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RESEARCH ARTICLE

Impact of root observation windows on plant performance, root development, and rhizosphere-microbial communities during late vegetative growth of field-grown winter wheat

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Abstract

Background and Aims Root observation windows (RW) installed in the field provide a tool for non-destructive monitoring of root development and rhizosphere processes. However, the highly invasive installation process, which requires cutting soil profiles, may affect plant development and, ultimately, the outcome of the experiments. This study systematically compares plant development with and without the installation of RW.

Methods Using the location of a long-term field experiment, the responses of winter wheat to different intensities of tillage, N-fertilization, and use of fungicides were compared for plants grown along root windows and in undisturbed control plots. Sampling was performed during late vegetative growth, six weeks after RW installation, with comparisons of shoot and root biomass, root length, mineral nutritional status, stress-related gene expression, stress metabolites, and the composition of microbial communities reflecting rhizosphere (RH) conditions.

Results The presence of RW did not significantly affect most of the parameters analyzed. As an exception, RW plants showed increased foliar concentrations of several nutrients (K, Mg, Ca, Cu, Mn), potentially attributable to reduced inter-plant competition because RW were installed along border rows of the experimental plots. By contrast, N-fertilization intensity and fungicide use affected plant biomass, root growth, and fungal communities. Tillage intensity primarily affected fine-root production,

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the composition of RH-microbial communities, and physiological stress indicators in leaf tissue.
Conclusion The results suggest only a limited influence on performance of winter wheat six weeks after RW installation under the selected experimental conditions, with plant responses and experimental results comparable to undisturbed controls.

Keywords Root windows · Winter wheat · Soil microbiota · Stress-related gene expression · Tillage intensity · Fertilization intensity

Introduction

Plant roots can modify the conditions in the surrounding soil, creating a specific soil compartment called the "rhizosphere." The respective root-induced modifications can shape physical soil properties, redox conditions, water and nutrient availability, and the composition and activity of rhizosphere microbial communities. Finally, these processes are important determinants of general plant performance, health, stress resilience, yield formation, and crop product quality. Consequently, understanding rhizosphere processes is an important prerequisite for developing rhizosphere management strategies to improve the efficiency and sustainability of agricultural production systems (Wang et al. 2022). Characterizing the properties of the rhizosphere bears various methodological challenges. Significant knowledge gaps concern the mechanistic linkages among the physical, chemical, and biological processes operating across different spatial and temporal scales. These processes are frequently investigated in model experiments under controlled conditions, but simply upscaling them to the root system, natural soil profile, and field scale remains difficult (Vetterlein et al. 2020).

Destructive sampling techniques based on root excavation typically cannot adequately account for temporal and spatial variations in rhizosphere processes. For non-destructive assessments of physicochemical changes along soil-grown roots, specialized culture vessels equipped with root observation windows (rhizoboxes) have been frequently employed (Engels et al. 2000; Fitz et al. 2006; Neumann 2006; Neumann et al. 2009), allowing repeated observations over longer periods under controlled conditions. However, due to size limitations, rhizobox techniques are usually restricted to early plant developmental stages. Although non-destructive monitoring of soil-grown roots is possible, rhizobox approaches remain model experiments in artificial soil culture systems with two-dimensional observation planes and are not directly comparable to natural growth conditions (Böhm 1984; Vetterlein et al. 2020).

In principle, it is possible to install root observation windows (RW) along soil profiles also in the field (Smit et al. 2000; Neumann et al. 2009; Vetterlein et al. 2020). In pioneer work, this technique was mainly employed for studies of root development and dates back to the early twentieth century (McDougall 1916; Böhm 1984). Later on, studies on root turnover (Teskey and Hinkley 1981; Keyes and Grier 1981), root branching (Sword 1998), monitoring of rhizosphere pH (Häussling et al. 1985), detection of enzyme activities (Dong et al. 2007; Meier et al. 2014), or microlysimetric collection of rhizosphere soil solutions (Dieffenbach and Matzner 2000) were performed with RWs permanently installed in forest ecosystems. More recently, planar imaging optodes were used to record changes in oxygen concentration in the rhizosphere of salt marsh plants (Ahkami et al. 2024) and in a vegetated Alaskan thermokarst bog to examine processes of methane oxidation and production (Turner et al. 2020). However, investigations of plant species in agricultural systems remain rare. Reports include the use of root windows for zymographic detection of rhizosphere phosphatase activities, monitoring rhizosphere pH with imaging optodes, and DGT imaging of P and Mn fluxes along the roots of field-grown maize plants (Vetterlein et al. 2020; Bilyera et al. 2022). Behr et al. (2024) and Francioli et al. (2025) used root windows in a long-term field experiment with winter wheat and maize to monitor root growth characteristics, perform HPLC–MS profiling of rhizosphere metabolites, and characterize rhizosphere microbial communities.

Installation of RW in the field requires cutting soil profiles with steel plates near the plants of interest. For seedlings derived from seeds sown along the RW, this approach enables repeated observations throughout the vegetation period (Vetterlein et al. 2020; Bilyera et al. 2022). However, for fast-growing plants (e.g., maize), the limited size of the observation plane can lead to excessive root proliferation along the window over time, resulting in overlapping rhizospheres. This can limit the differentiation of bulk soil from

rhizosphere soil and the identification of different root types with distinct functions (Bilyera et al. 2022). Moreover, concentrations of rhizodeposits along with related changes in rhizosphere-microbial activities and nutrient availability may be affected by excessive, artificial root growth.

Alternatively, observations may focus on root regeneration over time along the observation plane after RW installation, near intact plants. (Neumann et al. 2009; Behr et al. 2024; Francioli et al. 2025). However, cutting soil profiles near the test plants inevitably results in root cutting and wounding. These experimental artifacts may affect plant development in general, with the strongest effects expected during the time period immediately after RW Installation. Accordingly. Liu et al. (2023) reported that cutting parts of the root system of soil-grown wheat plants resulted in significant reductions of shoot dry matter and even longer-lasting effects on grain yield, particularly expressed under conditions of water limitation.

If the RWs are well-installed and insulated against fluctuations in temperature, measurements of soil temperature and soil moisture conditions behind root windows compared with undisturbed bulk soil usually revealed no distinct differences (Böhm 1979; Smit et al. 2000). A tight contact with the profile surface is essential to avoid the formation of air gaps caused by artificial root proliferation between the window and the soil profile which can affect root functions as well as the physicochemical conditions at the observation plane (Smit et al. 2000), with consequences for the availability of oxygen and water, redox state, microbial activity and nutrient solubility. Moreover, the increased mechanical impedance during root development forced along a solid plate may also induce alterations in root and shoot growth, root exudation, hormonal profiles, and the metabolome of root and shoot tissues (Boeuf-Tremblay et al. 1995; Jacobsen et al. 2021; Kumari et al. 2021). Consequently, root windows under field conditions do not represent completely undisturbed culture systems and are at risk of artifacts that affect soil conditions, root growth, and activity, with consequences for plant development, posing a potential bias in RW-based rhizosphere studies.

Recently, we used the RW approach to study the effects of tillage and fertilization intensity on plant performance, physiological stress indicators, rhizosphere metabolites, and the composition and functions of the rhizosphere microbiome under field conditions in winter wheat (Azarbad 2024; Behr et al. 2024). However, it remained unclear whether RW-derived data would be representative of wheat plants grown under completely undisturbed conditions in the central plots (CP) of the field experiment. Therefore, the previously published data (Behr et al. 2024) were compared here with data obtained from wheat plants grown in the same year in undisturbed plots of a long-term field experiment located in Bernburg, Germany (Deubel et al. 2011). It was hypothesized that this comparison would identify plant and microbiome responses attributable to disturbances caused by the installation of the root windows, rather than to the experimental treatments. To assess the potential impact of root window installation on plant performance, responses to the various experimental treatments were recorded for a range of indicators covering growth and the physiological status of plants growing along RW compared with undisturbed CP plants. These indicators comprised (i) shoot biomass production reflecting plant growth in general, that might be affected as a consequence of root damage during RW installation or the mechanical impedance of the solid observation window (Kumari et al. 2021; Liu et al. 2023); (ii) root growth characteristics such as root biomass, root length and diameter, and length of root hairs most likely affected by root cutting during the installation process; (iii) the plant nutritional status reflecting the capacity of roots for nutrient acquisition; (iv) expression of stress-related genes in the shoot tissue as well as profiles of hormones and selected stress metabolites were recorded to assess a potential physiological impact of systemic stress factors induced by root damage related with RW installation (Kumari et al. 2021). (v) The composition of fungal and bacterial/archaeal communities in the rhizosphere known to be affected by the physicochemical conditions in the rhizosphere (Huang et al. 2014; Zhalnina et al. 2018; Vieira et al. 2020) was recorded as an indirect indicator for potential rhizosphere effects induced by RW installation since assessments of root exudation and related alterations in rhizosphere chemistry are only possible in RW plants (Behr et al. 2024). The DNA-based characterization of the rhizosphere microbiome comprises not only active microorganisms and consequently reflects potential RW effects over extended time periods, Samplings were conducted during late vegetative

growth (heading stage, EC 59) when the most intense expression of root development and root activity can be expected prior to a shift of preferential carbon partitioning towards the formation of reproductive shoot organs and induction of root senescence during generative growth (Mattsson et al. 1993; Marschner 1995; Santangeli et al. 2024). Consequently, we expected that rhizosphere effects directly associated with the investigated agricultural management practices would be most clearly detectable at the selected stage of plant development, which may also provide sufficient time for recovery from initial disturbances caused by the RW installation process.

To the best of our knowledge, systematic comparisons of plants growing along RWs with undisturbed plants have not been published so far.

Materials and methods

Study site and experimental design

The study was conducted at a long-term field trial site established in 1992 by the Anhalt University of Applied Sciences in Bernburg, Germany (51.82°N, 11.70°E). A loess-chernozem soil on limestone characterizes the site and is subject to a crop rotation with grain maize (*Zea mays*), winter wheat 1 (*Triticum aestivum* post maize), winter barley (*Hordeum vulgare*), winter oilseed rape (*Brassica napus* ssp. *napus*), and winter wheat 2 (*Triticum aestivum* post-rapeseed). Plants were cultivated on five 1.2 ha-sized plots in four replicates, divided into sub-plots managed under conventional tillage (mouldboard plow, MP; 20–30 cm depth with soil inversion) or conservation tillage (cultivator, CT; 12–15 cm topsoil loosening). In addition, subplots were subjected to two nitrogen (N)-fertilization strategies: intensive (Int) N-fertilization with pesticide/growth regulator application according to the farmer´s practice, and 50% reduced extensive (Ext) N-fertilization without the addition of fungicides and growth regulators. More details regarding the study site and experimental setup are described by Deubel et al. (2011); Sommermann et al. (2018), and Babin et al. (2019). Details on agricultural management are summarized by Behr et al. (2024).

In the 2018/2019 growing season, at 6 months after sowing, RWs were installed in winter wheat 2 (cultivar Lemmy) on April 9th, 2019 (EC 29 end of tillering) in four replicates along the border rows of each treatment (MP-Ext, MP-Int, CT-Ext, CT-Int) according to the method of Neumann et al. (2009) and Behr et al. (2024). Briefly, steel plates (50 × 50 cm) were used to cut soil profiles to a depth of 50 cm at distances of approximately 10 cm from a row of 8–10 wheat plants. The profiles were then covered with plexiglass plates tightly fixed to the profile surface with vertical wooden timbers. Two layers of Styrofoam panels (10 cm diameter) were applied along the window, which was then covered from the top with black plastic foil, and the previously excavated field soil was loosely refilled. This setup has been designed to provide effective insulation against light, external water penetration, and temperature interference, ensuring optimal conditions for observing and analyzing the root growth. Detailed video documentation of the installation process is also available under: http://dicontrol.igzev.de/wp-content/uploads/2021/01/Root-windows_Moradtalab_Neumann_2020.mp4.

At final sampling on May 21 st 2019 (late heading stage, EC59), the plants had received a total N supply of 130 kg ha^{-1} for CT-Int and MP-Int versus 60 kg ha^{-1} for CT-Ext and MP-Ext. Sampling was performed in four replicates, with plants grown along root windows (RW) and from undisturbed central plots (CP) across four experimental treatments.

Sampling of above- and belowground plant material

For each replicate, six plants (RW) and 9 plants (CP) were excavated and divided into root systems and shoots. Shoot dry mass (SDM) and root dry mass (RDM) were determined after drying representative aliquots of the plant material at 65 °C until weight constancy was reached. Loosely adhering root-affected (RA) soil was obtained after shaking of roots and subsequently sieved to 2 mm and stored at −20 °C for further analyses (no sample was obtained for RW-RA-CT-Int-2). For rhizosphere (RH) microbiome analysis, the remaining roots were washed with sterile tap water. Microbial cells and strongly adhering soil particles were detached from 5 g of roots by three 1-min Stomacher treatments, and the RH pellets were recovered by centrifugation according to Schreiter et al. (2014). RH pellets were kept at −20 °C until TC-DNA extraction. The remaining root systems after Stomacher treatment were stored at 4 °C with

subsequent conservation in 30% (v/v) ethanol for the determination of total root length (RL) after digital scanning and subsequent analysis with the WinRhizo root analysis software (Regent Instruments, Quebec, Canada). Root hair length (RHL) was determined from high-resolution digital photographs of excavated root samples and subsequently analyzed with Zeiss Axiovision software (Zeiss, Oberkochen, Germany). In parallel, flag leaves from three plants per plot were collected and oven-dried at 65 °C for the determination of mineral nutrients.

Nutrient analysis

After grinding of dried flag leaves, 200–500 mg of the plant material was subjected to microwave digestion (Mars 6, CEM, Charlotte, NC, USA) with 5 mL HNO_3 (65%) and 3 mL H_2O_2 (30%) for 20–30 min. Phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) concentrations were determined via inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher Scientific, Waltham, USA). Total carbon (C) and N were determined via elemental analysis (Elementary Vario El cube, Elementar, Langenselbold, Germany).

RNA extraction, and analysis of gene expression and stress metabolites from flag leaves

In total, flag leaves from nine plants were collected per replicate in CP. Flag leaves were also collected from three plants per replicate grown in the RW variants. The flag leaves were cut into small pieces (5 cm) and immediately stored in RNAlater solution (Ambion, Life Technologies, Carlsbad, USA). The leaf tissues in RNAlater were stored overnight at 4 °C to allow thorough penetration of the tissue and then transferred to −20 °C for long-term storage. For RNA extraction, the leaf samples were retrieved with sterile forceps, excess RNAlater was quickly blotted away with a paper towel, and then the samples were submerged in liquid nitrogen and pulverized. RNA was extracted from 100 mg leaves with the RNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany) and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Thereafter, cDNA was synthesized from 2 µg of RNA with the High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA, USA) as described by Behr et al. (2024).

Gene expression of 30 genes from wheat (*Triticum aestivum* L.) associated with biotic and abiotic stress responses or involved in N-metabolism and Fe-transport was studied as described previously by Chowdhury et al. (2019) and Behr et al. (2024). Briefly, the reference genes coding for ubiquitin (*Ubi*) and elongation factor 1α (*EF1α*) were used as a reference, and the comparative C_T method (also known as the $\Delta\Delta C_T$ method) (Livak and Schmittgen 2001) was used for the qPCR assay. The target and endogenous control genes were validated, and only primers with 100% (±10%) efficiencies were used. For this purpose, the target gene primers and the endogenous controls were used to amplify RNA (cDNA) from winter wheat with a concentration range of 100 ng to 100 pg in a series of ten-fold dilutions in triplicate for each dilution. The qPCR was performed with Power SYBR Green Supermix using a peqSTAR 96Q thermal cycler (PEQLAB Biotechnologie GmbH, Erlangen, Germany) with three technical replicates as described previously (Chowdhury et al. 2019). A total of 28 primer pairs met the criteria and were selected for further analysis (Behr et al. 2024). The determination of hormones and stress metabolites was conducted as described by Behr et al. (2024).

Microbial community analysis

TC-DNA was extracted from complete RH pellets or 0.5 g RA soil (fresh weight) using the FastPrep FP24 bead-beating system (twice, every 30 s, 5.5 m s-1) and the FastDNA Spin Kit for soil (MP Biomedicals, Santa Ana, CA) according to the manufacturer's recommendations. Extracted TC-DNAs were purified using the GeneClean Spin Kit (MP Biomedicals, Santa Ana, USA). TC-DNA yield and quality were checked by agarose gel electrophoresis and stored at −20 °C.

The bacterial/archaeal community was profiled based on the amplification of the V3-V4 region of the 16S rRNA gene as described in Babin et al. (2021) using primers 341F/806R. Adding of Illumina sequencing adapters and sample-specific dual indexes and library preparation were performed as described in Fernandez-Gnecco et al. (2022). Sequencing was carried out on an Illumina MiSeq platform using Reagent Kit v2 (2×250 bp; Illumina, San Diego, USA).

16S rRNA gene reads were processed into amplicon sequence variants (ASVs) using DADA2 version 1.10.0 (Callahan et al. 2016). Taxonomic annotation of bacterial/archaeal ASVs was conducted with the SILVA SSU rel. 132 database (Quast et al. 2013) as described in Fernandez-Gnecco et al. (2022).

To account for PCR and sequencing artifacts, microbial ASVs with fewer than five reads across the entire data set were excluded from the analysis. Furthermore, ASVs classified as chloroplasts, mitochondria, or present in the negative control were discarded. This resulted in a final number of 13,138 ASVs and, on average, 32,246 quality reads per sample. Unassembled raw sequences were submitted to the NCBI Sequence Read Archive (root window: PRJNA1037063; central plot: PRJNA1109326.

Fungal community composition was assessed by high-throughput sequencing of the Internal Transcribed Spacer (ITS2) region amplified using primer pair ITS86F/ITS4 (Op de Beeck et al. 2014; White et al. 1990) as previously described (Babin et al. 2021; Behr et al. 2024). ITS2 sequencing on the Illumina ® MiSeq® platform in paired-end mode (2 × 300 bp) followed by taxonomic classification using a local GALAXY Bioinformatics Platform in combination with the fungal UNITE database v8.0 (UNITE Community 2019) was conducted as previously described in detail (Babin et al. 2021; Behr et al. 2024). Unassembled raw sequences were submitted to the European Nucleotide Archive (ENA) under the following BioProject accession number (PRJEB67553). The generation of the ASV abundance table was based on the SH numbers of the UNITE database as identifiers, and the summed sequences per assignment, which resulted in 1,734 fungal ASVs with an average of 89,919 reads per sample.

Data analyses

The growth traits of winter wheat and shoot nutrient concentrations were statistically analyzed using R software (v.4.3.2; R Core Team 2023). The main effect of the tillage practice, N-fertilization intensity, and RW installation, as well as the interaction effect between tillage and N-fertilization intensity, were analyzed by three-way Analysis of Variance (ANOVA) for SDM, RDM, RL, RH and shoot nutrients. Homoscedasticity and normal distribution of residuals were verified using the 'performance' package (v.0.10.5; Lüdecke et al. 2021). When ANOVA assumptions were not met, data transformation was applied using the 'rcompanion' package (v.2.4.34; Mangiafico 2023), following Tukey's ladder of power transformation method. Post-hoc analyses used Tukey's Honestly Significant Difference (HSD) test from the 'agricolae' package (v.1.3–7; de Mendiburu 2023). A threshold below a p-value of 0.05 was considered statistically significant. Data visualization was conducted using 'ggplot2' (version 3.4.4; Wickham 2016) and 'ggpattern' (version 1.0.1; FC and Davis 2022) packages. For gene expression PERMANOVA (10.000 permutations) analyses were performed based on Bray–Curtis dissimilarities calculated from ΔCt values of 28 genes in R (version 3.6.1) using package vegan and data were represented in form of a Principal coordinates analysis (PCoA).

Statistical analyses of microbial communities were performed in R (R Core Team 2019) including the packages vegan (Oksanen et al. 2019), phyloseq (McMurdie and Holmes 2013), RColorBrewer (Neuwirth 2014), ggplot2 (Wickham 2016), pheatmap (Kolde 2019), MASS (Venables and Ripley 2002).

PERMANOVA (10.000 permutations) and NMDS analyses were performed based on Bray–Curtis distances calculated from log-transformed relative abundances (bacteria/archaea) and count data (fungi).

Results

Six weeks after the RW installation, the plants reached the heading stage (EC 59), and new root development was detectable over the whole surface of the observation plane without artificial root growth outside the soil profile (Fig. 1). Roots were densely covered with root hairs (Fig. 1B). In all treatments, comparative samplings were performed for undisturbed control (CP) plants and plants growing along the RW.

Shoot and root growth

In all treatments, no significant differences for dry shoot biomass were recorded between RW plants and the undisturbed CP plants (Fig. 2A). Among the different treatments, shoot biomass in MP-Int (was significantly reduced by 17% (CP) and 28% (RW) compared with the CT-Ext treatment with reduced input

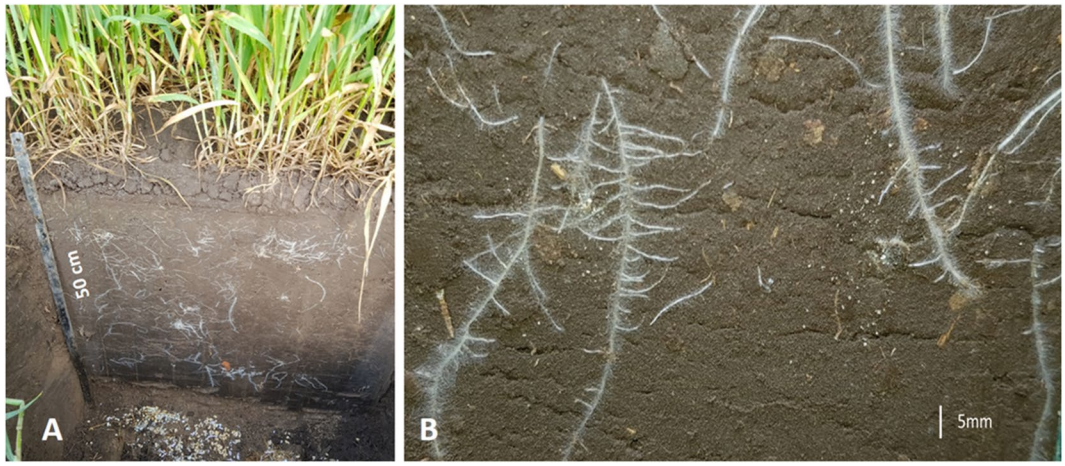

Fig. 1 Root development along the root observation plane of field-grown winter wheat (cv. Lemmy, EC 59) six weeks after installation of root windows (A). Close-up of fine roots with root hairs (B)

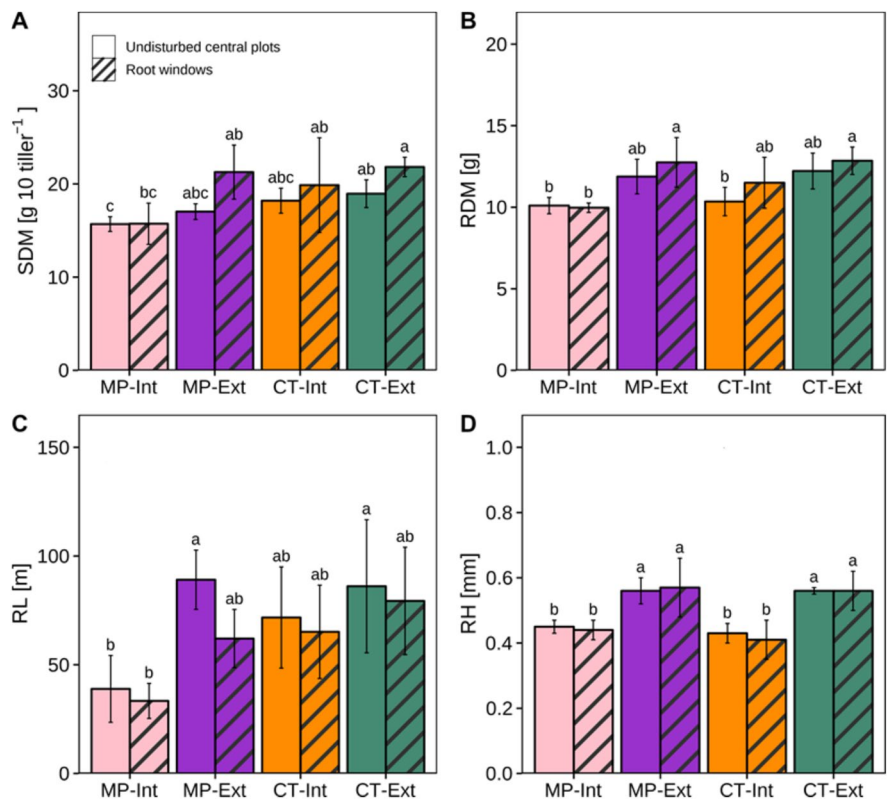

Fig. 2 Effect of long-term agricultural practice on A) shoot dry mass (SDM), B) root dry mass (RDM), C) total root length (RL), and, D) root hair length (RH) of winter wheat (cv. Lemmy; EC 59) affected by root window installation in comparison to undisturbed plants in central plots. MP- Mouldboard plow, CT- Cultivator tillage, Ext = Extensive N-fertilization intensity without fungicides and growth regulators, Int = Intensive N-fertilization intensity with fungicides and growth regulators. Data are adjusted to ten tillers, representing the average number of tillers per plant. Values are presented as means ± standard deviation of four replicates. Means with different letters are significantly different (Tukey-test, $p \leq 0.05$)

of N fertilizers, fungicides and energy for soil tillage. This effect coincided with intense visible symptoms of powdery mildew on leaves, particularly in the MP-Int treatment in both RW and CP plants (Suppl. Fig. S1).

A similar pattern was also observed for the root dry mass (RDM) and total root length (RL) of the excavated root systems, with no significant differences between RW and CP plants. In the MP-Int treatment, RDM decreased by 17% (CP) and 22% (RW) relative to CT-Ext. The RL showed an even more pronounced decline of 55% (CP) and 58% (RW) in the MP-Int treatment compared with CT-Ext (Fig. 2B-C). Additionally, intensive fertilization significantly reduced root hair length (RHL) compared with extensive fertilization in both tillage practices (Fig. 2D), whereas RW installation did not significantly affect RHL. Independent of the fertilization regime, tillage intensity affected fine root development, resulting in a significantly higher total length of fineroots (diameter < 0.2 mm) in CT compared with MP treatments, both in RW and CP plants (Supplementary Figure S2).

Plant nutritional status

The mineral nutritional status (Table 1) at flag leaf maturity was generally low or reached a critical level for the macronutrients nitrogen (N) and potassium (K), and for copper (Cu), as well as for zinc (Zn) as plant micronutrients. Compared to plants in the undisturbed CP, the foliar concentrations of K, magnesium (Mg), calcium (Ca), manganese (Mn), and Cu tended to be higher for all treatments in RW plants, although the respective differences were not significant in all cases.

Only in the CT-Ext treatment of RW plants, a reduction in N fertilization led to a notable 17% decrease in foliar N concentration compared to the CT-Int treatment. Similar trends were also recorded for MP-Ext vs. MP-Int (RW) and CT-Ext vs. CT-Int (CP). In RW, CT-Ext plants also showed significantly lower leaf concentrations of K, Mg, and sulfur (S) compared to CT-Int plants.

Cultivator tillage practice significantly reduced Mg (CT-Ext) and S concentrations (CT-Int and CT-Ext) in RW plants. In CP plants, CT significantly decreased Ca concentrations in CT-Int plants and Cu concentrations in CT-Ext plants. However, the nutrient concentrations did not decline below the deficiency thresholds reported for wheat.

Expression of stress-related genes and stress metabolites in the leaf tissue of winter wheat

As indicators for the physiological stress level of the investigated wheat plants, 28 target genes involved in nutrient transport/metabolism and (a)biotic stress responses, as well as regulatory and signal transduction genes, were selected for expression analyses in the tissue of flag leaves. The clustering of the farming practices (Treatment) showed a clear separation along the tillage practices, with an overall higher expression level for the MP treatments compared to a lower expression level for the CT treatments (Fig. 3A). The fertilization intensity (Int vs. Ext) had only a minor impact on the gene expression clustering pattern. CP and RW plants showed highly similar gene expression patterns in response to different farming practices. Clustering was also confirmed by PERMANOVA analysis (Table 2).

A principal coordinate analysis (PCoA) of gene expression levels confirmed the separation of samples by tillage practice and a very narrow clustering pattern between CP and RW samples, especially for MP-Int and MP-Ext treatments (Fig. 3B). A PERMANOVA analysis showed that tillage practice had the strongest influence on the gene expression and explained 64.1% of the variance. The location (CP vs. RW) did not show a significant influence (Table 2).

Similar to the expression of stress-related genes, the levels of physiological stress indicators, such as jasmonic and salicylic acids, proline, total antioxidants, and phenolics, and the activity of ascorbate peroxidase were reduced in the flag leaf tissue of wheat plants grown under reduced tillage intensity (CT) in both fertilization regimes. No significant differences were recorded between RW and CP plants (Table 3).

Composition of microbial communities Separate clustering of RH and RA samples was demonstrated by NMDS analysis, with distinct subclusters depending on tillage practice. Microbial communities from CP and RW samples were highly similar (Fig. 4). This was confirmed by PERMANOVA analysis, which revealed the habitat (RH vs. RA) and the tillage practice (MP vs. CT) as the main shaping factors, whereas the sampling location (CP vs. RW) only marginally influenced the microbial community composition (Table 4). This also became apparent at the level of the top 30 most

Table 1 Impact of long-term farming practices and root window (RW) installation on A) the nutrient status of winter wheat (cv. Lemmy) in comparison to the undisturbed plants in the central plots (CP). MP = mouldboard plow tillage, CT = cultivator tillage, Ext = extensive N-fertilization without fungicides and growth regulators, Int = intensive N-fertilization including fungicides and growth regulators, CP = undisturbed central plots. Different letters indicate significant differences between farming practices according to three-way ANOVA, Tukey test: $p \leq 0.05$. B) *P*-values from ANOVA show the main effects and interactions of tillage practice, N-fertilization, and RW installation

A)	Macronutrients [g kg^{-1} shoot DM]												Micronutrients [mg kg^{-1} shoot DM]							
	C_{total}		N_{total}		P		K		Mg		Ca		S		Cu		Fe		Mn		Zn	
Deficiency Threshold[#]			30.0		1.5		20.0		1.0		1.5		1.0		3.0		25.0		15.0		15.0	
MP-Int-RW	449.9	abc	37.13	a	2.14	ab	18.40	a	1.82	a	6.56	a	4.57	a	4.58	a	51.40	b	52.61	a	14.93	ab
MP-Ext-RW	450.4	abc	35.88	ab	2.54	a	17.25	ab	1.63	ab	5.69	bc	3.44	bcd	4.79	a	66.64	ab	52.90	a	15.82	ab
CT-Int-RW	453.8	a	37.88	a	2.39	ab	19.13	a	1.73	ab	5.91	ab	3.88	b	4.04	ab	67.64	ab	57.14	a	15.40	ab
CT-Ext-RW	448.8	bc	31.38	b	2.13	ab	15.65	bc	1.40	c	5.33	bcd	2.92	e	4.15	ab	100.87	a	55.31	a	16.58	ab
MP-Int-CP	450.0	abc	36.01	ab	2.04	b	12.03	d	1.55	bc	5.88	ab	3.79	bc	3.99	ab	56.14	ab	39.92	b	18.31	a
MP-Ext-CP	451.7	ab	37.89	a	2.39	ab	13.63	cd	1.38	c	5.05	cd	3.38	cd	4.69	a	89.81	ab	39.33	b	19.79	a
CT-Int-CP	451.1	ab	38.14	a	2.44	ab	14.30	cd	1.38	c	4.72	d	3.54	bcd	3.63	bc	61.46	ab	41.70	b	15.72	ab
CT-Ext-CP	446.2	c	35.14	ab	2.43	ab	12.15	d	1.39	c	4.93	d	3.19	de	3.08	c	52.29	ab	39.26	b	12.31	b

B)	C_{total}	N_{total}	P	K	Mg	Ca	S	Cu	Fe	Mn	Zn
Tillage practice (TP)	0.4255	0.3338	0.2941	0.9670	0.0004	< 0.0001	< 0.0001	< 0.0001	0.1572	0.2507	0.0059
Fertilization intensity (Fertl-int)	0.0084	0.0006	0.0767	0.0044	< 0.0001	< 0.0001	< 0.0001	0.3896	0.0761	0.4408	0.9661
Root Window (RW)	0.1659	0.1202	0.7022	< 0.0001	< 0.0001	< 0.0001	0.0250	0.0005	0.6177	< 0.0001	0.2583
TP x Fertl-int	0.0001	< 0.0001	0.0006	0.0012	0.8154	0.0036	0.6146	0.0170	0.2313	0.6411	0.1291
TP x RW	0.0197	0.6217	0.0298	0.3262	0.1986	0.5052	0.0075	0.1487	0.0112	0.6412	0.0008
Fertl-int x RW	0.6173	0.0104	0.4685	0.0211	0.0054	0.0545	< 0.0001	0.7471	0.1801	0.6179	0.1864
TP x Fertl-int x RW	0.7029	0.4800	0.2428	0.3989	0.0094	0.0855	0.5710	0.0392	0.2918	0.8409	0.0898

[#]Deficiency thresholds of macro- and micro-nutrients after Campbell (2000)

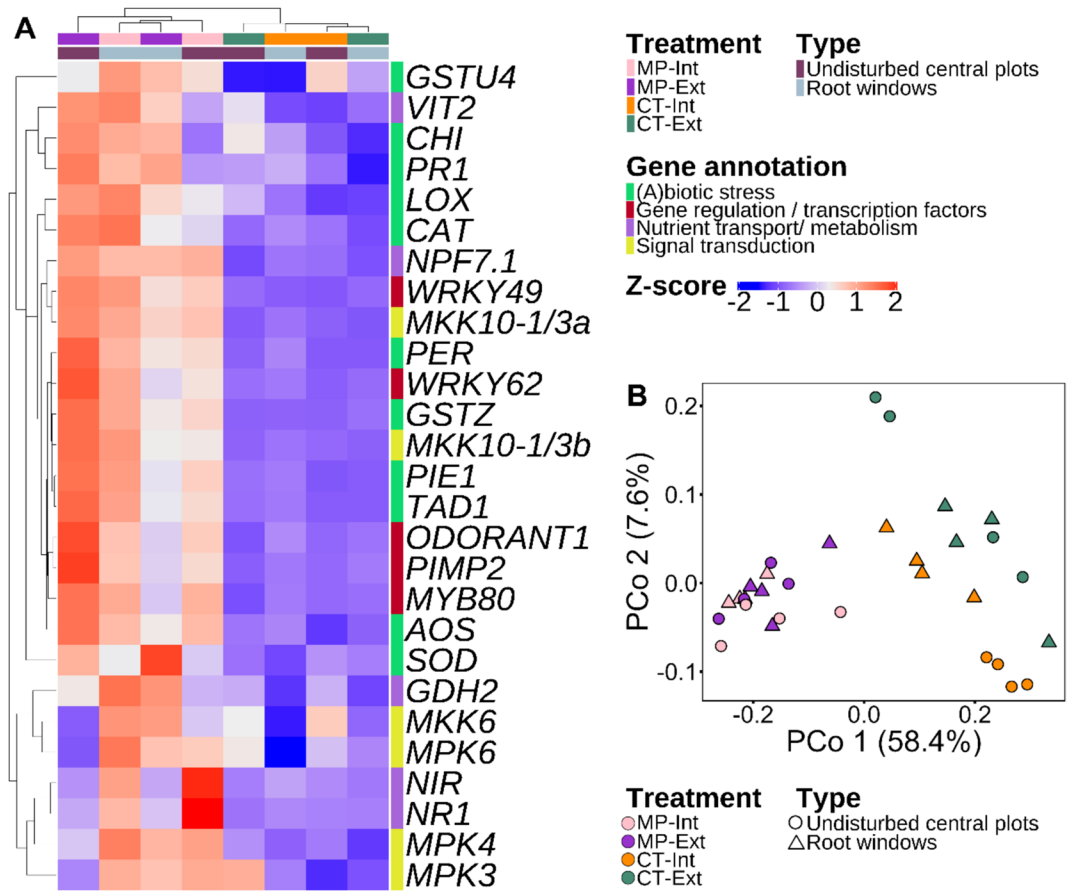

Fig. 3 Influence of farming practice and root window installation on gene expression in flag leaves of winter wheat (cv. Lemmy; EC 59). A) Heatmap of mean gene expression (ΔCq values) and B) Principal coordinates analysis (PCoA) based on the expression of 28 selected genes. MP = mouldboard plow, CT = cultivator tillage, Ext = extensive N-fertilization intensity without fungicides and growth regulators, Int = intensive N-fertilization intensity with pesticides and growth regulators

Table 2 PERMANOVA analysis based on Bray–Curtis distances (10,000 permutations) performed on gene expression profiles of 28 selected genes expressed in leaves of winter wheat grown in central plots or with root windows (location) under different management practices of LTE Bernburg. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$

Factor	Explained Variance (%)	P-Value
Tillage	64.1	< 0.001 ***
N-Fertilization intensity	2.8	< 0.05 *
Location (RW vs CP)	0.9	< 0.328
Tillage:Fertilization	2.3	< 0.086
Tillage:Location	2.6	< 0.069
Fertilization:Location	4.8	< 0.014 **
Residuals	22.5	

abundant bacterial/archaeal (Fig. 5) and fungal genera (Fig. 6). In both cases, clustering was according to the habitat, followed by tillage practice. The relative abundance of major genera was similar in CP and RW plants. Thus, the heatmap of the dominant bacterial genera (Fig. 5) revealed several taxa with higher relative abundance in the rhizosphere of CP and RW-grown wheat plants compared to root-affected soil, such as Allorhizobium, Neorhizobium, Pararhizobium, Rhizobium, Devosia, Pseudoxanthomonas, Saccharimonadales, Massilia, and *Pedobacter*. The heatmap of the 30 dominant fungal genera (Fig. 6) indicated a higher variability but showed that *Zymoseptoria*, *Cystofilobasidium,* and *Filobasidium* were enriched in the rhizosphere of both CP and RW-grown plants.

Table 3 Phytohormones [ng g^{-1} FW]: ABA = abscisic acid, SA = salicylic acid and JA = jasmonic acid; and stress-associated metabolites: T-AO = total antioxidants [% inhibition]; proline [mg g^{-1} FW], total phenolics [mg gallic acid equivalents g^{-1} FW]; APX = ascorbate peroxidase activity [units g^{-1} FW] in leaves of winter wheat (cv. Lemmy, EC 59) grown under different long-term agricultural practices. MP –mouldboard plow tillage, CT – cultivator tillage, Ext –extensive N-fertilization intensity without fungicides and growth regulators, and Int – intensive N-fertilization intensity with pesticides and growth regulators. Values are presented as means ± standard errors of four replicates. Means sharing different letters are significantly different by the Tukey-test ($P \leq 0.05$); FW = fresh weight

		MP-Int	MP-Ext	CT-Int	CT-Ext
Phytohormones					
ABA	RW	0.94 ± 0.17b	1.03 ± 0.21ab	1.39 ± 0.16a	1.25 ± 0.15ab
	CP	0.87 ± 0.12b	0.96 ± 0.17ab	1.29 ± 0.14a	1.17 ± 0.14ab
SA	RW	0,84 ± 0.08a	0.74 ± 0.06a	0.42 ± 0.12c	0.55 ± 0.05b
	CP	0.71 ± 0.05a	0-66 ± 0.06a	0.34 ± 0.06c	0.47 ± 0.04b
JA	RW	0.72 ± 0.12a	0.76 ± 0.07a	0.33 ± 0.03c	0.54 ± 0.08b
	CP	0.64 ± 0.06a	0.68 ± 0.08a	0.29 ± 0.03c	0.43 ± 0.04b
Stress indicators					
Proline	RW	4.20 ± 1.05a	4.79 ± 0.96a	2.26 ± 0.29c	2.68 ± 0.26b
	CP	3.71 ± 0.96a	4.41 ± 0.94a	1.82 ± 0.22c	2.42 ± 0.27b
Phenolics	RW	5.31 ± 1.12a	5.63 ± 1.11a	3.32 ± 0.90c	4.42 ± 0.84b
	CP	5.21 ± 1.12a	5.52 ± 1.11a	3.21 ± 0.91c	4.34 ± 0.90b
T-AO	RW	104.02 ± 0.21a	102.08 ± 0.35a	95.81 ± 0.28b	97.42 ± 1.57b
	CP	104.06 ± 0.20a	102.18 ± 0.23a	95.88 ± 0.23b	97.44 ± 1.66b
APX	RW	692.21 ± 1.34a	699.16 ± 1.14a	487.80 ± 0.75b	495.94 ± 0.80b
	CP	691.52 ± 1.11a	698.39 ± 1.15a	486.97 ± 0.71b	495.07 ± 0.77b

Additionally, we tested the effects of different agricultural practices (tillage, N-fertilization intensity) on microbial community composition separately in CP and RW samples. In both sampling locations, similar influences of tillage and N-fertilization intensity were observed. RW and CP analysis showed in agreement that tillage exhibited a much stronger effect on the microbial community than N-fertilization intensity, which was even more pronounced in RH in the case of bacterial/archaeal communities (Supplementary Tables S2 and S3).

Discussion

Implementing root windows (RWs) under field conditions is a labor-intensive task, yet it offers unique opportunities for non-destructive in situ observation of root growth characteristics and rhizosphere processes. Recently, we used the root window approach with winter wheat in a long-term field experiment to assess rhizosphere effects of varying tillage intensities, N fertilization, and pesticide use (Behr et al. 2024). Root windows were installed in early spring (EC 29 end of tillering), selecting plants with homogeneous regeneration after winter to reduce variability between replicates and to minimize the risk of excessive root growth at the observation plane, reported as a critical factor by Bilyera et al. (2022) after RW installation prior to sowing. However, RW installation along intact plants is a highly invasive process that requires cutting of soil profiles near the root systems of interest, which may result in root damage in some parts of the root system. Moreover, cutting the soil profile for RW installation may represent a disturbance to the long-term CT treatments similar to an intensive tillage event. This raises questions about the extent to which RW-based findings truly reflect natural conditions, as the installation process itself and the presence of RW during plant development may distort the results. To address this aspect, in the present follow-up study to Behr et al. (2024), we systematically compared RW plants with wheat plants grown in completely undisturbed central field plots (CP) subjected to the same treatments to identify potential effects induced by RW installation.

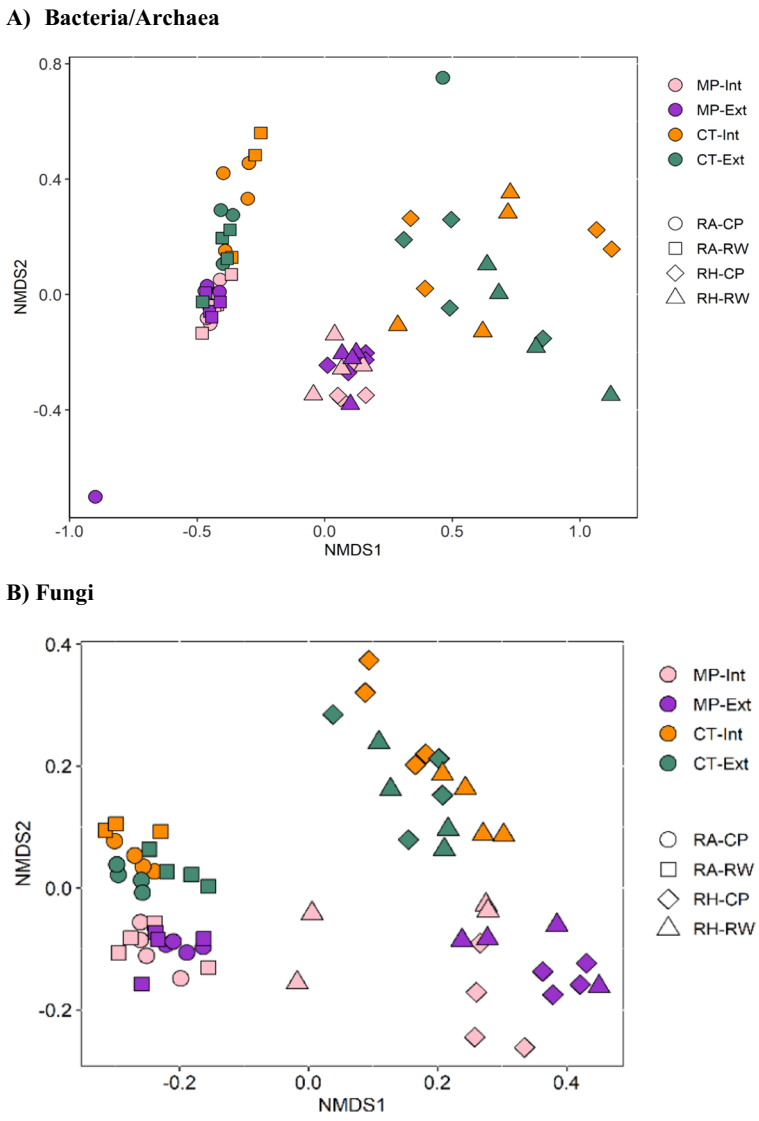

Fig. 4 Non-metric multidimensional scaling (NMDS) plots of bacterial/archaeal (A) and fungal communities (B) in root-affected soil (RA) and in the rhizosphere (RH) of winter wheat (cv. Lemmy) obtained from central plots and root windows (stress for both = 0.12). RW = root window; CP = central plot; MP = mouldboard plow; CT = cultivator tillage; Int = intensive N-fertilization including fungicides and growth regulators; Ext = extensive N-fertilization without fungicides and growth regulators

Table 4 PERMANOVA analysis based on Bray–Curtis distances (10,000 permutations) of bacterial/archaeal and fungal communities of winter wheat (cv. Lemmy; EC 59) obtained from undisturbed central plots (CP) and root windows (RW). Missing factor combinations are not significant. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$

Factor	Explained variance [%]	
	Bacterial/archaeal community	Fungal community
Location (RW vs CP)	1.3	1.6*
Habitat	23.5***	39.5***
Tillage practice	11.4***	12.3***
N-fertilization	2.2*	7.5***
Tillage practice x N-fertilization	1.8*	2.9**
Tillage practice x Habitat	3.9***	3.6***
Tillage practice x Location	1.3	1.8*
Habitat x Location	1.2	1.4*
Residuals	45.9	25.3

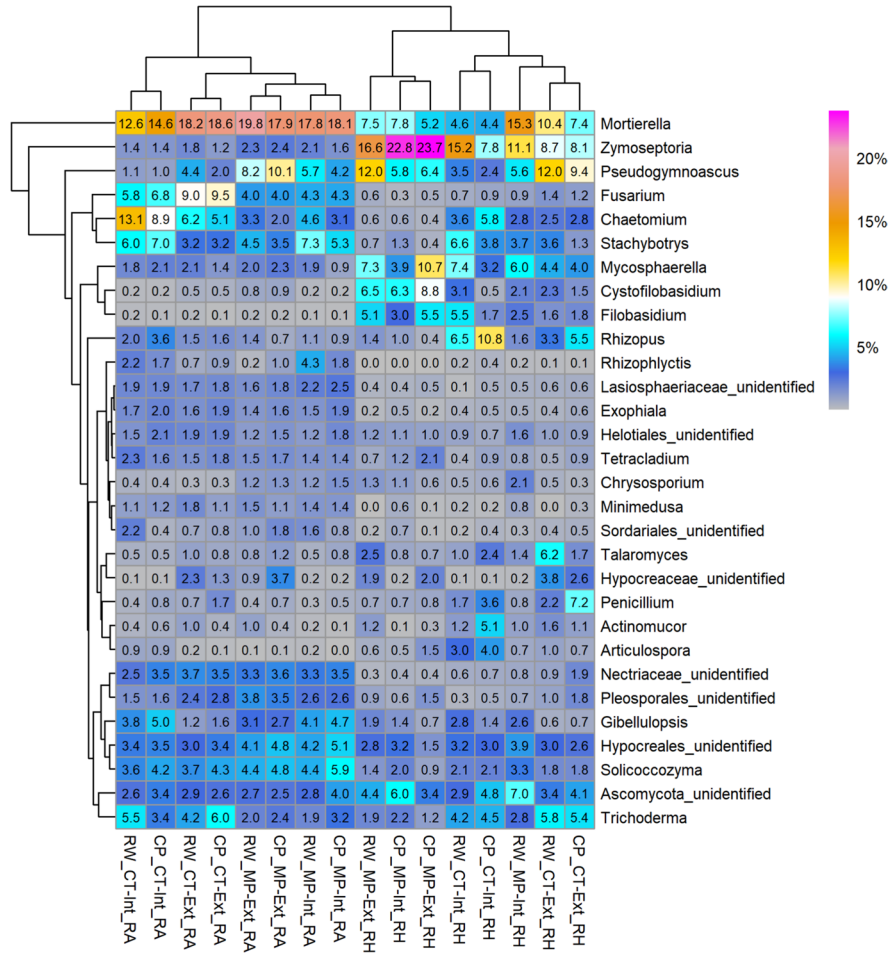

Fig. 5 Relative abundance distribution of the 30 most abundant fungal genera in the root-associated soil (RA) and rhizosphere (RH) of winter wheat (cv. Lemmy) obtained from central plots (CP) and root windows (RW). Values represent mean (n = 4). MP = mouldboard plouw; CT = cultivator tillage; Int = intensive N-fertilization including fungicides and growth regulators; Ext = extensive N-fertilization without fungicides and growth regulators

Effects on root growth and morphology.

Root development is the most likely factor directly affected by mechanical damage during RW installation close to intact plants. Additionally, subsequent regeneration of root growth along the RW can result in a large proportion of young roots with different physiological properties and growth characteristics compared with those of completely undisturbed plants (Hayes and Seastedt 1987). Moreover, regenerated roots growing along the solid observation window may encounter greater mechanical impedance than purely soil-grown roots. Accordingly, increased mechanical impedance of the substrate or root growth along mechanical barriers inhibited the growth of seminal roots in maize (Boeuf-Tremblay et al. 1995) and of primary roots in *Arabidopsis* and Tomato, while the formation of lateral roots and the growth of root hairs were stimulated (Jacobsen et al. 2021; Kumari et al. 2021).

In our study, root growth parameters were affected by both tillage intensity and N fertilization to a similar extent in RW and CP plants. The lowest values for root biomass, total root length, and root hair length were recorded in the MP-Int treatments. The reduction in root development most likely reflected an adaptive response to the high nutrient availability induced by the increased level of N-fertilization, stimulation of mineralization, improved soil incorporation of fertilizers, and increased nutrient availability from sub-surface soil layers triggered by increased tillage intensity (Wang et al. 2005; Nunes et al. 2020).. By contrast, conservation tillage significantly increased the length of fine roots (diameter < 0.2 mm), reflecting increased lateral root production. Hence, the observed root growth effects must be mainly attributed to the experimental treatments and were not significantly affected by the installation of root windows. In temperate climates, winter wheat usually

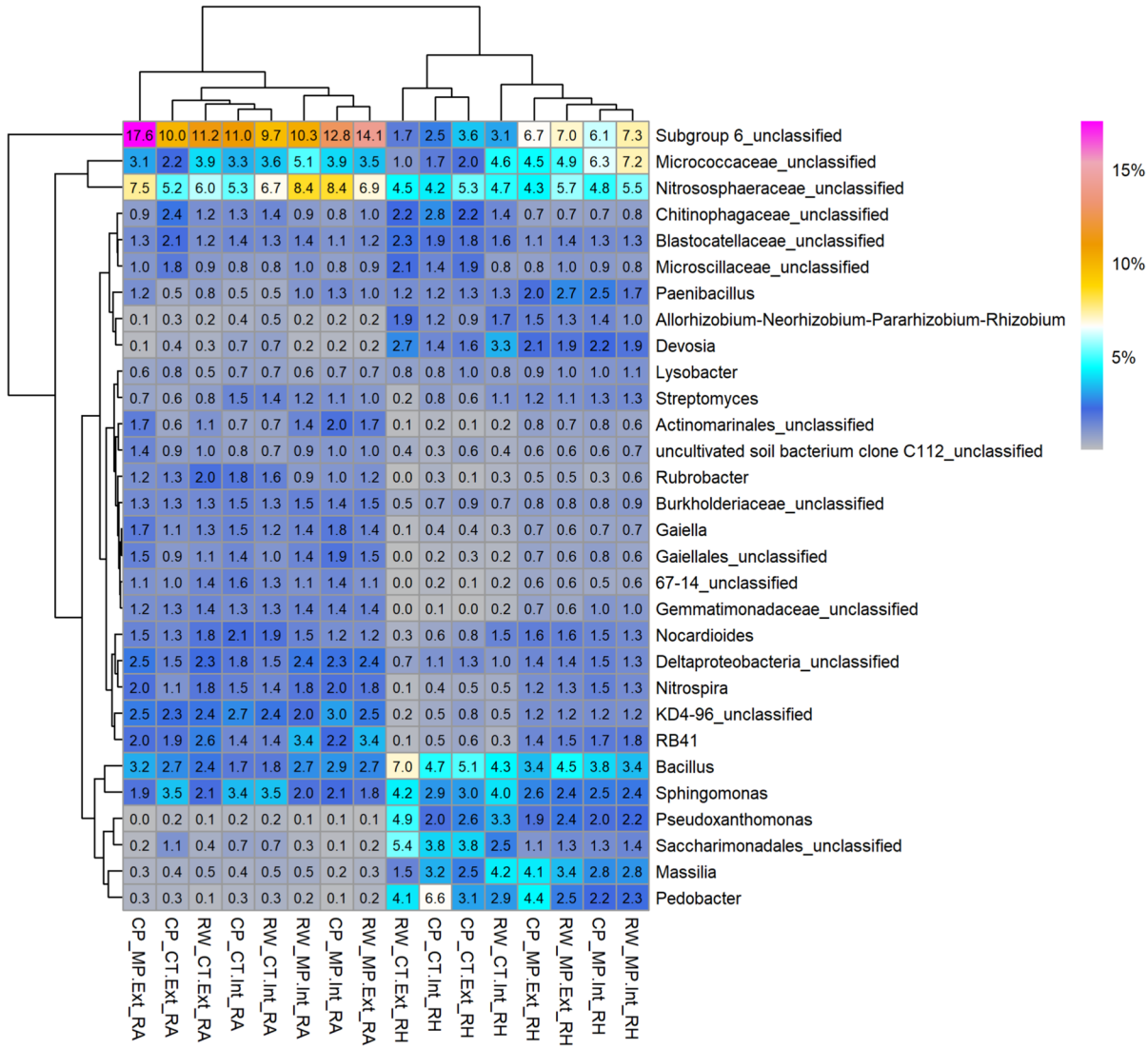

Fig. 6 Relative abundance distribution of the 30 most abundant bacterial/archaeal genera in the root-associated soil (RA) and rhizosphere (RH) of winter wheat (cv. Lemmy) obtained from central plots (CP) and root windows (RW). Values represent mean (n = 4). MP = mouldboard plough; CT = cultivator tillage; Int = intensive N-fertilization including fungicides and growth regulators; Ext = extensive N-fertilization without fungicides and growth regulators

shows only limited root development between autumn and early spring, followed by a phase of intense root proliferation in later stages of vegetative growth (Thorup-Christensen et al. 2009). Consequently, RW installation in early spring (as conducted in our study at the beginning of April) may reduce the risk of root damage by taking advantage of the poor root development at the time of RW installation, and ensure rapid root regeneration in the later stages of plant development, similar to undisturbed CP plants.

Systemic effects on shoot growth and physiology

Although root growth and morphology were obviously not affected by the presence of RW, initial effects of disturbance shortly after RW installation are likely to occur at the physiological level, with root-localized or transient stress responses. However, even in the long run, potential root damage during RW installation and subsequent alterations in root activity may also induce systemic

effects affecting aboveground plant parts and whole plant development. Accordingly, Liu et al. (2023) reported that partial cutting of roots in wheat was associated with a decline in shoot dry matter, particularly under water limitation. Additionally, increasing the mechanical impedance of the growth substrate affected not only root development but also reduced shoot dry matter production in maize, first detectable in the 5–6 leaf stage (Boeuf-Tremblay et al. 1995), and inhibited hypocotyl elongation in tomato seedlings (Kumari et al. 2021). By contrast, in our study, no significant differences in shoot dry matter production were recorded in RW compared with undisturbed CP plants in the late heading stage, but potential effects in later stages of plant development cannot be completely excluded. However, systemic metabolic modifications induced by stress factors are often detectable already before the expression of growth responses. In tomato shoots, increased ethylene emission (Roberts et al. 2002) and increased concentrations of stress hormones (abscisic, jasmonic, and salicylic acids) associated with declining levels of growth-related phytohormones (IAA, zeatin) and accumulation of various stress-related metabolites (e.g. trehalose, dopamine, caffeoylquinic acid, and suberic acid) were identified as responses to increased mechanical impedance of the growth substrate (Kumari et al. 2021). Similarly, root growth along mechanical plastic barriers (comparable to root windows) activated oxidative stress signaling in *Arabidopsis* (Jacobsen et al. 2021), and root exposure to increased mechanical impedance induced rapid reductions in leaf elongation of barley plants (Young et al. 1997). Therefore, it was of particular interest for the outcome of our study, to what extent comparable effects would still be detectable in the late vegetative growth state (EC 59) selected for plant and rhizosphere sampling. To identify latent systemic effects potentially induced by RW installation, we compared the expression of stress-related genes (Behr et al. 2024), hormonal profiles, and the accumulation of selected stress metabolites in flag leaf tissue of RW and CP plants. While conservation tillage significantly reduced the expression of 21 out of 28 stress-related genes (Behr et al. 2024), associated with lower levels of stress-related phytohormones (jasmonic and salicylic acids) and various physiological stress indicators (proline, total antioxidants, phenolics, activity of ascorbate peroxidase), no comparable differences were recorded between RW and CP plants. These findings demonstrate that, unlike tillage intensity, RW installation did not exert long-lasting latent systemic stress effects or stress memory detectable at the molecular level, consistent with the absence of growth responses.

As an exception, the nutritional status of RW plants was higher across a range of mineral nutrients (K, Mg, Ca, Cu, Mn) than in undisturbed controls. This observation was unexpected because the potential damage to roots from RW installation should instead cause limitations in nutrient acquisition. However, this effect is most likely not linked to disturbances caused by the RW installation process itself but rather to the RW's location. To enable RW samplings without interference in the undisturbed experimental plots and to avoid disturbances by RW installation in the plots with long-term reduced tillage, the RWs were generally located in the border rows of the experimental plots. Moreover, during RW installation, some plants located in front of the observation plane were removed by excavating the soil covering the steel plate used to cut the soil profile. Consequently, RW plants experienced less inter-plant competition for nutrient foraging and uptake than undisturbed CP plants growing in the center of the plots, likely resulting in improved uptake of certain mineral nutrients. However, the observed differences in nutrient accumulation were obviously not growth-limiting and did not translate into significant differences in shoot growth responses at least under the given experimental conditions. By contrast, a methodological bias may arise from those differences, particularly under conditions characterized by limited availability of the respective nutrients. An improved comparative analysis to assess potential border row effects would require including undisturbed plants grown in the border rows as well. Ideally, this should also comprise comparisons of the physicochemical conditions in the rhizosphere, such as nutrient availability, rhizosphere pH, hydraulic conductivity, and soil compaction effects, etc., although earlier studies (Böhm 1979; Smit et al. 2000) claimed no distinct differences in soil temperature and soil moisture conditions behind properly installed root windows compared with undisturbed bulk soil.

Effects on microbial communities

After a six-week cultivation period, the composition of microbial communities in the rhizosphere was only marginally affected by the presence of RW, and RW plants showed the same responses to different farming practices as undisturbed CP plants. Since root exudates and root-induced modifications of the physico-chemical conditions in the rhizosphere are important drivers shaping the assembly of rhizosphere microbiota (Huang et al. 2014; Zhalnina et al. 2018; Vieira et al. 2020), the high similarity of microbial communities in RW and CP plants may indicate that the installation of RWs did not significantly affect root exudation and rhizosphere chemistry. Consequently, the increased accumulation of various bioactive compounds (such as benzoxazinoids, trehalose, tryptophan, succinic acid), with known functions in rhizosphere-microbiome interactions (Sampedro et al. 2015; Vuroconda et al. 2016; Hu et al. 2018) detected by Behr et al. (2024) in the CT rhizosphere was most likely not affected by the RW-approach. However, it remains to be established to what extent the small differences detected for the composition of rhizosphere-microbial communities by PERMANOVA analysis may still be ecologically meaningful. Moreover, compositional similarity of microbial communities does not necessarily imply functional equivalence as an additional aspect to be considered in future comparative experiments.

The increased rhizosphere accumulation of bioactive compounds in CT plants was associated with enrichment of potential plant-beneficial microbial taxa and genes related to plant-beneficial functions (e.g., siderophore and spermidine production, trehalose synthase, ACC deaminase, Behr et al. 2024). Accordingly, a wide range of physiological stress markers were reduced in the shoot tissue of CT winter wheat, both in RW and CP plants. This scenario may indicate a reduced impact of stress factors on wheat plants grown in CT soil, most likely supported by beneficial interactions with the rhizosphere microbiome.

No effect of N-fertilization intensity on bacterial/archaeal communities in the rhizosphere of winter wheat was observed in RW and CP plants. In contrast, the fungal community was affected by N-fertilization intensity both in RW and CP. These results confirm previous observations in experiments with lettuce grown on the respective soils (Babin et al. 2021). Surprisingly, in RW and CP plants with high N supply (MP-Int), shoot biomass production was significantly lower than in CT-Ext-plants grown under reduced N-fertilization intensity. These findings suggest that differences in N availability did not directly cause the shoot growth responses. As an alternative explanation, the limitations of shoot biomass production in the MP-Int treatments with high N supply may be related to the observation of intense powdery mildew (*Blumeria graminis*) symptoms on leaves, as a biotrophic pathogen promoted by high N availability (Luo et al. 2021), reflecting an indirect effect of the nutritional status on plant growth and composition of the microbiome.

Concluding remarks

Taken together, the findings of this study suggest that during the six weeks after RW installation, the winter wheat plants had effectively compensated for root damage induced by the installation process and re-established rhizosphere conditions largely comparable to those of completely undisturbed plants with only limited differences at least under the investigated experimental conditions. The absence of RW effects on plant growth and stress-related gene expression demonstrates that RW installation also did not induce longer-lasting stress responses in the respective wheat plants. Hence, the RW approach could provide a tool for investigating rhizosphere processes under the conditions described in this study, yielding responses comparable to those expected from undisturbed plants. However, the time window required for regeneration may vary depending on the growth characteristics of the investigated plants or may be affected by external stress factors during the phase of plant establishment. Consequently, the findings cannot be generalized and need to be characterized for each specific case.

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Author contributions Loreen Sommermann (Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft), Jan Helge Behr (Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft), Narges Moradtalab (Data curation, Formal analysis, Investigation, Methodology, Writing – original draft), Saskia Windisch (Investigation, Methodology, Writing – review & editing), Soumitra Paul Chowdhury (Data curation, Formal analysis, Investigation, Methodology, Writing – original draft), Michael Rothballer (Project administration, Supervision, Writing – review & editing),Ingo Schellenberg (Project administration, Supervision, Writing – review & editing), Doreen Babin (Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing), Kornelia Smalla (Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing), Uwe Ludewig (Project administration, Supervision, Writing – review & editing), Günter Neumann (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing), Joerg Geistlinger (Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing), and Rita Grosch (Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing), Lorrie Maccario (Methodology – review & editing), SØren J. SØrensen (Methodology – review & editing).

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Data availability Unassembled raw ITS sequences were submitted to the European Nucleotide Archive (ENA; BioProject accession number PRJEB67553) and unassembled raw 16S rRNA gene sequences were submitted to NCBI Sequence Read Archive (SRA; Bioproject accession numbers PRJNA1109326 and PRJNA1037063.

Declarations

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

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