns similar to those observed in field conditions in the same loamy soil or in other studies, consistently higher in the topsoil than the subsoil (Svoboda et al. 2020; Zhao et al. 2023; Nguyen et al. 2024). However, the depth of our soil column (80 cm) may have caused root accumulation, concentrating processes that typically span tens of centimeters into the final few centimeters (Fig. 3).

### Genotypes strategies facing water deficit

In the present study, we observed that the average fRWU and root biomass for all plant modalities in the subsoil under WD increased (Fig. 3, 6). This strategy was more pronounced for DRS than SRS in monoculture, reflecting their natural niche preferences (Fig. S7). However, in the topsoil under WD, where water availability has been shown to decrease significantly (from pF 2.5 to 4), the fRWU increased on average. This increase was driven by maintained high contributions from DRS in monoculture and an increase in SRS in monoculture, with no significant change in root biomass (Fig. 8). This finding aligned with the extant literature, which also indicated that several grassland and crop species facing a water deficit showed a preference for maintaining or even increasing root water uptake from the topsoil, despite its lower soil water availability (Deseano Diaz et al. 2023; Prechsl et al. 2015; Angeletti et al. 2022). This finding subsequently invalidate the second hypothesis, which postulated that water resource availability may predict root water uptake, as proposed by Kulmatiski et al. (2017). Thus to correctly estimate root water uptake, we showed that it is essential to consider both the environmental factors, such as soil water availability, and plant individual root traits, including distribution of root biomass and morphology (Delval et al., 2024; Müllers et al. 2023; Rickard et al. 2025).

Furthermore, the root water uptake strategy in response to water deficit was not uniform, but rather contingent on the genotype-specific root traits and those of the neighboring plant. The water niches differences could be partially attributable to the distribution of fine root surface area (less than 250 µm), which is more abundant in the topsoil of the SRS than the DRS under WD (Fig. S4). This root trait may have resulted, for SRS under WD, in better root hydraulic conductance and greater root exudation in this soil layer, enhancing the soil's hydro-physical properties in the rhizosphere (Bramley et al. 2009; Doussan et al. 2024; Le Gall et al. 2024; McDougall and Rovira 1970). In addition, arbuscular mycorrhizal fungi (AMF), which have been shown to be able to improve RWU, have been found to be more prevalent in the topsoil of SRS monoculture, and conversely in the DRS monoculture subsoil (Zaman et al. 2024; Lattacher et al. 2025b).

Most articles on root traits suggest that deep roots improve the plant's resistance to drought (Uga et al. 2013; Shoaib et al. 2022; Kou et al. 2022; Odone et al. 2024; Bagale et al. 2025). However, this was not what we observed in our study. Although the root traits and RWU of DRS were oriented toward the subsoil, where water was more accessible, SRS oriented toward topsoil resisted better to water deficit, as evidenced for DRS by the higher reduction in the number of tillers and total leaf area from 35 days after sowing, i.e., nearly a week before the booting stage studied (Fig. 4). Similar evidences have been demonstrated for trees (Juglans regia) and perennials (Cichorium intybus L.) that increasing the proportion of deep root water uptake facing water deficit does not guarantee greater water deficit resistance (Sun et al. 2011; Rasmussen et al. 2020). To the best of our knowledge, this is the first demonstration of this phenomenon for the wheat. This observation could be explained by aboveground traits such as higher stomatal density for DRS than SRS, resulting in a reduce drought tolerance (Hasanuzzaman et al. 2023; Robertson et al. 2023)(Table S1). The deep-rooting genotype may have also incurred a higher metabolic cost and a greater investment in photoassimilates than SRS to maintain deep root activity, leading to reduced shoot growth or limited stomatal regulation in response to prolonged water deficit (Li et al. 2022; Tardieu 2012).

### A niche complementarity in the wheat genotype mixture?

The third hypothesis of our study focused on the conservation of contrasted root water uptake distribution of the two genotypes in the mixture, allowing for "hydrological niche segregation" (Zhao et al. 2024). In the mixture, the two genotypes exhibited distinct fRWU profiles corresponding to their respective preferred niches, a particularly significant characteristic under water-deficient conditions (Figs. 6 and S7). Lattacher et al. (2025b) used an independent method involving $^{15}$N labeling to demonstrate that the SRS genotype in monoculture extracted more nitrogen (enabled by the root water uptake, which contains nutrients) from the topsoil than the DRS, with a more pronounced effect in mixtures under water deficit conditions (Gorska et al. 2008; Plett et al. 2020). These observations supports the view that slight variations in root biomass distribution between two root systems developed in the same soil profile (Fig. 3) can have a significant impact on water uptake patterns (Kulmatiski et al. 2020). The maintenance, or even increase, of these contrasting nutrient and water uptake patterns by the genotypes allowed better spatial distribution of access to these resources under water deficit in the mixtures, which can partly explain the high or higher yield and protein quality in such condition (Vidal et al., 2020; Kong et al. 2023; Stefan et al. 2025).

Furthermore, it was observed that, in addition to niche complementarity in terms of access to water and nutrients, the SRS and DRS mixtures were associated with an increase in fRWU towards the subsoil layers and a decrease in the topsoil layer in situations of water deficit (Figs. 6 and S7) compared to the monocultures. In addition, a significantly higher number of seminal roots was observed in the mixture than in the two monocultures, regardless of water treatment (Table 2). According to Lynch (2022), this high number of taproots could have be linked to increased foraging in the topsoil, leading to increased competition at the surface and forcing a shift to deeper horizons. However, no significant faster diminution of water availability in the topsoil of the MIX treatment compared to the two monocultures was observed before the end of the elongation phase and until the beginning of the stem elongation phase (35DaS) (Fig. 2), which could have forced them to increase their contributions from the subsoil layers. Therefore, surface competition at early vegetative stages between individual plants would not be sufficient to fully explain the process of niche displacement towards the subsoil in mixture.

The emerging niche-shift in mixture towards subsoil layers would be therefore the result of the interaction of the two genotypes, compared to their behavior in monoculture. To our knowledge, this is the first documented instance of this phenomenon being observed among genotypes of the same species, specifically *Triticum aestivum* L. But this emerging pattern of water uptake niche-shift toward subsoil layers has been observed between different tree species (Demir et al. 2024), grassland species (Guderle et al. 2018) and crop species (Schmutz and Schöb 2023) compared to monocultures. In order to understand this phenomenon, the sole perspective of higher topsoil competition between individuals faced with the scarcity of the same resource to exploit (Tragedy of the Commons) during the early stages of surface development is not sufficient. (Montazeaud et al. 2025) showed that wheat variety mixtures actually reduced root competition for water and nitrogen between individuals during the early stages of development. Indeed, our study did not observe any greater initial depletion of soil water in mixed crops compared to monocultures, indicating that there was no early increase in water extraction competition. We therefore propose that mechanisms enabling recognition of whether another individual belongs to the same genotype may have been at work through chemical communication between roots, leading to changes in their individual physiology and function (e.g., number of shoots and primary roots, and deeper water uptake). In our study, this communication could have been mediated by soluble pathways, as demonstrated by Ghatak et al. (2025) for several dozen wheat genotypes. Each genotype exudes a distinct metabolome, capable of inducing a specific physiological response in neighboring plants of a different genotype (e.g., allelopathy or allelobiosis; Mathieu et al. (2025)). During the four days of fRWU measurement, if this communication existed, it could only have been maintained underground in a mixture, as each individual was isolated in two gas exchange chambers independent of each other.

The plasticity of the water uptake profile by roots is therefore not only determined by the root traits of the genotype and the characteristics of soil water availability (Fromm 2019), but also by the interaction with the root traits of neighboring plants, i.e., in this case, the cultivation practice of wheat in mixture or monoculture. This implies that when selecting wheat varieties for mixed cropping, characteristics can be selected not only in single-species trials but also in functionally complementary combinations. Genotype-specific root traits, such as the number of seminal roots or fine root surface area rather than root angle insertion, could be used in experimental and modeling approaches to design mixtures of root ideotypes that improve water use efficiency and yield stability compared to monocultures. However, we highlighted too that breeding deep-rooted systems does not guarantee better drought resistance and may even be disadvantageous, as in our case. To improve the plant's water deficit resistance, breeding efforts should consider both root and shoot traits. Thus, we call on the scientific community and breeding programs to provide increasingly comprehensive descriptions of crop root traits to enable the study, improvement, and use of lines that maintain or enhance beneficial plastic responses to climate change and ensure better yields through crop mixture systems.

## Conclusion

This study demonstrates that mixing wheat genotypes with contrasting root traits (deep- and shallow-rooting) leads to complementary root water uptake strategies, particularly under water deficit. Using stable isotope tracing ($\delta^2$H and $\delta^{18}$O) and high-resolution root imaging, we quantified distinct vertical RWU patterns: the shallow-rooting genotype (SRS) absorbed significantly more water from the top 7 cm of soil, while the deep-rooting genotype (DRS) showed greater uptake in deeper soil layers (69–80 cm), especially when water was limited.

In mixtures, these uptake patterns were preserved, with RWU shifting toward deeper layers under drought—by approximately 0.5% cm$^{-1}$—while decreasing in the topsoil. The number of crown roots was also higher in mixtures compared to monocultures, suggesting enhanced soil exploration despite similar total root biomass. To our knowledge, this is the first time that hydrological niche segregation and shift towards subsoil has been observed between genotypes of the same species. Furthermore, these findings suggest that contrasting wheat root trait mixture may enhance access to deeper water sources and root water uptake complementarity during periods of water deficit at this critical growth stage, which could potentially lead to an increase in yield.

This study highlights how fine-scale monitoring of root water uptake can reveal the benefits of intraspecific diversity for improving water use in cropping systems. However, it also demonstrates that high-resolution root trait data alone are insufficient to predict RWU distribution under heterogeneous matric potential conditions, emphasizing the need for direct or indirect RWU measurements through, for instance, stable isotope labelling experiments. However, the effect of water deficit on intraspecific root interaction during plant diurnal cycles, which allows potential hydraulic lifting to be observed, remains to be studied. In order to corroborate the outcomes of this controlled condition experiment comparing two wheat species with contrasting root systems, we recommended to pursue the research in real-world conditions and to increase the number of compared genotypes per root type (shallow/deep roots). Finally, the results of this study support the strategic design of genotype mixtures as a practical approach to enhance resilience to drought, offering potential benefits for yield stability and resource efficiency under climate change conditions.

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**Authors' contributions** Samuel Le Gall, Adrian Lattacher, Mona Giraud, Holger Pagel, Andrea Schnepf, Guillaume Lobet, Ellen Kandeler, Christian Poll, Mathieu Javaux and Youri Rothfuss developed the study concept. Samuel Le Gall, Dagmar van Dusschoten, Adrian Lattacher, Mona Giraud, Moritz Harings, Paulina Deseano Diaz, Guillaume Lobet, Christian Poll, Mathieu Javaux and Youri Rothfuss contributed to build the experimental design and conduct the study. Samir Alahmad and Lee Hickey developed and provided the required wheat genotypes. Samuel Le Gall and Moritz Harings constructed, automated and adjusted the entire experimental platform, according to the recommendations of Paulina Deseano Diaz, Dagmar van Dusschoten and Youri Rothfuss. Samuel Le Gall, Dagmar van Dusschoten, Adrian Lattacher, Mona Giraud, Moritz Harings, Ahmet Sircan and Youri Rothfuss conducted the column experiment in the climate chamber and collected the data. Daniel Pflugfelder and Dagmar van Dusschoten assisted Samuel Le Gall in performing and analyzing the MRI scans. Samuel Le Gall analyzed the samples, evaluated the data and performed statistics with the contribution of Christian Poll, Mathieu Javaux and Youri Rothfuss. Samuel Le Gall wrote the first draft of the manuscript under the supervision of Mathieu Javaux and Youri Rothfuss. All authors critically revised previous versions of the manuscript.

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**Data availability** The datasets generated during this study are available from the corresponding author on request.

**Code availability** This is not applicable to the manuscript.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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