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RESEARCH ARTICLE

Temporary growth cessation of wheat roots following defoliation

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Abstract

Background and aims Defoliation triggers the remobilisation of root reserves to generate new leaves which can affect root growth until the shoot resumes net assimilation. However, the duration of root growth cessation and its impact on resource uptake potential is uncertain.

Methods Winter wheat was established in a 4 m high outdoor rhizobox facility equipped with imaging panels, sensors, and access points for tracer-labelling. The wheat was defoliated in autumn at early tillering and roots were imaged at a high-time resolution and analyzed by deep learning segmentation. The water and nitrogen (N) uptake were measured using time-domain reflectometer (TDR) sensors and ^{2}H and ^{15}N isotopes.

Results Root penetration of wheat paused for 269 °C days (20 days) following defoliation after which it resumed at a similar rate to un-defoliated plants (1.8 mm °C days^{-1}). This caused a substantial decrease in root density with an associated reduction in water and N uptake at maturity, especially from deeper soil layers (>2 m).

Conclusions Our results have significant implications for managing the grazing of dual-purpose crops to balance the interplay between canopy removal and the capacity of deep roots to provide water and N for yield recovery.

Keywords Deep learning · Dual-purpose cropping · Image analysis · Stable isotope · TDR sensor

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Introduction

Plant root growth can be affected by the removal of the shoot canopy as the supply of photosynthates is interrupted, and remobilisation of reserves from roots is stimulated for the initiation of new leaf generations (Hay and Porter 2006). For example, under perennial pasture management optimising the time between grazing to recharge root reserves has been shown to be critical to support the re-growth of the shoot canopy (Moot et al. 2021). Studies of this type show that shorter intervals between defoliation lead

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to significantly smaller root reserves (Sim et al. 2015; Yang 2020), e.g., to below the critical 3 t dry matter after 28 d continuous grazing (Teixeira et al. 2007).

Defoliation of dual-purpose annual crops (e.g. wheat and canola) have revealed varying effects on root growth depending on how and when the shoot removal occurs. For example, when wheat crops were grazed/defoliated around growth stage Z30 (Zadoks et al. 1974) just prior to stem elongation and without removing the apical meristem, the effect on rooting depth was minimal (Virgona et al. 2006). However rooting depth was affected if grazing continued for longer periods and with higher stocking rate (Harrison et al. 2011). Reduction in rooting depth by 0.3 m at harvest was also shown, when the defoliation commenced at an earlier stage of wheat development (Z14, four-leaf stage) and was followed by two more defoliation events at the tiller and stem elongation (Z30) stage (Kirkegaard et al. 2015).

Unlike rooting depth, which is largely determined by the growth of seminal roots, root density in upper soil layers has been significantly reduced by grazing/defoliation. For example, Kirkegaard et al. (2015) observed decreased root density of wheat at 1.0–1.5 m depth after grazing compared to un-grazed treatments and assumed this was due to the reduced growth of nodal roots, which are initiated from tillers, and are often reduced in number under grazing (Paez-Garcia et al. 2019). In these studies, the authors assumed that the lower root density did not reduce the resource uptake potential of the defoliated crops, as even with grazing, the root length density remained greater than 1.0 cm cm^{-3} which is considered sufficient to extract most of the soil water within a given layer in a few days (Passioura 1983).

Reduced or deferred resource uptake can be one of the benefits of dual-purpose crops in semi-arid climates. For example, "water sparing" in the winter due to the reduced transpiration in grazed crops can conserve water to be used later in the sensitive flowering and grain-filling periods when rainfall is often low (Harrison et al. 2010). On the other hand, a recent review by Sprague et al. (2021) claimed that dual-purpose crops are less efficient in taking up fertilizer nitrogen (N) (14%) compared with grain-only crops (41%) owing to their smaller canopy size and reduced demand following defoliation. When the application of N fertilizer was delayed by two weeks, N use efficiency improved due to the increased canopy size. These interactions between seasonal conditions and the timing of grazing/fertilization generate an interplay between the effects on the demand for resources (i.e. canopy size) and resource supply (root system size).

These few studies, in which the effects of grazing crops on root depth and density were explored, have used destructive soil coring in the field at a few selected time points to monitor roots, water and N. Their results were limited by the effects of the high variability of root growth in structured field soils (Kirkegaard et al. 2008; White and Kirkegaard 2010). This led to insufficient sampling frequency to understand the dynamics of root growth following defoliation due to the laborious root washing, cleaning, and quantification procedure at each sampling. Rhizotrons and minirhizotrons provide an alternative approach to view root growth repeatedly in time non-destructively (Rewald and Ephrath 2013). Recent development in Machine Learning has made segmenting biological images faster and more accurate (Han et al. 2021, 2022b; Smith et al. 2022). Using this approach, a trained model can be further exploited to segment root images in large numbers with accuracy. Under these conditions, direct observation of root growth dynamics via image capture in high-time resolution can be carried out without the need for time-consuming manual counting.

Assuming that the shoots of young wheat plants are transferring up to 50% of assimilate to the roots (Gregory 2006) and that roots can serve as reserves for the initial shoot recovery, it is logical to assume that the root penetration rate may slow within a short time after defoliation until the leaves become the source of net assimilation again. In pot studies on vegetative canola (*Brassica napus*), McCormick et al. (2012) showed that following defoliation, the fine root growth (<2 mm) ceased, starch was remobilised from the taproots to the recovering shoot, the remaining leaves delayed senescence, and new leaves were thinner and with a high specific leaf area. Root growth resumed after plant allometry was restored. Likewise, a minimum of 300–350 C° days was required for the remobilisation of root reserves for shoot recovery when lucerne was defoliated in spring (Yang 2020). Understanding the dynamics of root and shoot responses to defoliation in dual-purpose wheat would be useful to plan grazing management strategies under different scenarios given the crops are

often grazed multiple times over several months, and recovery to produce high grain yield is a key aspect of their profitability (Dove and Kirkegaard 2014).

To understand the impacts of defoliation on the dynamics of root and shoot growth and resource capture on wheat, we used an outdoor rhizobox facility that facilitated direct measurement of roots, water and N using imaging, sensing and tracer labelling. Our aims were to: (1) measure the root growth dynamics of defoliated wheat at a high-time resolution scale to capture the effect on root depth and density up to 4 m depth; and (2) determine the effects of defoliation on the uptake potential of N and water throughout a full growing season using dual-labelling of ^{15}N and ^{2}H isotopes and water sensors.

Materials and methods

Study site and facility

We conducted an outdoor rhizobox-experiment at the University of Copenhagen, Taastrup (55°40′08.5”N 12°18′19.4″E), Denmark. A detailed description of this rhizobox facility is available in Thorup-Kristensen et al. (2020). In short, the laboratory consists of 24 soil-filled chambers (4 × 1.2 × 0.3 m) of which 12 were used for this experiment. Each chamber had 20-plexiglass panels arranged vertically, and they were covered by removable foamed PVC (Polyvinyl chloride) plates (Fig. 1) which allows us to (1) measure root density and depth by digital photography at frequent intervals; (2) install TDR (Time-domain reflectometry) water sensors at varying depth levels to monitor soil water; and (3) inject tracers (^{2}H and ^{15}N) to study the uptake of water and nutrients. Characteristics of the soil used in the rhizotrons are available in Table 1. Several previous studies describing the use of the facility for these purposes are available (Rasmussen et al. 2020a; Chen et al. 2021). Data on the monthly precipitation and

Table 1 Main characteristics of the soil used in the rhizotrons

Depth (m)	Organic matter (%)	Clay (%) <0.002 mm	Silt (%) 0.002–0.02	Fine sand (%) 0.02–0.2 mm
0–0.25	2.0	8.7	8.6	46.0
0.25–4.00	0.2	10.3	9.0	47.7

Source: Rasmussen et al. (2020a)

Fig. 1 12-rhizotron boxes at the study site (**a**) and wheat near maturity on 6 of the rhizotron boxes (**b**). Each box was divided into two chambers un-defoliated left, defoliated right (**c**). Each horizonal panel could be moved to the side to allow digital photograph of the soil faces to observe roots (**d**)

daily temperature were obtained at the weather station at the study site (Supp. Fig. 1). In short, the total precipitation received during the experiment period (Aug 2020 to Jul 2021) was 740 mm and the average monthly temperature was 9.6 °C. Daily soil temperature patterns (°C) in the rhizobox is shown in Supp. Fig. 2.

Experimental design

Winter wheat (*Triticum aestivum* L. cv. Ohio) was grown in the 2020–2021 season using 12 units at the rhizobox facility. The wheat plants were sown on Aug 21, 2020, at a rate of 41.7 g m^{-2} to establish 791 plants m^{-2} which were later thinned to 278 plants m^{-2} following the plant density adopted by Rasmussen and Thorup-Kristensen (2016) for early sown wheat in the field (240 plants m^{-2}). Fertilizer (N-P-K) was applied at rates of 66–8-40 kg ha^{-1} and 80–13-83 kg ha^{-1} in 2020 (Aug 20) and 2021 (Apr 2021) respectively. No irrigation was applied during the crop growth.

The wheat was defoliated on Sep 24, 2020 using scissors in six randomly chosen chamber units. This was 34 days (514 °C days) after sowing when the canopy height was 0.24 m and the wheat was at the early tillering stage, Zadok 14 (5–7 tillers). Shoots were cut 2 cm above the ground surface and the defoliated leaves were oven-dried at 75 °C for 48 hours for dry matter measurement. The wheat plants in the remaining six units were kept un-defoliated. The wheat was grown throughout the entire season until just prior to physiological maturity when the experiment was terminated early due to bird damage to the heads of the un-defoliated plants.

Image capture and processing

Root images

We measured the root growth of the wheat plants by performing a series of digital image campaigns (Table 2) using an Olympus TG-860 (OLYMPUS IMAGING CORP). For imaging we used a focal length of 3.7 mm, aperture f/3.5 and exposure time of 1/30. The resulting image size was 4608 × 1592 pixels with 72 DPI (dots per inch) in JPEG format. Canola had been grown in the 2019–2020 season prior to the experiment, and the remnant roots were visible at the outset of the experiment. To deal with this, we captured root images on the day of sowing on Aug 21, 2020, which we used for further processing to identify and exclude the remnant roots of canola from the images at further stages of root analysis. For the same reason, another set of reference images was taken one day prior to defoliation. After defoliation, we performed 11 continuous image campaigns in 2–3-day intervals from Sep 26 to Oct 16 followed by 7 more at 3–4 day intervals from Oct 20 to Nov 13. Two more imaging campaigns were carried out on Dec 2

Table 2 Activity, date, description of the experiment

Activity	Date	Description
Irrigation	2020-07-31 to 08–13	Automated irrigation for equalized water content between the rhizobox chambers monitored with TDR water sensor
Sowing	2020-08-21	Ohio sown at 417 kg ha^{-1}
Thinning	2020-09-03	Thinned to 278 plants m^{-2}
Fertilisation	2020-08-20 2021-04-12	N-P-K: 66–8-40 kg ha^{-1} N-P-K: 80–13-63 kg ha^{-1}
Defoliation	2020-09-24	6 randomly chosen unit defoliated to the ground
Root imaging	2020-08-21 2020-09-23 2020-09-26 to 12–15 2021-03-02 to 07–01	Reference imaging Pre-defoliation imaging 20 imaging campaign 8 imaging campaign
Canopy imaging	2021-04-12 to 07–01	5 imaging campaign
Isotope labelling	2021-05-26	^{15}N and ^{2}H at 2.9 m
Harvest	2021-07-12	Maturity

and Dec 15. We resumed root imaging after winter on Mar 2, 2021, and a further 8 campaigns were completed at approximately two-week intervals until the crop was harvested on Jul 1. The campaigns of frequent image capture produced 13,672 images for processing in total.

Prior to the root image analysis, we selected 1/10th the number of images from each campaign (1203 in total) for further image analysis. Images chosen were cropped into the size of 4008 × 1842 pixels by removing the panel borders at the image boundaries. We used the batch processing function of the IrfanView software (ver 4.59, 64-Bit). The resulting images covered 300 × 170 mm of the surface area of the imaged panels resulting in 119.04 pixels per cm - which was used as a calibration factor when estimating root length visible on the panel surface.

Shoot canopy images

In 2021, we also performed shoot canopy imaging to trace the shoot recovery after winter. Using the same digital camera, we imaged the shoot canopy directly above the rhizobox at approximately 30–50 cm vertical distance resulting in pixel dimensions of 4608 × 1592 (72 DPI). The imaging was repeated five times on Apr 12 (tillering), May 6 (stem elongation I), May 20 (stem elongation II), Jun 4 (booting), Jun 18 (anthesis) and Jul 1 (maturity) (Table 1). All 72 canopy images taken during the five campaigns were used for image analysis. Cropping the images for the region of interest was performed using the IrfanView software. In contrast to the root images, the canopy images contained artefacts such as soil at the top of rhizobox, rhizobox frames, green grass on the ground at the bottom of the rhizobox chambers etc. It was not possible to consistently cut out these artefacts, therefore, we let the further analytic process rule out these artefacts automatically (see below). We used the chamber surface area (0.36 m^2) as a reference to calculate the calibration factor (pixels per cm).

Image analysis using the RootPainter software

We used RootPainter software (Smith et al. 2022) for automated and accurate analysis of root and shoot growth. The software is built on Convolutional Neural Network (CNN) with a user-friendly interface allowing the non-specialist to train a model, i.e., learning from labelled examples. We used the free Google Colab notebook-based GPU (https://colab.research.google.com/drive/104narYAvTBt-X4QEDrBSOZm_DRaAKHtA; accessed on Sep 10, 2021) for training.

Training the model to measure wheat roots

Upon analysis, we followed the training protocol validated by Smith et al. (2022). Using "Create training dataset" function, we divided the images into tiles with a smaller size (1002 × 921 pixels) which decreased the need for annotation per image and time for image loading upon saving the annotation which can lead to more efficient training. We trained a model to segment only the wheat roots by excluding the remnant roots from canola grown in previous years. In 2020, the roots from young wheat were quite distinct from the remnant roots from canola. In 2021, however, some of the wheat roots discoloured and this required us to train the dataset acquired in 2021 separately to capture these roots. The roots from both species became visually similar over time, and we identified the wheat roots by comparing the images taken in 2021 to the images taken prior to the sowing in 2020 assuming the position of the older roots did not change (Supp. Fig. 3). For each training, we initiated corrective annotation after annotating 10 examples without using prediction. We trained the first model for 7 hours using 200 images, and 3 hours with 100 images for the second model.

Training model to measure the wheat shoot canopy ground cover

Using the same "Create training dataset" function, we tiled each image into five resulting in a total dataset size of 372 for training. We spent 3 h 40 m for training in total. The training was focused on detecting the shoot canopy of "green" wheat plants only and ruling out the artefacts.

Data extraction from the trained models

Root length extraction and root depth determination

After training, we segmented the original images to PNG format and extracted the root length visible at the

soil-panel interface using the calibration with the number of pixels. We calculated the root length data into meter (m) root per 1 m^2 of image area (m m^{-2}) in this study as the metric for root growth. We also recorded the depth of the deepest visible roots for each rhizobox unit to determine the maximum visible root depth for each time of observation. The root depth was plotted against the accumulated thermal time (°C days) until the early season in 2021. The daily temperature was obtained from the weather station at the study site, and for the thermal time, we assumed that the base temperature for the growth of wheat was 0 °C.

Ground cover extraction

We extracted the number of pixels from the canopy segmentation from which we calculated the area of shoot canopy per growing area (0.36 m^2) referred to hereafter as the ground cover index which at least during early stages is a proxy for the Leaf Area Index (LAI). Destructive sampling for repeated direct measures of LAI was not possible due to the limited number of plants in the boxes. Examples of root and shoot segmentations after the training are shown in Supp. Fig. 4.

Soil water content determination

The TDR sensors (TDR-315/TDR-315 L, Acclima Inc., Meridian, Idaho) were installed at 0.5, 1.4 and 2.3 m soil depths. The accuracy of the TDR sensors in this rhizobox condition was tested by Rasmussen et al. (2020a). Volumetric water content (VWC; %) was recorded at every 10 minutes on a datalogger (CR6, Campbell Scientific Inc., Logan, Utah), which was extracted as **.dat** format using LoggerNet software. VWC was averaged daily. To make the soil water content uniform between the chambers intermittent irrigation prior to sowing was applied between Jul 31 to Aug 13 in 2020. The water status was monitored using the water sensors.

To compare the two treatments, we calculated the differences in VWC for each year by subtracting the VWC values from the wettest to driest points in each year. We experienced a period of malfunctioning of the data-logging system from Jun 1 to Jun 21 in 2021 during which no measured data are available. Considering the plant demand and the pattern of VWC dynamics, we assumed that no greater or smaller values during that period would have occurred and for interpretation of the data and graphical depiction, we joined the data assuming a linear trend between the two-time points.

Isotope analysis and shoot measurement

Both treatments were dual-labelled using ^{15}N and ^{2}H isotopes by mixing 4.98 g of $Ca(^{15}NO_3)_2$ (>98 at% ^{15}N) with 450 mL of $^{2}H_2O$ (^{2}H content = 99.91%) and with 450 mL distilled water. The labelling was carried out at 2.9 m depth on May 26 in 2021 when the wheat plants were at the booting stage. Prior to the injection, 20 holes with a diameter of 0.5 cm were made in two rows across the chamber (10 per row) using a steel stick with a diameter of 0.5 cm. Labelling was done through these holes to which 5 ml of tracer was injected in 1 ml aliquots at 5, 10, 15, 20 and 25 cm from the vertical surface as the syringe was moved within the tube. This meant a total of 100 ml of the tracer mixture was applied per chamber.

Plant samples from each rhizobox chamber were taken at the time of harvest on Jul 12 in 2021. The entire biomass was cut at the ground surface. The plants were not fully mature at the time of final harvest, and the measurement on final harvest was confounded due to problems with bird damage, which was worse in the un-defoliated plants due to more advanced development. The non-grain biomass remained unaffected. Nevertheless, for isotopic analysis the plant parts were separated into kernels and shoot for the isotope analysis. As we measured concentration of these isotopes from each plant part, the bird damage did not influence the measurements. The samples were dried at 80 °C for 48 hours and powdered for further analysis. For the ^{15}N analysis, approximately 3 mg of the powdered samples were put in tin capsules which were placed in a 96-well plate and analysed using a continuous-flow isotope ratio mass spectrometer (IRMS) at the University of Copenhagen, Frederiksberg, Denmark and at the University of Göttingen for ^{2}H analysis. Isotope values of ^{15}N and ^{2}H in the samples were expressed in delta notation (δ) and calculated by the following equation.

$$\delta(‰) = \frac{\text{Rsample}}{\text{Rstandard}} - 1$$

For ^{15}N isotope, Rsample $\delta^{15}N$ is the ratio of the heavier to the lighter isotope of the sample, i.e. $^{15}N/^{14}N$ ratio, and the Rstandard is 0.0036765 and,

i.e. the natural abundance of ^{15}N. The delta notation values were converted to ^{15}N atomic % and the ^{15}N atomic % in excess by subtraction of the Rstandard. For ^{2}H isotope, Rsample $\boldsymbol{\delta}^{2}H$ is the ratio $^{2}H/^{1}H$, and the Rstandard for $\delta^{2}H$ is Vienna standard mean ocean water (Rstandard $\approx$ 1/6412). We used the original delta notation for $\boldsymbol{\delta}^{2}H$ (‰).

Statistical analysis

R version 4.1.0 (R Core Development Team 2021) was used for statistical analysis. The 22 observation points on root depth (m) recorded from the time of defoliation to the maximum rooting depth reached during the crop cycle (from Aug 21, 2020 to Mar 2, 2021) were used for linear regression analysis (lm function) against the thermal time (°C days) generating R^2 values. Linear regressions were run separately before and after the root penetration was resumed following defoliation. Significance of effects on root depth, root length, VWC, ground cover, final plant biomass and isotope measurement were estimated using a mixed effects model (Pinheiro and Bates 2000) based on the approximated degrees of freedom calculated by the package lmerTest accompanied by multiple comparisons (multcomp package) ($P \leq 0.05$).

Results

Root depth

On average, 16 g per chamber of leaf dry matter (standard error of ±0.46 g) was removed by defoliation, which was equivalent to 449 kg ha^{-1}. At the time of defoliation, the root depth of wheat plants in both treatments was 0.7 m (Fig. 2). Defoliation paused root penetration for 269 °C days (approximately 20 days) while the roots of un-defoliated plants continued to elongate downwards at the rate of 2.2 mm °C day^{-1} and reached a depth of 1.2 m

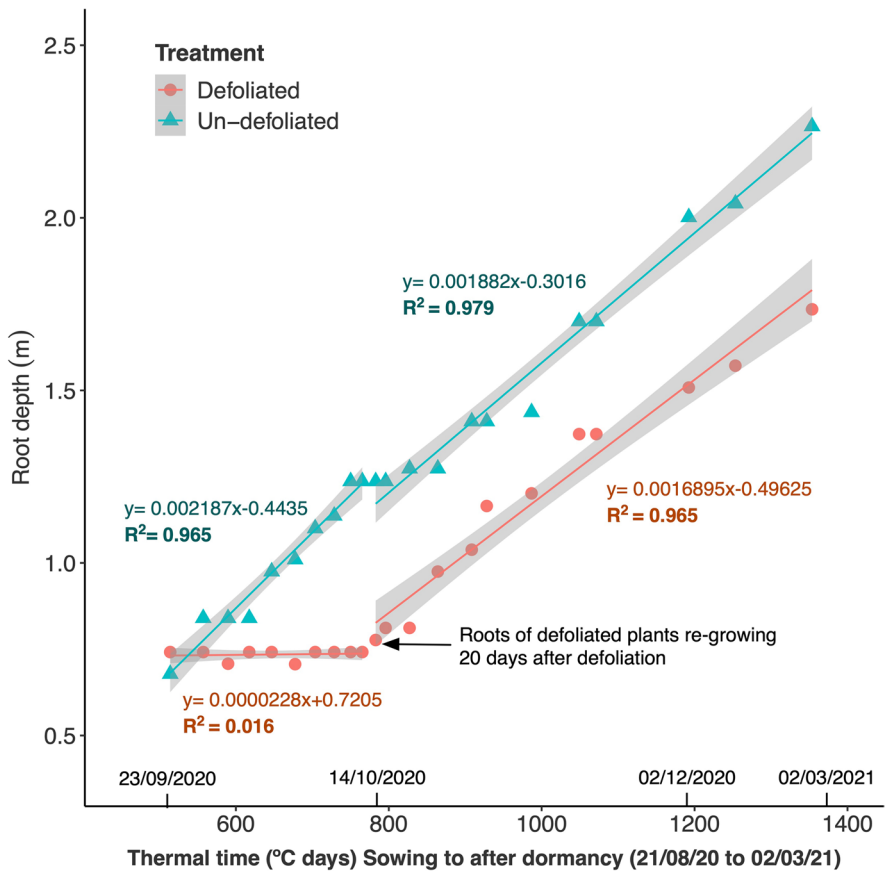

Fig. 2 Root depth over thermal time (°C days) from sowing to the early season in 2021 (2021-03-21). Linear regression analysis was fitted for the two separate periods for each treatment (1) sowing to the time of root re-growth and (2) after root regrowth to the maximum root depth observed in 2021

by the time the defoliated treatments resumed root growth. At this point, the recovering shoot canopy of the defoliated treatment was 21 cm in height (from a height of 2 cm when defoliated), while the un-defoliated plants were 31 cm high. After the root growth of the defoliated plants resumed, the root penetration rate of the two treatments was similar (1.68 mm for defoliated vs 1.88 mm for un-defoliated). An example of the pause and resumption of root elongation in an individual root tip is captured in the images shown in Fig. 3. This transient cessation of root growth generated a difference in root depth of 0.5 m which persisted into early March 2021 (Fig. 4). The effect diminished as the season progressed, and the effect became insignificant during most of the growth period in 2021. The final maximum root depth measured on Jul 1 was 1.76 m for defoliated and 2.03 m for un-defoliated.

Root length dynamics

Significant effects of defoliation on root length were observed 12 days after defoliation (Oct 6, 2020) in 0–0.4 m and 0.6–1.0 m layers (Fig. 5). In these layers, the effect persisted until the time of booting and anthesis in 2021, respectively. Defoliation delayed the time of root appearance into subsoil layers (1.2–1.6 m and 2.4–2.8 m) for 20 days. In the 1.2 m to 1.6 m layer, once the effect of defoliation manifested in Nov of 2020, it persisted until maturity in the next season in 2021 (Jul, 2021). At depths of 1.8–2.2 m, the roots of defoliated plants appeared only in a single replicate out of six in the initial three observations, and no significant effect of defoliation emerged until anthesis in 2021. The first appearance of roots from defoliated treatments at 2.4–2.8 m depth was on Apr 2, 2021, whereas roots in the un-defoliated treatment had already

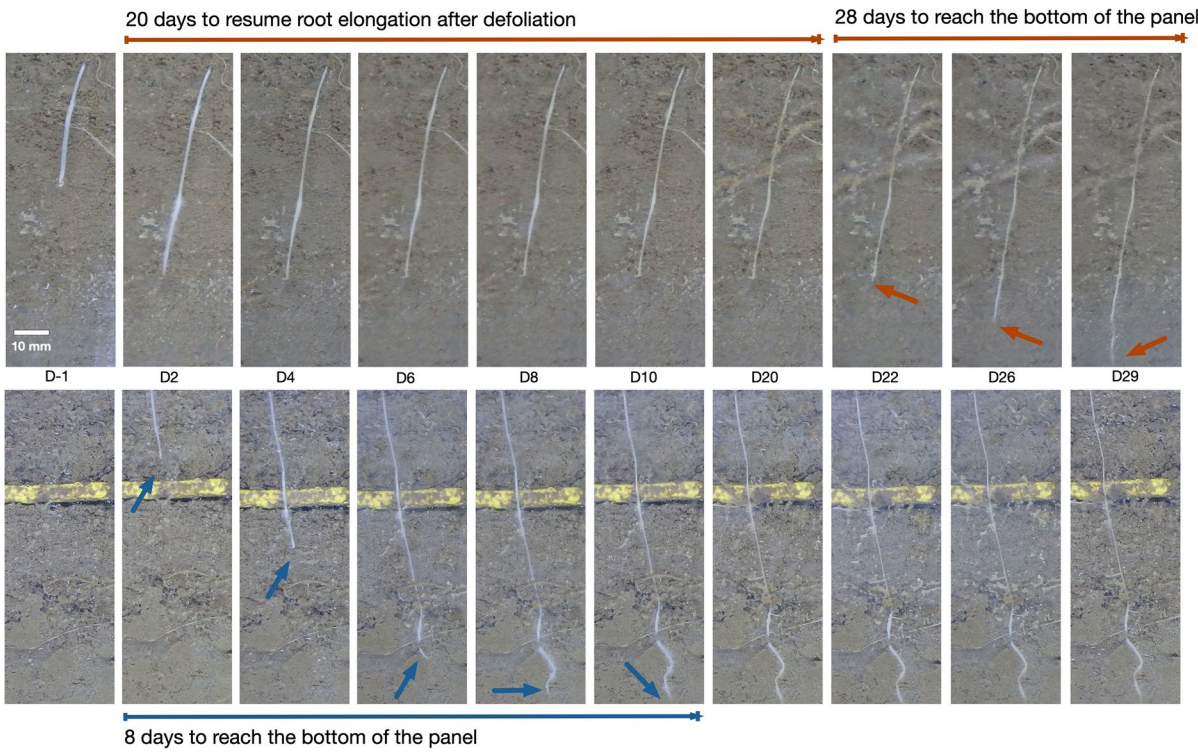

Fig. 3 An example of direct observation of paused and resumed elongation of a root tip 20 days after defoliation (top panel); while root growth continued without defoliation (lower panel). *D-1 = One day before defoliation; D2 = Two days after defoliation and so on

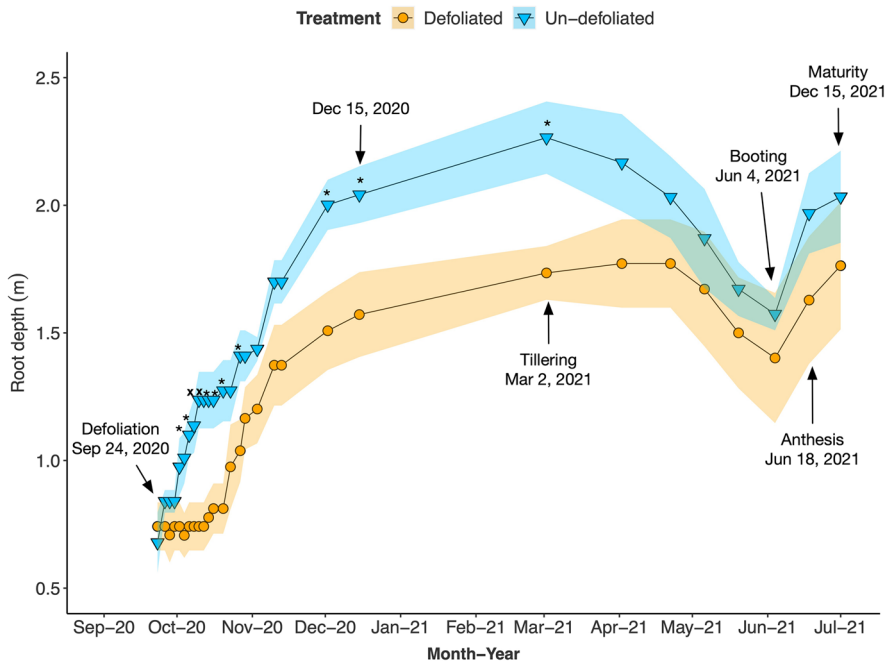

Fig. 4 Root depth dynamics of defoliated and un-defoliated treatments. Symbols indicate significant effects of defoliation (Mixed-effects model: $P<0.05$*; $P<0.01^{x}$; $P<0.001^{\dagger}$)

been present at that depth on Dec 15, 2020. However, no meaningful comparison in root length was possible due to the lack of data points from both treatments. Considering individual depth-levels, only the roots from the un-defoliated plants were present at the deepest layer (2.6–2.8 m; see Supp. Fig. 5).

Soil moisture content

In 2020 during early vegetative stages, defoliation reduced water use at 0.5 m (Fig. 6a). No difference between the treatments was evident at deeper layers at that stage and no distinctive change in VWC was observed at 2.3 m. In 2021, as the crops resumed growth in spring, the differences in water use between the treatments were more obvious in the shallower soil (0.5 m) in May and moved into deeper layers (1.4 m and 2.3 m) in June, and by then differences were only significant at the deepest layer (2.3 m) (Fig. 6b). In the upper layer (0.5 m) the apparent differences in water content had diminished in July, presumably as the defoliated plants were eventually able to dry the soil to a similar extent as the un-defoliated plants (i.e. to near the wilting point). Differences at deeper layers persisted to the final harvest.

Crop canopy, head number, ^{15}N and ^{2}H signature

Ground cover was at a peak around the time of Z39 (late in stem elongation; Jun 4) for both defoliated and un-defoliated treatments (Fig. 7a, b). The effect of defoliation was significant until anthesis (Jun 18), and no effect was evident at maturity (Jul 1) however the un-defoliated plants had commenced senescence earlier than the defoliated plants (see Jul 1 photo from Fig. 7a). Defoliation had substantially reduced head numbers at both anthesis and maturity (Fig. 7c). The effect of defoliation in reducing ^{15}N uptake from 2.9 m in the period after injection on May 26, 2021 to final harvest was significant regardless of the plant part analysed ($P<0.05$) as shown by ^{15}N at (%) (Fig. 8a). Un-defoliated plants showed significantly higher $\boldsymbol{\delta}^{2}H$ enrichment compared with defoliated plants, and the enrichment was higher for grains compared with straw part of the plants (Fig. 8b).

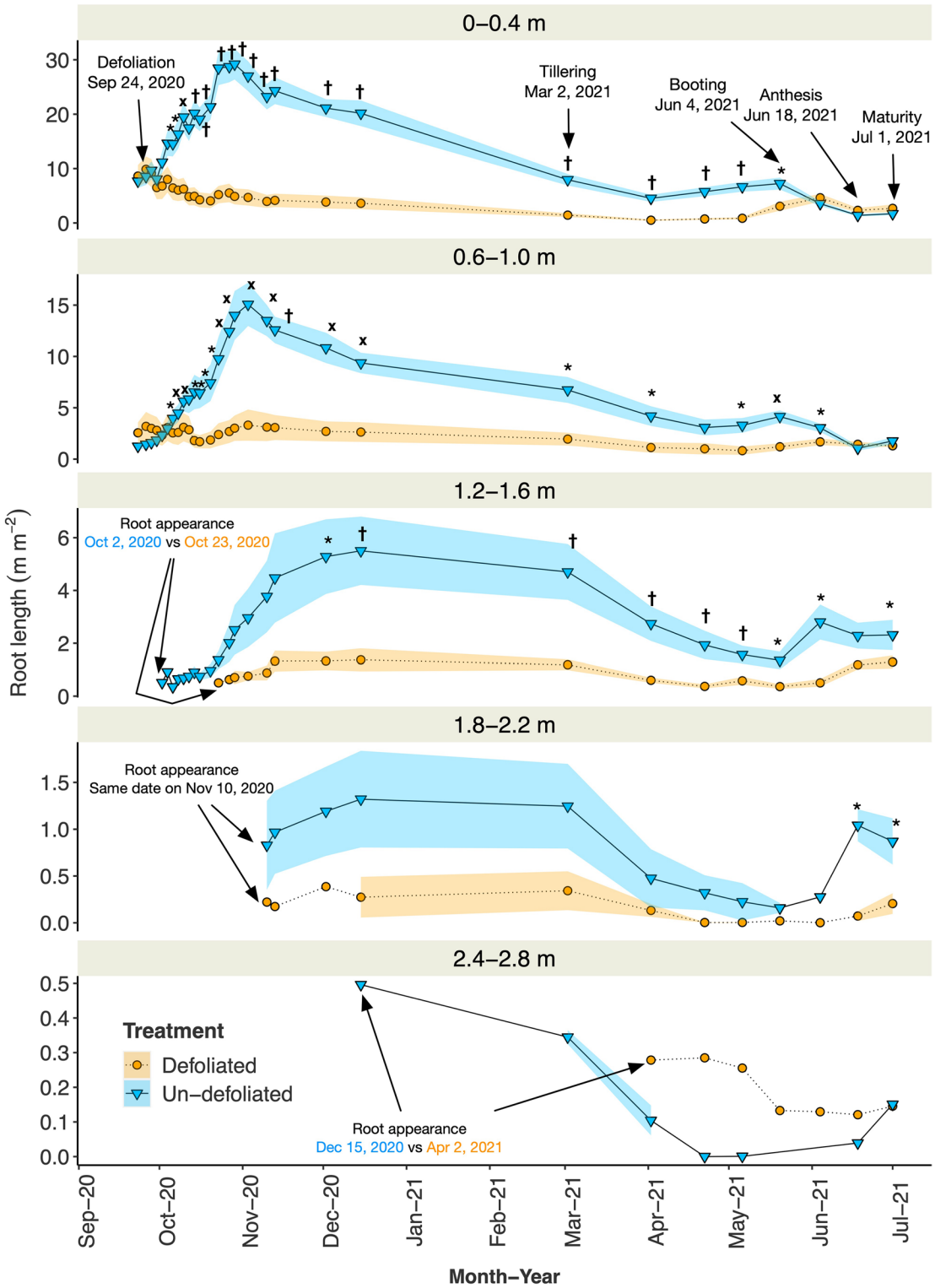

Fig. 5 Root length (m) of wheat from sowing (Aug 21, 2020) to harvest (Jul 1, 2021) at a range of depths 0.-0.4 m, 0.6–1.0 m, 1.2–1.6 m, 1.8–2.2 m and 2.4–2.8 m to 2.7. Symbols indicate significant effects of defoliation (Mixed-effects model: $P<0.05^{*}$; $P<0.01^{x}$; $P<0.001^{\dagger}$)

Discussion

Root growth paused for 20 days

Our determination on root growth was based on the observations of the visible roots on the transparent glass panels of the rhizotron, which we assume to be a representation of the root growth and responses within the rhizobox. While acknowledging the inherent limitations of all rhizotron platforms in that regard, we believe our central finding - that roots of defoliated wheat ceased root elongation temporarily is valid. This is further supported by the related measurements of reduced water and N uptake, and follows the general notion of shoot-root relationship following defoliation. The period of growth cessation identified (269 °C days or 20 days) and the subsequent impacts on rooting depth requires further validation in field soils to fully consider its practical implications, but our results pinpoint the likely timing of sampling required for such validation.

Defoliation causes the reversed translocation of root carbohydrates for rapid shoot recovery (Moot et al. 2021), leading to the slowed root depth penetration rate (Briske and Richards 1993). During the vegetative growth stages in wheat once the first few leaves have appeared, it has been reported that around 50% of assimilation is directed to root growth (Gregory 2006). Consequently, the removal of shoots during this early period would be expected to divert the allocation of assimilate to the shoots until a photosynthesising canopy is recovered (Moot et al. 2021). In the case of defoliated *Brassica napus* seedlings, McCormick et al. (2012) also found that existing assimilate stored as starch in the taproot was remobilised to the shoot, so that taproot weight declined during that period and no new fine roots were produced until allometry was restored.

Our observations provide direct evidence of cessation in wheat root growth until the shoot canopy had recovered from 2 cm height to 24 cm, when root growth resumed. The vegetative period in wheat is a stage of rapid root penetration and branching (Gregory 2006; Kirkegaard et al. 2007; Thorup-Kristensen et al. 2009) and roots continue downward growth until just after anthesis. It is reasonable to expect that significant defoliation by grazing in the period prior to stem elongation would reduce rooting depth, however previous field-based studies have not detected significant effects of grazing wheat on maximum rooting depth. For example, Kirkegaard et al. (2015) measured the rooting depth of wheat crops following defoliation in field conditions at three study sites. The results demonstrated that defoliation of wheat that commenced at the start of stem elongation did not significantly affect the rooting depth when measured at wheat maturity. This conclusion led the authors to form the opinion that there would be little impact of the grazing on rooting depth and resource capture which could explain the relatively limited impact of substantial grazing on crop yield provided grazing ceased prior to stem elongation. As roots could reach the same depth, resource capture may be deferred due to reduced shoot demand during recovery, but eventually would be likely to match that of the un-grazed crops. The exception to this was when grazing commenced very early (four leaf stage) and was repeated and prolonged (Kirkegaard et al. 2015) which reduced final rooting depth by 0.3 m. However, there is minimal biomass available for grazing at that time, and the risk of dislodging young plants is high, so this is not generally practical for commercial dual-purpose cropping. Nevertheless, that observation is consistent with the reduction in final rooting depth (0.33 m) caused by defoliation at four leaf stage in this experiment, although the difference was not significant due to the variation in maximum rooting depth. It is possible that previous field experiments failed to capture the effects of grazing on root growth if it was transient, and the crop ultimately recovered, as measurements of root growth in previous experiments began at least 60 days after defoliation/grazing (e.g. Virgona et al. 2006; Kirkegaard et al. 2015). This is equivalent to or longer than 450 °C days under Australia's milder winter conditions.

Smaller root system following defoliation

Defoliation substantially reduced the root length, however this effect varied among soil layers at different times throughout the season. On average, undefoliated plants had 5-fold higher root density compared with defoliated plants in shallower soil layers (<1 m) during the 2020 season. This was a greater reduction in root length compared with earlier studies. For example, grazed wheat/canola plants had a decrease in root length density (RLD) up to 50% following intensive sheep-grazing under water-limited

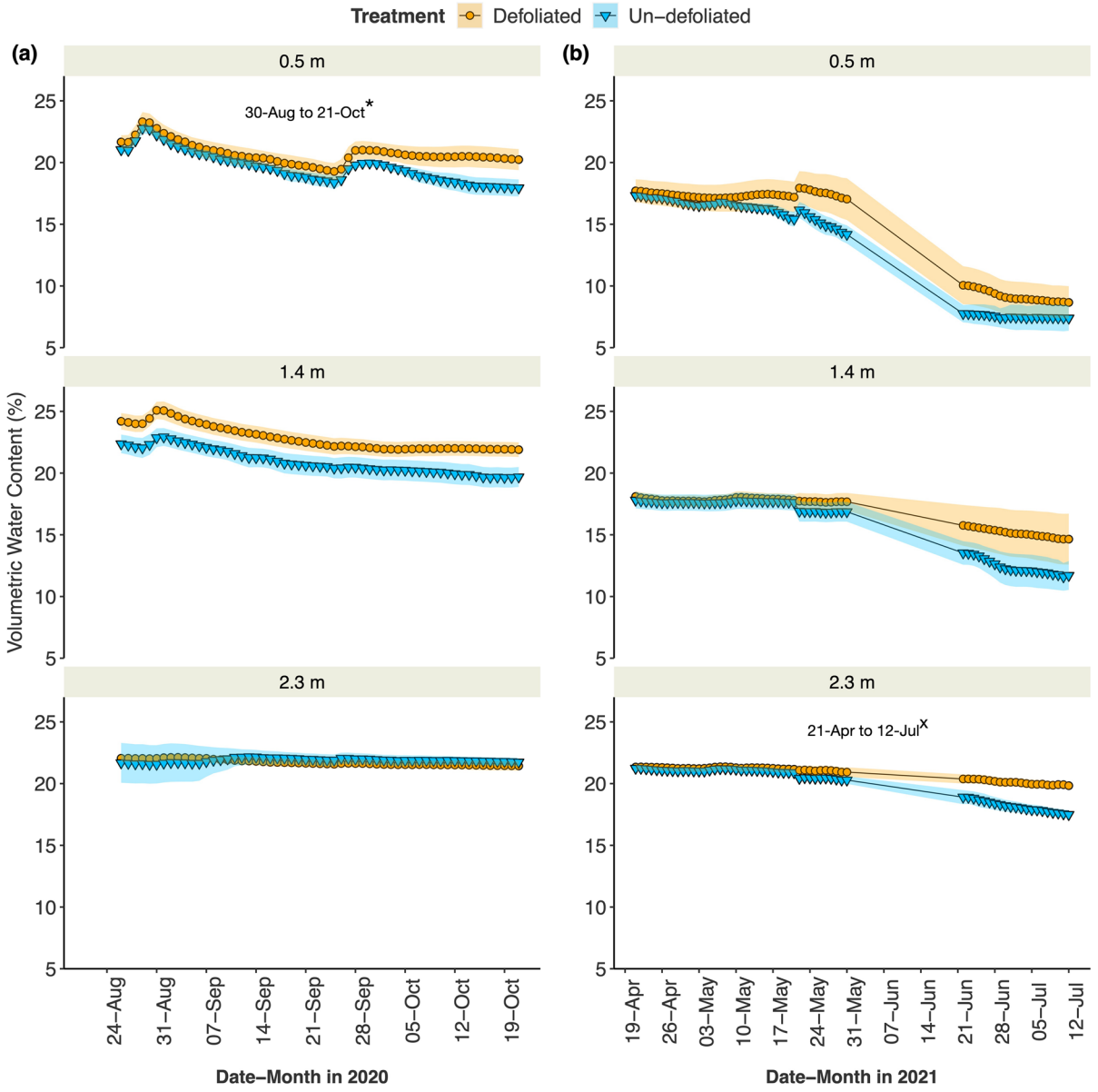

Fig. 6 Volumetric Water Content (VWC: %) measured at 0.5, 1.4 and 2.3 m in 2020 (**a**) and 2021 (**b**). The comparison was done using the differences in VWC from 30-Aug to 21 Oct and 21-Apr to 12-Jul in 2020 and 2021, respectively. Symbols indicate significant effects of defoliation (Mixed-effects model: $P < 0.05$*; $P < 0.01^{x}$; $P < 0.001^{\dagger}$)

conditions (Kirkegaard et al. 2015; Virgona et al. 2006). However, these studies did not measure root growth until after crop maturity, and it is not certain whether more significant reductions existed earlier in the season. This stronger effect may also be due to the commencement of defoliation at the early growth stage as smaller plants are more affected by defoliation in terms of root length (Sullivan et al. 2000).

The effect of defoliation on root density in deeper soil layers (1.2–1.6 m and 1.8–2.2 m) was evident in later periods compared to the shallower layers. This was due to the delayed appearance of roots, particularly for defoliated plants where only a few replicates had roots visible to test for significance. At 2.4–2.8 m depth, only one replicate from the defoliated treatment had roots present. Nevertheless,

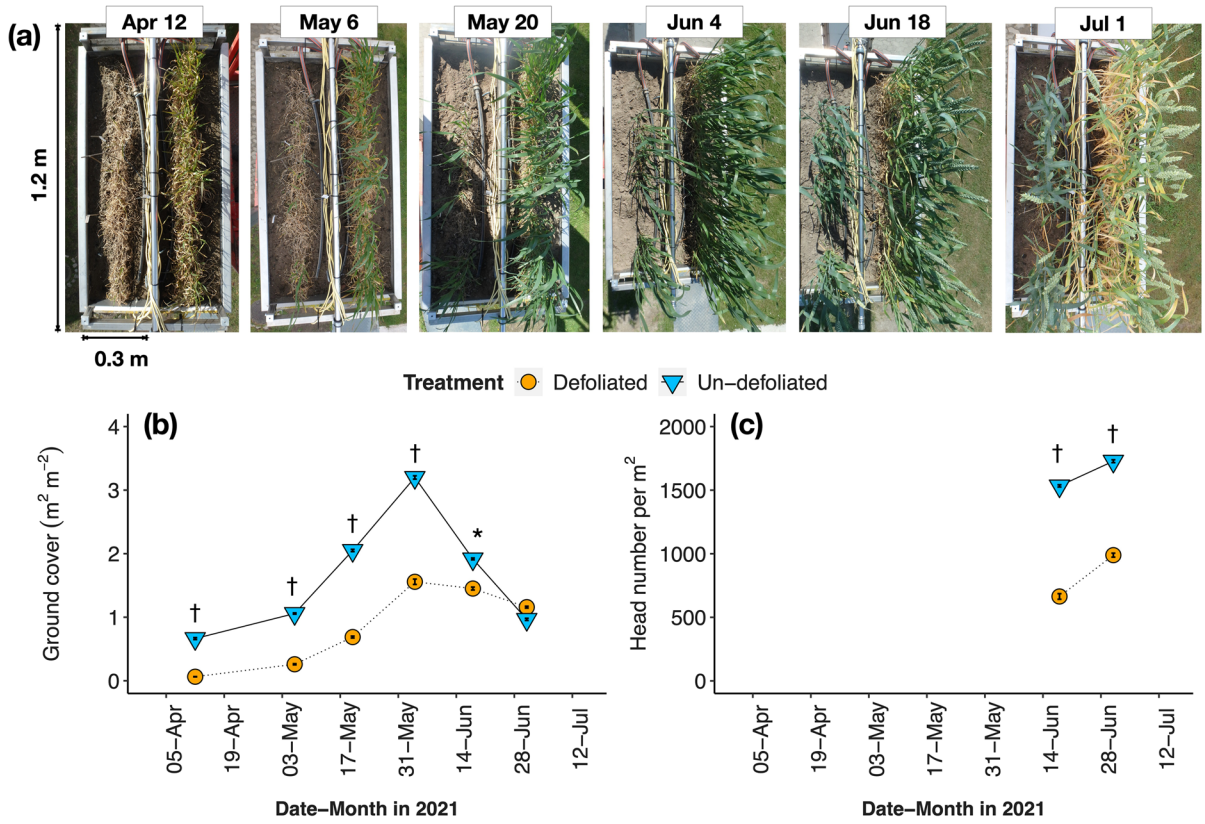

Fig. 7 Pictures of the wheat aboveground part taken in five dates (**a**), ground cover (**b**), and head number (**c**). Symbols indicate significant effects of defoliation (Mixed-effects model: $P<0.05$*; $P<0.01^{x}$; $P<0.001^{\dagger}$)

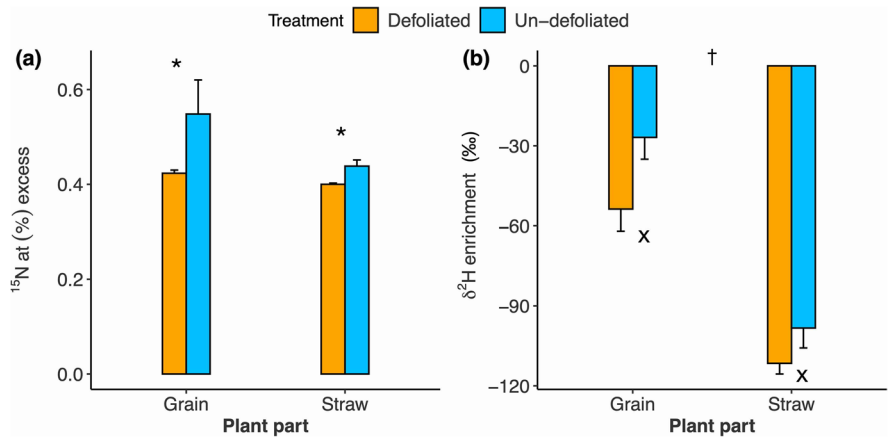

Fig. 8 ^{15}N at (%) excess (**a**), δ^2H enrichment (‰; **b**) as affected by defoliation. Symbols indicate significant effects of defoliation (Mixed-effects model: $P<0.05$*; $P<0.01^{x}$; $P<0.001^{\dagger}$)

there was a clear indication that defoliation delayed root penetration into deep layers, and when statistically testable, the effect persisted to crop maturity.

Root density of winter wheat increases as a function of photoperiod. Several studies indicate the maximum rooting density throughout the soil profile around the time following anthesis and persisting to maturity depending on the sowing time and seasonal conditions (e.g. Han et al. 2015). However, we observed a substantial decrease in root density/depth after the

winter of 2020. From this rhizobox study it is hard to conclude if this is an artefact of the rhizobox platform or could also occur under normal field conditions. Slowing of root development between stem elongation and heading has been observed in Australian and Indian wheat cultivars (Li and Richards 2019). The authors concluded that the shoot demand for resources increased during this period causing a transient reduction in allocation of photosynthate for root growth. However, this did not cause a substantial reduction in root density as shown in our experiments. We propose that there was an external factor unrelated to defoliation and associated with the growing conditions that has caused the decrease in root density observed in both defoliated and un-defoliated plants at that time. One clear possibility is related to cold stress during Jan-Feb in 2021, where the average daily air temperature fell below zero frequently. This cold stress caused shoot senescence plant death, which may account for the disappearance of roots from the rhizobox panels by reducing the nodal root density (Supp. Fig. 6). Another explanation could be that the fluctuating temperature of the rhizobox soil (Supp. Fig. 2) could have caused the freezing of the visible roots near the glass panels over the winter (Dec-Feb, 2021). As the temperature rose and the frozen roots thawed in the spring 2021, the visible roots might have gone through a faster decaying period, which caused the disappearance of the visible roots from the panel surface. As the season progressed new roots were observed on the panel, and this appearance of new roots continued even to June and July, 2021 – which contributed to the slight increase in apparent root length at maturity.

Despite this observation related to the specific conditions of the rhizobox soil, these potential artefacts do not change our major conclusions regarding the transient pause of root penetration following defoliation, and the subsequent reduction in root density related to defoliation. The observed effects of defoliation on roots were related to differences in water and nutrient uptake from the inner soil volume of the rhizobox (as discussed in the next section) providing evidence that observations of roots at the panel surface was reflecting similar effects within the rhizobox soil.

Decreased resource uptake following defoliation

Our results from the water sensors and the tracers show that defoliation can cause a reduction in water and N uptake. The sensors were able to capture the reduced water uptake by defoliated plants within 3 days after the shoot removal in 2020 presumably due to the reduced leaf size and transpiration potential. In 2021, the sensors documented the difference in water uptake from early May and it persisted until the end of the season although the effect was only significant at the deepest layer (2.3 m). Given that defoliated plants had lower root density in deeper soil layers (e.g. 1.8–2.2 m), this can be interpreted as a reduced uptake capacity following defoliation. ^{15}N uptake and ^{2}H enrichment also show evidence that having more roots at deeper layers increased deep N and water uptake at the end of crop development. We observed that the ^{2}H enrichment was greater in grains than in shoot biomass. This highlights the value of having the access to the deep-placed resource at the late growth stages of wheat. This notion has been well-established by the slower phenological development of early-sown winter wheat allowing deeper roots to capture more water during the reproductive stages (Lilley and Kirkegaard 2011; Hunt et al. 2019).

Resource uptake: demand vs capacity

In our study, defoliation (1) substantially reduced root system and plant vigour (ground cover) up to maturity; (2) reduced water and N uptake from deep soil layers. It is difficult to un-tangle the contribution of shoot demand vs. root uptake capacity on the actual resource uptake. Both effects would influence resource acquisition simultaneously. Firstly, the overall demand for the N and water would have been reduced for defoliated plants, and the pattern of demand through time would have changed. In fact, one effect of grazing dual-purpose crops in water-limited regions such as southern Australia is to defer water use from the milder winter period when water may be in excess to use it later in spring during the sensitive reproductive stages when conditions generally become hotter and drier, a phenomenon known as “water-sparing” (Virgona et al. 2006). The slower depth penetration of the root system and reduced root density would also mean access to deeper stored water becomes available to the grazed crops when it is needed for grain filling, avoiding the rapid exhaustion of stored soil water and “haying-off” which can occur under Australia’s semi-arid conditions. This reduced demand, however, can result in a disadvantage in terms of N use efficiency

(NUE). For example, Sprague et al. (2021) analyzed the data from 14 grazed crop experiments on canola, wheat and barley in southern Australia, and concluded that the uptake of N by defoliated crops was only half that of un-defoliated crops. The authors assumed that the low N demand by the smaller shoot canopy of the grazed crops was the key reason for low NUE, rather than a lack of supply or uptake capacity by the root system, although the roots were only monitored in a limited number of experiments.

Secondly, it is possible that improved resource uptake by un-defoliated plants due to the presence of more and deeper roots could play a role. In general, greater root density can indicate better resource exploitation potential. Interventions designed to facilitate deeper root growth such as early sowing (Thorup-Kristensen et al. 2009; Rasmussen and Thorup-Kristensen 2016) or improved subsoil structure through biopore creation (Gaiser et al. 2012; Han et al. 2015) have increased water, N and phosphorus (P) uptake of wheat. More fundamental studies using tracers often reveal a strong relationship between root density and tracer uptake from deep soil layers (Rasmussen et al. 2020b; Han et al. 2020, 2022a; Chen et al. 2021). In these European examples, the improved uptake was assumed to be associated with an increased density of roots which is important in the subsoil where general root density is low and often the root systems have limited time to exploit the available resources during late growth stages. In the experiment reported here, the reduced root density caused by defoliation could become a disadvantage for resource uptake as shown in our results by decreased water use and N at deep soil.

In contrast, for environments where the supply of the major resources (N and water) can be frequently limited by dry seasonal conditions, such an extensive root system can be a disadvantage. For example, Lilley and Kirkegaard (2016) conducted a long-term simulation analysis at eight sites in the semi-arid environment of Australia, and the crops with more extensive root systems dried out soil more quickly and only rarely provided yield benefits (3–10% of years tested). In these environments, it has been suggested that optimised root density, especially in topsoil layers, can reduce the need for the allocation of carbon to roots (Wasson et al. 2012). This is based on the notion that fewer roots (e.g. 1 cm per cm^3) are needed for sufficient plant available water uptake than is grown in upper soil layers in most cases (Passioura 1983). Under these conditions, the impact of reduced root density following defoliation may be minimal or even advantageous if water use is slowed and spared until more sensitive grain filling stages.

Yield penalty and other scenarios

Based on the trajectory of ground cover as a proxy of biomass production, and the difference in head numbers, there was a clear impact of defoliation on the assimilation of aboveground resources and crop yield due to defoliation (Kirkegaard et al. 2015). Yield penalties can occur in dual-purpose crops but the magnitude is often related to grazing time and intensity (Virgona et al. 2006; Kirkegaard et al. 2015), water (Zeleke 2019) and N availability (Sprague et al. 2021) and to the potential yield of the crop. Higher-yielding crops (>6 t ha^{-1}) are less likely to be able to recover biomass after grazing to support those higher yields and so defoliation impacts on yield potential will occur even without grazing beyond stem elongation and removing reproductive parts. In this experiment, given there were abundant soil resources and only one defoliation event was applied, growth and yield reduction would have stemmed from the aboveground and belowground capacity to capture the light and soil resources rather than any interactions with the timing of environmental stress. The revelation of significant and persistent impacts of grazing on root and shoot growth shown in this study, provides a basis to consider the likely implications of transient cessation of root growth under different resource supply scenarios.

Conclusions

We captured the pauses in root growth caused by early defoliation in wheat lasting 269 °C days. Defoliation also caused persistent reductions in shoot canopy, head number, root density and water/N uptake potential from depth, possibly aggravated by frost during the winter. Based on these observations, future research can focus on the likely impacts of defoliation on the dynamics of resource capture under different growing conditions to develop strategies for better management of dual-purpose crops.

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Author contributions EH, JK and KTK co-designed the research. EH conducted the research and prepared the manuscript. All authors contributed to the manuscript writing.

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Data availability The image datasets will be available upon request.

Declarations

Conflict of interest There is no competing interest in this study.

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