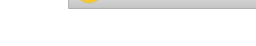

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RESEARCH ARTICLE

Shifts in plant functional trait dynamics in relation to soil microbiome in modern and wild barley

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Societal Impact Statement

Understanding domestication's impact on crop root traits and interactions with soil microbiomes is vital for improving crop resilience and agricultural sustainability. Using this knowledge to enhance root systems, reduce chemical inputs, and adapt crops to environmental stress will help to increase global food production, promote eco-friendly farming, and mitigate the effects of climate change. Additionally, identifying microorganisms specific to plant species may help in biodiversity conservation. Advancing scientific understanding and educating future generations on the intricate relationships between plants, soil, and microorganisms is integral to developing innovative, sustainable agricultural practices and improved food security.

Summary

- Domestication and intensive management practices have significantly shaped characteristics of modern crops. However, our understanding of domestication's impact had mainly focused on aboveground plant traits, neglecting root and rhizospheric traits, as well as trait–trait interactions and root-microbial interactions.
- To address this knowledge gap, we grew modern (*Hordeum vulgare* L. var. Barke) and wild barley (*Hordeum spontaneum* K. Koch var. *spontaneum*) in large rhizoboxes. We manipulated the soil microbiome by comparing disturbed (sterilized soil inoculum, DSM) versus non-disturbed (non-sterilized inoculum, NSM) microbiome. Results showed that modern barley grew faster and increased organic-carbon exudation (OC_{EXU}) compared to wild barley.
- Both barley species exhibited accelerated root growth and enhanced OC_{EXU} under DSM, indicating their ability to partially compensate and exploit the soil resources independently of microbes if need be. Plant trait network analysis revealed that modern barley had a denser, larger, and less modular network of microbes than wild barley indicating domestication's impact on trait–trait coordination. In addition, the relative abundance of bacteria did not vary between wild and modern barley rhizospheres; however, species-specific unique bacteria were identified, with stronger effects under DSM.

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- Overall, our findings highlight domestication-driven shifts in root traits, trait coordination, and their modulation by the soil microbiome.

KEYWORDS
bacterial diversity, domestication syndrome, exudation, network analysis, root growth rate, root traits, soil microbiome, trait-coordination

1 | INTRODUCTION

For centuries, artificial selection pressures through crop domestication as well as more recent breeding programs and agricultural management practices have led to significant changes in crop phenotypes based on human requirements, leading to "domestication syndrome" (DS). For instance, modern crop cultivars tend to grow faster and characterized by acquisitive traits due to homogenous environmental conditions and ample availability of soil resources (Martín-Robles et al., 2019; Roucou et al., 2018; Spor et al., 2020). It is evident from the literature that the DS has led to variable resource investments belowground (roots) to acquire soil resources by modern crop cultivars leading toward tradeoff in resource acquisition and use efficiency (Martín-Robles et al., 2019). Therefore, it is becoming increasingly important to understand how root traits have changed through crop domestication as they were not directly targeted in crop breeding programs for cereal crops (Milla et al., 2015). A better understanding of root traits will likely help us know whether and how modern crop cultivars are adapted to ever-changing environmental conditions and agricultural management practices. Given the importance of root traits to crops' ability to efficiently acquire soil resources, only a few attempts have been made which explicitly linked root phenotypes to plant performance (Guo et al., 2021; Schneider & Lynch, 2020). This is, in part because, firstly, it is extremely difficult to quantify various root traits due to technical challenges, and second, root traits for resource acquisition are more complex than aboveground traits (Isaac et al., 2021; Meister et al., 2014).

Various functional traits of roots have been shown to affect plant performance (Isaac et al., 2021; Milla et al., 2014; Yamauchi et al., 2021). For instance, root tissue density, mean root diameter, root N content, specific root length, and specific root area are well documented root traits forming a multidimensional root economic space, and determine plant's resource uptake capacity (Honvault et al., 2021; Kumar et al., 2020; Wen et al., 2020). A greater specific root length, specific root respiration, and root N content whereas a smaller root tissue density and mean root diameter have been shown to increase the soil exploration and exploitation by roots, and characterized as acquisitive root traits (Bergmann et al., 2020; Guo et al., 2021; Weemstra et al., 2016). Also, root-derived compounds (i.e., exudates) play a central role in nutrient acquisition by plants (Bilyera et al., 2021; Liu et al., 2022). Recently recognized as a functional root trait, root exudates are integral to the multidimensional root economic space, specifically influencing the acquisition of nitrogen (N) and phosphorus (P) resources (Wen et al., 2022). However, it is not clear whether and how the quality and quantity of root exudates co-vary with other root traits. Many studies indicate a close connection between root exudation, root nitrogen concentration, and specific root length, while root exudation is negatively associated with root diameter, tissue density, and root longevity (Bergmann et al., 2020; Sun et al., 2021; Wen et al., 2020). Interestingly, the exudation of enzymes from roots (especially phosphorus mobilizing enzymes such as phosphomonoesterases) to mobilize nutrients in the rhizosphere has been negatively correlated to root colonization by arbuscular mycorrhizal fungi (AMF) (Han et al., 2023; Honvault et al., 2021). These studies along with others show diverse root trait variations across plant species, revealing correlations and resource tradeoffs for efficient soil resource acquisition (X. Kong, et al., 2014; Milla et al., 2014; X-X. Wang et al., 2023; Wen et al., 2020). They also highlight that different plant species are able to adjust which strategies they use, depending on the potential for symbiosis, thus reaching the same outcomes (growth and reproduction) through different channels.

Next, the composition of microbial communities in the rhizosphere of wild progenitors and modern crop cultivars vary which directly feedback to plant fitness. Such variations in rhizosphere microbial communities are attributed to selection pressure, management practices, and root traits (especially root exudation). It is believed that wild plants profit more from rhizosphere microbes whereas intensive management of modern crop cultivars has led to the disruption of such root-microbial interactions (Martín-Robles et al., 2020, 2018; Pérez-Jaramillo et al., 2016). Empirical evidence has shown domestication-mediated disruption of plant-microbial interactions. For example, the responsiveness and efficiency of 27 modern crops to root colonization by AMF were found to be lower than of their wild counterparts (Martín-Robles et al., 2018). The composition of distinct bacterial communities in the rhizosphere of wild progenitors and domesticated crops has also been highlighted in other studies (Bulgarelli et al., 2015; Pérez-Jaramillo et al., 2017). In a study by (Martín-Robles et al., 2020) involving 10 crop species, it was observed that modern crops and their wild counterparts interact differently with soil biota. Specifically, when grown in soil conditioned by modern crops, the modern crops showed higher nematode infection and lower arbuscular mycorrhizal colonization of their roots. This highlights the contrasting ways in which these crops engage with soil microorganisms through a plant–soil feedback approach. They highlighted the microbial recruitments in the rhizosphere to be crop-specific and dependent on edaphic factors, which makes it difficult to utilize this knowledge for generalization for other crops. Altered microbial communities in the rhizosphere may also indirectly affect plant fitness by altering decomposers' activities and therefore nutrient cycling via soil organic matter decomposition (Kumar et al., 2016;

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Kuzyakov, 2002; Pausch et al., 2016). Therefore, it becomes crucial to investigate for specific crops how domestication has led to changes in root-microbial interactions and variation in plant traits and their coordination for resource acquisition. This information will help us to improve the nutrient acquisition of modern crop cultivars in a rapidly changing world where the ability to withstand harsher conditions (e.g., stress related to extreme weather events) is rapidly becoming more important for food security. By adopting management practices that favor positive rhizosphere interactions (Rillig et al., 2019) and incorporating functional traits (especially root traits) future crop breeding programs will be better equipped to increase the efficiency of crops to acquire soil resources in a world of global change.

In our study, we employed a comparative approach using modern and wild barley species to explore the impact of crop domestication on functional root traits and the composition of rhizosphere bacterial communities. Additionally, we employed soil sterilization to experimentally manipulate microbial life (as sterilization leads to reduced soil microbial diversity), investigating how it influences changes in root traits and bacterial communities.

We hypothesized the following:

- Intensive management and artificial selection pressures, whether direct or indirect, result in the development of acquisitive plant traits in modern barley, whereas wild barley tends to exhibit relatively more conservative traits.
- Wild barley demonstrates a stronger response to changes in soil microbiome compared to modern barley, possibly due to the tight co-evolutionary links between them. In contrast, domestication may have hindered such links in modern barley.
- Both modern and wild barley possess distinct species-specific bacterial communities in the rhizosphere.
- The coupling of plant traits is expected to be stronger with more interactions in wild barley compared to its modern counterpart.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

Topsoil (0–20 cm) was collected from a conventional agricultural field (latitude: 53.144472, longitude: 9.912944) near Lüneburg, Germany. General soil properties were as follows: soil pH: 4.9, soil organic matter content: 2.3%, total nitrogen content: 0.07%, total carbon content: 0.98%, and C to N ratio: 12:1. The soil was passed through a 2.5-mm sieve and γ-sterilized with 30-kGy radiation. To investigate the impact of the soil microbiome on root traits and the recruitment of species-specific bacterial communities in the rhizospheres, we utilized freshly collected field soil in two forms: disturbed soil microbiome (DSM) and non-disturbed soil microbiome (NSM). The DSM was created by subjecting the field soil to γ-sterilization (30 kG), resulting in a 56% reduction in microbial abundance as determined by microbial biomass estimation from chloroform fumigation extraction method (Vance et al., 1987). In comparison, the NSM retained its natural microbial composition. This controlled manipulation allowed us to examine the effects of disturbed and non-disturbed soil microbiomes on the observed changes in root traits and the establishment of species-specific bacterial communities. It must be noted that there were microbes present in the in DSM inoculum, however, their abundance was 56% lower. For this reason, we used the term "disturbed" instead of their non-existence. Thereafter, 10% of either DSM or NSM inoculum was mixed with 90% of γ-sterilized (30 kG) soil that was collected from the same field to fill the rhizoboxes. Filling the rhizoboxes 90% with γ-sterilized soil and adding 10% of either DSM or NSM inocula allowed us to keep all the abiotic properties of soil similar and investigate the effects of only DSM versus NSM inocula. Seeds of modern (*Hordeum vulgare* L. var. Barke) and wild barley (*H. spontaneum* K. Koch var. *spontaneum*) were surface sterilized with 70% ethanol and thoroughly rinsed with distilled water. Seeds were de-husked to facilitate germination for wild barley. Thereafter, seeds were soaked in distilled water overnight before transferring to petri-plates having sterilized moistened filter paper and allowed to germinate in a climate chamber. After germination (within 2 days), seedlings were transferred to rhizoboxes (one seedling per rhizobox). Each rhizobox ($58.0 \times 26.6 \times 2.0$ cm^3) contained a transparent window to non-destructively and dynamically monitor root development. Rhizoboxes were positioned at 45–50° angle from the table surface in the climate chamber that facilitated roots to grow toward the transparent window which helped in their visualization. The environmental conditions inside the climate chamber were kept constant and were as follows: light phase: 21.6 ± 1.1°C; dark phase: 17.2 ± 1.0°C; 16 h light/8 h dark; lamps: SANlight P4-serie, 400–760 nm; PAR: 302 ± 21 μmol m^{-2} s^{-1}. In total, the experiment consisted of two barley species (modern and wild barley) times two type of soil inocula (disturbed vs. non-disturbed) times five replicates of each treatment combination, giving us 20 experimental units.

2.2 | Root image acquisition and determination of root growth rate

Starting two days after seedling transfer to rhizoboxes (July 30, 2021), roots were pictured from the transparent window of the rhizobox with a digital camera (Canon EOS 5D Mark III) through a 28-mm lens. Pictures were taken on alternate days starting from August 2, 2021, until the roots reached the bottom of the rhizobox (August 18, 2021) giving a total of nine measurement times in 18 days. All the root pictures were cropped within ImageJ (Schindelin et al., 2012) to exclude rhizobox boundaries for further analysis. To detect all the roots of our images, we trained a convolutional neural network using RootPainter (Smith et al., 2022). For this purpose, we used RootPainter to generate a dataset of images by randomly selecting three sub-regions pe cropped image (width: 861 pixels, height: 897 pixels) and annotating the roots from this image dataset to improve the model until it was able to identify most of the roots in our images. After achieving satisfactory performance of the model, we used it to segment all of our original images and extract the roots present in them. Segmented

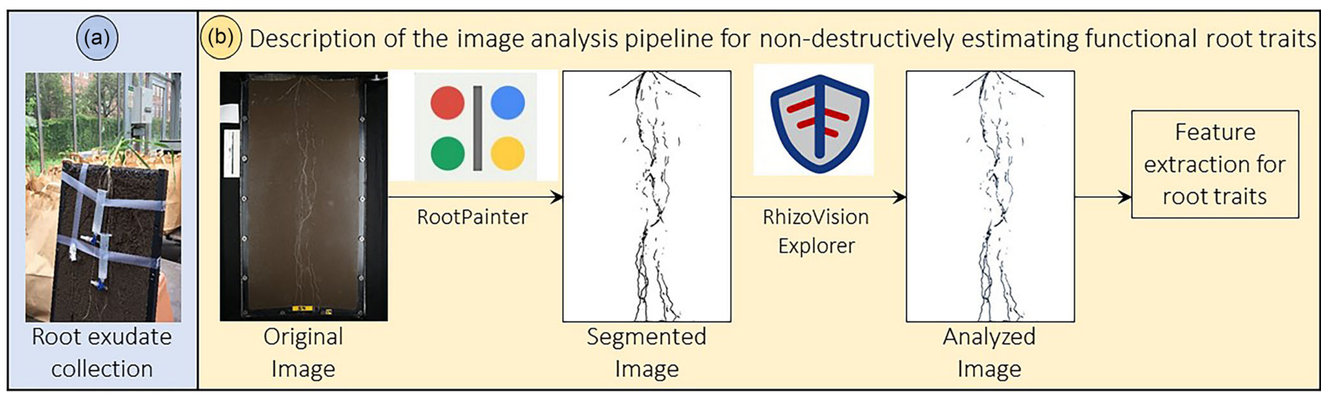

FIGURE 1 (a) Collection of exudates for intact roots as in R. P. Phillips et al., 2008 with modifications including the washing of the most distal roots before placing them in syringes filled with glass beads and C-free nutrient solution. (b) Image analysis pipeline for estimating the total visible root length to determine root growth rate. To detect roots, we trained a convolutional neural network using RootPainter followed by segmenting all images using the best model. Thereafter, the total visible root length from the segmented images was estimated with RhizoVision Explorer.

pictures were used to determine the total visible root length (VRL) by using RhizoVision Explorer by using the batch-processing mode (Seethepalli et al., 2021; Alonso-Crespo et al., 2023) (Figure 1a). Visible root length was plotted against time and the slope of this regression was used as a proxy for root growth rate (RGR).

$$\mathrm{RGR}\left(\mathrm{slope;cm\,day^{-1}}\right)=\frac{\Delta\,\mathrm{Visible\,root\,length\,(cm)}}{\Delta\,\mathrm{Time\,(day)}}$$

Afterward, plants were moved to the greenhouse facility of Leuphana University of Lüneburg on August 19, 2021 and allowed to acclimatize to the greenhouse conditions (comparable to climate chamber conditions: day/night temperature and relative humidity were 22/15.3°C and 60/73%, respectively) for two days before installing the root exudation traps.

2.3 | Collection of root exudates and analyses

On August 21, 2021, we installed the root exudation traps with modifications from (R. P. Phillips et al., 2008), where we removed the transparent window of the rhizobox, and the most distal part of the roots was carefully excavated from the soil and washed with distilled water. Then roots were put in the exudation traps (20-mL syringes) filled with glass beads and carbon (C)-free nutrient solution and allowed to acclimatize for 2 days (Figure 1b). On August 23, 2021, the nutrient solution from the exudation traps was sucked out and replaced with a new C-free nutrient solution. After 48 h (August 25, 2021), the solution from the exudation traps was collected. The trapped solution was immediately passed through a membrane filter (Captive Agilent Premium syringe filter with 0.7-µm glass fiber membrane) and stored in dry ice to transport to the lab and, thereafter, stored at −20°C. Two exudation traps were installed per rhizobox and pooled together to make one composite sample to obtain enough solution for subsequent lab analyses. Exuded organic C (OC_{EXU}) in trapped solution was measured with a multi N/C analyzer (multi N/C analyzer 2100S, Analytik Jena). Roots in the trap solutions were cut and scanned to determine the root surface area by using RhizoVision Explorer (Seethepalli et al., 2021).

We also measured the potential activity of the phosphomonoesterase (PHO_{EXU}) enzyme in the exudation solution by using a fluorogenic artificial substrate (Kumar & Pausch, 2022; Marx et al., 2001). For this, frozen exudate solutions were allowed to thaw first at 4°C and later at room temperature. Exudate solution aliquots were used to fluorogenically measure PHO activity. Fluorogenic methylumbelliferone (MUB)-based artificial substrate was used to measure the potential activity of PHO_{EXU} enzyme and each field replicate was measured in analytic triplicates and the potential activity was expressed in units of nmol MUB cleaved $mL^{-1}\ h^{-1}$.

2.4 | Harvest and plant measurements

Before destructively harvesting the plants at advanced tillering stage on August 25, 2021, we measured leaf chlorophyll content using a chlorophyll content meter (CCM-330; Opti-Sciences, Hudson, NY). For each plant, the top three fully expanded leaves were measured and for each leaf, three measurements were taken and averaged to account for spatial heterogeneity. Afterward, leaves were collected and dried at 70°C for 48 h to determine leaf N content, and the rest of the aboveground plant material was also harvested and dried at 70°C for 48 h. Total shoot biomass was measured on a dry weight basis.

Roots were excavated from rhizoboxes and rhizosphere soil was collected after vigorous shaking to remove most of the loosely bound soil. Thereafter, the root material was stored at −20°C before thoroughly washing the roots. Representative samples from washed roots were scanned at 1200 dpi resolution using a flatbed scanner (Epson Perfection V800 Photo, 8-bit grayscale images). Scanned root images were analyzed with RhizoVision Explorer with the following setting: Image thresholding level: 180, enable edge smoothing: true, edge

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smoothing threshold: 2, root pruning threshold: 15, dpi: 1200. We used each root diameter class to determine the following root traits: specific root length (root length divided by root dry biomass, SRL), specific root area (root area divided by root dry biomass, SRA), root tissue density (root dry biomass divided by root volume, RTD), and root length weighted average fine root diameter (FRD) as recommended by others (Rose, 2017).

2.5 | Root AMF colonization

AMF abundance in roots was determined as root length colonization in percent. Roots were cut into 1–1.5 cm fragments and 10% KOH was used to clear the roots. Then, roots were washed with distilled water, acidified with 1% HCl, and placed in a 2% blue ink solution for staining before clearing them with lactoglycerol (1:1:1) (J. M. Phillips & Hayman, 1970; Vierheilig et al., 1998). Root fragments were mounted on glass slides and root length colonization by AMF was quantified with the intersection method (McGonigle et al., 1990). All scanned and un-scanned roots were pooled together and dried at 70°C for 48 h to determine root biomass on a dry weight basis.

2.6 | Leaf and root C and N concentrations

Dried leaves and root tissues were ball-milled and total C and N concentrations were measured with an elemental analyzer (Vario EL Cube, Elementar, Langenselbold, Germany).

2.7 | Rhizospheric bacterial community composition

DNA was extracted from 0.5 g of soil using Nucleospin Soil Kit (Macherey Nagel) with buffer SL1 and SX according to manufacturers' instructions. Amplicon sequencing of the V4 hypervariable region of the 16S rRNA gene was performed on a MiSeq Illumina instrument (MiSeq Reagent Kit v3 [600 Cycle]; Illumina, San Diego, CA, USA) using the universal eubacterial primers 515F (Parada et al., 2016) and 806R, extended with sequencing adapters to match Illumina indexing primers. To identify potential contamination during DNA extraction and amplification, both extraction and PCR no template control samples were performed. PCR was done using NEBNext high fidelity polymerase (New England Biolabs, Ipswich, USA) in a total volume of 25 μL (5-ng DNA template, 12.5-μL polymerase, 5 pmol of each primer, 2.5 μL 3% BSA). PCR conditions were 5 min at 98°C; 25 cycles of 10 s at 98°C, 30 s at 55°C, 30 s at 72°C; 5 min 72°C. PCR products were purified using MagSi NGSprep Plus beads (Steinbrenner, Wiesenbach, Germany) and quantified via PicoGreen assay. Subsequently, indexing PCR was performed using the Nextera XT Index Kit v2 (Illumina, Inc. San Diego, CA, US) in a total volume of 25 μL (10 ng DNA template, 12.5 μL NEBNext high fidelity polymerase, 2.5 μL of each indexing primer) and the following PCR conditions: 30 s at 98°C; eight cycles of 10 s at 98°C, 30 s at 55°C, 30 s at 72°C; 5 min 72°C. Indexing PCR products were purified using MagSi NGSprep Plus beads, qualified and quantified via a Fragment Analyzer™ instrument (Advanced Analytical Technologies, Inc., Ankeny, USA), and pooled in an equimolar ratio of 4 nM.

Sequences were analyzed on the Galaxy web platform (https://usegalaxy.org; Afgan et al, 2016). FASTQ files were trimmed with a minimum read length of 50 using Cutadapt (Martin, 2011). Quality control was performed via FastQC (Andrews, 2010). For subsequent data analysis DADA2 pipeline (Galaxy Version 1.20) (Callahan, et al., 2016) was used with the following trimming and filtering parameters: 20 bp were removed n-terminally, and reads were truncated at position 240 (forward) and 180 (reverse), respectively, with an expected error of 3 (forward) and 5 (reverse). The resulting unique amplicon sequence variants (ASV) were assigned to the SILVA v138.1 (release 99%) reference database. To exclude potential contamination, ASV occurring in no template controls, as well as unassigned, mitochondrial and chloroplast, reads, and singletons (ASV represented by only one read) were removed from the dataset, resulting in an average read loss of 5.2%. To compare samples without statistical bias, 38,724 reads were subsampled in all samples, reflecting the lowest observed read number. Rarefaction analysis indicated that this sampling depth was sufficient for further analysis of samples (Figure S1). The sequence data obtained in this study are deposited in the short-read archive of NCBI under accession number PRNJA989406 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA989406?reviewer=ficg6d69j4e0tit266actkpjd9).

2.8 | Statistical analysis

Statistical analyses were done within R version 4.2.2 (R Core Team, 2022). First, we performed a principal component analysis (PCA) on above- and below-ground plant traits where values of individual traits were standardized using z-transformation by using the function PCA from the FactoMinerR package (Csardi & Nepusz, 2006). The explained variance from the first two PCs and individual plant trait loading weightage on them were extracted. To test the contribution of PCA loadings, a combination of a threshold selected using the number of dimensions (Richman, 1988) and a bootstrapped eigenvector method (Peres-Neto et al., 2003) were used. For plant traits and bacterial diversity indices (ASV Richness, Shannon diversity, and Pielou's evenness), we performed linear models to test the main effects of domestication (wild versus modern barley), soil microbiome (DSM versus NSM), and their interactions. The step-wise data exploration protocol from (Zuur et al., 2010) was followed to avoid common statistical errors in which, the mean–variance relationship from residual plots was visually checked. Measured plant and bacterial variables are presented as means with 95% confidence intervals that were computed by using a non-parametric bootstrap resampling with 10,000 iterations. The 95% confidence intervals are referred to as compatibility intervals (95% CI), henceforth (Amrhein & Greenland, 2018; Berner & Amrhein, 2022). Given the practice of using *p*-values ($\alpha = .05$) as dichotomous to test the null hypothesis

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and to favor “statistically significant” over “non-significant” results, we refrain from using the above-mentioned terms and mostly mentioned the mean differences between treatments and effect sizes wherever possible while interpreting our results (Berner & Amrhein, 2022; Halsey, 2019).

We performed trait–trait network analysis to investigate how multiple plant traits are interacting. In the network analysis, traits are assigned as nodes and their connections as edges. We extracted network parameters such as edge density, diameter, distance, and modularity which have clear ecological significance (Flores-Moreno et al., 2019). For instance, edge density is the ratio of present to total possible connections, ranging from 0 to 1 and traits with higher edge density are considered more efficient. Modularity determines connectivity among trait modules where trait networks with higher modularity have tighter traits within than between modules. Trait networks with shorter diameter and mean distance imply stronger coordination among various traits. For the trait–trait relationship, data was log-transformed followed by the calculation of correlation coefficients for both wild and modern barley separately for DSM and NSM. Trait network analysis was described by significant correlation coefficients and illustrated by using the igraph package (Csardi & Nepusz, 2006). Network properties such as edge density, network diameter, mean path length, and modularity were extracted (He et al., 2020; Xie et al., 2022). We excluded root AMF colonization data from correlation and trait networks as this dataset was comprised of many zero values.

The effect of DSM verus NSM and wild versus modern barley on overall bacterial community composition was analyzed via NMDS ordination of weighted uniFrac distance and PERMANOVA using the ordinate and adonis2 function of the vegan package (Oksanen et al., 2022), whereas, for the identification of biomarker taxa, a generalized linear model was used from MASS package (Ripley, 2023). Additionally, differences between log2fold changes were calculated and both LEfSe (Linear discriminant analysis Effect Size) and ANCOMBC (Analysis of Compositions of Microbiomes with Bias Correction) were used to validate the results. Multiple test correction was performed by p-value adjustment via the Benjamini-Hochberg method. Plots were created in R using ggplot2 (Wickham et al., 2023) and metacoder (Zachary et al., 2023) packages. The core microbiome of barley species under DSM and NSM was calculated using the core-function of the R package microbiome (Lahti & Shetty, 2017), which determines core microbiota across various abundance/prevalence thresholds with the blanket analysis (Figure S2). Based on these results, a core genus was defined as genus occurring with 0.1% in at least 75% of replicates per group. The shared core taxa were plotted as Venn diagram using eulerr package (Larsson, 2024).

3 | RESULTS

3.1 | Plant trait covariation

The PCA was comprised of four aboveground and eleven belowground plant traits where the first two principal components (PCs) explained almost half (~50%) of the total trait variation (Figure 2a). The first PC axis (PC1) explained 31.12% of total variation and was dominated by slow versus fast plant growth strategies, as indicated by high loadings of SRA, SRL, LNC, and PHO_{EXU}, which related positively and AMF, AGB, and LCC which related negatively on PC1. The second PC axis (PC2) explained 19% of the total variation and the only highly loaded variables on PC2 were FRD and RTD (Figure 2b).

3.2 | Individual plant trait response to domestication and soil microbiome

The four aboveground plant traits (dry shoot weight, LNC, LCC, and Chl) varied between wild and modern barley, however, we found no evidence of the effects of soil microbiome treatment (DSM versus NSM) on the aboveground traits measured (Figure 3a–d). For instance, the average shoot dry weight of wild barley was 0.87 g plant^{-1} and decreased by 32% ($p = .01$) for modern barley (Figure 3a). Similarly, the LCC was, on average, 41% for wild barley and decreased to 40.2% for modern barley ($p = .002$) (Figure 3b). In contrast, the LNC and Chl concentrations were greater in modern than wild barley. The LNC was, on average, 4.47% in wild barley and increased by 19% in modern barley ($p < .001$) (Figure 3c). The Chl concentration in wild barley leaves was 170.8 mg m^{-2}, whereas values for modern barley were up to 17% greater ($p = .02$) (Figure 3d).

In comparison to plant aboveground traits, the belowground traits were more variable and responded distinctly to soil microbiome for both wild and modern barley (Figure 3e–o). For instance, we found no evidence of the impact of soil microbiome (DSM versus NSM) and barley type (wild versus modern) on root dry weight, RCC, SRL, and FRD. However, our results showed that the RNC for wild barley was 1.15% and the values increased on average by 0.22% for modern barley ($p = .03$) (Figure 3g). Similarly, the SRA and PHO_{EXU} activity were greater for modern than wild barley with an increase of 15% for SRA ($p = .045$) (Figure 3j) and 71% for PHO_{EXU} ($p < .001$) (Figure 3n). Our results further indicated that the RTD, RGR, root AMF colonization, and OC_{EXU} responded to both barley species and soil microbiome. For instance, the root AMF colonization was close to 0 for both barley species under DSM but increased (up to 15%) only for wild barley under NSM (Figure 3h). The RTD for wild barley was 74.5 mg cm^{-3} and decreased on average by 10% for modern barley ($p = .03$). We found a very weak effect of soil microbiome on RTD where DSM led to a decrease of RTD by 9% (Figure 3k). In contrast, our results showed that the RGR and OC_{EXU} values were greater for modern barley and disturbing soil microbiome (DSM) had facilitative effects on them (values increased). For instance, DSM led to an increase in RGR by 36% ($p = .007$) (Figure 3o). Our results provided only weak evidence on differences in RGR between two barley species where the roots of modern barely tended to grow slightly faster than that of wild barley ($p = .08$) (Figure 3o). Lastly, the root OC_{EXU} was, on average, 0.98 µg-C h^{-1} cm^{-2} for wild barley and the values increased by 0.32 µg-C h^{-1} cm^{-2} for modern barley ($p = .07$)

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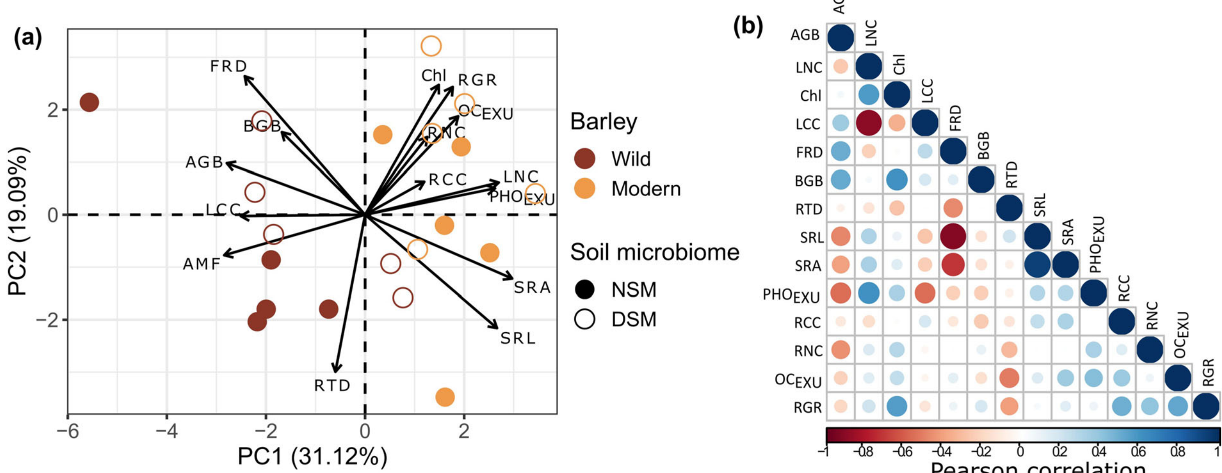

FIGURE 2 This figure shows the relationships between aboveground and belowground plant traits of wild and modern barley in both disturbed (DSM) and non-disturbed (NSM) soil microbiomes. (a) Principal component analysis (PCA) and (b) correlations of aboveground and belowground plant traits of wild and modern barley with DSM and NSM soil microbiomes. LCC: Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%), Chl: Leaf Chlorophyll Concentration (mg m^{-2}), RCC: Root Carbon Concetration (%), RNC: Root Nitrogen Concentration (%), AMF: Root length colonized by arbuscular mycorrhizal fungi (%), SRL: Specific Root Length (m g^{-1}), SRA: Specific Root Area (m^2 g^{-1}), RTD: Root Tissue Density (mg cm^{-3}), FRD: Fine Root Diameter (mm), OC_{EXU}: Exudation of Carbon (μg Carbon h^{-1} cm^{-2}), PHO_{EXU}: Activity of root exuded phosphomonoesterases enzyme (nmol ml^{-1} h^{-1} cm^{-2}), RGR: Root Growth Rate (cm day^{-1}), AGB: dry shoot weight (g $plant^{-1}$), BGB: Dry root weight (g $plant^{-1}$).

providing weak evidence of the difference between exudation rates of two barley species. Disturbing the soil microbiome led to an increase in the root exudation rate of organic C, on average, by 32% (Figure 3m).

3.3 | Impact of domestication and soil microbiome on plant trait–trait relationship

We found contrasting patterns of trait–trait relationships in network analysis as affected by both domestication and soil microbiome. Overall, we found a greater edge density (present edges to all possible edges within a trait network) for modern barley as compared to its wild counterpart (Figure 4). Next, the network diameter values were greater for modern barley than that for wild barley. We also found that the network diameter values increased for both barley species under DSM in comparison with NSM (Figure 4). Similarly, the mean path distance between various traits was greater for modern barley and the path distance further increased under DSM for both barley species (Figure 4). The modularity of the network was much higher for wild barley as compared to the modern barley and the impacts of soil microbiome were less pronounced (Figure 4).

3.4 | Impact of domestication and soil microbiome on rhizosphere bacterial community

Bacterial alpha diversity was significantly lower under DSM, whereas no effect of barley species was observed (Figure 5a–c). Similarly, NMDS ordination analysis of overall community composition indicated the occurrence of distinct bacterial communities as a result of soil microbiome whereas barley species did not differ (Figure 5d). This was confirmed by the calculation of log2-fold changes between soil microbiome and barley species (Figure 5e). Although our results showed no significant effect of barley on the relative abundance of bacterial taxa, core microbiome analysis revealed differences between barley species (Figure 5f and Table S1). Interestingly, the number of barley-specific taxa was dependent on soil microbiome: besides the 20 genera present in all groups, the effect of species was more pronounced under DSM treatment with four and five unique genera for modern and wild barley, respectively, and only five genera shared between both, whereas under NSM, almost all genera were shared between barley genotypes. The shared NSM taxa are mainly members of Proteobacteria (7 of 14 genera), Actinobacteria (3 of 14 genera), and Acidobacteria (2 of 14 genera), which was different from the shared genera under DSM with mainly Armatimonadota (2 of 5 genera). Wild barley-specific genera under DSM belong to members of Proteobacteria and Verrucomicrobia, whereas mainly Chloroflexi were unique for modern barley.

4 | DISCUSSION

We explored domestication-mediated changes in root and rhizosphere traits and trait–trait interactions of barley. Certainly, modern barley manifested fast-to-acquire (acquisitive) traits as compared to wild barely for both above- and belowground traits as indicated from PCA analysis (Figure 2a), supporting our first hypothesis. For example,

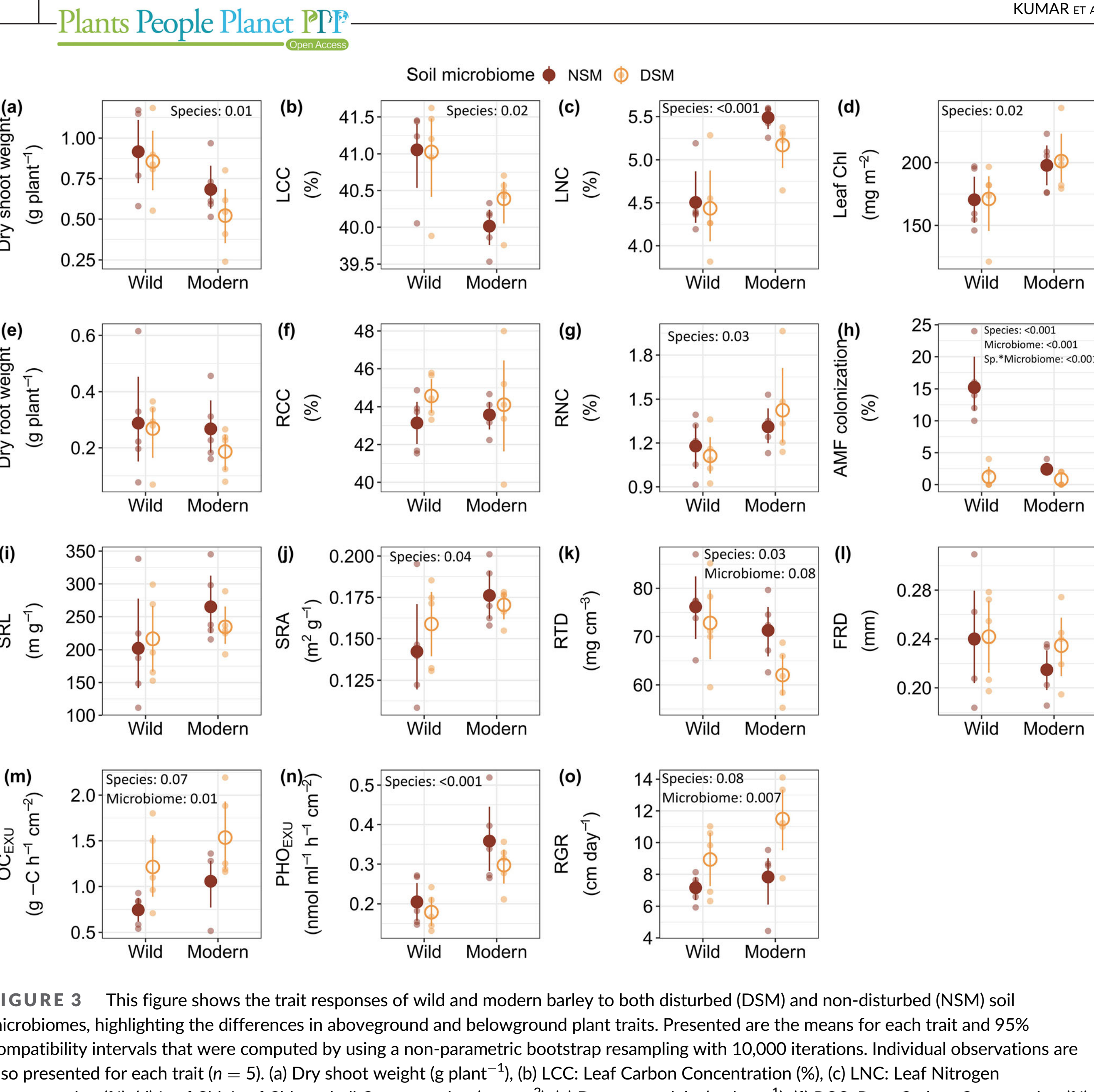

FIGURE 3 This figure shows the trait responses of wild and modern barley to both disturbed (DSM) and non-disturbed (NSM) soil microbiomes, highlighting the differences in aboveground and belowground plant traits. Presented are the means for each trait and 95% compatibility intervals that were computed by using a non-parametric bootstrap resampling with 10,000 iterations. Individual observations are also presented for each trait ($n = 5$). (a) Dry shoot weight (g plant^{-1}), (b) LCC: Leaf Carbon Concentration (%), (c) LNC: Leaf Nitrogen Concentration (%), (d) Leaf Chl: Leaf Chlorophyll Concentration (mg m^{-2}), (e) Dry root weight (g plant^{-1}), (f) RCC: Root Carbon Concetration (%), (g) RNC: Root Nitrogen Concentration (%), (h) AMF colonization: Root length colonized by arbuscular mycorrhizal fungi (%), (i) SRL: Specific Root Length (m g^{-1}), (j) SRA: Specific Root Area (m^2 g^{-1}), (k) RTD: Root Tissue Density (mg cm^{-3}), (l) FRD: Fine Root Diameter (mm), (m) OC_{EXU}: Exudation of Carbon (µg Carbon h^{-1} cm^{-2}), (n) PHO_{EXU}: Activity of root exuded phosphomonoesterases enzyme (nmol ml^{-1} h^{-1} cm^{-2}), and (o) RGR: Root Growth Rate (cm day^{-1}). Two-way ANOVA results are presented for each measured variable.

greater N concentration and leaf greenness (a proxy for chlorophyll concentration) but lower C concentration in leaves of modern barley suggest increased leaf metabolic rates (an acquisitive trait, sensu Henneron et al., 2020). Higher N concentrations are generally linked to higher photosynthetic rates and inversely linked to the life span of a leaf suggesting that a relationship exists between these leaf traits (Reich et al., 1999) and points toward leaf economic spectrum (LES) which describes physiological trade-offs among them (Wright et al., 2004). Our results are supported by previous findings by Roucou et al. (2018) where they found that modern "elite" wheat varieties (another very important cereal crop) possessed high N content and photosynthetic rates in their leaves as compared to their wild relatives and landraces. Next, we found that the shoot biomass of modern barley was lower than that of wild barley. Domestication and the introduction of new varieties through crop breeding programs have led to substantial changes in plant phenotypes. Modern varieties of major cereal crops show reduced branching and tillering but larger inflorescence and grain sizes (Ross-Ibarra et al., 2007; Wacker et al., 2002). Especially, after the first Green Revolution, dwarf and semi-dwarf varieties of various crops including modern barley (*H. vulgare* cv. Barke, a semi-dwarf variety) were introduced to lower lodging-associated yield losses. Lowering plant height by decreasing internode

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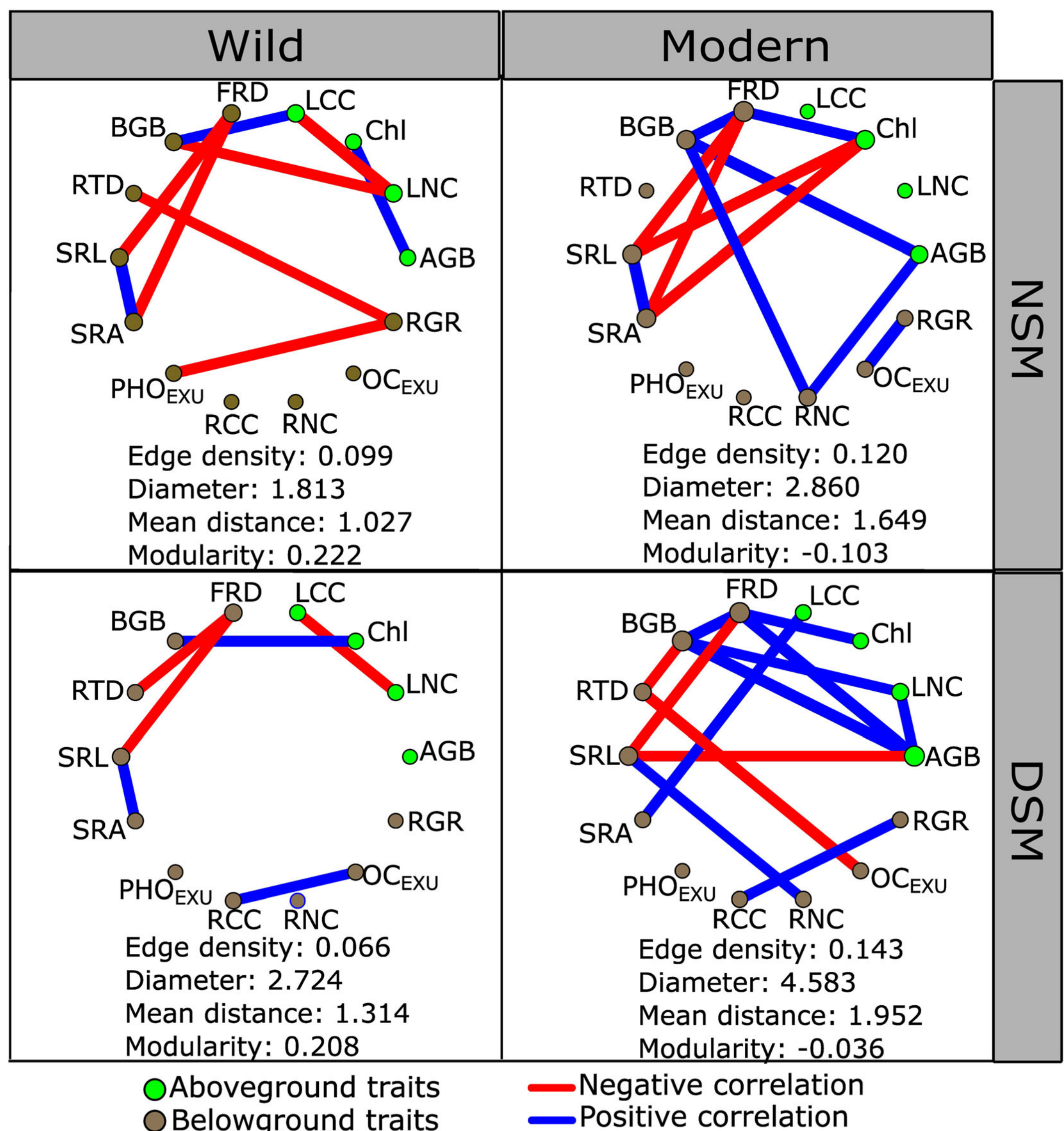

FIGURE 4 Trait correlation network of wild and modern barley with disturbed (DSM) and non-disturbed (NSM) soil microbiomes. Correlation coefficient threshold was set at 0.7 and only strong correlations ($p = .05$) are shown. Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%), Leaf Chl: Leaf Chlorophyll Concentration (mg m^{-2}), RCC: Root Carbon Concetration (%), RNC: Root Nitrogen Concentration (%), SRL: Specific Root Length (m g^{-1}), SRA: Specific Root Area (m^2 g^{-1}), RTD: Root Tissue Density (mg cm^{-3}), FRD: Fine Root Diameter (mm), OC_{EXU}: Exudation of Carbon (μg Carbon h^{-1} cm^{-2}), PHO_{EXU}: Activity of root exuded phosphomonoesterases enzyme (nmol ml^{-1} h^{-1} cm^{-2}), RGR: Root Growth Rate (cm day^{-1}), AGB: dry shoot weight (g $plant^{-1}$), BGB: Dry root weight (g $plant^{-1}$).

length, and therefore, lesser resource investments in vegetative tissues also contributed to an increased harvest index (proportion of grain yield to the total plant biomass production) (Bezant et al., 1996; J. Wang et al., 2014). Therefore, the lower shoot biomass of modern barley than that of wild barley support this notion and provide more evidence of fewer resource investments in vegetative structures. Previously, it has been shown that domestication-mediated changes in plant biomass are crop species dependent (Martín-Robles et al., 2019). Comparing 30 different modern crop cultivars and their putative wild progenitors, Martín-Robles et al. (2019) provided evidence that domestication led to an increase in plant biomass more so for larger crops (e.g., cucumbers and beans) whereas the opposite was true for small crops including barley, rucola, and white clover, further supporting our findings.

We found no effect of soil microbiome on plant biomass (root and shoot biomass) for both barley species, partly rejecting our second hypothesis where we expected stronger microbiome-mediated effects on plant biomass for wild barley. Our results contradict recent findings where soil microbes have been shown to decrease barley biomass independent of soil N availability indicating a net negative effect of microbes on plant biomass production (Munkager et al., 2021). The soil microbial inoculum in our study was collected from a conventional agricultural field site with intensive management history which could help explain the absence of microbial response for wild barley

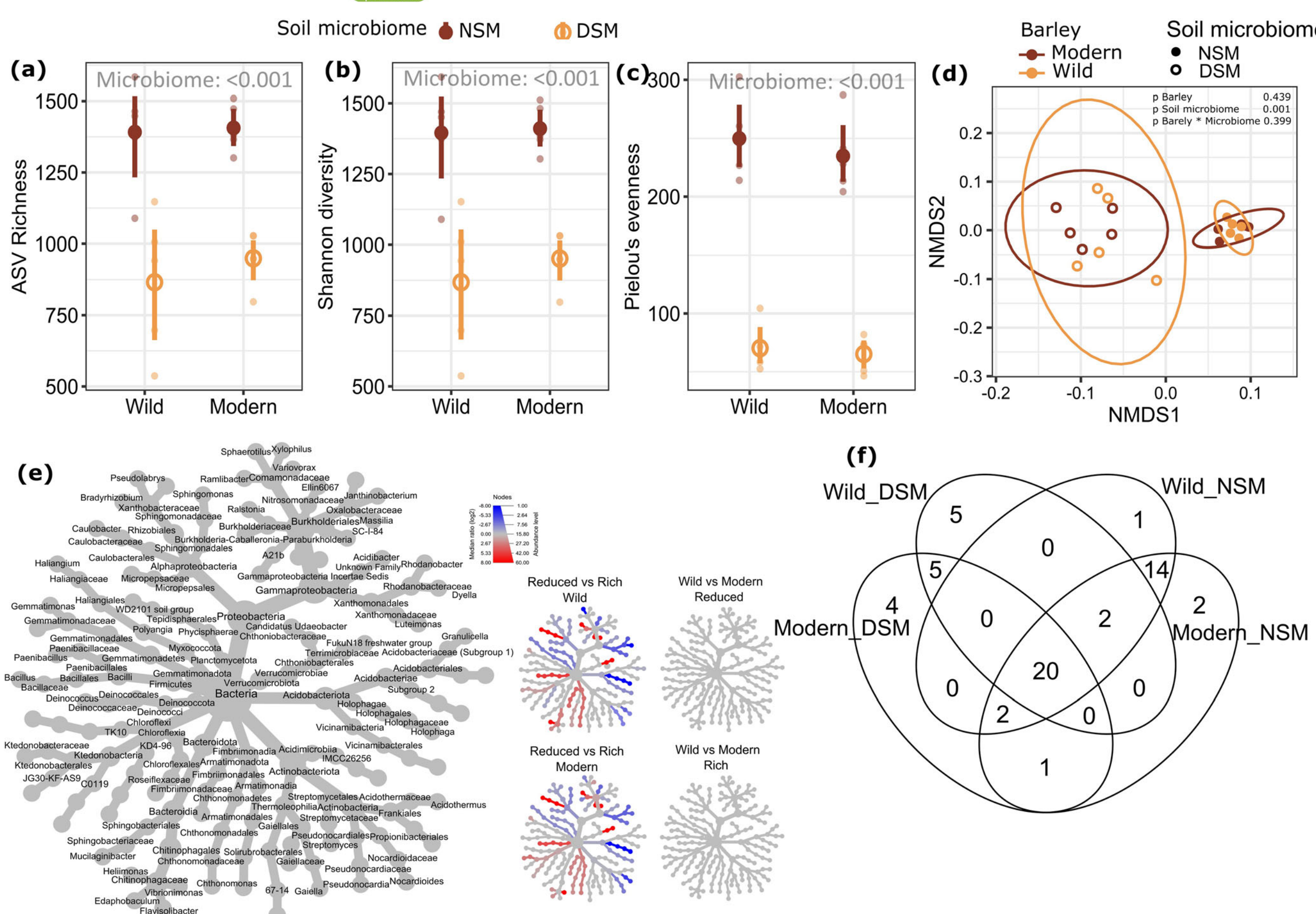

FIGURE 5 This figure compares the impact of disturbed (DSM) and non-disturbed (NSM) soil microbiomes on the bacterial communities in the rhizosphere of wild and modern barley. (a–c) Bacterial richness, diversity, and evenness in the rhizosphere of wild and modern barley as impacted by DSM versus NSM soil microbiomes. Presented are the means for each trait and 95% compatibility intervals that were computed by using a non-parametric bootstrap resampling with 10,000 iterations. Individual observations are also presented for each trait ($n = 5$). Two-way ANOVA results are presented for (a)–(c). (d) Nonmetric multidimensional scaling (NMDS) ordination of bacterial taxa in the rhizosphere of wild and modern barley as impacted by rich (filled circles) and reduced (open circles) soil microbial life. (e) Relative abundance of bacterial taxa.

biomass. Specific microbial taxa associated with wild barley in its natural habitat may simply not be present in the soil inoculum collected from conventional agricultural field with intensive management practices. This is also visible from the finding of no difference in alpha- and beta-diversity and the relative abundance of bacterial community in the rhizosphere of wild and modern barley (Figure 5a–e) thereby only partly rejecting our third hypothesis as we found some variation in crop-specific bacterial taxa under DSM as determined by using core microbiome analysis (Figure 5f). However, it has to be mentioned that the results of the core microbiome analysis used in this study have to be taken with caution. Although the chosen abundance-occurrence approach is more conservative than using occurrence or abundance only, the considered core taxa are dependent on the chosen thresholds for abundance and prevalence. Taken together with the small sample size ($n = 5$), the results should be interpreted with care. Soil sterilization has been shown to decrease both microbial abundance and diversity (Yang, Roy, et al., 2021; Yang, Ryo, et al., 2021) but microbial communities are able to recover especially if an inoculum of soil microbiota is used. The trajectory of microbial community recovery from disturbance through sterilization can be very different from the initial microbial communities (Yang, Ryo, et al., 2021). Moreover, distinct organic compounds released as root exudates may have acted as signaling molecules to attract specific bacterial taxa in the respective rhizospheres of wild versus modern barley (Kumar et al., 2018; Zhalnina et al., 2018). Next, we found that the root colonization by AMF was greater for wild than modern barley, but the percentage values were very low. This increase in root AMF colonization for wild barley did not translate into an additional benefit in terms of increased plant biomass as previously shown (Camenzind et al., 2016; Kumar et al., 2021). For modern barley, in contrast, domestication and artificial selection pressures (such as intensive management by using various agrochemicals and optimal irrigation especially after the first green revolution) may have disrupted the root-AMF interactions thereby leading to their lower responsiveness to AMF colonization. Our results are supported by a comprehensive study by Leff et al. (2017) on 33 sunflower genotypes with the varied extent of domestication where they found domestication-mediated variation in rhizosphere and seed-associated fungal taxa whereas root and

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rhizosphere-bacterial taxa were not affected as a function of domestication. Moreover, domestication-mediated decreases in root AMF colonization have previously been demonstrated (Martín-Robles et al., 2018; Spor et al., 2020). Next, under DSM, we found no evidence of root colonization by AMF, which along with a decreased bacterial richness and diversity in this treatment further supports our experimental manipulation of soil microbiome. Nonetheless, in consideration of our short-term study, the observed minimal root colonization by arbuscular mycorrhizal fungi is expected, particularly under DSM. Root colonization by AMF is not anticipated to be significant at this stage, especially without the use of AMF inoculum. For instance, previous studies have showed effective root AMF colonization in barley between 2 and 4 weeks, facilitated by inoculation (Grace et al., 2009; Vierheilig et al., 2000). Therefore, the minimal root AMF colonization observed in the present research reflects the natural establishment process of natural AMF communities. Therefore, these results should be considered carefully. Given the experimental timeline constraints in the present research, we emphasize the need for future investigations with an extended timeframe to comprehensively unravel fungal community dynamics.

We found the belowground traits to be more idiosyncratic supporting recent findings from Lozano et al. (2020) where they found the root traits of 24 grassland species (including grasses, forbs, and legumes) to be more variable than aboveground plant traits which further responded in a species-specific manner to soil resource availability (i.e., water). Contrary to our expectations, we found more trait correlations for modern barley than wild barley. At the root level, as compared to wild barley, roots of modern barley had greater RNC, grew faster, SRA values were greater, and exuded more organic compounds, whereas RTD decreased, all indicative of acquisitive strategies. For instance, greater RNC in modern barley may be indicative of high metabolic rates to warrant the quick acquisition of resources (Bergmann et al., 2020; Reich, 2014; Sun et al., 2021). The roots of modern barely grew faster and had greater SRA implying fast exploration strategies to acquire soil resources. Just like SRL, higher SRA has been interpreted as a larger soil exploration strategy with low resource investments (D. Kong et al., 2014; Lynch, 2015; McCormack et al., 2015). Faster root growth for modern barley may also be seen as an alternate strategy to explore more soil volumes for resources when root AMF colonization is minimal, in which, AMF can spread its hyphae far away from the nutrient depletion zone around roots (i.e., rhizosphere) to trade nutrients for C from plants (Kumar et al., 2019; Ma et al., 2018). For wild barley, on the other hand, to accommodate more AMF structures in the root cortex, increased root AMF colonization has often been linked to an increased FRD and decreased SRL (Bergmann et al., 2020; Kong et al., 2016; Ma et al., 2018). However, such covariation between these traits was not evident in the present study. This may be because root traits are multi-dimensional in contrast with leaf traits that fall across a slow versus fast leaf economic spectrum (Kramer-Walter et al., 2016; Weemstra et al., 2016). It is also important to note that in the present study, the root length colonization by AMF was still low (~15%) and the root cortex might be enough to accommodate such low AMF colonization without increasing the FRD. We are also aware that such differences in root AMF colonization between wild and modern barley should only be seen as the responsiveness of these barley species to AMF colonization and should be interpreted with caution. We further found that the RTD was smaller for modern barley as compared to its wild counterpart which aligns well as an acquisitive root trait and its negative relationship with RNC (on orthogonal planes in PCA axis, Figure 2a) (Kong et al., 2016, D. Kong et al., 2014). Lower RTD for modern barley accompanied by higher SRA and faster growth rate further hint toward an effective strategy to explore soil volume by lowering resource investments including respiration/maintenance costs (Huang et al., 2022; Lynch, 2018). Alternatively, as RTD is inversely linked to soil fertility levels (Ryser & Lambers, 1995), it is plausible that the modern barley in our experiment which is bred to perform better under high nutrient availability (under intensive agriculture) led to an overall decrease in RTD. Further, RTD and root growth rates are generally inversely linked supporting our results (Kramer-Walter et al., 2016). Higher RTD for wild barley, on the other hand, may hint toward a longer life span and slow growth strategy as previously documented in many studies (Kong et al., 2016; Reich, 2014; Roucou et al., 2018). Higher PHO_{EXU} activity in exudates accompanied by higher RNC but lower RTD for modern barley provide further evidence of a fast-to-acquire strategy. Further, higher PHO_{EXU} activity accompanied by less responsiveness to root AMF colonization for modern barley hints toward alternative nutrient (especially P) acquisition strategy and tradeoffs for their acquisition. These results are supported by recent findings from Han et al. (2022) where they found the root PHO activity to align with the fast growth strategy of roots and negatively related to root AMF colonization, among 20 co-occurring tree species. Resource tradeoffs among various traits for P acquisition across a range of crops have also been shown previously that were dependent on crop identity (Wen et al., 2019).

5 | CONCLUSIONS

Our comparative analysis revealed that modern barley displays more fast-to-acquire traits than its wild counterpart. Greater leaf N and chlorophyll content accompanied by faster root growth rate as well as greater OC_{EXU} and PHO_{EXU} exudation rates support previous evidence that modern crops are bred to perform optimally by quickly acquiring soil resources under intensive management practices. Further, our results highlighted a mismatch between above- and below-ground trait–trait coordination between modern and wild barley that was further intensified by soil microbiome. These results may have far-reaching implications. First, we need to understand such above- and below-ground trait coordination of modern crops to investigate how the "out-of-focus" root and root traits were impacted by domestication and management practices. This information will be crucial to promote sustainability in cropping systems through reduced external inputs and ability to withstand more extreme abiotic conditions once we identify the efficient root traits to acquire soil resources. Trait

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network analysis highlighted how domestication led to mismatches between above- and below-ground traits. These results provide important information for novel crop breeding programs focused on developing crops to perform optimally under reduced external inputs and high microbial diversity in soils.

AUTHOR CONTRIBUTIONS

AK led experimental conceptualization, investigation, formal analysis, visualization, writing, and supervision. OK contributed to the investigation, data collection, and writing. SG was involved in data collection, formal analysis, visualization, and writing. HC participated in data collection. IMA contributed to writing. SS played a key role in supervision and contributed to the formal analysis. MY was involved in data analysis. MB, MS, and VMT contributed in supervision. VMT played a key role in the conceptualization.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the figures and supplementary material of this article. The sequence data obtained in this study are deposited in the short-read archive of NCBI under accession number PRNJA989406 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA989406?reviewer=ficg6d69j4e0tit266actkpjd9).

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