Implications of domestication syndrome in barley for above- and belowground plant traits and microbial interactions

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Abstract

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 (*H. spontaneum* K. Koch var. *spontaneum*) in large rhizoboxes. We manipulated 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 $_{\mathrm{EXU}}$) compared to wild barley. Interestingly, both barley species exhibited accelerated root growth and enhanced OC $_{\mathrm{EXU}}$ 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 than wild barley indicating domestication's impact on trait coordination. Further, soil microbiome influenced specific network parameters. While the relative abundance of bacteria didn't vary between wild and modern barley rhizospheres, species-specific core bacteria were identified, with stronger effects under DSM. Overall, our findings highlight domestication-driven shifts in root traits, trait coordination, and their modulation by the soil microbiome.

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Abstract

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 (*H. spontaneum* K. Koch var. *spontaneum*) in large rhizoboxes. We manipulated 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. Interestingly, 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 than wild barley indicating domestication's impact on trait coordination. Further, soil microbiome influenced specific network parameters. While the relative abundance of bacteria didn't vary between wild and modern barley rhizospheres, species-specific core bacteria were identified, with stronger effects under DSM. Overall, our findings highlight domestication-driven shifts in root traits, trait coordination, and their modulation by the soil microbiome.

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

Introduction

For centuries, natural and 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. For instance, modern cultivars of various cereal crops (such as rice, wheat, and barley) possess an increased number of grains per panicle, larger seeds, uniform germination and flowering timings, reduced seed dormancy and dispersal, loss of shattering, and shorter longevity in contrast to higher photosynthetic rates and nitrogen content in leaves (Meyer, DuVal & Jensen 2012; Preece *et al.* 2017; Roucou *et al.* 2018). Modern crop cultivars also tend to grow faster due to homogenous environmental conditions and ample availability of soil resources, suggesting that they may have adapted toward quick-to-acquire (acquisitive) strategies. This so-called domestication syndrome has led to lesser resource investment in the physical and chemical defense system of modern crop cultivars (Turcotte, Turley & Johnson 2014; Simpson, Wade, Rees, Osborne & Hartley 2017; Fernandez, Saez, Quintero, Gleiser & Aizen 2021; Allaby, Stevens, Kistler & Fuller 2022). In a meta-analysis, Fernandez et al. (2021) provided strong evidence across a range of crops that plant damage by herbivory was higher in modern crop cultivars whereas the defense level was higher in their putative wild counterparts. They showed that, in modern crop cultivars, the average defense production lowered by only 10% whereas their susceptibility to herbivory increased by up to 430% in comparison with putative wild relatives. These results can be only partly explained by differential resource investments and their potential cost-benefit tradeoffs and sought for further empirical evidence to have a holistic understanding of mismatches for growth and defense mechanisms in modern crops in developing novel crop varieties (Tracy *et al.* 2020; York *et al.* 2022a; York, Griffiths & Maaz 2022b).

Nonetheless, despite recent research advancements in identifying such remarkable resource tradeoffs and altered phenotypes between modern crop cultivars and their putative wild relatives, most of such advancements have focused on aboveground plant traits whereas the belowground plant traits have not been given significant attention. We lack a clear understanding of whether and how crop domestication and breeding programs have led to changes in functional root traits, including their interactions with soil microbes. It is becoming increasingly important to understand how the traits have changed through crop domestication as they were not directly targeted in crop breeding programs for cereal crops (Milla, Osborne, Turcotte & Violle 2015) yet a range of different traits related to resilience in a changing world are set to gain in importance in agriculture. This 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 (Schneider & Lynch 2020; Guo *et al.* 2021). This is, in part because, firstly, it is extremely difficult to quantify various root traits due to technical challenges, and secondly, root traits for resource acquisition are more complex than aboveground traits (Meister, Rajani, Ruzicka & Schachtman 2014; Isaac*et al.* 2021). For instance, root exudates – chemical compounds released by roots, mediate numerous biogeochemical processes in the rhizosphere. Only recently, root exudation has been considered a functional root trait and linked to the multidimensional root economic space for the acquisition of soil resources, where, distinct functional roles of exudates have been proposed for N and P acquisition (Wen, White, Shen & Lambers 2022). However, it is not clear whether and how the quality and quantity of root exudates co-vary with other biological, morphological, and architectural root traits. A few studies which have been linking various root traits have shown that a root exudation and root N concentration as well as specific root length are closely connected, whereas root exudation is negatively associated with root diameter, tissue density, and root longevity (Bergmann *et al.*2020; Wen *et al.* 2020; Sun *et al.* 2021). 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) (Honvault *et al.* 2021; Han*et al.* 2023). These studies suggest the existence of large variations in root traits across plant species and indicate that root traits correlate with each other and resource tradeoffs among them exist to efficiently acquire soil resources (Kong, Zhang, De Smet & Ding 2014; Wen *et al.* 2020; Wang, Zhang, Wang, Rengel & Li 2023). 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, reproduction) through different channels.

Next, the assemblages 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 (Perez-Jaramillo, Mendes & Raaijmakers 2016; Martin-Robles*et al.* 2018, 2020). 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 was found to be lower than for their wild counterparts (Martin-Robles et al., 2018). The assemblage of distinct bacterial communities in the rhizosphere of wild progenitors and domesticated crops has also been highlighted in other studies (Bulgarelli *et al.* 2015; Perez-Jaramillo *et al.* 2017). Using a plant-soil feedback approach, Martin-Robles et al. (2020) showed across 10 crop species that modern crops and their wild counterparts recruited soil biota in opposing ways. 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 feedback to plant fitness by altering decomposers' activities and therefore nutrient cycling via soil organic matter decomposition (Kuzyakov 2002; Pausch *et al.* 2016; Kumar, Kuzyakov & Pausch 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 incorporation of functional traits (especially root traits) future crop breeding programs will be better equipped increase the efficiency of crops to acquire soil resources in a world of global change.

Therefore, through a comparative approach by using modern- and wild barley species, we investigated whether and how crop domestication has led to changes in functional root traits and assemblage of rhizosphere bacterial communities. We further determined whether there had been changes in root traits and assemblage of rhizosphere bacterial communities depending on soil microbial diversity for which we experimentally

manipulated soil microbial life by using soil sterilization.

We hypothesized that:

- Intensive management and 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.

Materials and methods:

Experimental setup

Topsoil (0-20 cm) was collected from a conventional agricultural field (latitude: 53.144472, longitude: 9.912944) near Luneburg, 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 (company info). To investigate the effects of soil microbiome on root traits and recruitment of species-specific bacterial communities in the rhizospheres, freshly collected field soil (five independent field replicates) with and without γ-sterilization was used as soil inoculum to introduce disturbed soil microbiome (DSM) and non-disturbed soil microbiome (NSM), respectively. For instance, the DSM inoculum had 56% lower microbial abundance than NSM as determined through microbial biomass estimation from chloroform-fumigation extraction method (Vance, Brookes & Jenkinson 1987). Only 10% of soil inoculum was mixed with 90% γ-sterilized soil that was collected from the same field as that of inoculum soil to fill the rhizoboxes. This allowed us to keep all the abiotic properties of soil in each rhizobox similar and investigate the effects of only DSM versus NSM. 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 x 26.6 x 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 towards 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 hr light/8 hr 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 versus non-disturbed) times five replicates of each treatment combination, giving us 20 experimental units.

Root image acquisition and determination of root growth rate

Starting two days after seedling transfer to rhizoboxes (30^{th} July 2021), roots were pictured from the transparent window of rhizobox with a digital camera (Canon EOS 5D Mark III) through a 28-mm lens. Pictures were taken on alternate days starting from 2^{nd} August 2021 until the roots reached the bottom of the rhizobox (18^{th} August 2021) giving a total of 9 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 generated a dataset of images by randomly selecting three sub-regions pe cropped image (width: 861 pixels, height: 897 pixels) and annotate 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 pictures were used to determine the total visible root length (VRL) by using RhizoVision Explorer using the batch-processing mode (Seethepalli*et al.* 2021) (Fig. 1a). Visible root length was plotted against time and the slope of this regression was used as a proxy for root growth rate (RGR).

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

greek Time (day)

Afterward, plants were moved to the greenhouse facility of Leuphana University of Lüneburg on 19$^{\text{th}}$ August 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.

Collection of root exudates and analyses

On 21$^{\text{st}}$ August 2021, we installed the root exudation traps with modifications from Phillips, Erlitz, Bier & Bernhardt 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 (Fig. 1b). On 23$^{\text{rd}}$ August 2021, the nutrient solution from the exudation traps was sucked out and replaced with a new C-free nutrient solution. After 48 hours (25$^{\text{th}}$ August 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 0.7µm 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 pooed 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 (Marx, Wood & Jarvis 2001; Kumar & Pausch 2022). 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}.

Harvest and plant measurements

Before destructively harvesting the plants at advanced tillering stage on 25$^{\text{th}}$ August 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. One subsample of rhizosphere soil was immediately frozen with dry ice for microbial analyses whereas the other subsample was stored at 4 °C for substrate-induced respiration

measurements. Thereafter, the rest of 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 (Seethepalli *et al.* 2021) with the following setting: Image thresholding level: 180, enable edge smoothing: true, edge 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; Rose & Lobet 2019).

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 and acidified with 1% HCl and placed in a 2% blue ink solution for staining before clearing them with lactoglycerol (1:1:1) (Phillips & Hayman 1970; Vierheilig, Coughlan, Wyss & Piche 1998). Root fragments were mounted on glass slides and root length colonization by AMF was quantified with the intersection method (McGonigle, Miller, Evans, Fairchild & Swan 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.

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).

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, Needham & Fuhrman 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: 30s at 98 @C; 8 cycles of 10 s at 98 @C, 30s at 55 @C, 30s at 72 @C; 5min 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 4nM.

Sequences were analyzed on the Galaxy web platform (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, 38724 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 (Supplementary Figure. 1). 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).

Statistical analysis

Statistical analyses were done within R version 4.2.2 (Team R Development Core, 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, Jackson & Somers 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, Ieno & Elphick 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 ($\alpha = 0.05$) as dichotomous to test the null hypothesis 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 (Halsey 2019; Berner & Amrhein 2022).

We performed 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, Wang & Li 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. The core microbiome of barley species under DSM and NSM was defined as genera occurring with 0.1% in at least 75% of replicates. Plots were created in R using ggplot2 (Wickham *et al.* 2023) and metacoder (Zachary, Grunwald & Gilmore 2023) packages.

Results

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 (Fig. 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 Ex_P, 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 (Fig. 2b).

Individual plant trait response to domestication and soil microbiome

The four aboveground plant traits (dry shoot weight, LNC, LCC, Chl) varied between wild and modern barley, however, we found no evidence of the effects of soil microbiome treatment (DSM versus NSM) on the above ground traits measured (Fig. 3a-d). For instance, the average shoot dry weight of wild barley was 0.87 g plant^{-1} and decreased by 32% ($p = 0.01$) for modern barley (Fig. 3a). Similarly, the LCC was, on average, 41% for wild barley and decreased to 40.2% for modern barley ($p = 0.002$) (Fig. 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 ($p < 0.001$) (Fig. 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 = 0.02) (Fig. 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 (Fig. 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 ($p = 0.03$) (Fig. 3g). Similarly, the SRA and PHO_{EXU} activity were greater for modern than wild barley with an increase of 15% for SRA ($p = 0.045$) (Fig. 3j) and 71% for PHO_{EXU} ($p< 0.001$) (Fig. 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 (Fig.3h). The RTD for wild barley was 74.5 mg cm^{-3} and decreased on average by 10% for modern barley ($p = 0.03$). We found a very weak effect of soil microbiome on RTD where DSM led to a decrease of RTD by 9% (Fig. 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% ($p = 0.007$) (Fig. 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 ($p= 0.08$) (Fig. 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 ($p = 0.07$) 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% (Fig. 3m).

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 (Fig. 4, Table 1). 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 (Fig. 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 (Fig. 4, Table 1). 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 (Fig. 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

(Fig. 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 (Fig.5d). This was confirmed by the calculation of log2-fold changes between soil microbiome and barley species (Fig 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 (Fig 5f, Supplementary Table. 1). Interestingly, the number of barley-specific taxa was dependent on soil microbiome: besides a large core of 20 genera present in all groups, the effect of species was more pronounced under DSM treatment with four and five specific 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 rich core consists mainly of 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 specific for modern barley.

Discussion

We explored domestication-mediated changes in root and rhizosphere traits and trait-trait interactions of barley. Such changes in belowground traits have poorly been understood for modern crops because most literature on functional traits and domestication syndrome has focused mainly on aboveground plant traits that are of agronomical interest (for instance, seed size and number) (Meyer et al., 2012; Ren et al., 2020). Recently, more attention is given to improve our understanding whether domestication and artificial/natural selection have also generated indirect selection of 'out-of-focus' plant traits and root-microbial interactions (Martín-Robles et al., 2020, 2019). This is particularly important to improve sustainability in cropping systems by identifying and incorporating efficient plant traits for resource acquisition in novel breeding programs as well as their contribution to modulating soil health (García-Palacios et al., 2018; Milla et al., 2015; Schmidt et al., 2016).

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 (Fig. 2a), supporting our first hypothesis. For example, 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, and therefore, acquisitive trait, at the expense of leaf longevity and structural defense. 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 towards 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 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; 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, Martin-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 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 bacterial community structure in the rhizosphere of wild and modern barley (Fig. 5a-e) thereby only partly rejecting our third hypothesis as we found some variation in crop-specific core bacterial taxa under DSM as determined by using core microbiome analysis (Fig 5f). Soil sterilization has been shown to decrease both microbial abundance and diversity (Yang, Roy, Veresoglou & Rillig 2021a; Yang, Ryo, Roy, Hempel & Rillig 2021b) 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 *et al.* 2021b). Moreover, distinct organics released as root exudates may have acted as signaling molecules to attract specific bacterial taxa in the respective rhizospheres of wild versus modern barley (Zhalnina *et al.* 2018; Kumar, Shahbaz, Blagodatskaya, Kuzyakov & Pausch 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 indirect selection pressures (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 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.

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 organics, 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., 2020). 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 (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; Mccormack et al., 2019; 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, Fig. 2a) (Kong et al., 2014, 2016). 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., 2021; Lynch, 2018). Alternatively, as RTD is inversely linked to soil fertility levels (Ryser and 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 towards 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 towards alternative nutrient (especially P) acquisition strategy and tradeoffs for their acquisition. These results are supported by recent findings from Han et al. (2021) 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).

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 organic C and PHO 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 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.

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Data availability

Should the manuscript be accepted for publication, the presented data will be submitted to BonaRes data repository (https://datenzentrum.bonares.de/data-portal.php).

Author contributions

	AK	OK	SG	HC	IMA	SS	MY	MB	MS	VMT
Conceptualization	Lead									Equal

	AK	OK	SG	HC	IMA	SS	MY	MB	MS	VMT
Investigation	Lead	Equal								Equal
Data collection	Equal	Equal	Equal	Equal						
Formal analysis	Lead		Equal		Equal		Equal			
Visualization	Lead	Equal	Equal							
Writing	Lead	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal	Equal
Supervision	Lead					Equal		Equal	Equal	Equal

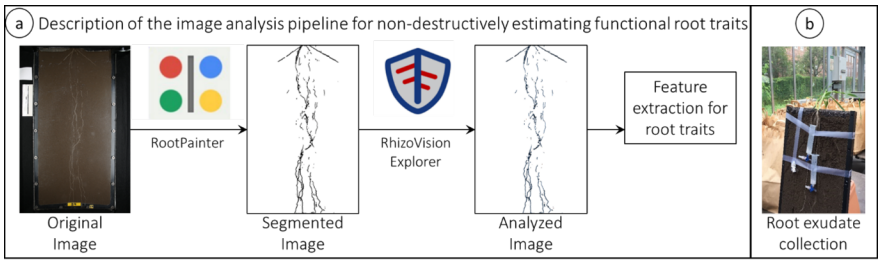

Fig 1. (a) Image analysis pipeline for estimating the total visible root length to determine root growth rate. (b) Collection of root exudates for intact roots. For detail, see material and method section.

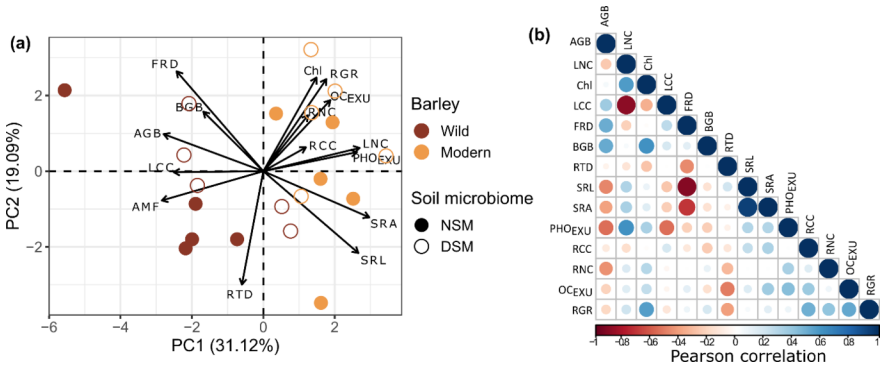

Fig. 2. (a) Principal component analysis (PCA) and (b) correlations of aboveground and belowground plant traits of wild and modern barley with disturbed (DSM) and non-disturbed NSM) soil microbiome. Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%), Leaf Chl: Leaf Chlorophyll Concentration (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 $plant^{-1}$).

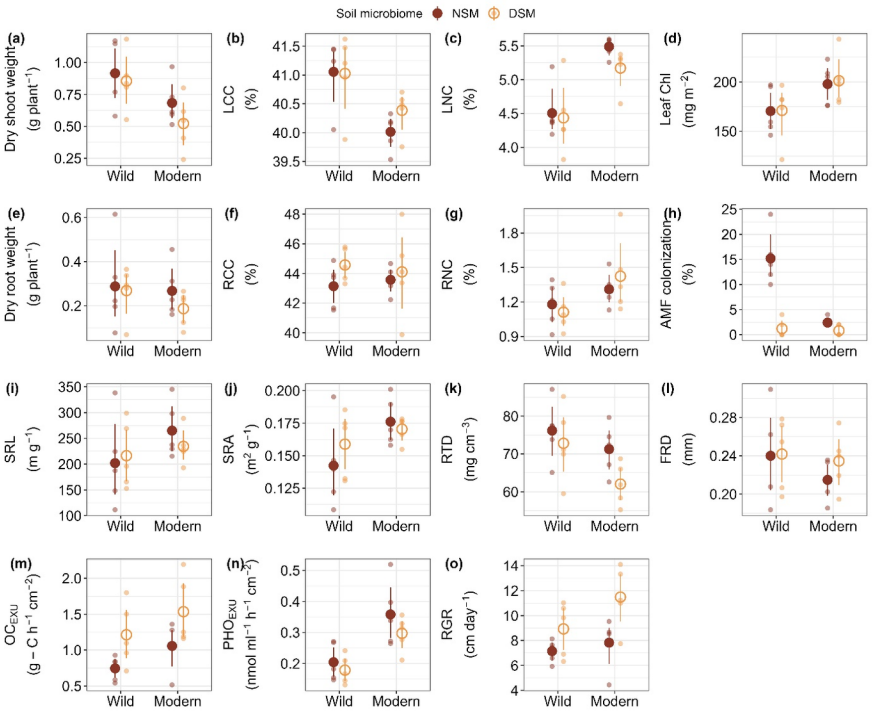

Fig. 3. Aboveground and belowground trait responses of wild and modern barley to disturbed (DSM) and non-disturbed NSM) soil microbiome. Presented are the means for each trait and 95% compatibility intervals that were computed by using a non-parametric bootstrap resampling with 10,000 iterations. Individual observations are also presented for each trait (n = 5). Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%), Leaf Chl: Leaf Chlorophyll Concentration (mg m^{-2}), RCC: Root Carbon Concetration (%), RNC: Root Nitrogen Concentration (%), AMF colonization: Root length colonized by arbuscular mycorrhizal fungi (%), SRL: Specific Root Length (m g^{-1}), SRA: Specific Root Area (m^2g^{-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 $plant^{-1}$). For descriptive statistics, see table 1.

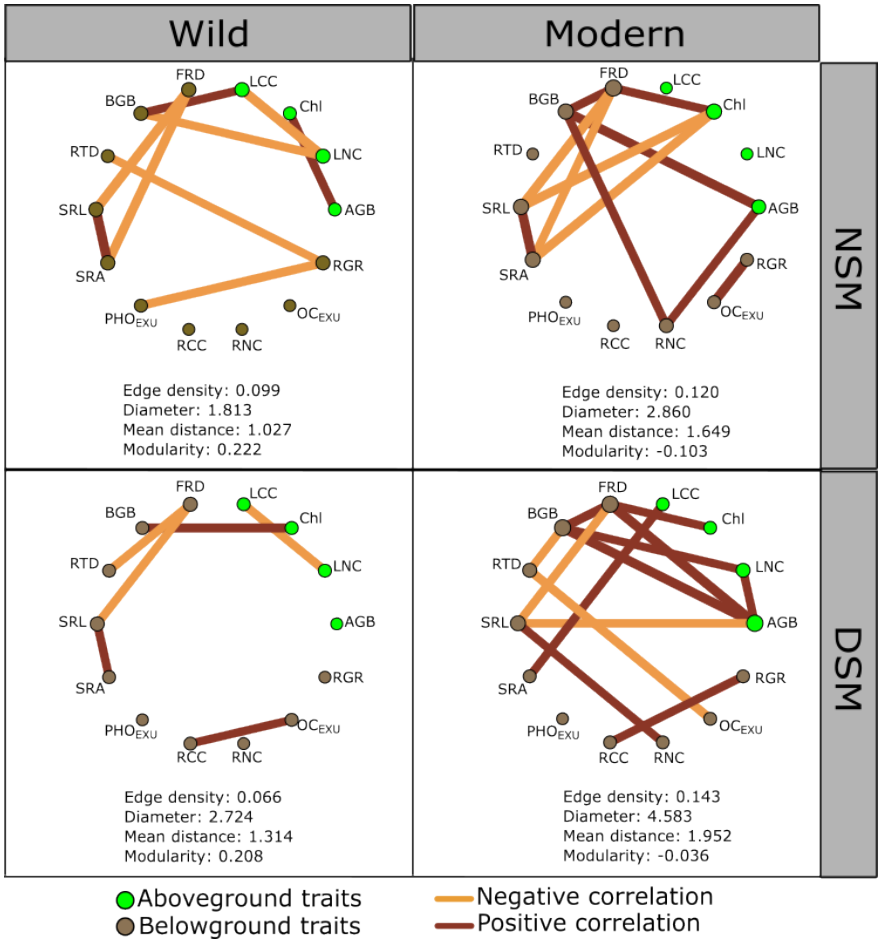

Fig. 4. Trait correlation network of wild and modern barley with disturbed (DSM) and non-disturbed (NSM) soil microbiome. Correlation coefficient threshold was set at 0.7 and only strong correlations (p = 0.05) are shown. Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%), Leaf Chl: 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^2g^{-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 $plant^{-1}$).

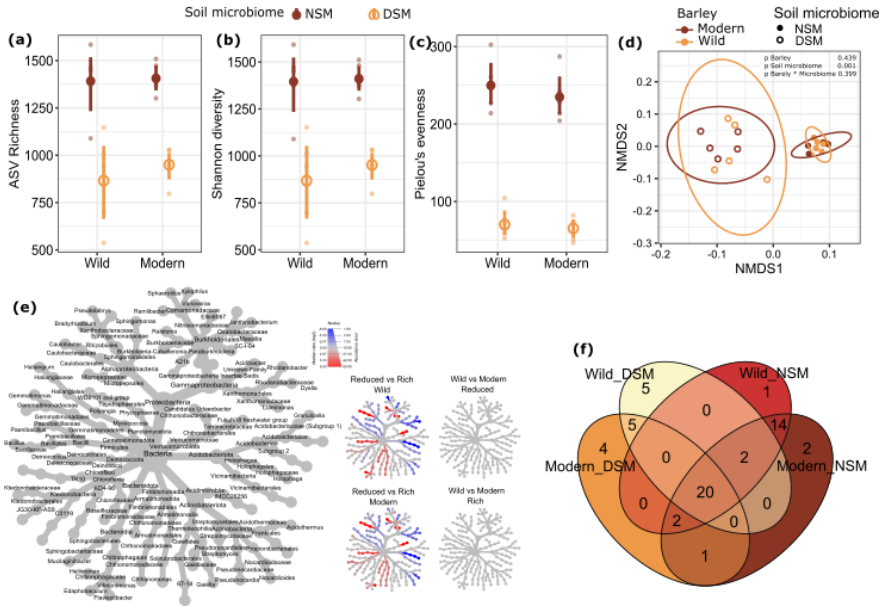

Fig. 5. (a-c) Bacterial richness, diversity, and evenness in the rhizosphere of wild and modern barley as impacted by soil microbiome (DSM versus NSM). Presented are the means for each trait and 95% compatibility intervals that were computed by using a non-parametric bootstrap resampling with 10,000 iterations. Individual observations are also presented for each trait (n = 5). (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.

	CHL	**LNC**	**LCC**	**AGB**	**RNC**	**RCC**
Barley species (B)	**0.02****	**< 0.001****	**0.002****	**0.01****	**0.03****	0.98
	(F1,16 = 6.57)	(F1,16 = 26.42)	(F1,16 = 13.43)	(F1,16 = 8.42)	(F1,16 = 5.06)	(F1,16 =
Soil microbiome (SM)	0.86	0.26	0.46	0.27	0.81	0.24
	(F1,16 = 0.03)	(F1,16 = 1.32)	(F1,16 = 0.56)	(F1,16 = 1.29)	(F1,16 = 0.05)	(F1,16 =
B x SM	0.9	0.46	0.39	0.61	0.37	0.58
	(F1,16 = 0.01)	(F1,16 = 0.55)	(F1,16 = 0.76)	(F1,16 = 0.26)	(F1,16 = 0.84)	(F1,16 =

Table 1: Linear model ANOVAs for the effects of barley species (B, modern versus barley), soil microbiome (SM, DSM versus NSM), and their interactions (B x SM) on Leaf Carbon Concentration (%), LNC: Leaf Nitrogen Concentration (%),CHL: Leaf Chlorophyll Concentration (mg m^{-2}), RCC: Root Carbon Concetration (%), RNC: Root Nitrogen Concentration (%), AMF colonization: Root length colonized by arbuscular mycorrhizal fungi (%), SRL: Specific Root Length (m g^{-1}), SRA: Specific Root Area (m^2g^{-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 $plant^{-1}$). Bold letters with ** and * indicate significane at $p < 0.05$ and 0.1, respectively. Presented are p and F values.

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